University of Reading

Novel Strategies to Mitigate Snakebite Burden in India

A thesis submitted to the University of Reading in partial fulfilment for the Degree of Doctor of Philosophy

School of Chemistry, Food, and Pharmacy

Anika Salim

January 2024

﴿ بِسْمِ اللهِ الرَّحْمٰنِ الرَّحِيْم ﴾

In the name of God, the Most Gracious, the Most Merciful

Contents

Theme 1: Clinical management of SBE

Theme 2: SBE mitigation and public awareness

Appendices

Acknowledgements

First and foremost, I must thank and praise God, for His guidance and the opportunity to pursue my education further.

I want to acknowledge the extraordinary debt I owe to my supervisors Professor Sakthivel Vaiyapuri and Professor Ketan Patel who have guided me as a scientist and researcher. They have supported every hare-brained crazy idea and experiment, and stood in support of me, their patience and faith unwavering. They have been instrumental in my personal development, and I will always be grateful.

Professor Sakthivel Vaiyapuri's impact on my life is far greater than a purely academic one. I have learnt so much more from him, his ideals, influence, critique, extensive discussions (and arguments) around our work (and timelines!), passion for snakebite research and saving lives are the reasons I completed this work. He put his trust in me and gave me the freedom and flexibility to explore all my scientific ideas. It has been an honour to have been supervised by you.

I would like to thank Harry Fonseca Williams, Dina Abdulrahman I. Albadawi, Medha Sonavane, Adam Gadd, Loki Guo, Hussain Bin Haidar, Ali A Kh R Alqallaf, Soheil Gilabadi, Jomanah A Kh R Alqallaf, Soofia Khatibi, Jarred Williams, Jose De Almeida, Alex Crump and all my colleagues and collaborating partners in India and around the world, who supported me in this research and thesis.

On a more personal note, I would like to thank my family - my parents Mohammed Salim, and Rafia Khanum, my brothers Atif Salim, Asim Salim Khan, and Akil Salim Khan, my husband Marco Daniyal Claassen, my second parents Marianne and John Claassen for their prayers, support, encouragement, love and for all the sacrifices they have made for me to complete this journey and to whom I am eternally grateful.

DECLARATION

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Anika Salim

Abbreviations

3FTXs	Three finger toxins
AI	Artificial Intelligence
AIDS	Acquired Immunodeficiency Syndrome
ASHA	Accredited Social Health Activist
ASV	Anti-snake venom
AYUSH	Ministry of Ayush
BPL	Below Poverty Line
CDC	Centers for Disease Control and Prevention
CHC	Community Health Centres
EU	Europe
FRU	First Referral Units
GDP	Gross Domestic Product
GIS	Geographic Information System
GMP	Good Manufacturing Principles
HCSAP	Health Canada Special Access Program
HDI	Human Development Index
HIV	Human Immunodeficiency Virus
HWC	Health and Wellness Centres
ISCICS	Irula Snake Catchers' Industrial Cooperative Society
kDa	Kilodalton
LMIC	Low Middle-Income Country
MMR	Maternal mortality ratio
NASBR	North American Snakebite Registry
NDHM	National Digital Health Mission
NGAL	Neutrophil Gelatinase–Associated Lipocalin
NHPS	National Health Protection Scheme
NSSO	National Sample Survey Organisation
NTD	Neglected Tropical Disease
PLA_2s	Phospholipase A_2
PHC	Primary Health Centres
PMJAY	Pradhan Mantri Jan Arogya Yojana
PTSD	Posttraumatic stress disorder
RSBY	Rashtriya Swasthya Bima Yojana
SARPA	The Snakebite Assistant
SBE	Snakebite envenomation
SDH	Sub-divisional Hospitals
SVDK	Snake Venom Detection Kit
SVMP	Snake venom metalloproteinases
SVSP	Snake venom serine proteinases
UK	United Kingdom
UN	United Nations
UNDP	United Nations Development Programme
USA	United States of America
VDK	Venom Detection Kit
WBCT	Whole Blood Clotting test

WHO	World Health Organization
₹	Indian Rupee

Abstract

Snakebite envenoming (SBE) is a neglected tropical disease affecting the rural impoverished communities in many tropical and subtropical countries. Around 5.4 million people are affected by snakebites each year resulting in around 1.8-2.7 million cases of envenoming, 140,000 deaths and triple the number of amputations and permanent disabilities. India is considered the 'snakebite capital' of the world and it is estimated to account for up to 50% of these snakebite statistics. Unfortunately, accurate epidemiological field data on SBE like most other neglected tropical diseases is lacking, and often remains incomplete; this can be attributed to multiple reasons such as a lack of policy, inadequate funding to conduct the research, limited medical and health infrastructure for data collection and monitoring and difficulties in ascertaining reporting data in rural areas to measure the true impact of SBE. Delays in seeking prompt hospital treatment are one of the primary causes of SBE-induced deaths and disabilities. The root causes for patients to defer and postpone hospital treatment are many and complex, from strong confidence in traditional healers for treating SBE to fear of high treatment costs in hospitals. The clinical presentation following SBE of many snake species across India can be quite different, but there are no defined diagnostic tests or biomarkers available to aid prompt diagnosis of SBE specifically in rural healthcare settings. Notably, healthcare professionals in rural settings often lack confidence and training in managing SBE. Hence, in the first theme of this study, we sought to develop the use of novel biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) in Russell's viper bites, determine the most effective yet affordable antibiotics for SBE, evaluate the treatment costs for SBE in private tertiary care hospitals and establish knowledge gaps and training needs for healthcare professionals to pinpoint areas of focus to improve the clinical management of SBE in India. In the second theme of this study, we aimed to determine the level of snake phobia in Indian communities, understand the challenges faced by snake rescuers who play a vital role in mitigating SBE burden while saving lives, and the necessity to improve public awareness about SBE. Overall, this study has underpinned various aspects of SBE, and these findings will form a strong base to develop several health policies to mitigate the SBE burden in rural India.

Publications and Presentations

The following publications originated within the timeframe of my PhD:

Neutrophil gelatinase–associated lipocalin acts as a robust early diagnostic marker for renal replacement therapy in patients with Russell's viper bite–induced acute kidney injuries. Senthilkumaran, S., Patel, K., Salim, A., Vijayakumar, P., Williams, H. F., Vaiyapuri, R., & Vaiyapuri, S. (2021). Toxins, 13(11), 797.

The effectiveness of antibiotics in managing bacterial infections on bite sites following snakebite envenomation. Senthilkumaran, S., Salim, A., Almeida, J. R., Williams, J., Vijayakumar, P., Thirunavukarasu, A. & Vaiyapuri, S. (2023). Toxins, 15(3), 190. Equal first author.

Identifying key factors contributing to treatment costs for snakebite envenoming in private tertiary healthcare settings in Tamil Nadu, India. Salim, A., Williams, J., Abdel Wahab, S., Adeshokan, T., Almeida, J. R., Williams, H. F., & Vaiyapuri, S. (2023). PLOS Neglected Tropical Diseases, 17(10), e0011699.

Multifaceted community health education programs as powerful tools to mitigate snakebite-induced deaths, disabilities, and socioeconomic burdens. Vaiyapuri, S., Kadam, P., Chandrasekharuni, G., Oliveira, I. S., Senthilkumaran, S., Salim, A., & Pucca, M. B. (2023). Toxicon: X, 17, 100147.

Repurposing cancer drugs batimastat and marimastat to inhibit the activity of a group I metalloprotease from the venom of the western diamondback rattlesnake, *Crotalus atrox*. Layfield, H. J., Williams, H. F., Ravishankar, D., Mehmi, A., Sonavane, M., Salim, A. & Vaiyapuri, S. (2020) Toxins, 12(5), 309.

Russell's viper envenomation induces rectus sheath haematoma. Senthilkumaran, S., Almeida, J. R., Williams, J., Salim, A., Williams, H. F., Thirumalaikolundusubramanian, P., & Vaiyapuri, S. (2023). Toxicon, 224, 107037.

Publications under review

Determining the knowledge gaps and training requirements amongst Indian healthcare professionals for snakebite clinical management

Assessing the prevalence of snake phobia among the general population in India

Challenges in rescuing snakes to save lives from snakebites in Tamil Nadu, India

Oral presentations at conferences

Salim, A., Analysis of snake phobia in a snakebite endemic country, India. Venoms & Toxins, 10th International Toxicology Meeting, Oxford, UK, 2023.

Salim, A., Challenges in rescuing venomous snakes to save lives in India. Venoms & Toxins, 9th International Toxicology Meeting, Oxford, UK, 2022.

Salim, A., Neutrophil Gelatinase-Associated Lipocalin Acts as a Robust Early Diagnostic Marker for Renal Replacement Therapy in Patients with Russell's Viper Bite-Induced Acute Kidney Injuries. Celebrating Excellence in Research Engagement and Impact, Research Excellence Awards, University of Reading, 2022.

Salim, A., Neutrophil gelatinase-associated lipocalin acts as a robust early diagnostic marker for renal replacement therapy in patients with Russell's viper bite-induced acute kidney injuries. Reading School of Pharmacy PhD Showcase, University of Reading 2022

Salim, A., Novel Strategies to mitigate snakebite-induced socioeconomic burden on rural communities. Graduate School Doctoral Research Conference, Research for a Better World Competition, University of Reading, 2022

Salim, A., Determining the knowledge gaps and training requirements among Indian healthcare professionals to improve snakebite management. Venoms & Toxins, 8th International Toxicology Meeting, Oxford, UK, 2021.

Salim, A., Snakebite management amongst Indian healthcare professionals. Reading School of Pharmacy PhD Showcase, University of Reading 2021.

Salim, A., Development of novel therapeutic strategies for snakebite-induced muscle damage. Reading School of Pharmacy PhD Showcase, University of Reading 2020.

Posters

Salim, A., Snakebite Prevention and Awareness for Children in Tamil Nadu, India - Graduate School's Research Poster and Research Film competition, University of Reading, 2022

Communications in magazines

Salim, A. and **Vaiyapuri, S**., Venomous Snakebites, a community education programme. University of Reading Graduate School Doctoral Research Highlights 2022

Funding and Awards

Salim, A. and **Vaiyapuri, S**., Early Career Researcher (ECR) Best Research Output Prize for the Agriculture, Food and Health theme, University of Reading, 2022.

Salim, A. and **Vaiyapuri, S**., University of Reading Graduate School: Research for a Better World Competition, Global Category, Winner, 2022.

Salim, A. and **Vaiyapuri, S**., The Friends of the University of Reading Grant for snakebite awareness poster development. 2021.

Chapter 1: Introduction

Chapter 1: Introduction

1.1) Neglected Tropical Diseases (NTDs)

NTDs are chronic and debilitating conditions that mostly affect the impoverished communities in rural regions of developing countries. The definition and inclusivity of NTDs have been expanded over time to include parasitic, helminthic, protozoan, bacterial, fungal as well as ectoparasitic infections [1] [2]. Their diversity means that their epidemiology, risk factors and prevention and control mechanisms vary widely. It is noteworthy that NTDs can be prevented and treated via relatively simple public health measures [3]. When considering the scale of NTDs globally, the World Health Organisation (WHO) ranks Southeast Asia as the most populous region for NTD interventions that are required to reach around 857 million people, tailed by the African Region of 584 million people. The Americas, Europe, Eastern Mediterranean and Western Pacific regions account for 212 million people requiring interventions, equivalent to 12.9% of the global total [4]. Moreover, more than 1 billion people equating to a sixth of the world's total population are afflicted by one or more NTDs [4]. These diseases have vast economic implications for the developing world, as the resultant clinical sequelae, disabilities and long-term health implications affect the economic productivity of communities and in turn the respective countries and their growth [5, 6]. A key distinguishing feature is their lack of visibility for public health policies, and as a result, accurate data on disease burden is often missing and it lowers their ranking as a public health priority to address them. Thus, they remain neglected at various strata within the health architecture including at the community, national and international levels [7]. Reducing and/or eliminating NTDs requires reliable epidemiological data, adequate political support, and health infrastructure and accessible and affordable treatments for patients. Prevention is always better than cure, so due emphasis must also be placed on public health awareness strategies for NTDs.

1.2) Snakebite Envenoming (SBE) as an NTD

In 2017, the WHO's Strategic and Technical Advisory Group for Neglected Tropical Diseases prompted the WHO to formally recognise SBE as an NTD. This in turn advised the implementation of a globally coordinated plan of action to set forth strategies to reduce the mortality and disability from SBE by 50% by 2030. Thus enshrining SBE as not only a healthcare priority but as a significant topic of ethical and moral duty for the wider international scientific and medical communities to solve this issue [8] [9] [10] [11]. SBE is considered a high-priority NTD affecting mostly the impoverished communities in many tropical and subtropical countries. Around 5.4 million people are affected by snakebites (all bites including from non-venomous snakes and dry bites from venomous snakes), resulting in 1.8-2.7 envenomings (i.e. SBE when the venom is injected into the body of victims),

140,000 deaths and 400,000 permanent disabilities each year. SBE predominantly affect poor rural communities and instigates significant socioeconomic impacts in low and middle-income countries across Asia, Africa and Latin America [8]. The issue of SBE particularly affects developing countries has a multitude of reasons and it is thought that around 95% of deaths and disabilities originate from these countries although some well-developed countries such as the USA and Australia also have SBE incidents (**Figure 1**) [12].

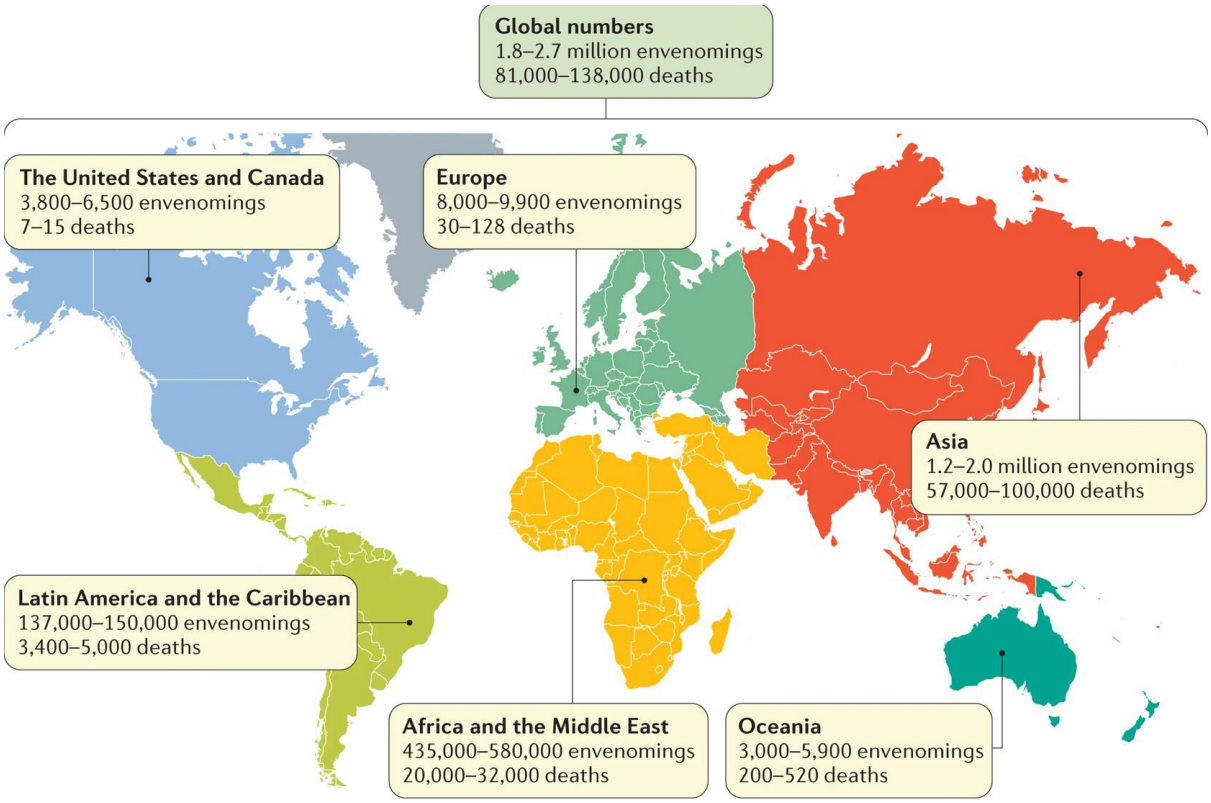


Figure 1: The Global distribution of estimated SBE incidents and mortalities. This image was taken from Gutiérrez, J. *et al.* (2017) [13].

1.2.1) SBE in developed countries

North America mainly consists of the United States of America (USA) and Canada both of which have vast and distinct landscapes from desert to prairie, forests, and arctic mountain ranges. The snake fauna in the USA is quite diverse with around 100 species, of which around 20 are considered venomous. Most of these belong to either the *Viperidae* or *Elapidae* family [14]. It is estimated that in the USA, there are approximately 5,000–10,000 emergency department visits because of snakebites and around 50% of these are usually due to venomous snakes. The most common cause of bites is due to

rattlesnakes (*Crotalus sp.*) followed by copperheads (*Agkistrodon contortrix*), cottonmouths (*Agkistrodon piscivorus*) and coral snakes (*Micrurus fulvius*) [15] [16]. In 2013, the American College of Medical Toxicology established the North American Snakebite Registry (NASBR), a physician-led endeavour aiming to track SBE profiles and outcomes in patients with the hope of it being a national database [17]. It is important to note that despite endemic venomous species, SBE statistics in the USA include bites from several non-native snake species such as exotic venomous snakes held in captivity as pets and in zoos [18] [17]. SBE in the USA is not a public health concern due to the availability of two regulatory-approved antivenoms such as CroFab® and Anavip®, both of which are effective with minimal adverse events, widely available and accessible [19] [20]. Moreover, the health infrastructure in the USA allows patients to have access to emergency care, trained healthcare providers to manage their clinical symptoms and adequate insurance to cover the treatment costs when needed.

Canada is thought to have around 26 species of snakes of which 3 are venomous, all of which are members of the pit viper (*Crotalinae*) subfamily. These include the western rattlesnake (*Crotalus oreganus*), the prairie rattlesnake (C*rotalus viridis*), and the eastern massasauga rattlesnake (*Sistrurus catenatus*) [14]. The frequency of snakebites in Canada is low and conservative estimates from 2009 to 2015 show that around 99 envenomations were reported [21]. Canada, like the USA, also has a highly developed healthcare infrastructure and the Health Canada Special Access Program (HCSAP) can promptly offer both CroFab® and Antivipmyn™ to patients, so SBE is not a public health concern [21].

Europe (EU) is a comparatively smaller continent of around 50 countries, with equally diverse landscapes as smaller geographic areas form unique sub-regions, e.g. the Iberian peninsula [22]. Currently, there are around 57 species of snakes in Europe, belonging to six different families, *Colubridae*, *Erycidae*, *Natricidae*, *Psammophiidae*, *Typhlopidae* and *Viperidae* [23] [24]. Nearly all venomous snakes in the EU belong to the *Viperidae* family [25]. Out of the 14 viper species, six are thought to be of medical importance as they represent the greatest number of bites. These include the horned viper (*Vipera ammodytes*), asp viper (*Vipera aspis*), European adder (*Vipera berus*), Lataste's viper, (*Vipera latastei*), Basquian viper (*Vipera seoanei*) and meadow viper, (*Vipera ursinii*) [26] [24]. Very few countries (such as France and Sweden) in the EU track SBE data [27]. According to current data, the EU has around 7500 cases of snakebites every year, of which around 1000 result in envenoming with very few deaths [24] [27]. Most SBE events in the EU do not require immunotherapy, however, several antivenoms are available specifically against the vipers. Therefore, the patients and health systems are adequately prepared to manage SBE in the EU [28].

Australia is one of the largest countries in Oceania with a unique snake fauna of around 156 species, 90 of which are venomous [29]. However, there is only one snake that can be considered to be the most medically important and this is the eastern brown snake (*Pseudonaja textilis*), belonging to the

Elapidae family [30]. The relative SBE incidence and mortality rates remain low in Australia, and this can be attributed to low levels of snake encounters principally due to the low population ratio compared to land mass, well-trained healthcare professionals to manage envenomation, use and availability of snake detection kits along with an adequate and effective supply of antivenoms [31]. Most importantly the Australian healthcare system has implemented robust infrastructure such as the Royal Flying Doctor Service of Australia and clinical training for all health professionals to manage SBE events successfully along with envenoming from other animals, e.g. spiders.

The impact of SBE in the developed world is of minimal health concern, due to fewer venomous species of medical importance, reduced human-snake interactions, urban development, excellent transport, and logistics along with effective health infrastructure, trained healthcare professionals and effective antivenoms. Thus, SBE in the developed world is not a public health concern. Moreover, any potential risk of SBE has been effectively mitigated with the availability of polyvalent antivenom, snakebite detection kits (in Australia) and effective public awareness ensuring prompt access to treatment.

1.2.2) SBE in developing countries

There are many definitions of what constitutes a developing country but according to the United Nations Development Programme (UNDP), the most simplistic approach to define this concept is the Human Development Index (HDI) which is a measure of development relative to other countries [32]. Developing countries often have the lowest HDI which manifests as less industrialised, less developed infrastructure and economies and this translates into low financial growth and per capita income. The underlying question of why the developing world is still developing has been under much scrutiny by scientists for decades and many theories proposed for why this is the case [33, 34]. There is no doubt that countries closer to the equator are not only climatically stable but also more populous and poorer, with high disease and pathogen burden. The world's equatorial regions are represented by countries in Latin America, Africa, and Southeast Asia. Some countries are far better than others in terms of Gross Domestic Product (GDP) per capita, yet they remain below the global average and the lowest GDP per capita is found in Africa and Southeast Asia [33]. GDP is a determinant of a country's economic growth, which can be continuously stable, accelerated or decelerated in certain financial contexts and is important to the country's development. Economic growth can mean better investment into public services and infrastructure development creating more industrialised nations which in turn continues further growth and results in a higher relative location in the HDI [35].

Unfortunately, SBE disproportionately affects the developing world in comparison to more developed and industrialised nations. To determine why SBE thrives in such countries we must strive to understand what could have led to this. The true global incidence rate of SBE is underreported. Of 237 countries (193 recognised by the United Nations as well as including all other observer states, self-ruling territories and sovereign states) in the world, only 45 countries were identified as having published literature with data on snakebites, 58 countries were recorded as having no venomous snakebites, and 92 countries were found to have no data on snakebites [36]. Thus, the lack of data on SBE burden is not surprising when considering its NTD status. The true representation of SBE is negatively affected by the lack of field data, reporting statistics, national databases, and epidemiological research. This poses further challenges to solving the issue, meeting the WHO targets, and saving lives. The landscape of poverty and financial inequality in low to middle-income developing countries is a complex issue and studies have highlighted that political instability (conflict), poor governance, lack of investment in education, healthcare, and economic and agricultural developments negatively impact their growth. In addition, potential inequalities in gender, caste, religion and tribal affiliation can prevent access to vital resources [37]. The limited knowledge and lack of access to healthcare compels patients to seek traditional treatments embedded within their cultural context, and so again any deaths and disabilities here remain unrecorded. The developing world bears the brunt of NTDs such as SBE compared to the developed world. The regions with the highest SBE burden are tropical Southeast Asia specifically the Indian subcontinent, Latin America and sub-Saharan Africa [36] [38] [39]. Consequently, the majority of the ~5.4 million people suffering from snakebites annually will be residing here and so the ramifications of envenomings are greater here as a public health concern. Here, I will briefly review the SBE context in the developing world.

Latin America and the Caribbean consist of a cluster of many countries yet despite common customs they have individual differences from independent geographies, climates, governance, and cultures. It is estimated that around up to ~150,000 envenoming cases occur per year in this region, yet the true magnitude is likely to be higher due to the lack of reporting from rural indigenous areas [40]. Latin America has an extraordinary snake fauna yet the snakes of the *Bothrops* genus are the most medically important, with the Fer-de-lance (*Bothrops atrox*) being considered the most medically significant snake contributing to most SBE fatalities in this region [41]. The Instituto Butantan in Brazil and Instituto Clodomiro Picado in Costa Rica have been instrumentally active in advancing antivenom therapy for many decades along with newer entities such as Instituto Bioclon in Mexico to tackle SBE. Despite the efforts of publicly funded institutions to control tropical NTDs, SBE is still a public health concern, and one main contributory factor is the inability to adequately supply antivenom to meet the demand along with the logistics and public health awareness in providing antivenom to remote communities [42].

The burden of SBE in Africa and in particular sub-Saharan Africa is a significant public health issue compounded by various factors which also contribute to the high burden of other NTDs across the continent. The ability to quantify the SBE burden is hindered by the lack of robust reporting mechanisms and the nature of the disease itself [43]. At present it is estimated that up to ~580,000 envenomings occur annually with much of these transpiring in rural areas amongst agricultural workers with males being predominantly at high-risk [44]. There are around 110 venomous snake species in Africa of which the WHO considers 24 species to be the most medically significant. These include species from the *Bitis, Dendroaspis, Echis* and *Naja* genera [45]. As with other regions that score low on the HDI, the African region struggles with many issues such as patient access to care, underdeveloped healthcare infrastructure, affordability of treatment and access to effective antivenoms. Indeed, the WHO predicts that a complete supply failure of antivenom is a looming reality here unless action is taken by the wider scientific and pharmaceutical communities [45]. Thus, treating and preventing SBE in Africa poses immense challenges and has the potential to trigger a humanitarian crisis if suitable antivenoms are not produced to meet the demands.

Much like SBE in the African context, the accurate SBE burden in Southeast Asia remains difficult to capture. However, it is assumed that this region has the highest risk of SBE in comparison to the others simply due to its high population density [46]. The current estimates suggest that up to 2 million envenomings occur annually. There are around 45 medically important venomous snakes in Asia with the Russell's viper (*D. russelii*), saw-scaled viper (*E. carinatus*) and hump-nosed pit vipers (*H. hypnale*) responsible for the highest associated mortalities and morbidities in the region [47]. Unlike in sub-Saharan Africa, domestically manufactured antivenoms are available although often not with the highest level of efficacy [48]. A majority of SBE-associated deaths in these countries are thought to be premature due to the lack of access to affordable and effective clinical care.

This thesis will principally focus on the SBE burden in India and novel strategies to mitigate this. Here I describe how and why India has inherited the moniker of the 'snakebite capital of the world', the unique issues surrounding healthcare and what can be done to reduce the burden of SBE and save lives as well as continue to preserve the unique reptile ecosystem.

1.3) India, the 'snakebite capital' of the world

India is the 'snakebite capital' of the world as it is predicted to have over one million bites per year leading to around 58,000 deaths, which largely affects rural populations specifically working-age adults (**Figure 2**) [49]. The true estimates of SBE are predicted to be much greater. SBE deaths and disabilities in India need to be significantly reduced and prevented. Several actions can be taken to address the root causes contributing to the high rates of SBE-induced deaths and disabilities. The large diverse geographical spread of the population, lack of adequate infrastructure in rural areas and other

important health and disease burdens often push the priority of tackling SBE to the bottom of the NTD list.

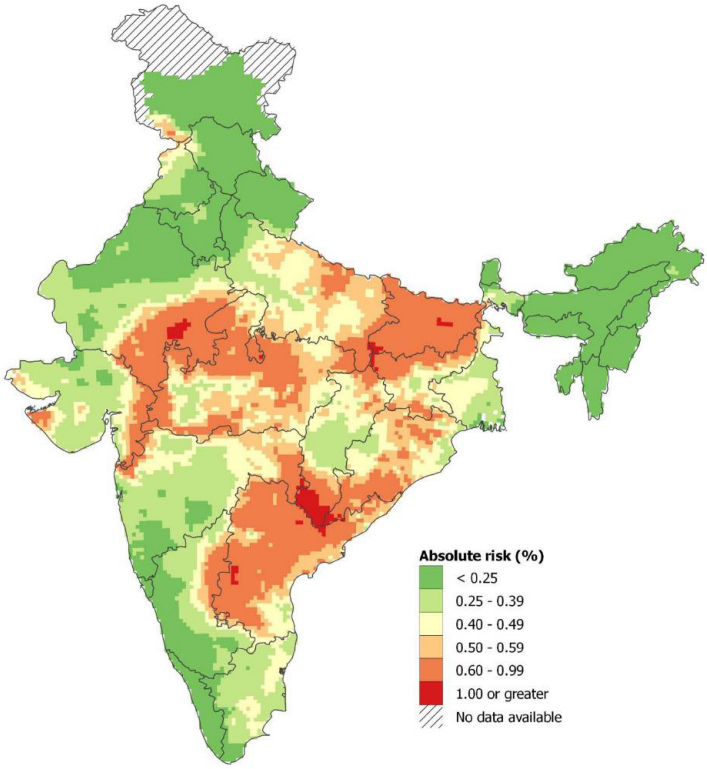


Figure 2: The risk of SBE-induced mortalities in the Indian population compiled with data collected from 2004-2013. This image was taken from Suraweera *et al.* (2020) [50]. This map illustrates the spatial distribution and level of the risk posed across India. Areas coloured red/orange show the highest level of risk.

1.3.1) Medically Important Snakes of India

There are three major families of venomous snakes that are responsible for most SBE incidents in India. These are *Elapidae* (Indian cobra, and krait), *Viperidae* (Russell's and saw-scaled vipers) and *Hydrophidae* (sea snakes). In India, over 200 species of snakes have been identified, 52 of which are venomous, and they mainly include the 'big four' snakes, which are responsible for most snakebites in India. The 'big four' snakes comprised of the common krait (*Bungarus caeruleus*), Indian cobra (*Naja naja*), Russell's viper (*Daboia russelii*) and saw-scaled viper (*Echis carinatus*) (**Figure 3**) [51].

Figure 3: The 'big four' snakes of India. A) saw-scaled viper, B) Indian cobra, C) krait and D) Russell's viper. Images were provided by Prof. Sakthivel Vaiyapuri as part of the 'Venomous Snakebites: Rapid Action Saves Lives programme [52].

1.3.2) The socioeconomic impact of snakebites in India

The National Sample Survey Organization (NSSO, 71st Round) formed by the Government of India conducted surveys to assess disease prevalence and morbidities [53]. Here, it was noted that out-of-pocket expenses by patients constitute 64.2% of the financing for the healthcare sector. Moreover, only 28.6% of the total health expenditure is financed by the government itself [54]. Overcrowding, administrative inefficiencies, and lack of adequate clinical professional development as a result of the pressurised public health sector impact the government's ability to grow, develop as well as sustain healthcare for the masses [55] [55-57] [58]. This in turn affects the ability of the Indian government to comprehensively implement effective healthcare interventions which ultimately affect patients, not only concerning their treatment but also their healthcare-seeking behaviour. Consequently, the barrier to accessing any potential benefits the local healthcare system has to offer is impacted and inadequate frameworks often push patients to source alternative routes such as expensive private healthcare services. These serve to provide more assurance on the quality of their care to the patient, their family and communities [54]. The private healthcare sector in India is growing and accounts for 80% of the total outpatient and 60% of the total in-patient care in India [53]. This means that patients including

SBE victims are not completely absolved from paying for healthcare services. Hence, there is an immediate need for improved financing mechanisms and medical insurance for SBE and ensuring a smoother flow of the already existing policies and framework to make it easier for afflicted communities to benefit and mitigate their socioeconomic burden [59].

Normally, socioeconomically disadvantaged communities will be impacted more considerably compared to other sectors of society. An unintended medical event such as SBE can be catastrophic for poor families and their future [60] [61] [62]. Often poor people seek healthcare to a lesser extent and rely on cheaper locally available traditional therapies not only because of a lack of access to funds but also due to inadequate infrastructure in the rural public healthcare sector, difficulties in accessing public transport and a lack of awareness of access to hospitals. Indigenous medicine plays an important role in the health structure of developing countries, not just in India but also in sub-Saharan Africa as well as Latin America. It is important to note that Indian Systems of Medicine play a key role in providing healthcare to vast members of the population who may not otherwise have access to traditional care and represent a cost-effective economic alternative. SBE treatment is entrenched in folklore, culture and traditional healers [63] [64]. Moreover, poor people often have a higher rate of illnesses compounded by a lack of education, and more complex health needs because of delaying or avoiding hospital treatment, and lack of facilities, health knowledge and healthy nutrition. All these factors are heightened in resulting SBE clinical manifestations and complications.

India as a country has the highest rate of out-of-pocket health expenditures across the Asian continent. It is estimated that 8% of the population falls into poverty due to these out-of-pocket expenses. This leads to the poor becoming poorer [65]. Historical research conducted by our group involving household surveys in Tamil Nadu, India has provided a spotlight on the socioeconomic ramifications of SBE on rural agricultural communities. These surveys highlighted that none of the SBE victims interviewed had medical insurance and their medical costs could be upwards of ₹350,000 (£4,858/$4223.36), with 75% of victims going on to seek treatment at private hospitals. The financial impact of this was crippling on the families' already stretched finances, especially when one considers the meagre daily wage for subsistence farming and agricultural workers. This meant that over 40% of victims went either into debt to pay for loans acquired for SBE treatment or sold their valuables and belongings to offset the costs of these loans. This involved selling stored crops, livestock, land, property, and vehicles as well as sometimes removing children from formal education so that they could save on costs and gain employment to help appease the family's financial hardship [66]. Notably, in 2022, ₹11,924 (£112.89/$143.88) was the average monthly income per agricultural household in Tamil Nadu according to the Ministry of Agriculture & Farmers Welfare, India [67]. Therefore, even a bill of ₹100,000 is almost equivalent to a whole year's worth of wages for such workers. Rural and agricultural communities have a higher risk of SBE simply as an occupational hazard, and these are the communities

that can least afford treatment. Out-of-pocket expenses due to an SBE are inevitable and thus, some patients forego earlier treatments until conditions worsen to necessitate clinical intervention. This not only results in increased medical complications for the patient but also increased health expenditures because of unnecessary delays. Greater awareness of these implications is needed at the community level to reduce the socioeconomic burden on patients.

1.4) The Indian Healthcare System & Infrastructure

The foremost challenge for any country is equitable public expenditure and this challenge is only more pronounced in low to middle-income countries with minimal resources but heavy public expenditure burdens. Adequate resourcing of education, health care, social welfare and safety are stretched and can be exacerbated by natural disasters, conflict, and political and financial instabilities. Adapting and ensuring resources are used efficiently and effectively to affect measured change and provide resilience at a grassroots level presents many obstacles. According to the World Bank classifications, India is ranked as a lower-middle-income country (LMIC). With vast economic growth, health systems and their infrastructure are required to grow at an equal rate to keep up with sustained growth. However, India has a unique epidemiological scenario and is currently facing a triple burden of diseases including reproductive health (maternal mortality), child health and nutrition. This is along with other unresolved major communicable diseases and a high burden of non-communicable diseases (NCDs) [68]. Evidence from the World Bank and the Indian government suggests a stagnation in government spending on healthcare when considering India's Gross Domestic Product (GDP); which also varies when reflecting on state and central-level support. When India is compared to other Asian countries such as China, the total health expenditure as a proportion of GDP to population size is significantly lower [69]. The healthcare infrastructure of developing countries is lacking and its creation and implementation require substantial time, investment and complex partnerships to ensure functionality, including but not limited to urban planning as well as transport logistics [70]. This can also be regionally specific, for example, ensuring clean water facilities, electricity, cold storage, and supply chains. Not only does this require political will and expenditure but also the administrative investment of management to ensure facilities operate efficiently to meet the country's health goals [71].

Hence, with a diverse population of more than 1.4 billion people from various ethnic groups, religions, tribes, castes and classes as well as gender-based disparities, providing adequate affordable healthcare for everyone in India is not an easy task [72] [73] [74]. Since the Indian Independence Act was passed in 1947, the first national health policy of India was officially developed in 1983 to ensure primary healthcare services to all members of the population by the year 2000 [68]. Based on India’s economic development over time and its mass population, much progress has been made in developing its health infrastructure and patient access to healthcare services to support the mass population. Some

states perform better than others. Some less developed states and areas require improvement in their infrastructure [75]. India has a dual healthcare system consisting of a private as well as a public healthcare system. The latter is governed by various ministries of central and state governments. The presence of a dual healthcare system means the private sector is largely unregulated with varying standards and quality of care. These are also usually located in larger urban areas with high population densities and are not always available to the rural poor communities [68].

The public healthcare system in India provides free healthcare and is structured across tiers (**Figure 4**). These include tertiary care facilities such as district hospitals and medical college hospitals at the highest level delivering the most specialist care, followed by community health centres (CHC), sub-divisional hospitals (SDH) and primary health centres (PHC). The village-level support is usually delivered by accredited social health activists (ASHA - community health workers). It is important to note that ASHA's health workers are usually volunteers and are not trained to support SBE activities [76-78]. Antivenom is provided free of charge to SBE patients in the public health sector, however, as it is administered intravenously only hospitals which have the appropriate facilities and expertise can administer it. Therefore, only some PHCs, SDHs and CHCs can provide effective antivenom treatment. It is important to note that due to a lack of administrative logistics and adequately trained staff such facilities often refer patients to tertiary care facilities where they can access this care. Hence, some patients prefer to simply present at tertiary facilities by acquiring suitable transport, resulting in longer travel times over difficult terrain and potentially increased treatment costs due to medical complications due to delayed treatment.

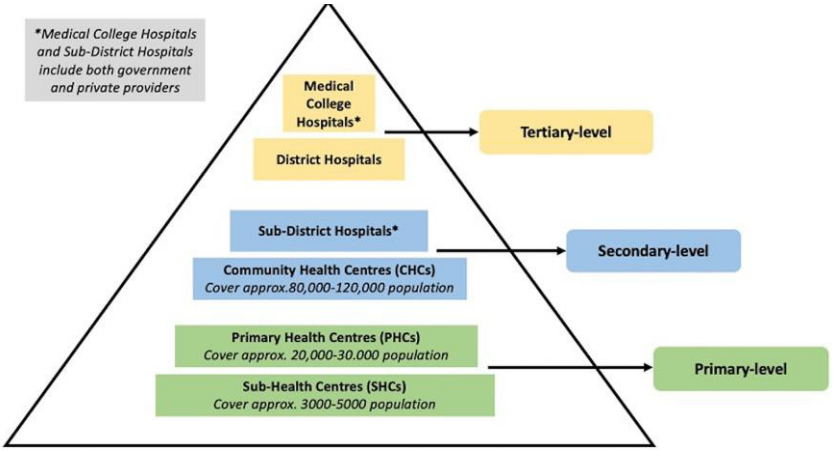


Figure 4: A schematic diagram of the Indian healthcare structure detailing, primary, secondary, and tertiary level care and facilities included therein along with the population service coverage. Image taken from Humphries *et al.* (2020) [79].

The Government of India created several health insurance schemes and funded the National Health Protection Scheme (NHPS) along with the Rashtra Swasthya Bima Yojana (RSBY) which was set up by the Ministry of Labour and Employment, to allow access to social security for those below the poverty line. Similarly, several state governments introduced the Chief Minister's Comprehensive Health Insurance schemes to support poor people to access prompt healthcare. This highlighted focus on the social determinants of health aimed to alleviate the risk of health spending leading to poverty as well as improve health-seeking behaviour amongst rural agricultural communities. However, not all state government insurance schemes are as easily accessible to those who need it. Often patients are required to present identification and payslips and maybe even require a bank account to access such schemes. In addition, not all healthcare providers including private hospitals are approved by these schemes. Therefore, the patients could be denied care as hospitals would be unwilling to admit patients with such insurance. Thus, these schemes all had varying degrees of complexities and success depending on the state. They shed light on the need for improved healthcare in rural areas where poor communities can benefit without cost as a barrier to appropriate healthcare [80].

The RSBY was eventually superseded by the Ayushman Bharat Pradhan Mantri Jan Aarogya Yojana (PMJAY) in 2017, an ambitious national program for universal health coverage, designed to meet the sustainable development goals (SDGs) organised by the UN [81]. This also served to provide more comprehensive measures in care by creating health and wellness centres (HWCs) and by reviewing and improving the original PHCs to expand healthcare services and care. The aim was to provide healthcare and social security to one of the most populous nations in the world along with the largest health insurance scheme with coverage to the most deprived populations [81]. It is important to also note that the government of India has formally recognised the indigenous medical systems and practices in India which fall under the umbrella of the Ministry of Ayush or AYUSH which stands for Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homeopathy [82]. The goal is to not only preserve ancient systems of medicine, research, and development but to also improve health and provide patients with an alternative for their care to strengthen healthcare for Indians [63].

Despite the impressive goals of the Indian Government and the PMJAY, sufficient coverage for poor rural agricultural communities afflicted by SBE is often not sufficient to receive full support. Economic and organisational disparities also remain among Indian states where some have done better than others [83]. Most importantly widespread knowledge amongst communities afflicted by SBE of the PMJAY, their ability to qualify as below poverty line persons, acquisition of services and healthcare insurance and reimbursement present a great challenge to such people. This leads them to not only seek ineffective traditional treatments but also to delay accessing treatment entirely due to fear of unaffordability. Current healthcare awareness and messaging are often ineffective, confusing, and bureaucratic, resulting in low uptake and reduced impact on improving the health of some of the most

vulnerable members of the population. Most importantly, the level of coverage provided by the schemes does not adequately cover the costs of SBE at a patient level. Thus, a single snakebite incident can have vast and longer-lasting socioeconomic ramifications [66] [67]. Moreover, education on access to healthcare is needed across the wider Indian community, especially for those at risk of SBE. The public healthcare sector is often lacking investment due to a chronic lack of government expenditure and other more pressing health priorities which in turn shunt SBE to the bottom of the priority list. In India, the need for healthcare has outpaced the growth and investment in the healthcare system itself. A simpler, effective, and accessible level of insurance which grants access to all treatment facilities is therefore needed.

1.5) Snake Venoms

Venomous snakes inject their venom using modified teeth known as fangs. The venom is produced in the venom glands which are modified salivary glands and housed in the venom ducts until use. The functional role of this venom apparatus and its anatomy has been the subject of much discourse. Since venomous snakes are considered to have evolved from their non-venomous counterparts, it provided them with an evolutionary adaptive advantage over time from prey capture to defence. This can be seen across all venomous snakes considering the anatomical features of fang structure, surrounding musculature and venom reservoirs [84] [85]. Snake species can also be further divided into whether they are front-fanged snakes (vipers that have erectile fangs and elapids that contain fixed fangs) or rear-fanged snakes (such as boomslang, a colubrid snake). The mechanics of venom delivery from both types of snakes remain similar. Upon contact, the snake uses its fangs to cause injury to the skin and the venom is injected into the body through open grooves on the surface of the fangs [86]. A bite by a venomous snake that does not result in any clinical manifestations is called a ‘dry bite’. The estimations of how many venomous snakebites are likely to be ‘dry bites’ are not available, and it needs greater scientific evaluation across several snake species.

Venoms are modified forms of saliva secreted from the venom glands and are composed of a blend of complex molecules forged over time in an evolutionary ‘arms race’ of increasing toxicity with the primary function being to aid the killing, immobilisation and or digestion of the targeted prey [87] [88]. The wide variety of pharmacological and toxicological effects can be attributed to the diverse array of toxins present in venoms. Snake venoms can vary at an inter-species level as well as intra-species level. The venom composition also varies based on age, gender, geographical location, diet, and seasonality. This variability underlies toxin diversity and multifunctionality and is of great importance for antivenom production and treatment and diagnosis of envenomation [89]. Venoms are comprised of around 50-200 components distributed in dominant and secondary families as multiple proteins and

peptides. The dominant families of toxins that mediate multiple pharmacological effects alone or in combination include phospholipase A_2 (PLA_2s), snake venom metalloproteinases (SVMPs), snake venom serine proteases (SVSPs) and three-finger peptides (3FTXs). Secondary families of toxins include cysteine-rich secretory proteins, L-amino acid oxidases, C-type lectins, disintegrins, and natriuretic peptides [90] [91].

1.5.1) PLA_2s

These enzymes have a molecular mass of around 13–15 kDa and are classified as groups I, II and IIE. Despite their similarities in structure and catalytic properties, they produce a wide spectrum of pharmacological effects. Several PLA_2s are considered to exert strong myotoxic/cytotoxic effects leading to necrosis of tissues and neurotoxic effects can be attributed to the modulation of pre-synaptic pathways by targeting nerve terminals at neuromuscular junctions although the precise mechanisms of action are yet to be fully understood [90] [92].

1.5.2) SVMPs

These are zinc-dependent proteinases with molecular weights ranging from 20 to 110 kDa. SVMPs are classified into P-I to P-IV depending on their domain organisation. The simplest class, P-I, contains only a metalloproteinase domain. P-II contains a metalloproteinase domain followed by a disintegrin domain. P-III consists of a metalloprotease domain, as well as disintegrin-like and cysteine-rich domains. P-IV consists of a metalloprotease domain as well as disintegrin-like, cysteine-rich domains and two C-type lectin-like domains connected by disulphide bonds. SVMPs are known to play multiple roles including the degradation of fibrinogen, collagen, basement membrane and capillaries resulting in extensive tissue damage [91] [93] [94] [95]. Similarly, they alter blood coagulation by targeting several clotting factors.

1.5.3) SVSPs

These enzymes belong to the S1 family of serine proteinases with two distinct structural domains with molecular weights ranging from 26 to 60 kDa. SVSPs are mostly found in viper venoms. Their main mode of toxic action is by altering the haemostatic system of the bite victims, preventing or activating the coagulation cascade to induce bleeding, venom-induced consumption coagulopathy and random blood clotting in the vasculature [90] [93].

1.5.4) 3FTXs

These toxins consist of 58 to 81 amino acid residues and possess a three-finger fold structure stabilized by disulphide bridges. These are non-enzymatic toxins that often bind post-synoptically at neuromuscular junctions inducing flaccid paralysis associated with SBE. Some of the 3FTXs have diverse muscarinic receptor targets with many targeting the nicotinic acetylcholine receptor of vertebrate skeletal muscle. They are also involved in enzyme inhibition, cardiotoxicity, cytotoxicity, ion channel blocking and anticoagulation [90] [96].

1.6) Clinical features of SBE

The variability of venom toxins can lead to a wide variety of clinical complications in SBE patients. Clinically, snake venoms can be classified as haemotoxic, neurotoxic, cytotoxic, and nephrotoxic with the predominant effects depending on the family of offending snakes. For example, the venoms of vipers are mostly haemotoxic and cytotoxic, whereas the venoms of elapids are mainly neurotoxic. However, such simplification may be considered superficial as the diversity of the toxic components of any given snake venom can result in an array of pathologically diverse consequences [97] [98] [99] [100].

1.6.1) Haemotoxicity

Haemotoxicity is one of the most common clinical signs in SBE victims due to their cardiovascular specifically haemostatic effects. Cardiovascular effects can be characterised by a dramatic fall in blood pressure or blood clotting abnormalities caused by different venom toxins. Haemostatic effects consist of local and systemic haemorrhage whereby clinical presentation includes spontaneous bleeding from the gums, wounds, bite site, gastro-intestinal and/or genito-urinary tracts, haematemesis and haemoptysis. The resulting symptomology can result in death amongst patients due to hypotension as well as haemorrhage [98].

1.6.2) Neurotoxicity

Neurotoxicity is a common feature of envenomings especially from elapid snakes such as kraits, cobras, and coral snakes. Presentation is usually acute neuromuscular paralysis, which requires mechanical ventilation as well as other supplementary care and prolonged hospital stay, all of which contribute to a significant cost for patients. The pathophysiological mechanisms remain unclear for venom-induced neurotoxicity. The variations between individual patients and their clinical presentations can result in varying degrees of associated neurological manifestations.

1.6.3) Cytotoxicity

SBE causes local tissue damage such as necrosis of the skin and skeletal muscle, which is an unfortunate outcome for most victims. Especially if the treatment is delayed, the muscle damage can eventually lead to significant tissue loss as well as possible amputation and subsequent disabilities. Venom-induced muscle damage is still poorly understood and antivenom is not able to neutralize toxins in tissues. The rapid onset of necrosis in tissues presents a major challenge in developing novel therapeutics that can neutralise venom toxins and limit these cytotoxic effects [101] [102].

1.6.4) Nephrotoxicity

The kidneys are vital organs removing biological waste products and excess fluid from the human body and helping to maintain our blood pressure. They are also important in maintaining the delicate balance of key minerals such as sodium and potassium which allows other tissues across our body to work effectively. As a result, our kidneys are highly vascularized organs, and it is estimated that they receive 20 per cent of the body's blood during cardiac output [103]. This makes these organs highly prone and sensitive to venom toxicity. The effects of snake venoms and their subsequent damage to the kidneys have been well-known since the 1930s. Clinical manifestations in kidneys following SBE can vary widely from mild proteinuria to complete renal failure requiring haemodialysis. A variety of pathological lesions have been witnessed in patients along with myoglobinuria due to myonecrosis and associated tubular necrosis leading to renal failure after SBE [104]. Envenomation from vipers and other snakes with haemotoxic capacity is most associated with impacting renal homeostasis and is responsible for the myriad of clinical renal pathologies observed in SBE victims. These can be caused due to tissue necrosis of the tubules, haemodynamic fluctuations, and immunological reactions all of which contribute to nephrotoxicity [105]. It is noteworthy that renal failure is the primary cause of death in patients bitten by *Bothrops* species [106]. The subsequent course of treatment for patients exhibiting renal failure is medically complicated and dialysis is often needed, elevating treatment costs for patients and requiring lengthy hospital stays. The exact mechanisms that maintain our complex vasculature in the kidneys are not well understood and there is a lack of evidence on the mechanisms of action relating to nephrotoxic abnormalities. It is presumed to occur because of the cascade of haemotoxic and cytotoxic effects of SBE. The exact impact of toxins here requires much further investigation [107]. Notably, it is critical to develop novel biomarkers to identify renal damage in advance and to provide earlier treatment to prevent excessive kidney damage as well as reduce subsequent treatment costs.

1.6.5) Clinical features of the Indian 'Big Four' snakes

Clinicians in India along with those in much of the developing world primarily rely on patient envenomation symptomology when it comes to treating patients and administering the appropriate antivenom. As the 'big four' snakes are responsible for most bites in India it is important to understand their envenomation effects. Despite the high variability of venom composition as well as geographical variations amongst the same species, the 'big four' snakes have some key symptomatic presentations that set them apart and can serve to aid clinicians in developing their treatment regimen for SBE patients.

The grade and range of clinical symptoms observed in most vipers such as Russell's viper and saw-scaled viper bites are usually accompanied by key haemotoxic features such as haemorrhage and various coagulopathies along with acute kidney injury. These can be attributed to the various levels of SVMPs found in their venoms. An important event in Russell's viper envenomation is the formation of microthrombi activated by the coagulation cascades and subsequent platelet adhesion, which causes depositions in blood vessels of the kidneys leading to renal impairment, acute kidney injury and even organ failure in other parts of the body [98] [108] [109] [110] [111] [112]. Saw-scaled viper bites can present with more developed coagulopathies compared to Russell's viper envenomation, and this can include gingival bleeding, bleeding from the bite site, blistering, tissue necrosis as well as ecchymosis. Acute renal failure and hypotension have also been reported following bites from saw-scaled vipers [98] [110] [111].

The clinical manifestations of patients bitten by kraits have not been entirely elucidated. Krait bites often occur at night and are most acquired by people who sleep on the floor. So, the majority of these bites are thought to occur when a person moves in their sleep causing the snake to strike. These bites are not considered to be painful and therefore, they can go unnoticed by the patient who continues to sleep. Patients usually present with common neurological symptoms, respiratory distress, ptosis, and various forms of paralysis which can attributed to the high levels of PLA_2s and three-finger toxins in this venom. Symptoms may also include vomiting, abdominal pain, dysphagia, drowsiness, delirium, and advancement into a deep coma. The symptom of most clinical concern is descending paralysis which can start in as little as under 30 minutes, and unless mechanical ventilation is sought it can lead to life-threatening respiratory paralysis [113] [114].

Patients bitten by the Indian cobra can also present with respiratory distress, ptosis and various forms of paralysis which can again be attributed to high levels of PLA_2s, and three-finger toxins which are the main molecular components causing neurotoxicity [114]. The neurological effects of cobra bites are attributed to the blocking of synaptic transmissions at the neuromuscular junctions by cobrotoxins.

Drugs such as anticholinesterases can be administered to patients in returning neuromuscular function after such bites [115]. Cobra bites can also result in swelling, cardiotoxicity, and tissue necrosis [116].

It is important to note that the effects of a bite from the same snake species can vary, as venom constituents may vary geographically, seasonally, and ontogenetically. Therefore, not all snakes of the same species can have the same number of toxins and thus clinical effects [115]. The molecular complexity of venom can produce a variety of more unique symptoms that may also exist outside of the common parameters presented [108]. Medical events after SBE are a result of a multitude of chemical processes and cannot always be assumed to be attributed directly to the venom toxins. The ability to detect and effectively treat envenomation effects requires thorough clinical education about the venoms of different snakes as well as robust training for healthcare professionals to manage SBE victims. Moreover, appropriate diagnostic tests/kits are needed to accurately diagnose SBE and to provide prompt treatment options.

1.6.6) Issues with the current clinical management of SBE in India

Immediate first aid along with antivenom administration to neutralise the venom toxins following SBE are of paramount importance in mitigating SBE-induced clinical ramifications. However, these as standalone treatments do not resolve all the clinical effects of envenoming. Venom toxins can still cause damage to cells, muscle tissue and organs as some toxins can be in tissues and could be hidden from antivenom neutralisation. Thus, effective treatment also involves alternative interventions such as wound care, fasciotomies, surgical debridement, skin grafts, haemodynamics, endotracheal intubation, and mechanical ventilation at the same time as antivenom administration. Later, when patients are more clinically stable and somewhat recovered, they may also need rehabilitative therapy and physiotherapy to make an effective recovery. Similarly, further pharmaceutical interventions such as antibiotic administration in dealing with local infections, anticholinesterase inhibitors to combat the effect of neurotoxic venomous snakes and hypo/hypertensive therapeutics for patients are needed. Standard treatment protocols for Indian snakes are not available to most healthcare professionals. Evidence-based clinical care protocols along with regionally specific guidelines are needed to standardise care along with educating healthcare professionals on the variations in clinical management practice.

1.7) Indian Antivenoms

Albert Calmette discovered and pioneered the principles of antivenom serotherapy in 1894. Since then very little has changed in terms of developing novel antivenom therapeutics and antivenom remains the only effective current therapeutic intervention [117] [118]. Antivenoms are produced by repeatedly

immunizing large mammals such as horses and sheep to build up an antibody response against venom components. The blood drawn from those immunised animals is then used to purify antibodies and formulate antivenoms [119] [120]. Antivenoms may be species-specific (monovalent/monospecific) or effective against several species (polyvalent/polyspecific) [121]. Even though antivenom is the only effective antidote for SBE, its administration alone does not solve all clinical manifestations induced by SBE. Indian polyvalent antivenoms are exclusively produced against the venoms collected from the snakes of Tamil Nadu by the Irula Snake Catchers' Industrial Cooperative Society (**Figure 5**), the only venom supplier to all Indian antivenom companies. Currently, India has several antivenom manufacturers such as Vins Bioproducts Limited (Hyderabad, Andra Pradesh), Premium Serums and Vaccines (Pune, Maharashtra), Haffkine Biopharmaceuticals (a Govt. of Maharashtra's company, Mumbai) and Bharat Serum and Vaccines Limited (Mumbai, Maharashtra) to produce the polyvalent antivenoms. The antivenom is available both in liquid and lyophilised (powdered) form which allows it to be stored easily without the need for refrigeration [122].

Figure 5: The Irula Snake Catchers' Industrial Cooperative Society, licensed by the Tamil Nadu Wildlife Department of India to catch snakes and extract the venom to aid in the production of Indian antivenom. Images kindly provided by The Madras Crocodile Bank Trust & Centre for Herpetology.

The Wildlife Protection Act in 1972 secured snakes as protected species which meant acquiring venoms for the quantities needed for commercial antivenom production was impacted. This also reduced the ability to source snake venoms from different geographical areas across the country, which directly impacts the effectivity of antivenoms as snake species in different geographical locations have shown to have varying toxin profiles [123] [114]. Thus, the current antivenom often fails to offset intra- and inter-species venom variations found across India. This results in multiple vials of antivenoms being

administered to patients, which subsequently increases the risk of serum sickness and anaphylaxis and potentially death [114] [124] [125] [126].

Antivenom is available for free in government hospitals in India and the cost of one vial (100 mg of antibodies) ranges from ₹500 to ₹700. A national snakebite protocol has been in place since 2007 which guides physicians on the rational use of antivenoms [127]. However, to ensure successful treatment, patients require good-quality antivenoms. The neutralising potency of antivenom has come into question for the venoms of the cobra and krait, and this presents challenges for the patient and clinical professionals as the marketed efficacy is often not met [114]. Thus, current Indian antivenoms are not always efficacious across the country, resulting in multiple vials being administered to the patient and escalating treatment costs for the patient.

The WHO's guidelines for the production, control and regulation of snake antivenoms provide technical expertise on antivenom manufacture and the need for adherence to Good Manufacturing Principles (GMP) [128]. However, India may need a similar regulatory policy on the production and quality of antivenoms. These antivenoms have not been clinically validated by undergoing a clinical trial process, and therefore, there is a lack of evidence on the consistency of their safety and efficacy. Thus, better regulations on antivenom production in India are needed along with independent assessment and evaluation of their preclinical efficacy [129]. There is also a need for set specifications for the potency, efficacy, dose, and safety of antivenom products. The manufacturing process of antivenom across India also needs to consider regional variability; upscaling and improving current antivenoms is a tentative solution to alternative therapeutics. Although antivenoms occupy an important space for the treatment of SBE victims, they are not the final solution, and they present limitations to treatment methodologies in practice. Often patients with respiratory, circulatory, and renal failure will require multiple clinical interventions including urgent resuscitation as well as antivenoms. They can result in reactions such as pruritus, urticaria and in extreme circumstances fatal anaphylaxis. The underlying mechanism of these acute reactions in certain individuals is unknown [130] [131].

Many barriers exist in countries such as India hindering the development of efficacious antivenom therapies and alternative therapies. In addition, the scientific ability to unravel the complexities of medically relevant toxins and neutralise them effectively requires a high level of investment in venom research. The advent of venomics, and toxico-genomics, despite their infancy, has helped researchers begin to lay the foundational groundwork for novel therapies, with the goal being to develop effective recombinant antivenoms against snakebites [132].

1.8) Barriers to accessing effective SBE care in India

As the majority of snakebites occur in poor rural areas with limited infrastructure and lack of access to transport, the high rate of SBE mortalities and morbidities can also be attributed to the delays in reaching health facilities in time. This is in addition to other factors such as refusal to seek clinical therapy, accepting their fate post-SBE and fear of extreme treatment costs. These could be prevented if communities and patients had awareness of basic first aid procedures for SBE as well as closer access to adequate facilities with trained healthcare professionals to deliver this as needed. An SBE's patients' first port of call is usually a PHC, however, many of these in India lack adequately trained staff with the expertise to deal with these patients. This means the patient is often onward bound to more developed facilities such as tertiary hospitals, further delaying time to treatment from bite, or to seek alternative traditional treatments. This increases the risk of dying in transit to referral hospitals further away. The National Health Mission, part of the Ministry of Health & Family Welfare, Government of India published guidelines for the management of snakebites to standardise treatment across India [133]. However, the awareness of these protocols is lacking, and the clinical expertise needed to implement these guidelines is usually ignored. As many clinical professionals are unaware of such protocols, this has wider implications for SBE patients. There is also no definitive SBE curriculum in the Indian medical and paramedical courses. Any official SBE training is left to be obtained as part of their continued professional development or clinical exposure in high-burden areas. As India is such a vast country with diverse landscapes and snake fauna, regional and cultural training materials are also lacking.

Currently, clinical professionals across India review patient signs and symptoms to make the best clinical judgements along with the whole blood clotting test (WBCT) to diagnose viper bites and to decide if there has been envenomation. Despite the protocols in place, there is no uniform application, and thus SBE is treated according to the clinical team or leading clinicians' discretion. As SBE is not a recorded health incident this makes tracking the numbers of bites and lack of field data more apparent. Patients, their families and or communities often capture the offending snake, dead or alive and bring it to the treatment centre to aid with diagnosis. There is no evidence to suggest that this is an effective strategy or helpful to healthcare professionals in being able to treat patients. In turn, this damages the reptile fauna as it leads to the indiscriminate killing of snakes with no real impact on the clinical management and prognosis of patients. The rural healthcare professionals in India are not adequately trained to deal with SBE. The awareness and knowledge of providing even basic first aid to snakebite victims is often lacking, let alone administering antivenom or dealing with the multitude of clinical symptoms. Therefore, robust training for healthcare professionals is critical in reducing the SBE burden.

1.9) Lack of accurate data on SBE in India

A consequence of SBE being an NTD is that it succumbs to the same fate as the other NTDs. The lack of a sufficient health infrastructure means that the recording and reporting of SBE are not performed robustly [50]. Therefore, complete, current, and accurate epidemiological data on SBE is deficient. Baseline epidemiological and ecological data in many cases remains incomplete. This can be attributed to limited public health and medical infrastructure, compounded by public attitudes and healthcare-seeking behaviours following bites [49]. This in turn means that an accurate picture of SBE from incidents along with wider epidemiological and contributing socioeconomic data is missing. To meet the WHO goals, realistic baseline data is needed to ensure that the SBE issue is given the due care and attention it deserves. This requires an in-depth evaluation of the awareness of SBE in endemic communities and improving their medical literacy to mitigate these risks, understanding, and averting the socioeconomic impacts of an SBE event. This involves assessing and improving the clinical management of SBE as well as alleviating the final cost to patients with limited bureaucracy and acceptable insurance coverage. One must review and analyse all branching factors to determine the key and impactful areas of concern to provide individualistic models of input to improve the situation at a grassroots level. This comes under the WHO bracket of strengthening healthcare systems as well as encouraging partnerships, coordination, and resources between state and national levels. This also requires funding and collaboration to ensure investment not only monetarily but also from a resourcing perspective is provided. Planning, monitoring, and evaluation are key components in assessing any field intervention.

There are many gaps in obtaining a complete understanding of the impact of SBE due to a lack of understanding of even the underlying ecology and habitat of snakes [134]. It must be recognised that often, there is little data amplified by the lack of importance for investment into such demanding tasks as material collection for epidemiological purposes. A fifth of reptiles are data deficient; with snakes presenting their difficulties in monitoring populations e.g., low population densities, insufficient recaptures, and crypsis [135]. Moreover, many variables impact SBE data such as human population dynamics, demography, activity patterns, seasonality, and snake ecology. In some communities, SBE has a higher prevalence during the rainy seasons. These further highlight the multifaceted components that underpin our understanding of the complexities of SBE and its subsequent prevention. India has varying climates and distinct geographical areas and sub-regions, and therefore, there is also great snake species variability. Current field data from India regarding SBE is limited and it does not represent the true scale of the issue [36] [39] [49]. The National Digital Health Mission (NDHM) was launched by the Indian government, and it aims to create a process whereby health data is digitised to allow for process management and information exchange between the creation of various registries for the healthcare system. This would allow for improved communication between health service providers,

laboratories, and pharmacies to support clinical decisions. The implementation of the NDHM is meant to assist India in tackling NTDs as well as making a more developed robust healthcare system with evidence-based strategies [136] [137]. The successes of the NDHM and India's strategic strengthening and support for NTDs have been achieved in areas such as the National Tuberculosis Elimination Programme and in reducing the Maternal Mortality Ratio (MMR) [138] [139]. Considerable progress has been made when the supporting architecture has been constructed to implement, maintain, deliver, and conduct surveillance on these health issues. Much of the same is also required to mitigate SBE risk and reduce the SBE burden.

1.10) Indian health needs and healthcare diplomacy

Providing adequate and effective healthcare to a country of over one billion people is not easy for any government. The current drive by the Indian government to improve the healthcare sector is commendable yet it requires more resourcing, appropriate trained professionals, and public health expenditure to be increased. Ensuring adequate data collection on health priorities such as SBE is accurate and reliable can aid with subsequent healthcare diplomacy and warranting policy changes in healthcare relating to SBE for the benefit of all and not just endemic communities and diseases. There are a multitude of complex facets to the SBE issue in India, which include but are not limited to the venomous snakes of medical importance, four of which cause the most fatalities. The socioeconomic impact on the rural population which suffers greatly because of such unexpected health events can lead to financial and psychological devastation for generations. The lack of well-developed healthcare infrastructure and logistics, lack of access to care and insurance coverage for patients along with the lack of knowledge amongst healthcare professionals on clinical management of SBE and ineffective antivenoms are some of the major barriers that need urgent attention. Greater public health awareness of venomous snakes and their ecology across all sectors of society, improvements in health-seeking behaviour by endemic communities without fear of financial impact over short- and long-term treatment, greater mental health and disability-related financial support, compulsory training for all healthcare providers and reviewing antivenom manufacturing to create efficacious antivenom therapy are greatly needed. Consequently, SBE requires a high level of political commitment and expenditure in a vast country such as India where there are competing health priorities. The use of performance-linked mechanisms, intelligence and metrics serves to not only meet the WHO sustainability goals but also to reduce the burden of SBE in India and make effective progress.

Next, I will discuss the potential development and advancement of new therapies, improving the utilisation of current ones and innovative modelling to not only progress clinical outcomes for SBE patients but to also reduce the risk of SBE in the first place.

1.10.1) Plant-derived SBE therapeutics

The use of plants for medicinal purposes by early humans has been in practice since prehistoric times, and this echoes into modern medicine and helped form the principles of early drug and pharmaceutical discovery. The use of biological therapeutic interventions to resolve clinical unmet needs to improve our health drives discovery. Considering the global scope of SBE and the communities it impacts, the use of traditional plants and herbal therapies against SBE has proved largely ineffective [140]. It is a common misconception that certain plants may contain compounds that can act to neutralise venom toxins. Globally, it has been estimated that around 700 plants have been identified for use as traditional herbal medicine for SBE [141]. The Indian medical system of naturopathic and traditional medicine so far has not yielded any scientifically effective or commercially viable therapeutic interventions against SBE [140] [142] [143] [144] [145]. There is much more work and funding needed to evaluate potential plant-derived therapeutics for SBE [145].

1.10. 2) Snake Venom Detection Kits

Currently, Australia is the only country in the world that utilises a snake venom detection kit (SVDK), which serves to aid first responders and healthcare professionals in determining which antivenom should be utilised in the event of SBE. There is no venom detection kit available for medically important Indian snakes. Ensuring funding and manufacture of an SVDK for Indian snake venoms to be reliably detected poses immense challenges. For example, ensuring a high level of sensitivity and specificity for snake species, reliability and speed of detection would be of paramount importance.

1.10. 3) Biomarker discovery

A range of diagnostic biomarkers could be used to aid in the detection of envenomation pathology allowing healthcare providers to effectively diagnose and treat patients with targeted therapeutics to avoid wider consequences. Identifying suitable biomarkers for Indian SBE patients could aid in their prognosis as well as monitor progressive symptoms of various tissue damage. So far, no clinically reliable biomarker for the Indian ‘big four’ snake-induced pathology has been identified. Investigating potential clinical biomarkers requires clinical studies and trials with an effective population sample size, in-depth pharmacokinetic evaluation, and effective analysis. These are not only time-consuming endeavours but also costly as well as clinically resource-heavy.

1.10. 4) mHealth and SBE

The growing availability and access to the internet globally, its rapid growth and the use of mobile communication devices particularly smartphones have opened a new world of opportunities for use in health care, otherwise known as 'mHealth' (mobile health). The use of mHealth can aid in delivering medical care and is becoming popular in underserved and remote areas and can be driven in the developing world with the expansion of telecoms coverage. The use of mobile applications, artificial intelligence (AI) software and websites can aid in acquiring accurate reliable data relating to SBE. A few mobile applications have already been created such as The Snakebite Assistant, SARPA and Snakehub, to name only a few; yet these are only as useful as the communities' willingness and ability to adapt and use such technologies. Newer technologies, such as the utilisation of drone delivery models, AI, and machine learning, can also be utilised to future-proof technological advances and automate processing quickly for deprived communities. Predictive modelling, access to monitoring and data capture and storage could prove invaluable to the SBE cause [146] [147] [148].

1.10. 5) Geospatial tools and a Geographic Information System (GIS) approach

As the variability of snakes of medical importance differs per country in line with the scope of their unique ecological and environmental specifications, geospatial tools become increasingly important in gathering reliable field data [149] [150]. The utilisation of these tools can allow us to review, monitor and evaluate the impact of climate change, and population growth encroachment into reptile habitats by humans. This can allow us to assess infrastructure and transport logistics to build a more accurate image of our interaction with the reptile environment and any potential patterns in snakebites e.g., seasonality, altitude etc. This can then aid the Indian government to selectively allocate and deploy resources where needed to 'hotspots' and areas of greater need and plan more effective interventional and prevention strategies [8] [151].

1.11) The evolution of snakes

Evolutionary science about reptiles is constantly developing with new information and discoveries. It is believed that the earliest known species of reptile was discovered around 240 million years ago and this forms the ancestral basis for all of the world's lizards and snakes [152]. Snakes evolved independently from lizards around 168 million years ago during the Jurassic period [153]. Ecologically speaking snakes are distributed globally on every continent except Antarctica. Phylogenetics and taxonomy practises are used to study the relationship between species and their evolutionary history. The animal kingdom is organised phylogenetically, and snakes exist within the

Reptilia class. The classification of snakes (*Serpentes*) began with Linnaeus in 1758, illustrating the taxonomic hierarchy of the higher order (*Caenophidia*) containing the order *Colubroidea*. The order *Colubroidea* can further divide venomous snakes into *Viperidae*, *Elapidae*, *Lamprophiidae* and *Colubridae* families [154-156]. There are approximately 2670 species of snakes, 85% of which are located within the *Colubroidea* [156]. Most importantly this group contains around 600 snake species that are considered to be venomous and around 200 medically important venomous snakes [156] [157]. The WHO has identified 109 venomous snake species that have the highest medical importance overall globally and these are classified into category 1. A further 142 species have been classified into category 2 as they impact humans to a lesser extent yet nevertheless remain important [8]. Snakes of medical importance are determined by various considerations such as the chemical constituents of their venom, the level of induced fatalities and the level of medical attention needed after the bite. The clinical effects of subsequent envenomation, any long-term medical complications and antivenom therapy needed as well as the population density at risk of bites are all critical factors in determining these classifications [158] [159].

1.11. 1) Human-snake interactions and the psychological impact of SBE

SBE disproportionately affects developing countries and is often considered an occupational hazard for rural poor communities relying on agricultural labour (**Figure 6**) [8] [84] [160]. With human and urban development encroaching further into natural habitats, home to snake species, this leads to increased human-snake interactions and predictably more bites and negative consequences. One such negative consequence is the indiscriminate killing of snakes for fear of envenomings or to aid clinicians with diagnosis following SBE. Such disruption of the reptile fauna may have disastrous ecological effects as snakes are vital predatory species keeping the checks and balances of prey at equilibrium.

Figure 6: Snakes and humans have a shared landscape, here Russell's vipers are present in a rice paddy cultivated by Indian women, highlighting the close association between rural agricultural communities and snakes. Image kindly provided by Gnaneswar Chandrasekharun.

Progressive habitat destruction along with indiscriminate killing of snakes may drive valuable ecological species into extinction with untold environmental consequences. Snakes are viewed as dangerous pests requiring control and so 'snake rescuers', such as governmental forestry officers or known members of the community with such wildlife capture skills are often called to remove the snake and relocate them away from human habitation. These community members may not have the education, knowledge, or skills to be able to correctly distinguish between venomous and nonvenomous species, putting their own lives at risk to save others and preserve India's snake fauna. Currently, there is a gap between the conservation needs of snake species and human-snake conflict resolutions that exist in rural, impoverished communities. Moreover, the challenges faced by snake rescuers have never been studied properly. Since the Indian Wildlife Protection Act 1972 protects all Indian fauna, no snake species in India can be handled, held, provided veterinary care, and transported (or exported) without the permission of the relevant government forest department. Safe snake rescue therefore requires expertise and training, especially in handling venomous snakes. A snake rescue can pose challenging scenarios as handling animals requires the safety of the rescuer to be maintained along with the welfare of the animal itself. It is important to note that snake rescue will at times require specialised equipment such as snake hooks, bags, and ventilated plastic boxes with clip-locked lids for venomous snake capture.

Adequate knowledge of the law, specialised training, and tools for such rescuers along with appropriate credentials are needed to conduct, regulate, and detail such public service efforts. Suitable operational procedures on the release protocols for snakes are currently unavailable and need to be

developed for Indian snakes. Ecological education on snake habitat relocation is imperative as past research with radio telemetry has confirmed the high rate of mortality of relocated snakes [161] [162]. Protocols rooted in ecological science are important to conserve ecosystems as releasing snakes into unsuitable habitats also places predatory pressure on ecosystems not used to such snakes [163].

Snake rescuers play a commendable role within the communities they serve by risking their lives to save others as well as snakes. They face many challenges such as stigma in their line of work, lack of compensation, acknowledgement, and fundamental accreditation for their services. As this is an ongoing conflict engaging with the challenges faced by snake rescuers and aiming to alleviate these by local government can allow snake rescuers to operate in an unobstructed role as agents for change. Official recognition can allow the Indian government to harness their efforts in capturing vital reptile data and statistics on snakes aiding conservation efforts along with imparting fundamental ecological and environmental and basic first aid information to the communities they serve in how to reduce SBE, especially since prevention is always better than cure.

Snake species pre-existed to early humanity and so their entrenchment into modern human mythology, legend and folklore is not unremarkable. There are deep evolutionary origins for human's irrational fear of snakes and it is thought this was developed amongst our ancestors as an adaptive protective mechanism from venomous snakes for survival [164]. Hence, humans may have evolved to be predisposed to acquire a fear of snakes and this has been proposed by psychologists such as Martin Seligman with his preparedness theory of phobias which asserts that humans have acquired specific phobias due to inheriting a special sensitivity to stimuli (e.g., snakes) which once (and in some way still do) represented a severe threat [165] [166]. SBE pathology is not restricted to clinical symptoms and recent research has identified the long-term ramifications of the psychological trauma of SBE, such as the prevalence of post-traumatic stress disorder (PTSD), anxiety disorders (such as Ophidiophobia fear of snakes) and other acute and delayed stress symptoms and psychosocial impairment, putting some of society's most vulnerable communities at risk of impact to their quality of life [167] [168]. Specific phobias related to a fear of certain animals such as snakes fall under the bracket of a generalised anxiety disorder. These can cause severe emotional responses to a perceived threat. Living with such phobias can be an incredibly debilitating condition and the acquisition of these phobias has been often linked to negative experiences [169]. Thus an SBE event can trigger a snake phobia to develop, which can have long-lasting mental health implications [167].

Mental health care services are often under-resourced and even more so in developing countries [170]. A better understanding of the emotional and psychological factors involving human conflict such as the development and presence of specific phobias to snakes in India is needed to elaborate our understanding, as well as prevent the unnecessary killing of snakes. Moreover, clinical management of

SBE must also encompass the psychological complications which may not be apparent immediately but develop over time. Community mental health support workers would play an intrinsic role in improving the quality of life for SBE victims by providing such support where needed. Therefore, it is important to measure the level of snake phobia in Indian communities and develop strategies to mitigate such phobia.

1.12) Public Awareness of SBE

Effective communication is essential to build better and healthier societies not just for the present but also for the future. A key goal of public health awareness is their reach, accessibility, and ability to communicate to key audiences how to make the best health decisions for themselves, their families and in turn communities. The primary aim is to enhance and improve health and to allow and influence the 'patient' to make better health decisions. The field of public health communications overlaps, with informatics, marketing and advertising and these domains change with societal progress and more recently with advanced technology, access to it via social media [171]. We must respect that simply indicating to members of the public to change their behaviours will not always be the most effective manner of solving complex health issues [172]. Creating interventions for health concerns for the wider public is an altruistic endeavour and making society better as a whole despite with no underlying commercial motivation. By merging strategies of creative marketing, human behaviour and psychology, public health awareness can and should appeal to the masses to whom the campaign is directed [173].

The best campaigns should engage as many individuals as possible across diverse populations, sociodemographic groups, and orientations, to accomplish the wider health goals and instil the desired change. Specific criteria need to be established to evaluate the social intervention programme and if policy establishment would be beneficial here, for example, the use of seat belts as a legislative health measure has saved many lives. There are usually two interventional approaches, the first is to reduce, prevent or modify behaviours that lead to diseases and the second is to focus on increasing behaviours socially and environmentally to promote health. Examples of effective healthcare campaigns that utilise these approaches were those to reduce and stop smoking in the 1970s [174] and AIDS/HIV awareness campaigns of the 1980s [175]. It is important to appreciate that there are many lifestyle choices, personal, social and religious beliefs as well as macroeconomic issues that underpin affecting change for any health-related intervention [176].

Public health awareness campaigns can be diverse and often overlap other health measures. A formative study in the field of public health awareness was the Stanford Five-City Project, which evaluated health education and communication for the prevention of cardiovascular disease, a leading

cause of death globally. This study was instrumental as they assessed interventions such as mass media alone along with mass media and interpersonal contacts and highlighted that risk reduction behaviours could be acquired through mass media alone. This illustrates that we must not exclude social support structures and community-led interventions, and the impact of social marketing on health promotion campaigns. It is important to note that the diversity in populations has meant one must consider different cultural practices when it comes to prevention methods and to be effective one must be strategic [177].

Increasingly, public health communication interventions on both local and national levels have been adopting social marketing and branding strategies. These are proving to be highly effective as they can develop persuasive messaging and effective strategies [178] [179]. A public health awareness campaign is not effective if the strategy chosen produces short-term results, one must have long-term goals in place to tip the balance in favour of the health goal. The ability to identify and create a concept linked to the health measure in question allows consumer equity to be sought and this in turn relates to a greater uptake in the marketplace; this in turn aids in sustaining the longevity of the desired health behaviour that is meant to benefit the target population. It is important to note that here we are not striving to sell a product or service in comparison to competitors, but we are aiming to earn emotional space within a target population as a preferred healthcare option. Distinctive features of a campaign allow ease of recognition and long-term sustainability, and this entails the usage of multimedia media images such as those used commercially to create brand awareness; what do we want from our target audience? and what do we need? Key concepts involve an initial needs analysis, audience targeting, product design testing, distribution, and evaluation. This is analogous to designing an effective public health campaign, which involves gathering and analysing epidemiological data, identifying the population most at risk and using appropriate multimedia to tailor the health messages to the audience. The next step is evaluating the impact of these messages on the rate of incidence.

The WHO is targeting a reduction in the number of SBE-induced deaths and disabilities by 50% by 2030 [8]. One of the core principles of the strategy for the prevention and control of SBE was to improve community education awareness about risk and prevention. Different rural communities across India have discrete sociocultural perceptions of SBE as well as holding varying spiritual relevance and importance to SBE, its causes, prevention, and treatment [180]. Engaging and empowering communities to become active participants in their healthcare discourse plays a vital role in risk reduction and thus saving lives, and we have already witnessed this in our earlier research into SBE awareness campaigns in Tamil Nadu, India [181]. Risk reduction of snakebites also crosses over to the prevention of other NTDs such as malaria, dengue, and soil-transmitted helminthiasis [182]. Activating a ground-up approach allows us to cast the net of prevention wider as it focuses on implementation methods at a community level. Moreover, empowered and engaged communities function more effectively with better health outcomes [181] [52]. Engaging communities is not without its challenges, for example, strategies that work in one setting may not work in another, hence strong

community-led participation is needed. The local community can be considered the first responders in the line of care and so ensuring local communities have the basic skills and knowledge as first responders for pre-hospital care until more developed medical care is available is essential to preserving life [183]. We need to ensure communities have the tools and capabilities to make informed decisions to prevent snakebites and improve the outcomes of those affected. [181]

1.12.1) Children's Snakebite awareness

Women, children, tribal communities, ethnic minorities, and remote communities are always most at risk of NTDs. Children are a vulnerable group of society, not just physically but as they are unable to protect their health and do not choose their environment. The contributory risk factors for children affected by SBE are higher, so they can be considered to be one of the most vulnerable populations [8]. There is a distinct lack of epidemiological field data reviewing SBE in children and field studies with children present limitations as data collected is either from secondary sources e.g., parents/relatives, or self-reported and relies on the child's ability to recall information. Current data indicates that males and working-age adults are most at risk of snakebites [66] [67] [52]. However, few studies have highlighted that children are also highly vulnerable to snakebites, specifically pre-pubescent children historically are at greater risk [184] [185]. The implications of childhood envenomation can be considered greater presumably because of the small body mass, action of venom on their developing bodies, subsequent medical complications, and the high risk of fatality. The risks associated with SBE in children may therefore be more serious for them than for adults [186].

To impact sustained and future-proof change, children must be considered when conducting campaigns driving public health initiatives (**Figure 7**). Children are a unique and specific target population so effective child-centric techniques should be developed and launched to be effective. Aiming campaigns at children before they become working-age adults encourages unprompted awareness, for example, similar to washing hands after using the bathroom. This provides them with the will and control to transform their well-being. To date, there have been no SBE public awareness campaigns for children and materials developed by NGOs are often passive and not specific or strategic enough. An effective public campaign for children can work towards ensuring the risk reduction of SBE cases moving to adulthood.

Figure 7: Prof. Sakthivel Vaiyapuri providing community snakebite awareness education as part of the Venomous Snakebites: Rapid Action Saves Lives programme to children at a secondary school in Tamil Nadu, India. The image was kindly provided by Prof. Sakthivel Vaiyapuri.

Successful and effective campaigns for children's health measures have been conducted in the past, for example, the VERB™ campaign launched in 2002 by the Centres for Disease Control (CDC) in the USA, utilised a social media marketing approach to encourage physical activity amongst children. This campaign also set out to establish collaborative partnerships with national and local organisations and communities to ensure goals were met. They applied strategic and commercial marketing techniques which were consistent over time. The awareness and impact of the programme were measured along with health outcomes and unprompted awareness was used as a tool for how well the campaign performed over the years. However, the high level of success of the VERB™ campaign can also be attributed to its sizeable funding and investment as the program received congressional funding of $125 million in the first year alone [187]. These studies show how delivering key health messages early in life backed by political will, adequate funding, brand marketing and commercial strategies can achieve long-term health goals in populations. To achieve the WHO's strategic goals between 2019 and 2030, they have anticipated a total cost of $136.76 million to be delivered over three phases, this budget is set aside for the first 2–4 years and will determine whether support can be fulfilled for the complete 12-year strategy. This financial plan will account for the pilot phase, scale-up phase and full rollout of all activities from engaging communities, safe treatment, strengthening health systems and increasing partnership and resourcing [8]. If one compares the budget of the successful VERB™ campaign with that of the availability of WHO funding, it is clear that public awareness campaigns for SBE and campaigns for children in mind fall significantly short of what would be needed to affect demonstrable change.

Creating, proliferating as well as accessing public health information is challenging in developing countries with high illiteracy and low literacy rates, this is especially pronounced in children who often do not have access to mainstream education. Effective educational tools along with innovative approaches can help change the health outcomes of these vulnerable groups [188]. Any public health awareness and educational strategies for SBE must consider these factors when targeting populations. Measuring and quantifying the impact of such campaigns also poses some difficulty, and there are limitations to how the influence is measured thoughtfully, some suggestions include measured uptake in hospital cases, application of correct first aid measures and a reduction in time taken to pursue medical care.

There are many factors and limitations to consider when designing and implementing a potential public health awareness campaign for SBE, however, to achieve the goals set by the WHO as well as to reduce the burden of SBE in India, effective mass campaigns must be devised to save lives now and in the future.

1.13) The rationale of this project

SBE in India is a major public health issue, especially in rural agricultural communities, which represent the poorest of the poor amongst the general population. India alone accounts for approximately 50% of the global SBE-induced deaths and disabilities and the true extent may be even higher. NTDs such as SBE lack directly allocated funding simply due to the nature of other diseases taking public health priority. The lack of reliable clinical and epidemiological data has meant that this has not received the suitable governance needed to work towards a change and improve outcomes for patients. To resolve this, we must have a multifaceted, holistic, one-health approach at every level possible.

At present education and training on SBE is not compulsory across India for any healthcare professionals. Considering SBE can be a complex medical event requiring professional intervention, there is no standard of care especially when considering the varied medical presentations in patients because of envenomings. Therefore, improved training for all healthcare workers is needed especially at the grassroots level amongst community healthcare workers who are likely to be the first responders. Effective first aid and acquiring prompt treatment help to save patients' lives. The lack of education on the management of SBE is further complicated by a lack of diagnostics tests or venom detection kits to aid healthcare workers in the diagnosis of the offending snake and administering effective treatment. Further investigation is needed on potential biomarkers that can be used to aid the detection of specific venom and manage clinical events before they escalate.

The lack of SBE awareness amongst communities prevents patients from accessing prompt treatment, which in turn exacerbates their treatment costs leading to wider socioeconomic ramifications for patients and their families and communities. The ability to underpin and determine key factors that lead to exaggerated costs in these health settings is incredibly important to mitigate the financial burden an SBE event can have as well as the long-lasting psychological trauma post-treatment.

1.14) Aim and objectives

This project aims to develop evidence-based strategies to improve various aspects of the clinical management of SBE and mitigate human-snake conflicts and SBE burden by improving public awareness.

The key objectives of this project are to;

1) To determine fundamental factors that contribute to exacerbated treatment costs for SBE and suitable biomarkers and antibiotics to manage this condition.
2) To determine the knowledge gaps and training needs for healthcare professionals in SBE management in India.
3) To assess snake phobia and the challenges faced by snake rescuers and develop public awareness tools to mitigate the SBE burden.

References

1. Hotez, P.J., et al., *What constitutes a neglected tropical disease?* PLoS Negl Trop Dis, 2020. **14**(1): p. e0008001.
2. D, M., *Neglected tropical diseases.* Community Eye Health, 2013. **26**(82): p. 21-4.
3. Kuper, H., *Neglected tropical diseases and disability-what is the link?* Trans R Soc Trop Med Hyg, 2019. **113**(12): p. 839-844.
4. WHO, *Global report on neglected tropical diseases*. 2023, World Health Organisation: Geneva, Switzerland.
5. Herricks, J.R., et al., *The global burden of disease study 2013: What does it mean for the NTDs?* PLoS Negl Trop Dis, 2017. **11**(8): p. e0005424.
6. Turner, H.C., et al., *Are current preventive chemotherapy strategies for controlling and eliminating neglected tropical diseases cost-effective?* BMJ Glob Health, 2021. **6**(8).
7. WHO, *World Health Organization Neglected tropical diseases: hidden successes, emerging opportunities*. 2009: Geneva, Switzerland.
8. WHO, *World Health Organization: Snakebite envenoming: a strategy for prevention and control* 2019.
9. WHO, *Report of the tenth meeting of the WHO Strategic and Technical Advisory Group for Neglected Tropical Diseases*. 2017, World Health Organization.
10. WHO, *Resolution WHA71.5. Addressing the burden of snakebite envenoming*. 2018, World Health Organization: Geneva, Switzerland.
11. Bhaumik, S., et al., *How and why snakebite became a global health priority: a policy analysis.* BMJ Glob Health, 2023. **8**(8).
12. Chippaux, J.P., *Snakebite envenomation turns again into a neglected tropical disease!* J Venom Anim Toxins Incl Trop Dis, 2017. **23**: p. 38.
13. Gutierrez, J.M.C., J. J. Habib, A. G. Harrison, R. A. Williams, D. J. Warrell, D. A., *Snakebite envenoming.* Nat Rev Dis Primers, 2017. **3**: p. 17063.
14. Wright, A.H.W.a.A.A., *List of the Snakes of the United States and Canada by States and Provinces.* The American Midland Naturalist, 1952. **48**(3): p. 574-603
15. Mary Elizabeth O'Neil, K.A.M., Julie Gilchrist, Edward J. Wozniak, *Snakebite Injuries Treated in United States Emergency Departments, 2001–2004.* Wilderness & Environmental Medicine, 2007. **18**(4): p. 281-287.
16. Phillips, C., et al., *Snakebites and climate change in California, 1997-2017.* Clin Toxicol (Phila), 2019. **57**(3): p. 168-174.
17. Ruha, A.M., et al., *The Epidemiology, Clinical Course, and Management of Snakebites in the North American Snakebite Registry.* J Med Toxicol, 2017. **13**(4): p. 309-320.
18. Minton, S.A., *Bites by non-native venomous snakes in the United States.* Wilderness and Environmental Medicine, 1996. **4**: p. 297-303
19. Brandehoff, N., et al., *Total CroFab and Anavip Antivenom Vial Administration in US Rattlesnake Envenomations: 2019-2021.* J Med Toxicol, 2023. **19**(3): p. 248-254.
20. Wilson, B.Z., et al., *Initial Experience with F(ab')2 Antivenom Compared with Fab Antivenom for Rattlesnake Envenomations Reported to a single poison center during 2019.* Toxicon, 2022. **209**: p. 10-17.
21. Curran-Sills, G. and J. Kroeker, *Venomous Snakebites in Canada: A National Review of Patient Epidemiology and Antivenom Usage.* Wilderness Environ Med, 2018. **29**(4): p. 437-445.
22. Mutabdzija, G., *Regional Geography of Europe; From geographical to innovative regions*. 2018, Columbia, SC: Amazon.
23. Freitas, I., et al., *Evaluating taxonomic inflation: towards evidence-based species delimitation in Eurasian vipers (Serpentes: Viperinae).* Amphibia-Reptilia, 2020. **41**(3): p. 285-311.
24. Di Nicola, M.R., et al., *Vipers of Major clinical relevance in Europe: Taxonomy, venom composition, toxicology and clinical management of human bites.* Toxicology, 2021. **453**: p. 152724.
25. de Haro, L. and D. Boels, *European Snakes*, in *Critical Care Toxicology*. 2017. p. 2441-2452.

26. Paolino G, D.N.M., Pontara A, Didona D, Moliterni E, Mercuri SR, Grano M, Borgianni N, Kumar R, Pampena R., *Vipera snakebite in Europe: a systematic review of a neglected disease.* J Eur Acad Dermatol Venereol, 2020. **34**(10): p. 2247-2260.
27. Chippaux, J.P., *Epidemiology of snakebites in Europe: a systematic review of the literature.* Toxicon, 2012. **59**(1): p. 86-99.
28. Kurtovic, T., et al., *Comparison of Preclinical Properties of Several Available Antivenoms in the Search for Effective Treatment of Vipera ammodytes and Vipera berus Envenoming.* Toxins (Basel), 2021. **13**(3).
29. Williams, D., W. Wuster, and B.G. Fry, *The good, the bad and the ugly: Australian snake taxonomists and a history of the taxonomy of Australia's venomous snakes.* Toxicon, 2006. **48**(7): p. 919-30.
30. Shea, G.M., *The distribution and identification of dangerously venomous Australian terrestrial snakes.* Aust Vet J, 1999. **77**(12): p. 791-8.
31. Welton, R.E., D. Liew, and G. Braitberg, *Incidence of fatal snake bite in Australia: A coronial based retrospective study (2000-2016).* Toxicon, 2017. **131**: p. 11-15.
32. Programme, U.N.D. *Human Development Index (HDI)*. 2023 [cited 2023; Available from: https://hdr.undp.org/data-center/human-development-index#/indicies/HDI.
33. Van de Vliert, E.D., Serge, *Hell on earth? Equatorial peaks of heat, poverty, and aggression.* Behavioral and Brain Sciences, 2017. **40**.
34. Rinderu, M.I., B.J. Bushman, and P.A. Van Lange, *Climate, aggression, and violence (CLASH): a cultural-evolutionary approach.* Curr Opin Psychol, 2018. **19**: p. 113-118.
35. Kira, A.R., *The factors affecting Gross Domestic Product (GDP) in developing countries: The case of Tanzania.* . European Journal of Business and Management, 2013. **5**(4): p. 148-158.
36. Kasturiratne A, W.A., de Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, Savioli L, Lalloo DG, de Silva HJ, *The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths.* PLoS Med, 2008. **5**(11): p. e218.
37. Cohen, D., & Soto, M., *Why Are Poor Countries Poor?* Econometric Society, Latin American Meetings, 2004. **75**.
38. Gutiérrez, J.M., *Snakebite envenoming in Latin America and the Caribbean*, P.V. Gopalakrishnakone, C. W.; Seifert, S. A.; Tambourgi, D. V., Editor. 2017. p. 51-72
39. Jha, P., et al., *Prospective study of one million deaths in India: rationale, design, and validation results.* PLoS Med, 2006. **3**(2): p. e18.
40. Gutierrez, J.M., *Reducing the impact of snakebite envenoming in Latin America and the Caribbean: achievements and challenges ahead.* Trans R Soc Trop Med Hyg, 2014. **108**(9): p. 530-7.
41. Nunez, V., et al., *Snake venomics and antivenomics of Bothrops atrox venoms from Colombia and the Amazon regions of Brazil, Peru and Ecuador suggest the occurrence of geographic variation of venom phenotype by a trend towards paedomorphism.* J Proteomics, 2009. **73**(1): p. 57-78.
42. Gutierrez, J.M. and H.W. Fan, *Improving the control of snakebite envenomation in Latin America and the Caribbean: a discussion on pending issues.* Trans R Soc Trop Med Hyg, 2018. **112**(12): p. 523-526.
43. Halilu, S., et al., *Snakebite burden in Sub-Saharan Africa: estimates from 41 countries.* Toxicon, 2019. **159**: p. 1-4.
44. Chippaux, J.P., *Estimate of the burden of snakebites in sub-Saharan Africa: a meta-analytic approach.* Toxicon, 2011. **57**(4): p. 586-99.
45. WHO, *Target product profiles for animal plasma-derived antivenoms Antivenoms for treatment of snakebite envenoming in sub-Saharan Africa*. 2023: WHO, Geneva.
46. Alirol, E., et al., *Snake bite in South Asia: a review.* PLoS Negl Trop Dis, 2010. **4**(1): p. e603.
47. Patikorn, C., et al., *Estimating economic and disease burden of snakebite in ASEAN countries using a decision analytic model.* PLoS Negl Trop Dis, 2022. **16**(9): p. e0010775.
48. Soopairin, S., C. Patikorn, and S. Taychakhoonavudh, *Antivenom preclinical efficacy testing against Asian snakes and their availability in Asia: A systematic review.* PLoS One, 2023. **18**(7): p. e0288723.

49. Mohapatra, B., et al., *Snakebite mortality in India: a nationally representative mortality survey.* PLoS Negl Trop Dis, 2011. **5**(4): p. e1018.
50. Suraweera, W., et al., *Trends in snakebite deaths in India from 2000 to 2019 in a nationally representative mortality study.* Elife, 2020. **9**.
51. Ian D. Simpson, R.L.N., *Snakes of Medical Importance in India: Is the Concept of the "Big 4" Still Relevant and Useful?* Wilderness & Environmental Medicine, 2007. **18**(1): p. 2-9.
52. Samuel, S.P., et al., *Venomous snakebites: Rapid action saves lives-A multifaceted community education programme increases awareness about snakes and snakebites among the rural population of Tamil Nadu, India.* PLoS Negl Trop Dis, 2020. **14**(12): p. e0008911.
53. India, G.o., *National Sample Survey Organisation (2014) Social consumption in India, Health (NSSO 71st round, January–June 2014).* . 2014, New Delhi, India: National Sample Survey Organisation, Ministry of Statistics and Programme Implementation.
54. Kaur, B., *Disasters and Exemplified Vulnerabilities in a Cramped Public Health Infrastructure in India.* International Journal of Disaster Risk Management, 2020. **2**(1): p. 15-22.
55. Kumar, R., *Achieving Universal Health Coverage in India: The Need for Multisectoral Public Health Action.* Indian J Community Med, 2020. **45**(1): p. 1-2.
56. Deepak, K.K., Y. Kumar, and B.V. Adkoli, *Extending professional education to health workers at grass root level: an experience from all India institute of medical sciences, new delhi.* Indian J Community Med, 2014. **39**(1): p. 38-42.
57. Misra, A., D.C. Yadav, and T. Kole, *Emergency care in India beyond 75 years of independence - problems and solutions.* J Glob Health, 2023. **13**: p. 03015.
58. Sharma, D.C., *India still struggles with rural doctor shortages.* Lancet, 2015. **386**(10011): p. 2381-2.
59. Babu, B. and Y. Kusuma, *Universal health coverage in India: A move with hope and despair.* International Journal of Medical Science and Public Health, 2016. **5**(3).
60. Babu, B.V., et al., *Primary healthcare services among a migrant indigenous population living in an eastern Indian city.* J Immigr Minor Health, 2010. **12**(1): p. 53-9.
61. Brinda, E., Rajkumar, A., Enemark, U., Prince, M., & Jacob, K., *Nature and determinants of out-of-pocket health expenditure among older people in a rural Indian community.* International Psychogeriatrics, 2012. **24**(10): p. 1664-1673.
62. Awasthi S, P.V., *Family Expenditure on Sickness Episodes of Pre-School Children in Urban Slums of Lucknow, North India.* Tropical Doctor, 1998. **28**(3): p. 141-146.
63. Samal, J., *Situational analysis and future directions of AYUSH: An assessment through 5-year plans of India.* J Intercult Ethnopharmacol, 2015. **4**(4): p. 348-54.
64. Ravishankar B, S.V., *Indian systems of medicine: a brief profile.* Afr J Tradit Complement Altern Med., 2007. **4**(4): p. 319-37.
65. Yadlapalli S. Kusuma, B.V.B., *The costs of seeking healthcare: Illness, treatment seeking and out of pocket expenditures among the urban poor in Delhi, India.* Health and Social Care in the Community, 2019. **27**(6): p. 1401-1420.
66. Vaiyapuri, S., et al., *Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India.* PLoS One, 2013. **8**(11): p. e80090.
67. Williams, H.F., et al., *Challenges in diagnosing and treating snakebites in a rural population of Tamil Nadu, India: The views of clinicians.* Toxicon, 2017. **130**: p. 44-46.
68. Bank, T.W. *The World Bank In India*. 2023 [cited 2023 October]; Available from: https://www.worldbank.org/en/country/india.
69. Acharya D, V.G., Muraleedharan VR, Dheenadayalan DS and Dash U *Do the Poor Benefit from Public Spending on Healthcare in India? Results from Benefit (Utilisation) Incidence Analysis in Tamil Nadu and Orissa* C.f.R.o.E.H.S. (CREHS), Editor. 2011, Indian Institute of Technology Madras: Chennai, India.
70. Aggarwal, S., *The long road to health: Healthcare utilization impacts of a road pavement policy in rural India.* Journal of Development Economics, 2021. **151**.
71. Mittal, Y.K., et al., *Delay factors in the construction of healthcare infrastructure projects: a comparison amongst developing countries.* Asian Journal of Civil Engineering, 2020. **21**(4): p. 649-661.

72. Mukherjee, S., *Conceptualisation and classification of caste and tribe by the Census of India.* J Anthropol Surv India 2013. **62**: p. 805-20.
73. Bhasin, M.K., *Morphology to molecular anthropology: Castes and tribes of India. International* Journal of Human Genetics, 2009. **9**(3-4): p. 145-230.
74. Patra, S. and M.D. Bhise, *Gender differentials in prevalence of self-reported non-communicable diseases (NCDs) in India: evidence from recent NSSO survey.* Journal of Public Health, 2016. **24**(5): p. 375-385.
75. Ghosh, D., & Dinda, S., *Health Infrastructure and Economic Development in India*. Health Economics and Healthcare Reform: Breakthroughs in Research and Practice. 2018: IGI Global.
76. Chokshi, M., et al., *Health systems in India.* J Perinatol, 2016. **36**(s3): p. S9-S12.
77. Bhandari, L., & Dutta, S., *Health infrastructure in rural India.* India infrastructure report, 2007: p. 265-85.
78. Hati, K.K., & Majumder, R., *Health Infrastructure, Health Outcome and Economic Wellbeing: A District Level Study in India.* 2013.
79. Humphries, C., et al., *Investigating discharge communication for chronic disease patients in three hospitals in India.* PLoS One, 2020. **15**(4): p. e0230438.
80. India, G.o. *Rashtriya Swasthya Bima Yojana*. 2016 [cited 2023 October]; Available from: https://www.india.gov.in/spotlight/rashtriya-swasthya-bima-yojana.
81. India, G.o. *Pradhan Mantri Jan Arogya Yojana (PM-JAY)*. 2019 [cited 2023 October]; Available from: https://nha.gov.in/PM-JAY.
82. Government of India, T.N.P.o.I. *Ministry of Ayush*. 2023 [cited 2023 October]; Available from: https://ayush.gov.in/index.html.
83. Datt, G. and M. Ravallion, *Why Have Some Indian States Done Better than Others at Reducing Rural Poverty?* Economica, 2003. **65**(257): p. 17-38.
84. Warrell, D.A., *Snakebite.* Lancet 2010. **375**: p. 77-88.
85. Kardong, K., *THE EVOLUTION OF THE VENOM APPARATUS IN SNAKES FROM COLUBRIDS TO VIPERIDS & ELAPIDS.* Mem. Inst. Butantan, 1982. **46**: p. 106-118.
86. Young, B.A.a.H., Florian and Friedel, Paul and Rammensee, Sebastian and Bausch, Andreas and van Hemmen, J. Leo, *Tears of Venom: Hydrodynamics of Reptilian Envenomation.* Phys. Rev. Lett., 2011. **106**(19).
87. Barlow, A., et al., *Coevolution of diet and prey-specific venom activity supports the role of selection in snake venom evolution.* Proc Biol Sci, 2009. **276**(1666): p. 2443-9.
88. Fry, B.G., et al., *Molecular evolution and phylogeny of elapid snake venom three-finger toxins.* J Mol Evol, 2003. **57**(1): p. 110-29.
89. Gutiérrez JM, C.J., Habib AG, Harrison RA, Williams DJ, Warrell DA, *Snakebite envenoming.* Nat Rev Dis Primers, 2017. **14**(3).
90. Ferraz, C.R., et al., *Multifunctional Toxins in Snake Venoms and Therapeutic Implications: From Pain to Hemorrhage and Necrosis.* Frontiers in Ecology and Evolution, 2019. **7**.
91. Rucavado, J.M.G.a.A., *Snake venom metalloproteinases: Their role in the pathogenesis of local tissue damage.* Biochimie, 2000. **82**(9–10): p. 841-850.
92. Kini, R.M., *Structure-function relationships and mechanism of anticoagulant phospholipase A2 enzymes from snake venoms.* Toxicon, 2005. **45**(8): p. 1147-61.
93. Fox, J.W. and S.M. Serrano, *Structural considerations of the snake venom metalloproteinases, key members of the M12 reprolysin family of metalloproteinases.* Toxicon, 2005. **45**(8): p. 969-85.
94. Takeda, S., H. Takeya, and S. Iwanaga, *Snake venom metalloproteinases: structure, function and relevance to the mammalian ADAM/ADAMTS family proteins.* Biochim Biophys Acta, 2012. **1824**(1): p. 164-76.
95. Ullah, A., et al., *Purification, crystallization and preliminary X-ray diffraction analysis of a class P-III metalloproteinase (BmMP-III) from the venom of Bothrops moojeni.* Acta Crystallogr Sect F Struct Biol Cryst Commun, 2012. **68**(Pt 10): p. 1222-5.
96. Heyborne, W.H. and S.P. Mackessy, *Identification and characterization of a taxon-specific three-finger toxin from the venom of the Green Vinesnake (Oxybelis fulgidus; family Colubridae).* Biochimie, 2013. **95**(10): p. 1923-32.

97. Adukauskienė D, V.E., Adukauskaitė A., *Venomous snakebites.* Medicina (Kaunas), 2011. **47**(8): p. 461-7.
98. Slagboom, J., et al., *Haemotoxic snake venoms: their functional activity, impact on snakebite victims and pharmaceutical promise.* Br J Haematol, 2017. **177**(6): p. 947-959.
99. Antonypillai, C.N., et al., *Hypopituitarism following envenoming by Russell's vipers (Daboia siamensis and D. russelii) resembling Sheehan's syndrome: first case report from Sri Lanka, a review of the literature and recommendations for endocrine management.* QJM, 2011. **104**(2): p. 97-108.
100. Massey, D.J., et al., *Venom variability and envenoming severity outcomes of the Crotalus scutulatus scutulatus (Mojave rattlesnake) from Southern Arizona.* J Proteomics, 2012. **75**(9): p. 2576-87.
101. Azofeifa, K., Y. Angulo, and B. Lomonte, *Ability of fucoidan to prevent muscle necrosis induced by snake venom myotoxins: comparison of high- and low-molecular-weight fractions.* Toxicon, 2008. **51**(3): p. 373-80.
102. Laing, G.D., et al., *Inflammatory pathogenesis of snake venom metalloproteinase-induced skin necrosis.* Eur J Immunol, 2003. **33**(12): p. 3458-63.
103. Sequeira Lopez ML, G.R., *Development of the renal arterioles.* J Am Soc Nephrol. , 2011. **22**(12): p. 2156-65.
104. Varagunam T, P.R., *Bilateral cortical necrosis of the kidneys following snakebite.* Postgrad Med J. , 1970. **46**(537): p. 449-51.
105. Sitprija, V., *Snakebite nephropathy.* Nephrology (Carlton), 2006. **11**(5): p. 442-8.
106. H.A., M.A.T.H.a.R., *Pathology of sea-snake poisoning.* Br Med J., 1961. **1**(5235): p. 1290-3.
107. Chugh, K.S., *Snake-bite-induced acute renal failure in India.* Kidney Int, 1989. **35**(3): p. 891-907.
108. Senthilkumaran, S., et al., *An Unnecessary Russell's Viper Bite on the Tongue Due to Live Snake Worship and Dangerous First Aid Emphasise the Urgent Need for Stringent Policies.* Toxins (Basel), 2022. **14**(12).
109. Warrell, D.A., *Snake venoms in science and clinical medicine 1. Russell's viper: biology, venom and treatment of bites.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1989. **83**(6): p. 732-740.
110. Gutierrez, J.M., et al., *Hemorrhage Caused by Snake Venom Metalloproteinases: A Journey of Discovery and Understanding.* Toxins (Basel), 2016. **8**(4): p. 93.
111. S.A.M. Kularatne, S.S., S.C. Medagedara, K. Maduwage, A. de Silva, *Revisiting saw-scaled viper (Echis carinatus) bites in the Jaffna Peninsula of Sri Lanka: distribution, epidemiology and clinical manifestations.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2011. **105**(10): p. 591-597.
112. Adhikari, R.B., et al., *Clinico-epidemiology and management of Russell's viper (Daboia russelii) envenoming in dogs in Sri Lanka.* Toxicol Rep, 2019. **6**: pp. 809-818.
113. Ramaswamy, M., S. Duraikannu, and C. Solaimuthu, *The Prevalence of Indian Common Krait Envenomation and Its Clinical Complications among the Rural Populations of India.* Journal of Drug Delivery and Therapeutics, 2018. **8**(2).
114. Senji Laxme, R.R., et al., *Biogeographical venom variation in the Indian spectacled cobra (Naja naja) underscores the pressing need for pan-India efficacious snakebite therapy.* PLoS Negl Trop Dis, 2021. **15**(2): p. e0009150.
115. Ranawaka, U.K., D.G. Lalloo, and H.J. de Silva, *Neurotoxicity in snakebite--the limits of our knowledge.* PLoS Negl Trop Dis, 2013. **7**(10): p. e2302.
116. Saluba Bawaskar, H., & Himmatrao Bawaskar, P., *Envenoming by the common krait (Bungarus caeruleus) and Asian cobra (Naja naja): clinical manifestations and their management in a rural setting.* Wilderness & environmental medicine, 2004. **15**(4): p. 257-266.
117. A., C., *L'immunisation artificielle des animaux contre le venin des serpents, et la thérapeutique expérimentale des morsures venimeuses.* C R Soc Biol. , 1894. **46**: p. 120–4.
118. Bochner, R., *Paths to the discovery of antivenom serotherapy in France.* J Venom Anim Toxins Incl Trop Dis, 2016. **22**: p. 20.

119. León, G., et al., *Current technology for the industrial manufacture of snake antivenoms.* Toxicon, 2018. **151**: p. 63-73.
120. Gutiérrez JM, L.G., Lomonte B, Angulo Y, *Antivenoms for snakebite envenomings.* Inflamm Allergy Drug Targets. , 2011. **10**(5): p. 369-80.
121. O'Leary, M.A. and G.K. Isbister, *Commercial monovalent antivenoms in Australia are polyvalent.* Toxicon, 2009. **54**(2): p. 192-195.
122. Herpetology, M.C.B.T.a. C.f. *The Irulas*. 2023 [cited 2023 October]; Available from: https://madrascrocodilebank.org/the_iruals.
123. Mukherjee, A.K., *Species-specific and geographical variation in venom composition of two major cobras in the Indian subcontinent: Impact on polyvalent antivenom therapy.* Toxicon, 2020. **188**: p. 150-158.
124. Maduwage, K., et al., *Efficacy of Indian polyvalent snake antivenoms against Sri Lankan snake venoms: lethality studies or clinically focussed in vitro studies.* Sci Rep, 2016. **6**: p. 26778.
125. Chakma, J.K., et al., *White paper on venomous snakebite in India.* Indian J Med Res, 2020. **152**(6): p. 568-574.
126. Madhushani, U., et al., *Effect of Indian Polyvalent Antivenom in the Prevention and Reversal of Local Myotoxicity Induced by Common Cobra (Naja naja) Venom from Sri Lanka In Vitro.* Toxins (Basel), 2021. **13**(5).
127. ID, S., *Snakebite management in India, the first few hours: a guide for primary care physicians.* J Indian Med Assoc., 2007. **105**(6): p. 324-328.
128. WHO, *Guidelines for the production, control and regulation of snake antivenom immunoglobulins, Annex 5, TRS No 1004*. 2017: Geneva, Switzerland.
129. Senji Laxme, R.R., et al., *Beyond the 'big four': Venom profiling of the medically important yet neglected Indian snakes reveals disturbing antivenom deficiencies.* PLoS Negl Trop Dis, 2019. **13**(12): p. e0007899.
130. Theakston, R.D., D.A. Warrell, and E. Griffiths, *Report of a WHO workshop on the standardization and control of antivenoms.* Toxicon, 2003. **41**(5): p. 541-57.
131. de Silva, H.A., N.M. Ryan, and H.J. de Silva, *Adverse reactions to snake antivenom, and their prevention and treatment.* Br J Clin Pharmacol, 2016. **81**(3): p. 446-52.
132. Pucca, M.B., et al., *History of Envenoming Therapy and Current Perspectives.* Front Immunol, 2019. **10**: p. 1598.
133. *Standard Treatment Guidelines, Management of Snake Bite*, M.o.H.F. Welfare, Editor. 2017, Government of India: New Delhi, India.
134. Bolon, I., et al., *Snakebite in domestic animals: First global scoping review.* Prev Vet Med, 2019. **170**: p. 104729.
135. Ward, R.J., et al., *Optimising monitoring efforts for secretive snakes: a comparison of occupancy and N-mixture models for assessment of population status.* Sci Rep, 2017. **7**(1): p. 18074.
136. India, G.o. *The Ayushman Bharat Digital Mission (ABDM)*. 2023 [cited 2023 October]; Available from: https://abdm.gov.in/.
137. Salve, P.S., S. Vatavati, and J. Hallad, *Clustering the envenoming of snakebite in India: The district level analysis using Health Management Information System data.* Clinical Epidemiology and Global Health, 2020. **8**(3): p. 733-738.
138. Khanna, A., R. Saha, and N. Ahmad, *National TB elimination programme - What has changed.* Indian J Med Microbiol, 2023. **42**: p. 103-107.
139. Joe, W., Sharma, S., Sharma, J., Shanta, Y. M., Ramanathan, M., Mishra, U. S., & Sri, B. S., *Maternal mortality in India: a review of trends and patterns*. Esocialsciences Working Papers, 2015(No. 353).
140. Chatterjee, I., A.K. Chakravarty, and A. Gomes, *Daboia russellii and Naja kaouthia venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla Hemidesmus indicus R.Br.* J Ethnopharmacol, 2006. **106**(1): p. 38-43.
141. S.S, S.V.a.S., *Anti-Snake Venom Activity of the Leaves and Stem Bark Extract of Alstonia Venenata R.Br. By In Vitro and In Vivo Methods in Swiss Albino Mice.* EAS Journal of Pharmacy and Pharmacology, 2019. **1**(6).

142. Alam, M.I. and A. Gomes, *Snake venom neutralization by Indian medicinal plants (Vitex negundo and Emblica officinalis) root extracts.* J Ethnopharmacol, 2003. **86**(1): p. 75-80.
143. Krishnan, S.A., et al., *Studies on the neutralizing effect of Ophiorrhiza mungos root extract against Daboia russelii venom.* J Ethnopharmacol, 2014. **151**(1): p. 543-7.
144. Mahalingam, P., *In vitro antitoxin activity of aqueous extracts of selective medicinal herbs against Naja naja venom.* 2018.
145. Gomes, A., S. Ghosh, and A. Gomes, *Why there are no effective herbal antidotes against snake venom available in India?* Indian J Med Res, 2020. **151**(6): p. 525-527.
146. Nyaaba, A.A. and M. Ayamga, *Intricacies of medical drones in healthcare delivery: Implications for Africa.* Technology in Society, 2021. **66**.
147. Kamalraj, R., *Deep Learning Model for Identifying Snakes by using Snakes' Bite Marks.* International Conference on Computer Communication and Informatics, 2020: p. 1-4.
148. Kirkham, G., *Snakes and smartphones: exploring transdisciplinary design collaborations for the governance of snakebite.* cultural geographies, 2023.
149. Hansson, E., et al., *Using geographical information systems to identify populations in need of improved accessibility to antivenom treatment for snakebite envenoming in Costa Rica.* PLoS Negl Trop Dis, 2013. **7**(1): p. e2009.
150. Anna M. Molesworth, R.H., R. David, G. Theakston, David G. Lalloo, *Geographic information system mapping of snakebite incidence in northern Ghana and Nigeria using environmental indicators: a preliminary study.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2003. **97**(2): p. 188-192.
151. Pintor, A.F.V., et al., *Addressing the global snakebite crisis with geospatial analyses - Recent advances and future direction.* Toxicon X, 2021. **11**: p. 100076.
152. Tiago R. Simões, M.W.C., Mateusz Tałanda, Massimo Bernardi, Alessandro Palci, Oksana Vernygora, Federico Bernardini, Lucia Mancini & Randall L. Nydam, *The origin of squamates revealed by a Middle Triassic lizard from the Italian Alps 557, .* Nature, 2018. **557**: p. 706–709.
153. Da Silva, F.O., et al., *The ecological origins of snakes as revealed by skull evolution.* Nat Commun, 2018. **9**(1): p. 376.
154. Alex Pappachen James, B.M., Sherin Sugathan and Dileep Kumar Raveendran, *Discriminative histogram taxonomy features for snake species identification.* Human-centric Computing and Information Sciences, 2014. **4**(3).
155. Pyron, R.A., et al., *Effectiveness of phylogenomic data and coalescent species-tree methods for resolving difficult nodes in the phylogeny of advanced snakes (Serpentes: Caenophidia).* Mol Phylogenet Evol, 2014. **81**: p. 221-31.
156. Burbrink, F.T., & Crother, B. I., *Evolution and taxonomy of snakes. Reproductive biology and phylogeny of snakes*. Vol. 9. 2011.
157. Chippaux, J.P., *Snake venoms and envenomations* 2006: Krieger Publishing
158. MD., M., *What is the most dangerous snake?* J Venom Anim Toxins Incl Trop Dis, 2013. **19**(19).
159. A., S., *Dangerous snakes, deadly snakes and medically important snakes.* J Venom Anim Toxins Incl Trop Dis., 2013. **19**(26).
160. Longbottom, J., et al., *Vulnerability to snakebite envenoming: a global mapping of hotspots.* Lancet, 2018. **392**(10148): p. 673-684.
161. Cornelis, J., *Killing them softly: a review on snake translocation and an Australian case study.* Herpetological Journal, 2021(Volume 31, Number 3): p. 118-131.
162. Barve, S., Bhaisare, D., Giri, A., Shankar, P. G., Whitaker, R., & Goode, M, *A preliminary study on translocation of "rescued" King Cobras (Ophiophagus hannah).* Hamadryad, 2013. **36**(6): p. 80-86.
163. Wolfe, A.K., P.A. Fleming, and P.W. Bateman, *Impacts of translocation on a large urban-adapted venomous snake.* Wildlife Research, 2018. **45**(4).
164. Weiss, L., P. Brandl, and D. Frynta, *Fear reactions to snakes in naive mouse lemurs and pig-tailed macaques.* Primates, 2015. **56**(3): p. 279-84.
165. Lobue V, D.J., *Detecting the snake in the grass: attention to fear-relevant stimuli by adults and young children. .* Psychol Sci. , 2008. **19**(3): p. 284-9.

166. Seligman, M.E.P., *Phobias and preparedness.* Behavior Therapy, 1971. **2**(3): p. 307-320.
167. Williams, S.S., et al., *Delayed Psychological Morbidity Associated with Snakebite Envenoming.* PLOS NEGLECTED TROPICAL DISEASES, 2011. **5**(8).
168. Habib, Z.G., et al., *Posttraumatic stress disorder and psycho-social impairment following snakebite in Northeastern Nigeria.* Int J Psychiatry Med, 2021. **56**(2): p. 97-115.
169. Weems, C.F., et al., *Physiological response and childhood anxiety: association with symptoms of anxiety disorders and cognitive bias.* J Clin Child Adolesc Psychol, 2005. **34**(4): p. 712-23.
170. Bannister, R., *Underfunded mental healthcare in the NHS: the cycle of preventable distress continues.* BMJ, 2021. **375**: p. n2706.
171. Parvanta, C.P., David E. Nelson, Sarah A. Parvanta, Richard N. Harner, *Essentials of Public Health Communication*. 29 Sept 2010: Jones & Bartlett Publishers.
172. Tesh, S.N., *Hidden Arguments: Political Ideology and Disease Prevention Policy*. 1988: Rutgers University Press.
173. Guttman, N., *Public Health Communication Interventions*. 2000: SAGE.
174. Durkin, S., E. Brennan, and M. Wakefield, *Mass media campaigns to promote smoking cessation among adults: an integrative review.* Tob Control, 2012. **21**(2): p. 127-38.
175. Noar, S.M., et al., *A 10-Year Systematic Review of HIV/AIDS Mass Communication Campaigns: Have We Made Progress?* Journal of Health Communication, 2009. **14**(1): p. 15-42.
176. M A. Winkleby, C.B.T., D Jatulis, and S P Fortmann, *The long-term effects of a cardiovascular disease prevention trial: the Stanford Five-City Project.* American Journal of Public Health, 1996. **86**: p. 1773-1779.
177. Osteria T, S.G., *The impact of religion and cultural values on AIDS education programs in Malaysia and the Philippines.* AIDS Educ Prev. , 1991 **3**(2): p. 133-46.
178. Slater, M.D., K. Kelly, and R. Edwards, *Integrating Social Marketing, Community Readiness and Media Advocacy in Community-Based Prevention Efforts.* Social Marketing Quarterly, 2016. **6**(3): p. 124-137.
179. Basu, A. and J. Wang, *The role of branding in public health campaigns.* Journal of Communication Management, 2009. **13**(1): p. 77-91.
180. Chaaithanya, I.K., et al., *Perceptions, awareness on snakebite envenoming among the tribal community and health care providers of Dahanu block, Palghar District in Maharashtra, India.* PLoS One, 2021. **16**(8): p. e0255657.
181. Vaiyapuri, S., et al., *Multifaceted community health education programs as powerful tools to mitigate snakebite-induced deaths, disabilities, and socioeconomic burden.* Toxicon X, 2023. **17**: p. 100147.
182. Kadam, P., et al., *Approaches for implementing society-led community interventions to mitigate snakebite envenoming burden: The SHE-India experience.* PLoS Negl Trop Dis, 2021. **15**(2): p. e0009078.
183. Harrison, R.A. and J.M. Gutierrez, *Priority Actions and Progress to Substantially and Sustainably Reduce the Mortality, Morbidity and Socioeconomic Burden of Tropical Snakebite.* Toxins (Basel), 2016. **8**(12).
184. Kshirsagar VY, A.M., Colaco SM, *Clinical profile of snake bite in children in rural India.* Iranian Journal of Pediatrics, 2013. **23**(6): p. 632-6.
185. Jamieson R, P.J., *An epidemiological and clinical study of snake-bites in childhood.* Med J Aust., 1989. **150**(12): p. 698-702.
186. Harbi, N.a., *Epidemiological and clinical differences of snake bites among children and adults in southwestern Saudi Arabia.* J Accid Emerg Med, 1999. **16**(6): p. 428-30.
187. Huhman, M.E., et al., *The Influence of the VERB campaign on children's physical activity in 2002 to 2006.* Am J Public Health, 2010. **100**(4): p. 638-45.
188. Julia Bello-Bravo, I.B., *Animated Videos as a Learning Tool in Developing Nations: A Pilot Study of Three Animations in Maradi and Surrounding Areas in Niger.* EJISDC The Electronic Journal of Information Systems In Developing Countries, 2017. **55**(1).

Theme 1: Clinical Management of SBE

Theme 1: Clinical Management of SBE

SBE is a complex medical scenario that requires well-trained healthcare professionals, at every level of patient presentation. One way of aiding healthcare professionals in managing the clinical manifestations of SBE is the use of biomarkers to help detect medical complications earlier, this supports and helps reduce overall treatment costs for patients by reducing the clinical burden of complex care due to an SBE event. Our work has highlighted how the detection of NGAL in Russell's viper bite patients can assist clinicians in being able to detect AKI earlier, initiating RRT sooner, improving clinical outcomes for patients and reducing extensive hospital stays which in turn contributed to a reduction in the final treatment costs. High treatment costs are also exacerbated by prescribing costs in hospitals which can escalate depending on the level of care patients require. Antibiotic interventions are usually required during wound infections from SBE; however, they are often administered as a standard of care to prevent any bacterial infection from developing without a confirmed clinical diagnosis. Our work illustrates that it is vital to ensure the most cost-effective antibiotic therapy is provided to patients only upon a clinically confirmed diagnosis of bacterial infection. This in turn reduces the risk of global antibiotic resistance as well as prevents patient treatment costs from cumulatively increasing. The fear of high treatment costs and lack of trust in medical professionals prevents patients from seeking prompt treatment which in turn leads to more complex care needed at a later stage as well as a larger socioeconomic burden to the patient. Identifying key factors contributing to the overall treatment costs for SBE patients is central to understanding the downstream socioeconomic impact a snakebite can have on patients, their families, and endemic communities. However, treating SBE for Indian healthcare professionals poses many difficulties as there is a lack of training and education which means professionals do not have the adequate skills or confidence to manage patients. Our work aims to understand and baseline the foundational knowledge of healthcare providers across all levels of the Indian healthcare infrastructure. Here we have highlighted that there is a fundamental lack of understanding of even basic first aid amongst healthcare professionals as well as a poor understanding of the environmental factors leading to SBE. The length of service in the profession and exposure to SBE patients does not necessarily mean a wider insight into clinical care or management. By identifying areas of concern, we can focus efforts and work with key policyholders to ensure appropriate educational curricula and professional training are developed for healthcare professionals. Moreover, our work can assist with informing public health policy changes and the development of appropriate medical insurance so that vulnerable communities can seek access to unobstructed care promptly without the fear of financial ruin thus avoiding complex medical manifestations later and saving lives.

Chapter 2

Neutrophil gelatinase-associated lipocalin acts as a robust early diagnostic marker for renal replacement therapy in patients with Russell's viper bite–induced acute kidney injuries.
Subramanian Senthilkumaran, Ketan Patel, Anika Salim, Pradeep Vijayakumar, Harry F. Williams, Rajendran Vaiyapuri, Ravi Savania, Namasivayam Elangovan, Ponniah Thirumalaikolundusubramanian, M. Fazil Baksh, and Sakthivel Vaiyapuri. Toxins (Basel). 2021 Nov 12;13(11):797.

The rationale for this study

The variations in the biochemical properties of snake venom toxins can result in a myriad of clinical complications for patients. Even bites from the same snake species can present different complications in patients. To date, no specific clinical tests have been developed in India to aid SBE detection and prognosis of these medical complications in patients. Therefore, clinicians deploy a battery of tests and diagnostic procedures such as laboratory tests e.g. blood and urine biochemical tests and different imaging techniques to aid in the clinical management of SBE patients. To improve the clinical management of patients, there is a great need to better understand and investigate potential biomarkers that could be used to facilitate the clinical management of SBE patients.

SBE-associated AKI can mostly be attributed to bites from the *Viperidae* family, and this complication can be frequently observed in patients who are bitten by Russell's viper in India. AKI is a life-threatening clinical complication and potentially fatal. Patients who develop AKI require complex and lifesaving care with renal replacement therapy. Currently, the development of AKI in SBE patients is only ascertained based on the creatinine levels which may take up to 48 hours to increase. This not only prolongs the duration of complex clinical management for patients but also contributes to high treatment costs for the patient depending on the level of injury they acquire.

NGAL has received increasing attention over the years as an early biomarker for AKI. Since Russell's viper patients are at risk of AKI it was proposed that investigating the use of NGAL compared to serum creatinine could explore the use of NGAL as a potential early biomarker for SBE-induced AKI. Hence, in this study, 309 confirmed Russell's viper patients were recruited following the inclusion/exclusion criteria and obtaining their consent. Their NGAL levels were tested at admission by collecting their blood samples along with serum and urine creatinine levels at various time points.

This study indicated that gender, age and time of receiving antivenom are not predictors of the AKI grades in patients. Serum creatinine cannot be considered to be a reliable marker of AKI due to the length of time required for elevated levels to be seen in patients, which can be up to 48 hours. NGAL

can act as an early biomarker for AKI and an NGAL value of above 494 ng/dL on admission indicates that renal replacement therapy should be initiated for patients immediately without waiting for the creatinine levels to increase. Further work developing this clinical study to encompass a larger patient population as well as those who may have other underlying health conditions needs to be undertaken to achieve a consensus on the robust use of NGAL in SBE patients in clinical settings.

My contribution to this chapter (50%)

Our collaborators in India (Subramanian Senthilkumaran, Rajendran Vaiyapuri, Namasivayam Elangovan, Ponniah Thirumalaikolundusubramanian) along with Sakthivel Vaiyapuri, Ravi Savania and Ketan Patel at the University of Reading, UK conceptualised and carried out this study by collecting data from the patients.

I organised, cleaned and analysed the raw data at the University of Reading. The statistical analysis plan was developed with Sakthivel Vaiyapuri which included exploratory data modelling. This involved identifying the key properties of the data and which differentiators to utilise to apply the most appropriate modelling strategy. The Youden's index was applied to the data by Fazil Baksh. I completed the visualisation, programming, and preparation of the study figures and the smoothed density and Sankey plots were prepared by Fazil Baksh. Manuscript preparation, writing, editing, and review were performed by Anika Salim and Sakthivel Vaiyapuri.

toxins

MDPI

Article

Neutrophil Gelatinase–Associated Lipocalin Acts as a Robust Early Diagnostic Marker for Renal Replacement Therapy in Patients with Russell's Viper Bite–Induced Acute Kidney Injuries

Subramanian Senthilkumaran [1,2,†], Ketan Patel [3,†], Anika Salim [4,†], Pradeep Vijayakumar [4], Harry F. Williams [5], Rajendran Vaiyapuri [5], Ravi Savania [4], Namasivayam Elangovan [1], Ponniah Thirumalaikolundusubramanian [6], M. Fazil Baksh [7] and Sakthivel Vaiyapuri [4,*]

1 Department of Biotechnology, School of Biosciences, Periyar University, Salem 636011, Tamil Nadu, India; maniansenthil76@gmail.com (S.S.); elangovannn@gmail.com (N.E.)
2 Emergency Department, Manian Medical Centre, Erode 638001, Tamil Nadu, India
3 School of Biological Sciences, University of Reading, Reading RG6 6UB, UK; ketan.patel@reading.ac.uk
4 School of Pharmacy, University of Reading, Reading RG6 6UB, UK; anika.salim@pgr.reading.ac.uk (A.S.); pradeep.vijayakumar@pgr.reading.ac.uk (P.V.); r.savania@reading.ac.uk (R.S.)
5 Research and Development Department, Toxiven Biotech Private Limited, Coimbatore 641042, Tamil Nadu, India; harry@toxiven.com (H.F.W.); raj@toxiven.com (R.V.)
6 Research Department, Trichy SRM Medical College Hospital & Research Centre, Trichy 621105, Tamil Nadu, India; ponniah.tks@gmail.com
7 Department of Mathematics and Statistics, University of Reading, Reading RG6 6UR, UK; m.f.baksh@reading.ac.uk
* Correspondence: s.vaiyapuri@reading.ac.uk
† These authors contributed equally to this study.

Abstract: Snakebite-induced acute kidney injury (AKI) is frequently observed in patients following bites from vipers such as Russell's viper (*Daboia russelii*) in India. Currently, the levels of serum creatinine are mainly used as a marker to determine the necessity for renal replacement therapy (RRT) (haemodialysis) in severe cases of AKI. However, it takes up to 48 h to ascertain a distinct change in creatinine levels compared to its baseline level upon admission. The time lost between admission and the 48 h timepoint significantly affects the clinical management of snakebite victims. Moreover, early diagnosis of AKI and decision on the necessity for RRT in snakebite victims is critical in saving lives, reducing long-term complications, and minimising treatment costs arising from expensive haemodialysis. Neutrophil gelatinase–associated lipocalin (NGAL) has been recently studied as a robust early marker for AKI in non-snakebite patients. However, its suitability for clinical use in snakebite victims has not been rigorously established. Here, we demonstrate the clinical significance of plasma NGAL as a robust marker for RRT following AKI using a large cohort (309) of Russell's viper victims without any pre-existing health conditions. NGAL levels upon admission are positively correlated with creatinine levels at 48 h in different stages of AKI. Overall, NGAL acts as a robust early marker to ascertain the need for RRT following Russell's viper bites. The quantification of NGAL can be recommended as a routine test in hospitals that treat snakebites to decide on RRT at early time points instead of waiting for 48 h to confirm the increase in creatinine levels. The diagnostic use of NGAL in Russell's viper victims with pre-existing comorbidities and for other vipers should be evaluated in future studies.

Keywords: snakebite envenomation; Russell's viper; viper bites; acute kidney injury; renal biomarker; neutrophil gelatinase–associated lipocalin; NGAL; renal replacement therapy

Key Contribution: Russell's viper bite-induced AKI is frequently observed in victims, and it needs urgent medical attention. Using a large clinical study, we demonstrate that NGAL acts as a robust early diagnostic marker to ascertain the need for RRT in patients with AKI following Russell's viper bites.

Citation: Senthilkumaran, S.; Patel, K.; Salim, A.; Vijayakumar, P.; Williams, H.F.; Vaiyapuri, R.; Savania, R.; Elangovan, N.; Thirumalaikolundusubramanian, P.; Baksh, M.F.; et al. Neutrophil Gelatinase–Associated Lipocalin Acts as a Robust Early Diagnostic Marker for Renal Replacement Therapy in Patients with Russell's Viper Bite–Induced Acute Kidney Injuries. *Toxins* **2021**, *13*, 797. https://doi.org/10.3390/toxins13110797

Received: 16 October 2021
Accepted: 10 November 2021
Published: 12 November 2021

1. Introduction

Snakebite envenomation (SBE) is a high priority, neglected tropical disease that predominantly affects rural communities living in developing countries such as India [1,2]. Among the Indian 'Big Four' snakes (Russell's viper, Indian cobra, common krait, and saw-scaled viper), Russell's viper (*Daboia russelii*) is responsible for most incidents and subsequent deaths and disabilities in rural India [3–6]. In addition to haemotoxic, neurotoxic, and myotoxic envenomation effects, nephrotoxicity in the form of acute kidney injury (AKI) in victims following Russell's viper bites is frequently (around 15% of SBE victims [7]) observed [8,9]. AKI is also one of the key factors that contribute to SBE-induced deaths and long-term complications [10]. The severity of AKI following Russell's viper bites varies widely depending on the amount of venom injected; locality of the offending snake; age, body mass, and existing health conditions of victims; and notably, the time delay between the bite and hospital treatment [11]. The proportion of patients with AKI (including non-SBE cases) requiring renal replacement therapy (RRT, which indicates the necessity for haemodialysis) varies from 25% to 100% [7,12,13]. In current clinical practice, the levels of serum creatinine, blood urea nitrogen, urinary albumin/proteins, and urine outputs are considered as biomarkers to determine the functional status of kidneys and severity of AKI [14]. However, these conventional markers are not ideal to establish the injuries arising from haemodynamic changes in kidneys that lead to variations in glomerular filtration rate, particularly during acute damage [14,15]. Moreover, serum creatinine and blood urea levels do not change promptly at the early phases of AKI, as the individuals with normal renal function have a functional reserve [16]. Thus, the glomerular filtration rate measured based on serum creatinine or other methods may not precisely reflect the early AKI [17]. It is also important to note that serum creatinine levels do not rise until over 50% of the renal glomeruli are affected. The elevation of serum creatinine appears from approximately 24 to 48 h following the renal injury/damage and thus, it does not aid in confirming SBE-induced AKI at early stages [15,16]. Generally, the serum creatinine levels are monitored for up to 48 h to confirm elevated levels before a decision is made regarding the need for RRT. Therefore, this type of clinical decision may be too late for SBE victims as the progression of pathological complications following SBE can be rapid. A robust early diagnostic tool to ascertain the necessity for RRT and subsequent timely intervention/treatment is likely to enhance the outcomes of SBE victims and reduce the substantial treatment costs arising from expensive haemodialysis.

Neutrophil gelatinase–associated lipocalin (NGAL), a 25 kDa protein belonging to the lipocalin family, has received significant attention in recent years as an early marker for AKI, as its levels are elevated in blood and urine much earlier than those of serum creatinine [18]. NGAL is expressed and released mainly from kidneys, neutrophils, epithelial cells, and the liver in response to various pathological conditions including AKI, inflammation, infection, and intoxication. The elevated NGAL level during AKI reflects structural injuries to the kidneys in contrast to serum creatinine, which demonstrates their functional status [18]. The clinical relevance of NGAL for SBE has not been explored until recently. In our preliminary studies, we reported the occurrence of elevated levels of NGAL following Russell's viper bites long before the clinical manifestations and conventional serum creatinine levels [19–21]. Similarly, a few other studies have reported the prominence of plasma and urinary NGAL in SBE victims to determine the severity of AKI [9,22]. However, the clinical relevance of NGAL as a robust early biomarker to ascertain the need for RRT following Russell's viper bites specifically in South India has not been effectively established. Hence, we have conducted a rigorous clinical study with a large cohort of Russell's viper bite victims and vigorous exclusion and inclusion criteria and demonstrate the significance of plasma NGAL as a robust early biomarker for AKI to determine the need for RRT in these patients.

2. Results

2.1. Males and Working-Age Groups Are Largely Affected by Russell's Viper Bites

Patients who had pre-existing diabetes, hypertension, and renal diseases as well as other infectious diseases were not included in this study (more details are provided in methods section). Following our exclusion and inclusion criteria, totally, 309 patients were recruited in this study, and they included 227 (73.5%) males and 82 (26.5%) females. They were further assigned into appropriate grades [by following the criteria provided by the acute kidney injury network (AKIN)] based on their serum creatinine levels or its fold increase at 48 h from baseline level creatinine at admission: grade 0 (101 patients)—74 (73.3%) males and 27 (26.7%) females; grade 1 (93)—72 (77.4%) males and 21 (22.6%) females; grade 2 (73)—49 (67.1%) males and 24 (32.9%) females; grade 3 (35)—26 (74.3%) males and 9 (25.7%) females; grade X (7)—6 (85.7%) males and 1 (14.3%) female (Figure 1A). These data suggest that males were more affected by Russell's viper bites than females in this study cohort.

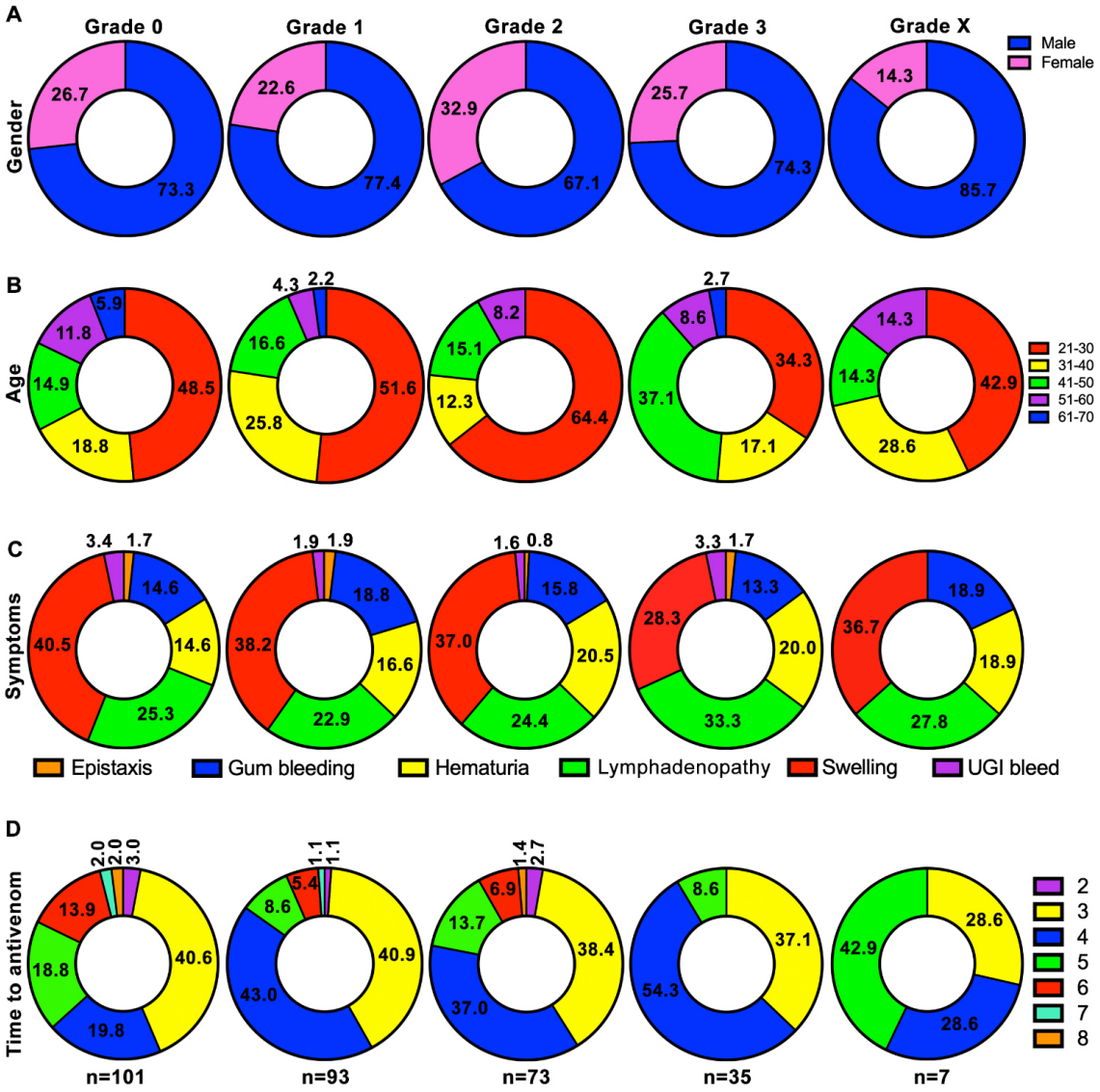


Figure 1. Characteristics of study population in different grades. (**A**) The distribution of males and females by grade. (**B**) Age distribution of patients in different grades. (**C**) Distribution of classical clinical symptoms of Russell's viper bite in each category. (**D**) Distribution of elapsed time (hours) from bite to antivenom administration in different categories. This study only included patients who arrived at the hospital within 8 h following the bite. Multinomial logistic regression models were used to test whether gender, age, clinical symptoms, and time to antivenom were predictors of grade. The numbers shown on figure panels indicate the percentage in respective categories.

To determine the specific age groups that were largely affected by Russell's viper bites, the age of study participants was analysed. Their age ranged from 21 to 70 years with a mean and median of 34.88 (SD = 11.23) and 30 years (IQR: 26 to 42 years), respectively. As shown in Figure 1B, the patients between 21 and 50 years old were mainly affected by Russell's viper bites in all grades. Specifically, the age group of 21–30 included 51.4% of total victims. These data confirm that the working-age groups of this cohort were largely affected by Russell's viper bites.

The clinical symptoms of Russell's viper bites were then analysed in these patients. Here, most patients in all grades displayed classical Russell's viper bite symptoms of swelling, lymphadenopathy, haematuria, and bleeding around their gums (Figure 1C). A small number of patients developed epistaxis and upper gastrointestinal bleeding. There was no significant difference detected among any of these symptoms between the patients in various grades.

Delay in seeking antivenom treatment results in excessive skeletal muscle and kidney damage among SBE victims. In this study, although we recruited patients who arrived at the hospital within 8 h following bites, the arrival time was very similar (no significant difference) in all the grades (mean arrival time: grade 0 = 4.1 h; grade 1 = 3.8 h; grade 2 = 3.9 h; grade 3 = 3.7 h; grade X = 4.1 h) (Figure 1D). Based on the clinical symptoms and clotting parameters (specifically, prothrombin time/international normalised ratio of clotting test (PT/INR)), the patients received antivenom treatment. As shown in Figure 1D, all patients in this study received intravenous infusion of polyvalent antivenom raised against the Indian 'Big Four' snakes (Bharat Serums and Vaccines, India). The antivenom doses varied from 10 (100 mL) to 30 (300 mL) vials based on the severity of symptoms and clotting complications. The antivenom administration was started with 10 vials followed by monitoring of clotting parameters for every 6 h and additional infusion of 5–10 vials if PT/INR was still prolonged. In this study, none of the patients received more than 30 vials of antivenom.

The urine output in patients who were in grade 0 was normal (i.e., >100 mL/hour). However, the urine output was reduced in patients in other grades: grade 1: 50–60 mL/hour; grade 2: 30–40 mL/hour; grade 3: 20–25 mL/hour. All the patients in grade 3 were treated with 4 to 6 cycles of haemodialysis based on their creatinine levels at 48 h. The same number of cycles was used for patients in grade X based on their other critical conditions (as detailed in the methods section).

Together, these data confirm that gender, age, clinical symptoms, and time to antivenom were not significant predictors of grade classifications (X^2 = 56.7859, df = 52, and p = 0.301) for AKI. These suggest that the severity of bite and elevation of creatinine levels were likely to be due to other factors such as the amount of venom injected in these patients.

2.2. Creatinine Levels Are Significantly Increased over Time in Grades 1–3

To determine the status of serum creatinine over time following the bite, its levels were measured at 0, 12, 24, and 48 h from admission. Patients' profile plots of serum creatinine for different grades are provided in Figure 2. While there was no significant change (t = 0.870; p = 0.384) in the mean level of creatinine in grade 0 over the study period (Figure 2A), the mean rates of change in creatinine levels for all other grades were significantly different from that of grade 0 (p values < $2e^{-16}$ for grades 1–3 and X). The estimated mean increase in creatinine level in grade 1 was 0.273 mg/dL/day (SE = 0.0096) (Figure 2B), grade 2 was 0.468 mg/dL/day (SE = 0.0152) (Figure 2C), grade 3 was 0.634 mg/dL/day (SE = 0.0348) (Figure 2D), and grade X was 0.315 mg/dL/day (SE = 0.0310) (Figure 2E). These data suggest that the antivenom treatment did not completely prevent the progression of AKI (as measured via creatinine levels) and the necessity for haemodialysis among these patients, especially in grade 3 and X.

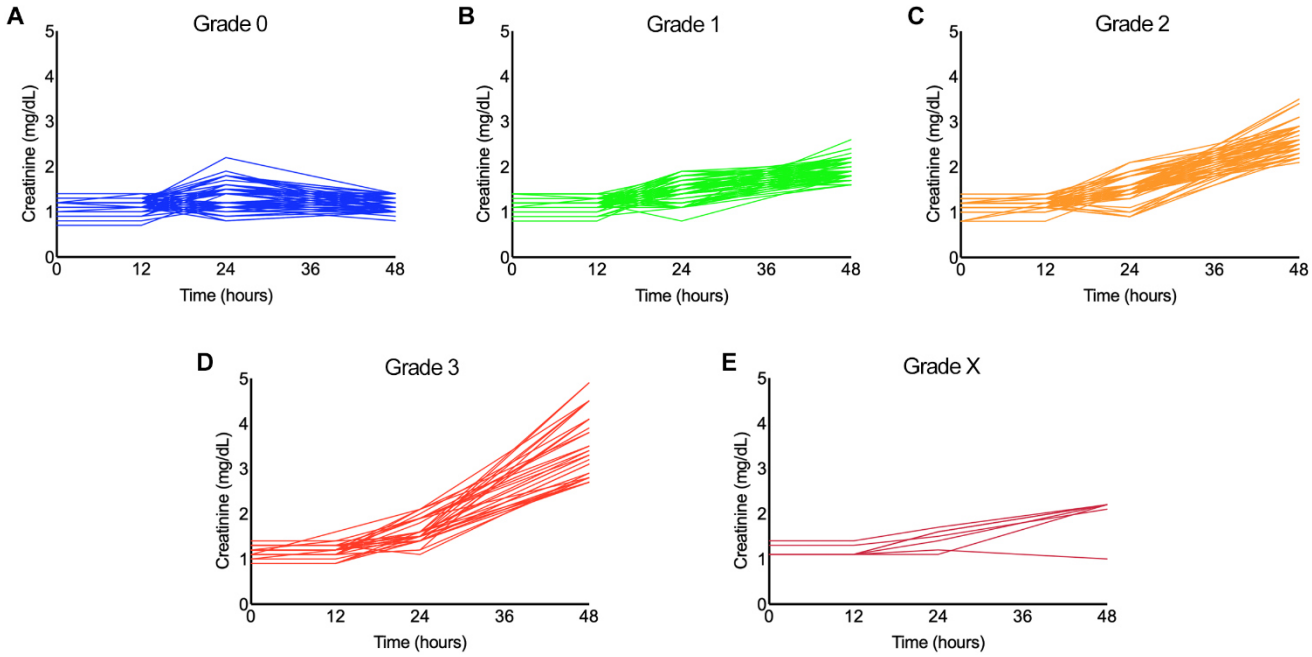


Figure 2. Patient profile plots of serum creatinine levels measured over 48 h in different grades. The levels of serum creatinine were measured at 0, 12, 24, and 48 h following admission for all patients in grade 0 (**A**), grade 1 (**B**), grade 2 (**C**), grade 3 (**D**), and grade X (**E**). For each category, we tested whether there is a significant change in mean creatinine level over time using generalised linear mixed effects models.

2.3. NGAL Acts as a Robust Biomarker for Grades 3 and X

To determine whether NGAL could acts as an early diagnostic marker for Russell's viper bite–induced AKI that necessitates RRT, its plasma level was measured in all the patients upon admission (i.e. 0 h). NGAL levels varied widely among different grades: grade 0—170 to 430 (mean 228.9; SD = 61.27); grade 1—210 to 450 (mean 346.7; SD = 45.63); grade 2—180 to 450 (mean 387.7; SD = 48.72); grade 3—500 to 700 (mean 633.7; SD = 43.40); grade X—590 to 680 (mean 620.6; SD = 27.91) (Figure 3A). NGAL was a highly significant predictor for creatinine levels at 48 h and positively correlated with the elevation of creatinine levels when adjusted for gender, age, symptoms, and time to antivenom (F = 540.6; df = 1, 282; $p = 2.2e^{-16}$) (Figure 3B). However, NGAL levels were not significantly positively correlated with time to antivenom treatment. The Youden index was used to identify the best cut-off level for plasma NGAL to decide on the need for RRT in Russell's viper bite victims. The mean value for this index based on 5000 bootstrap samples was found to be 493.75 ng/dL (SD = 20.02) with a sensitivity and specificity of 98% (SD = 0.03) and 100% (SD = 0.01), respectively, with an area under the curve (AUC) of 1.0. The accuracy was 99% (SD = 0.01). Hence, a cut-off value of >494 ng/dL of NGAL may act as a best indicator to ascertain the necessity for haemodialysis.

Furthermore, the levels of blood glucose (Figure 4A), urea (Figure 4B), INR (Figure 4C), sodium (Figure 4D), and potassium (Figure 4E) did not significantly differ between grades. Similarly, they did not significantly associate with the levels of NGAL in all grades (p values of test for joint association with NGAL were as follows: grade 0: F = 1.7784, df = (5,82), $p = 0.1264$; grade 1: F = 0.3313, df = (5,74), $p = 0.8925$; grade 2: F = 1.4198, df = (5,55), $p = 0.2316$; grade 3: F = 0.0734, df = (5,16), $p = 0.9955$).

Overall, these data suggest that an NGAL value of above 494 ng/dL upon admission indicates the necessity for RRT among Russell's viper bite victims with AKI. Hence, RRT can be initiated in these patients earlier instead of waiting for 48 h for creatinine levels to get increased as an indicator.

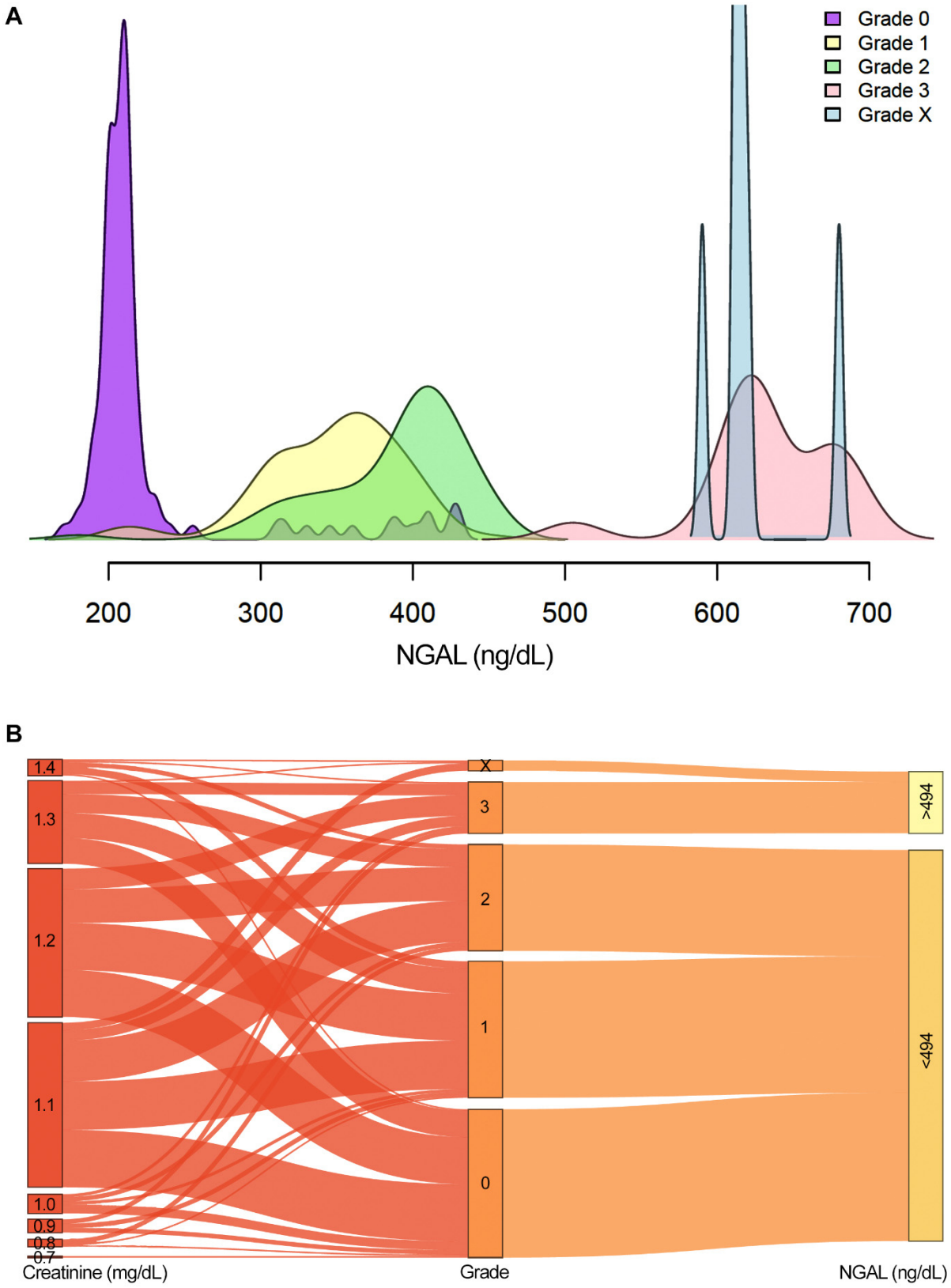


Figure 3. NGAL levels measured upon admission in different grades. (**A**) Smoothed density plots of the NGAL levels of the study population measured upon admission for different grades. Negligible overlap was observed in the NGAL values of grades 3 and X with other grades. (**B**) Sankey plot illustrates the association between serum creatinine levels at 0 h with NGAL levels measured at the same time point in different grades. All patients in grades 3 and X had more than 494 ng/dL of NGAL upon admission, while their creatinine levels at time 0 did not align with the grade classification at 48 h. The association between creatinine values at 48 h and NGAL was analysed using a linear model and adjusted for gender, age, clinical symptoms, and time to antivenom.

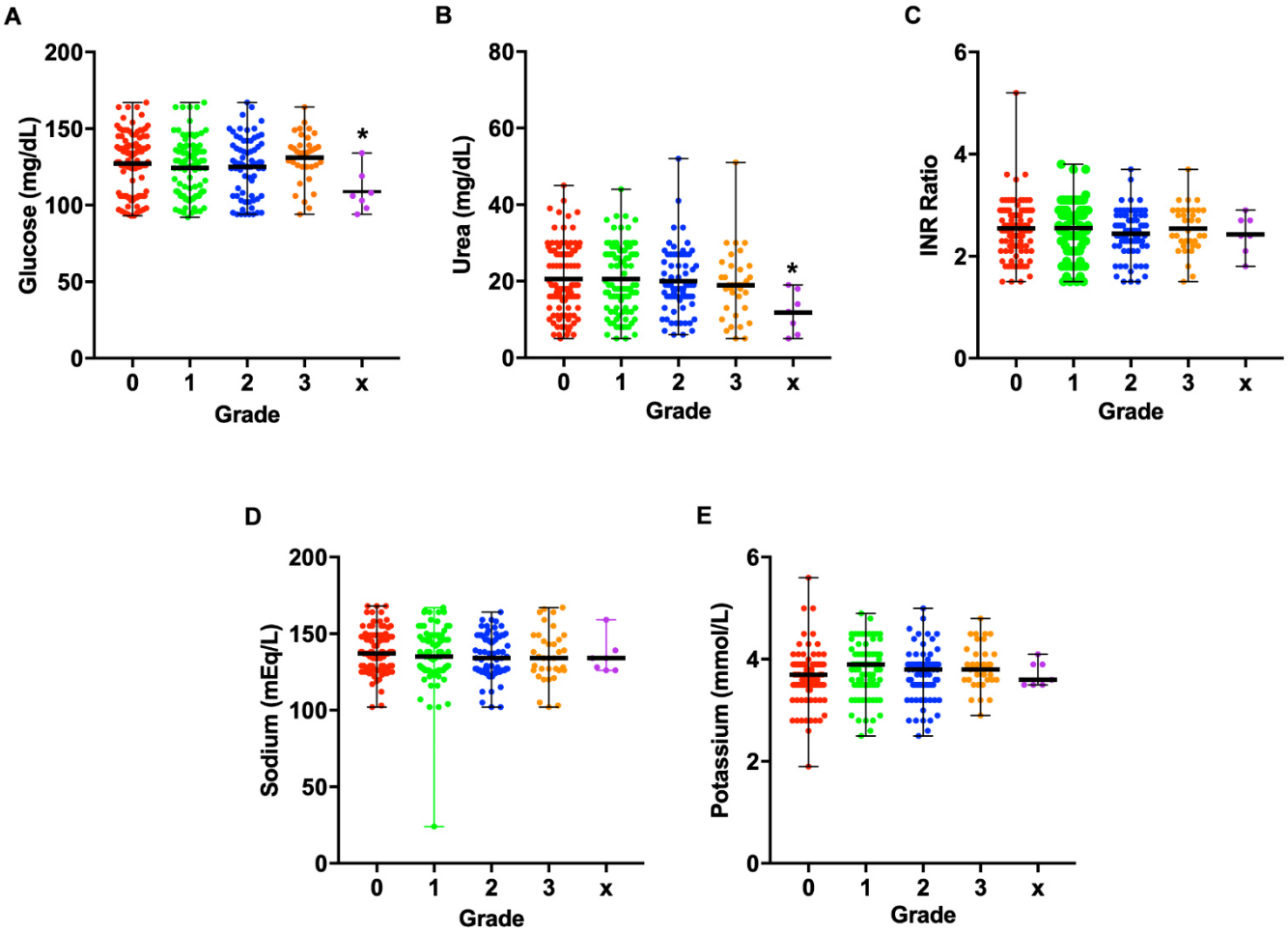


Figure 4. Distinct parameters relevant to AKI measured in different grades. Blood glucose (**A**), urea (**B**), INR ratio (**C**), sodium (**D**), and potassium (**E**) levels were measured in the study population among different grades at the time of admission. These values did not significantly differ between grades. There was no association between NGAL and these clinical parameters as analysed using a linear model, adjusted for gender, age, clinical symptoms, and time to antivenom.

3. Discussion

SBE is a major public health issue in rural tropics as it causes a significant number of deaths, permanent disabilities, and economic loss among rural communities [2,4]. Notably, the World Health Organisation has classified SBE as a high-priority, neglected tropical disease with a strategic map that aims to reduce SBE-induced deaths and disabilities by half before 2030 [23]. SBE victims (especially following viper bites) frequently develop AKI due to the direct actions of venom toxins on kidney tubules, decreased renal, or intrarenal perfusion because of venom-induced hypotension, inflammation, and oedema in the tubulointerstitial region, nephropathy following extensive haemolysis and/or rhabdomyolysis, infection due to the bite, and reduced filtering capacity of the glomerulus due to venom-induced hypoperfusion [11]. Since this wide range of SBE-induced complications in kidneys develops within a short period, AKI acts as a critical factor for SBE-induced deaths and long-term complications such as chronic kidney disease, prehypertension, and hypertension [10]. To retrieve the normal functions of kidneys, RRT as well as other supportive treatment measures are being used for SBE victims based on the severity of damage. Notably, pre-existing health conditions such as diabetes, hypertension, acute/chronic renal diseases, and inflammation in SBE victims may significantly increase the susceptibility to

develop AKI and the necessity for extensive RRT. Currently, serum creatinine levels are mainly used to ascertain the necessity for RRT in SBE victims [15]. In most cases, urine outputs are also considered when deciding on RRT, although this is not a useful factor in all cases. Serum creatinine levels represent the normal functions of kidneys by measuring the glomerular filtration rate, which conventionally reduces with age. An abnormal rate for certain age groups is most likely to indicate unusual kidney function [17]. However, measuring serum creatinine levels is time consuming, and the levels are affected by other factors such as diet, exercise, or reduced muscle mass, suggesting that it is not a powerful predictor for true reduction in glomerular filtration rate [24]. Notably, it takes up to 48 h to exhibit an elevated level following AKI, and therefore, it is not an ideal marker to detect early AKI, for example, in SBE. Hence, it is crucial to develop a reliable biomarker that can detect AKI at its early stage in SBE victims.

NGAL has been studied as a valuable diagnostic marker for AKI over the recent years [25,26]. Based on the nature of the damage, either plasma or urinary NGAL is being considered as a specific biomarker to detect AKI [27–29]. We have previously proposed NGAL as an early marker for Russell's viper bite–induced AKI [19–21]. In this study, using a large cohort of Russell's viper bite victims who arrived at the hospital within 8 h with normal serum creatinine levels, we demonstrate that NGAL acts as a robust diagnostic marker to ascertain the need for RRT among patients who were classified in grades 3 and X of AKI. The elevated level of NGAL was prominent at the time of admission, while it took up to 48 h for creatinine levels to increase. Previously, urinary NGAL has been identified as a useful marker for AKI among Russell's viper bite victims in Sri Lanka [9]. Another study identified urinary NGAL as a good marker for *Bothrops* species–induced AKI although the authors concluded that serum creatinine is the best marker [22]. A range of studies has explored the use of NGAL as an early and effective biomarker for AKI although there are certain limitations in its use under diverse settings. Some studies have reported NGAL to be a definitive biomarker for AKI [30–32] while others have acknowledged the utility of NGAL but do not yet consider it to be a gold standard marker for early detection of AKI [33,34]. One of these studies has suggested that urinary NGAL is not a robust biomarker for AKI, although it used a smaller cohort of patients who were already critically ill with sepsis, and it did not measure plasma NGAL [33]. Similarly, another study has highlighted the different molecular forms of urinary and plasma NGAL, and therefore, the authors considered that this is not a perfect marker for early AKI detection [34]. A multicentre study with a large patient cohort confirmed the utility of NGAL values for detecting AKI [35]. The use of NGAL-deficient mice demonstrated the strong relationship between NGAL and progression of diabetic nephropathy and confirmed NGAL as a useful biomarker [36]. Based on the existing literature, it is apparent that the use of NGAL as an early diagnostic marker may vary depending on the nature of AKI and associated comorbidities. However, this present study (which included 309 patients) has confirmed NGAL as a perfect marker for early detection of AKI among Russell's viper bite victims who did not have any previous history of medical conditions. Comorbidities such as chronic kidney disease [37,38], diabetes [39], hypertension, and other cardiovascular diseases [40] were reported to increase the levels of plasma/urinary NGAL.

Currently, there is no standard cut-off value for plasma NGAL to decide on RRT in patients following viper (including Russell's viper) bites. Since plasma NGAL is being enthusiastically considered as a point of care test in various clinical settings, it is important to identify the best cut-off value to decide on RRT at least in the context of SBE. Here, we identified a plasma NGAL value of 494 ng/dL as the best cut-off value because of its high specificity and sensitivity among the study cohort. Although there was no clear defining range for NGAL values among different grades, the patients (in grades 0–2) with a plasma NGAL value of below 500 ng/mL did not require RRT while patients in grade 3 and X who displayed an NGAL value of over 500 ng/dL certainly needed RRT in this study. The patients in grade X required RRT due to critical conditions that they developed although their serum creatinine levels were not increased. This demonstrates the significance

of NGAL for SBE victims even if they do not display an elevated value of serum creatinine until 48 h after the bite. In addition to NGAL and/or serum creatinine values, it is important for the clinicians to continuously monitor the clinical signs and symptoms as well as other biochemical parameters to ascertain the need for RRT in exceptional situations where NGAL may fail to detect early SBE-induced AKI. Although the time to antivenom administration did not vary significantly between the patients in different grades in this study, the early arrival to the hospital is highly recommended to receive the antivenom treatment, which prevents a range of venom-induced complications. Moreover, the progression of AKI in SBE victims also relies on the amount of venom injected (or the severity of bite), and toxins composition.

In this study, males were more affected by Russell's viper bites than females and this could be related to increased farming and other outdoor activities for men as reported previously [5]. Although the time delay between the bite and treatment is a critical factor in augmenting SBE-induced complications, here, we did not see any difference in the severity of AKI based on the time to antivenom treatment within 8 h. Previous studies, which have analysed patients who arrived up to 72 h following the bite, have demonstrated the time delay as a critical factor in induing AKI following viper envenomation [7,13]. Moreover, there was no significant difference between the glucose, sodium, potassium, urea, and INR levels among different grades, although this might be different when measured at later time points. Early detection of AKI using NGAL as a biomarker will aid in initiating early RRT to prevent further progression of renal damage. Moreover, this will significantly reduce the cost of treatment arising from RRT (dialysis). There was no death found in our study despite many patients suffering severe AKI in grades 3 and X. Since, NGAL values were reported to increase in patients with other health conditions such as cancer and inflammatory diseases [41], the pre-existing comorbidities in SBE victims are likely to influence the levels of NGAL.

To the best of our knowledge, this is a large study wherein plasma NGAL values were determined for 309 Russell's viper bite victims with robust inclusion and exclusion criteria. Based on this study, we propose plasma NGAL as a reliable biomarker to diagnose and triage AKI among Russell's viper bite victims at the time of admission much before the elevation of serum creatinine levels. This will allow the clinicians to initiate appropriate care including RRT at the earliest to reduce extensive renal damage and associated deaths following envenomation. Notably, early intervention for AKI with RRT will significantly reduce the treatment costs. Further studies are required to confirm whether NGAL can be used as a biomarker for AKI developing from the bites of other snake species in different countries. It is also important to ascertain whether NGAL can be used as a marker for Russell's viper bites from different regions within [42] and outside of India such as Myanmar and Sri Lanka as intra-specific and regional variations were found in their venom compositions, especially in specimens that were found outside of India [43,44]. The minimum cut-off values of plasma NGAL may also differ among different ethnic groups living in geographically diverse areas. Hence, further studies to ascertain these factors will facilitate the use of NGAL as a point of care test for worldwide use.

4. Methods

4.1. Study Design

This prospective study was carried out at the Emergency Department of Manian Medical Centre, Tamil Nadu (a large state in South India with a high burden of SBE) from June 2018 to May 2021. This study was approved by the Institutional Ethical Review Committee at Toxiven Biotech Private Limited (reference number: ICMR—Toxiven Ethics 2018–001/002), Tamil Nadu. Informed written consent was obtained from every participant before their enrolment in this study. In total, 309 victims who were confirmed as Russell's viper bite victims (based on dead/live specimens brought to the hospital and/or classical clinical symptoms) were included in this study. All patients were above 20 years of age, and we did not receive any patients with the age of <20 years old who met the inclusion criteria.

These patients were presented at the hospital within 8 h following the bite, and their serum creatinine level was less than 1.5 mg/dL upon admission. Patients with bites from species other than Russell's viper were excluded from this study. Furthermore, patients whose serum creatinine level was more than 1.5 mg/dL upon admission or who had arrived at the hospital after 8 h following bites were excluded from this study. Similarly, the patients who had pre-existing renal diseases, diabetes, hypertension, sepsis or systemic infectious diseases, haemodynamic instability of any cause, exposure to nephrotoxic drugs/chemicals, or other biological toxins and injuries or other comorbidities with or without medications were excluded from this study.

4.2. Data Collection

All the patients who were included in this study were subjected to detailed clinical examination and basic laboratory investigation at the time of arrival to the emergency department. Blood samples collected upon admission were used to estimate the level of plasma NGAL using the standardised Triage® NGAL tests (Biosite Inc., San Diego, CA, USA). The demographic (e.g., age and gender), clinical (time of bite to calculate the interval between bite and antivenom administration and clinical symptoms such as swelling, lymphadenopathy, bleeding in gums, epistaxis, upper gastrointestinal bleeding, and haematuria) and laboratory [e.g., the levels of serum creatinine, plasma NGAL, blood glucose, urea, sodium, potassium, and the international normalised ratio (INR) of blood clotting] data of all victims were collected and thoroughly verified by the authors prior to systematic recording of data for further analysis. All the patients were monitored hourly for urine output, and their serum creatinine levels were measured using the modified Jaffe method according to the manufacturer's instructions at 0, 12, 24, and 48 h following admission. Patients were treated according to the standard protocols including for the antivenom administration and supportive measures. During their stay at the hospital, none of these patients developed hypotension, hypoxia, dehydration, or sepsis or received any nephrotoxic agents. Normal levels of serum creatinine and urine output were ensured prior to their discharge from the hospital.

4.3. Classification of Patient Groups

The grades of AKI were determined using serum creatinine levels at different time points based on the criteria provided by the acute kidney injury network (AKIN): grade 0 (or no AKI) had a serum creatinine level of <1.5 mg/dL or less than 1.5-fold increase at 48 h compared to their baseline level; grade 1—serum creatinine level of 1.5 to 2.1 mg/dL or 1.5- to 1.9-fold increase at 48 h; grade 2—serum creatinine level of 2.2 to 2.6 mg/dL or 2.0- to 2.9-fold increase at 48 h; grade 3—serum creatinine level of >2.6 mg/dL or >3-fold increase at 48 h. RRT was initiated in those patients with AKIN stage 3. A total of seven patients (grade X) received RRT based on their critical conditions instead of serum creatinine levels: three of them displayed volume overload due to hugely reduced or no urine output, two patients had severe acidosis, one suffered anuria, and another had severe rhabdomyolysis.

4.4. Statistical Analysis

Multinomial logistic regression models were used in the hypothesis test of whether gender, age, clinical symptoms, and time to antivenom are predictors of grade, and generalised linear mixed effects models were used to model change in creatinine levels over time. All statistical analyses were performed using the R statistical package (Version 3.6.2, R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism (Version 7, GraphPad Software, San Diego, CA, USA).

Author Contributions: Conceptualization, S.S., N.E., P.T., and S.V.; methodology, S.S., P.T., K.P. and S.V.; software, M.F.B. and S.V.; validation, S.S., P.T., K.P., M.F.B., R.S., and S.V.; formal analysis, A.S., P.V., M.F.B. and S.V.; investigation, S.S., N.E., P.T., K.P., and S.V.; resources, S.S., S.V., M.F.B., R.S., H.F.W., and R.V.; data curation, S.S., H.F.W., R.V. and S.V.; writing—original draft preparation, P.V., A.S., S.S., S.V., R.S., S.V. and M.F.B.; writing—review and editing, S.V., P.T., K.P., M.F.B., R.S. and

S.V.; visualization, A.S., S.V. and M.F.B.; supervision, S.S. and S.V.; project administration, S.V., P.T. and S.S.; funding acquisition, S.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Royal Society Leverhulme Trust Senior Research Fellowship for S.V. (SRF\R1\201102).

Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Ethics Committee of Toxiven Biotech Private Limited (2018-001/002 on the 1 May 2018).

Informed Consent Statement: Written informed consent was obtained from all patients involved in the study.

Data Availability Statement: All data associated with this study are provided in this article.

Acknowledgments: We thank all the study participants who supported this study.

Conflicts of Interest: The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Kasturiratne, A.; Wickremasinghe, A.R.; de Silva, N.; Gunawardena, N.K.; Pathmeswaran, A.; Premaratna, R.; Savioli, L.; Lalloo, D.G.; de Silva, H.J. The global burden of snakebite: A literature analysis and modelling based on regional es-timates of envenoming and deaths. *PLoS Med.* **2008**, *5*, e218. [CrossRef]
2. Williams, H.F.; Layfield, H.J.; Vallance, T.; Patel, K.; Bicknell, A.B.; Trim, S.A.; Vaiyapuri, S. The Urgent Need to Develop Novel Strategies for the Diagnosis and Treatment of Snakebites. *Toxins* **2019**, *11*, 363. [CrossRef]
3. Samuel, S.P.; Chinnaraju, S.; Williams, H.F.; Pichamuthu, E.; Subharao, M.; Vaiyapuri, M.; Arumugam, S.; Vaiyapuri, R.; Baksh, M.F.; Patel, K.; et al. Venomous snakebites: Rapid action saves lives-A multifaceted community education programme increases awareness about snakes and snakebites among the rural population of Tamil Nadu, India. *PLoS Negl. Trop. Dis.* **2020**, *14*, e0008911. [CrossRef] [PubMed]
4. Vaiyapuri, S.; Vaiyapuri, R.; Ashokan, R.; Ramasamy, K.; Nattamaisundar, K.; Jeyaraj, A.; Chandran, V.; Gajjeraman, P.; Baksh, M.F.; Gibbins, J.M.; et al. Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India. *PLoS ONE* **2013**, *8*, e80090. [CrossRef] [PubMed]
5. Mohapatra, B.; Warrell, D.A.; Suraweera, W.; Bhatia, P.; Dhingra, N.; Jotkar, R.M.; Rodriguez, P.S.; Mishra, K.; Whitaker, R.; Jha, P.; et al. Snakebite Mortality in India: A Nationally Representative Mortality Survey. *PLOS Negl. Trop. Dis.* **2011**, *5*, e1018. [CrossRef]
6. Suraweera, W.; Warrell, D.; Whitaker, R.; Menon, G.; Rodrigues, R.; Fu, S.H.; Begum, R.; Sati, P.; Piyasena, K.; Bhatia, M.; et al. Trends in snakebite deaths in India from 2000 to 2019 in a nationally representative mortality study. *eLife* **2020**, *9*, e54076. [CrossRef]
7. Harshavardhan, L.; Lokesh, A.J.; Tejeshwari, H.L.; Halesha, B.R.; Siddharama, S. A study on the acute kidney injury in snake bite victims in a tertiary care centre. *J. Clin. Diagn. Res.* **2013**, *7*, 853–856. [CrossRef]
8. Alfred, S.; Bates, D.; White, J.; Mahmood, M.A.; Warrell, D.A.; Thwin, K.T.; Thein, M.M.; Sint San, S.S.; Myint, Y.L.; Swe, H.K.; et al. Acute Kidney Injury Following Eastern Russell's Viper (*Daboia siamensis*) Snakebite in Myanmar. *Kidney Int. Rep.* **2019**, *4*, 1337–1341. [CrossRef] [PubMed]
9. Ratnayake, I.; Mohamed, F.; Buckley, N.A.; Gawarammana, I.B.; Dissanayake, D.M.; Chathuranga, U.; Munasinghe, M.; Maduwage, K.; Jayamanne, S.; Endre, Z.H.; et al. Early identification of acute kidney injury in Russell's viper (*Daboia russelii*) envenoming using renal biomarkers. *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007486. [CrossRef] [PubMed]
10. Priyamvada, P.S.; Jaswanth, C.; Zachariah, B.; Haridasan, S.; Parameswaran, S.; Swaminathan, R.P. Prognosis and long-term outcomes of acute kidney injury due to snake envenomation. *Clin. Kidney J.* **2019**, *13*, 564–570. [CrossRef]
11. Sarkar, S.; Sinha, R.; Chaudhury, A.R.; Maduwage, K.; Abeyagunawardena, A.; Bose, N.; Pradhan, S.; Bresolin, N.L.; Garcia, B.A.; McCulloch, M. Snake bite associated with acute kidney injury. *Pediatric. Nephrol.* **2021**. [CrossRef]
12. Vikrant, S.; Jaryal, A.; Parashar, A. Clinicopathological spectrum of snake bite-induced acute kidney injury from India. *World J. Nephrol.* **2017**, *6*, 150–161. [CrossRef] [PubMed]
13. Singh, R.R.; Uraiya, D.; Kumar, A.; Tripathi, N. Early demographic and clinical predictors of developing acute kidney injury in snake bite patients: A retrospective controlled study from an Indian tertiary care hospital in North Eastern Uttar Pradesh India. *Indian J. Crit. Care Med.* **2016**, *20*, 404–408. [CrossRef] [PubMed]
14. Vaidya, V.S.; Ferguson, M.A.; Bonventre, J.V. Biomarkers of acute kidney injury. *Annu. Rev. Pharm. Toxicol.* **2008**, *48*, 463–493. [CrossRef] [PubMed]
15. Bagshaw, S.M.; Gibney, R.T. Conventional markers of kidney function. *Crit. Care Med.* **2008**, *36*, S152–S158. [CrossRef]
16. Endre, Z.H.; Pickering, J.W. Biomarkers and creatinine in AKI: The trough of disillusionment or the slope of enlightenment? *Kidney Int.* **2013**, *84*, 644–647. [CrossRef] [PubMed]

17. Endre, Z.H.; Pickering, J.W.; Walker, R.J. Clearance and beyond: The complementary roles of GFR measurement and injury biomarkers in acute kidney injury (AKI). *Am. J. Physiol. Ren. Physiol.* **2011**, *301*, F697–F707. [CrossRef] [PubMed]
18. Bolignano, D.; Donato, V.; Coppolino, G.; Campo, S.; Buemi, A.; Lacquaniti, A.; Buemi, M. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. *Am. J. Kidney Dis.* **2008**, *52*, 595–605. [CrossRef]
19. Senthilkumaran, S.; Thirumalaikolundusubramanian, P.; Elangovan, N. Neutrophil gelatinase-associated lipocalin as an early diagnostic biomarker of acute kidney injury in snake bite. *J. Emergencies Trauma Shock* **2019**, *12*, 260–262. [CrossRef]
20. Snthilkumaran, S.; Manimaran, D.; Thirumalaikolundusubramanian, P.; Elangovan, N. Neutrophil Gelatinase-Associated Lipocalin as an Early Marker of Acute Kidney Injury in Snake Bites Victims. *Int. J. Theor. Appl. Sci.* **2017**, *9*, 178–180.
21. Thamarai, R.; Sivakumar, K. Plasma neutrophil gelatinase associated lipocalin as an early biomarker of acute kidney injury in snake bite. *J. Evol. Med. Dent. Sci.* **2014**, *3*, 14737–14746.
22. Albuquerque, P.L.M.M.; da Silva Junior, G.B.; Meneses, G.C.; Martins, A.M.C.; Lima, D.B.; Raubenheimer, J.; Fathima, S.; Buckley, N.; Daher, E.D.F. Acute Kidney Injury Induced by Bothrops Venom: Insights into the Pathogenic Mechanisms. *Toxins* **2019**, *11*, 148. [CrossRef] [PubMed]
23. Chippaux, J.-P. Snakebite envenomation turns again into a neglected tropical disease! *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2017**, *23*, 38. [CrossRef]
24. Samra, M.; Abcar, A.C. False estimates of elevated creatinine. *Perm J.* **2012**, *16*, 51–52. [CrossRef] [PubMed]
25. Matsa, R.; Ashley, E.; Sharma, V.; Walden, A.P.; Keating, L. Plasma and urine neutrophil gelatinase-associated lipocalin in the diagnosis of new onset acute kidney injury in critically ill patients. *Crit. Care* **2014**, *18*, R137. [CrossRef] [PubMed]
26. Khawaja, S.; Jafri, L.; Siddiqui, I.; Hashmi, M.; Ghani, F. The utility of neutrophil gelatinase-associated Lipocalin (NGAL) as a marker of acute kidney injury (AKI) in critically ill patients. *Biomark Res.* **2019**, *7*, 4. [CrossRef]
27. Schley, G.; Köberle, C.; Manuilova, E.; Rutz, S.; Forster, C.; Weyand, M.; Formentini, I.; Kientsch-Engel, R.; Eckardt, K.-U.; Willam, C. Comparison of Plasma and Urine Biomarker Performance in Acute Kidney Injury. *PLoS ONE* **2015**, *10*, e0145042. [CrossRef]
28. Mahmoodpoor, A.; Hamishehkar, H.; Fattahi, V.; Sanaie, S.; Arora, P.; Nader, N.D. Urinary versus plasma neutrophil gelatinase-associated lipocalin (NGAL) as a predictor of mortality for acute kidney injury in intensive care unit patients. *J. Clin. Anesth* **2018**, *44*, 12–17. [CrossRef]
29. Li, Y.M.; Li, Y.; Yan, L.; Wang, H.; Wu, X.J.; Tang, J.T.; Wang, L.L.; Shi, Y.Y. Comparison of urine and blood NGAL for early prediction of delayed graft function in adult kidney transplant recipients: A meta-analysis of observational studies. *BMC Nephrol.* **2019**, *20*, 291. [CrossRef]
30. Zhang, Y.; Li, J.; Li, F.; Qi, X.; Zhang, J. Neutrophil gelatinase-associated lipocalin accurately predicts renal tubular injury in patients with chronic hepatitis B treated with nucleos(t)ide analogs. *Hepatol. Res.* **2018**, *48*, 144–152. [CrossRef]
31. Yi, A.; Lee, C.H.; Yun, Y.M.; Kim, H.; Moon, H.W.; Hur, M. Effectiveness of Plasma and Urine Neutrophil Gelatinase-Associated Lipocalin for Predicting Acute Kidney Injury in High-Risk Patients. *Ann. Lab Med.* **2021**, *41*, 60–67. [CrossRef] [PubMed]
32. Zhang, J.; Han, J.; Liu, J.; Liang, B.; Wang, X.; Wang, C. Clinical significance of novel biomarker NGAL in early diagnosis of acute renal injury. *Exp. Med.* **2017**, *14*, 5017–5021. [CrossRef] [PubMed]
33. Törnblom, S.; Nisula, S.; Petäjä, L.; Vaara, S.T.; Haapio, M.; Pesonen, E.; Pettilä, V.; Laru-Sompa, R.; Pulkkinen, A.; Saarelainen, M.; et al. Urine NGAL as a biomarker for septic AKI: A critical appraisal of clinical utility—Data from the observational FINNAKI study. *Ann. Intensive Care* **2020**, *10*, 51. [CrossRef] [PubMed]
34. Glassford, N.J.; Schneider, A.G.; Xu, S.; Eastwood, G.M.; Young, H.; Peck, L.; Venge, P.; Bellomo, R. The nature and discriminatory value of urinary neutrophil gelatinase-associated lipocalin in critically ill patients at risk of acute kidney injury. *Intensive Care Med.* **2013**, *39*, 1714–1724. [CrossRef]
35. Srisawat, N.; Praditpornsilpa, K.; Patarakul, K.; Techapornrung, M.; Daraswang, T.; Sukmark, T.; Khositrangsikun, K.; Fakthongyoo, A.; Oranrigsupak, P.; Praderm, L.; et al. Neutrophil Gelatinase Associated Lipocalin (NGAL) in Leptospirosis Acute Kidney Injury: A Multicenter Study in Thailand. *PLoS ONE* **2015**, *10*, e0143367. [CrossRef] [PubMed]
36. Liu, X.; Zhao, X.; Duan, X.; Wang, X.; Wang, T.; Feng, S.; Zhang, H.; Chen, C.; Li, G. Knockout of NGAL aggravates tubulointerstitial injury in a mouse model of diabetic nephropathy by enhancing oxidative stress and fibrosis. *Exp. Med.* **2021**, *21*, 321. [CrossRef]
37. Patel, M.L.; Sachan, R.; Misra, R.; Kamal, R.; Shyam, R.; Sachan, P. Prognostic significance of urinary NGAL in chronic kidney disease. *Int. J. Nephrol. Renov. Dis.* **2015**, *8*, 139–144. [CrossRef]
38. Castillo-Rodriguez, E.; Fernandez-Prado, R.; Martin-Cleary, C.; Pizarro-Sánchez, M.S.; Sanchez-Niño, M.D.; Sanz, A.B.; Fernandez-Fernandez, B.; Ortiz, A. Kidney Injury Marker 1 and Neutrophil Gelatinase-Associated Lipocalin in Chronic Kidney Disease. *Nephron* **2017**, *136*, 263–267. [CrossRef]
39. Papadopoulou-Marketou, N.; Margeli, A.; Papassotiriou, I.; Chrousos, G.P.; Kanaka-Gantenbein, C.; Wahlberg, J. NGAL as an Early Predictive Marker of Diabetic Nephropathy in Children and Young Adults with Type 1 Diabetes Mellitus. *J. Diabetes Res.* **2017**, *2017*, 7526919. [CrossRef]
40. Malyszko, J.; Bachorzewska-Gajewska, H.; Malyszko, J.S.; Pawlak, K.; Dobrzycki, S. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in hypertensive and normotensive patients with coronary artery disease. *Nephrology* **2008**, *13*, 153–156. [CrossRef]
41. Ning, M.; Mao, X.; Niu, Y.; Tang, B.; Shen, H. Usefulness and limitations of neutrophil gelatinase-associated lipocalin in the assessment of kidney diseases. *J. Lab. Precis. Med.* **2018**, *3*. [CrossRef]

42. Pla, D.; Sanz, L.; Quesada-Bernat, S.; Villalta, M.; Baal, J.; Chowdhury, M.A.W.; León, G.; Gutiérrez, J.M.; Kuch, U.; Calvete, J.J. Phylovenomics of *Daboia russelii* across the Indian subcontinent. Bioactivities and comparative in vivo neutralization and in vitro third-generation antivenomics of antivenoms against venoms from India, Bangladesh and Sri Lanka. *J. Proteom.* **2019**, *207*, 103443. [CrossRef] [PubMed]
43. Faisal, T.; Tan, K.Y.; Tan, N.H.; Sim, S.M.; Gnanathasan, C.A.; Tan, C.H. Proteomics, toxicity and antivenom neutralization of Sri Lankan and Indian Russell's viper (*Daboia russelii*) venoms. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2021**, *27*, e20200177. [CrossRef] [PubMed]
44. Tan, K.Y.; Tan, N.H.; Tan, C.H. Venom proteomics and antivenom neutralization for the Chinese eastern Russell's viper, Daboia siamensis from Guangxi and Taiwan. *Sci. Rep.* **2018**, *8*, 8545. [CrossRef] [PubMed]

Chapter 3

The effectiveness of antibiotics in managing bacterial infections on bite sites following snakebite envenomation. Subramanian Senthilkumaran, Anika Salim, José R. Almeida, Jarred Williams, Pradeep Vijayakumar, Angayarkanni Thirunavukarasu, Markellos Alexandros Christopoulos, Harry F. Williams, Ponniah Thirumalaikolundusubramanian, Ketan Patel and Sakthivel Vaiyapuri. (2023). Toxins, 15(3), 190.

The rationale for this study

Antibiotic resistance is a looming public health concern and potentially at a crisis point globally due to the overuse as well as misuse of these medications. This is also compounded by the lack of development into new drugs and antibiotics to combat microbial infections. Lack of effective antibiotics for the treatment and prevention of infections can seriously harm and reduce our ability to clinically treat and manage them increasing the risk of death and disability.

A common secondary complication from SBE is wound infection, and ASV cannot combat this. Broad-spectrum antibiotics are often administered to patients without confirming if there is indeed a clinical need for them, which contributes to higher treatment costs for patients. At present there is limited information available on the common bacterial profiles in SBE patients with infected wounds. A better understanding of the common bacterial wound infections in patients can aid us in developing better antibiotic application strategies in clinical settings, and efficient and effective treatment protocols, reducing costs for treatment and associated complex wound care for patients. In this study, 242 Russell's viper patients with a visible SBE wound upon admission at our collaborating hospitals consented to participate following the inclusion/exclusion guidelines. The wound at the bite site was then swabbed for microbial and sensitivity/resistance culturing.

This study highlighted that a variety of bacterial strains can be found in the wounds of SBE patients, three gram-positive strains and four gram-negative strains were identified with *Staphylococcus aureus* being the most observed bacteria. A total of 189 Russell's viper patients exhibited bacterial growth, and gender did not influence the variety of bacterial growth, whereas age did have some influence on bacterial growth. Our sensitivity and resistance testing revealed the most and least effective antibiotics depending on the observed growth of the bacterial strain and highlighted the importance of clinical professionals to better understand the variety of bacteria present in potential wound infections. A better understanding of the most effective antibiotics against the relevant strain can help patients, reduce their treatment costs overall as well as prevent any unnecessary contributions to worldwide antibiotic resistance.

My contribution to this chapter (50%)

Our collaborators in India (Subramanian Senthilkumaran, Angayarkanni Thirunavukarasu, Ponniah Thirumalaikolundusubramanian) along with Sakthivel Vaiyapuri and Ketan Patel at the University of Reading, UK conceptualised and carried out this study by collecting data from the patients. An initial review of the data was conducted by Pradeep Vijayakumar and a primary analysis of the data was conducted by Markellos Alexandros Christopoulos at the University of Reading.

I organised, cleaned and analysed the raw data at the University of Reading. The statistical analysis plan was developed with Sakthivel Vaiyapuri which included exploratory modelling of the data to determine the various associations between bacterial strain and gender and age and evaluate resistance and susceptibility. I expanded the interpretation of the data and completed the visualisation, programming, and preparation of all the study figures and tables. Manuscript preparation, writing, editing, and review were performed by Anika Salim and Sakthivel Vaiyapuri.

toxins

MDPI

Article

The Effectiveness of Antibiotics in Managing Bacterial Infections on Bite Sites following Snakebite Envenomation

Subramanian Senthilkumaran [1,†], Anika Salim [2,†], José R. Almeida [2], Jarred Williams [2], Pradeep Vijayakumar [2], Angayarkanni Thirunavukarasu [1], Markellos Alexandros Christopoulos [2], Harry F. Williams [3], Ponniah Thirumalaikolundusubramanian [4], Ketan Patel [5] and Sakthivel Vaiyapuri [2,*]

1 Manian Medical Centre, Erode 638001, Tamil Nadu, India
2 School of Pharmacy, University of Reading, Reading RG6 6UB, UK
3 Toxiven Biotech Private Limited, Coimbatore 641042, Tamil Nadu, India
4 The Tamil Nadu Dr M.G.R Medical University, Chennai 600032, Tamil Nadu, India
5 School of Biological Sciences, University of Reading, Reading RG6 6UB, UK
* Correspondence: s.vaiyapuri@reading.ac.uk
† These authors contributed equally to this study.

Abstract: Snakebite envenomation (SBE) is a life-threatening medical emergency with a high mortality rate. Common secondary complications following SBE, such as wound infections, are significant due to their impact on worsening local tissue damage and causing systemic infection. Antivenoms are not effective to treat wound infections following SBE. Moreover, in several rural clinical settings, broad-spectrum antibiotics are often used without clear guidelines or based on limited laboratory data, resulting in undesirable side effects and exacerbated treatment costs. Therefore, robust antibiotic strategies should be developed to tackle this critical issue. Currently, there is limited information available on the bacterial profiles of SBE-induced infections and antibiotic susceptibility. Hence, it is essential to improve the knowledge of bacterial profiles and their antibiotic sensitivity in SBE victims to develop better treatment strategies. This study aimed to address this issue by examining the bacterial profiles of SBE victims with a specific focus on Russell's viper envenomation. The most frequently found bacteria in the bites of SBE victims were *Staphylococcus aureus*, *Klebsiella* sp., *Escherichia coli*, and *Pseudomonas aeruginosa*. Linezolid, clindamycin, colistin, meropenem, and amikacin were some of the most effective antibiotics for commonly grown bacteria in SBE victims. Similarly, ciprofloxacin, ampicillin, amoxiclave, cefixime, and tetracyclin were the least effective antibiotics for common bacteria found in the wound swabs of SBE victims. These data provide robust guidance for infection management following SBE and offer useful insights to aid in designing effective treatment protocols for SBE with serious wound infections in rural areas where laboratory facilities may not be readily available.

Keywords: antibiotics; Russell's viper; *Daboia russelii*; snakebite envenomation; wound infections; antibiotic sensitivity; antibiotic resistance

Key Contribution: This study demonstrates the types of bacteria that are commonly found in wounds caused by SBE, specifically Russell's viper bites, and how well these bacteria respond to different antibiotic treatments. The findings from this study will be important when making decisions about how to treat SBE-induced wound infections in patients.

check for updates

Citation: Senthilkumaran, S.; Salim, A.; Almeida, J.R.; Williams, J.; Vijayakumar, P.; Thirunavukarasu, A.; Christopoulos, M.A.; Williams, H.F.; Thirumalaikolundusubramanian, P.; Patel, K.; et al. The Effectiveness of Antibiotics in Managing Bacterial Infections on Bite Sites following Snakebite Envenomation. *Toxins* **2023**, *15*, 190. https://doi.org/10.3390/toxins15030190

Received: 15 February 2023
Revised: 28 February 2023
Accepted: 1 March 2023
Published: 3 March 2023

1. Introduction

Snakebite envenomation (SBE) is a serious medical emergency that often results in deaths, disabilities, and socioeconomic consequences in impoverished communities of developing countries [1]. India accounts for a high SBE incidence and mortality (58,000 deaths every year) rate, and this can be attributed to widespread myths/misconceptions about snakes and snakebites as well as inadequate public awareness to seek prompt hospital

treatment following bites [2,3]. Russell's viper (*Daboia russelii*) appears to be the most medically important venomous snake in India, as it causes a majority of hospital admissions, deaths and permanent disabilities [4]. Its venom is rich in phospholipases A_2 and proteolytic enzymes and causes a wide range of debilitating effects on the body of victims that require timely administration of antivenom and other relevant clinical interventions [5]. Secondary complications arising in patients bitten by Russell's viper may require additional and complementary therapies, such as antibiotics, haemodialysis, analgesics, and transfusion of plasma/blood cells to achieve a favourable clinical outcome [6,7]. Therefore, it is important to understand the secondary complications induced by SBE to develop better treatment strategies.

Microbial (specifically bacterial) infections are a common secondary consequence of SBE, especially in viper bites, due to the extensive local tissue damage [8,9]. These infections can lead to serious complications such as exacerbating necrosis, systemic infections, and septic shock [10]. The cause of these infections can be diverse, including the snake's oral microbiome, the environment, and opportunistic bacteria growing on human skin [11]. A recent study found the presence of several drug-resistant bacteria in the oral microbiome of a Russell's viper and the inefficacy of conventionally used antibiotics such as amoxiclave and penicillin [12]. However, a range of antibiotics are often used in several clinical settings, specifically in rural areas, without appropriate justification to treat SBE-induced bacterial infections, and this frequently results in exacerbated treatment costs and unwarranted side effects. Moreover, the use of antivenom is ineffective in treating SBE-induced microbial infections. These issues highlight the urgent need for further research on this clinically relevant but understudied medical complication associated with SBE. This will pave the way to developing improved and cost-effective clinical management strategies for SBE with robust guidance to tackle a wide range of SBE-induced complications, including infection. In this study, we document the bacterial profiles in local bite sites of 266 SBE victims and provide guidance for the effective management of SBE-induced infections which would be mainly useful in under-resourced clinical settings in rural areas.

2. Results

2.1. Study Population

To determine the bacterial profiles of SBE-induced infections and their antibiotic sensitivity, we recruited SBE victims from January 2021 to December 2022 at Manian Medical Centre, Tamil Nadu following exclusion and inclusion criteria (as detailed in methods). Following an initial screening, a total of 266 SBE victims (180 (67.7%) males; 86 (32.3%) females (Figure 1A)) were included in this study. The patients' cohort comprised 0 males (0%) and 4 females (1.5%) aged from 0 to 10; 4 males (1.5%) and 7 females (2.6%) aged 11–20, 15 (5.6%) males and 3 (1.1%) females aged 21–30; 20 males (7.5%) and 10 females (3.8%) aged 31–40; 37 males (13.9%) and 19 (7.1%) females aged 41–50; 49 males (18.4%) and 25 (9.4%) females aged 51–60; 38 (14.3%) males and 10 (3.8%) females aged 61–70; 15 males (5.6%) and 5 females (1.9%) aged 71–80; and 2 males (0.8%) and 3 females (1.1%) aged 81–90 years (Figure 1A). The youngest patient included in this study was 8 years old and the oldest patient was 85 years old, with a mean of 50.8 (SD = 16.42) and median of 53 years (IQR: 42 to 62 years). For the Russell's vipers, the minimum age was 8 years old, and the maximum age was 56, with a mean of 50.7 (SD = 16.9) and a median of 52 (IQR: 41.5 to 63 years).

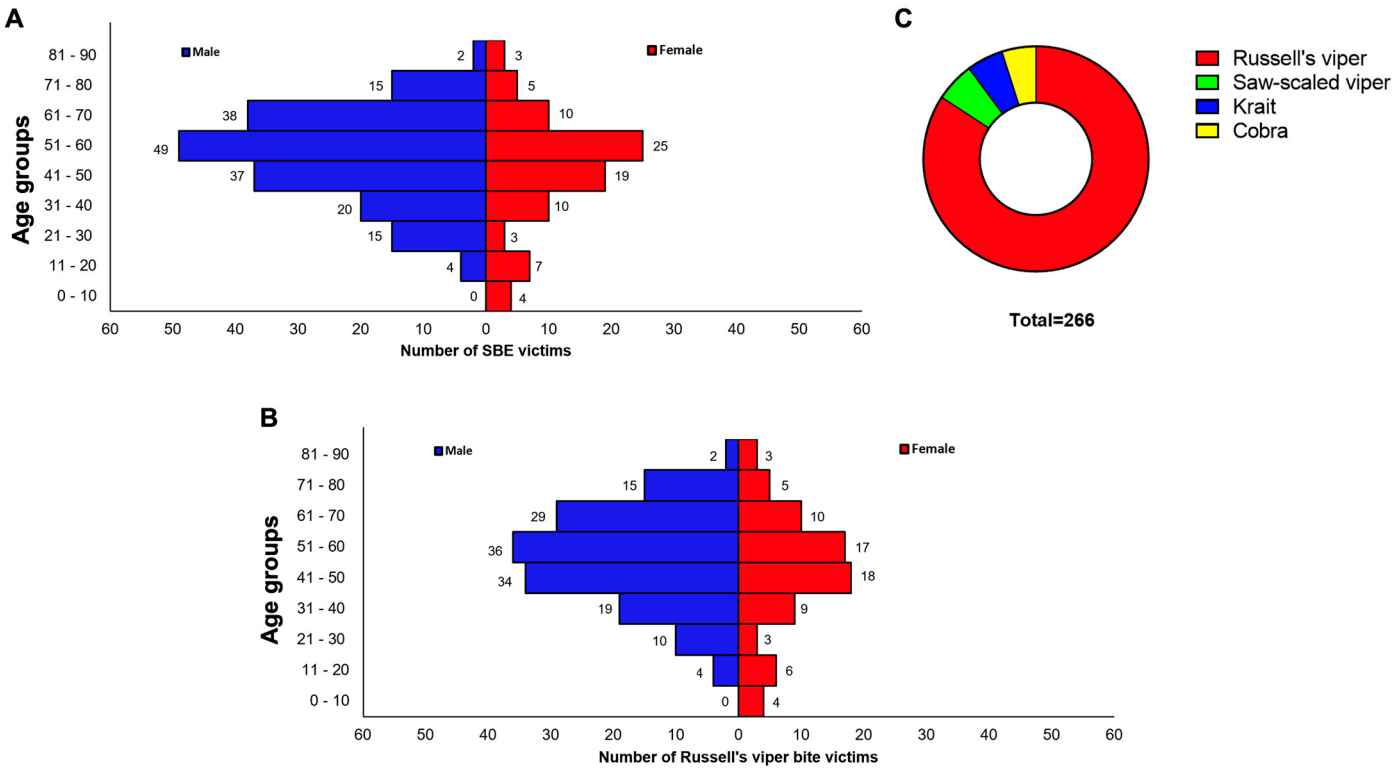


Figure 1. Characteristics of SBE victims included in this study. (**A**) The total number of SBE victims included in this study was organised based on their gender and age groups. (**B**) Different snake species involved in bites of total SBE victims. (**C**) Russell's viper bite victims were classified based on their gender and age groups.

Notably, this study population included a total of 224 Russell's viper bite victims (Figure 1B) with 42 victims bitten by other snakes. The distribution of patients across different age groups in Russell's viper bite victims is similar to the total number of patients (Figure 1C). Due to the high number, we performed a rigorous analysis of bacterial infections from Russell's viper bite envenomation compared to the others. The 42 patients bitten by other snakes included 13 by cobras (*Naja naja*), 14 by kraits (*Bungarus caeruleus*), and 15 by saw-scaled vipers (*Echis carinatus*).

2.2. Diverse Bacterial Strains Were Identified in Wound Swabs from SBE Victims

The bacterial strains in wound swabs collected from SBE victims were identified using standard bacterial culture methods in a clinical laboratory associated with the hospital. The bacterial growth was identified in 219 patients; in others, there was no visible growth found. A diverse range of bacteria was identified in these patients (Figure 2A). Most samples displayed a single bacterial growth, whilst 22 patients showed the growth of two different bacteria. These results indicate that *Staphylococcus aureus* was the most observed bacteria as it was found in 74 (31%) patients. This was followed by *Klebsiella* sp. [41 patients (17%)], *Pseudomonas aeruginosa* [34 patients (14%)], and *Escherichia coli* [33 patients (14%)]. All other (six species) bacteria were identified in a small number of patients (Figure 2A). The total bacterial population included three Gram-positive strains (*Enterococcus* sp., *Staphylococcus aureus* and *Streptococcus pyogenes*) and four Gram-negative strains (*Acinetobacter* sp., *Citrobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Proteus vulgaris*). A total of 189 Russell's viper bites and 30 patients bitten by other snakes displayed bacterial growth. The patients bitten by Russell's viper displayed a similar pattern of bacterial profiles to the total patient population (Figure 2B). The most common strains present in Russell's viper patients were *Staphylococcus aureus* (n = 58, 31%), *Klebsiella* sp. (n = 36, 19%), *Escherichia coli* (n = 32, 17%), and *Pseudomonas*

aeruginosa (*n* = 27, 14%). Similarly, the least common strains were *Acinetobacter* sp. (*n* = 13; 7%), *Proteus vulgaris* (*n* = 8; 4%), *Citrobacter* sp. (*n* = 1; 1%), and *Streptococcus pyogenes* (*n* = 1; 1%). Of the thirteen patients bitten by cobra (*N. naja*), ten patients presented with *Staphylococcus aureus* and two with *Klebsiella* sp. One patient did not show any bacterial growth. No secondary bacterial strains were found in these patients. Of the patients bitten by kraits (*B. caeruleus*) (*n* = 14), three patients presented with *Staphylococcus aureus*, two with *Klebsiella* sp., and one with *Pseudomonas aeruginosa*. No bacterial growth was found in eight patients. On the other hand, in the analysis of the bacterial data of saw-scaled vipers (*E. carinatus*) bite patients (*n* = 15), the following bacteria were identified: *Pseudomonas aeruginosa* (6), *Staphylococcus aureus* (3), *Proteus mirabilis* (1), *Escherichia coli* (1) and *Klebsiella* sp. (1). Three patients did not present any bacterial growth. One of these patients with *Staphylococcus aureus* exhibited a secondary strain which was found to be *Citrobacter* sp. (1). These data demonstrate the common bacterial profiles found in SBE victims, and this is not hugely different for Russell's viper bite victims.

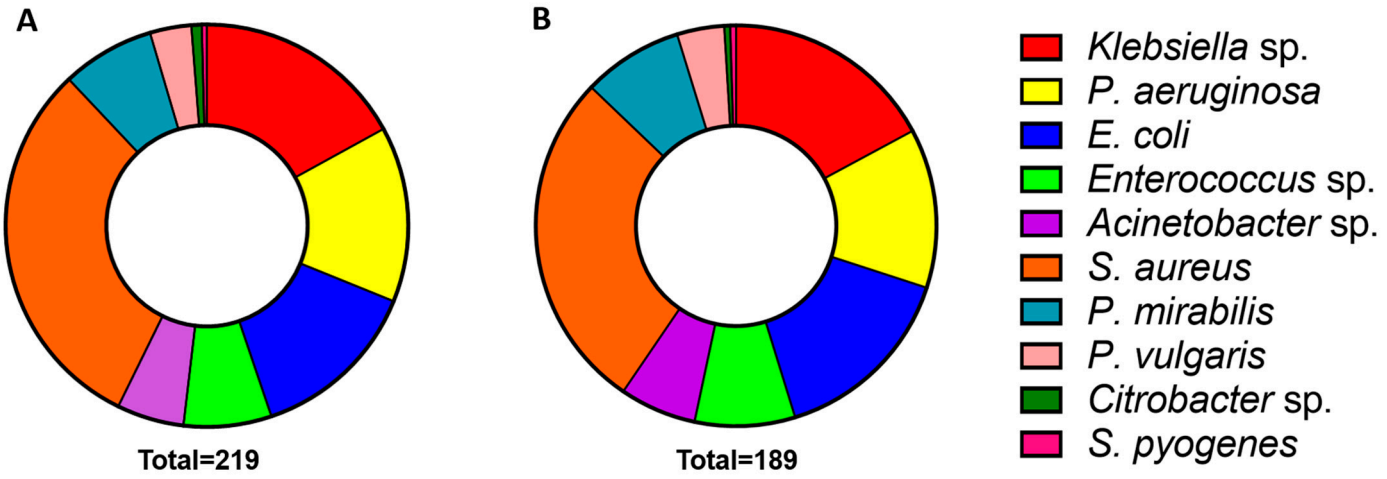


Figure 2. Summary of diverse bacterial strains identified in wound infections of total SBE (**A**) and Russell's viper bite victims (**B**).

2.3. Gender Does Not Correlate with the Growth of Diverse Bacterial Strains, but Age Does

To determine if gender can influence the type of bacteria growing in patients' wounds, further analysis was performed. A chi-square test was used to examine the association between most bacterial strains present and gender. However, Fisher's exact test was used where the sample numbers were small (*Enterococcus* sp. and *Proteus mirabilis*). The results (Table 1) demonstrate that there is no association between gender and type of bacterial strains found in SBE victims. However, the relationship between *Enterococcus* sp. ($p = 0.05$) and *Pseudomonas aeruginosa* ($p = 0.08$) and gender appeared to be close to significant.

Table 1. Association between gender and occurrence of different bacterial strains.

Strain *	Female (*n* = 63)	Male (*n* = 126)	*p*-Value
AC	4 (6%)	9 (7%)	0.84
EC	12 (19%)	20 (16%)	0.58
EN	2 (3%)	15 (12%)	0.05
KL	10 (16%)	26 (21%)	0.43
PA	13 (21%)	14 (11%)	0.08
PM	3 (5%)	14 (11%)	0.19
SA	22 (34%)	36 (29%)	0.37

* AC—*Acinetobacter* sp., EC—*Escherichia* coli, EN—*Enterococcus* sp., KL—*Klebsiella* sp., PA—*Pseudomonas aeruginosa*, PM—*Proteus mirabilis* and SA—*Staphylococcus aureus*.

We then analysed the association between age and bacterial strains. These results (Table 2) indicated that *Escherichia coli* ($p = 0.02$) and *Staphylococcus aureus* ($p = 0.02$) strains varied significantly between age groups. *Escherichia coli* was more commonly (27 out of 35 patients) observed in the age category of above 50 years old, whereas *Staphylococcus aureus* was commonly (37 out of 58 patients) observed in patients of below 50 years old. The other bacterial strains did not vary significantly between different age groups. These data demonstrate that there is an influence of age in specific types of bacteria growing in SBE victims, while gender may not be a concern.

Table 2. Association between age and occurrence of diverse bacterial strains.

Strain *	Age $\leq$ 20 (n = 10)	Age 21–30 (n = 12)	Age 31–40 (n = 22)	Age 41–50 (n = 44)	Age 51–60 (n = 48)	Age 61–70 (n = 33)	Age 71+ (n = 20)	p-Value
AC	1 (10%)	0 (0%)	2 (9%)	2 (5%)	5 (10%)	2 (6%)	1 (5%)	0.88
EC	0 (0%)	1 (8%)	1 (5%)	6 (7%)	13 (27%)	9 (27%)	5 (25%)	0.02
EN	0 (0%)	1 (8%)	1 (5%)	6 (14%)	3 (6%)	4 (12%)	2 (10%)	0.74
KL	2 (20%)	3 (25%)	9 (41%)	5 (12%)	7 (15%)	5 (15%)	2 (25%)	0.12
PA	2 (20%)	2 (17%)	3 (14%)	3 (14%)	7 (15%)	8 (24%)	2 (10%)	0.51
PM	0 (0%)	0 (0%)	0 (0%)	9 (21%)	4 (8%)	2 (6%)	2 (10%)	0.12
SA	5 (50%)	5 (42%)	6 (27%)	21 (48%)	13 (27%)	4 (12%)	4 (20%)	0.02

* AC—*Acinetobacter* sp., EC—*Escherichia coli*, EN—*Enterococcus* sp., KL—*Klebsiella* sp., PA—*Pseudomonas aeruginosa*, PM—*Proteus mirabilis* and SA—*Staphylococcus aureus*. The p values shown in bold denote the statistical significance for the association of those bacterial strains with the age of victims.

2.4. Bacterial Sensitivity and Resistance to Antibiotics in Russell's Viper Victims

Due to the large number of patients in Russell's viper cohort compared to other snakes, we used only these data to analyse the sensitivity of bacterial strains to a range of antibiotics (Figure 3A). The data demonstrate that *Staphylococcus aureus* is most sensitive to linezolid (96%), amikacin (90%), clindamycin (84%), and colistin (66%). Similarly, the top four most effective antibiotics for the other most commonly identified bacterial strains such as *Klebsiella* sp. (doxycycline hydrochloride (62.5%), tetracyclin (59.4%), netilmycin (50%), and amikacin (50%)), *Pseudomonas aeruginosa* (colistin (100%), amikacin (91.7%), cefepime (87.5%), imipenem (87.5%), meropenem (87.5%), and cefeparazone sulbactum (83.3%)), and *Escherichia coli* (meropenem (78.6%), piperacillin tazobactum (75%), amikacin (67.9%) and netilmycin (64.3%)), were identified. The sensitivity of other bacterial strains to various antibiotics is shown in Figure 3A.

Similarly, the least effective antibiotics against bacterial strains grown in Russell's viper bite victims were analysed (Figure 3B). The top four antibiotics that are resistant to *Staphylococcus aureus* include ciprofloxacin (76%), oxacillin (50%), gentamycin (46%), and amoxiclave (42%). *Klebsiella* sp. were resistant to ampicillin (97%), amoxiclave (84%), cefixime (84%), and piperacillin (81%). *Escherichia coli* were resistant to ampicillin (96%) piperacillin (86%), cefuroxime (86%), and ceftazidime (86%). Notably, *Pseudomonas aeruginosa* was fully resistant to several antibiotics such as amoxiclave (100%), ampicillin (100%), cefixime (100%), cefuroxime (100%), colistin (100%), tetracyclin (100%), and ampicillin sulbactum (95.8%). The resistance of other bacterial strains to various antibiotics is shown in Figure 3B.

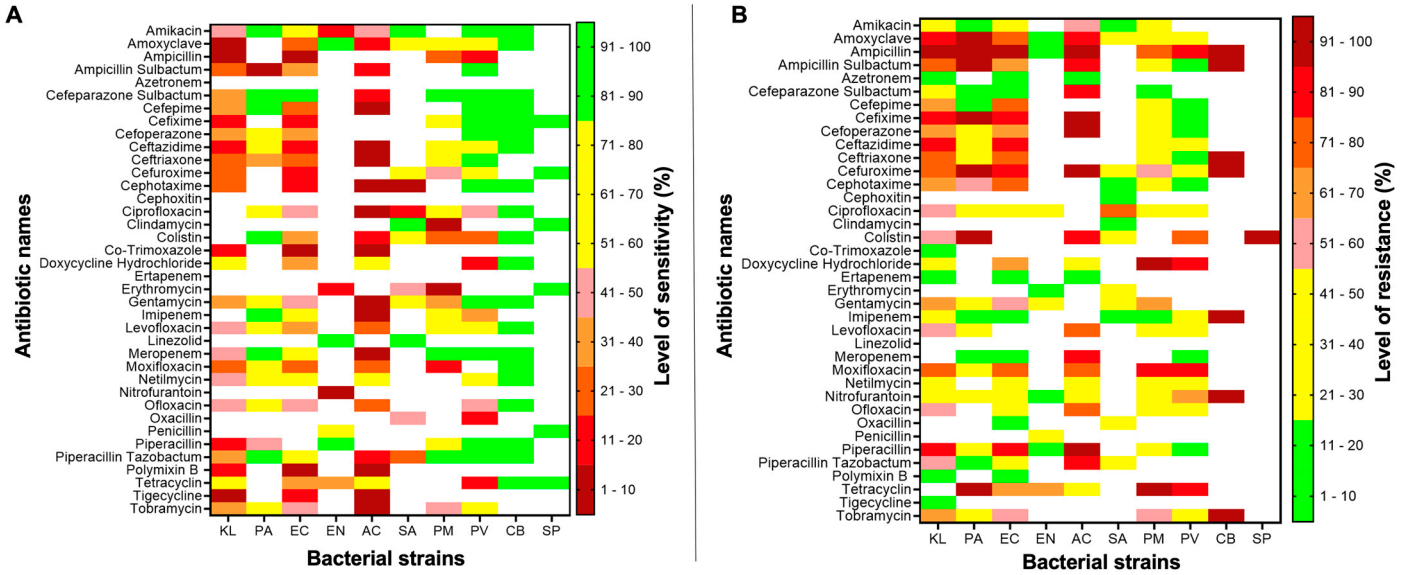


Figure 3. Antibiotic sensitivity data for different bacterial strains grown on bite sites of Russell's viper bite victims. The sensitivity (**A**) and resistance (**B**) of diverse bacterial species grown at the bite sites of Russell's viper victims for a broad range of antibiotics tested in this study. The scales shown in the figure indicate the level of sensitivity in (**A**) and resistance in (**B**) for antibiotics. KL—*Klebsiella* sp.; PA—*Pseudomonas aeruginosa*; EC—*Escherichia coli*; EN—*Enterococcus* sp.; AC—*Acinetobacter* sp.; SA—*Staphylococcus aureus*; PM—*Proteus mirabilis*; PV—*Proteus vulgaris*; CB—*Citrobacter*; SP—*Staphylococcus pyogenes*. White squares indicate the antibiotics that were not tested for the given bacterial strain.

2.5. The Cost Analysis of All Antibiotics Indicates Their Significance in SBE Treatment

The total costs of a 5-day course of antibiotics were calculated based on a twice-daily dosing (Figure 4). Three of the antibiotics (cefixime, cefuroxime, and ciprofloxacin) were provided as tablets, while all others were given as an intravenous infusion. The cheapest course of antibiotics was ciprofloxacin with the cost in Indian Rupees being INR 32. The most expensive antibiotics course was polymixin B, as it costs INR 10,000. The most effective antibiotic (linezolid) for *Staphylococcus aureus* costs INR 1490 for a 5-day course. Similarly, for *Klebsiella* sp., the effective antibiotic, doxycycline hydrochloride, costs around INR 4660. Colistin was identified as the most effective antibiotic against *Pseudomonas aeruginosa*, and it costs around INR 8600. For *Escherichia coli*, with the effective antibiotic, meropenem, treatment costs INR 2700. All the costs of different antibiotics are shown in Figure 4. The analysis of this cost data highlights that out of the ten bacterial strains identified, eight bacteria showed >80% sensitivity to at least one antibiotic tested. *Klebsiella* sp. and *Acinetobacter* sp. did not display >80% sensitivity to any antibiotic tested. Of the 38 antibiotics tested, 27 presented with at least 80% sensitivity to at least one bacterial strain. In this study, the patients received different antibiotics based on their level of sensitivity to the bacterial strain identified and the cost of antibiotics, which significantly varied (Figure 4).

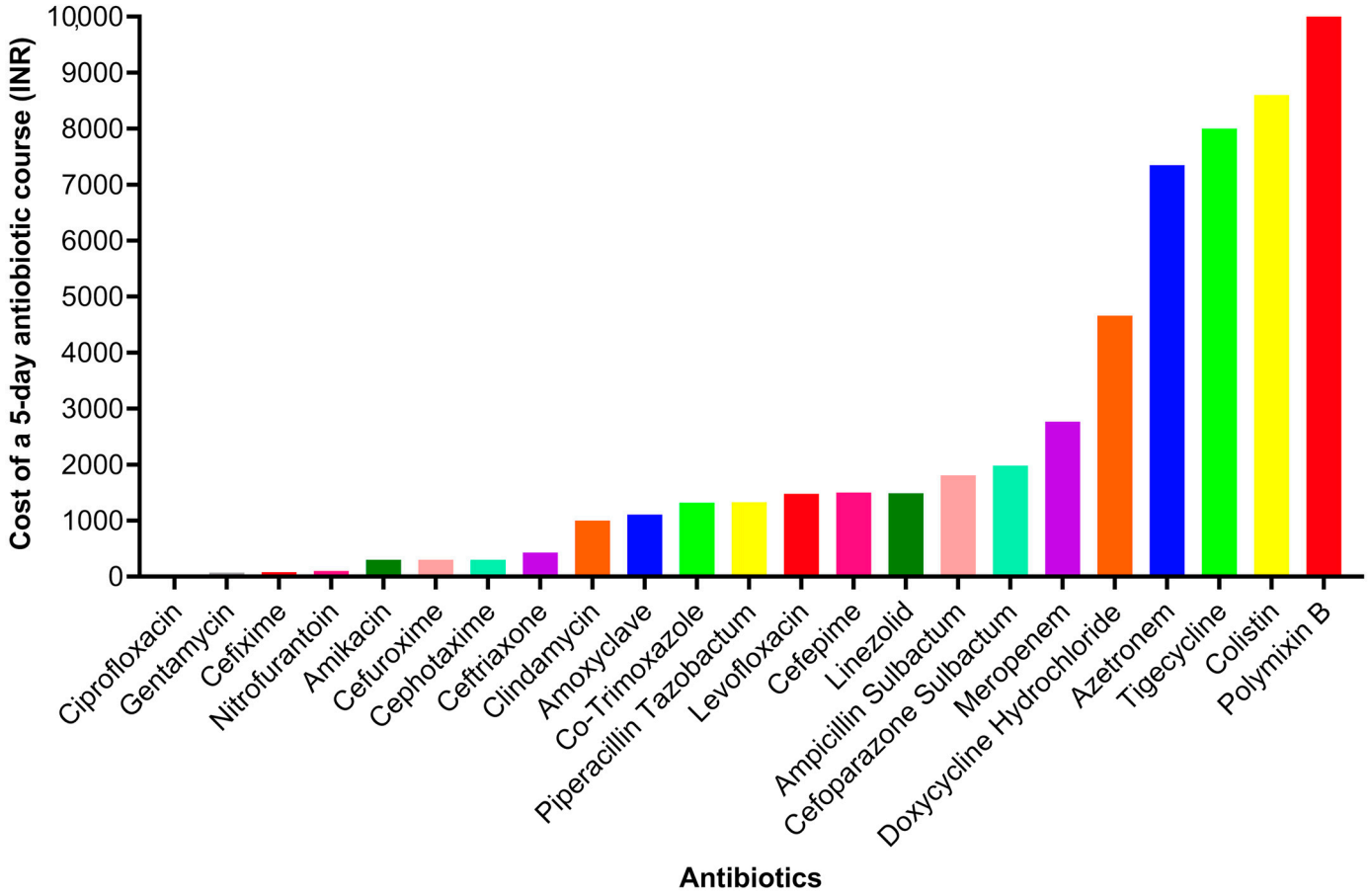


Figure 4. Cost of a 5-day antibiotic course (two doses daily) for different antibiotics that were used in SBE victims in this study.

3. Discussion

SBE is an acute medical emergency that can cause a range of different complications within the body [13]. Some of these complications are due to the direct actions of venom toxins and others develop as secondary complications to envenomation effects. Snake venoms (specifically from vipers such as the Russell's viper) contain a combination of toxins that damage various cells and proteins, leading to blood clotting disorders, acute inflammation, and extensive damage to local tissues [14,15]. The local tissue damage creates an environment that is favourable for the growth of multiple pathogenic microorganisms, which can come from the environment, the skin, or even the oral cavity of the snake [11]. Indeed, the mouth of the Russell's viper snake contains diverse types of bacteria, including antibiotic-resistant strains [12]. Additionally, the use of alternative and non-validated treatment methods such as plant extracts, cow dung, and other forms of traditional remedies in SBE-affected areas are common in rural areas [16]. These methods may also significantly increase the risk of developing microbial infections. Therefore, the clinical management of SBE should consider the risk of wound infections, including from antibiotic-resistant bacterial strains, and hence develop robust antibiotic strategies as part of the treatment protocol to successfully treat victims [17].

The use of antibiotics as a preventative measure following envenomation by snakes is controversial or unnecessary, and therefore, they should only be used when there is confirmation of a wound infection [18,19]. Overuse or unnecessary administration of antibiotics in SBE victims can contribute to antimicrobial resistance, undesirable side effects, and increased treatment costs [20,21]. Moreover, wound infections that are not treated quickly can lead to serious complications such as abscess, gangrene, and necrosis, which may require surgical intervention, adding a strain on already fragile healthcare systems

with limited resources and poor infrastructure [22]. Hence, it is critical to improve our understanding of microbial/bacterial populations that grow in SBE-induced infections and develop robust strategies to tackle this issue. In this study, we analysed the types of bacteria and their sensitivity and resistance to a wide range of antibiotics using data collected from SBE victims who displayed visible wounds upon admission to a hospital. This study is aimed towards developing an empirical treatment regimen that can bring several potential benefits to SBE victims, including rapid response to severe infections, improving victim care in healthcare settings with limited resources, and avoiding the overuse or unnecessary use of ineffective antibiotics that can lead to resistance and increased treatment costs.

A total of 10 bacterial strains were identified in the wound swabs obtained from SBE victims in this study. *Staphylococcus aureus*, *Klebsiella* sp., *Pseudomonas aeruginosa*, and *Escherichia coli* were identified as the most common bacteria in SBE victims. These results are similar to previous reports describing the bacterial profiles of SBE-induced wounds in other countries and species of snakes [23,24]. For example, a study carried out in Taiwan evaluating the secondary infection in patients bitten by *Naja atra* identified a total of 23 bacterial strains [22]. Another study performed in Brazil described the presence of 54 different bacterial strains in abscesses developing at the bite site [25]. Some studies have shown the predominance of aerobic Gram-negative bacteria [22,25,26]. However, our results indicate the presence of certain specific Gram-positive and Gram-negative bacterial strains as the most commonly found bacteria in SBE victims in an Indian hospital. A previous study that analysed SBE victims over approximately 5 years (2003–2008) in an Indian hospital showed a higher prevalence of Gram-positive strains (53%) [27]. Garg et al. (2009) reported that *Staphylococcus aureus* (32%) was the most frequent microorganism followed by *Escherichia coli* (15%). Our data analysis highlighted the occurrence of *Staphylococcus aureus* in 31% of victims followed by a Gram-negative bacterium, *Escherichia coli*, in 17% of victims. Some of the bacteria identified in our study coincide with those previously reported in the oral cavity of Russell's viper [12]. The mouth of Russell's viper comprises a series of pathogenic bacteria that include *Staphylococcus* sp., *Enterococcus* sp., *Lysinobacillus* sp., *Escherichia coli*, *Pseudomonas* sp., *Salmonella* sp., *Proteus* sp., *Providencia* sp., *Morganella* sp., and *Alcaligenes* sp. However, the bacterial profiles or epidemiology of bacteria in wound infections have a different pattern of distribution to the bacteria found in the oral cavity of this snake. This suggests that some bacterial infections in SBE patients may be associated with the snake's oral microflora but may not be all of them. Therefore, other sources of microbes, including the skin of the patient and any first aid or traditional treatment methods, were used post-snakebite. Moreover, the mouth of different snakes may have various microbial patterns which can influence the infection in patients. During this study period, there was no correlation found between the bacteria identified from the patient swabs and the nosocomial culture (no bacterial growth observed) that was obtained using swabs from theatres, intensive care units, and wards.

Antibiotic resistance is a pressing global issue that affects the wider clinical practice [28]. The inadequate efficacy and unnecessary use of antibiotics in treating SBE-induced infections can lead to serious consequences, including undesirable side effects [26]. Our study on antibiotic resistance and sensitivity to a wide range of bacteria through a robust assessment using a large cohort of patients provides practical guidelines for selecting effective antibiotics to treat SBE-induced infections in victims. Our results showed that antibiotics such as linezolid, amikacin, clindamycin, piperacillin tazobactum, cefeparazone sulbactum, and colistin are some of the most effective to treat a range of bacteria identified in the wound swabs of SBE victims. Similarly, ampicillin, cefuroxime, cefixime, and amplicillin sulbactum are some of the least effective antibiotics for bacterial strains identified. This is in accordance with a previous study [12] that analysed the susceptibility of bacteria from Russell's viper's mouth as it includes ampicillin, cefpodoxime, amoxiclave, oxacillin, and penicillin as resistant antibiotics. These data provide a comprehensive profile of sensitivity and resistance to a range of antibiotics based on the type of bacteria. These data can be used as a potential guide to determine the antibiotic regime for SBE victims.

Vulnerable populations with scarce economic resources face high treatment costs for SBE, which can be largely impacted by secondary effects [1]. A retrospective analysis performed in Kenya has highlighted that SBE-related complications have significant consequences on medical/pharmacy costs and supportive therapies that are key determinants for the total high treatment costs [29]. However, the number of investigations focused on the estimations of the cost of SBE treatments is very limited. The economic impact of antibiotic therapy remains widely unknown [2,29,30]. Here, we showed that the treatment costs can vary drastically depending on the antibiotics used. These findings draw attention to the necessity of designing effective and affordable antibiotic treatment strategies that can reduce the cost and, consequently, the burden that this issue represents for the population in low-income regions. More robust studies are required to tackle this issue across the world and develop appropriate treatment guidelines and policies.

4. Conclusions

This study highlights the importance of understanding the bacterial community in wound infections from SBE victims, specifically Russell's viper bite victims. Moreover, antibiotic sensitivity tests were performed to improve treatment plans and clinical guidelines. In rural areas with limited resources, identifying microbes in SBE victims can be challenging and time-consuming, hindering prompt treatment. Therefore, a systematic review of microbial data from low-resource settings is necessary to establish efficient management protocols. However, this study has limitations due to its size, despite having the most data from one of the most medically significant snake species (Russell's viper) in the country. Indeed, the development of visible wounds from other snakebite victims was minimal. Therefore, further studies are needed to fully understand the bacterial communities in the remaining three deadliest Indian snakes. This study used a robust automated culture technique for rapid bacterial identification, and this may not be available in rural healthcare settings. Moreover, some studies that used mass spectrometry-based methods have been proposed as faster and more sensitive alternatives for diagnosing bacterial infections, and should be considered in future studies. In conclusion, a better understanding of microbial/bacterial communities that are responsible for SBE-induced infections and their sensitivity/resistance to a range of antibiotics will significantly improve the treatment and reduce treatment costs for SBE victims.

5. Materials and Methods

5.1. Data Collection

This prospective study was conducted from January 2021 to December 2022 at Manian Medical Centre (a snakebite referral hospital), Erode, Tamil Nadu, India. All patients of any age who displayed a visible wound or bleeding upon admission were included in this study. The patients bitten by only the 'Big Four' snakes were included, and others were excluded. The identity of the snakes was confirmed based on dead/live specimens brought by the victims or their family members, and/or clinical symptoms displayed. A trained herpetologist has analysed the identification of snakes where specimens were available based on their morphological features. A total of 266 patients were included in this study. Due to the low number of cases of snakes other than Russell's viper, the data obtained from Russell's viper bite victims (242 cases) was used for most of the analysis in this study to draw firm conclusions.

Sterile cotton microbiological swabs (HiMedia, India) were used to collect the wound exudates at the bite site of patients and immediately sent [in HiCulture^{TM} swab transport media containing Dey-Engley neutralising broth, (HiMedia, India)] for microbial culture in the laboratory associated with the hospital. The swabs were streaked using the quadrant method on blood and Macconkey agar media plates. They were incubated at 37 °C for 48 h for the bacteria to grow. If there was no bacterial growth observed at this stage, the plates were monitored for another 48 h with an examination every 12 h. Then, single well-isolated colonies of bacteria were selected for further microbial characterisation using

a Gram stain kit (HiMedia, India) and a range of biochemical tests [e.g., Indole, citrate, methyl red, oxidase, triple sugar iron, and coagulase tests using HiIMViC^{TM} Biochemical test kits] according to the manufacturer's guidelines (HiMedia, India).

Similarly, the isolated colonies were grown in separate Muller Hinton agar plates and used for antibiotic sensitivity tests using a range of antibiotic discs in line with the manufacturer's (HiMedia, India) guidelines. Briefly, the susceptibility test measures the zone of clearance for each antibiotic disc following the guidelines from the Clinical & Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The zone of clearance or antibiotic susceptibility was measured using an automatic analyser (Vitek 2 compact analyser, Biomerieux, India). Similarly, this analyser has calculated the minimum inhibitory concentrations (MIC) for susceptible antibiotics based on the zone of clearance observed. If there was no zone of clearance, then those bacteria were considered resistant to that antibiotic. To determine the nosocomial infections, the swabs from operation theatres, intensive care units, and wards were routinely tested for every 15 days. There was no bacterial growth identified from these swabs as the hospital is maintaining stringent surface cleaning protocols.

5.2. Statistical Methods

All statistical analyses were performed using SPSS version 26 (IBM, Portsmouth, UK) and R version 4.1.2 (Lucent technologies Ltd, Manchester, UK) to evaluate the association between the age category and gender of the patients and the presence of various strains of bacteria. Due to the relatively small patient sample within each age group, the analysis for age was performed using Fisher's exact test. A chi-square test was used to study the association with the bacterial strain presence.

Author Contributions: Conceptualization, S.S. and S.V.; methodology, S.S., A.S. and S.V.; validation, S.S., A.S., H.F.W., A.T., K.P. and S.V.; formal analysis, S.S., A.S. and S.V.; investigation, S.S., A.S., A.T., M.A.C. and S.V.; resources, S.S. and S.V.; data curation, S.S., A.S., P.V., P.T. and K.P.; writing—original draft preparation, S.S., A.S., J.R.A., J.W. and S.V.; writing—review and editing, S.S., A.S., J.R.A., J.W., H.F.W., K.P. and S.V.; visualization, S.S., A.S. and S.V.; supervision, S.S. and S.V.; project administration, S.S. and S.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by funding from the Medical Research Council, UK (Project grant reference: MR/W019353/1 and Integrative Toxicology Training Partnership—PhD studentship).

Institutional Review Board Statement: This study was performed according to the Declaration of Helsinki and approved by the Institutional Ethics Committee of Toxiven Biotech Private Limited (2019-001/002 on the 11 October 2019).

Informed Consent Statement: Informed consent was obtained from all study participants involved in the study to anonymously analyse and publish the data.

Data Availability Statement: Not applicable—as all data from this study are included within this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gutiérrez, J.M.; Calvete, J.J.; Habib, A.G.; Harrison, R.A.; Williams, D.J.; Warrell, D.A. Snakebite envenoming. *Nat. Rev. Dis. Prim.* **2017**, *3*, 17063. [CrossRef] [PubMed]
2. Vaiyapuri, S.; Vaiyapuri, R.; Ashokan, R.; Ramasamy, K.; Nattamaisundar, K.; Jeyaraj, A.; Chandran, V.; Gajjeraman, P.; Baksh, M.F.; Gibbins, J.M.; et al. Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India. *PLoS ONE* **2013**, *8*, e80090. [CrossRef] [PubMed]
3. Samuel, S.P.; Chinnaraju, S.; Williams, H.F.; Pichamuthu, E.; Subharao, M.; Vaiyapuri, M.; Arumugam, S.; Vaiyapuri, R.; Baksh, M.F.; Patel, K.; et al. Venomous snakebites: Rapid action saves lives—A multifaceted community education programme increases awareness about snakes and snakebites among the rural population of Tamil Nadu, India. *PLoS Negl. Trop. Dis.* **2021**, *14*, e0008911. [CrossRef] [PubMed]
4. Glaudas, X. Proximity between humans and a highly medically significant snake, Russell's viper, in a tropical rural community. *Ecol. Appl.* **2021**, *31*, e02330. [CrossRef]

5. Senthilkumaran, S.; Miller, S.W.; Williams, H.F.; Savania, R.; Thirumalaikolundusubramanian, P.; Patel, K.; Vaiyapuri, S. Development of Wunderlich syndrome following a Russell's viper bite. *Toxicon* **2022**, *215*, 11–16. [CrossRef]
6. Senthilkumaran, S.; Miller, S.W.; Williams, H.F.; Vaiyapuri, R.; Savania, R.; Elangovan, N.; Thirumalaikolundusubramanian, P.; Patel, K.; Vaiyapuri, S. Ultrasound-guided compression method effectively counteracts Russell's viper bite-induced pseudoaneurysm. *Toxins* **2022**, *14*, 260. [CrossRef]
7. Arathisenthil, S.V.; Senthilkumaran, S.; Vijayakumar, P.; Savania, R.; Williams, H.F.; Elangovan, N.; Bicknell, A.B.; Patel, K.; Trim, S.A.; Thirumalaikolundusubramanian, P.; et al. Rapid development of a salivary calculus in submandibular gland and its potential causes in a young victim following Russell's viper bite. *Toxicon* **2022**, *206*, 85–89. [CrossRef]
8. Sadeghi, M.; Barazandeh, M.; Zakariaei, Z.; Davoodi, L.; Tabaripour, R.; Fakhar, M.; Zakariaei, A. Massive cutaneous complications due to snakebite: A case report and literature review. *Clin. Case Rep.* **2021**, *9*, e04129. [CrossRef]
9. Resiere, D.; Mehdaoui, H.; Névière, R.; Olive, C.; Severyns, M.; Beaudoin, A.; Florentin, J.; Brouste, Y.; Banydeen, R.; Cabié, A.; et al. Infectious complications following snakebite by *Bothrops lanceolatus* in Martinique: A case series. *Am. J. Trop. Med. Hyg.* **2020**, *102*, 232–240. [CrossRef]
10. Chuang, P.C.; Lin, W.H.; Chen, Y.C.; Chien, C.C.; Chiu, I.M.; Tsai, T.S. Oral bacteria and their antibiotic susceptibilities in Taiwanese venomous snakes. *Microorganisms* **2022**, *10*, 951. [CrossRef]
11. Houcke, S.; Resiere, D.; Lontsingoula, G.R.; Cook, F.; Lafouasse, P.; Pujo, J.M.; Demar, M.; Matheus, S.; Hommel, D.; Kallel, H. Characteristics of snakebite-related infection in French Guiana. *Toxin* **2022**, *14*, 89. [CrossRef] [PubMed]
12. Panda, S.K.; Padhi, L.; Sahoo, G. Evaluation of cultivable aerobic bacterial flora from Russell's viper (*Daboia russelii*) oral cavity. *Microb. Pathog.* **2019**, *134*, 103573. [CrossRef]
13. Senthilkumaran, S.; Almeida, J.R.; Williams, J.; Salim, A.; Williams, H.F.; Thirumalaikolundusubramanian, P.; Patel, K.; Vaiyapuri, S. Russell's viper envenomation induces rectus sheath haematoma. *Toxicon* **2023**, *224*, 107037. [CrossRef] [PubMed]
14. Senji Laxme, R.R.; Khochare, S.; Attarde, S.; Suranse, V.; Iyer, A.; Casewell, N.R.; Whitaker, R.; Martin, G.; Sunagar, K. Biogeographic venom variation in Russell's viper (*Daboia russelii*) and the preclinical inefficacy of antivenom therapy in snakebite hotspots. *PLoS Negl. Trop. Dis.* **2021**, *15*, e0009247. [CrossRef] [PubMed]
15. Kalita, B.; Patra, A.; Mukherjee, A.K. Unraveling the proteome composition and immuno-profiling of Western India Russell's viper venom for in-depth understanding of its pharmacological properties, clinical Manifestations, and effective antivenom treatment. *J. Proteome Res.* **2017**, *16*, 583–598. [CrossRef] [PubMed]
16. Steinhorst, J.; Aglanu, L.M.; Ravensbergen, S.J.; Dari, C.D.; Abass, K.M.; Mireku, S.O.; Adu Poku, J.K.; Enuameh, Y.A.K.; Blessmann, J.; Harrison, R.A.; et al. 'The medicine is not for sale': Practices of traditional healers in snakebite envenoming in Ghana. *PLoS Negl. Trop. Dis.* **2021**, *15*, e0009298. [CrossRef]
17. Esmaeilishirazifard, E.; Usher, L.; Trim, C.; Denise, H.; Sangal, V.; Tyson Gregory, H.; Barlow, A.; Redway Keith, F.; Taylor John, D.; Kremyda-Vlachou, M.; et al. Bacterial adaptation to venom in snakes and Arachnida. *Microbiol. Spectr.* **2022**, *10*, e02408-21. [CrossRef]
18. Resiere, D.; Gutiérrez, J.M.; Névière, R.; Cabié, A.; Hossein, M.; Kallel, H. Antibiotic therapy for snakebite envenoming. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2020**, *26*, e20190098. [CrossRef]
19. Bhaumik, S.; Gopalakrishnan, M.; Kirubakaran, R.; Jagnoor, J. Antibiotics for preventing wound infections after snakebite. *Cochrane Database Syst. Rev.* **2022**, *7*, 1–14. [CrossRef]
20. Patiño, R.S.P.; Salazar-Valenzuela, D.; Robles-Loaiza, A.A.; Santacruz-Ortega, P.; Almeida, J.R. A retrospective study of clinical and epidemiological characteristics of snakebite in Napo Province, Ecuadorian Amazon. *Trans. R. Soc. Trop. Med. Hyg.* **2022**, *117*, 118–127. [CrossRef]
21. Sørensen, C.V.; Knudsen, C.; Auf dem Keller, U.A.D.; Kalogeropoulos, K.; Gutiérrez-Jiménez, C.; Pucca, M.B.; Arantes, E.C.; Bordon, K.C.F.; Laustsen, A.H. Do antibiotics potentiate proteases in hemotoxic snake venoms? *Toxins* **2020**, *12*, 240. [CrossRef] [PubMed]
22. Mao, Y.C.; Liu, P.Y.; Hung, D.Z.; Lai, W.C.; Huang, S.T.; Hung, Y.M.; Yang, C.C. Bacteriology of *Naja atra* snakebite wound and its implications for antibiotic therapy. *Am. J. Trop. Med. Hyg.* **2016**, *94*, 1129–1135. [CrossRef]
23. Chen, C.M.; Wu, K.G.; Chen, C.J.; Wang, C.M. Bacterial infection in association with snakebite: A 10-year experience in a northern Taiwan medical center. *J. Microbiol. Immunol. Infect.* **2011**, *44*, 456–460. [CrossRef]
24. Wagener, M.; Naidoo, M.; Aldous, C. Wound infection secondary to snakebite. *S. Afr. Med. J.* **2017**, *107*, 315–319. [CrossRef]
25. Jorge, M.T.; Ribeiro, L.A.; da Silva, M.L.; Kusano, E.J.; de Mendonça, J.S. Microbiological studies of abscesses complicating *Bothrops* snakebite in humans: A prospective study. *Toxicon* **1994**, *32*, 743–748. [CrossRef] [PubMed]
26. Sachett, J.A.G.; da Silva, I.M.; Alves, E.C.; Oliveira, S.S.; Sampaio, V.S.; do Vale, F.F.; Romero, G.A.S.; dos Santos, M.C.; Marques, H.O.; Colombini, M.; et al. Poor efficacy of preemptive amoxicillin clavulanate for preventing secondary infection from *Bothrops* snakebites in the Brazilian Amazon: A randomized controlled clinical trial. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005745. [CrossRef] [PubMed]
27. Garg, A.; Sujatha, S.; Garg, J.; Acharya, N.S.; Chandra Parija, S. Wound infections secondary to snakebite. *J. Infect. Dev. Ctries* **2009**, *3*, 221–223. [CrossRef]
28. Almeida, J.R.; Palacios, A.L.V.; Patiño, R.S.P.; Mendes, B.; Teixeira, C.A.S.; Gomes, P.; da Silva, S.L. Harnessing snake venom phospholipases A(2) to novel approaches for overcoming antibiotic resistance. *Drug Dev. Res.* **2019**, *80*, 68–85. [CrossRef]

29. Okumu, M.O.; Patel, M.N.; Bhogayata, F.R.; Ochola, F.O.; Olweny, I.A.; Onono, J.O.; Gikunju, J.K. Management and cost of snakebite injuries at a teaching and referral hospital in Western Kenya. *F1000Res* **2019**, *8*, 1588. [CrossRef]
30. Kasturiratne, A.; Lalloo, D.G.; Janaka de Silva, H. Chronic health effects and cost of snakebite. *Toxicon X* **2021**, *9*, 100074. [CrossRef]

Chapter 4

Identifying key factors contributing to treatment costs for snakebite envenoming in private tertiary healthcare settings in Tamil Nadu, India. Anika Salim, Jarred Williams, Samir Abdel Wahab, Tade Adeshokan, José R. Almeida, Harry F. Williams, Rajendran Vaiyapuri, Subramanian Senthilkumaran, Ponniah Thirumalaikolundusubramanian, Ketan Patel, M. Fazil Baksh, Matthew R. Lewin, Sakthivel Vaiyapuri. (2023). PLOS Neglected Tropical Diseases, 17(10), e0011699.

The rationale for this study

Snakebite patients delay seeking treatment for their condition for a variety of reasons, whether this be religious, social, or accepting their fate from a karmic perspective. Anecdotal reports however suggest that the fear of high treatment costs following care is a principal determinant in causing them to delay seeking treatment. Treatment delays following snakebites can increase the risk of the patient developing medically severe complications that require more complex and specialist medical care, and this in turn can result in a hefty medical bill for the patient. High medical bills following snakebites have vast socio-economic manifestations for some of the poorest members of society and can lead to complete financial devastation and destitution.

This study aimed to identify which key factors play a role in contributing to high treatment costs for SBE patients and what average costs were incurred to quantify the financial impact on patients. Thus, patient admission data on 913 SBE cases was collated from 10 private tertiary hospitals that treat snakebite patients. The data was classified according to the various cost categories such as hospital, pharmacy, investigation, and laboratory bills.

This study suggests that most patients incur average treatment costs of around INR 100,000 which is equivalent to $1200/ £1000. Extreme treatment costs are experienced when more complex medical events occur requiring lengthy stays and costly treatment such as prolonged ventilation and or renal replacement therapy such as dialysis. Our investigations highlight the scale of financial ramifications as a result of SBE to some of the poorest members of society as well as the need to protect such patients with adequate policy and patient health insurance coverage.

My contribution to this chapter (60%)

Our collaborators in India (Rajendran Vaiyapuri, Subramanian Senthilkumaran and Ponniah Thirumalaikolundusubramanian) along with Sakthivel Vaiyapuri and Ketan Patel at the University of Reading, UK conceptualised and carried out this study by collecting data from the patients. An initial review of the data and primary analysis was conducted by Samir Abdel Wahab and Tade Adeshokan at the University of Reading.

I organised, cleaned and analysed the raw data at the University of Reading. The statistical analysis plan was developed with Sakthivel Vaiyapuri and M. Fazil Baksh which included exploratory modelling of the data and application of various post-hoc analyses to determine statistically significant results. The statistical modelling methodology and programming were also reviewed by Dr. Alain F. Zuur a senior statistician and Dr. Elena N. Ieno a biologist to help determine the correct statistical modelling approach to account for snakebites from the various snakes and their respective treatment costs. The analysis was interpreted by myself with advice from Dr. Alain F. Zuur and Dr. Elena N. Ieno and I completed the visualisation, programming, and preparation of all study figures and tables. Manuscript preparation, writing, editing, and review were performed by Anika Salim, Sakthivel Vaiyapuri. José R. Almeida, Jarred Williams, Harry F. Williams and Matthew R. Lewin.

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Identifying key factors contributing to treatment costs for snakebite envenoming in private tertiary healthcare settings in Tamil Nadu, India

Anika Salim[1], Jarred Williams[1], Samir Abdel Wahab[1], Tade Adeshokan[1], José R. Almeida[1], Harry F. Williams[2], Rajendran Vaiyapuri[2], Subramanian Senthilkumaran[3], Ponniah Thirumalaikolundusubramanian[4], Ketan Patel[5], M. Fazil Baksh[6], Matthew R. Lewin[7], Sakthivel Vaiyapuri[1]*

1 School of Pharmacy, University of Reading, Reading, United Kingdom, **2** Toxiven Biotech Private Limited, Coimbatore, Tamil Nadu, India, **3** Manian Medical Centre, Erode, Tamil Nadu, India, **4** The Tamil Nadu Dr M. G.R Medical University, Chennai, Tamil Nadu, India, **5** School of Biological Sciences, University of Reading, Reading, United Kingdom, **6** Department of Mathematics and Statistics, University of Reading, Reading, United Kingdom, **7** California Academy of Sciences, San Francisco, California, United States of America

* s.vaiyapuri@reading.ac.uk

OPEN ACCESS

Citation: Salim A, Williams J, Abdel Wahab S, Adeshokan T, Almeida JR, Williams HF, et al. (2023) Identifying key factors contributing to treatment costs for snakebite envenoming in private tertiary healthcare settings in Tamil Nadu, India. PLoS Negl Trop Dis 17(10): e0011699. https://doi.org/10.1371/journal.pntd.0011699

Editor: Wuelton M. Monteiro, Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, BRAZIL

Received: June 23, 2023

Accepted: October 5, 2023

Published: October 16, 2023

Data Availability Statement: All data from this study are included within this manuscript.

Funding: We would like to thank the Medical Research Council, UK (Grant reference: MR/W019353/1 and ITTP PhD Studentship) for their funding support to S.V. The funder had no role in the study design, data collection and analysis, decision to publish or the preparation of the manuscript.

Abstract

Background

India suffers ~58,000 annual deaths due to snakebites. The 'Big Four' snakes (Russell's viper, Indian cobra, common krait, and saw-scaled viper) that are responsible for most bites cause diverse clinical effects. Delayed treatment increases the risk of serious complications and treatment costs. Although government hospitals offer free treatment for snakebites in India, most patients opt for private healthcare, which is an out-of-pocket expense as they often lack health insurance coverage. This study aims to analyse snakebite treatment costs in private tertiary care hospitals in Tamil Nadu, India and identifies the key factors contributing to treatment costs.

Methodology/Principal findings

The treatment cost details for 913 snakebite victims were collected from 10 private tertiary care hospitals across Tamil Nadu. The data were classified into hospital, pharmacy, investigation, and laboratory costs, and analysed to determine various factors that contribute to the costs. The results demonstrate that the average treatment costs vary widely for different snakes. The hospital and pharmacy costs are higher than investigation and laboratory costs for all snakebites. Notably, Russell's viper bites cost significantly more than the bites from other snakes. Overall, the type of snake, nature of complications, specialist treatments required, and arrival time to hospitals were identified as some of the key factors for higher treatment costs.

Competing interests: The authors have declared that no competing interests exist.

Conclusions/Significance

These data demonstrate that ~80% of snakebite patients can be treated with INR 100,000 (~GBP 1000 or USD 1200) or less. This study emphasises the urgent need to improve rural medical care by providing appropriate training for healthcare professionals and essential resources to facilitate early assessment of patients, administer the initial dose of antivenom and refer the patients to tertiary care only when needed. Moreover, the outcome of this study forms a basis for developing appropriate policies to regulate snakebite treatment costs and provide affordable medical insurance for vulnerable communities.

Author summary

Snakebite envenoming (SBE) predominantly affects poor communities living in rural areas of developing countries. As SBE induces a wide range of pathological effects in patients, they need a broad spectrum of treatment approaches to tackle those issues. Therefore, antivenom alone is not sufficient to treat SBE patients. In India, most people (including SBE patients) seek treatments from private healthcare settings, although government hospitals provide free treatments. Hence the treatment costs pose a significant burden on the SBE victims and their families leading to severe socioeconomic impacts. To develop better policies to support the clinical management of SBE, it is critical to estimate the costs of SBE treatment in private healthcare settings. In this study, we analysed the treatment costs of 913 snakebite patients who were treated in 10 different private tertiary care hospitals in Tamil Nadu, India, and identified various factors that contribute to treatment costs. For example, snake type, specialist treatments and the time of arrival to the hospital following bites are some of the key factors leading to increased treatment costs. These results form the basis for developing policies to regulate SBE treatment costs, provide health insurance coverage and ensure timely treatment for SBE victims in rural healthcare settings.

Introduction

Snakebite envenoming (SBE), a high-priority neglected tropical disease [1], is considered a disease of poverty as it predominantly affects rural populations living in developing countries such as India [2–5]. SBE has been estimated to cause around 150,000 deaths and 500,000 permanent disabilities every year worldwide [2, 6, 7]. India alone accounts for around 58,000 deaths every year due to SBE [8,9]. A household survey in Tamil Nadu, India [10] found that up to 79% of envenomings occurred when patients were performing agricultural fieldwork and SBE induces significant socioeconomic impacts. However, the true magnitude of SBE and its socioeconomic ramifications on rural communities are not yet fully understood [2,10,11]. In India, the 'Big Four' snakes [Russell's viper (*Daboia russelii*), Indian cobra (hereafter, referred to as cobra) (*Naja naja*), common krait (*Bungarus caeruleus*), and saw-scaled viper (*Echis carinatus*)] are responsible for most of the incidents, resulting deaths, and disabilities [10,12–14]. Notably, many patients do not seek appropriate hospital treatment promptly after bites as they rely on locally available traditional healers and a range of often dangerous/inappropriate first aid [10,12,15,16]. The challenges in accessing transport facilities, emergency services and correct treatments in rural areas are also major constraints in seeking prompt treatment for SBE

[10,16–18]. These factors contribute to significant delay in seeking treatment and result in worse-than-necessary complications which pose additional challenges for the clinical management of SBE as well as subsequent economic losses for generations [10,14,16,18].

Although antivenom is essential for treating SBE patients, it is not the only treatment that can save lives [18–20]. For example, airway and respiratory compromise in SBE patients suffering from neurotoxic envenomings can be managed almost entirely by mechanical ventilation where no antivenom is available or the antivenom is not effective [20–23]. Tissue necrosis around the bite site may require operative interventions such as local debridement and fasciotomy along with possible amputation where attempts at limb salvage are not viable or successful [18,19]. A wide range of antibiotics is used to combat bacterial infections arising from SBE and patients who experience acute kidney injury often need dialysis [20,24–26]. Thus, a notable variety of treatment methods is required to counteract the broad spectrum of clinical effects induced by SBE. All these treatment methods increase the final treatment costs for SBE patients. In countries like India, a significant proportion of people prefer to seek medical treatment in private healthcare settings even though the state and/or central governments provide free treatment in their healthcare centres [10,12,15,18,27]. Most rural people in India do not have health insurance policies to cover their medical treatment [10,12].

Since SBE largely affects agricultural populations who struggle to earn enough money for their everyday survival, the treatment costs for a single SBE event can significantly alter their lives through resulting socioeconomic impacts [4,10]. Some of the socioeconomic impacts include the loss of or changing occupation, inability to work, loss of properties, jewellery, and savings, and removing children from their education to send them to work to meet the family's financial needs [10,28]. Notably, some people are reluctant to seek hospital treatments only because of the high treatment costs, and certain people regret taking hospital treatments after surviving SBE because of the long-term financial consequences [10]. Hence, it is critical to not only save their lives from SBE but also minimise the treatment costs and resulting socioeconomic ramifications. SBE treatments are mostly provided by tertiary care hospitals that possess the necessary facilities, equipment, and expertise [18]. Hence, the treatment costs may vary based on the settings that the SBE patients choose, and often they visit more than one facility [18]. However, to improve clinical management and develop relevant policies, it is critical to understand the various factors involved in the treatment costs for SBE in private healthcare settings. To the best of our knowledge, no specific studies were performed to address this gap in the existing knowledge to determine the average treatment costs for SBE, and their justification, and identify the key factors contributing to high treatment costs.

To address this critical issue in the SBE field, this study aimed to determine the direct costs of treating SBE patients and key factors that increase treatment costs by analysing data from a large (913) cohort of SBE patients who were treated in different private tertiary care hospitals across Tamil Nadu, India. The data from this study indicates that on average, most SBE patients can be treated with roughly INR 100,000 (~GBP 1000 or USD 1200) or less, and hospital and pharmacy costs play critical roles in overall treatment costs. These results shed light on this poorly studied aspect of SBE and create awareness amongst SBE patients, clinicians, healthcare policymakers and insurance providers about the significance of timely treatments for SBE, the various components involved in clinical management and the justification for treatment costs.

Methods

Ethics statement

This study was approved by the Institutional Ethical Review Committees at Toxiven Biotech Private Limited, Tamil Nadu (reference number: 2019-001/002) and the University of Reading

(reference: UREC 23/05). The study was performed in line with the guidelines provided by the Indian Council for Medical Research and the Declaration of Helsinki. Before enrolment in this study, informed written consent was obtained from every participant or their legal guardians.

Patient and public involvement statement

The patients and members of the public were not directly involved in the study design, data collection, analysis and writing of the manuscript mainly due to their limited availability to attend several meetings that are often arranged during day times when they are also occupied with their daily work. However, all patients provided written consent to collect these data and to publish them in scientific journals. Involvement in this study was completely voluntary and participants could withdraw their consent at any time during this study. The participation did not result in any implications for their treatment and outcomes. We will ensure that the results of this study are disseminated to study participants and wider communities through scientific publications, which might be followed by press releases in media in the local language and English.

Study design and data collection

This study was carried out in 10 different private tertiary care hospitals located in various cities/towns across Tamil Nadu (a large state in South India with a high burden of SBE) in India. These hospitals were not selected to represent the whole state although they are distributed across the state. They were selected using the convenience sampling approach mainly due to their popularity in treating snakebites and easy access to snakebite patients. Indeed, four of them are snakebite (Note: the term 'SBE' refers to actual envenoming and 'snakebite' includes all bites including dry and non-venomous bites) referral hospitals where patients are routinely referred from other healthcare settings. The data were equally distributed across four snakebite referral hospitals (150 from each) and other hospitals (50 from each except one hospital where 63 patients were recruited due to the availability of many patients bitten by snakes other than Russell's viper). The hospital authorities wish to remain anonymous due to the sensitive nature of the data provided on treatment costs, and therefore, we are unable to reveal their names, precise locations, and treatment costs in individual hospitals. Data were collected between January 2019 and June 2021. All the data sets were received in an anonymised format without any personal details of the patients.

All patients bitten by venomous species presenting at the hospital were assigned a patient number, so their details could be anonymised. In total, 927 patients were admitted following venomous bites, of which 913 suffered from snakebites, 1 centipede, 9 unknown insect bites and 4 scorpion bites. Patients who were bitten by non-snake species were excluded from this study. Hence, a total of 913 patients were included for further analysis. The details of their treatments (except basic details to ascertain if they visited another medical facility and received any treatment specifically antivenom) and costs before admission to these hospitals were not collected in detail as this was not the scope of this study. Therefore, this study presents only the data that were collected from the study hospitals.

All the patients included in this study were subjected to detailed clinical examination and laboratory investigation at the time of arrival at the emergency department of each hospital. Demographic data and patient anamnesis upon presentation were collected. In some cases, the patients or their relatives brought dead or live snakes for identification. A range of clinical symptoms (a total of 45) was determined by clinicians to be used for clinical assessments. Patients were treated according to each hospital's standard of care including antivenom administration and supportive measures.

Classification of patients

The offending snake species was determined mainly based on the presenting clinical symptoms and the dead/live specimens brought to the hospitals in some cases and categorised as Russell's viper, saw-scaled viper, common krait, cobra, or non-venomous snakes. Since the clinicians in selected hospitals treated snakebite victims for several years, they were familiar with the common symptoms of envenomation from Big Four snakes. However, in cases where clinicians could not clearly identify the snake species based on the information provided by patients or their relatives and presenting symptoms, the bite was classified as an unknown category. In some cases, the patients were shown images of commonly available snakes around their regions and asked to identify the offending species. While this approach helped to identify the offending snake in some cases in line with their symptoms, in other cases, this was not helpful. For example, patients who identified multiple snakes as potentially responsible for the bite were categorised as being of unknown origin. Therefore, this classification method aided in identifying appropriate offending species to calculate their treatment costs as accurately as possible.

Classification of clinical manifestations

Upon consultation with clinicians, a range of clinical signs and symptoms were reviewed to provide the appropriate level of diversity in symptomology following bites from Indian venomous snakes. These include the presence of puncture wounds/fang marks, bleeding, pain, changes in blood pressure, muscle weakness, blurred vision, vomiting, numbness, swelling, sweating, ptosis, skin discolouration, difficulty breathing, necrosis, epigastric burning sensation, giddiness, difficulties in swallowing, absence of urine output, cellulitis, generalised itching, haematuria, abdominal pain, fever, chills, unhealing wound, burning sensation at the bite site, sore throat, heaviness of head, chest pain, tickling sensation, tiredness, restlessness, haematemesis, haemoptysis, dyspnoea, diplopia, oliguria, tremors, shivering, xerostomia, frothing, cold extremities, pulmonary oedema and gum bleeding. The total number of signs and symptoms experienced by each victim was analysed in this study.

Classification of total treatment costs

The direct treatment cost data were divided into four categories. (1) Hospital cost which was defined by the patient's duration of hospitalisation and the type of procedures/treatment received (including intensive care unit, ward stay, use of medical equipment e.g., ventilation support and operative interventions). (2) Pharmacy costs which covered the cost of medications received including antivenom, antibiotics and blood products along with consumables used during their treatment. (3) Investigation costs were the fees charged for the care provided by the healthcare team including the expenses charged by specialist clinicians to visit the patient from other organisations/hospitals. (4) The laboratory costs covered the expenses for different laboratory tests (such as haematological analysis and microbial culture) and specialist investigations [e.g., computed tomography (CT), magnetic resonance (MRI) and ultrasound imaging] conducted on the patients. A total treatment cost was calculated by adding up each of these individual parts. All the prices charged to participants in this study were mentioned in the form of Indian rupees (INR).

Statistical analysis

A generalised linear model with a gamma distribution and a log-link function for the mean component was used to model treatment costs with the snake, age, sex, number of vials of

antivenom and interactions as covariates. This modelling approach is due to the fact that the treatment costs are positive, and it allows us to address the observed heterogeneity in the data detected during the model validation process. Before fitting any model, data exploration was applied using the protocols that were previously described [29, 30]. The presence of outliers and collinearity were investigated. One way ANOVA was performed to compare the treatment costs for different snake species. The Wald test was used to evaluate the effects of gender, age, and the number of antivenom vials on total treatment costs. The descriptive analysis was used for calculating the mean, median and standard deviation for different variables. Similarly, percentages, ranges and ratios were calculated to summarise the distribution of data where necessary.

All statistical analyses were performed using the R (Version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria) and SPSS (Version 27, IBM, USA) statistical packages and GraphPad Prism (Version 7, GraphPad Software, San Diego, CA, USA).

Results

Characteristics of the study population

In total, 913 snakebite patients who were admitted to 10 different private tertiary care hospitals in Tamil Nadu were included in this study (**Fig 1A**). Among the study population, 600 (65.7%) males and 313 (34.3%) females were included indicating that males are more vulnerable to snakebites than females, probably due to their increased involvement in agricultural activities. Further classification of these data based on the type of snake involved in the incident revealed that 355 [38.9% of total incidents: 242 (68.2%) males and 113 (31.8%) females] patients were bitten by Russell's vipers (**Fig 1B**), 133 [14.6% of total incidents; 103 (77.4%) males and 30 (22.6%) females] patients were bitten by common kraits, 108 [11.8% of total incidents; 70 (64.8%) males and 38 (35.2%) females] patients were bitten by saw-scaled vipers and 69 [7.6% of total cases; 43 (62.3%) males and 26 (37.7%) females] patients were bitten by cobras. Moreover, 87 [9.5% of total incidents; 47 (54%) males and 40 (46%) females] patients were bitten by non-venomous snakes such as Indian rat snakes (*Ptyas mucosa*), wolf snake (*Lycodon aulicus*), and common bronzeback tree snake (*Dendrelaphis tristis*). A total of 161 patients [17.6% of total cases; 95 (59%) males and 66 (41%) females] were bitten by unidentified snake species as their identities could not be ascertained based on the available details. This category may include a few cases of Malabar (*Craspedocephalus malabaricus*), hump nosed (*Hypnale hypnale*) and bamboo (*Craspedocephalus gramineus*) pit vipers as these cases are also being reported in distinct parts of Tamil Nadu (information gathered from collaborating hospitals).

To determine the specific age groups that are most affected by snakebites, the data were categorised based on the age of the patients. In total, 38 (4.2% of total incidents) people were in the age range of 0–10, 92 (10%) in 11–20, 143 (15.7%) in 21–30, 200 (21.9%) in 31–40, 196 (21.5%) in 41–50, 146 (16%) in 51–60, and 98 (10.7%) were above 60 years old (**Fig 1A**). The youngest patient included in this study was 2 years old and the oldest patient was 91 years old with a median of 40 years (IQR: 28 to 51; SD = 16.67) and a mean of 39.9. For Russell's viper bites, the minimum age was 2.5 years old, and the maximum age was 91 with a median age of 40 and a mean of 40.6 (IQR 29 to 51; SD = 16.2) (**Fig 1B**). For common krait bites, the minimum age was 11 and the maximum age was 81 with a median age of 47 and a mean of 46.9 (IQR 38 to 55; SD = 13.9). For cobra bites, the minimum age was 4 years old, and the maximum age was 80 with a median age of 35 and a mean of 36.2 (IQR 21 to 52; SD = 18.5). For saw-scaled viper bites, the minimum age was 2 years old, and the maximum was 70 with a median age of 41.5 and mean of 41.8 (IQR 31 to 54; SD = 15.4). For non-venomous snakebites, the minimum age was 3 years old, and the maximum age was 75 with a median age of 33 and a

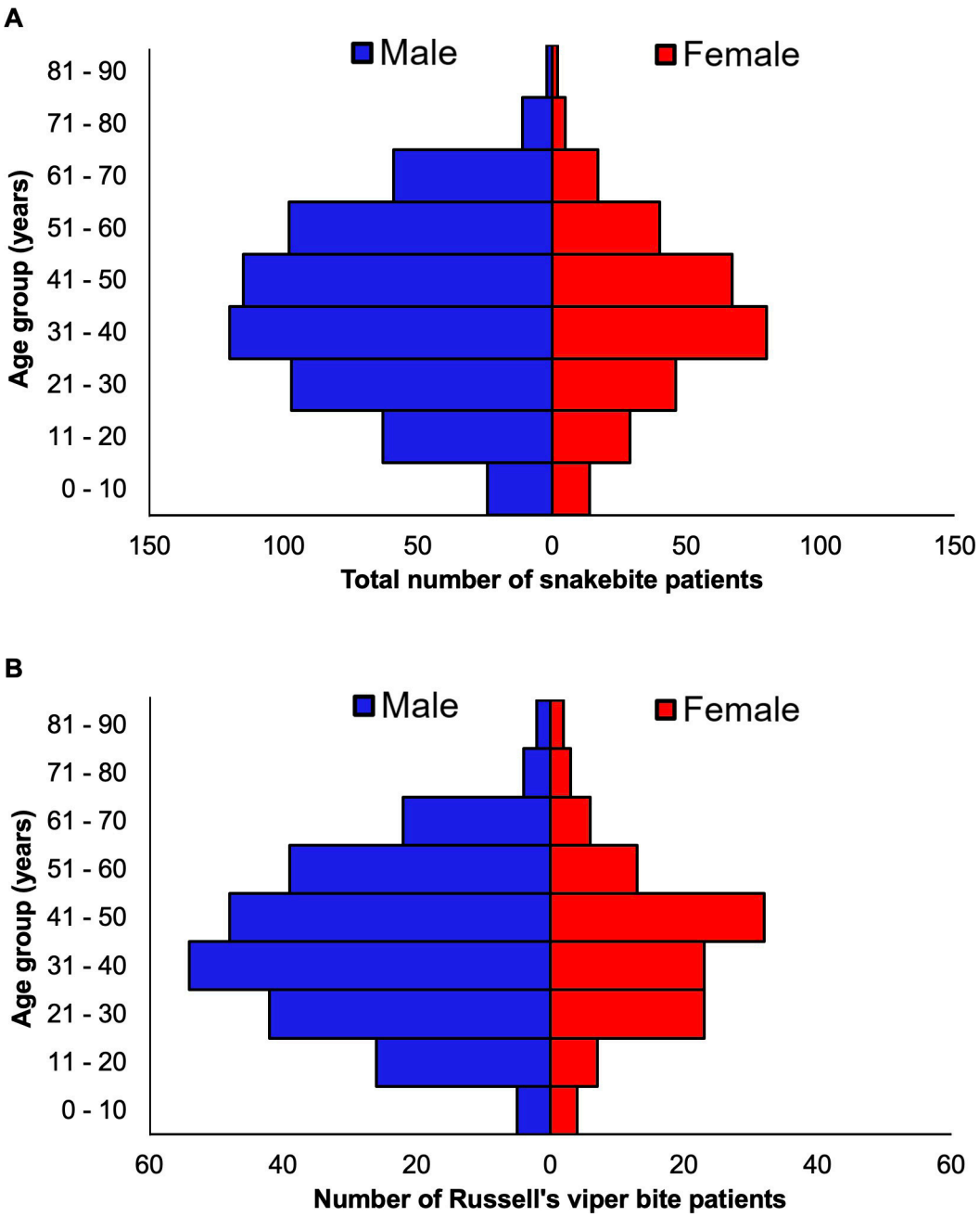


Fig 1. Age and gender profile of snakebite patients. (**A**) A graph showing the age and gender distribution of the 913 total snakebite patients included in this study. (**B**) The age and gender distribution of 355 Russell's viper bite patients.

https://doi.org/10.1371/journal.pntd.0011699.g001

mean of 32.2 (IQR 19.5 to 31.9; SD = 16). For unknown snakes, the minimum age was 2 years old, and the maximum age was 75 with a median age of 39 and a mean of 37.2 (IQR 24 to 50; SD = 17.7). This data indicates that males of working age (from 21 to 60) are most likely to suffer snakebites.

We then analysed the most common signs and symptoms that the patients displayed upon admission to the study hospitals. Russell's viper patients experienced pain [342 (96.3% of total Russell's viper bites)], swelling [286 (80.6%)], vomiting [173 (48.7%)], blurred vision [119

(33.5%)] and bleeding [117 (33%)] as the most prevalent signs/symptoms. Common krait bites induced ptosis [62 (46.6% of total krait bites)] and blurred vision [50 (37.6%)]. Cobra bites induced pain [67 (97.1% of total cobra bite patients)], swelling [42 (60.9%)], vomiting [28 (40.6%)], blurred vision [22 (31.9%)] and ptosis [21 (30.4%)]. Saw-scaled viper bites induced pain [107 (99% of total saw-scaled viper patients)], swelling [66 (61.1%)], and blurred vision [30 (27.8%)]. Non-venomous snakes mainly induced pain [72 (82.8% of total non-venomous cases)]. Bites from snakes within the unknown category induced pain [131 (81.4% of unknown bite cases)], swelling [84 (52.2%)], vomiting [54 (33.5%)], and bleeding [30 (18.6%)].

Russell's viper bites incur higher treatment costs than bites from other snake species

The total treatment costs were classified into hospital, investigation, laboratory, and pharmacy costs. The total treatment costs for Russell's viper bites ranged from INR 2,502 to INR 694,620 with an average of INR 111,496 (median value of INR 79,260) (Fig 2A). The costs for common krait bites ranged from INR 10,504 to INR 856,688 with an average cost of INR 47,276 (median of INR 21,378). For cobra bites, the costs ranged from INR 11,029 to INR 309,683 with an average of INR 86,833 (median of INR 63,893). Treating saw-scaled viper bites costs between INR 5,814 to INR 105,223 with an average of INR 28,863 (median of INR 24,546). The costs of non-venomous bites ranged from INR 2,937 to INR 108,057 with an average of INR 17,554 (median of INR 15,162). The unknown snake category costs ranged from INR 2,101 to INR 674,884 with an average of INR 77,342 (median of INR 36,804). These data suggest that the average treatment costs for Russell's viper bites are higher than the costs for treating bites from other snake species.

Further evaluation of treatment costs revealed that hospital (Fig 2B) and pharmacy (Fig 2C) costs are considerably higher than the laboratory (Fig 2D) and investigation (Fig 2E) costs. For example, the hospital costs ranged from INR 500 to INR 357,300 (average of INR 50,739) and pharmacy costs ranged from INR 1,111 to INR 253,713 (average of INR 41,638) for Russell's viper patients. However, the average costs of investigation (INR 100 to 106,000) and laboratory tests (INR 191 to 68,307) were only INR 8,742 and INR 10,377, respectively, for Russell's viper patients. These data suggest that hospital and pharmacy costs are the most significant factors contributing to the overall treatment costs for snakebite patients. The average percentages of different treatment costs compared to the total costs for bites from diverse snake species are shown in Table 1.

Age of patients and snake species act as significant predictors of treatment costs

When the total treatment costs for males and females were compared, there was no significant difference between the cohorts, indicating that gender is not a significant predictor for total treatment cost [$X^2 = 0.07$; df = 1; p = 0.79] (Fig 3A). However, when the total treatment costs of different age groups were compared, age was determined to be a significant predictor for the total treatment cost [$X^2 = 6.54$; df = 1; p = 0.01] (Fig 3B). Similarly, when the treatment costs of patients from different snake species were compared, the offending snake was confirmed to be a significant predictor of total treatment cost [$X^2 = 332.9$; df = 5; $p<0.0001$]. The results indicate that all snake species' total treatment costs vary significantly from each other. However, the total treatment costs are not significantly different between Russell's viper and the cobra, or between the unknown category and cobra. The data also indicate that the overall treatment cost decreases with age in both males and females (Fig 3B).

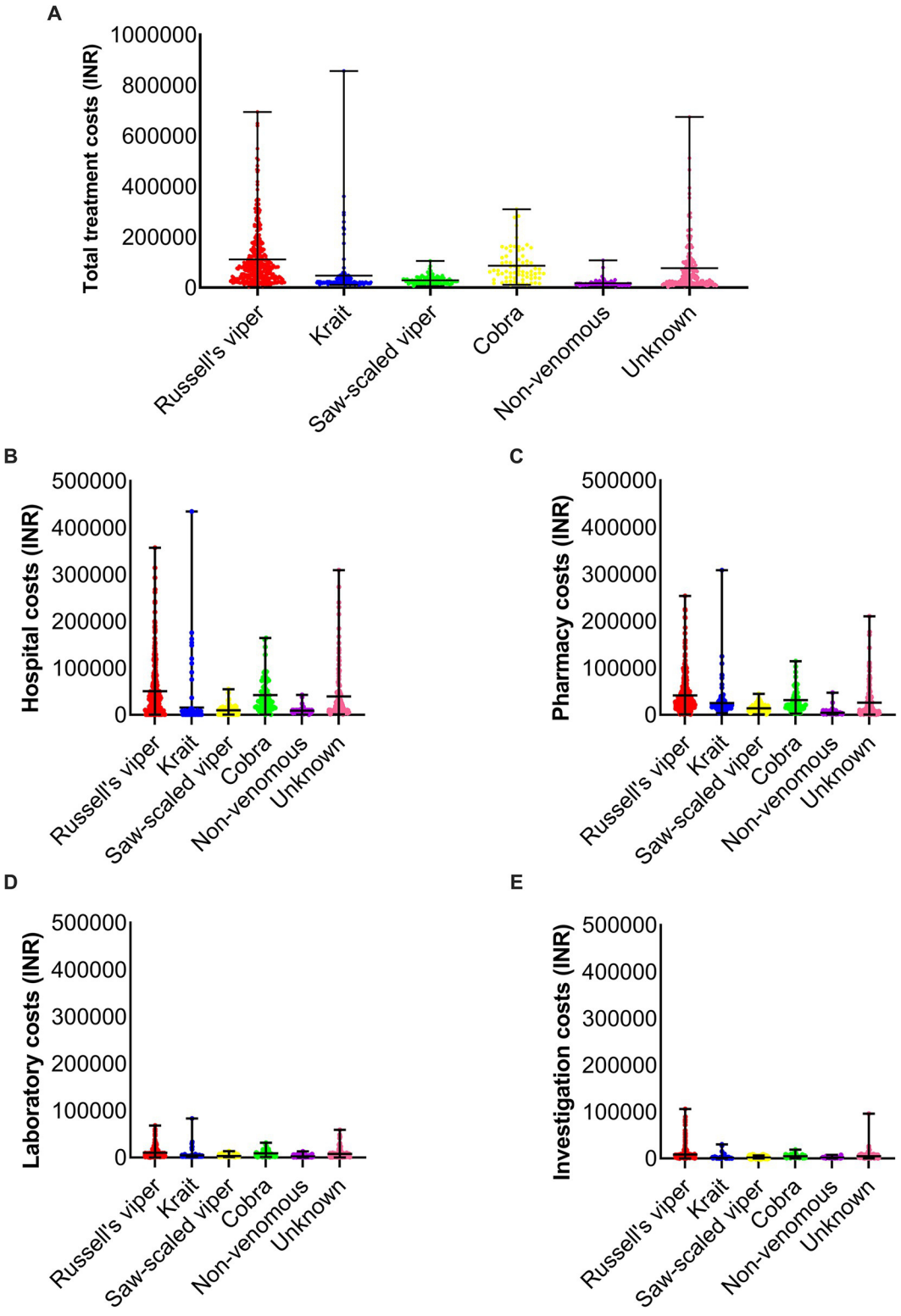


Fig 2. The breakdown of total treatment costs based on the type of snake species and nature of expenses. (**A**) Total treatment costs of patients bitten by different snake species. The total costs for the hospital (**B**), pharmacy (**C**), laboratory (**D**) and investigation (**E**) were also analysed individually.

https://doi.org/10.1371/journal.pntd.0011699.g002

Table 1. Average treatment costs of different categories of total treatment costs for each snake. All the values are shown in percentages of total treatment costs for each snake.

	Average costs of different categories of total treatment costs (%)			
Snake	**Hospital**	**Pharmacy**	**Laboratory**	**Investigation**
Common krait	33.50	53.50	10	3
Cobra	48.5	36	10	5.4
Saw-scaled viper	34	48	10.8	6.2
Russell's viper	45.5	37.3	9.3	7.8
Non-venomous	50.9	26	13.2	9.9
Unknown	50.7	33.4	9.7	6

https://doi.org/10.1371/journal.pntd.0011699.t001

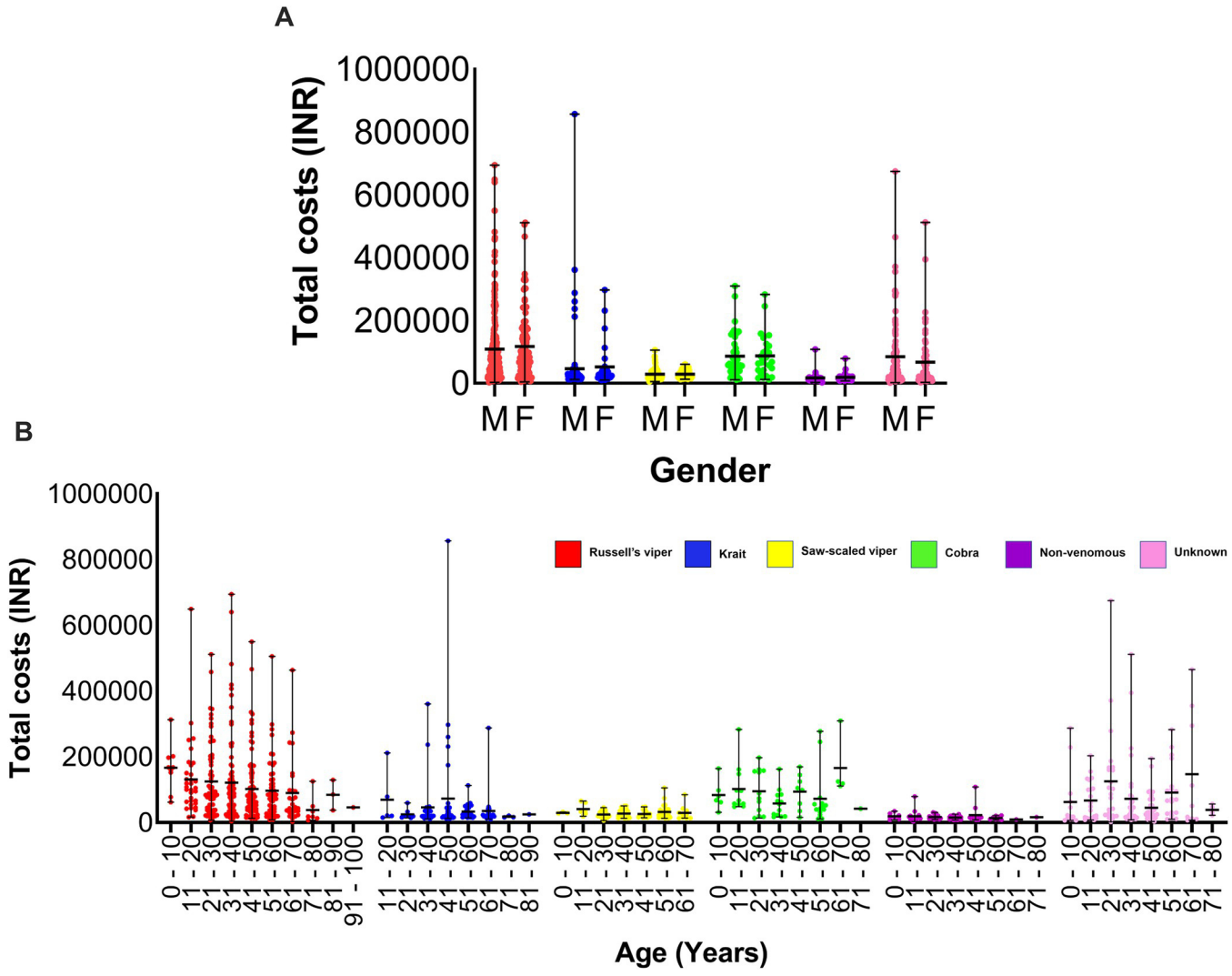


Fig 3. The relationship between the gender and age of snakebite patients and their total treatment costs. (**A**) The relationship between the gender of the patient and their total treatment costs for different snake species. (**B**) The patient's age and the total treatment costs for different snakebites.

https://doi.org/10.1371/journal.pntd.0011699.g003

Table 2. Some of the specialist treatments given to patients who were bitten by different snakes and their total treatment costs. The values shown are average total treatment costs presented in INR, and the values in brackets represent the number of patients who received those treatments. ICU-intensive care unit, PCV-packed red cell volume and FFP-fresh frozen plasma.

	Average total treatment cost (INR)					
Specialist treatment	**Russell's viper**	**Common krait**	**Cobra**	**Saw-scaled viper**	**Non-venomous**	**Unknown**
For Allergy	59,644 *(5)*	24,299 *(4)*	79,430 *(2)*	32,457 *(2)*	20,180 *(3)*	50,059 *(4)*
Dialysis	172,018 *(13)*	20,432 *(2)*	39,524 *(1)*	22,908 *(3)*	17,435 *(4)*	138,829 *(4)*
For Parotitis	292,502 *(1)*	-	-	-	21,254 *(1)*	-
For Jaundice	292,502 *(1)*	-	-	-	-	-
ICU	188,950 *(26)*	212,056 *(7)*	68,019 *(6)*	40,736 *(9)*	13,218 *(7)*	83,776 *(17)*
Blood transfusion	139,541 *(55)*	112,307 *(18)*	83,215 *(13)*	39,959 *(12)*	16,504 *(16)*	74,104 *(26)*
Surgeries, e.g., wound debridement	133,148 *(38)*	23,373 *(14)*	91,002 *(7)*	28,334 *(13)*	18,115 *(6)*	62,633 *(18)*
Tracheostomy	-	-	-	17,586 *(2)*	-	87,120 *(2)*
PCV	160,644 *(2)*	-	37,404 *(1)*	16,286 *(1)*	-	261,989 *(2)*
FFP	-	12,988 *(1)*	-	16,286 *(1)*	11,895 *(1)*	261,989 *(2)*

https://doi.org/10.1371/journal.pntd.0011699.t002

Impact of specialist treatments on total treatment costs

All associated treatment costs for Russell's viper bites are higher than the bites from other snakes, which can be attributed to the specialist treatments that these patients received (Table 2). For example, 13 patients who were bitten by Russell's viper received dialysis and therefore, their average total treatment costs were INR 172,018 which is higher than the average (INR 111,496) total costs for Russell's viper patients. A single patient who developed parotitis along with excessive levels of bilirubin paid a total treatment cost of INR 292,502. A total of 26 patients bitten by Russell's viper stayed in intensive care units and they paid a total of INR 188,950. 55 Russell's viper patients received blood transfusions and paid a total average cost of INR 139,541. 38 Russell's viper patients had some form of operating intervention such as wound debridement and paid an average cost of INR 133,148. Similarly, seven cobra patients had wound debridement and they paid INR 91,002 which is higher than the total average (INR 86,833) costs for treating cobra patients (Table 2). Moreover, two patients bitten by saw-scaled vipers developed anaphylaxis and they paid higher (INR 32,457) than the average (INR 28,863) total treatment costs. Nine saw-scaled viper patients who stayed in intensive care units paid a total of INR 40,736 which is higher than the total average costs (Table 2). 12 patients of saw-scaled vipers had blood transfusions, and they paid INR 39,959, which is also higher than the average total treatment costs. 17 patients bitten by unknown snakes stayed in the intensive care units and paid an average of INR 83,766 which is more than the average (INR 77,342) total treatment costs. Similarly, two patients of unknown snake categories had a tracheostomy and therefore their total treatment costs were increased to INR 87,120, higher than the total average costs.

The quantity of antivenom vials used is not a key factor for the total treatment cost

We then analysed the data to determine if the volume of antivenom administered is a key predictor of total treatment costs for SBE. The number of vials of antivenom used for Russell's viper patients varied from 3 to 50 vials (average 25; median 30). Cobra bites required 5 to 35 vials (average 16; median 15), common krait bites used 5 to 20 vials (average 16; median 20), saw-scaled viper bites used 2 to 10 vials (average 5; median 5), non-venomous snakebites used 3 to 5 vials (average 4.5; median 5) and the unknown category required 3 to 35 vials (average 17; median 15) (Fig 4A). The number of antivenom vials received by patients varies significantly depending on the offending snake ($X^2 = 382.485$, df = 5, p = <0.0001). Neither the age

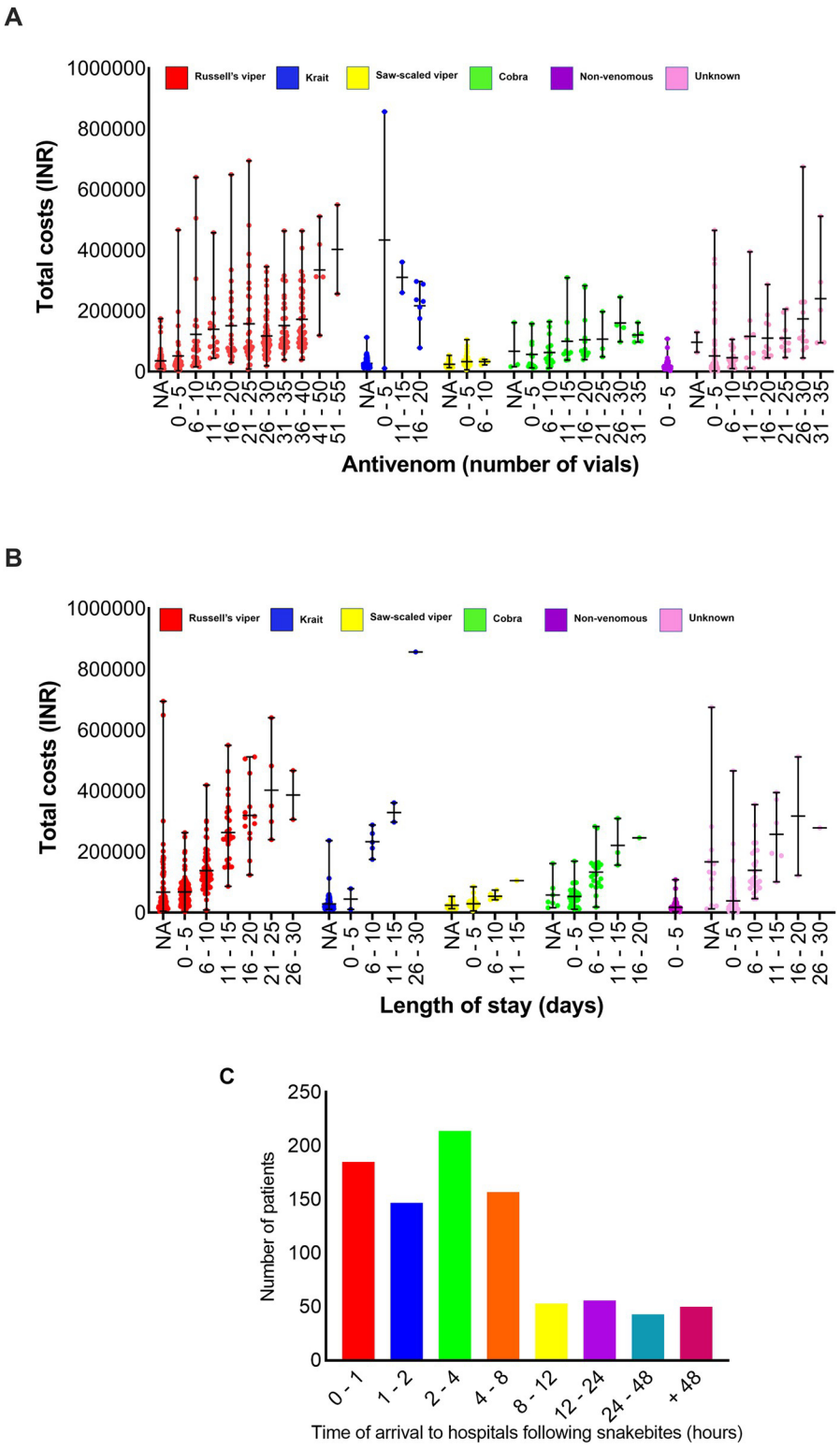


Fig 4. Impact of the number of vials of antivenom used, and the length of hospital stay in snakebite treatment costs. (**A**) The relationship between the number of antivenom vials received by snakebite patients and their corresponding total treatment costs for the species in question. (**B**) The length of hospital stay in days for snakebite patients and the corresponding total treatment costs. NA—'not available' indicates that for a small number of patients, accurate details are not available. (**C**) The number of patients arrived at the hospital at different time points following the snakebites.

https://doi.org/10.1371/journal.pntd.0011699.g004

($X^2 = 0.00$, df = 9, p = 0.9407) nor the gender ($X^2 = 1.484$, df = 1, p = 0.223) of the patients had any significant bearing on the number of vials received. However, males received 11% (or 1.1 times) more vials than females although this was not found to be statistically significant due to large variations within the data set. The total number of antivenom vials used was calculated as a proportion of the total treatment cost, and it was found to be a lesser contributor to the total treatment cost. In most hospitals, antivenom was provided for around INR 700/vial. Therefore, its contribution to the total treatment cost ranged from INR 1,400 to INR 35,000. Compared to the total treatment costs, this is only a small factor in determining the final treatment costs in tertiary healthcare settings. The cost of an average number of antivenom vials received by Russell's viper bite patients represents 15.7% (INR 17,500) of the total treatment cost. Similarly, it accounts for 13.1%, (INR 11,410) of cobra, 24.3% (INR 11,480) of common krait, 12.1% (INR 3,500) of saw-scaled viper, 17.9% (INR 3,150) of non-venomous and 15.7% (INR 12,110) of unknown category treatment costs. Notably, if patients received up to 10 vials of antivenom in the primary or local healthcare settings prior to their admission in tertiary care settings, then a significant decrease in the number of vials received at the tertiary private hospitals was observed ($X^2 = 10.95$, df = 9, p = 0.0024).

Impact of hospital stay, time of bites and time taken to reach hospitals on treatment costs

For Russell's viper bites, the minimum stay in the hospital was 30 minutes and the maximum stay was 30 days (average 6.8 days; median 5) (Fig 4B). Similarly, patients bitten by cobras required a stay of 0.5 to 18 days (average 5.3; median 5). Common krait bite patients required between 1 to 26 days (average 9.7; median 9), non-venomous bites ranged from 2 hours to 4 days (average 1.4; median 1), and unknown bite patients ranged from one hour to 27 days (average 4; median 3). Although the length of stay in hospitals can contribute to increased treatment costs, this alone is not the sole predictor of total treatment costs for any specific snake. For example, the patients bitten by common kraits stayed an average of 9 days which is higher than any other snakebite patients but paid comparatively lower treatment costs. On the other hand, Russell's viper bite patients who had shorter stays in the hospital paid more treatment costs than patients bitten by common kraits. Therefore, there are other contributory factors that determine the overall treatment costs for snakebite patients.

Out of the 913 patients, 252 went directly to tertiary care hospitals from where the data were collected in this study. 661 patients visited at least one local primary or secondary healthcare setting prior to reaching these tertiary care hospitals. Moreover, 185 patients arrived at the hospitals within one hour following the bite, 147 arrived between 1 and 2 hours, and 214 arrived between 2 to 4 hours following the bites (Table 3). Therefore, a total of 546 (60%)

Table 3. Total average treatment costs for snakebite patients who arrived at hospitals at different times following bites. The total treatment costs are shown in INR, and the number of patients in each category was shown within the brackets.

	Average total treatment cost (INR)					
Time to Hospital (hours)	**Russell's viper**	**Common krait**	**Cobra**	**Saw-scaled viper**	**Non-venomous**	**Unknown**
0–1	102,277 *(56)*	39,779 *(38)*	82,898 *(15)*	25,615 *(24)*	18,675 *(20)*	80,652 *(32)*
1–2	120,366 *(58)*	35,199 *(22)*	69,623 *(13)*	30,245 *(21)*	14,600 *(15)*	53,955 *(18)*
2–4	110,624 *(87)*	48,095 *(31)*	97,119 *(16)*	28,106 *(23)*	19,783 *(18)*	80,368 *(39)*
4–8	115,202 *(60)*	34,741 *(24)*	58,066 *(14)*	30,163 *(19)*	15,065 *(11)*	63,718 *(29)*
8–12	85,032 *(21)*	66,690 *(9)*	113,061 *(4)*	31,417 *(4)*	21,793 *(7)*	93,014 *(8)*
12–24	126,879 *(22)*	30,425 *(6)*	136,822 *(4)*	31,325 *(9)*	16,569 *(4)*	132,935 *(11)*
24–48	124,590 *(20)*	21,469 *(1)*	277,473 *(1)*	28,460 *(1)*	18,782 *(8)*	60,110 *(12)*
+ 48	101,134 *(25)*	436,326 *(2)*	37,404 *(1)*	28,863 *(6)*	10,946 *(4)*	82,501 *(12)*

https://doi.org/10.1371/journal.pntd.0011699.t003

patients arrived at hospitals within the recommended 4-hour period following bites [15]. In addition, 157 (17%) arrived at hospitals between 4 and 8 hours after bites. The remaining patients arrived at various time points (Fig 4C). These data suggest that a majority of SBE patients are seeking treatment at hospitals within the recommended time either in local primary/secondary or tertiary healthcare settings.

We found that the time of arrival is not a significant predictor of total treatment costs for most SBE patients (Table 3). However, the data indicate that Russell's viper patients who arrived after 12 hours following bites paid more (INR 126,879) than the total average costs (INR 111,496) for Russell's viper patients although it is not significant. Similarly, common krait patients who arrived eight hours following bites paid more (INR 66,690) than the total average (INR 47,276) treatment costs. Cobra patients who arrived after 4 hours following bites paid more (INR 124,942) than the average total costs (INR 86,833). Saw-scaled viper patients who arrived four hours following bites paid more (INR 30,968) than the average total treatment costs (INR 28,863). Notably, there was a significant difference ($p<0.0001$; $df = 7$; $f = 7.155$) when comparing the total treatment costs for common krait patients who arrived after 48 hours following bites (INR 436,326) with patients who arrived within one hour (INR 39,779) following bites although there were only two patients admitted to the hospitals after 48 hours. Similarly, there was a significant ($p = 0.0291$; $df = 7$; $f = 2.343$) difference in total treatment costs of cobra patients who arrived after 24 hours (INR 277,473) compared to patients who arrived within one hour (INR 82,898) although only one cobra patient arrived after 24 hours. These data reemphasise that the delay in seeking hospital treatment is a key factor in contributing to increased treatment costs.

Moreover, when analysing the total treatment costs of patients who were bitten during day times (between 6 am and 6 pm), early evenings (6 pm to 10 pm) and late night (10 pm to 6 am), there was no significant difference in the total treatment costs for Russell's viper, saw-scaled viper, and cobra patients although the values are high for cobra and saw-scaled viper patients who arrived after 10 pm (Table 4). However, common krait patients who were bitten after 10 pm paid significantly more (INR 138,575) than the total average treatment costs (INR 47,276). The increase in treatment costs in late nights could be due to high transport costs, and investigation charges as the specialists may need to come from home or other hospitals for expert investigation and emergency intubation. As the common krait is a nocturnal snake, its bites are likely to be high at night times leading to late-night hospital admissions.

The hospital care for nearly 80% of SBE patients cost INR 100,000 or less for their treatments

Finally, the total amount of treatment costs was analysed for all snakebite patients. This reveals that 711 (78%) patients paid INR 100,000 (~GBP 1000 or USD 1200) or less for their hospital

Table 4. Total average treatment costs for snakebite patients who were bitten at different times of the day/night. The treatment costs are shown in INR and the number of patients is shown in brackets.

	Average total treatment cost (INR)					
Time of bite	**Russell's viper**	**Common krait**	**Cobra**	**Saw-scaled viper**	**Non-venomous**	**Unknown**
06:00–10:00	98,601 *(67)*	20,342 *(14)*	82,233 *(14)*	29,400 *(17)*	14,649 *(15)*	62,869 *(24)*
10:00–14:00	115,039 *(70)*	32,840 *(27)*	71,657 *(18)*	25,471 *(23)*	17,749 *(18)*	103,124 *(31)*
14:00–18:00	132,837 *(69)*	31,865 *(34)*	97,357 *(13)*	28,567 *(26)*	16,439 *(19)*	72,987 *(29)*
18:00–22:00	112,646 *(98)*	40,165 *(38)*	87,910 *(16)*	29,468 *(24)*	20,787 *(29)*	66,320 *(56)*
22:00–02:00	107,039 *(20)*	168,272 *(7)*	117,142 *(6)*	34,046 *(9)*	10,206 *(5)*	87,585 *(9)*
02:00–06:00	86,575 *(17)*	108,878 *(12)*	13,655 *(1)*	27,891 *(6)*	21,767 *(1)*	55,543 *(6)*

https://doi.org/10.1371/journal.pntd.0011699.t004

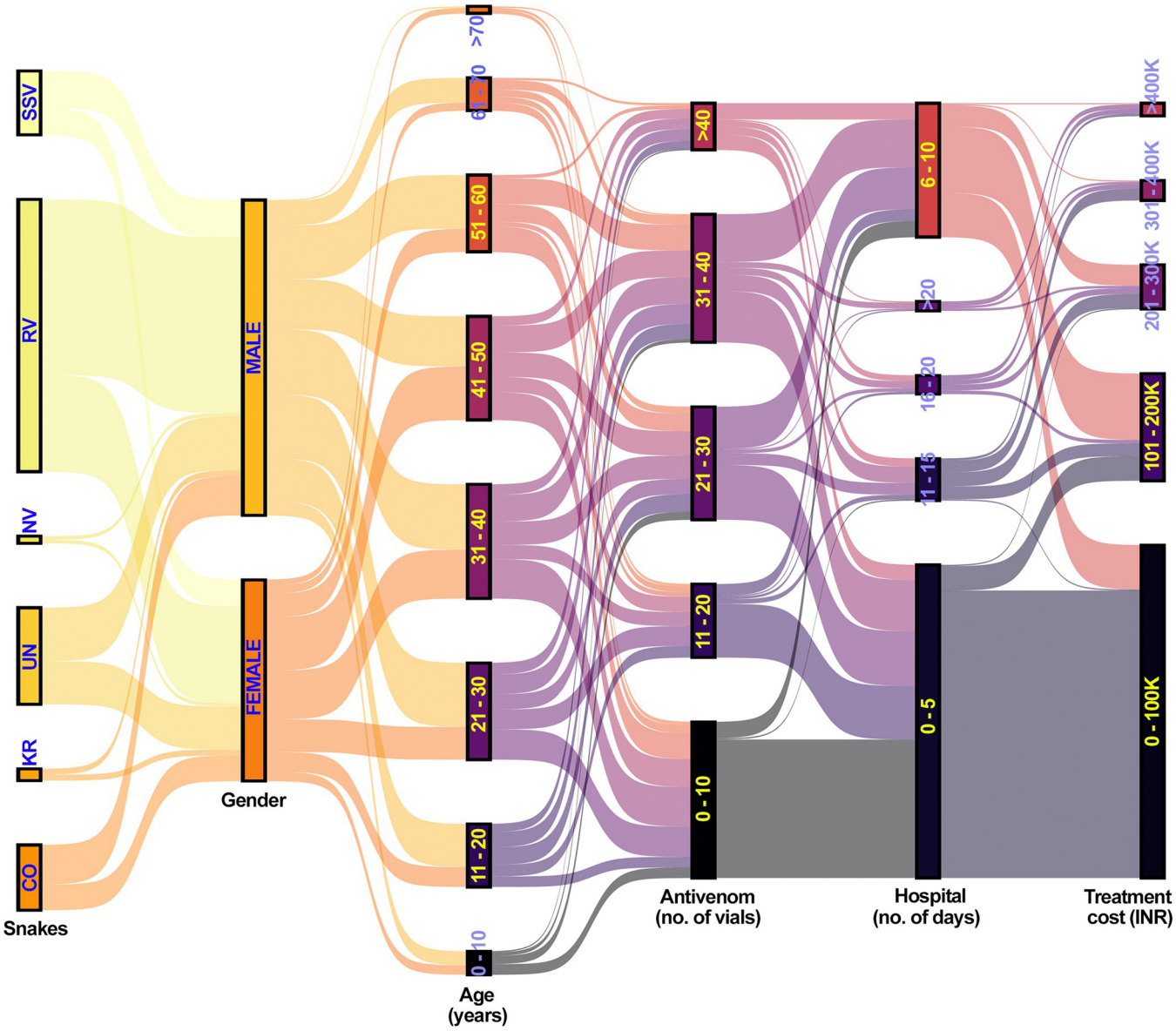


Fig 5. A Sankey plot showing the relationships between treatment costs and various parameters analysed in this study. RV—Russell's viper, CO—cobra, KR—common krait, UN—unknown, NV—non-venomous snakes and SSV—saw-scaled viper.

https://doi.org/10.1371/journal.pntd.0011699.g005

treatment. This includes 223 (63%) Russell's viper, 49 (71%) cobra, 123 (93%) common krait, 107 (99%) saw-scaled viper, 86 (99%) non-venomous and 123 (76%) unknown bite patients. 120 (13%) patients paid between INR 100,001 to INR 200,000 for their treatment. 47 (5%) patients paid between INR 200,001 to INR 300,000, and 19 (2%) paid INR 300,001 to INR 400,000 for their treatments. The higher cost of more than INR 400,000 was paid by a minority of patients. These data suggest that despite gender, age, the offending snake species, time of the bite, time taken to reach tertiary care hospitals, administration of antivenom and specialist treatments used, around 80% of patients can be treated with INR 100,000 or less in private tertiary healthcare settings (Fig 5).

Discussion

SBE affects rural communities in several countries specifically in South Asia, Africa and Central and South America [2, 3]. The World Health Organisation (WHO) has developed a strategic road map comprising four key pillars to reduce SBE-induced deaths and disabilities by half in 2030. To achieve this ambitious target, we need to initiate trans-disciplinary research on various aspects associated with SBE to establish its true impact on vulnerable communities. Notably, strengthening the health system is one of the key pillars of the WHO's SBE strategies. Therefore, it is critical to understand the wider context of healthcare systems, their operations, training needs and the costs of treating SBE in vulnerable countries [31]. Healthcare costs are a major issue in many parts of the world specifically in low- and middle-income countries [32]. While some countries provide healthcare free of charge for SBE patients, in other places people pay for their treatment out of pocket. Among poor communities living in rural areas of developing countries, the affordability of healthcare is a major issue [32]. Since SBE is an unexpected accident as well as an occupational health hazard, the treatment costs can cause significant socioeconomic impacts on patients and their families for generations [10,12,33]. As shown in our previous studies [10,18], SBE treatment costs vary widely based on the nature of the bite and the type of treatments involved. In most cases, the patients do not have any health insurance to cover their treatment costs [10]. This results in patients taking out loans for treatment costs from private lenders as the nationalised banks often do not offer such loans to cover medical expenses. The subsequent economic hardships force some patients to sell their homes, valuables, and other assets to cover costs [10]. In some cases, they are forced to withdraw their children from education to start working to bring in more money to cover household costs and alleviate the financial burden that SBE has caused. Hence, SBE treatment costs act as a key factor in instigating socioeconomic impacts on patients and their families, inducing a cycle of poverty [10,11]. However, the availability of data on a range of treatments involved and their costs for SBE in many parts of the world specifically in India is highly limited [34]. Therefore, this study analysed the treatment costs in a broad sample of private tertiary healthcare settings in Tamil Nadu, India to establish the key factors associated with SBE treatment costs that will aid in developing strategies to minimise these costs and enable SBE patients to seek prompt treatment.

India suffers around 58,000 annual deaths due to SBE even though the medical facilities are advanced in urban areas [8]. Healthcare in India is provided by both government and private parties including some charitable organisations [12]. In India, the state and central governments have an excellent architecture in their healthcare system including village health nurses, primary health centres, taluk hospitals, district general hospitals and medical college hospitals. These healthcare facilities have the required facilities and expertise to provide free healthcare for everyone [12]. For a range of different reasons, a vast majority of people seek treatment in private healthcare settings even for mild ailments. Similar to the government's healthcare system, private parties run small primary care clinics in rural areas and secondary (with limited facilities), tertiary (hosts all necessary facilities, equipment and expertise) and super speciality (well-advanced facilities, equipment and expertise) hospitals in urban areas [12, 35]. In addition, local pharmacies are mostly managed by private individuals, and they often treat mild ailments with over-the-counter medications. People must pay for their treatments and medicines in all private healthcare settings. Based on the type of illness, nature of treatments and the provider used, the treatment costs vary widely [35]. Due to the broad range of complications arising from snake venoms, SBE patients are mostly treated in tertiary healthcare settings to tackle the issues promptly.

In this study, the data collected from participating private tertiary care hospitals were divided into several categories (hospital, pharmacy, investigation, and laboratory costs) to underpin the key contributors to the overall treatment costs. This has simplified the discussion about the nature of treatments involved and the rationale for expensive treatments. The hospital costs that cover intensive/critical care units, wards, operating theatres, and surgical procedures appeared to be the major contributor to total treatment costs. Pharmacy cost as a category covered all medications used during the treatment of SBE patients including antivenom and was the second significant contributor to SBE treatment costs. Each vial of antivenom only costs around INR 700 to INR 1,000 but additional costs such as antibiotics, blood products, consumables and other relevant materials are key contributors to treatment costs. Since the government sets the price for antivenom in India, this helps to minimise the costs of antivenom to the patients. The laboratory investigation and the fees for clinicians including specialists were found to be the least significant contributors to the total treatment costs compared to hospital and pharmacy costs. In this study, nearly 95% of people did not have medical insurance and therefore, they had to pay the full treatment costs. However, a small number of people had medical insurance although it did not cover the full treatment costs, so they still had to contribute towards the total treatment costs. In some hospitals, small discretionary discounts were offered based on the financial situation of patients, however, this is not the most common option offered to all patients.

Among the different snakebites analysed in this study, Russell's viper bites appear to incur greater treatment costs compared to the other snakes analysed. The amount of venom injected and therefore, resulting complications following Russell's viper bites vary widely. In some patients, a small amount of tissue damage and coagulation abnormalities occur, however, in others, it results in extensive tissue damage which demands specialist interventions. Surgical procedures such as debridement, fasciotomy and amputation require operating theatres and consumables, which results in increased costs. For example, a simple debridement and fasciotomy may cost around INR 5000 to cover staff and operating theatre costs. However, amputation of a limb may increase this cost up to ten times (INR 50,000). Due to the impacts of Russell's viper venom on the coagulation system, the patients often need plasma or whole blood transfusions, and other related products [36]. Notably, the unknown category shown in this study is likely to include many bites from Russell's vipers as these are most frequently encountered. The bites from cobra and common krait often may need mechanical ventilation support for days to weeks [37]. While cobra patients may need ventilation only for a few days, common krait bite patients may need up to a week or longer [14,20]. The clinicians believe that at least 1 in 10 SBE patients needs ventilation support as part of their treatment. The ventilation charges vary widely from around INR 3500 to INR 20,000 every day based on the type of hospital and the time. This ultimately increases the total treatment costs for SBE patients. For example, a patient who arrives at a hospital promptly (within 30 minutes) after the bite with only prolonged coagulopathy without any other complications may cost around INR 20,000 to INR 25,000 to treat. However, a patient with local swelling, fasciotomy and 20 vials of antivenom is likely to cost around INR 35,000. Acute kidney injury necessitates dialysis which costs around INR 2000 to INR 5,000 (based on the hospital) per cycle, with patients often requiring 3 to 15 cycles over two to three weeks. Hospitals normally charge around INR 2000 to INR 10,000 per day for intensive care units, and the SBE (specifically Russell's viper) patients may need care in this unit for 3 to 8 days. Similarly, for wards, hospitals charge anything between INR 500 to INR 3000 based on the type of ward the patients choose. Generally, treating saw-scaled viper bites in Tamil Nadu costs less as it often requires less than the minimum 10 vials of antivenom for SBE in the rest of India and pain management for these bites is effective. The treatment for non-venomous snakebites costs around INR 1000 to INR 2000 as they

only necessitate monitoring of the patients for up to 24 hours and some basic measurement of blood parameters along with infection management when needed. The data from this study suggest that for any venomous snakebites, the delay in seeking treatment may exacerbate complications and further increase treatment costs. Therefore, SBE patients should seek immediate medical care after the bite to minimise the threat to life, complications, and subsequent treatment costs. This has also been reported in a previous study where longer hospital stays resulted in higher treatment costs [38].

Snakebite patients and their families and communities have different perspectives in seeking prompt hospital treatment. In some places, snakebite has been considered a fate, and therefore, they should not seek any treatment. For several individuals, the traditional treatment is the only solution for SBE although this significantly delays the hospital treatment [16,39,40]. Similarly, there are several other misconceptions about snakes and SBE treatments [41]. Notably, several people are intimidated to seek prompt medical care due to high treatment costs in private hospitals [10]. Therefore, more healthcare policies should be developed to tackle various issues associated with SBE treatment costs. For example, intense public awareness activities are required to educate people about the identification of locally available medically important venomous snakes and encourage patients to seek prompt care as arriving at the hospitals earlier will reduce the treatment costs [12,15,27,40,42]. The delay in seeking treatment is not only exacerbating envenomation effects and treatment costs, but it is also significantly increasing the long-term health consequences, which may ultimately result in further socioeconomic impacts [43]. Notably, the policies to provide free medical care in private healthcare settings or health insurance to fully cover the treatment for SBE will significantly encourage people to arrive at hospitals promptly after the bite. It is often inevitable to arrive at late night due to the nature of SBE (e.g., common krait bites at night times), and therefore, clinicians and hospitals should consider charging the same amount even for patients who are arriving at late night as SBE mainly affects poor rural communities. The use of intensive care units is also critical and indeed, some patients can be managed with only supportive measures such as ventilation support in such units even in the absence of antivenoms [23]. Therefore, the hospitals should consider reducing the charges for intensive care units for SBE. People prefer immediate access to emergency services, which are often difficult to reach late at night. Notably, there are numerous remote and tribal villages not just in India but also in other countries such as Brazil [44,45] and Kenya [39] without proper road facilities where people have to carry the SBE patients (as well as others) to the nearest facilities. Although there may not be an immediate solution for this issue, rural healthcare workers should be trained to empower them to provide appropriate first aid and administer a few vials of antivenom in the closest setting that is possible, before transporting the patients to distant hospitals [39,46,47]. Training for rural healthcare professionals is also critical for the early assessment of patients, ascertaining envenoming compared to the dry and non-venomous bites [48] and their timely referral to tertiary care settings [27,49]. Early intervention using appropriate adjunct therapy may also significantly reduce the envenomation effects and subsequent treatment costs [34]. Governments, non-governmental organisations and private hospitals could offer SBE first aid kits containing some key materials such as pressure bandages, gauze cloths, and painkillers to support the patients in such remote locations [46]. This study has noted that pharmacy costs are high, and this can be minimised if some leading pharmaceutical companies offer key medications such as antivenoms, antibiotics and blood products at a reduced cost exclusively for SBE patients. It is also important to ensure adequate antivenom production and efficient supply including in rural healthcare settings to allow easy access [17,50–52].

Due to the complex nature of this study, it has several limitations. Since the treatments in government hospitals are provided free of cost to patients, we did not collect the actual costs

incurred by the governments to treat SBE in these settings as this was outside of the scope of this work. However, it would have been helpful to analyse these data to determine the contributions of governments to tackle SBE. This study was conducted by collecting only direct treatment costs from 913 snakebite patients who were treated in 10 different private tertiary care hospitals within Tamil Nadu. Therefore, future studies with more patients and hospitals including the ones managed by the governments and charitable organisations are required to get a better understanding of the treatment costs for SBE across Tamil Nadu. Similar studies are also warranted in other states of India as the healthcare system may vary widely across the country. Moreover, we mainly analysed the data under four major categories due to the small sample size specifically for snakes other than Russell's viper. A larger sample size would allow further characterisation of the treatment costs, allowing for a more in-depth analysis to be performed on the specific factors associated with increased treatment costs. We also did not thoroughly analyse the number of antivenom vials administered in local healthcare centres prior to arriving at these tertiary care hospitals due to the poor memory of patients or the unavailability of these data in several cases. These data will add more to estimate the overall treatment costs for SBE. Therefore, data and costs presented in this study should only be considered as guidance for further studies and policy development as this may vary widely in different hospitals in India and other countries. Moreover, inflation, increasing costs of medications and equipment and availability of medications may ultimately play a role in the increased treatment costs for SBE. The indirect costs associated with SBE treatments were not analysed in this study, and this is another key factor that should be considered in future studies. This study did not discriminate between brands of antivenom used as national standards dictate the potency requirements across brands. Additionally, we did not attempt to analyse the social patterns associated with gender, age, or occupational differences in the presentation of different kinds of snakebites although these are areas of interest for epidemiological and educational purposes. The use of clinical symptoms by treating clinicians was mainly used to ascertain the snake species, and this could have been inaccurate in some cases. Notably, women may not have visited hospitals in a similar manner to men although there is no evidence to ascertain this notion. However, this is another key factor that should be addressed in future.

Conclusions

Overall, this study suggests that there are numerous factors associated with the treatment costs for SBE in private tertiary care hospitals. However, it appears that a vast majority (~80%) of SBE patients can be treated with around INR 100,000 or less. Further increases in costs are attributable to complications resulting in operative interventions, dialysis, prolonged ventilation support, extended hospitalisation, and medications. Major healthcare insurance providers could consider SBE as a disease of poverty and offer cheap insurance schemes to cover these people from unexpected SBE treatment costs. Members of the public should also consider taking out these insurance schemes by paying a small fee, protecting themselves from SBE treatment costs. Necessary awareness should be created for members of the public to emphasise the need for taking such medical insurance to access prompt care. Moreover, there are currently no strict regulations or guidelines for SBE treatment costs in private healthcare settings. This results in hospitals deciding on the treatment cost themselves and can often appear unjust to the patients. While patients struggle to survive SBE complications, the treatment costs often come as another major shock, leading to serious downstream socioeconomic impacts. The societal impacts arising from SBE-associated financial burden can be a contributor to causing a shift in the social status of patients and their families within their communities for generations to come. Therefore, the health authorities could investigate this system and try to balance

it. The prime minister's and chief minister's comprehensive insurance schemes in India could also be extended to more people and cover the entire treatment costs associated with SBE as it mostly affects poor rural farmers. India is a nation that largely relies on its agricultural and related workforce, and therefore, protecting these key workers from unexpected medical costs such as due to SBE is a priority. A key finding of the study is consistent with other studies in that antivenom is a significantly minor driver of costs while still being the only pharmacological preparation known to be effective in the treatment of snakebites [34,53]. The outcomes of this study may be useful to most if not all healthcare systems worldwide to improve the clinical management and accessible treatment for SBE. However, there may be several region- and country-specific issues that need to be considered while adapting these data for different regions, but these data will act as a basis for further discussions. In addition, this data will form the basis to support the policy recommendations within the countries and by the WHO to facilitate accessible treatments for SBE. Some of the key policy recommendations would be to provide health insurance and key medications free of cost or at a cheaper rate for all vulnerable communities. Together, these policies may aid in achieving the goals of the WHO to reduce SBE-induced deaths and disabilities by 50% in 2030 [34,53,54].

Acknowledgments

We would like to thank Professor Jose Maria Gutierrez for critically reviewing this article. We also thank Alain F. Zuur and Elena N. Ieno for providing guidance and support on data exploration, regression and gamma generalised linear models using R. We express our sincere gratitude to the staff who supported the data collection in various hospitals and staff members at Toxiven Biotech for collating these data for further analysis.

Author Contributions

Conceptualization: Anika Salim, Subramanian Senthilkumaran, Ponniah Thirumalaikolundusubramanian, Ketan Patel, Sakthivel Vaiyapuri.

Data curation: Anika Salim, Harry F. Williams, Rajendran Vaiyapuri, Subramanian Senthilkumaran, Ponniah Thirumalaikolundusubramanian, Sakthivel Vaiyapuri.

Formal analysis: Anika Salim, Jarred Williams, Samir Abdel Wahab, Tade Adeshokan, José R. Almeida, Harry F. Williams, Rajendran Vaiyapuri, M. Fazil Baksh, Sakthivel Vaiyapuri.

Funding acquisition: Ketan Patel, Sakthivel Vaiyapuri.

Investigation: Ponniah Thirumalaikolundusubramanian, Sakthivel Vaiyapuri.

Methodology: Anika Salim, Samir Abdel Wahab, Tade Adeshokan, Rajendran Vaiyapuri, Sakthivel Vaiyapuri.

Project administration: Harry F. Williams, Rajendran Vaiyapuri, Sakthivel Vaiyapuri.

Resources: Subramanian Senthilkumaran, M. Fazil Baksh, Sakthivel Vaiyapuri.

Software: Anika Salim, M. Fazil Baksh, Sakthivel Vaiyapuri.

Supervision: Sakthivel Vaiyapuri.

Validation: Anika Salim, Ponniah Thirumalaikolundusubramanian, Ketan Patel, M. Fazil Baksh, Matthew R. Lewin, Sakthivel Vaiyapuri.

Visualization: Anika Salim, José R. Almeida, Sakthivel Vaiyapuri.

Writing – original draft: Anika Salim, Jarred Williams, Samir Abdel Wahab, Tade Adeshokan, José R. Almeida, Harry F. Williams, Rajendran Vaiyapuri, Matthew R. Lewin, Sakthivel Vaiyapuri.

Writing – review & editing: Anika Salim, Jarred Williams, Matthew R. Lewin, Sakthivel Vaiyapuri.

References

1. Chippaux JP. Snakebite envenomation turns again into a neglected tropical disease! J Venom Anim Toxins Incl Trop Dis. 2017; 23:38. Epub 20170808. https://doi.org/10.1186/s40409-017-0127-6 PMID: 28804495
2. Gutierrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, Warrell DA. Snakebite envenoming. Nat Rev Dis Primers. 2017; 3:17063. Epub 20170914. https://doi.org/10.1038/nrdp.2017.63 PMID: 28905944
3. Williams HF, Layfield HJ, Vallance T, Patel K, Bicknell AB, Trim SA, et al. The Urgent Need to Develop Novel Strategies for the Diagnosis and Treatment of Snakebites. Toxins (Basel). 2019; 11(6). Epub 20190620. https://doi.org/10.3390/toxins11060363 PMID: 31226842
4. Harrison RA, Hargreaves A, Wagstaff SC, Faragher B, Lalloo DG. Snake envenoming: a disease of poverty. PLoS Negl Trop Dis. 2009; 3(12):e569. Epub 20091222. https://doi.org/10.1371/journal.pntd.0000569 PMID: 20027216
5. Alirol E, Sharma SK, Bawaskar HS, Kuch U, Chappuis F. Snake bite in South Asia: a review. PLoS Negl Trop Dis. 2010; 4(1):e603. Epub 20100126. https://doi.org/10.1371/journal.pntd.0000603 PMID: 20126271
6. Kasturiratne A, Wickremasinghe AR, de Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, et al. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Med. 2008; 5(11):e218. https://doi.org/10.1371/journal.pmed.0050218 PMID: 18986210
7. Longbottom J, Shearer FM, Devine M, Alcoba G, Chappuis F, Weiss DJ, et al. Vulnerability to snakebite envenoming: a global mapping of hotspots. Lancet. 2018; 392(10148):673–84. Epub 20180717. https://doi.org/10.1016/S0140-6736(18)31224-8 PMID: 30017551
8. Suraweera W, Warrell D, Whitaker R, Menon G, Rodrigues R, Fu SH, et al. Trends in snakebite deaths in India from 2000 to 2019 in a nationally representative mortality study. Elife. 2020;9. Epub 20200707. https://doi.org/10.7554/eLife.54076 PMID: 32633232
9. Collaborators GBDSE. Global mortality of snakebite envenoming between 1990 and 2019. Nat Commun. 2022; 13(1):6160. Epub 20221025. https://doi.org/10.1038/s41467-022-33627-9 PMID: 36284094
10. Vaiyapuri S, Vaiyapuri R, Ashokan R, Ramasamy K, Nattamaisundar K, Jeyaraj A, et al. Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India. PLoS One. 2013; 8(11):e80090. Epub 20131121. https://doi.org/10.1371/journal.pone.0080090 PMID: 24278244
11. Babo Martins S, Bolon I, Alcoba G, Ochoa C, Torgerson P, Sharma SK, et al. Assessment of the effect of snakebite on health and socioeconomic factors using a One Health perspective in the Terai region of Nepal: a cross-sectional study. The Lancet Global Health. 2022; 10(3):e409–e15. https://doi.org/10.1016/S2214-109X(21)00549-0 PMID: 35180422
12. Vaiyapuri S, Kadam P, Chandrasekharuni G, Oliveira IS, Senthilkumaran S, Salim A, et al. Multifaceted community health education programs as powerful tools to mitigate snakebite-induced deaths, disabilities, and socioeconomic burden. Toxicon X. 2023; 17:100147. Epub 20221226. https://doi.org/10.1016/j.toxcx.2022.100147 PMID: 36632238
13. Senji Laxme RR, Khochare S, de Souza HF, Ahuja B, Suranse V, Martin G, et al. Beyond the 'big four': Venom profiling of the medically important yet neglected Indian snakes reveals disturbing antivenom deficiencies. PLoS Negl Trop Dis. 2019; 13(12):e0007899. Epub 20191205. https://doi.org/10.1371/journal.pntd.0007899 PMID: 31805055
14. Chakma JK, Menon JC, Dhaliwal RS, Indian Council of Medical R. White paper on venomous snakebite in India. Indian J Med Res. 2020; 152(6):568–74. https://doi.org/10.4103/ijmr.IJMR_3377_20 PMID: 34145096
15. Samuel SP, Chinnaraju S, Williams HF, Pichamuthu E, Subharao M, Vaiyapuri M, et al. Venomous snakebites: Rapid action saves lives-A multifaceted community education programme increases awareness about snakes and snakebites among the rural population of Tamil Nadu, India. PLoS Negl

Trop Dis. 2020; 14(12):e0008911. Epub 20201231. https://doi.org/10.1371/journal.pntd.0008911 PMID: 33382715

16. Schioldann E, Mahmood MA, Kyaw MM, Halliday D, Thwin KT, Chit NN, et al. Why snakebite patients in Myanmar seek traditional healers despite availability of biomedical care at hospitals? Community perspectives on reasons. PLOS Neglected Tropical Diseases. 2018; 12(2):e0006299. https://doi.org/10.1371/journal.pntd.0006299 PMID: 29489824
17. Cristino JS, Salazar GM, Machado VA, Honorato E, Farias AS, Vissoci JRN, et al. A painful journey to antivenom: The therapeutic itinerary of snakebite patients in the Brazilian Amazon (The QUALISnake Study). PLOS Neglected Tropical Diseases. 2021; 15(3):e0009245. https://doi.org/10.1371/journal.pntd.0009245 PMID: 33661895
18. Williams HF, Vaiyapuri R, Gajjeraman P, Hutchinson G, Gibbins JM, Bicknell AB, et al. Challenges in diagnosing and treating snakebites in a rural population of Tamil Nadu, India: The views of clinicians. Toxicon. 2017; 130:44–6. Epub 20170224. https://doi.org/10.1016/j.toxicon.2017.02.025 PMID: 28238804
19. Russell JJ, Schoenbrunner A, Janis JE. Snake Bite Management: A Scoping Review of the Literature. Plast Reconstr Surg Glob Open. 2021; 9(4):e3506. Epub https://doi.org/10.1097/GOX.0000000000003506 PMID: 33936914.
20. Menon JC, Joseph JK, Jose MP, Dhananjaya BL, Oommen OV. Clinical Profile and Laboratory Parameters in 1051 Victims of Snakebite from a Single Centre in Kerala, South India. J Assoc Physicians India. 2016; 64(8):22–9. PMID: 27762105
21. Prasarnpun S, Walsh J, Awad SS, Harris JB. Envenoming bites by kraits: the biological basis of treatment-resistant neuromuscular paralysis. Brain. 2005; 128(Pt 12):2987–96. Epub 20050929. https://doi.org/10.1093/brain/awh642 PMID: 16195243
22. Punde DP. Management of snake-bite in rural Maharashtra: a 10-year experience. Natl Med J India. 2005; 18(2):71–5. PMID: 15981441
23. Lang HJ, Amito J, Dünser MW, Giera R, Towey R. Intensive-care management of snakebite victims in rural sub-Saharan Africa: An experience from Uganda. South Afr J Crit Care. 2020; 36(1). Epub 20200730. https://doi.org/10.7196/SAJCC.2020.v36i1.404 PMID: 37304251
24. Senthilkumaran S, Salim A, Almeida JR, Williams J, Vijayakumar P, Thirunavukarasu A, et al. The Effectiveness of Antibiotics in Managing Bacterial Infections on Bite Sites following Snakebite Envenomation. Toxins (Basel). 2023; 15(3). Epub 20230303. https://doi.org/10.3390/toxins15030190 PMID: 36977081
25. Brenes-Chacón H, Ulloa-Gutierrez R, Soriano-Fallas A, Camacho-Badilla K, Valverde-Muñoz K, Ávila-Agüero ML. Bacterial Infections Associated with Viperidae Snakebites in Children: A 14-Year Experience at the Hospital Nacional de Niños de Costa Rica(†). Am J Trop Med Hyg. 2019; 100(5):1227–9. https://doi.org/10.4269/ajtmh.18-1015 PMID: 30915952
26. Yeh H, Gao S-Y, Lin C-C. Wound Infections from Taiwan Cobra (Naja atra) Bites: Determining Bacteriology, Antibiotic Susceptibility, and the Use of Antibiotics-A Cobra BITE Study. Toxins. 2021; 13(3):183. https://doi.org/10.3390/toxins13030183 PMID: 33801318
27. Kadam P, Ainsworth S, Sirur FM, Patel DC, Kuruvilla JJ, Majumdar DB. Approaches for implementing society-led community interventions to mitigate snakebite envenoming burden: The SHE-India experience. PLOS Neglected Tropical Diseases. 2021; 15(2):e0009078. https://doi.org/10.1371/journal.pntd.0009078 PMID: 33630848
28. Magalhães SFV, Peixoto HM, de Almeida Gonçalves Sachett J, Oliveira SSAlves ECdos Santos Ibiapina HN, et al. Snakebite envenomation in the Brazilian Amazon: a cost-of-illness study. Transactions of The Royal Society of Tropical Medicine and Hygiene. 2020; 114(9):642–9. https://doi.org/10.1093/trstmh/traa005 PMID: 32239168
29. Zuur AF, Ieno EN. A protocol for conducting and presenting results of regression-type analyses. Methods in Ecology and Evolution. 2016; 7(6):636–45. https://doi.org/10.1111/2041-210X.12577.
30. Zuur AF, Ieno EN, Elphick CS. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution. 2010; 1(1):3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x.
31. Barnes K, Ngari C, Parkurito S, Wood L, Otundo D, Harrison R, et al. Delays, fears and training needs: Perspectives of health workers on clinical management of snakebite revealed by a qualitative study in Kitui County, Kenya. Toxicon: X. 2021; 11:100078. https://doi.org/10.1016/j.toxcx.2021.100078 PMID: 34401745
32. Fazal F, Saleem T, Ur Rehman ME, Haider T, Khalid AR, Tanveer U, et al. The rising cost of healthcare and its contribution to the worsening disease burden in developing countries. Ann Med Surg (Lond). 2022; 82:104683. Epub 20220915. https://doi.org/10.1016/j.amsu.2022.104683 PMID: 36148082
33. Franco MVS, Alexandre-Silva GM, Oliveira IS, Santos PL, Sandri EA, Cerni FA, et al. Physical and social consequences of snakebites in the Yanomami indigenous community, Brazil: Report of two

cases. Toxicon. 2022; 214:91–2. Epub 20220521. https://doi.org/10.1016/j.toxicon.2022.05.008 PMID: 35609827

34. Herzel BJ, Samuel SP, Bulfone TC, Raj CS, Lewin M, Kahn JG. Snakebite: An Exploratory Cost-Effectiveness Analysis of Adjunct Treatment Strategies. Am J Trop Med Hyg. 2018; 99(2):404–12. Epub 20180531. https://doi.org/10.4269/ajtmh.17-0922 PMID: 29869597

35. Kasthuri A. Challenges to Healthcare in India—The Five A's. Indian J Community Med. 2018; 43 (3):141–3. https://doi.org/10.4103/ijcm.IJCM_194_18 PMID: 30294075

36. Warrell DA. Snake venoms in science and clinical medicine. 1. Russell's viper: biology, venom and treatment of bites. Trans R Soc Trop Med Hyg. 1989; 83(6):732–40. https://doi.org/10.1016/0035-9203(89)90311-8 PMID: 2533418

37. Sehgal IS, Gandra RR, Dhooria S, Aggarwal AN, Prasad KT, Muthu V, et al. A randomised trial of adaptive support ventilation in patients with neuroparalytic snake envenomation. Br J Anaesth. 2022; 128(3): e232–e4. Epub 20220110. https://doi.org/10.1016/j.bja.2021.12.015 PMID: 35027167

38. Okumu M, Patel M, Bhogayata F, Ochola F, Olweny I, Onono J, et al. Management and cost of snakebite injuries at a teaching and referral hospital in Western Kenya [version 1; peer review: 2 approved, 2 approved with reservations]. F1000Research. 2019; 8(1588). https://doi.org/10.12688/f1000research.20268.1 PMID: 31824667

39. van Oirschot J, Ooms GI, Okemo DJ, Waldmann B, Reed T. An exploratory focus group study on experiences with snakebites: health-seeking behaviour and challenges in rural communities of Kenya. Transactions of The Royal Society of Tropical Medicine and Hygiene. 2021; 115(6):613–8. https://doi.org/10.1093/trstmh/trab059 PMID: 33836536

40. Chaaithanya IK, Abnave D, Bawaskar H, Pachalkar U, Tarukar S, Salvi N, et al. Perceptions, awareness on snakebite envenoming among the tribal community and health care providers of Dahanu block, Palghar District in Maharashtra, India. PLOS ONE. 2021; 16(8):e0255657. https://doi.org/10.1371/journal.pone.0255657 PMID: 34351997

41. Wood L, Ngari C, Parkurito S, Barnes K, Otundo D, Misiani DA, et al. "Then they prayed, they did nothing else, they just prayed for the boy and he was well": A qualitative investigation into the perceptions and behaviours surrounding snakebite and its management in rural communities of Kitui county, Kenya. PLOS Neglected Tropical Diseases. 2022; 16(7):e0010579. https://doi.org/10.1371/journal.pntd.0010579 PMID: 35793372

42. Abdullahi A, Yusuf N, Debella A, Eyeberu A, Deressa A, Bekele H, et al. Seasonal variation, treatment outcome, and its associated factors among the snakebite patients in Somali region, Ethiopia. Frontiers in Public Health. 2022;10. https://doi.org/10.3389/fpubh.2022.901414 PMID: 36276393

43. Kasturiratne A, Lalloo DG, Janaka de Silva H. Chronic health effects and cost of snakebite. Toxicon: X. 2021;9–10:100074. https://doi.org/10.1016/j.toxcx.2021.100074 PMID: 34355162

44. Murta F, Strand E, de Farias AS, Rocha F, Santos AC, Rondon EAT, et al. “Two Cultures in Favor of a Dying Patient”: Experiences of Health Care Professionals Providing Snakebite Care to Indigenous Peoples in the Brazilian Amazon. Toxins. 2023; 15(3):194. https://doi.org/10.3390/toxins15030194 PMID: 36977085

45. Maciel Salazar GK, Saturnino Cristino J, Vilhena Silva-Neto A, Seabra Farias A, Alcântara JA, Azevedo Machado V, et al. Snakebites in "Invisible Populations": A cross-sectional survey in riverine populations in the remote western Brazilian Amazon. PLOS Neglected Tropical Diseases. 2021; 15(9):e0009758. https://doi.org/10.1371/journal.pntd.0009758 PMID: 34499643

46. Pochanugool C, Wilde H, Bhanganada K, Chanhome L, Cox MJ, Chaiyabutr N, et al. Venomous Snakebite in Thailand II: Clinical Experience. Military Medicine. 1998; 163(5):318–23. https://doi.org/10.1093/milmed/163.5.318

47. Ooms GI, van Oirschot J, Waldmann B, von Bernus S, van den Ham HA, Mantel-Teeuwisse AK, et al. The Current State of Snakebite Care in Kenya, Uganda, and Zambia: Healthcare Workers' Perspectives and Knowledge, and Health Facilities' Treatment Capacity. The American Journal of Tropical Medicine and Hygiene. 2021; 104(2):774–82. https://doi.org/10.4269/ajtmh.20-1078 PMID: 33236717

48. Pucca MB, Knudsen C S. Oliveira I, Rimbault C, A. Cerni F, Wen FH, et al. Current Knowledge on Snake Dry Bites. Toxins. 2020; 12(11):668. https://doi.org/10.3390/toxins12110668 PMID: 33105644

49. Chaudhari TS, Patil TB, Paithankar MM, Gulhane RV, Patil MB. Predictors of mortality in patients of poisonous snake bite: Experience from a tertiary care hospital in Central India. Int J Crit Illn Inj Sci. 2014; 4 (2):101–7. https://doi.org/10.4103/2229-5151.134145 PMID: 25024937

50. Nduwayezu R, Kinney H, Amuguni JH, Schurer JM. Snakebite Envenomation in Rwanda: Patient Demographics, Medical Care, and Antivenom Availability in the Formal Healthcare Sector. The American Journal of Tropical Medicine and Hygiene. 2021; 104(1):316–22. https://doi.org/10.4269/ajtmh.20-0976 PMID: 33146107

51. Monteiro WM, Farias ASd, Val F, Neto AVS, Sachett A, Lacerda M, et al. Providing Antivenom Treatment Access to All Brazilian Amazon Indigenous Areas: 'Every Life has Equal Value'. Toxins. 2020; 12 (12):772. https://doi.org/10.3390/toxins12120772 PMID: 33291444

52. Patikorn C, Ismail AK, Zainal Abidin SA, Othman I, Chaiyakunapruk N, Taychakhoonavudh S. Potential economic and clinical implications of improving access to snake antivenom in five ASEAN countries: A cost-effectiveness analysis. PLOS Neglected Tropical Diseases. 2022; 16(11):e0010915. https://doi.org/10.1371/journal.pntd.0010915 PMID: 36383562

53. Hamza M, Idris MA, Maiyaki MB, Lamorde M, Chippaux JP, Warrell DA, et al. Cost-Effectiveness of Antivenoms for Snakebite Envenoming in 16 Countries in West Africa. PLoS Negl Trop Dis. 2016; 10 (3):e0004568. Epub 20160330. https://doi.org/10.1371/journal.pntd.0004568 PMID: 27027633

54. Williams DJ, Faiz MA, Abela-Ridder B, Ainsworth S, Bulfone TC, Nickerson AD, et al. Strategy for a globally coordinated response to a priority neglected tropical disease: Snakebite envenoming. PLoS Negl Trop Dis. 2019; 13(2):e0007059. Epub 20190221. https://doi.org/10.1371/journal.pntd.0007059 PMID: 30789906

Chapter 5

Determining the knowledge gaps and training requirements amongst Indian healthcare professionals for the clinical management of snakebite envenoming. Salim, A., Williams, H. F., & Vaiyapuri, S. (2023).

The rationale for this study

A variety of healthcare professionals from remote healthcare workers to clinicians attend to snakebite patients across the care pathway and each one plays a vital role in ensuring the patient survives. To reduce the snakebite disability and fatality burden, adequately trained healthcare professionals are essential and integral to the healthcare system. Currently in India, snakebite clinical management education is not mandated in the healthcare curriculum and medical education is varied across the country. This study was set out to determine the baseline knowledge of all clinical professionals across India when it comes to treating snakebites to investigate where further work may be needed to improve training for key professionals. We received 480 responses from across India and highlighted how the majority of healthcare professionals have not received adequate training on attending to snakebite patients nor are the majority confident in treating patients. This study aims to help facilitate the development of targeted training and resources as well as serve to highlight the need for embedding SBE management training for all healthcare professionals.

My contribution to this chapter (60%)

This study was conceptualised by Sakthivel Vaiyapuri and myself, whereby past historical surveys on clinical snakebite management in India among clinical professionals were reviewed and analysed. A pilot study and questionnaire were developed at the University of Reading and validated by our collaborating teams across India. I then programmed and launched the final study survey as well as disseminated the survey across our collaborating network.

I organised, cleaned and analysed the raw data at the University of Reading. The statistical analysis plan was developed with Sakthivel Vaiyapuri which included exploratory data modelling. This involved reviewing the various healthcare professional, grouping their roles, their length of service and correct responses to the survey. The data was categorised according to binary variables as well as ordinal variables and appropriate statistical modelling of the data was conducted. I completed the visualisation, programming, and preparation of all study figures and tables. Manuscript preparation, writing, editing, and review were performed by Anika Salim and Sakthivel Vaiyapuri.

Determining the knowledge gaps and training requirements amongst Indian healthcare professionals for the clinical management of snakebite envenoming

Abstract

Snakebite envenoming (SBE) is a high-priority neglected tropical disease that affects mostly impoverished communities in many tropical and subtropical countries. Delays in seeking hospital treatment are the primary cause of snakebite-induced deaths and disabilities. Rural healthcare professionals are not fully trained to understand nor confident enough to clinically manage the SBE victims and as a result, they refer patients to distant tertiary healthcare facilities. This exacerbates the complications exhibited and becomes a bigger challenge to treat as well as costly to the patient. To improve health outcomes following SBE, it is important to better understand the challenges faced by healthcare professionals in providing treatment for SBE victims. Here, we designed an online survey for all Indian healthcare professionals to determine the current state of their knowledge of clinical management of SBE and their training requirements. This survey was disseminated to healthcare professionals in India through various methods. We received 480 responses from 28 out of the 36 Indian states. The data demonstrates that many healthcare professionals did not receive adequate education and training on SBE during their university education and workplace training. Over half of them are not confident in managing SBE patients. Most healthcare professionals stated that they require training in various elements of SBE management, and if they receive such training, they will feel confident in treating SBE victims including in rural healthcare settings. The outcomes of this study will facilitate the development of targeted training materials to address knowledge gaps and training needs to empower healthcare professionals and reduce the SBE burden in rural India.

Introduction

Snakebite envenoming (SBE) is a high-priority neglected tropical disease that affects around 5.4 million people each year worldwide, resulting in approximately 140,000 deaths and 500,000 permanent disabilities. SBE disproportionately affects those in rural, agricultural communities. India has the highest rate of SBE-related deaths in the world and as a result, it accounts for nearly half of the global burden of snakebite deaths (~58,000 deaths every year) [1] [2] [3] [4]. India is home to over 300 species of snakes, of which 60 are venomous. However, the 'big four' snakes; Russell's viper (*Daboia russelii*), Indian cobra (*Naja naja*), common krait (*Bungarus caeruleus*), and saw-scaled viper (*Echis carinatus*) are considered to cause most of these deaths and disabilities [5, 6]. Specifically, Russell's viper causes around 60-70% of incidents and resulting deaths and disabilities in the country. The World Health Organisation's global strategy for prevention and control of SBE aims to halve the number of deaths and disabilities by 2030 [7]. One of their key objectives is to ensure patients have access to effective antivenom treatment and prompt medical care. Currently, the only standardised materials available in India (along with other countries) are the WHO Guidelines for the Clinical Management of Snakebites in the Southeast Asia Region and various regional guidelines at the state level. In 2022, the Government of India declared the launch of a national programme for the prevention and control of SBE [8]. Unfortunately, there are many challenges that both healthcare professionals and patients face in India for SBE treatment. One principal component is the availability of adequately trained healthcare professionals to provide prompt treatment for SBE victims in rural healthcare settings. Currently, there is no standard or government-mandated medical education, training or continuing medical education that ensures SBE management is acquired as a core skill for healthcare providers across India.

Moreover, medical education and training in India varies across states and union territories, with government and private institutions and colleges.

As a result, SBE victims may interact with many professionals along their route depending on their treatment-seeking behaviour, from traditional healers, and rural healthcare workers to government primary healthcare centres to tertiary care hospitals. The skills necessary for effective treatment of SBE amongst these professionals may vary widely. The lack of adequately trained and informed healthcare professionals protracts and compounds the SBE prognosis to patients. Due to the lack of diagnostic kits, healthcare professionals in India must rely on envenomation signs and symptoms along with clinical tests to manage the patients. The growing access to the internet globally and rapid growth in the use of mobile communication devices particularly smartphones has opened a new world of opportunities for use in healthcare, otherwise known as 'mHealth' (mobile health). The use of mHealth can aid in delivering medical care and is becoming popular in underserved areas and driven in the developing world with the expansion of telecom coverage. The use of mobile applications, artificial intelligence software and websites can aid in snake identification, but the use of these by healthcare professionals in effectively treating SBE along with efficacy has not been evaluated. To promote such advances in healthcare, it is critical to understand the knowledge gaps and training requirements for healthcare professionals in India. Hence, to determine the knowledge gaps and training requirements of healthcare professionals for the clinical management of SBE, we devised an online survey for all healthcare professionals from varying sectors including doctors, surgeons, nurses, pharmacists, and community healthcare workers across the whole of India. This study explored for the first time, the SBE practice amongst all sectors of healthcare management across India and identified concerns, knowledge gaps, areas of improvement and where training is needed for each sector. The data demonstrated various gaps in SBE knowledge and training needs for healthcare professionals to improve the clinical management of SBE in India, specifically in rural settings.

Results

Study population

We received 480 responses from a range of healthcare professionals across India, working in 28 out of the 36 Indian states and union territories. The highest responses were received from Tamil Nadu 270 (56.3%), Maharashtra 36 (7.5%), Karnataka 32 (6.7%) and Kerala 31 (6.5%). We received no responses from 8 states, mainly union territories (**Figure 1A**). Of the 480 participants, 243 (50.6%) were males and 230 (47.9%) were females with only 7 participants (1.5%) preferring not to mention their gender. The number of males and females was not significantly different indicating the gender balance of this study (**Figure 1B**). The age range of participants varied with the greatest number of responses, 189 (39.4%) were from the age range 26-35 years, followed by 18-25 years 126 (26.3%) and 36-45 years 103 (21.5%) correlating with working-age groups (**Figure 1C**). 212 (44.2%) participants were considered as doctors, 36 (7.5%) nurses, 28 (5.8%) medical officers, 27 (5.6%) technicians, 25 (5.2%) pharmacists and 152 (31.7%) stated 'other' which consisted of roles such as researchers, research associates, professors, and radiologists (**Figure 1D**). The range of educational qualifications among the participants varied, 131 (27.3%) participants selected 'other' as their highest qualification which consisted of PhD's, 117 (24.4%) with MD, 72 (15%) MBBS, 57 (12%) MSc specialised courses, 26 (5.4%) BSc specialised courses, 21 (4.4%) BSc Nursing, 13 (2.7%) with M. Pharm, 8 (1.7%) with B. Pharm, 7 (1.5%) with an MSc in Nursing and 3 (0.6%) with D. Pharm as their highest educational qualification.

140 (29.2%) participants had been in their current profession for over 10 years, 93 (19.4%) had been in between 5-10 years, 37 (7.7%) 4 years, 36 (7.5%) 3 years, 63 (13.1%) 2 years, 31 (6.5%) 1 year and 80 (16.7%) less than one year (**Figure 1E**). The length of time in their respected profession varied

significantly across the different professional groups ($\chi 2$ = 23.5, df=5, *p*= <0.001). 283 (59%) stated they work in private healthcare, 166 (34.6%) work in government healthcare facilities, 15 (3.1%) in other organisations including those freelancing and studying and 8 (1.7%) were working at an NGO.

233 (48.5%) participants had access to first aid facilities, 166 (34.6%) had ambulance service, 154 (32%) had pharmacy services, 153 (31.9%) had emergency care facilities, 136 (28.3%) had clinical laboratory diagnostics, 130 (27%) had intensive care units, 116 (24.2%) surgical facilities, 115 (24%) had X-Ray facilities, 112 (23.3%) had ventilation support, 107 (22.3%) had ultrasound, 103 (21.5%) had CT, 102 (21.3%) had physiotherapy, 102 (21.3%) had counselling services, 97 (20.2%) had haemodialysis access, 96 (20%) had orthopaedics; 95 (20%) had paediatrics, 95 (20%) had MRI, 92 (19.2%) had antivenom, and 87 (18.1%) had rehabilitation services (**Figure 1F**).

Snakebite awareness varies between professions

To determine the general awareness of snakebites among healthcare professionals, they were asked several questions relating to their awareness. 397 (82.7%) participants were aware of snakebites, 36 (7.5%) were not aware and 44 (9.1%) were not sure about snakebites within their geographical areas. The awareness of snakebites among the participants across professions varied significantly ($\chi 2$ = 33.3, df=5, p= <0.001). Pharmacists and the 'other' profession groups were less likely to be aware of snakebites. Our analysis also highlighted that there is no significant difference in snakebite knowledge across all the healthcare professionals when length of tenure was factored in, this means a doctor with >10 years' experience does not perform better or worse than a junior doctor with < 1 years' experience. Moreover, 214 (44.6%) participants were aware of venomous snakes in their area but 55 (11.5%) were not sure and 34 (7.1%) were not aware at all. 158 (33%) were aware of non-venomous snake species with 60 (12.5%) not aware and 89 (18.5%) not sure. The general knowledge about snakes among study participants varied significantly between the professions [awareness of venomous snakes ($\chi 2$ =25.3, df=5, p= <0.001) and awareness of non-venomous snakes ($\chi 2$ =16.9, df=5, p= 0.005)]. The pharmacists and other professional groups were least likely to be aware of venomous snakes. Nurses were most aware of non-venomous snakes.

Only a limited number of healthcare professionals were able to identify the 'big four' snakes

The 'big four' are the four most important venomous snakes responsible for most snakebites in India. Participants were asked if they had heard of the 'big four' to test their knowledge on this specific aspect. Only those who had heard of the 'big four' were allowed to participate to see if they could correctly name them as well as identify and distinguish between the specific snake species via a series of photographs. Participants who had not heard of the 'big four' were routed to progress to further sectors of the survey and so were prevented from accessing these questions.

Out of our 480 healthcare professionals, 295 (61.5%) heard of the 'big four' snakes. Technicians and other professions were least likely to have heard of the 'big four', with only around half of these groups having heard of them [technicians 14 (52%) and other 74 (49%)]. This contrasted with around 70% of the other professions [doctors 144 (69%), nurses 26 (72%) medical officers 20 (71%), pharmacists 17 (71%). These participants were asked to confirm if they could name them. 293 (99% of 295) were able to name the Indian cobra, 263 (89.1%) for Russell's viper, 255 (86.4%) for the common krait and 229 (77.6%) for the saw-scaled viper. The ability to name the 'big four' snakes varied significantly across the professions, Indian cobra ($\chi 2$ = 22.6, df=5, p= <0.001); saw-scaled viper ($\chi 2$ = 21.3, df=5, p= <0.001); Russell's viper ($\chi 2$ = 22.9, df=5, p= <0.001) and common krait ($\chi 2$ = 26.4, df=5, p= <0.001). Nurses were more likely to correctly name the 'big four' snakes, whereas technicians and 'other' professions were the least likely to name these snakes. Other snakes that were mistaken for the 'big four' included the Malabar pit viper (*Trimeresurus malabaricus*) [35 (11.9%)], black mamba

(*Dendroaspis polylepis*) [33, (11.2%)], hump-nosed pit viper (*Hypnale hypnale*) [22 (7.5%)], Indian wolf snake (*Lycodon aulicus*) [19 (6.4%)], rat snake (*Ptyas mucosa*) [15 (5.1%)], eastern coral snake (*Micrurus fulvius*) [8 (2.7%)], and the eastern brown snake (*Pseudonaja textilis*) [4 (1.4%)].

Only 138 (46.8%) of 295 participants who heard about 'big four' snakes, could correctly identify the common krait, 269 (91.2%) could correctly identify the cobra, 165 (55.9%) could correctly identify Russell's viper and 107 (36.3%) could correctly identify the saw-scaled viper. There was no significant difference between professions for identifying the common krait and Russell's viper. However, differences were observed for the other snakes [Indian cobra, ($\chi2 = 11.6$, df=5, p=0.04); and saw-scaled viper ($\chi2 = 15.2$, df=5, p= 0.01)]. For snakes where there were differences, nurses generally had the highest level of identification ability, particularly for the rat snake, saw-scaled viper, and Indian wolf snake, where their identification was much better than all other professions **(Table 1)**.

Environmental and ecological preventative measures

Participants were then asked to rate how useful certain measures were to prevent snakebites as well as test their understanding of how the times of day and seasons can affect snakebite incidence. 298 (62%) participants agreed that keeping a sanitary local environment was useful along with 153 (31.9%) sleeping under a mosquito net, 309 (64.4%) wearing shoes/boots, 254 (52.9%) sleeping on a raised bed, 303 (63.1%) good outside lighting, using a torch at night 306 (63.8%), 309 (64.4%) keeping doors closed, 162 (33.8%) feeding pets outside, 232 (48.3%) using screens on doors and windows, 272 (56.7%) keeping grass short and 360 (75%) blocking cavities in housing/sheds/walls/roofs to prevent from snakebites. Most of these measures did not vary significantly between the professions. However, differences were observed for sleeping under a mosquito net ($\chi2 = 17.8$, df=5, p= 0.003) and good outside lighting ($\chi2 = 14.1$, df=5, p= 0.02). For mosquito nets, around half of nurses 18 (51%) indicated this useful, along with 62 (43%) of 'other' professions, with higher values than for any other professions. Doctors, nurses, and other professionals were most likely to indicate that good outside lighting was useful **(Figure 2)**. When asked if participants were aware that mice and rats attract snakes, 381 (79.4%) agreed that they were. 423 (88.1%) agreed correctly that snakebites are most common during the monsoon and post-monsoon seasons. 251 (52.3%) stated that most bites occur at night, 167 (34.8%) stated anytime, 129 (26.9%) stated evening, 49 (10.2%) stated morning and 32 (6.7%) afternoons.

Snakebite first aid

Participants were asked a series of questions on whether they thought all snakes were venomous, knew the differences between a dry bite and envenomation and if they were aware of basic diagnostic criteria and first aid techniques to be used in the primary instance of snakebite. 431 (89.8%) stated that not all snakes are venomous, and 31 (6.5%) said that believed they are all venomous. 310 (64.6%) did not believe envenoming occurs in every bite, 49 (10.2%) stated yes and 114 (23.8%) were unsure. Moreover, only 238 (49.6%) participants were able to correctly identify the correct definition of a dry bite, as a bite by a venomous animal in which no venom is released. 128 (26.7%) believed that the sighting, picture, and capture, killing of a snake was mandatory for a snakebite diagnosis, and 283 (59%) did not believe this was the case. 275 (57.3%) of our participants believed that fang mark identification was mandatory for a snakebite diagnosis, and 137 (28.5%) stated this was not the case. When asked about the pressure immobilisation technique 352 (73.3%) were aware of this first-aid technique. When asked what first aid techniques are useful in the event of a snakebite 325 (67.7%) participants stated immobilisation as a useful first aid, 243 (50.6%) tight bands (tourniquets), 159 (33.1%) splint application, 81 (16.9%) sucking the venom out of the bite wound, 66 (13.8%) incisions, 61 (12.7%) calcium carbonate application to the bite wound, 54 (11.3%) plant extract/secretion application to bite wound, 23 (4.8%) stated plant extract secretion oral intake, 11 (2.3%) calcium

carbonate oral intake and 8 (1.7%) suggested walking and moving would be appropriate. All measures relating to snakebite first aid and management education varied significantly across professions. Doctors, nurses, and medical officers were most likely to be aware of pressure mobilisation and splint application (over 85% of all these groups), compared to other professions ($\chi2 = 60$, df=5, p= <0.001). 424 (88.3%) participants agreed a snakebite patient should be transported to the hospital as soon as possible after the bite.

Training and education for snakebite management

The participants were asked to self-classify how they felt about their level of knowledge on snakebite management. 44 (9.2%) stated it was excellent, 122 (25.4%) stated it was good, 124 (25.8%) stated it was fair, 139 (29%) participants stated it was limited, 42 (8.8%) stated it was poor and 9 (1.9%) said none. Their knowledge of snakebite management varied significantly across different professional groups ($\chi2 = 43.8$, df=5, p=<0.001). The knowledge of SBE was highest for nurses with 23 (63.9% of total nurses) suggesting excellent or good. Only 86 (40.6% of doctors) doctors, 10 (37%) technicians, 10 (35.7%) medical officers, 8 (32%) pharmacists and 29 (19%) for 'other' professions said that their level of knowledge is excellent or good. 179 (37.3%) participants received their training at university, 98 (20.4%) as part of their continuing medical education, 58 (12.1%) during their workplace introductory training, 19 (4.0%) at school and 6 (1.3%) stated elsewhere including conferences and by case study discussions. Most [91 (19.0%)] participants received training between 1 to 5 hours, 62 (12.9%) less than 1 hour, 49 (10.2%) stated it was a few days, 28 (5.8%) stated 6 - 10 hours, 13 (2.7%) over 20 hours and 9 (1.9%) stated 11-20 hours. When asked if they would consider themselves confident in managing and treating snakebite patients, 159 (33.1%) stated yes, 55 (11.5%) said no and 79 (16.5%) stated they were not sure. Notably, 297 (61.9%) participants stated that they needed additional training to manage snakebites. The professions did not vary in terms of their need for training, with a high proportion of all professions suggesting that they needed training. When asked what type of snakebite management training was needed, most [240 (50%)] participants stated first aid, 171 (35.6%) for clinical management, 121 (25.2%) for specialised treatment, 105 (21.9%) for theoretical knowledge, and 64 (13.3%) stated surgical procedures. Overall, 282 (94.9% of those who needed training) participants would be happy to treat snakebite patients if they were provided training. The professions did not vary in terms of the need for training for surgical procedures, clinical management, and others. Pharmacists and other professions were most likely to indicate that they needed specialised training (**Table 2**).

Snakebite management at the workplace

Participants were asked if they treated snakebites and if yes, select signs and symptoms along with other effects associated with Indian snakes. A few non-Indian snakes were also included as a quality control measure to identify participants who may not be fully engaged. Questions on best clinical management practices were asked along with which tests they would use to diagnose a snakebite. 206 (42.9%) stated that they treated snakebites. 50 (24.3%) of them had seen 300+ patients, 7 (3.4%) had seen 200+ patients, 11 (5.3%) had seen 100+ patients, 15 (7.3%) had seen 51-100 patients, 20 (9.7%) had seen 26-50 patients and 94 (45.6%) had seen between 1-25 snakebite patients in their career so far. The six professional groups varied significantly for whether they treated snakebites ($\chi2 = 114.6$, df=5, p= <0.001), number of snakebite cases treated across their whole career ($\chi2 = 39.7$, df=5, p= <0.001) and number of snakebite patients treated in the last year ($\chi2 = 35.3$, df=5, p= <0.001). Doctors [129 (61%)], nurses [25 (69%)] and medical officers [20 (71%)] were treating snakebites at work. When asked which of the 'big four' snakes cause the most bites in their region, 134 (65.0%) stated the Indian cobra, 114 (55.3%) stated Russell's viper, 110 (53.4%) stated the common krait and 71 (34.5%) stated

the saw-scaled viper. Other snakes included the rat snake (*Ptyas mucosa*), Indian wolf snake (*Lycodon aulicus*), hump-nosed pit viper (*Hypnale hypnale*), Malabar pit viper (*Trimeresurus malabaricus*).

When asked on average how often they attend to snakebite patients at their place of work, 24 (11.7%) stated every few hours, 34 (16.5%) stated daily, 27 (13.1%) stated every week and 48 (23.3%) stated every month. When asked the average length of time after the bites that patients take to arrive at the hospitals, 40 (19.4%) stated less than one hour, 83 (40.3%) stated between 1-5 hours, 21 (10.2%) said 6-10 hours, 14 (6.8%) stated 11-24 hours, 7 (3.4%) said 25-48 hours, 3 (1.5%) 49-72 hours and 5 (2.4%) +72 hours. The ideal bite-to-treatment time was stated as less than one hour by 172 (83.5%) participants, 1-5 hours by 11 (5.3%), 11-24 hours by 3 (1.5%), 48 hours by 4 (1.9%) and 72 hours by 3 (1.5%). They were asked what parts of the body they had witnessed snakebites on, in most cases 190 (92.2%) stated it was the lower limbs, 104 (50.5%) stated the upper limbs, 41 (19.9%) stated the torso, 17 (8.3%) said head and 14 (6.8%) claimed neck. 137 (66.5%) of these participants were aware of the WHO Guidelines for the 'Clinical Management of Snakebites in the Southeast Asia Region'. When asked if they referred to the WHO Guidelines during their practice, 159 (77.2%) stated they did. When asked to provide reasons for why they did not refer to the guidelines, participants varied in their responses, some were not aware of them, unable to access them or had their hospital guidelines. We also asked participants if they felt the guidelines needed to be revised, and many of them said yes to developing more specific guidelines for Indian states by covering more than just the 'big four' snakes and providing more detailed clinical complications and management protocols.

For the ability to identify Russell's viper bite-induced symptoms, statistically significant differences between professions were found for swelling ($\chi2 = 21.5$, df=5, p= 0.001), blistering ($\chi2 = 21.6$, df=5, p=0.001), bleeding gums ($\chi2 =14.8$, df=5, p= 0.01) and dark coloured urine ($\chi2 = 15.4$, df=5, p=0.009). This means that doctors are more likely to be able to correctly identify Russell's viper bite compared to others. Similar results were observed for the saw-scaled viper. For Indian cobra, the identification abilities varied significantly between groups for ptosis ($\chi2 = 32.5$, df=5, p= <0.001), unconsciousness ($\chi2 =14.6$, df=5, p= 0.01), swallowing difficulties ($\chi2 = 13.8$, df=5, p= 0.02), paralysis of the body ($\chi2 = 14.5$, df=5, p=0.01) and respiratory paralysis ($\chi2 = 31.8$, df=5, p= <0.001). Here, doctors and medical officers typically had the best identification rates. For Krait, none of the signs/symptoms for this snake varied among the professions, which illustrates that healthcare professionals are unable to identify some of the key signs and symptoms of this snake. There was no significant difference between professions in being able to identify the krait as neurotoxic, but there was significance in them being able to identify the cobra as neurotoxic ($\chi2 = 27.4$, df=5, p= <0.001). There was a significant difference in professions identifying Russell's viper as haemotoxic ($\chi2 = 23.5$, df=5, p= <0.001), yet there was no significance when looking at the haemotoxic effects of the saw-scaled viper.

171 (83%) participants heard of the 20 Minute Whole Blood Clotting Test (20WBCT) test and 161 (78.2%) knew how to perform this test. When asked to clarify which of the tests they would conduct for snakebite patients, 145 (70.4%) said 20WBCT, 104 (50.5%) mentioned INR, 96 (46.6%) PT, 93 (45.1%) ptosis test, 84 (40.8%) blood count, 79 (38.3%) aPTT, 75 (36.4%) Blood urea nitrogen (BUN) test, 67 (32.5%) haemoglobin test, 47 (22.8%) blood glucose test, 45 (21.8%) toxicology tests, 41 (19.9%) liver enzyme analysis, 23 (11.2%) biopsy and 13 (6.3%) stated others including ECG, breath holding tests, D-dimer test and a fibrinogen test. The doctors were most likely to use 20WBCT, ptosis test and aPTT compared to others.

Knowledge about antivenom and its administration

In total, 191 participants who knew about antivenom were allowed to continue answering questions relating to antivenom usage. Most 108 (56.5%) participants stated they use lyophilised antivenom products, 99 (51.1%) used liquid antivenom, and others chose various answers which are not correct but we included them as distractors; 4 (2.1%) said that they used tablets, 4 (2.1%) capsules, 3 (1.6%) said implants and patches, 2 (1.0%) inhaler, 2 (1.0%) drops and 1 (0.5%) stated suppositories. 170 (89%) of participants strongly believe that envenomation symptoms could be cured by antivenom. 172 (90.1%) agreed that the priority for the patient is to receive antivenom as soon as possible to neutralise the venom. 158 (82.7%) participants administered antivenom themselves. 159 (83.2%) stated they had a suitable supply of antivenom. Participants were asked in how many cases they have administered or attended to patients who were treated with antivenom. 48 (25.1%) 300+ patients, 8 (4.2%) 200+ patients, 8 (4.2%) stated +100 patients, 7 (3.7%) stated 51-100 patients, 21 (11.0%) stated 21-50 patients, 21 (11.0%) said 11-20 patients and 68 (35.6%) said 1-10 patients. The professional groups varied significantly for the level of knowledge about antivenom ($\chi2 = 57.9$, df=5, p= <0.001) and the number of patients treated with antivenom ($\chi2 = 41.8$, df=5, p= <0.001). Nurses and pharmacists had the highest number of patients treated with antivenom. We also asked how many vials of antivenom you use on average for patients bitten by the 'big four' snakes. 40 (20.9%) highlighted 6-10 vials for Russell's viper. 49 (25.7%) said 6-10 vials for krait. 53 (27.7%) said 6-10 vials for saw-scaled viper. 44 (23.0%) said 6-10 vials for Indian cobra. 155 (81.2%) participants stated that IV infusion is the most appropriate route for administering antivenom. However, 55 (28.8%) stated slow push IV injection, 11 (5.8%) stated IM injection, 5 (2.6%) stated topically and 1 (0.5%) stated oral administration. Most [151 (79.0%)] participants stated that they use visible neurological signs e.g., ptosis as a sign for administering antivenom, but 148 (77.5%) stated uncoagulable blood determined by 20WBCT, 131 (68.6%) said systemic bleeding, 115 (60.2%) said evidence of descending paralysis, 107 (56.0%) ophthalmoplegia, 98 (51.3%) haemoptysis, 82 (42.9%) patient unconsciousness and 21 (11.0%) stated patient request. The uncoagulable blood ($\chi2 = 84.3$, df=5, p= <0.001), visible neurological signs ($\chi2 = 30.2$, df=5, p= <0.001), and descending paralysis ($\chi2 = 22.6$, df=5, p= <0.001) were proven to be significant criteria for administering antivenom by these participants **(Table 3)**. We then tested the knowledge about antivenom-induced adverse reactions. Most [144 (75.4%)] participants confirmed that they induce allergic reactions sometimes, 17 (8.9%) stated always, 19 (9.9%) stated they were not sure and 4 (2.2%) stated never. 120 (62.8%) participants witnessed antivenom-induced allergic reactions during their practice. When asked how many cases of allergic reactions to antivenom they had witnessed in their career thus far, 74 (38.7%) stated between 1-5, 18 (9.4%) 6-10, 11 (5.8%) 11-20, 7 (3.7%) 21-30, 4 (2.1%) 31-40, 2 (1.0%) 41 to 50 and 15 (7.9%) +50. Most [149 (78.0%)] participants stated itching as an adverse reaction along with others including rash [135 (70.7%)], urticaria [133 (69.6%)], anaphylactic shock [111 (58.1%)], fever [68 (35.6%)], headaches [57 (29.8%)], cough [37 (19.4%)] and brown urine [34 (17.8%)]. When it comes to the differences between professions regarding information on adverse reactions to antivenom, these were not statistically significant for most of the criteria analysed. However, significant differences were observed for urticaria ($\chi2 = 31.8$, df=5, p= <0.001) and anaphylactic shock ($\chi2 = 42.7$, df=5, p= <0.001). Doctors were the most likely group to identify both adverse reactions. We also wanted to enquire how are potential adverse reactions to antivenom managed. 97 (50.8%) stated adrenaline administration, 79 (41.4%) stated continue antivenom with a corticosteroid, 74 (38.7%) stated stop antivenom use, 28 (14.7%) said to continue with antivenom and 8 (4.8%) were not sure. The options of continuing antivenom with corticosteroids and adrenaline varied significantly between professions. Doctors were most likely to recommend both strategies, with medical officers giving similar responses to doctors for corticosteroids.

Discussion

SBE is a major public health burden in India. However, there is no robust national-level reporting and surveillance system to monitor SBE incidence rates and subsequent deaths and disabilities. Since access to medical treatment is possible in most regions provided by the governments, there is scope to prevent SBE-induced deaths and disabilities through timely and appropriate intervention. Despite this, the clinical management in rural areas is poor and the SBE burden prevails due to a lack of essential knowledge on SBE and efficient first aid skills [13]. Often most patients receive poor and inefficient basic first aid only to report later to health facilities with advanced symptoms and consequently have worse health outcomes [9]. This is compounded by the fact that clinical management in secondary and tertiary hospitals varies especially in India as it has no officially set curriculum or workplace training for effective clinical management of SBE [14]. Therefore, it is critical to determine the knowledge gaps and training requirements for healthcare professionals and develop appropriate materials/facilities for them to provide better treatment for SBE. A few surveys have been carried out in the past across the whole of India for the clinical management of SBE. However, these surveys focussed almost exclusively on clinicians and were not robust enough to cover the whole country or a state [11], [12]. Therefore, in this study, we deployed a unique survey by targeting all healthcare professionals from varying backgrounds in India and captured a comprehensive snapshot of their knowledge and understanding of SBE management. This was key to identifying areas of greatest need, focus and attention for regional improvements but also for designing targeted training to ensure patients are given the most appropriate treatment to reduce the rate of SBE-induced deaths, disabilities, and socioeconomic impacts.

We had equal representation from both genders with most participants considering themselves as doctors/surgeons/specialists. The length of time in the profession did not indicate any better SBE education or knowledge. Most of our participants also had access to basic first aid facilities, with just under half of them working at facilities that have intensive care units at their place of work and ventilation support available for patients. Most of our participants were aware of snakebites occurring in their work locality but they were not aware of the varying venomous and non-venomous snakebites. Despite most of the professionals being aware of the term 'big four' snakes, they were unable to correctly name/identify them from pictures. It is important to note that the use of 'big four' snakes has been called into question, as there is a chance that patients presenting at hospitals could have been bitten by other medically important venomous snakes in India [6]. However, we believe that this should be emphasised in education and workplace training. It was clear from the data that the healthcare professionals were unable to identify key environmental protective measures against snakebites. This shows that even at the professional level communities in India, remain unaware of the associations between their environment and the threat of snakebites. Healthcare professionals must convey the importance of snakes to the ecosystem and their dangers to patients with aftercare measures to avoid being re-bitten and to empower them to make changes in their environment for their families and communities. Therefore, more community-level developments and social work are needed to educate them about snakes and their significance [15]. Healthcare professionals should also be educated about this importance as they are first responders to snakebites.

Immediate and appropriate first aid is essential to preventing SBE-induced complications. Our results highlighted that despite healthcare professionals being aware that patients must access prompt care immediately following snakebites, they did not know effective first aid measures for patients. For example, many healthcare professionals believe that tourniquet application is an effective first aid measure. Most SBE patients arrive at healthcare facilities within the critical window of between 1-4 hours, with the limiting factor preventing access being transportation and delays in transport logistics

to the hospital. There are currently no standardised diagnostics tests on the market that are effective at detecting the offending snake in India. Therefore, the ability to identify key clinical symptoms is important in the treatment of patients. The doctors were mostly able to identify these for snakes such as Russell's viper, cobra, and saw-scaled viper, but not for krait. Most healthcare professionals were unable to correctly categorise the key envenomation effects such as the neurotoxic and haemotoxic effects as well as neuromuscular paralysis. This links to their difficulty in being able to designate appropriate clinical tests. Due to the complexity of venom toxins, SBE patients have the potential of presenting with multiple biological effects and so doctors perform a battery of tests to combat and alleviate these symptoms. Our data show that our healthcare professionals found it difficult to correctly identify which tests are best suitable for SBE patients. Even though most healthcare professionals who treat snakebites are aware of antivenom and its ability to treat SBE, the identification criteria to determine its administration was lacking along with the management of potential anaphylactic reactions. This can lead to further medical complications as well as increasing treatment costs for patients and their families [9] further inducing socioeconomic effects [10].

It is clear from our data that healthcare professionals surveyed in India rate their knowledge of snakebites as limited and most agreed they would be confident in treating patients if training was provided. Notably, nurses play a critical role in delivering SBE treatment as they seem to have the most exposure to patients and play a key role in providing them with treatment. It would be beneficial to consider specific SBE training for nurses along with other health care professionals. The top areas of concern are basic first-aid measures, clinical management, and specialised treatments. Empowering healthcare professionals ensures better patient safety and survival outcomes. Education, training and continued professional development in this field are much needed and should be supported from all levels from policy down to the grassroots. Our survey highlights areas of concern but also the need for more robust and pragmatic teaching approaches that encompass a holistic approach that can benefit patients in the long term as well as prevent future re-occurrence. Healthcare professionals especially in endemic areas such as India should also strive to educate their patients on first aid measures, so they can do so themselves and raise greater prevention awareness in the communities [16] [13] [10]. Medical education relating to SBE management has been a persistent issue for over 20 years with very few initiatives ever to address it appropriately [17]. SBE treatment across various regions in India is highly variable and effective treatment protocols and issues regarding antivenom efficacy prevail in rural settings. Providing patients with the best possible care is a key objective for any healthcare system, and this in turn is very much dependent on the people who work within these systems. Healthcare workers in SBE endemic regions must be aware of the basic first aid requirements and work collaboratively with their local population by providing appropriate environmental and ecological recommendations where needed. The ability to provide first aid by any healthcare professional whether it be a community healthcare worker or local nurse has great significance for SBE patients. Adequate treatment response can make the difference between life, disability, or death (WHO, 2020).

Limitations of the survey

Although this study provides current insights into the knowledge base of healthcare professionals across India, a few limitations should be noted. This research was performed based on the data collected via an online platform and it required participant reflection. As a result, some might have over or underestimated or even inferred responses. We appreciate that the participants were voluntarily derived from online respondents and may not be representative of the full spectrum of healthcare professionals across India, allowing for possible selection bias in our results. The sampling strategy employed a social media marketing approach whereby all platforms were utilised where possible to disseminate the survey to as many professionals as possible. We have seen a higher rate of response

from the states in which we have collaborative networks, resulting in an unequal distribution and were unable to control if multiple responses were received from the same individuals as the survey was anonymous and did not capture personal data or IP addresses.

To conclude, the SBE burden in India does not correspond with the educational resources allocated to strengthening the healthcare system. Standardised SBE management training should be part of the Indian medical curriculum and given to all healthcare professionals as well as part of continuing professional development. The results of our survey provide a better understanding of the key areas in need of focus, such as providing adequate first aid courses as well as suitable materials for clinicians to be able to recognise the signs and symptoms of snakebites. A comprehensive and targeted education with ongoing professional development is needed to aid the reduction of associated SBE-induced disabilities and fatalities.

Methods

Survey Design

The survey was designed using Joint Information Systems Committee (JISC) online surveys referencing the WHO Guidelines for the clinical management of snakebites in the Southeast Asia Region (WHO, 2016) in addition to consultation with our collaborators in snakebite research and clinicians at TCR Multispeciality Hospital, Manian Medical Centre and Trichy SRM Medical College Hospital and Research Centre (Tamil Nadu, India). The survey was tested internally and validated externally by a small number (n=10) of clinicians who regularly treat SBE and later those who did not (n=42). Based on their collective feedback, the survey was refined and launched. The survey flow was customised to allow continuation, branching, blocking, and termination based on the answers of the participants. Thus, each section of the survey was designed carefully considering the varying levels of participant experience within the survey and ensured certain criteria were met before proceeding. A given question could be shown or hidden based on the response to another question within the same section to avoid any bias in their answers. The accessibility of the survey on multiple devices including Android, iPhone and electronic tablets was thoroughly tested to ensure maximum participation.

There were nine sections in the survey which presented a range of questions in the following critical areas: 1) socio-demographic questions identifying, gender, age, and occupation details. 2) general knowledge – reviewing their general awareness of snakes and snakebites at their place of work. 3) Knowledge of snake species – only those who were aware of the 'big four' were asked to identify the snakes further in a series of photographs. 4) Environmental and ecological preventive measures – to determine their understanding of such measures that could be used to prevent snakebites. 5) Appropriate first aid for snakebites – evaluating their awareness of basic first aid measures and techniques. 6) Snakebite management education & training – only those who studied snakebite management as part of their education were asked to provide further insights into the training/knowledge they received. Only those who stated they would like further training were asked what forms of training they needed. 7) Interaction with snakebite victims – only those who selected that they treated snakebites were permitted to proceed further with specific questions on clinical management. The others were taken to the final page to close the survey. 8) Clinical management and investigation of snakebites – a deeper dive was conducted into clinical management best practices with specific questions on signs and symptoms of snakebites as well as clinical effects of the various medically important snake species. 9) Knowledge of antivenom and its administration – only those who confirmed they knew what antivenom was could continue further.

Data Collection

This prospective study was conducted from August 2020 and March 2021, and we aimed to recruit at least 500 varying healthcare professionals across all Indian states and union territories with representation from each state. The survey was self-administered and open to all healthcare professionals from any field with adequate English knowledge, over the age of 18, residing and working in India. There were no other inclusion or exclusion criteria. Participants were not compensated as participation was voluntary and unchallenging as they were being asked to reflect on their snakebite knowledge. The respondents were recruited through the Internet and social media platforms. To ensure participation from a variety of demographic, socioeconomic and educational backgrounds invitations to the survey were posted on various forums. Participants were also encouraged to share the survey via their specific professional networks such as the Indian Medical Association. Participants could not save the survey and come back to it at a later stage to avoid duplication and potential bias from reviewing snakebite materials.

Ethical considerations

The survey was hosted on the Jisc Online Survey platform, ensuring GDPR compliance and certified to ISO 27001 standards. This study was approved by the Institutional Ethical Review Committees at Toxiven Biotech Private Limited, Tamil Nadu (reference number: 001/2019) and the University of Reading (reference: UREC 23/05). The study was performed in line with the guidelines provided by the Indian Council for Medical Research and the Declaration of Helsinki. Before participation, a brief introduction to the purpose and aims of the survey were provided and participants were asked to confirm consent if they were willing to proceed. All responses were anonymous, and the demographic details of all respondents were preserved. No personal or identifiable information was collected. There were no ethical issues or risks to participants because of taking part.

Patient and public involvement statement

This study was aimed at healthcare professionals in India so patients and lay members of the public were not directly involved in the study design and data collection. The outcomes of this study however can prompt and inform better research and care for snakebite patients, as we can develop a collaborative partnership amongst healthcare professionals and their wider communities via scientific publications, which might be followed by press releases in media in the local language and English.

Statistical Methods

The healthcare professionals were categorised into six groups for analysis; 1) Doctors which included surgeon/specialist consultants, 2) Nurses which included any registered midwives and community healthcare workers, 3) Medical officers who were separated into a category as they specifically work in rural areas, 4) Medical technicians to include medical assistant, pharmacy technicians, hospital technicians, physician's assistant, and any other assistant healthcare worker. 5) Pharmacists and 6) 'others' to include all other categories such as physiotherapists. All analyses were compared between the different professions. All questionnaire responses were categorical. Binary variables and questions without a natural ordering were compared between professions using the Chi-square test. Ordinal variables were compared between groups using the Kruskal-Wallis test. Moreover, for each outcome two sets of analyses were performed, an unadjusted comparison, not accounting for experience and an adjusted comparison, allowing for experience in the profession. Here, binary outcomes were analysed using logistic regression, whilst ordinal variables were compared between groups using ordinal logistic regression. Several regression models were fitted to the data collected with each regression model using different sets of independent variables. For some outcomes, all responses within a single profession had the same outcome. In this instance, it is not mathematically possible to

properly perform the regression analyses, and so no additional analysis was performed for these outcomes. The results generally show little difference between the unadjusted and adjusted results, this suggests that although the professions vary in terms of their experience, this does not impact on the difference in outcomes between professions. As such in this paper we will only focus on the unadjusted results. All statistical analyses were performed using the R (Version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria) [18], Stata Statistical Software (Version 11.2, StataCorp, College Station, Texas) [19], and GraphPad Prism (Version 7, GraphPad Software, San Diego, CA, USA) [20].

Acknowledgements

We would like to thank Professor Wolfgang Wüster, Steve Spawls and Peter Uetz at the Reptile Database for their advice and permission to use the snake pictures in this study. We express our sincere gratitude to all the healthcare professionals, collaborators, and Priyanka Kadam from SHE India who supported this study by participating in and disseminating the survey.

Figures

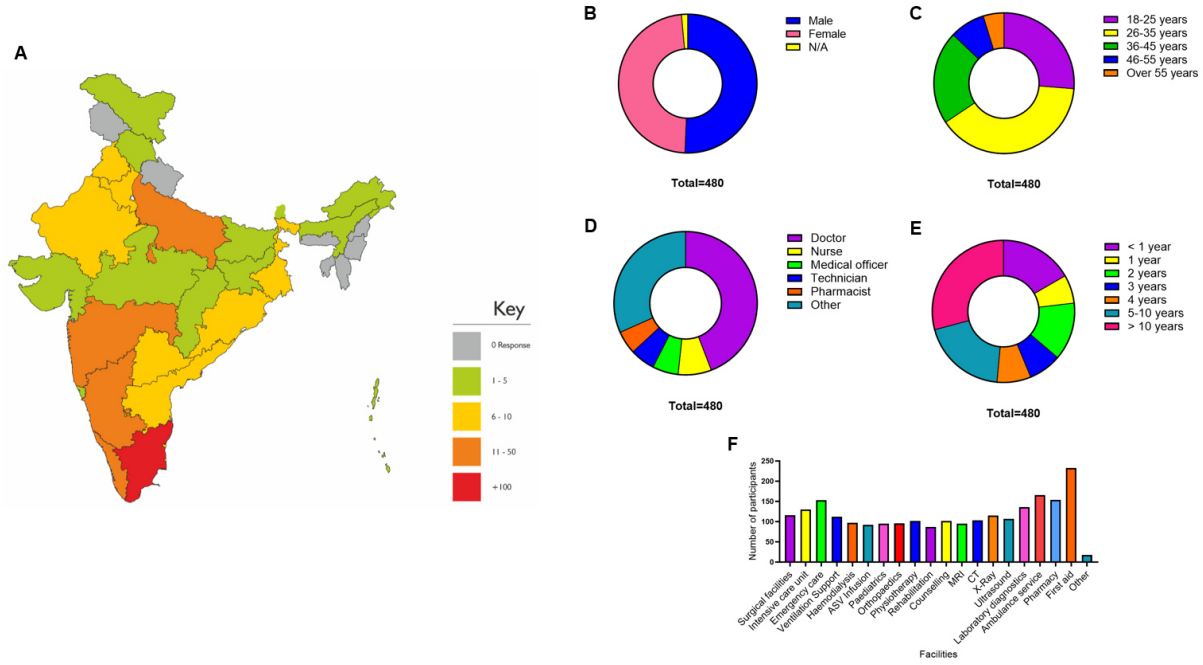


Figure 1: A) Geographical map of Indian states showing the response rate to the survey. States that provided the greatest numbers of responses are coloured red, whilst the least are green and then grey for no response received. Demographic characteristics of healthcare professionals included in this study (B) the gender and (C) age groups of participants. D) The distribution of healthcare professionals from each sector and (E) the number of years of experience in their stated profession. F) The varying facilities available to healthcare professionals at their place of work.

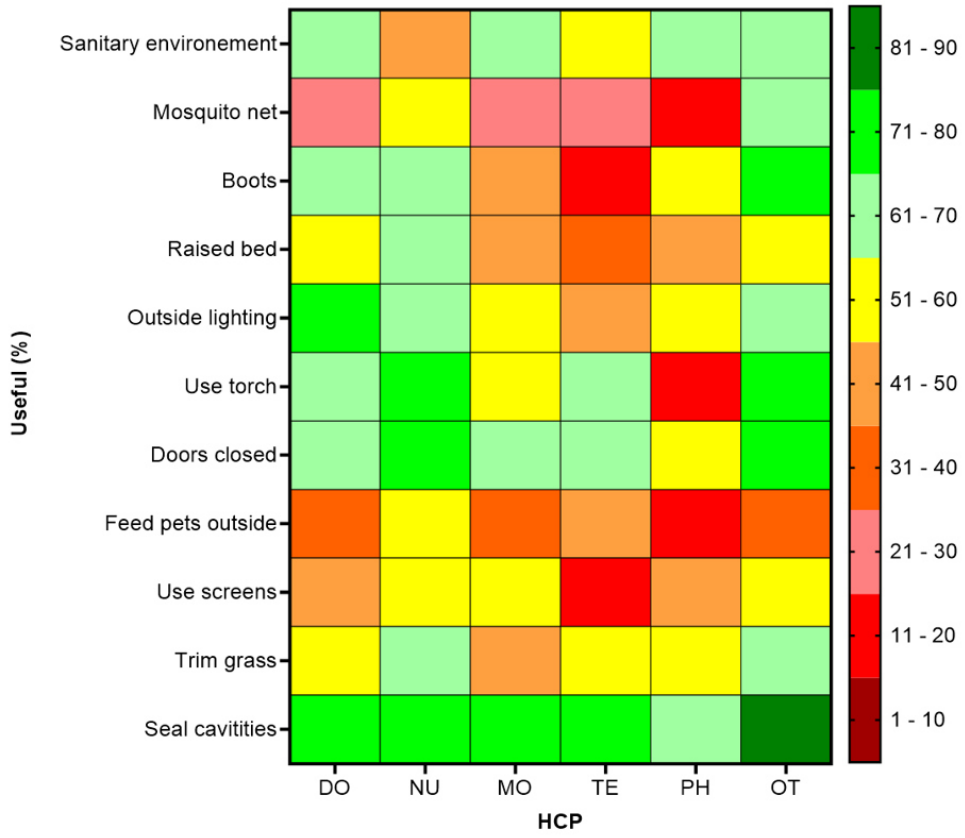


Figure 2: Useful ecological and environmental measures to prevent SBE noted by healthcare professionals. The scales shown in the figure indicate the level of usefulness for each environmental measure as determined by each of the healthcare professional groups. DO- doctors, NU- nurses, MO- medical officers, TE-technicians, PH-pharmacists, and OT-others. The red and green squares indicate the percentage of professionals that considered each measure useful.

Table 1: Association between healthcare professionals and their ability to name and identify the 'big four'.

Question	Category	Doctors n (%)	Nurses n (%)	Med. Offic. n (%)	Technicians n (%)	Pharmacists n (%)	Other n (%)	χ^2 (*)	P-value
Heard of 'big four'	Yes	144 (69%)	26 (72%)	20 (71%)	14 (52%)	17 (71%)	74 (49%)	19.3	**0.002**
	No	66 (31%)	10 (28)	8 (29%)	13 (48%)	7 (29%)	77 (51%)		
Name 'big four' (#)	Cobra	145 (69%)	26 (72%)	19 (68%)	14 (52%)	18 (72%)	71 (47%)	22.6	**<0.001**
	Saw-scaled viper	115 (54%)	23 (64%)	16 (57%)	11 (41%)	13 (52%)	51 (34%)	21.3	**<0.001**
	Russell's viper	134 (63%)	24 (67%)	18 (64%)	13 (48%)	13 (52%)	61 (40%)	22.9	**<0.001**
	Krait	135 (64%)	22 (61%)	17 (61%)	12 (44%)	11 (44%)	58 (38%)	26.4	**<0.001**
Identify krait	No	73 (53%)	8 (31%)	12 (60%)	9 (64%)	9 (50%)	39 (53%)	6.2	0.28
	Yes	64 (47%)	18 (69%)	8 (40%)	5 (36%)	9 (50%)	34 (47%)		
Identify cobra	No	8 (6%)	2 (8%)	4 (21%)	2 (14%)	4 (22%)	4 (5%)	11.6	**0.04**
	Yes	135 (94%)	24 (92%)	15 (79%)	12 (86%)	14 (78%)	69 (95%)		
Identify rat snake	No	98 (77%)	8 (32%)	16 (89%)	9 (64%)	13 (72%)	42 (59%)	26.2	**<0.001**
	Yes	29 (23%)	17 (68%)	2 (11%)	5 (36%)	5 (28%)	29 (41%)		

Identify Russell's viper	No	58 (42%)	8 (31%)	10 (50%)	10 (71%)	7 (41%)	31 (40%)	6.7	0.24
	Yes	81 (58%)	18 (69%)	10 (50%)	4 (29%)	10 (59%)	42 (58%)		
Identify saw-scaled viper	No	84 (65%)	7 (28%)	13 (68%)	11 (79%)	12 (71%)	43 (60%)	15.2	**0.01**
	Yes	46 (35%)	18 (72%)	6 (32%)	3 (21%)	5 (29%)	29 (40%)		
Identify Indian wolf snake	No	95 (81%)	4 (16%)	12 (71%)	10 (71%)	13 (72%)	55 (76%)	43.4	**<0.001**
	Yes	23 (19%)	21 (84%)	5 (29%)	4 (29%)	5 (28%)	17 (24%)		

Analysis using Chi-square test
(#) Separate analyses for each snake
(*) Chi-square values with 5 degrees of freedom

Table 2: The association between healthcare professionals and their need for snakebite management training.

Question	Category	Doctors n (%)	Nurses n (%)	Med. Offic. n (%)	Technicians n (%)	Pharmacists n (%)	Other n (%)	$\chi^{2\,(*)}$	P-value
Surgical procedures	Not required	191 (90%)	33 (92%)	24 (86%)	24 (89%)	22 (88%)	122 (80%)	8.5	0.13
	Required	21 (10%)	3 (8%)	4 (14%)	3 (11%)	3 (12%)	30 (20%)		
Clinical Management	Not required	139 (66%)	24 (67%)	19 (68%)	14 (52%)	17 (68%)	96 (63%)	2.4	0.78
	Required	73 (34%)	12 (33%)	9 (32%)	13 (48%)	8 (32%)	56 (37%)		
Specialised Treatment	Not required	192 (91%)	29 (81%)	20 (71%)	21 (78%)	16 (64%)	101 (66%)	12.6	**0.03**
	Required	20 (9%)	7 (19%)	8 (29%)	6 (22%)	9 (36%)	51 (34%)		
First aid	Not required	146 (69%)	24 (67%)	20 (71%)	12 (44%)	9 (36%)	29 (19%)	99.8	**<0.001**
	Required	66 (31%)	12 (33%)	8 (29%)	15 (56%)	16 (64%)	123 (81%)		
Theoretical	Not required	193 (91%)	34 (94%)	22 (79%)	18 (67%)	17 (68%)	91 (60%)	59.5	**<0.001**
	Required	19 (9%)	2 (6%)	6 (21%)	9 (33%)	8 (32%)	61 (40%)		
Other training	Not required	211 (99.5%)	36 (100%)	28 (100%)	27 (100%)	25 (100%)	151 (99%)	-	1.00
	Required	1 (0.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)		

Analysis using Chi-square test, except for 'other training where Fisher's exact test was used. (*) Chi-square values with 5 degrees of freedom

Table 3: Association between healthcare professionals and the criteria for administering ASV.

Question	Category	Doctors n (%)	Nurses n (%)	Med. Offic. n (%)	Technicians n (%)	Pharmacists n (%)	Other n (%)	$\chi^{2\,(*)}$	P-value
Uncoagulable blood	No	3 (3%)	17 (71%)	3 (16%)	1 (14%)	4 (80%)	3 (50%)	84.3	**<0.001**
	Yes	115 (97%)	7 (29%)	16 (84%)	6 (86%)	1 (20%)	3 (50%)		
Visible neurological signs	No	7 (6%)	7 (29%)	5 (26%)	4 (57%)	2 (40%)	3 (50%)	30.2	**<0.001**
	Yes	111 (94%)	17 (71%)	14 (74%)	3 (43%)	3 (60%)	3 (50%)		
Descending paralysis	No	30 (25%)	10 (42%)	10 (53%)	6 (86%)	4 (80%)	4 (67%)	22.6	**<0.001**
	Yes	88 (75%)	14 (58%)	9 (47%)	1 (14%)	1 (20%)	2 (33%)		
Systemic bleeding	No	21 (18%)	10 (42%)	7 (37%)	4 (57%)	3 (60%)	3 (50%)	16.3	**0.006**
	Yes	97 (82%)	14 (58%)	12 (63%)	3 (43%)	2 (40%)	3 (50%)		

Analysis using Chi-square test. (*) Chi-square values with 5 degrees of freedom

References

1. Chippaux, J.-P., A. Massougbodji, and A.G. Habib, *The WHO strategy for prevention and control of snakebite envenoming: a sub-Saharan Africa plan.* JOURNAL OF VENOMOUS ANIMALS AND TOXINS INCLUDING TROPICAL DISEASES, 2019. **25**.
2. Kasturiratne A, W.A., de Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, Savioli L, Lalloo DG, de Silva HJ, *The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths.* PLoS Med, 2008. **5**(11): p. e218.
3. JP, C., *Snake-bites: appraisal of the global situation.* Bull World Health Organ, 1998. **76**(5): p. 515-24.
4. Mohapatra, B., et al., *Snakebite mortality in India: a nationally representative mortality survey.* PLoS Negl Trop Dis, 2011. **5**(4): p. e1018.
5. Kochar DK, T.P., Norris RL, Sabir M, Nayak KC, Agrawal TD, Purohit VP, Kochar A, Simpson ID., *Rediscovery of severe saw-scaled viper (Echis sochureki) envenoming in the Thar desert region of Rajasthan, India.* Wilderness Environ Med, 2007. **18**(2): p. 75-85.
6. Ian D. Simpson, R.L.N., *Snakes of Medical Importance in India: Is the Concept of the "Big 4" Still Relevant and Useful?* Wilderness & Environmental Medicine, 2007. **18**(1): p. 2-9.
7. WHO. *WHO launches global strategy for prevention and control of snakebite envenoming*. Snakebite envenoming, A strategy for prevention and control, Executive Summary 2019 22 October 2023]; Available from: https://www.who.int/news/item/23-05-2019-who-launches-global-strategy-for-prevention-and-control-of-snanebite-envenoming.
8. Mandaviya, M., *Dr. Mansukh Mandaviya Chairs 7th Meeting of Mission Steering Group for NHM*, P.I. Bureau, Editor. 2022.
9. Anika Salim, J.W., Samir Abdel Wahab,Tade Adeshokan,José R. Almeida,Harry F. Williams,Rajendran Vaiyapuri,Subramanian Senthilkumaran,Ponniah Thirumalaikolundusubramanian,Ketan Patel,M. Fazil Baksh,Matthew R. Lewin,Sakthivel Vaiyapuri, *Identifying key factors contributing to treatment costs for snakebite envenoming in private tertiary healthcare settings in Tamil Nadu, India.* PLoS Negl Trop Dis, 2023. **17**(10): p. e0011699.
10. Vaiyapuri, S., et al., *Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India.* PLoS One, 2013. **8**(11): p. e80090.
11. Fung HT, L.S., Lam KK, Kam CW, Simpson ID, *A survey of snakebite management knowledge amongst select physicians in Hong Kong and the implications for snakebite training.* Wilderness Environ Med., 2009. **20**(4): p. 364-70.
12. Simpson, I.D., *A study of the current knowledge base in treating snake bite amongst doctors in the high-risk countries of India and Pakistan: does snake bite treatment training reflect local requirements?* Trans R Soc Trop Med Hyg, 2008. **102**(11): p. 1108-14.
13. Williams, H.F., et al., *Challenges in diagnosing and treating snakebites in a rural population of Tamil Nadu, India: The views of clinicians.* Toxicon, 2017. **130**: p. 44-46.
14. Gajbhiye, R.K., H. Munshi, and H.S. Bawaskar, *National programme for prevention & control of snakebite in India: Key challenges & recommendations.* Indian J Med Res, 2023. **157**(4): p. 271-275.
15. Martin, G., et al., *Implications of global environmental change for the burden of snakebite.* Toxicon X, 2021. **9-10**: p. 100069.
16. Samuel, S.P., et al., *Venomous snakebites: Rapid action saves lives-A multifaceted community education programme increases awareness about snakes and snakebites among the rural population of Tamil Nadu, India.* PLoS Negl Trop Dis, 2020. **14**(12): p. e0008911.
17. Theakston, R.D., D.A. Warrell, and E. Griffiths, *Report of a WHO workshop on the standardization and control of antivenoms.* Toxicon, 2003. **41**(5): p. 541-57.
18. *R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing*. 2020 Version 4.1.2].
19. StataCorp. *Stata Statistical Software*.
20. Prism, G. *GraphPad Software*. Version 7.

Theme 2: SBE mitigation and public awareness

Theme 2: SBE mitigation and public awareness

The human-snake conflict is becoming increasingly important to mitigate the risk of SBE. Public awareness on the prevention of neglected tropical diseases such as SBE is limited due to the lack of importance of the disease along with the lack of funding and political will to invest in such campaigns. Public health awareness campaigns are incredibly important if we wish to reduce the unnecessary high burden of disability and deaths due to SBE. By raising awareness, we encourage communities to ask questions, dispel myths, eliminate inaccurate information, and ensure patients can access the correct information, seek the right care, and even change behaviour that is beneficial to their health. Access to education, especially health education in resource-limited communities is challenging for local populations. Intervention programmes must take into consideration adults with poor literacy rates along with children who may not have access to educational resources. We aimed to create a range of engaging materials and tools to aid increased learning goals across our target populations to mitigate SBE risk and empower communities. The materials produced were designed thoughtfully to ensure they were culturally acceptable across a range of diverse communities and that the central messaging was conveyed to adults and children alike. The Government of Tamil Nadu, India has since endorsed the use of these materials, which aids efforts in popularising any awareness campaigns being conducted. Snake rescuers put their own lives at risk to save lives in their communities and play an underappreciated but vital role in reptile conservation. Our study revealed that appropriate training and accreditation are needed to avoid stigmatisation as well as eliminate unnecessary injury and deaths. Understanding these challenges faced by snake rescuers helps frame conversations around the development of public policy in snake conservation and SBE prevention. Appropriately trained rescuers can work with their local communities to impart vital knowledge that can help mitigate further snakebite deaths. Another facet of this conflict is the mental health burden an SBE event can have on patients. We aimed to understand the complex nature of our relationships with snakes and how this can serve to educate healthcare professionals on the trauma many patients may face post-bite. Our work on snake phobia highlights that males are unusually more snake-phobic compared to females. This facilitates greater conversations around mental healthcare and treatment encompassing the psychological ramifications of an SBE event to improve patient outcomes to prevent any long-lasting trauma. Our work has highlighted that a holistic one-health approach is needed to tackle the issue of SBE from the ground up and to consider each key stakeholder from awareness amongst rural communities and snake rescuers to healthcare providers and local government and the part they can all play to prevent SBE events.

Chapter 6

Multifaceted community health education programs as powerful tools to mitigate snakebite-induced deaths, disabilities, and socioeconomic burdens. Sakthivel Vaiyapuri, Priyanka Kadam, Gnaneswar Chandrasekharuni, Isadora S Oliveira, Subramanian Senthilkumaran, Anika Salim, Ketan Patel, Jacqueline de Almeida Gonçalves Sachett, Manuela B Pucca. (2023). Toxicon: X, 17, 100147.

The rationale for this study

Public awareness of SBE is widely lacking and to date, no government-sponsored SBE public health campaign has been officially launched in any country to combat the high levels of death and disability. Raising awareness of how to prevent SBE and providing endemic communities with the knowledge and tools about how to access safe care and provide first aid is important to prevent unnecessary treatment delays, complications and avoidable deaths. Intervention programmes must be designed with their respective community in mind and the impact of such programmes must also be tracked so that the assessment of their success and implementation can be measured.

This study aimed to investigate health education programmes across Latin America, principally Brazil and India and how they serve to prevent SBE. Utilising diverse methods for wide reach in local languages and using pictures for people with low literacy levels has shown that we can prevent SBE. Following government endorsement of our materials in Tamil Nadu, India, we have demonstrated that improving awareness is critical to mitigating the SBE burden.

My contribution to this chapter (30%)

All authors contributed equally to the conceptualisation of this manuscript along with the manuscript preparation, writing, editing, and review. I completed the visualisation, design and preparation of the snakebite prevention posters.

Toxicon:X 17 (2023) 100147

Contents lists available at ScienceDirect

Toxicon: X

journal homepage: www.journals.elsevier.com/toxicon-x

Multifaceted community health education programs as powerful tools to mitigate snakebite-induced deaths, disabilities, and socioeconomic burden

Sakthivel Vaiyapuri [a,*], Priyanka Kadam [b], Gnaneswar Chandrasekharuni [c], Isadora S. Oliveira [d], Subramanian Senthilkumaran [e], Anika Salim [a], Ketan Patel [f], Jacqueline de Almeida Gonçalves Sachett [g], Manuela B. Pucca [h]

[a] School of Pharmacy, University of Reading, Reading, RG6 6UB, UK
[b] Snakebite Healing & Education Society, Mumbai, India
[c] Madras Crocodile Bank Trust, Mamallapuram, Tamil Nadu, India
[d] Department of Biomolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
[e] Manian Medical Centre, Erode, Tamil Nadu, India
[f] School of Biological Sciences, University of Reading, Reading, RG6 6UB, UK
[g] Nurse School, State University of Amazonas, Manaus, Amazonas, Brazil
[h] Medical School, Federal University of Roraima, Boa Vista, Brazil

ARTICLE INFO

Handling Editor: Ray Norton

Keywords:
Snakebite envenoming
Community health education
Socioeconomic impact
Public awareness
Rural communities
Healthcare professionals
health policies

ABSTRACT

Snakebite envenoming (SBE) predominantly affects rural impoverished communities that have limited access to immediate healthcare. These communities often hold numerous myths/misbeliefs about snakes and SBE. Moreover, healthcare professionals who practice in rural regions often work in unstable situations with limited medical infrastructure and therefore, lack sufficient knowledge/experience and confidence in the clinical management of SBE. Due to the lack of reliable statistics on the true burden of SBE, developing health policies for this condition by relevant authorities may be difficult. Hence, it is critical to improve awareness about SBE among rural communities, healthcare professionals and health authorities using robust multifaceted community health education approaches. Here, we describe the design, development, implementation, and impact of distinctive community health education approaches that we used in India and Brazil. A wide range of educational tools including information leaflets, posters, pocket guides, learning materials for healthcare professionals and short/long video documentaries were developed in local languages and used to engage with target communities through direct assemblies as well as mass/traditional and social media. Notably, we used diverse methods to determine the impact of our programs in improving awareness, treatment-seeking behaviour, and clinical practice. The people-centred approaches that we used were inclusive and highly impactful in instigating fundamental changes in the management of SBE among rural communities. The resources and approaches presented in this article can be easily adapted for wider use in other countries in order to collectively reduce SBE-induced deaths, disabilities and socioeconomic ramifications.

1. Introduction

Snakebite envenoming (SBE) is a high-priority neglected tropical disease that predominantly affects rural communities living in low- and middle-income countries in Asia, Latin America, and Africa (Gutiérrez et al., 2017; Williams et al., 2019). SBE causes around 140,000 deaths and 500,000 permanent disabilities annually worldwide (Kasturiratne et al., 2008; Longbottom et al., 2018). Notably, SBE instigates substantial socioeconomic impacts on victims, their families and society through resulting consequences including deaths, permanent disabilities, psychological morbidity, and significant treatment cost (Vaiyapuri et al., 2013; Franco et al., 2022). Many people including children survive SBE but may suffer from permanent disabilities that need long-term or lifelong care. The majority of SBE victims are from underprivileged backgrounds with limited access to advanced healthcare due to geographic and economic circumstances (Harrison et al., 2009; Vaiyapuri et al., 2013; Cristino et al., 2021). Although SBE is a treatable condition, the victims often do not seek prompt treatment in appropriate

* Corresponding author.
E-mail address: s.vaiyapuri@reading.ac.uk (S. Vaiyapuri).

https://doi.org/10.1016/j.toxcx.2022.100147
Received 31 October 2022; Received in revised form 13 December 2022; Accepted 20 December 2022
Available online 26 December 2022

medical settings due to various factors (Harrison et al., 2009; Williams et al., 2017). For example, most victims may not have access to immediate medical or transport facilities, seek treatments from locally available traditional healers and practice inappropriate first aid measures. These factors exacerbate SBE-induced complications which impact the likely success of any treatment and inflate the treatment cost (Williams et al., 2017). Hence, encouraging, and empowering SBE victims to seek prompt hospital treatment is key to mitigating SBE-induced deaths and disabilities (Samuel et al., 2020). Similarly, improving primary healthcare settings with adequate facilities to handle SBE victims will be an important step in tackling this issue. SBE can also be prevented by taking simple but significant precautionary measures such as using appropriate clothing, footwear and the use of torches when going out in the dark (Kadam et al., 2021).

The World Health Organisation (WHO) has developed a strategic roadmap to reduce SBE-induced deaths and disabilities in half by 2030 (Williams 2019). The WHO's four key pillars of their strategy to decrease the burden of SBE include (1) empowering and engaging the communities, (2) ensuring safe and effective treatment, (3) strengthening health systems, and (4) increasing partnerships, coordination, and resources with strong collaborations. To achieve these goals, robust multifaceted/trans-disciplinary approaches with a broad spectrum of stakeholders are required (Gutiérrez et al., 2022), especially to empower and engage communities with the right information on the prevention and control of the SBE (Samuel et al., 2020; Kadam et al., 2021). Here, we describe the design and development of resources for multifaceted community health education programs and their implementation in India and Brazil. Importantly, we measure their impact by studying behavioural changes among rural populations in these countries. Moreover, we emphasise the need for integrated responses from scientists, research organisations and non-governmental organisations (NGOs) in influencing policies and guidelines for SBE in collaboration with local governments and other relevant authorities.

2. The necessity to improve SBE awareness

The epidemiology of SBE is difficult to establish, which makes it a challenging task to control and mitigate incidents in comparison to other common and tropical diseases. SBE mostly affects workers and their families who rely on agriculture-related occupations in rural and tribal areas (Vaiyapuri et al., 2013; Gutiérrez et al., 2017; Babo Martins et al., 2019). The daily activities of rural dwellers expose them to snakes and therefore the likelihood of SBE. Many SBE victims may have little or no access to immediate medical assistance, health care or appropriate educational programs, which often delay the essential treatment and augment SBE-induced complications (Williams et al., 2017). The literacy rate among rural communities may be another limiting factor which warrants additional support as well as access to care closer to their living areas. Moreover, several rural and tribal villages may not have appropriate road or transport facilities, making it difficult for them to access healthcare even if they chose to. The availability of local traditional healers and their familiarity with the communities is likely to be another concern as SBE victims are often encouraged or forced to seek treatments from them instead of modern medical assistance (Nann 2021). While the traditional healers may have experience in treating some ailments including a dry bite from a venomous snake or non-venomous snakebites (Pucca et al., 2020), they often refer the patients or stop treating them when they develop significant envenomation symptoms. Since SBE can induce a range of complications in the body (Warrell 2010), it needs proper care in an appropriate medical setting that contains the required facilities for treatment including ventilation support. Therefore, it is crucial to educate these communities about the importance of snakes to the ecosystem, how to avoid conflicts with snakes and the necessity to seek prompt and suitable medical care for SBE. The use of inclusive and targeted tools specifically designed to meet the needs of rural communities and circumstances is key for engagement and ultimately in realising impact (Samuel et al., 2020). Moreover, community engagement activities require individuals, local government/health authorities or NGOs who are familiar with the communities they serve in order to develop trust in the programme that is being advocated, otherwise, they may not engage or implement changes due to a lack of trust in external people or agencies (Kadam et al., 2021).

The only effective treatment available for SBE are the antivenoms produced against the venom(s) of one or a few medically significant snakes in specific regions/countries. Although antivenoms are known to induce some adverse effects such as anaphylaxis and serum sickness, their administration is critical to save lives (de Silva et al., 2016). Governments in several countries provide antivenoms free of charge. However, the healthcare professionals who practice in rural areas often do not have the confidence in handling SBE victims and administering antivenoms (Williams et al., 2017). Therefore, they often refer the patients to distant tertiary care settings, which may take several hours to reach. This delays the essential treatment and augments the complications. Moreover, the primary healthcare settings in rural regions may not be suitably equipped with all the necessary facilities, discouraging healthcare professionals from treating SBE victims. However, they need to understand that providing essential first aid and at least a few vials of antivenom will significantly improve the survival rate. Therefore, in addition to the vulnerable communities, healthcare professionals need urgent training and support to improve their awareness and skills to confidently provide immediate medical support for SBE victims.

The lack of reliable statistics on country specific SBE incidents is a significant limiting factor in developing appropriate health policies with dedicated funding support. Moreover, there are no specific mechanisms to collect SBE statistics in many countries, particularly where many people seek treatments from private healthcare and locally available traditional healers. Similarly, the availability of healthcare insurance for people in rural areas and the affordability for these people if available, is likely to be another issue. A single but significant medical (e.g., SBE) treatment cost can alter the lives of an individual and their family (Vaiyapuri et al., 2013). Lack of infrastructure, the unavailability of continuing medical education (CME) training for medical staff and appropriate security measures for the medical staff when patients' condition worsens are likely to be some of the key factors for a high number of referrals to tertiary hospitals and resulting deaths. Moreover, there is a need for local emergency response teams within the communities and local government administration. A robust ambulance service system in remote locations with short response times could be another key element in saving lives of SBE victims. Hence, it is important to raise awareness among government officials including healthcare authorities as well as insurance providers to develop robust policies to improve the healthcare systems and their affordability for SBE victims from all regions.

Due to the nature of community health education programs, the timeline to impact through behavioural and policy changes may be a slow process which might take a few years with the persistent influence of government agencies, rural health workers and members of the communities. However, providing key awareness through multifaceted approaches at different levels is essential to change the treatment-seeking behaviour of rural communities and their lifestyle to prevent SBE incidents (Samuel et al., 2020). Although people may take some time to adapt, they will become accustomed to the new lifestyle and eventually change their behaviour. For example, everyone knows that electricity is dangerous, however, they learned how to live with it. Similarly, community health education programs will empower communities to live with venomous snakes, reducing human-snake conflicts and incidence rates, mitigating the consequences of SBE.

3. SBE in India and Brazil

In this article, we mainly focus on the community health education programs that we performed in India and Brazil. Therefore, we outline

the context of SBE in India and Brazil in more detail to illustrate its burden and the need for robust community education programs.

India is the largest democratic country in the world with an estimated population of 1.39 billion (Ellis-Petersen, 2022)(India, 2022a). It is comprised of 28 states and 8 union territories (Government of (India, 2022b)). Each state is subdivided into several administrative districts and further split into taluks that comprise several rural panchayat villages. The majority of people in India may live in rural areas, and they are most likely to be involved in agricultural activities (Government of India, 2022a). Therefore, they may encounter snakes often at the workplace and around their living areas. A previous study demonstrated that small and medium-sized villages with up to 250 houses are likely to encounter snakes more frequently than larger villages and urban areas with more than 250 houses (Vaiyapuri et al., 2013). Generally, India is considered 'the capital of SBE' due to the high number of incidents and deaths. India accounts for around 58,000 SBE-induced deaths every year (Suraweera et al., 2020; Roberts et al., 2022; Livemint, 2022) although the actual number may undoubtedly be much higher as many victims do not seek hospital treatment or die on the way to hospitals (Vaiyapuri et al., 2013). Such deaths are likely to be unaccounted for as there are no available records in hospitals, local police stations or traditional treatment centres (Kadam et al., 2021). Moreover, a large proportion of SBE victims may seek treatment in private healthcare settings, where the incidence rates may not be recorded. More than 60 species of venomous snakes were identified in India. However, the 'Big Four' snakes including *Daboia russelii* (Russell's viper), *Naja* (Indian cobra), *Bungarus caeruleus* (common krait), and *Echis carinatus* (saw-scaled viper) are responsible for the majority of incidents and resulting deaths, disabilities and socioeconomic ramifications (Vaiyapuri et al., 2013; Chakma et al., 2020; Samuel et al., 2020). The state governments in India provide free healthcare for people through diverse settings ranging from primary health centres (PHC), community health centres (CHC), sub-divisional hospitals (SDH) and rural hospitals (RH) to tertiary care and medical college hospitals at the district level. Village-level support from accredited social health activists (ASHA - community health workers) may generally be absent in most cases as SBE is not included in the list of incentive activities for ASHAs. The state governments provide antivenom free of charge in tertiary care and other hospitals with facilities to administer intravenously. SBE treatment is also available in selective PHCs, SDHs and CHCs. However, due to the unstable infrastructure and insufficient number of staff, these healthcare facilities often prefer to refer patients to tertiary care hospitals. In addition, private healthcare is widely available across the country where people pay for their treatments. There are several ambulance providers in India including the service provided by the government, that respond to SBE emergencies via toll-free helpline numbers (specifically 108) (Gimkala et al., 2016). Access to ambulances and their response times may vary among states due to several factors such as the number of ambulances available, geographical terrain, time to reach the location, and distance of the location. However, a previous study reported that around 28,000 SBE victims in 2014 were transported to hospitals within 1 h of response time (from the time of the call to hospital admission) by emergency ambulance services which were available through the toll-free number 108 in 10 Indian states and 2 union territories (Gimkala et al., 2016). Despite this, a large proportion of victims may choose to use their own transportation due to a lack of awareness, the reliability of ambulance services and delayed arrivals of ambulance vehicles. India has a polyvalent antivenom produced against the 'Big Four' snakes and it is used for all envenomation cases. There are currently seven antivenom producers in India and the cost of antivenom is controlled by the Government of India (Whitaker et al. 2012). Antivenom is listed as an essential drug in all states and union territories except Ladakh. Health is considered a state matter in India, and therefore, the compensation for deaths and disability, claiming processes and the departments that manage them may vary in every state. The government's standard guidelines and protocols are available to support the clinical management of SBE in the country (National Health Mission, 2016). Moreover, some state governments provide insurance coverage for SBE treatments [e.g., the Tamil Nadu government provides the Chief Minister's Comprehensive Health Insurance Scheme (Government of Tamil Nadu, 2009)]. However, to benefit from such insurance, patients may need a bank account and seek treatment in a healthcare centre that is approved to use such a health insurance scheme. Several private hospitals either may not be approved or willing to get approval for government insurance policy schemes. Patients seeking treatment in such facilities must pay for the treatment.

Brazil is the fifth largest country in the world with an estimated population of over 215 million people (Government of Brazil, 2022). The country is made up of 26 states and one federal district (Government of Brazil, 2022). In Brazil, SBE is a major public health issue, and it affects numerous rural communities. An estimated 30,000 SBE incidents occur every year in this country resulting in approximately 4000 deaths (Schneider et al., 2021). Most SBE incidents are caused by snakes of the *Crotalus*, *Lachesis*, *Micrurus*, and *Bothrops* genera although *Bothrops* species (lanceheads) cause more incidents than the others since they are found throughout Brazil (Silva et al., 2019). The Ministry of Health in Brazil is responsible for managing SBE in the country, including the distribution of antivenom to main hospitals free of charge. Private hospitals may not be able to buy antivenoms for SBE treatment. However, the limited supply of antivenom may result in difficulties in accessing it by populations living in remote areas (e.g., tribal communities living in the Amazon Forest). Hence, the treatment may not reach all SBE victims when necessary (Cristino et al., 2021). Moreover, Brazil has other issues such as its biome diversity, the richness of snake species, and the gaps in socioeconomic indicators across areas, making it difficult to control and map the SBE issues. Thus, developing community health education programs in vulnerable regions and medical care settings for prevention, first-aid, and training has been demonstrated to be a significant strategy in mitigating SBE. The North region of Brazil encounters the most SBE incidents due to logistical, cultural, and financial difficulties as well as limited access to antivenom. For example, nearly one-third of total SBE incidents in Brazil were reported in the North region including Roraima and Amazonas states (Schneider et al., 2021). Data shows an uneven distribution of SBE issues within the country. Roraima is Brazil's northernmost state, presenting typical vegetation of the Amazon rainforest, the tropical climatic conditions observed here provide suitable habitat for a diversity of snakes throughout its territory. The stable annual temperatures (average of 27 °C) and high humidity provide the perfect conditions for reptiles to reproduce and thrive. Hence, Roraima is the state with the highest SBE incidence in Brazil, accounting for around 70 incidents per 100,000 people in contrast to other states (e.g., Amazonas state accounts for 50 SBE incidents per 100, 000 people) (Alcântara et al., 2018). However, the actual number of SBE incidents in Roraima and Amazonas is likely to be much higher. Although underreported SBE incidents are a significant issue in all Brazilian territories, Roraima can be highlighted due to the scarcity of research investment, a high number of indigenous communities (more than 40% of the state), and the migration of people from Venezuela, since Roraima is the main gate to Brazil from Venezuela. In addition, the state has the least developed economy in the country and a fragile health system, lacking doctors and medical supplies in multiple regions. The State of Amazonas has the second-highest incidence of SBE cases within the Northern region (Schneider et al., 2021). It is noteworthy that the incidence of SBE has been constantly increasing since 2000, this may be due to deforestation, which makes the population more vulnerable. The loss of habitat resulting from deforestation displaces snakes and forces them into new habitats and into conflict with humans, increasing the prevalence of *Bothrops* species within habituated areas, these species are responsible for the most SBE cases (Alcântara et al., 2018; Beck et al., 2022). In Amazonas states, the estimated annual costs associated with SBE are over $6 million (USD) dollars, of which nearly $3 million is due to loss of productivity because of premature death and $1.5 million is due to lost productivity resulting from permanent disabilities

(Magalhães et al., 2020).

4. A range of approaches used to educate rural communities

Here, we describe the community health education programs that have been developed and successfully implemented by various

Fig. 1. Snakebite information poster in Hindi developed by the SHE-INDIA organisation.

organisations in India and Brazil to promote awareness about snakebites. The details of the different approaches used, the level of outreach, and their impacts are documented.

Snakebite Healing and Education Society (SHE-INDIA) (www.she-india.org) in India has been performing a wide range of community awareness activities to improve basic knowledge and treatment-seeking behaviour of rural communities for SBE. SHE-INDIA developed community awareness tools in line with the needs of the target audience. Their short videos of one to 6 min with essential information about SBE and its effects have been highly engaging and impactful in communities (SHE, 2020). Their videos were translated into multiple regional languages, which is an important element in India as it contains several states with different languages, traditions, and cultures. Notably, SHE-INDIA produced an advocacy film, 'The Dead Don't Talk' (SHE-INDIA 2018; SHE-INDIA, 2022a) which became a powerful advocacy tool for SBE in India. The film incorporated interviews with SBE victims and their families to portray their experiences following the incidents. The stories about SBE victims whose lives were dramatically changed due to SBE effectively demonstrated the necessity to seek immediate medical treatment and highlighted its resulting socioeconomic burden. SHE-INDIA has also produced posters (Fig. 1) and short videos to educate the communities about the prevention of SBE and appropriate first aid in 12 regional languages. The content is freely available on their website and social media pages. SBE awareness information and mitigation strategies are shared on their Facebook page (SHE-INDIA, SHE-INDIA), LinkedIn and Twitter. Similarly, SHE-INDIA has been involved in programs broadcasted by All India Radio (AIR 2022), podcasts, Tedx Talks, National level panel discussions (Council 2022) and a rural media platform (Gaon Connection TV, 2022).

In addition, SHE-INDIA developed Venom Response Teams comprising local animal rescuers, community educators and self-help groups in rural areas to support SBE victims and their families immediately after the incidents to ensure they seek prompt medical treatment. In collaboration with corporate companies and educational institutions, SHE-INDIA organised several film screenings, workshops, and seminars where SBE information has been disseminated as well as exchanging ideas from various groups and countries to learn successful approaches used in different regions. In several regions, animal rescue groups and local NGOs that work with the forest departments were utilised to disseminate key information about SBE. Their tools also covered instructions to stabilise SBE victims following bites and ensure immediate healthcare has been received. Since usage of the internet and social media is prominent throughout India, SHE-INDIA has utilised various social media platforms to exchange information about SBE and engage with the victims' families and local communities to spread science-based awareness information and ideas to reduce human-animal conflicts to further prevent SBE. This has been an impactful and accessible method utilised in their awareness campaigns. SHE-INDIA collaborates with several corporate companies to fund their projects with the strict understanding that the collaboration is a charitable activity and that the programs will not promote the company's products. The organisation has worked in 13 states of India and collaborated with the state-level health and forest departments to work on SBE prevention, control and capacity building of Primary Health centres and Community Health Centre doctors and nurses. The CMEs conducted by SHE-INDIA use theory and practical training through simulation models to teach the use of a suction catheter, bag valve mask and laryngeal mask airways to nurses and tracheal intubation to PHC and CHC doctors. SHE-INDIA has also created a WhatsApp group with clinicians, herpetologists, teachers, and social activists from different states to guide and support rural healthcare professionals in the management of SBE.

The Madras Crocodile Bank Trust (MCBT) (https://madrascrocodilebank.org) is another organisation that has conducted valuable community-based studies to assess and improve the knowledge of SBE among rural communities. MCBT developed street plays and games for children promoting interactive learning about snakes and their safety measures. MCBT's Snakebite Mitigation Project conducts SBE awareness programs in 11 Indian states and has reached an estimated population of over 1.6 million through online activities and in-person assemblies in various locations. The videos (Sargunaraj, 2019) produced by MCBT were widely distributed and played a key role in several education programs. MCBT distributed educational handouts and installed over 400 A1-size posters with SBE information in various schools across India (Fig. 2). As a part of the project, walls of several schools and public areas were painted with SBE prevention and first aid information. MCBT is constantly developing efficient and sustainable educational models/tools to improve SBE awareness among rural communities. MCBT recently conducted a study to assess the communities outlook on protective equipment such as gumboots, torchlights, and mosquito nets and its ability to prevent SBE (Malhotra et al., 2021). The study highlighted the benefits and barriers of using the equipment which can be used to modify future programmes to ensure greater success. MCBT also conducts specialised training and capacity-building programs for the staff of the Forest and Fire Departments as well as snake rescuers across India.

The University of Reading (UoR) (www.reading.ac.uk) in the United Kingdom has developed a multifaceted community health education program, namely 'Venomous Snakebites: Rapid Action Saves Lives' to specifically improve SBE awareness, education and clinical management in Tamil Nadu, a large state in Southern India (Samuel et al., 2020). In collaboration with various stakeholders including clinicians, conservationists, journalists, NGOs, local government officials and educational organisations, the UoR team have made remarkable advances in improving the knowledge surrounding SBE and the treatment-seeking behaviour of victims and their families. Through direct assemblies, we reached over 50,000 students in schools, colleges, and universities. Similarly, through direct engagement activities in rural villages, we reached over 350,000 people throughout Tamil Nadu. Using lectures, video documentaries, information leaflets, pocket guides and posters, the UoR team has increased the SBE knowledge following their campaign activities compared to the level of knowledge that people had prior to participating in said activities. The unique SBE information leaflets that they recently developed using cartoons that are inclusive and representative of a range of ethnicities helping to engage with the target population (Fig. 3) are critical for SBE awareness activities. We also made animation videos using these cartoons to engage with targeted communities (University of Reading, 2022a &b). Participants knowledge retention was analysed, and significant information retention was documented even 12 months after participation in campaign activities. In addition to direct engagement, the team has used mass media in the state and country to publish numerous articles with essential information about SBE prevention and first aid. The combined readership of only the top few media providers suggests that the key messages would have reached over 70 million people within the country. Similarly, short, and long video documentaries were broadcasted through various television channels and podcasts via radio. One of these video documentaries reached over 4 million people during its broadcast over two weekends during peak hours. Notably, they developed a dedicated Facebook page (Vaiyapuri, 2018) to disseminate their materials, and events and engage with members of communities to answer their queries relating to SBE. This platform has also enabled them to disseminate short video documentaries to the specifically targeted rural population of Tamil Nadu. They also engaged several other social media-based platforms to disseminate key information about SBE. To ensure the continuous education, dissemination of information and monitoring of impact, they recruited over 120 snake rescuers and volunteers as 'local champions' in rural villages, providing them with an adequate supply of materials. Recently, the UoR team engaged with school students and villagers in remote trial regions providing them with torches, school bags and geometry boxes with information about SBE. These materials were gratefully received and were critical in ensuring engagement with these populations. They were not only useful to spread the message, but also beneficial in promoting the adoption of

Fig. 2. Snakebite Educational Programs from the Madras Crocodile Bank Trust. The campaign team has engaged with school children (**A**) and villagers (**B**) using information posters prepared in local languages (**C**).

appropriate preventive and first aid measures when bitten by snakes. This approach has substantially increased their reach and impact in improving SBE awareness and changing treatment-seeking behaviour. In summary, the UoR activities have led to the engagement of a substantial number of people within Tamil Nadu, and more widely in India within a short time through effective multifaceted community education approaches.

A few community education strategies have been developed in the northern region of Brazil and they were explained in detail in a previous study (Beck et al., 2022). In addition, the community education program coordinated by the Snakebite Roraima Research Group (www.snakebiteroraima.com) has made significant efforts in the state of Roraima and all over Brazil. The group covers a 'Snakebite Prevention and Control Program' (SPCP) to provide basic information on SBE preventative measures in different Roraima communities: rural populations, indigenous people, military personnel (army), and Venezuelan migrants (Fig. 4). In addition, the current activity of the group focuses on disseminating SBE knowledge throughout Brazil using their exclusive social media channel. The Snakebite Roraima group allows healthcare providers and students to benefit from their experience and improve their knowledge regarding the clinical management of SBE and prevention strategies. The Snakebite Roraima group indicators report that more than 20,000 people benefited from their program within different regions.

5. Methods to improve the skills and knowledge of healthcare professionals

SHE-INDIA organisation has been working with healthcare professionals in India to improve SBE treatment and outcomes. To build the nationwide SBE treatment capacity, SHE-INDIA recruited experts who routinely treat SBE to train numerous doctors, nursing staff and other rural healthcare workers in PHCs, sub-district hospitals, CHCs, and rural and district hospitals, improving their confidence in handling SBE victims in rural settings. SHE-INDIA organisation has also produced awareness films covering the importance of stabilising patients and transporting them to the local primary health centres, helping rural healthcare professionals in providing initial first aid and appropriate support for the victims before referring them for further treatment in tertiary care settings (SHE-INDIA 2021a; SHE-INDIA 2021b). This training ensured that referrals to tertiary care hospitals was reserved for critical cases.

The Venomous Snakebites: Rapid Action Saves Lives campaign has organised symposiums on clinical management of SBE to improve the skills and knowledge of rural healthcare professionals. These events attracted numerous clinicians, nurses, pharmacists, specialists, and medical officers. They covered a range of topics and medical aspects such as first aid, surgical procedures, kidney damage and issues with non-venomous snakebites for the clinical management of SBE. They also discussed the current issues with the standard protocols and guidelines provided by various healthcare authorities. These events enabled campaigners to create a network of experts which act as a forum to facilitate further discussion and support in handling SBE victims, their common symptoms, and rare complications. Notably, this network of healthcare professionals forged collaborative clinical research on various aspects of SBE using samples obtained from victims.

In Brazil, the Snakebite Roraima educational program is making huge efforts to improve knowledge about SBE at the clinical level, ensuring the adequate use of antivenoms through a Snakebite Training Program. The training programmes take undergraduate students from medical and nursing schools, physicians, nurses, and other healthcare staff (e.g., pharmacists, physiotherapists, and healthcare agents) from hospitals and basic healthcare units of the state and train them on the use of antivenoms and proper management of SBE victims. The existing guidelines from the Ministry of Health in Brazil have general recommendations for the clinical management of SBE, but it does not address pre-hospital care, first aid, storage, preparation or administration of antivenoms, wound care, auxiliary treatment for different local and systemic manifestations, referral to more complex health services, discharge criteria, determination of clotting time or the need to report cases to epidemiological surveillance systems. Hence, an integrated project in Brazil brought together a group of specialists (from the Dr Heitor Vieira Dourado Tropical Medicine Foundation (FMT-HVD), a tertiary care hospital for SBE in the Brazilian Amazon, and the Butantan Institute, the largest producer of antivenoms in the country to create SBE management guidelines and validate its contents. Their collective efforts resulted in new guidelines covering all these aspects to facilitate the clinical management of SBE including in rural settings (Rocha et al., 2022).

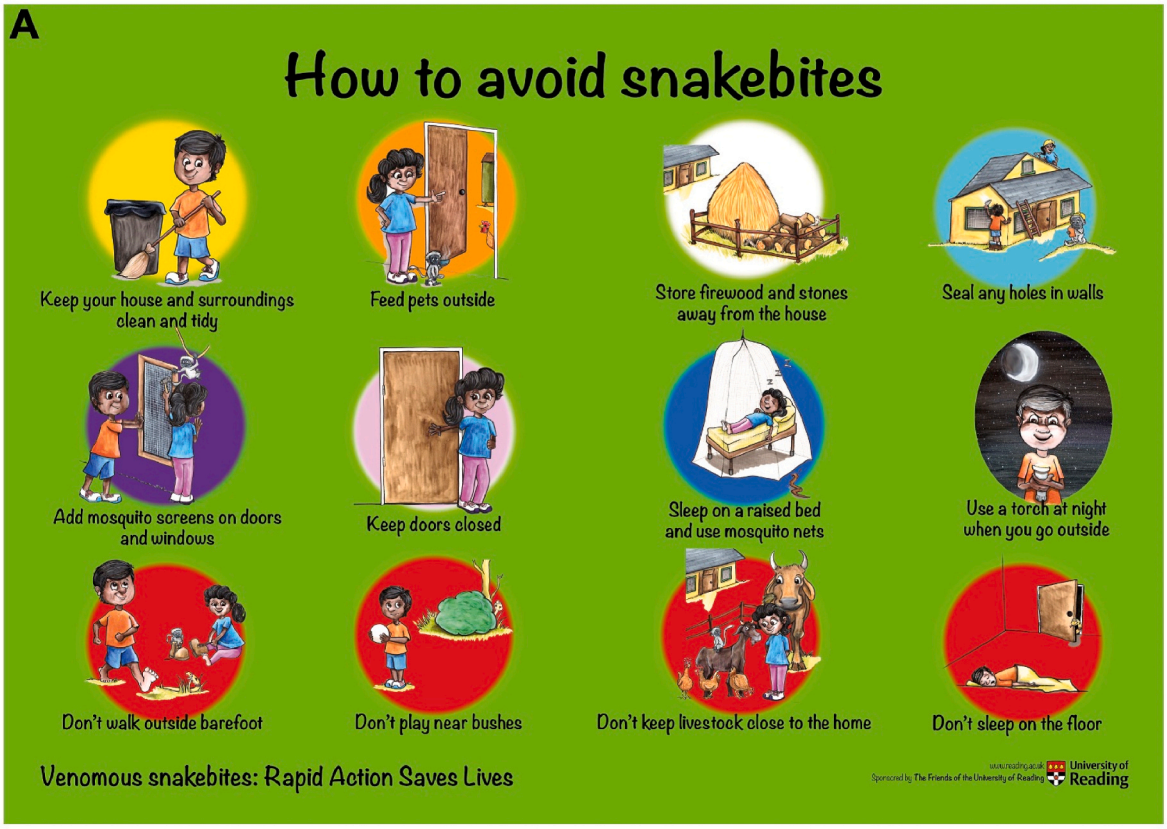


Fig. 3. Highly engaging children-friendly SBE information poster prepared by the University of Reading. The Venomous Snakebites: Rapid Action Saves Lives team at the University of Reading has developed highly engaging posters on how to avoid snakebites (**A**) and what to do if bitten by snakes (**B**) using inclusive cartoon images that represent diverse ethnicities.

Fig. 4. Snakebite Roraima Educational Program. (**A**) Snakebite training exclusively for Amazon Health Professionals was performed by Snakebite Roraima and FMT-HVD (2021). (**B**) Prevention campaigns disseminated through mass media. (**C**) Snakebite management training for Roraima's army (2022). (**D**) A fireman and a student learning how to handle a snake. (**E**) Snakebite training was performed for firefighters (2022). (**F**) Snakebite prevention campaign performed in an indigenous community (2022). (**G**) Snakebite Roraima illustrated, and printed material in different languages, including indigenous.

6. Engagements with local authorities to improve policies for SBE

Effective strategies to work with various stakeholders from diverse backgrounds within the government and NGOs to improve guidance and support for SBE awareness and prevention through education and learning are key to achieving a reduction in SBE incidences and resulting deaths. To ensure SBE is included in the existing national health programs in different countries, intervention is necessary both at the policy and field levels (Chakma et al., 2020). Hence, advocacy is critical to

highlight the burden of SBE and the status of the vulnerable/affected population (livemint, 2022).

In India, the SHE-INDIA organisation collaborates with various stakeholders at central and state-level health and forest departments. They identified partnering with health and forest departments at the state and district level as the most successful model to be implemented at the grassroots level through their activities. At the district level, stakeholders including the panchayat leaders, the ASHA network and the Anganwadi workers (rural childcare centres) are important avenues through which community awareness messages can be delivered as these stakeholders work directly with the families at the village level and communities trust them. SHE-INDIA, therefore, engages the target population through local stakeholders to reduce the SBE burden. Moreover, they worked with district-level health teams to conduct CME programs for SBE based on the principles of emergency medicine that provide simulation-based practical training on performing intubation, the use of laryngoscope and suction tube, and the use of face masks and Ambu bags to provide ventilatory support and stabilise SBE patients.

The MCBT is working with government and non-government stakeholders to improve policies for SBE in India. MCBT has advised the Indian Council for Medical Research on publishing a white paper on SBE. Notably, MCBT is the NGO representative to the National Consultation on Prevention and Control of Snakebites by the Ministry of Health and Family Welfare, Government of India. The consultation has concluded to implement dedicative and monitoring strategies to mitigate SBE across the country. These strategies will be implemented across several state National Health Missions. MCBT produced a policy film to highlight the significance of SBE in northeast India. The film was shot in this region to realistically demonstrate the extent of the problem. This film shed light on the challenges with accessing good healthcare provision. MCBT and SHE-INDIA are regularly advocating for SBE to be declared a 'Notifiable Disease' in India under the Epidemic Diseases Act of 1987 (Government of India, 1987) as then it can receive much more attention from the government.

The team led by the UoR has engaged with the government health and other relevant authorities in Tamil Nadu at various levels. Their vision is to improve awareness, education, clinical management, and affordable care for SBE within this state. The UoR team organised conferences with relevant stakeholders to discuss various aspects of SBE, venom toxicology and developed strategic maps to achieve their vision. Their engagement with healthcare professionals and experts who are involved in medical education identified gaps in currently used curricula for medical and allied healthcare courses relating to SBE education, skills development, and training. As a result, they are in the process of developing curricula and training programs to improve education and training for students who are studying medical and allied healthcare programs. Their further engagement with professionals in the fields of agriculture, law, homoeopathy, and Ayurveda identified various aspects that can be improved by introducing SBE education and awareness in these areas. Furthermore, in discussion with educationists who teach a wide range of life science courses, they identified the areas where venom toxinology along with SBE education can be introduced to reveal the therapeutic potential of molecules present in venoms while educating them about SBE. As their initial step, they are involved in introducing SBE education and awareness in secondary school curricula in discussion with the Ministry of Education at the Government of Tamil Nadu. Discussions with key stakeholders from the Ministry of Health at the Government of Tamil Nadu identified the issues around collecting accurate statistics, antivenom production, its availability within the state and the necessity for improved SBE training for rural healthcare professionals to provide prompt treatment for victims and make referrals only when necessary. To reduce the burden arising from treatment costs in private hospitals, the team has engaged with a leading healthcare insurance provider and identified cheap insurance policies that enable victims to seek prompt treatment in private healthcare settings without worrying about the treatment costs.

In the Amazon region, the education of professionals has been taking place through partnerships with municipalities in the state of Amazonas and other states in the northern region. Thus, with the construction and validation of the snakebite management guide, a work team was established between the FMT-HVD and Butantan Institute, with the support of the Brazilian Ministry of Health. Although many activities have been performed by the team of the FMT-HVD, the success of research with the Ministry of Health is still emerging. Limited research funding for neglected areas such as the Northern region, has resulted in poor investment into SBE policies in recent years. Although some public employees are dedicated to carry out campaigns and projects to mitigate SBE, funding is frequently denied making it impossible to carry out their actions.

7. Impact of community health education programs

Measuring the impact following public awareness activities is a challenging task especially in large countries. SHE-INDIA organisation provides their awareness materials in multiple languages free of charge through their online and social media platforms. The direct requests and number of views/downloads demonstrates the impact their tools have. There is also the free distribution and use of educational materials by groups that have procured the content through the NGOs working with SHE-INDIA. The impact in those cases can only be extrapolated by observing the health-seeking behaviour of the local populace following bites. The engagement with local villagers and students in educational organisations is demonstrated through the number of people who attended the events. In the last decade, SHE-INDIA has performed more than 1200 workshops. Through the Venom Response Team in local areas, SHE-INDIA envisages improving the recovery of SBE patients confirming that the recovery rate is higher than that before the development of such local teams.

The MCBT has conducted questionnaire-based surveys to assess the impact of their education programs. They conducted surveys pre- and post-education programs to understand the base knowledge of the people and to evaluate how much knowledge has been gained through involvement in activities. The surveys were conducted at different time intervals to get more diverse results. Based on these results, there has been a significant improvement in awareness in communities that attended the educational outreach programs. The survey had simple questions, translated into regional languages to get more accurate responses from beneficiaries. Audio and video testimonials were also recorded to assess impact. In online platforms, MCBT's work had reached over 1 million views. MCBT's work was recognised by several regional governing bodies and taken as a model to upscale.

The UoR has utilised robust methods to measure the impact of its activities in Tamil Nadu. For example, to measure the impact of SBE awareness among students and villagers, they employed a pictorial questionnaire with specific tasks on SBE prevention and first aid (Samuel et al., 2020). This questionnaire was used before their activities to capture their prior knowledge of SBE, immediately after their activities to determine their knowledge change, and after 12 months to analyse their long-term retention of knowledge. This method demonstrated the impact of their activities in significantly increasing the knowledge of villagers and students immediately after their activities, and more than 85% of people retained the knowledge 12 months later. To analyse the treatment-seeking behaviour of SBE victims and their relatives, they analysed data from a snakebite referral hospital before and after their activities. Remarkably, following their activities, a majority of SBE victims arrived at the hospital within 4 h following the bites and without practising any inappropriate first aid. This earlier arrival in the hospital resulted in early discharge and reduced treatment costs. This data demonstrated the translation of knowledge into treatment-seeking behaviour of villagers following SBE. The knowledge, skills and training they provided resulted in a change in clinical practice at various places as demonstrated by the number of questions that they received

through their network forums and personal emails sent to the team. Following the discussion with the Ministry of Health, the Government has increased the family's annual income level to be eligible for the Tamil Nadu Chief Minister's Comprehensive Insurance Scheme for people who earn a specific amount of money every year. The insurance provides treatment coverage for SBE along with a range of other illnesses in private hospitals in Tamil Nadu.

In the Amazon region, each municipality often has only one hospital at its headquarters, where SBE victims should travel to receive antivenom treatment. There is no antivenom available in rural health facilities where most incidents occur. Thus, access to antivenom is very limited due to long distances, and travel to the nearest hospital can even take days. Instead of being distributed to rural and indigenous health facilities, where most basic health problems are resolved by nurses, antivenom treatment is generally available only at the municipal headquarters, where physicians are present. Therefore, the decentralisation of antivenom treatment, which aims to promote the immediate and effective action of health professionals in the place where it occurs, will favour the prompt treatment and prevention of SBE-induced deaths and disabilities. This proposed action consists of care in health units under the supervision of non-medical professionals, when there is no doctor available, and this can be performed by a properly trained nurse. This is expected to increase the accessibility to antivenom treatment, reduce the time interval between the bite and treatment, and consequently, have a better prognosis for victims. As a first step in the decentralisation process, the specialist team guides the management of SBE and antivenom treatment using multidisciplinary care protocols. Moreover, there were a few campaigns relating to venomous animals led by the Ministry of Health through the *Programa Nacional de Controle do Ofidismo* (PNCO, National Program for the Control of Snakebites) in 1986. In 1989, this program was updated to the *Programa Nacional de Controle dos Acidentes por Animais Peçonhentos* (PNCAAP, National Program for the Control of Accidents by Venomous Animals). As a result of Snakebite Programs, the Ministry of Health recently launched the 5th edition of the *Guia de Vigilancia em Saúde* (GVS, Health Surveillance Guide), which elucidates strategies for surveillance, prevention and control of selected diseases important to public health, including SBE, leprosy, tuberculosis, and dengue (Malhotra et al., 2021). In parallel, researchers and professors residing in areas of the Brazilian Amazon Forest continue to perform SBE educational projects using their initiatives and funding. Although still modest, the number of SBE incidents in Brazil has begun to decrease in the last 3 years because of their educational projects.

8. Limitations

The approaches and resources described in this article were successfully developed and used in India and Brazil. Although these methods along with resources can be easily translated to any other countries or specific communities, modifications may be necessary to meet the needs of target populations. Moreover, such interventions must involve local people or groups who are trusted by rural communities as without their support, it may be difficult to engage and impact the population with awareness activities. Therefore, it is important for experts who can provide proper scientific, clinical, and epidemiological advice to engage with local people, volunteers or NGOs who are interested in these activities to design and develop people-centred approaches to improve the impact. Such methods are more likely to impact behavioural changes among rural people to reduce SBE and seek prompt medical treatments following bites. In addition, SBE researchers and clinicians should understand that making policy changes to include SBE in the existing health programs and impact on societies may take a long time due to the complexities associated with healthcare systems and the priorities of the governments during annual budgetary allocation for health risks in rural populations. Researchers must carefully design the impact measurement methods and tools at an earlier stage when they decide on the activities and target populations. It is critical to demonstrate the impact of any activities with rural communities to pave the way for future events and changes. The researchers should also be aware of ethical considerations, the necessity to obtain consent from target people or patients, when necessary, seeking appropriate permissions from relevant authorities, copyright issues when using licensed materials and risks in using media and social media. The funding for such awareness activities should also be carefully considered during the design of the project as abandoning the activities without completion is likely to cause reputational damage to the institutions and researchers, which may affect their future engagement with those communities.

9. A brief summary of similar snakebite health education activities in other countries

While much focus of this paper has been on the efforts to tackle SBE in India and Brazil, there are many other non-profit organisations, initiatives and charities that help to tackle the SBE problem worldwide. Asia, south America, and Africa are the major three hotspots for SBE, due to their abundance of venomous snakes' life, poor infrastructure and healthcare provision associated with their low socioeconomic status. In these parts of the world a large majority of people live and work in rural areas, in agriculture and in close proximity to snakes. It has been estimated that 1 million snakebites occur every year in sub-Saharan Africa, with 100,000–500,000 envenomations and 10,000–30,000 deaths (Chippaux, 2011). This number is greatly underreported for the same reasons described above in India and Brazil. Antivenom use within sub-Saharan countries depends on its accessibility including financial burden. In some parts of sub-Saharan Africa such as the Democratic Republic of Congo, antivenom is not available, in others, it is not available in remote settings where most bites occur and many antivenoms are unsuitable as they have been raised against snakes that are not from Africa or have been poorly purified (Chippaux, 2011; Warrell, 2008). The Health Action International (HAI) is an independent non-profit organisation that advocates access to effective quality and affordable medicines for all. Their HAI snakebite project works in collaboration with the Global Snakebite Initiative in sub-Saharan Africa, specifically Uganda, Zambia and Kenya to help mitigate SBE. Their project focusses on gathering data surrounding SBE, healthcare provision and antivenom treatment to gain a better understanding of the true burden faced by people in these countries. They have created civil society driven multi-stakeholder groups of snakebite experts that utilise the data collected to call for changes in policy. Education outreach projects provide local communities with the tools and resources needed to learn how to minimise conflict with snakes and reduce SBE, meanwhile teaching them how to treat SBE victims and what to do in the case of a snake bite. Their goals are to empower the people through awareness and education, make SBE reporting mandatory, ensure antivenom is of high quality, financially affordable and available to all. As with most initiatives, they have identified the importance of educating health care workers about SBE and how to properly care for their patients. This care extends beyond the primary health care provision and into the rehabilitation of patients that develop long-term morbidities following SBE, helping to improve their quality of life (HAI, 2022). In addition, another team of researchers introduced the use of a 'snake song' that was produced in Tamil (as well as in Zulu) to engage with children and educate them about what to look for and why should they care about SBE in a musically engaging manner (Erickson et al., 2020).

10. Future directions

As highlighted in the WHO's strategic roadmap, empowering and engaging the communities and collaborating with Government and non-government stakeholders is a critical approach to mitigating SBE incidents and reducing the deaths, disabilities, and socioeconomic impacts on victims and their families. In recent years, several researchers have

been involved in public engagement activities with rural communities in certain countries. However, more activities and a greater range of professionals and organisations including faith healers, alternative medicine practitioners, religious leaders, educationists, students, local champions, NGOs, hospitals, health workers and community centres should be involved and motivated to disseminate SBE awareness and mitigate the disease burden. Notably, various funding agencies across the world should understand the importance of multifaceted public engagement activities to mitigate SBE and save lives and consider funding such activities. It has been demonstrated that these activities are relatively cheap, but are powerful tools to mitigate SBE incidents, deaths, and disabilities. Involving vulnerable communities and understanding their needs will also improve research success in developing better diagnostic and therapeutic approaches for SBE. Overall, we conclude that multifaceted community health education programs in collaboration with numerous stakeholders will create a measurable impact and reveal the complexities/issues that need to be addressed in those communities.

Ethical statement

This research was conducted according to the Declaration of Helsinki and the ethical guidelines of the relevant institutions. The awareness activities and design and distribution of materials do not have any ethical concerns. However, the data collected from people and patients were covered under institutional ethical approvals as necessary.

Credit author statement

Sakthivel Vaiyapuri: Study design, Formal analysis, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Priyanka Kadam: Study design, Formal analysis, Validation, Investigation, Resources, Data curation, Writing – original draft, Visualization, Supervision, Gnaneswar Chandrasekharuni: Study design, Formal analysis, Validation, Investigation, Resources, Data curation, Writing – original draft, Visualization, Supervision, Isadora S. Oliveira: Resources, Data curation, Writing – original draft, Visualization, Subramanian Senthilkumaran: Validation, Investigation, Resources, Data curation, Anika Salim: Study design, Formal analysis, Validation, Investigation, Resources, Ketan Patel: Formal analysis, Resources, Writing – review & editing, Jacqueline de Almeida Gonçalves Sachett: Study design, Formal analysis, Resources, Writing – review & editing, Manuela B. Pucca: Study design, Formal analysis, Validation, Investigation, Resources, Data curation, Writing – original draft, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

The MCBT would like to thank Romulus Whitaker, Dr Darez Ahmed, Allwin Jesudasan, Ajay Kartik, Wilbur Sargunaraj, Peter Christopher, Indira Naidu, Zai Whitaker, Pramila Rajan and Dr Anita Malhotra for their support for their education programs. They would also like to thank Srinivasan Services Trust - TVS, USV Pvt Ltd, Infosys foundation, Google, King Cobra Conservancy and Hamish Ogston Foundation, UK for their funding support.

The UoR team would like to thank all clinicians, NGOs, snake rescuers, journalists traditionl and social media channels and UoR's Press office, Research Engagement team and all the others who support this program with funding and moral support. The team would also like to thank Mr Jarred Williams for his timely support with the final proofreading and editing of this manuscript. We also would like to thank Mr Alex Crump for his support with illustrations for snakebite information leaflets.

In Brazil, this study was supported by *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, São Paulo Research Foundation, scholarship to ISO 2020/13176–3), and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, The National Council for Scientific and Technological Development, scholarship to MBP 307184/2020–0. JAGS 311434/2021–5). The author JAGS was funded by Fundação de Amparo à Pesquisa do Estado do Amazonas (call No. 011/2021-PCGP/FAPEAM, call No. 010/2021- CT&I ÁREAS PRIORITÁRIAS and POSGRAD) and by the Ministry of Health, Brazil (proposal No. 733781/19–035).

References

Air, A., 2022. National Programme of Talks (English) II Snakebite Envenoming in India: Emerging Out of Shadows. https://www.youtube.com/watch?v=RDDS5CWwACQ. Accessed on the 10th of December 2022.

Alcântara, J.A., et al., 2018. Stepping into a dangerous quagmire: macroecological determinants of Bothrops envenomings, Brazilian Amazon. PLoS One 13 (12), e0208532.

Babo Martins, S., et al., 2019. Snakebite and its impact in rural communities: the need for a One Health approach. PLoS Neglected Trop. Dis. 13 (9), e0007608.

Beck, T.P., et al., 2022. Mapping of clinical management resources for snakebites and other animal envenomings in the Brazilian Amazon. Toxicon X 16, 100137.

Chakma, J.K., et al., 2020. White paper on venomous snakebite in India. Indian J. Med. Res. 152 (6), 568–574.

Chippaux, J.P., 2011. Estimate of the burden of snakebites in sub-Saharan Africa: a meta-analytic approach. Toxicon 57 (4), 586–599.

Council, I., 2022. Paradigm Shift: Conventional to Next Generation Snakebite Management in India. https://www.youtube.com/watch?v=BaBWuSUIsZU. Accessed on the 10th of December 2022.

Cristino, J.S., et al., 2021. A painful journey to antivenom: the therapeutic itinerary of snakebite patients in the Brazilian Amazon (The QUALISnake Study). PLoS Neglected Trop. Dis. 15 (3), e0009245.

de Silva, H.A., et al., 2016. Adverse reactions to snake antivenom, and their prevention and treatment. Br. J. Clin. Pharmacol. 81 (3), 446–452.

Ellis-Petersen, H., 2022. India Faces Deepening Demographic Divide as it Prepares to Overtake China as the World's Most Populous Country. The Guardian. https://www.theguardian.com/world/2022/nov/14/india-faces-deepening-demographic-divide-as-it-prepares-to-overtake-china-as-the-worlds-most-populous-country. Accessed on the 14th of December 2022.

Erickson, L.T., et al., 2020. The 'Snake song': a pilot study of musical intervention in Eswatini. Rural Rem. Health 20 (3), 5494.

Franco, M.V.S., et al., 2022. Physical and social consequences of snakebites in the Yanomami indigenous community, Brazil: report of two cases. Toxicon 214, 91–92.

Gimkala, A., Rao, G.V.R., Bharti, O., 2016. Transporting snake bite victims to appropriate health facility within golden hour through toll free emergency ambulance service in India, save lives. International Journal of TROPICAL DISEASE & Health 17.

Government of Brazil, 2022. Instituto Brasileiro de Geografia e Estatistica, Government of Brazil. https://www.ibge.gov.br/en/home-eng.html. Accessed on the 14th of December 2022.

Government of India, 1987. Epidemic Diseases Act, 1987. https://legislative.gov.in/sites/default/files/A1897-03.pdf. Accessed on the 12th of December 2022.

Government of Tamil Nadu, 2009. Chief Minister's Comprehensive Health Insurance Scheme. Government of Tamil Nadu. https://www.cmchistn.com. Accessed on the 14th of December 2022.

Gutiérrez, J.M., et al., 2017. Snakebite envenoming. Nat. Rev. Dis. Prim. 3 (1), 17063.

Gutiérrez, J.M., et al., 2022. Understanding and tackling snakebite envenoming with transdisciplinary research. PLoS Neglected Trop. Dis. 16 (11), e0010897.

Hai, 2022. Snake Bite Treatment and Prevention:. https://haiweb.org/projects/snakebite-treatments-prevention/. accessed 13/12/2022.

Harrison, R.A., et al., 2009. Snake envenoming: a disease of poverty. PLoS Neglected Trop. Dis. 3 (12), e569.

India, G. o, 2022a. Census Tables. Government of India.

India, G. o, 2022b. States of India. Government of India.

Kadam, P., et al., 2021. Approaches for implementing society-led community interventions to mitigate snakebite envenoming burden: the SHE-India experience. PLoS Neglected Trop. Dis. 15 (2), e0009078.

Kasturiratne, A., et al., 2008. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Med. 5 (11), e218.

Livemint, 2022. Snakebites Kill 58,000. https://www.livemint.com/news/india/snakebites-kill-58-000-govt-seeks-national-action-plan-11660755643291.html. Accessed on the 12th of December 2022.

Longbottom, J., et al., 2018. Vulnerability to snakebite envenoming: a global mapping of hotspots. Lancet 392 (10148), 673–684.
Magalhães, S.F.V., et al., 2020. Snakebite envenomation in the Brazilian Amazon: a cost-of-illness study. Trans. R. Soc. Trop. Med. Hyg. 114 (9), 635–642.
Malhotra, A., et al., 2021. Promoting co-existence between humans and venomous snakes through increasing the herpetological knowledge base. Toxicon X 12, 100081.
Nann, S., 2021. How beliefs in traditional healers impact on the use of allopathic medicine: in the case of indigenous snakebite in Eswatini. PLoS Neglected Trop. Dis. 15 (9), e0009731.
National Health Mission, 2016. Standard Treatment Guidelines: Management of Snake Bite. Government of India. https://nhm.gov.in/images/pdf/guidelines/nrhm-guidelines/stg/Snakebite_QRG.pdf. Accessed on the 14th of December 2022.
Pucca, M.B., et al., 2020. Current knowledge on snake dry bites. Toxins 12 (11).
Roberts, N.L.S., et al., 2022. Global mortality of snakebite envenoming between 1990 and 2019. Nat. Commun. 13 (1), 6160.
Rocha, G.D.S., et al., 2022. Validation of a culturally relevant snakebite envenomation clinical practice guideline in Brazil. Toxins 14 (6).
Samuel, S.P., et al., 2020. Venomous snakebites: rapid action saves lives-A multifaceted community education programme increases awareness about snakes and snakebites among the rural population of Tamil Nadu, India. PLoS Neglected Trop. Dis. 14 (12), e0008911.
Sargunaraj, W., 2019. The Pambu Goes Hissu. https://www.youtube.com/watch?v=QbuttE7T9s4. Accessed on the 14th of December 2022.
Schneider, M.C., et al., 2021. Overview of snakebite in Brazil: possible drivers and a tool for risk mapping. PLoS Neglected Trop. Dis. 15 (1), e0009044.
SHE-INDIA, 2018. The Dead Don't Talk. https://www.youtube.com/watch?v=q9wk-NkpKo8. Accessed on the 10th of December 2022.
SHE-INDIA, 2020. Snakebite - Be informed, Be safe. https://www.youtube.com/watch?v=CE3xbWkqa54. Accessed on the 10th of December 2022.
SHE-INDIA, 2021a. Catch a breath, save a life. https://www.youtube.com/watch?v=XI5ImT3rbs4. Accessed on the 10th of December 2022.
SHE-INDIA, 2021b. DISFIGURED, let us make them count. https://www.youtube.com/watch?v=53gJEXFl_jQ. Accessed on the 10th of December 2022.
SHE-INDIA, 2022a. The Dead Don't Talk. https://www.facebook.com/DeadDontTalk. Accessed on the 10th of December 2022.
Silva, J.L.D., et al., 2019. The deadliest snake according to ethnobiological perception of the population of the Alto Juruá region, western Brazilian Amazonia. Rev. Soc. Bras. Med. Trop. 53, e20190305.
Snakebite Roraima, 2022. https://www.youtube.com/c/SnakebiteRoraima. Accessed on the 14th of December 2022.
Suraweera, W., et al., 2020. Trends in snakebite deaths in India from 2000 to 2019 in a nationally representative mortality study. Elife 9.
University of Reading, 2022a. What to do if a snake bites you. https://www.youtube.com/watch?v=y2WaybA3_Fk. Accessed on the 13th of December 2022.
University of Reading, 2022b. How to Avoid Snakebites. https://www.youtube.com/watch?v=69Ean1ZKi3Y. Accessed on the 13th of December 2022.
Vaiyapuri, S., 2018. Venomous snakebites: rapid action saves lives. https://www.facebook.com/venomoussnakebite. Accessed on the 10th of December 2022.
Vaiyapuri, S., et al., 2013. Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India. PLoS One 8 (11), e80090.
Warrell, D.A., 2008. Unscrupulous marketing of snake bite antivenoms in Africa and Papua New Guinea: choosing the right product—'what's in a name? Trans. R. Soc. Trop. Med. Hyg. 102 (5), 397–399.
Warrell, D.A., 2010. Snake bite. Lancet 375 (9708), 77–88.
Whitaker, R., Whitaker, S., 2012. Venom, antivenom production and the medically important snakes of India. Curr. Sci. 103, 635–643.
Williams, D., 2019. Snakebite envenoming - a strategy for prevention and control. The World Health Organisation. ISBN: 978 92 4 151564 1 https://www.who.int/publications/i/item/9789241515641 (Accessed on the 27th December 2022).
Williams, H.F., et al., 2017. Challenges in diagnosing and treating snakebites in a rural population of Tamil Nadu, India: the views of clinicians. Toxicon 130, 44–46.
Williams, H.F., et al., 2019. The urgent need to develop novel strategies for the diagnosis and treatment of snakebites. Toxins 11 (6).

Chapter 7

Assessing the prevalence of snake phobia among the general population in India. Anika Salim, Gnaneswar Chandrasekharuni, José R Almeida, Rajendran Vaiyapuri, Harry F. Williams, Sundhararajan Arumugam, Subramanian Senthilkumaran, Ketan Patel, Timothy Williams, András Norbert Zsidó, and Sakthivel Vaiyapuri

The rationale for this study

Animal phobias, such as fear of snakes are some of the most prevalent forms of anxiety disorders. So far very little is known about the presence of snake phobia in a snakebite-endemic country and how this impacts the mental health of communities afflicted by snakebites. In this study, we aimed to estimate the level of snake phobia using a psychometric questionnaire the SNAQ12 which has demonstrated great consistency as an effective tool to measure snake phobias. Anecdotal data suggests that phobias of snakes can also lead to indiscriminate killing of snakes which has vast impacts on the reptile fauna resulting in potentially damaging effects on their respective ecosystem.

In this study, we deployed the SNAQ12 questionnaire across Tamil Nadu and India in both the Tamil and English language. We received 2030 responses to our survey and illustrated that the SNAQ12 has great internal consistency for use in Indian populations. These results highlighted that males are unusually more phobic than females and age has no impact on having a snake phobia. This means the SNAQ12 can be used in India and by healthcare professionals such as clinicians for SBE patients to provide appropriate mental health aftercare, preventing the development of a snake phobia. This can lead to the development of robust mental health strategies in SBE-afflicted communities which can aid the prevention of indiscriminate killing of snakes.

My contribution to this chapter (60%)

This study was conceptualised by myself and Sakthivel Vaiyapuri in conjunction with advice from Timothy Williams and András Norbert Zsidó who provided permission for the use of the SNAQ12 in India and its translation into Tamil.

The SNAQ12 survey was programmed and launched online by me at the University of Reading and disseminated via social media by our collaborators in India; Gnaneswar Chandrasekharuni, Rajendran Vaiyapuri, Sundhararajan Arumugam and Subramanian Senthilkumaran.

I organised, cleaned, analysed and interpreted the raw data at the University of Reading. The statistical analysis plan was developed by me, András Norbert Zsidó and Sakthivel Vaiyapuri. The reliability of the survey to determine the level of snake phobia across India was tested by me using Cronbach's alpha and McDonald's omega. The confirmatory analysis was developed and conducted by myself and András Norbert Zsidó. I completed the visualisation, programming, and preparation of all the study figures and tables. Manuscript preparation, writing, editing, and review were performed by Anika Salim and Sakthivel Vaiyapuri.

Assessing the prevalence of snake phobia among the general population in India

Anika Salim[1], Gnaneswar Chandrasekharuni[2], José R Almeida[1], Rajendran Vaiyapuri[3], Harry F. Williams[3], Sundhararajan Arumugam[4], Subramanian Senthilkumaran[5], Ketan Patel[6], Timothy Williams[7], András Norbert Zsidó[8], and Sakthivel Vaiyapuri[1]

[1]School of Pharmacy, University of Reading, Reading, UK

[2]Madras Crocodile Bank Trust, Chennai, Tamil Nadu, India

[3]Toxiven Biotech Private Limited, Coimbatore, Tamil Nadu, India

[4]Trichy SRM Medical College Hospital and Research Centre, Trichy, Tamil Nadu, India

[5]Manian Medical Centre, Erode, Tamil Nadu, India

[6]School of Biological Sciences, University of Reading, Reading, UK

[7]Department of Psychology, University of Reading, Reading, UK

[8]Institute of Psychology, University of Pécs, H-7624 Pécs, Ifjúság Str.6, Hungary

Abstract

A specific phobia is an anxiety disorder that is characterised by persistent and excessive fear in the presence of the object of the phobia. Animal phobias are the most prevalent forms of specific phobia among humans. Fear of snakes is present in non-human primates which suggests its evolutionary origins as the ability to detect the threat of snakes was critical for survival. Snake phobia is a critical factor in protecting snakes and mitigating snakebite burden. To date, only one standardised psychometric test [the Snake Questionnaire (SNAQ) developed in 1974] has been used to quantify snake phobia. Here, we estimated the level of snake phobia in India, where snakebites are highly prevalent using a modified version of the SNAQ (SNAQ12), which has previously demonstrated internal consistency, excellent reliability, and good discrimination between phobics and non-phobics in Hungary although it has never been tested among the general population in a snakebite-endemic country. We received a total of 2032 responses, comprising 1086 [53.4%] males and 946 [46.6%] females. The results demonstrated good internal consistency in determining phobia amongst the population. The data suggests that males are more likely to be snake-phobic than females, in contrast to previous research that suggested that females are usually more snake-phobic. The use of SNAQ12 allowed us to easily discriminate between individuals with phobia and non-clinical controls. This tool can be used as part of the 'One Health' approach to better understand the relationships between snake phobia and snakebites and their impact on the mental health and well-being of vulnerable populations.

Introduction

Snakebite envenoming (SBE) is a high-priority neglected tropical disease, that affects around 5.4 million people with approximately 150,000 deaths and 500,000 permanent disabilities every year worldwide [1-3]. India is the snakebite 'capital' of the world as it accounts for nearly half of the global burden of SBE-induced deaths, although the actual numbers are thought to be greatly underestimated due to the lack of reliable data [4-6]. Despite the high burden, very little is known about the fear of snakes among the general population living in rural areas in India as well as mental health sequelae caused by SBE in victims and their families [7]. A previous study highlighted the risk of Post Traumatic Stress Disorder (PTSD) in SBE patients and the necessity to establish better mental health safeguarding for them as this is often overlooked [7, 8]. Moreover,

the relationships between snake phobias and their direct/indirect influences on SBE burden and snake conservation have not been analysed previously.

According to the World Health Organisation (ICD-11 WHO), phobias are anxiety disorders, evoked in people in situations that can be well-defined and pose no danger to the person with the phobia [9]. The spectrum of anxiety or fear-related disorders is classified into different subtypes. Specific phobia is a major subtype that is restricted to highly specific situations, for example, an extreme irrational fear of specific animals [9, 10]. Specific phobias are one of the most prevalent lifetime mental health disorders with prevalence rates ranging from 2.6% to 12.5% [11] and they cause long-term distress to many sufferers [9, 10, 12-14]. Snake phobia (ophidiophobia, a clinically relevant snake phobia) is a fear of snakes and is alleged to represent half of all animal phobias [15-17]. Indeed, our evolutionary origins may be responsible for this irrational fear as it might have provided our ancestors with an adaptive protective mechanism from venomous snakes for survival [18]. Hence, humans might have evolved to be predisposed to acquire a fear of snakes [19]. This has also been proposed in Seligman's preparedness theory [20] which asserted that humans had acquired specific phobias due to inheriting a special sensitivity to stimuli (e.g., snakes) which represented a severe threat. Therefore, this evolutionary susceptibility to acquiring such an irrational fear can be easily induced in humans although it is resistant to treatment [20].

To date, very little is known about the prevalence of snake phobias among humans specifically in snakebite-endemic countries, where the risks of SBE-induced deaths, disabilities and socioeconomic impacts are high. Snake phobia results in poor management of the human-snake conflict and mental health prognosis in SBE victims. Therefore, it is imperative to estimate snake phobia among vulnerable populations and develop appropriate initiatives to reduce such fear of snakes to save snakes as they play an important part in our ecosystem and mitigate the SBE burden. Despite its high prevalence, only one test to measure snake phobia was developed by Klorman and colleagues in 1974 [21]. This was then revised by Zsido *et al.* 2018 [22] to create the SNAQ12, which is a shorter psychometric test for use in clinical settings and this has been proven to be effective, economical, and efficient in Hungary. However, the SNAQ12 has not been tested extensively in wider populations in other countries, specifically where snakebites are prevalent. Here, we validated and used the SNAQ12 in India (with a specific focus on Tamil Nadu), where the SBE burden is high.

Results

Study population

The SNAQ12 questions were available in both English and Tamil and were developed as an online survey which can be completed via any device with internet access. Following validation, the questionnaire was circulated to members of the public and students in colleges and universities via multiple routes (more details are provided in the methods section). A total of 2032 responses were received across a wide geographical distribution of Tamil Nadu (a major state in India with a high SBE burden) as well as other parts of India (**Figure 1A**). Tamil Nadu is comprised of 38 districts, and we received responses from all districts confirming the representative nature of the data. The following districts in Tamil Nadu displayed the highest responses: Coimbatore [689 (33.9%)], Chennai (the capital city of Tamil Nadu) [180 (8.9%)], Tiruchirappalli [123 (6.1%)] and Salem [89 (4.4%)]. A total of 256 responses were received from other Indian states.

Of the 2032 participants, 1086 (53.4%) were males and 946 (46.6%) were females. Moreover, 979 (48.2%) [464 (22.8%) males and 515 (25.3%) females] participants were in the age group of 18 to 25 years old, 263 (12.9%) [147 (7.2%) males and 116 (5.7%) females] were between 26 and 30 years, 215 (10.5%) [102 (5%) males and 113 (5.5%) females] were between 31 and 35 years. 180 (8.8%) [105 (5.16%) males and 75 (3.69%) females] were between 36 and 40 years, 156 (7.6%) [97 (4.77%) males and 59 (2.9%) females] were between 41 and 45 years, 87 (4.2%) [54 (2.65%) males and 33 (1.62%) females] were between 46 and 50 years, 55 (2.7%) [45 (2.21%) males and 10 (0.49%) females] were between the ages of 51 and 55, 32 (1.5%)

[22 (1.08%) males and 10 (0.49%) females] were between the ages of 56 and 60, 36 (1.7%) [26 (1.27%) males and 10 (0.49%) females] were between the ages of 61 and 65, 16 (0.7%) [14 (0.68%) males and 2 (0.09%) females] between the ages of 66 and 70 and 13 (0.6%) [10 (0.49%) males and 3 (0.14%) females] participants were over the age of 70 (**Figure 1B**). When the age groups were ranked with the number of participants, the median age of female participants was between 26 and 30 years and males was between 31 and 35 years.

Of the 2032 participants, 1606 (79%) had tertiary university level education, 340 (16.7%) had secondary school education, 32 (1.6%) had primary school education, 21 (1%) had technical/vocational training and 33 (1.6%) had no formal education (**Figure 1C**).

Confirmatory factor analysis ascertains the reliability of the SNAQ12

To determine the reliability of the SNAQ12 among this study population, a confirmatory factor analysis was performed using the diagonally weighted least squares (DWLS) estimator. For model fit, the comparative fit index (CFI), the Tucker-Lewis index (TLI), the root mean square error of approximation (RMSEA), and the standardized root mean squared residual index (SRMR) were used. They yielded an adequate level of fit on this sample (CFI = .992, TLI = .989, RMSEA = .057 95% CI = .051 to .064, and SRMR = .057). The factor loadings varied between .63 and .84. These results indicate that the one-factor solution fits the data and the SNAQ12 is a reliable tool in this study population to measure fear of snakes.

Moreover, to demonstrate the internal consistency of the SNAQ12 among this study population, Cronbach's alpha which calculates the pairwise correlations between items in a survey was used and it measured 0.84 ($0.8 \leq \alpha < 0.9$). Similarly, the McDonald's omega score was measured as 0.84. This illustrates that the SNAQ12 has good internal consistency in measuring snake phobia among this study population in India.

Males are more snake-phobic than females

The responses from all study participants for different questions are shown in **Figure 2**. The overall average score was 7.5 for all 2032 participants with a median score of 8 (SD - 3.26). 1061 (52.2%) participants were identified as having a potential snake phobia as they scored 8 or above on the SNAQ 12 with an average score of 10 and a median score of 10 (SD - 1.5). A total of 971 (47.8%) participants were identified as having no snake phobia as they scored less than 8, with the average score across this population of 4.6, with a median score of 5 (SD - 2.1).

Furthermore, the average score for the female population (946) was 6.4 with a median of 6 (SD - 3.3). Among them, 359 (38% of the total 946) females were considered to have a phobia as they scored an average of 9.8, a median of 10 (SD - 1.4) and 587 (62% of the total 946) females were considered to have no phobia with an average score of 4.3, a median of 5 (SD - 2.1) (**Table 1**). Similarly, 1086 participants were males, and their average score was 8.4, a median of 9 (SD - 3). 702 (64.6% of the total 1086) males were considered to have a snake phobia and their average score was 10.2 with a median of 10 (SD - 1.4). However, 384 (35.4% of the total 1086) males were considered to have no phobia as they had an average score of 5 with a median of 6 (SD - 1.9) (**Table 1**). Notably, there is a significant difference in the average scores for both males and females ($p=<0.0001$; $t=14.231$; $df=2030$) suggesting that males (~65%) are more snake-phobic than females (38%).

Snake phobia is not dependent on age group

We then analysed the presence of snake phobia among different age groups (**Table 2**). The results demonstrate that 495 of 18-25 (50.6% of a total 979), 146 of 26-30 (55.5% of 263), 107 of 31-35 (49.8% of 215), 97 of 36-40 (50.6% of 180), 82 of 41-45 (52.6% of 156), 45 of 46-50 (51.7% of 87), 29 of 51-55 (52.7% of 55), 15 of 56-60 (47% of 32), 27 of 61-65 (75% of 36), 12 of 66-70 (75% of 16), and 6 of >70 (46% of 13) were identified as phobic for snakes (**Figure 3**). Although the percentage of people who are phobic of snakes in the age range of 61-70 is high, there was no significant difference between the average scores across the age groups. These data suggest that age does not impact the presence of snake phobia in this study population. A

correlation analysis was also conducted and found that there was no significant correlation between the age groups amongst the total population and average SNAQ scores [$r(2032)=.01$, P=.53]. However, when comparing the phobias between males (**Figure 3A**) and females (**Figure 3B**) among different age groups, males are significantly more phobic than females in all age groups. The correlation analysis found that there was no significant correlation between the different age groups within the male population [$r(1084) = -.05$, P = .07 and in females $r(944) = -.01$, P = .64].

The level of education does not impact snake phobia

We then analysed the impact of education on developing snake phobia among the study participants. 843 (52.5% of the total of 1606) people who received university-level education were found to have snake phobia [average score - 10.1; median - 10; SD - 1.5]. Similarly, 179 (52.6% of 340) who received secondary school level education were snake phobic [average score - 9.9; median - 10; SD - 1.4]. Moreover, 16 (50%) [average score - 10.3; median - 10; SD - 1.6] that received primary school education, 10 (47,6%) [average score - 9.6; median - 9.5; SD - 1.6] that received technical/vocational training and 13 (39.4%) [average score - 10.2; median - 10; SD - 1.5] who have not received any formal education were found to have a snake phobia. There was no significant difference between the average SNAQ scores across the various educational groups. This suggests that the level of education does not impact the presence of snake phobia in this study population.

Discussion

SBE is a predominant occupational health hazard in rural, impoverished, agricultural communities in developing countries [6, 23]. The fear of snakes that develops naturally among rural populations persists throughout their lifetime, and it often affects their mental health and well-being. Although most people are aware that not all snakes are venomous or dangerous, their fear forces them to kill any snake that they see in their dwellings as well as in their natural habitats [24]. This has immense implications for human-snake conflicts and reptile conservation, particularly in vulnerable and diverse biomes, as snakes play a vital predatory role in our ecosystems and food webs albeit not least in balancing rodent populations in agricultural communities. This in turn may reduce the population of snakes and ultimately increase the population of rodents which can affect crop production [25]. Moreover, the fear of snakes often leads to increasing human-snake conflicts, SBE burden, and perpetuation of the cycle of poverty due to long-term complications that are deeply associated with economic and social ramifications [26]. For example, some people in rural communities are reluctant to improve their awareness about snakes and SBE mainly because of their phobia of snakes [27]. Thus, the global SBE crisis is a potentially lethal and debilitating consequence derived from a network of multiple interconnected factors that exacerbate the vulnerability of rural communities in low-resource regions [28]. In this multidimensional scenario, understanding the overlap and domino effect between human activities, distribution and ecology of snakes, fear, human-wildlife interactions, and snakebites should be a fundamental piece of the global plan of action against this neglected disease. Additionally, the relationship between these key factors is complex, highly dynamic, and context-dependent, which must take into account the religious and cultural aspects of the most affected populations. Mythical and religious beliefs play a key role in the development of snake phobia and consequently, in SBE burden [29]. As a result, synergistic actions, and efforts, such as conflict prevention strategies, community-based programs, sustainable coexistence promotion techniques, snake conservation initiatives and multilevel investigations of the different factors or priority elements mentioned above are crucial to minimise the huge impact of this challenge [30]. Understanding the scope of snake phobias in snakebite endemic communities allows us to develop targeted educational strategies to improve community understanding and implement productive approaches to snake conservation that not only promote positive wildlife interactions but also minimise snakebite incidents. Therefore, it is critical to tackle this issue by estimating snake phobia and developing better strategies to reduce the fear among vulnerable populations, enhance the conservation of snakes and improve the clinical management of SBE.

The data from this study emphasises that males are more likely to be snake-phobic across all age groups compared to females. The age and level of education appeared to not impact the development of snake phobia. This is contrary to most previous studies where the female members of a population were usually reported to be most phobic of snakes [14, 16, 31]. In fact, when it comes to the epidemiology of anxiety disorders, they are often significantly more prevalent in females compared to males. It is widely accepted that common fears have a higher incidence in childhood and then they rapidly taper off during adolescence and post-puberty [31]. However, our data highlight that the presence of a snake phobia does not decrease with age, in either males or females. When comparing both genders and their respective age groups, males proved to be more significantly phobic compared to females across all ages. It has been reported that the presence of sex differences in specific phobias is more apparent after puberty and the ratings are usually lower when age is increased [31]. The developmental process of adolescent fear responsiveness and acquisition has not been greatly characterised [32]. Unlike other anxiety disorders, it is generally assumed that phobias can be acquired at any time, from childhood fear acquisition, to adolescence, and adulthood. Therefore, age is not a restriction to developing, maintaining, and eliminating the fear of snakes as demonstrated in this study. Similarly, many participants in this study received university-level education, but still, most of them were found to be phobic of snakes. This suggests that education is not a factor in developing or removing phobia, and the current education may not help alleviate the fear of snakes among the general population. A more specific curriculum to alleviate the fear of snakes would aid in reducing snake phobia among students, and they may continue to live without the fear for the rest of their lives. A gentle harmless exposure of snakes to people who are phobic is also likely to reduce their fear of snakes [24]. In a specific population of Nigeria, the increased exposure to snakes has proven to have reduced phobia among the communities although a single snakebite resulted in the opposite effect and killing of snakes [24]. Similarly, a guided internet-delivered exposure strategy has been proposed and evaluated as a promising solution [33, 34]. The findings of this study support its potential and applicability to reduce snake phobia. In a clinical setting, other management strategies may also be useful, such as cognitive behavioural interventions and pharmacotherapy.

Many theories have been put forward for why humans develop or are predisposed to specific snake phobias including the conditioning theory of fear acquisition, social learning theory and the preparedness theory [35-37]. Familial transmission or increased frequency of social or indirect adverse exposure could account for why males in this study population have a higher incidence of phobia. For example, witnessing a reaction to a snake by someone suffering from snake phobia and higher exposure to snakes due to increased presence in agricultural activities could explain the higher phobic incidence among males. Fear of snakes has long been associated with evolutionary origins in protecting and ensuring our survival. The snake detection theory even suggests that the evolution of the primate visual system was innately adapted to detect threats such as snakes better in the environment [38]. Therefore, the ability of our ancestors to identify a snake and potential risk at any age would have been critical to early human survival. This could explain why there was no significance in snake phobia across the age groups in this study. Behavioural and electrophysiological research and the use of event-related potentials and earlier posterior negativity amplitude tests with brain imaging over the past decade have aided in improving our understanding of snake phobias. These studies have illustrated how snakes are not only easily identified by humans but also by primates held in captivity who have never been exposed to a real snake. This provides evidence of the fact that humans are naturally predisposed to rapidly identify a snake [38, 39].

Several studies have highlighted that there is a close association between specific phobias and other psychiatric and non-psychiatric disorders [40]. Indeed, specific phobias can result in co-morbidities such as cardiovascular disease, migraine and thyroid disorders [22]. SBE can be considered a traumatic episode for a patient and developing/having a snake phobia can worsen this situation. Therefore, effective, and economical psychometric tools that have been appropriately validated among vulnerable populations are vital for healthcare providers and mental health professionals when working with SBE patients to provide better care. The clinical assessments of snake phobia have mainly been conducted in developed countries where SBE is not a major concern. For the first time, in this study, the SNAQ12 has been used on the general population of

India where SBE is an endemic medical issue. The SNAQ12 is a modified version of the original SNAQ with only 12 questions and the reliability and efficiency of this tool have been established previously in Hungary [22]. We developed the SNAQ12 in a bilingual format which can be easily accessed and completed online using any device. The ease of use, lack of interpretability issues and low economic footprint mean the SNAQ12 can be a useful assessment tool for researchers, clinicians, and caregivers to better understand the level of snake phobia in patients and improve treatment outcomes. This will also improve the treatment-seeking behaviour and reduce the long-term impacts on SBE patients as well as others.

Our perceptions and interactions with the environment influence our relationships with our communities. Human-animal conflict is more common now than ever before and negative emotions and experiences are thought to underpin the maintenance and longevity of specific phobias including snake phobia [41]. Our intrinsic negative attitudes to snakes resulting in humans attacking and killing them have been one of the causes of the great losses to our reptile biodiversity. Killing snakes has a downstream ecological impact on the environment. Due to the threat that snakes pose in communities where SBE is common, they are more likely to be killed [42]. Therefore, it is critical to conserve snakes to promote their support in agriculture while saving lives from snakes. The deployment of SNAQ12 in assessing the attitudes of populations to snakes and subsequent actions may also aid in reducing the killing of snakes by vulnerable communities. Better education about the importance of snakes among children and adolescent students in schools and colleges/universities will further promote the cohabitation of humans and snakes. Overall, this study provides an overview of the status of snake phobia in India, which is considered the capital of SBE due to the high number of deaths. Using SNAQ12 in other parts of India as well as other countries will further establish the relationships between snake phobia and SBE burden.

Limitations

Despite obtaining a high response rate, this study has several limitations. The SNAQ12 was completed online using electronic devices and therefore, this might have prevented people who do not have access to such devices from completing the survey. Moreover, some people might have felt uncomfortable completing a study that is related to snakes. Similarly, this survey was not possible to complete without an assistant for people who were unable to read Tamil or English. Due to ethical concerns, this study only collected responses from people who were aged 18 and older. As specific phobias are some of the most prevalent anxiety disorders in children, this should be considered in future studies along with surveying adolescents to estimate the level of phobia in under-18s/non-adults. In addition, it would be better to include an equal number of males and females in different age groups to quantify their overall SNAQ scores. Future research is also required to establish the relationship between snake phobia and snakebite burden among vulnerable populations.

Materials and Methods

Translation and validation of SNAQ12

The original SNAQ (Klorman et al., 1974) is a 30-item self-reported measure of fear and phobia of snakes. A shorter version of this survey, the SNAQ12 was developed and validated by researchers in the Hungarian language and was shown to have excellent psychometric properties (Zsido *et al*. 2018). The SNAQ-12 is a 12-item questionnaire employing a discriminatory scale, where participants indicate whether or not they agree with a statement (**supplementary information**). This test has excellent discriminatory power, hence it is useful as a diagnostic tool for snake phobia. Individuals with scores of ≥8 on the SNAQ-12 are considered to be phobic to snakes. Therefore, the SNAQ12 can be recommended for use in clinical practice for fast and accurate estimations of a respondent's fear of snakes. The copyright author provided written consent for the SNAQ12 to be used in this study and to be translated into Tamil. The SNAQ12 was adapted and translated into the Tamil language by lead authors who are fluent in both Tamil and English. The validation of SNAQ12 was then carried out by our colleagues in India by collecting responses and feedback from 100 people. Based on their feedback,

an expert panel of researchers ensured the translation was as close as possible to the original SNAQ12 and accessible to both genders and ages. The online survey incorporated both the English and Tamil texts for all participants.

Data collection

This study was performed according to the Declaration of Helsinki and approved by the Institutional Ethics Committee of Toxiven Biotech Private Limited (reference: ICMR-Toxiven Ethics 2022/001) and the University of Reading Research Ethics Committee (reference: UREC 23/05). Informed consent was obtained from all study participants involved in the study to anonymously analyse and publish the data. This prospective study was conducted between September 2022 and February 2023, and we aimed to recruit at least 2000 participants across Tamil Nadu. Participants living in other Indian states were also allowed to participate following exclusion (anyone aged less than 18 and inability to read and provide consent) and inclusion (anyone aged over 18 with the ability to read and provide consent) criteria. Adults over the age of 18 were invited, and there were no other inclusion or exclusion criteria. Participants were not compensated as participation was completely voluntary. The survey was administered online using Jisc Online Surveys (JISC 2020) and respondents were recruited through social media and our academic and social networks in India. The link to the survey with a short and informative description of the study was posted on various forums, social networks, and mailing lists to ensure maximal participation. To ensure participation from a variety of demographic, socioeconomic and educational backgrounds, invitations to the survey were posted on various forums and mailing lists. Participants were also directly approached in several colleges in Tamil Nadu.

Statistical methods

All statistical analyses were performed using SPSS (version 26) (IBM, UK), JASP (version 0.17.2) (JASP team 2023, Amsterdam, The Netherlands), GraphPad Prism (version 8.0.0) (GraphPad Prism Inc, USA) and Jamovi (version 2.3) (www.jamovi.org).

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank all the study participants for their time and for completing the SNAQ12 in this study. We also extend our thanks to several collaborators, snake rescuers, NGOs and volunteers for disseminating this survey among students and members of the public. We are grateful to the Medical Research Council, UK (Grant reference: MR/W019353/1 and ITTP PhD Studentship) for their funding support.

References

1. Kasturiratne A, Wickremasinghe AR, de Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, et al. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Med. 2008;5(11):e218. doi: 10.1371/journal.pmed.0050218. PubMed PMID: 18986210; PubMed Central PMCID: PMCPMC2577696.
2. Longbottom J, Shearer FM, Devine M, Alcoba G, Chappuis F, Weiss DJ, et al. Vulnerability to snakebite envenoming: a global mapping of hotspots. The Lancet. 2018;392(10148):673-84. doi: 10.1016/S0140-6736(18)31224-8.
3. Gutiérrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, Warrell DA. Snakebite envenoming. Nat Rev Dis Primers. 2017;3:17063. Epub 20170914. doi: 10.1038/nrdp.2017.63. PubMed PMID: 28905944.
4. Suraweera W, Warrell D, Whitaker R, Menon G, Rodrigues R, Fu SH, et al. Trends in snakebite deaths in India from 2000 to 2019 in a nationally representative mortality study. eLife. 2020;9:e54076. doi: 10.7554/eLife.54076.
5. Mohapatra B, Warrell DA, Suraweera W, Bhatia P, Dhingra N, Jotkar RM, et al. Snakebite Mortality in India: A Nationally Representative Mortality Survey. PLOS Neglected Tropical Diseases. 2011;5(4):e1018. doi: 10.1371/journal.pntd.0001018.
6. Vaiyapuri S, Vaiyapuri R, Ashokan R, Ramasamy K, Nattamaisundar K, Jeyaraj A, et al. Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India. PLoS One. 2013;8(11):e80090. Epub 20131121. doi: 10.1371/journal.pone.0080090. PubMed PMID: 24278244; PubMed Central PMCID: PMCPMC3836953.
7. Millán-González R, Monge-Morales LF, De La Cruz-Villalobos N, Bonilla-Murillo F, Gutiérrez JM. Bothrops asper bite and post-traumatic stress disorder in Costa Rica: Report of two cases. Toxicon. 2023;231:107199. Epub 20230614. doi: 10.1016/j.toxicon.2023.107199. PubMed PMID: 37328114.
8. Habib ZG, Salihu AS, Hamza M, Yakasai AM, Iliyasu G, Yola IM, et al. Posttraumatic stress disorder and psycho-social impairment following snakebite in Northeastern Nigeria. Int J Psychiatry Med. 2021;56(2):97-115. Epub 20200326. doi: 10.1177/0091217420913400. PubMed PMID: 32216497.
9. Eaton WW, Bienvenu OJ, Miloyan B. Specific phobias. Lancet Psychiatry. 2018;5(8):678-86. doi: 10.1016/s2215-0366(18)30169-x. PubMed PMID: 30060873; PubMed Central PMCID: PMCPMC7233312.
10. LeBeau RT, Glenn D, Liao B, Wittchen H-U, Beesdo-Baum K, Ollendick T, et al. Specific phobia: a review of DSM-IV specific phobia and preliminary recommendations for DSM-V. Depression and Anxiety. 2010;27(2):148-67. doi: https://doi.org/10.1002/da.20655.
11. Wardenaar KJ, Lim CCW, Al-Hamzawi AO, Alonso J, Andrade LH, Benjet C, et al. The cross-national epidemiology of specific phobia in the World Mental Health Surveys. Psychological Medicine. 2017;47(10):1744-60. Epub 2017/02/22. doi: 10.1017/S0033291717000174.
12. Becker ES, Rinck M, Türke V, Kause P, Goodwin R, Neumer S, et al. Epidemiology of specific phobia subtypes: findings from the Dresden Mental Health Study. Eur Psychiatry. 2007;22(2):69-74. Epub 20061208. doi: 10.1016/j.eurpsy.2006.09.006. PubMed PMID: 17157482.
13. Grenier S, Schuurmans J, Goldfarb M, Préville M, Boyer R, O'Connor K, et al. The epidemiology of specific phobia and subthreshold fear subtypes in a community-based sample of older adults. Depress Anxiety. 2011;28(6):456-63. Epub 20110311. doi: 10.1002/da.20812. PubMed PMID: 21400642.
14. Fredrikson M, Annas P, Fischer H, Wik G. Gender and age differences in the prevalence of specific fears and phobias. Behaviour Research and Therapy. 1996;34(1):33-9. doi: https://doi.org/10.1016/0005-7967(95)00048-3.
15. Klieger DM. The Snake Anxiety Questionnaire as a Measure of Ophidophobia. Educational and Psychological Measurement. 1987;47(2):449-59. doi: 10.1177/0013164487472017.
16. Polák J, Sedláčková K, Nácar D, Landová E, Frynta D. Fear the serpent: A psychometric study of snake phobia. Psychiatry Res. 2016;242:163-8. Epub 20160531. doi: 10.1016/j.psychres.2016.05.024. PubMed PMID: 27280527.
17. Davey GCL. Self-reported fears to common indigenous animals in an adult UK population: The role of disgust sensitivity. British Journal of Psychology. 1994;85(4):541-54. doi: https://doi.org/10.1111/j.2044-8295.1994.tb02540.x.
18. Weiss L, Brandl P, Frynta D. Fear reactions to snakes in naïve mouse lemurs and pig-tailed macaques. Primates. 2015;56(3):279-84. doi: 10.1007/s10329-015-0473-3.
19. LoBue V, DeLoache JS. Detecting the Snake in the Grass:Attention to Fear-Relevant Stimuli by Adults and Young Children. Psychological Science. 2008;19(3):284-9. doi: 10.1111/j.1467-9280.2008.02081.x. PubMed PMID: 18315802.

20. Seligman MEP. Phobias and preparedness. Behavior Therapy. 1971;2(3):307-20. doi: https://doi.org/10.1016/S0005-7894(71)80064-3.
21. Klorman R, Weerts TC, Hastings JE, Melamed BG, Lang PJ. Psychometric description of some specific-fear questionnaires. Behavior Therapy. 1974;5(3):401-9. doi: https://doi.org/10.1016/S0005-7894(74)80008-0.
22. Zsido AN, Arato N, Inhof O, Janszky J, Darnai G. Short versions of two specific phobia measures: The snake and the spider questionnaires. Journal of Anxiety Disorders. 2018;54:11-6. doi: https://doi.org/10.1016/j.janxdis.2017.12.002.
23. Harrison RA, Hargreaves A, Wagstaff SC, Faragher B, Lalloo DG. Snake envenoming: a disease of poverty. PLoS Negl Trop Dis. 2009;3(12):e569. Epub 20091222. doi: 10.1371/journal.pntd.0000569. PubMed PMID: 20027216; PubMed Central PMCID: PMCPMC2791200.
24. Coelho CM, Polák J, Suttiwan P, Zsido AN. Fear inoculation among snake experts. BMC Psychiatry. 2021;21(1):539. doi: 10.1186/s12888-021-03553-z.
25. Kontsiotis VJ, Rapti A, Liordos V. Public attitudes towards venomous and non-venomous snakes. Science of The Total Environment. 2022;831:154918. doi: https://doi.org/10.1016/j.scitotenv.2022.154918.
26. Landry Yuan F, Devan-Song A, Yue S, Bonebrake TC. Snakebite Management and One Health in Asia Using an Integrated Historical, Social, And Ecological Framework. Am J Trop Med Hyg. 2021;106(2):384-8. Epub 20211206. doi: 10.4269/ajtmh.21-0848. PubMed PMID: 34872063; PubMed Central PMCID: PMCPMC8832943.
27. Vaiyapuri S, Kadam P, Chandrasekharuni G, Oliveira IS, Senthilkumaran S, Salim A, et al. Multifaceted community health education programs as powerful tools to mitigate snakebite-induced deaths, disabilities, and socioeconomic burden. Toxicon: X. 2023;17:100147. doi: https://doi.org/10.1016/j.toxcx.2022.100147.
28. Warrell DA, Williams DJ. Clinical aspects of snakebite envenoming and its treatment in low-resource settings. Lancet. 2023;401(10385):1382-98. Epub 20230314. doi: 10.1016/s0140-6736(23)00002-8. PubMed PMID: 36931290.
29. Senthilkumaran S, Arathisenthil SV, Williams J, Williams HF, Thirumalaikolundusubramanian P, Patel K, et al. An Unnecessary Russell's Viper Bite on the Tongue Due to Live Snake Worship and Dangerous First Aid Emphasise the Urgent Need for Stringent Policies. Toxins (Basel). 2022;14(12). Epub 20221122. doi: 10.3390/toxins14120817. PubMed PMID: 36548714; PubMed Central PMCID: PMCPMC9787415.
30. Malhotra A, Wüster W, Owens JB, Hodges CW, Jesudasan A, Ch G, et al. Promoting co-existence between humans and venomous snakes through increasing the herpetological knowledge base. Toxicon X. 2021;12:100081. Epub 20210826. doi: 10.1016/j.toxcx.2021.100081. PubMed PMID: 34522881; PubMed Central PMCID: PMCPMC8426276.
31. Agras S, Sylvester D, Oliveau D. The epidemiology of common fears and phobia. Comprehensive Psychiatry. 1969;10(2):151-6. doi: https://doi.org/10.1016/0010-440X(69)90022-4.
32. Stenson AF, Nugent NR, van Rooij SJH, Minton ST, Compton AB, Hinrichs R, et al. Puberty drives fear learning during adolescence. Dev Sci. 2021;24(1):e13000. Epub 20200728. doi: 10.1111/desc.13000. PubMed PMID: 32497415; PubMed Central PMCID: PMCPMC9206403.
33. Andersson G, Waara J, Jonsson U, Malmaeus F, Carlbring P, Ost LG. Internet-based exposure treatment versus one-session exposure treatment of snake phobia: a randomized controlled trial. Cogn Behav Ther. 2013;42(4):284-91. doi: 10.1080/16506073.2013.844202. PubMed PMID: 24245707.
34. Hunt M, Bylsma L, Brock J, Fenton M, Goldberg A, Miller R, et al. The role of imagery in the maintenance and treatment of snake fear. J Behav Ther Exp Psychiatry. 2006;37(4):283-98. Epub 20060213. doi: 10.1016/j.jbtep.2005.12.002. PubMed PMID: 16473325.
35. McNally RJ. Preparedness and phobias: a review. Psychol Bull. 1987;101(2):283-303. PubMed PMID: 3562708.
36. Rachman S. The conditioning theory of fearacquisition: A critical examination. Behaviour Research and Therapy. 1977;15(5):375-87. doi: https://doi.org/10.1016/0005-7967(77)90041-9.
37. Bandura A. Social learning theory. Englewood Cliffs, NJ: Prentice Hall. 1977.
38. Isbell LA. Snakes as agents of evolutionary change in primate brains. J Hum Evol. 2006;51(1):1-35. Epub 20060320. doi: 10.1016/j.jhevol.2005.12.012. PubMed PMID: 16545427.
39. Kawai N, Koda H. Japanese monkeys (Macaca fuscata) quickly detect snakes but not spiders: Evolutionary origins of fear-relevant animals. J Comp Psychol. 2016;130(3):299-303. Epub 20160414. doi: 10.1037/com0000032. PubMed PMID: 27078076.
40. Sancassiani F, Romano F, Balestrieri M, Caraci F, Di Sciascio G, Drago F, et al. The Prevalence of Specific Phobia by Age in an Italian Nationwide Survey: How Much Does it Affect the Quality of Life? Clin

Pract Epidemiol Ment Health. 2019;15:30-7. Epub 20190220. doi: 10.2174/1745017901915010030. PubMed PMID: 30972140; PubMed Central PMCID: PMCPMC6407652.
41. Zsidó AN, Inhof O, Kiss BL, Bali C, March DS. Threatening stimuli have differential effects on movement preparation and execution—A study on snake fear. People and Nature. n/a(n/a). doi: https://doi.org/10.1002/pan3.10500.
42. Pandey DP, Subedi Pandey G, Devkota K, Goode M. Public perceptions of snakes and snakebite management: implications for conservation and human health in southern Nepal. J Ethnobiol Ethnomed. 2016;12(1):22. Epub 20160602. doi: 10.1186/s13002-016-0092-0. PubMed PMID: 27255454; PubMed Central PMCID: PMCPMC4891849.

Figures

Figure 1

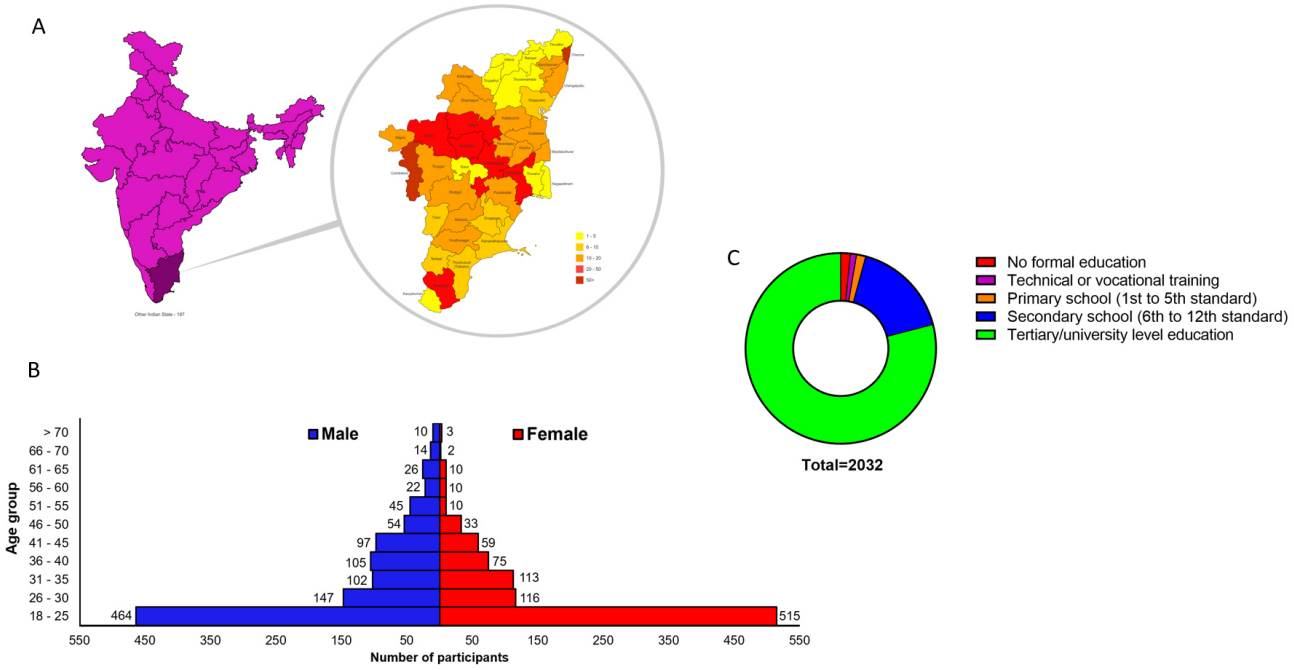


Figure 1: **Characteristics of the study population**. **A**) map of India showing the location of Tamil Nadu and a geographical heatmap displaying the number of participant responses from the 38 districts in Tamil Nadu. Districts are colour-graded according to the number of responses received. **B**) the total number of participants in this study based on their gender and age groups. **C**) the distribution of participants based on their level of education.

Figure 2

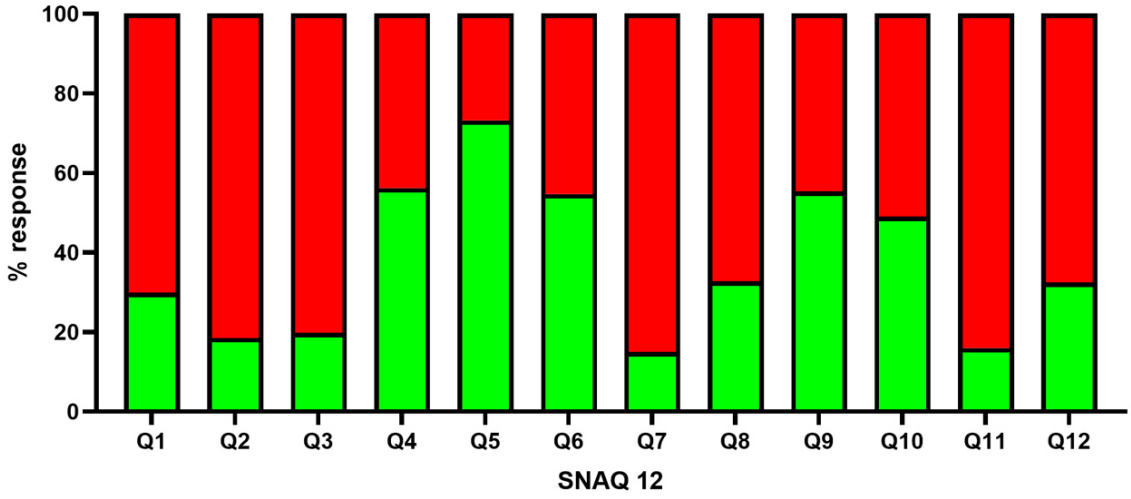


Figure 2: **Total responses for individual questions in SNAQ12**. The total percentage of responses from participants for each SNAQ12 question is shown. Green indicates the percentage of participants that answered 'yes' and red indicates the percentage of participants that answered 'no' to each question.

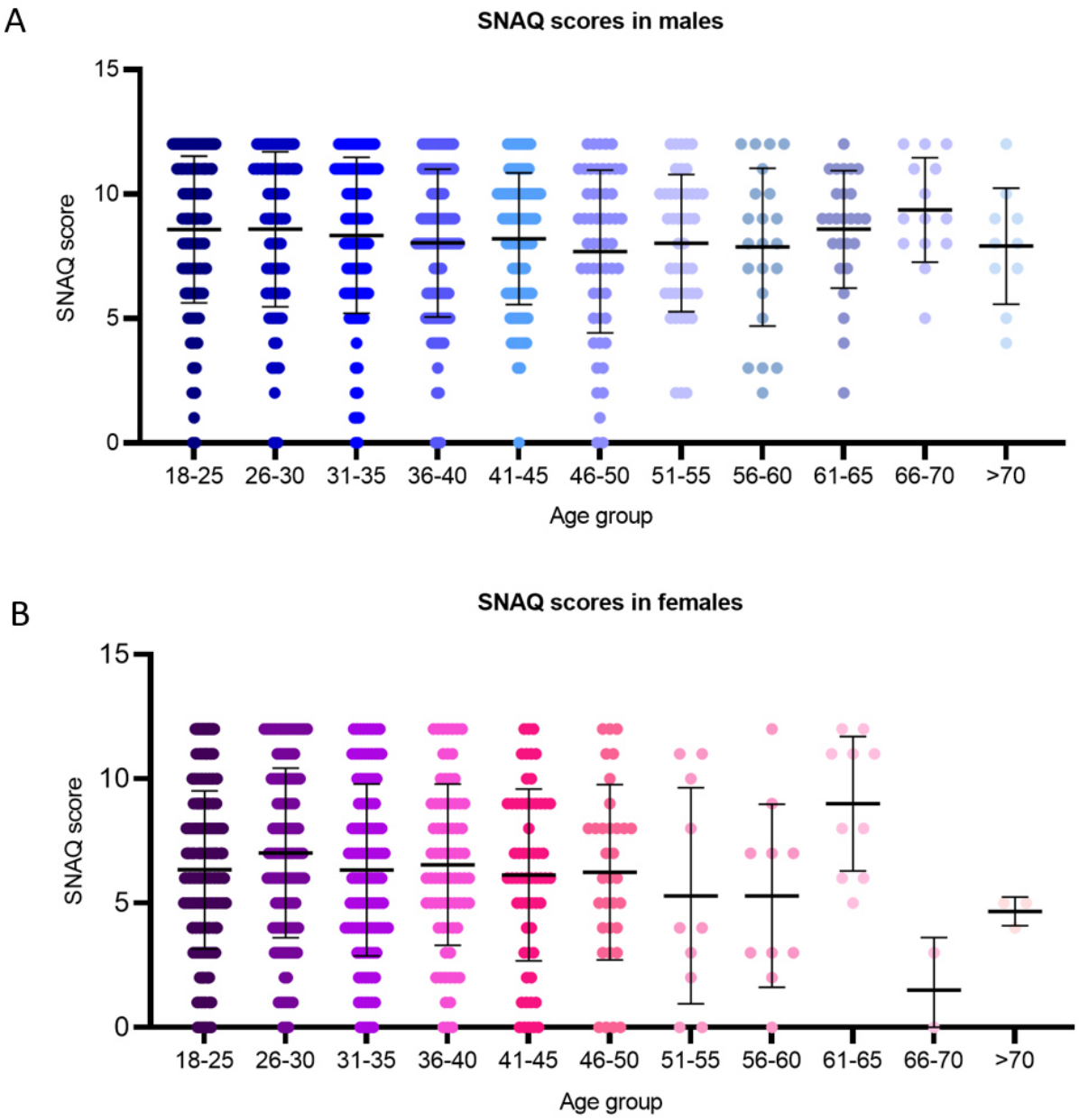


Figure 3: The distribution of SNAQ12 scores in **A**) males and **B**) females across their age groups.

Tables

Table 1: Mean and median SNAQ scores of males and females in the study population. This highlights the number of phobic and non-phobic individuals as well as the mean and median age groups of males and females.

	Mean score	**Median score**	**STDEV**	**Phobia ≥8**	**No Phobia <8**
Total number (2032)	7.5	8	3.3	1061	971
Mean and median age group: **26-30**	7.9	8	3.3	146	117
Total number of females (946)	6.4	6	3.3	359	587
Median age group of females: **18-25**	6.3	6	3.2	190	325
Mean age group of females: **26-30**	7	7	3.4	51	65
Total number of males (1086)	8.4	9	3	702	384
Median age group of males: **26 - 30**	8.6	9	3.1	95	52
Mean age group of males: **31 - 35**	8.3	9	3.1	65	37

Table 2: Mean and median SNAQ scores of different age groups in the study population. This highlights the number of phobic and non-phobic individuals in different age groups.

Group	**N**	**Mean**	**Median**	**SD**
Total	2032	7.5	8	3.3
Total non phobic	971	4.6	5	2.1
Total phobic	1061	10	10	1.5
Age groups	**N**	**Mean**	**Median**	**SD**
18 - 25	**979**	**7.4**	**8**	**3.3**
18-25 non phobic	484	4.6	5	2.1
18-25 phobic	495	10	10	1.5
26 - 30	**263**	**7.9**	**8**	**3.3**
26 - 30 non phobic	117	4.7	5	2.1
26 - 30 phobic	146	10.4	11	1.4
31 - 35	**215**	**7.3**	**7**	**3.4**
31 - 35 non phobic	108	4.4	5	2.2
31 - 35 phobic	107	10.2	11	1.5
36 - 40	**180**	**7.4**	**8**	**3.2**
36 - 40 non phobic	83	4.6	5	2
36 - 40 phobic	97	9.8	9	1.5
41 - 45	**156**	**7.4**	**8**	**3.1**
41 - 45 non phobic	74	4.7	5	2.1
41 - 45 phobic	82	9.9	10	1.3
46 - 50	**87**	**7.1**	**8**	**3.4**
46 - 50 non phobic	42	4.2	5	2.4
46 - 50 phobic	45	9.8	10	1.5
51 - 55	**55**	**7.5**	**8**	**3.2**
51 - 55 non phobic	26	4.7	5	2.2
51 - 55 phobic	29	10.1	10	1.2

56 - 60	**32**	**7**	**7**	**3.5**
56 - 60 non phobic	17	4.4	3	2.3
56 - 60 phobic	15	10.1	10	1.6
61 - 65	**36**	**8.7**	**9**	**2.4**
61 - 65 non phobic	9	5.3	6	1.6
61 - 65 phobic	27	9.8	10	1.4
66 - 70	**16**	**8.4**	**9**	**3.4**
66 - 70 non phobic	4	3.8	4	2.9
66 - 70 phobic	12	9.9	9.5	1.6
> 70	**13**	**7.1**	**7**	**2.5**
> 70 non phobic	7	5.3	5	1.2
> 70 phobic	6	9.3	9	1.5

Supplementary information

SNAQ-12

Please work through the next 12 statements. For each statement, indicate whether you rather agree or rather disagree. If you agree with the statement answer with YES, if you disagree with the statement answer with NO. Do not think too much about it – your initial responses are usually the best. Thank you.

1	I would feel some anxiety holding a toy snake in my hand	Yes	No
2	If a picture of a snake appears on the screen during a TV show/movie, I turn my head away	Yes	No
3	I dislike looking at pictures of snakes in magazines/newspapers	Yes	No
4	I am terrified by the thought of touching a harmless snake	Yes	No
5	If someone says that there are snakes anywhere, I become alert	Yes	No
6	When I see a snake, I feel tense and restless	Yes	No
7	I feel unwell/sick when I see a snake	Yes	No
8	The way snakes move is repulsive	Yes	No
9	If I came upon a snake in a forest/woods, I would probably run	Yes	No
10	I'm more afraid of snakes than any other animal	Yes	No
11	I would prefer not to finish a story if something about snakes was introduced	Yes	No
12	Even if I was late for a very important appointment, the thought of snakes would stop me from taking a shortcut through an open field	Yes	No

Chapter 8

Challenges in rescuing snakes to save lives from snakebites in Tamil Nadu, India

The rationale for this study

Snake rescuers are community volunteers who play an important role in preserving snakes by capturing them to reduce panic and potential SBE in the communities they serve. This role is incredibly risky and life-threatening when considering the venomous snakes of India and has great implications for the rescuer if they are bitten. Moreover, as this profession is not officially recognised by the local state government nor is the responsibility of 'snake capture' distinguished as a significant role within the forestry department this leaves rescuers without much legislative protection and subject to potential abuse.

In this study, we aimed to understand the key challenges faced by snake rescuers and their needs to be able to conduct this vital service role. In total 152 snake rescuers from across Tamil Nadu, India were interviewed following consenting procedures. Notably most snake rescuers were young males with the majority indicating their passion and interest in snakes and their conservation as being a motivating driver for their work. Few had received formal training on either non-venomous or venomous snakes and most felt they needed additional training for safe snake handling. Moreover many felt hindered by the lack of official recognition for their work and the provision of licensures and identity cards by the local government would allow them to continue to provide their services without fear or worry. Snake rescue in India can be considered a high-risk occupation and ensuring volunteers have appropriate training, equipment, health insurance and endorsed licenses by their local government can ensure communities can have access to authorised and trained professionals to safely remove snakes, prevent SBE and conserve snakes.

My contribution to this chapter (60%)

This study was conceptualised by Sakthivel Vaiyapuri in conjunction with advice from our collaborating partners in India. Open discourse with collaborators, the wildlife departments and snake rescuers were conducted to ascertain a framework of key information that could evaluate and determine the challenges faced by snake rescuers.

The survey was programmed and launched online by myself and disseminated by Sakthivel Vaiyapuri in India. I organised, cleaned and analysed the raw data at the University of Reading. The statistical

analysis plan was developed with Sakthivel Vaiyapuri which included exploratory data modelling to determine the key challenges, requirements, and significance of appropriate snake-handling equipment along with their professional tenure which may impact the risk of snakebites. I completed the visualisation, programming, and preparation of all the study figures and tables. Manuscript preparation, writing, editing, and review were performed by Anika Salim and Sakthivel Vaiyapuri.

Challenges in rescuing snakes to save lives from snakebites in Tamil Nadu, India

Abstract

Human-snake conflicts are a common occurrence worldwide with snakebites causing over 140,000 deaths and 500,000 permanent disabilities annually. India alone accounts for around 58,000 deaths every year. As human developments rapidly expand into suburban and rural areas, snakes are being displaced and incidences of residents finding snakes within their dwellings are increasing. Most people have an innate fear of snakes, compounded by centuries of negative influence from culture and mythology manifesting in people often attempting to kill snakes. Snake rescuers are volunteers who remove and relocate snakes to safe areas away from humans. This is a risky job that poses potentially fatal implications if bitten. These volunteers mostly receive no financial compensation for their time or transportation costs, but they chose to do it for their love of snakes, conservation, and to help others. However, they often receive no formal training resulting in removing snakes without adequate safety equipment and face several challenges in doing this task. In this study, we interviewed several snake rescuers in Tamil Nadu, India and analysed their challenges and needs for rescuing snakes effectively and safely. The results demonstrate that most people received no formal training and often they are bitten by snakes. They highlighted the urgent need for formal training for snake rescuers, safety equipment and standard protocols for rescuing snakes. These results demonstrate that snake rescuing should be appropriately regulated by the authorities and formal training along with necessary equipment should be provided to them to empower them to safely remove snakes from human dwellings to save both snakes and humans.

Introduction

Snakebite envenoming (SBE) is a high-priority neglected tropical disease that results in around 140,000 deaths and 500,000 permanent disabilities worldwide every year. India is the epicentre of this global issue, with an estimated 58,000 annual deaths [1] [2]. The 'big four' snakes including Russell's viper (*Daboia russelii*), the Indian cobra (*Naja naja*), the saw-scaled viper (*Echis carinatus*) and the common krait (*Bungarus caeruleus*) are regarded as being responsible for the most bites and fatalities in India. However, there are nearly 300 venomous and non-venomous snakes identified in India [3] [4]. The climate in India provides the perfect conditions for reptiles to thrive, but the high abundance of snakes and a large human population result in frequent human-snake conflicts. The cohabitation between humans and snakes often results in snakes being found inside people's houses, among livestock or near dwellings. In the summer, houses are often cooler than outside, providing a suitable place for snakes to stay cool. In the winter, the houses are often warmer than outside, providing warmth and comfort from the cold for snakes. Food scraps, waste, open sewage, and firewood stacks amongst others attract small rodents, frogs, and toads to human dwellings, which in turn attract snakes. Snakes are an effective tool for pest control, but a venomous snake near humans is an unfortunate accident waiting to happen. Snakes often have a negative connotation among rural communities worldwide as for centuries people have been taught that snakes bring death and misfortune. Poor understanding and knowledge of venomous and non-venomous snake species and their importance to the environment result in all snakes, even those that are harmless being regarded as dangerous and need removing (mostly killing). A large proportion of snakebites occur when attempting to remove or kill the snake as the snake feels threatened and will attempt to protect itself. Through education and public awareness activities, rural communities are learning that there are safer, less detrimental ways to remove snakes from their homes.

Snake rescuers are becoming increasingly common worldwide, and they mostly perform rescues voluntarily. They often face numerous challenges and lack formal training and recognition from the authorities and the public. For example, snake removals are risky as often the snakes are stressed and feel threatened resulting in a high chance of being bitten. Snake rescuers require an in-depth knowledge of snake species in

their area of operations, their behaviours and how to safely handle potentially deadly snakes. Despite the associated risks, many snake rescuers do not charge any money for removals and often cover large distances to reach callouts without assistance for transportation costs. Currently, there are no recognised qualifications or training requirements to become a snake rescuer in most places. Therefore, many rescuers are putting themselves at risk, performing snake removals without appropriate training and safety equipment. While most snake rescuers are sensible and perform this service for selfless reasons, some are utilising the opportunity to gain recognition, celebrity status and popularity on social media. Videos of people free handling and performing dangerous acts with venomous snakes are not uncommon and are a poor influence on many who see this and wish to replicate it. Risks can be mitigated with appropriate training, experience, and handling equipment. Therefore, it is important to gauge the views of snake rescuers and determine the key barriers to performing their work to develop evidence-based strategies for safely rescuing snakes and saving lives keeping communities safe and preserving the reptile fauna. In this study, we interviewed 152 snake rescuers in Tamil Nadu, India to establish their knowledge of snakes, challenges, and needs for effective and safer snake rescuing. These data demonstrate several key challenges and an urgent need to develop training and standard operating procedures for snake rescuers in Tamil Nadu.

Results

Demographics of snake rescuers

In total, 152 snake rescuers were interviewed following written informed consent using an online questionnaire. The interviewees were from a wide geographical distribution across Tamil Nadu. The responses were received from 27 out of the 38 districts, with no responses from 11 districts. Notably, 24.7% of respondents were from the Coimbatore district, 11.3% from Erode district and the same number were from the Madurai district. The remaining 52.7% of participants were distributed throughout the other 24 districts within Tamil Nadu (**Figure 1A**). Of the 152 snake rescuers, most of them were males [144 (94.7%)] and only a small number of them were females [8 (5.3%)] (**Figure 1B**). Notably, most rescuers were from the younger age groups [26.3% (40 rescuers) in 18-25 and 28.9% (44) were in 26-30], and a small number of rescuers were in other age groups (**Figure 1C**). No responses were received from people aged over 55 years indicating the involvement of youngsters in performing this task. Most [110 (72.4%)] rescuers had received a tertiary (university level) education, while others have secondary level education (6^{th} to 12^{th} standard) [33 (21.7%)]. One (0.7%) participant had primary school education (1^{st} to 5^{th} standard), three (2%) had either technical/vocational training, one (0.7%) received no formal education and four (2.6%) people were not willing to disclose their educational qualifications. Notably, 14 (9.2%) rescuers had a primary occupation in fire and rescue service, 13 (8.6%) were engaged in their own business, nine (5.9%) were working as engineers or technicians and five (3.3%) were engaged in agriculture, while others were engaged in different occupations. Most rescuers indicate that they perform snake rescues due to their interest in snakes [110 (72.4%)] and/or their conservation [105 (69.1%)]. The other reasons for them rescuing snakes were to help communities [37 (24.3%)], due to the impact of snakebites on society [30 (19.7%)], inspired by other snake rescuers [23 (15.1%)], have suffered from snakebite themselves [9 (5.9%)], not afraid of snakes [9 (5.9 %)], and due to family [7 (4.6%)] or religious [2 (1.3%)] reasons. Most participants [50 (32.9%)] had been involved in snake rescues for 10+ years, while others were involved in this task for varying periods [21 (13.8%) for six-10 years, 35 (23%) for four-six years, 25 (16.4%) for two-four years, 10 (6.6%) one-two years, 8 (5.3%) six months - one year, and 3 (2%) one - five months]. To determine if the snake rescuers are associated with any authorised organisations, their affiliations with such organisations were analysed. 82 (54%) participants are associated with the Tamil Nadu government forest department as snake rescuers and 29 (19%) are employed by the government in an official capacity. 75 (49.3%) rescuers are associated with non-governmental organisations as snake rescuers and others are working as independent rescuers. Interestingly, 127 (83.6%) rescuers are also involved in conserving other animals. These data indicate that young males are mostly involved in snake rescues with good education, and there are several reasons for them performing this task.

Snake rescuers are often educated and trained by herpetologists or other rescuers

Snake rescuers should be confident in identifying the species of snake that they are rescuing and determine whether the species is venomous or not. 145 (95.4%) rescuers stated they were confident in differentiating venomous and non-venomous snakes in their regions. They were then asked if they had received any education for handling venomous and/or non-venomous snakes. 93 (61.2%) participants received education on how to rescue both venomous and non-venomous snakes. Four (2.6%) received education for only non-venomous snake rescue and another three (2%) received education for only venomous snake rescue. 52 (34.2%) participants received no education on snake rescue. The participants who received education for both venomous and non-venomous snakes were asked how their training had been conducted. 68 (44.7%) received both practical and theoretical training for rescuing non-venomous snakes. 21 (13.8%) received only practical training and three (2%) received only theoretical training for rescuing non-venomous snakes. Most of these participants [58 (38.1%)] were trained by herpetologists or snake handling specialists, 36 (23.7%) were trained by other snake rescuers, 16 (10.5%) were trained by their friends, 15 (9.9%) by ecologists, 12 (7.9%) were trained by other means including self-training, by their parents, or fire and rescue officials. Three (2%) were trained by veterinary care providers. For handling venomous snakes, 62 (40.8%) participants received both practical and theoretical training. 20 (13.2%) received only practical training and eight (5.3%) received only theoretical training. 59 (38.8%) were trained by herpetologists or snake-handling specialists. 33 (21.7%) were trained by other snake rescuers, 19 (12.5%) were trained by their friends, 11 (7.2%) were trained by ecologists and others were trained by themselves, fire and rescue officials or veterinary care providers. Participants were then asked if they train others when they are rescuing snakes. For non-venomous snakes, 51 (33.6%) of these participants trained others. 45 (29.6%) participants that train others for handling venomous snakes.

Most participants [85 (56%)] needed additional training for effective and safe handling of venomous and non-venomous snakes and the remaining rescuers felt they did not need any additional training. 48 of these rescuers would like both practical and theoretical training, 10 would like only practical training and others stated only theoretical training on venomous snake handling was required. 49 rescuers also required both practical and theoretical training, 9 needed only practical training and 3 required only theoretical training for non-venomous snake rescue.

Working practices of snake rescuers vary widely

Most rescuers [96 (63.2%)] were mainly contacted through the fire and rescue service. 51 (33.6%) rescuers were contacted by in-person messengers, 42 (27.4%) received phone calls from members of the public, 37 (24.3%) received phone calls from any local authorities and 11 (7.2%) were contacted through social media. 59 (38.8%) were contacted through other ways that were not included here. Notably, rescuers were contacted by more than one route on several occasions. 73 (48%) rescuers received more than four calls per day, 35 (23%) received 2-3 calls per day, 10 (6.6%) received one call per day, 16 (10.5%) 2-4 times per week, 7 (4.6%) once per week, 6 (3.9%) more than four times per month, 2 (1.3%) 1-3 times per month and one (0.7%) received calls once per month during peak seasons (usually monsoon period). During non-peak seasons, 9 (6.3%) rescuers were called more than 4 times per day, 14 (9.8%) were called 2-3 times per day, and 22 (15.4%) were called once per day. Moreover, 2 (1.4%) rescuers were called more than 4 times per week, 11 (7.7%) were called 2 to 4 times per week, and 37 (25.9%) were called once per week. 3 (2.1%) rescuers were called more than four times per month, 10 (7%) were called 1-3 times per month, 29 (20.3%) once per month, and 6 (4.2%) rescuers never received any calls during low peak seasons. Most participants [66 (43.4%)] work alone when they rescue snakes. However, 39 (25.7%) stated that they work alone sometimes but mostly they will have an assistant to aid with the removal. Further, 46 (30.3%) rescuers never work alone, and they always have an assistant with them. 107 (70.4%) rescuers stated that they are unable to rescue snakes sometimes. 99 (65.1%) rescuers stated that due to their work commitments, they were unable to attend the rescue calls. 35 (23%) rescuers were unable to attend the rescue calls due to the distance of the location of the rescue from their homes. 31 (20.4%) rescuers were unable to attend due to time constraints, 16 (10.5%) couldn't do so due

to family commitments, 15 (9.9%) due to lack of transport, 15 (9.9%) due to lack of money, 11 (7.2%) due to lack of equipment, 7 (4.6%) misunderstandings of the locations, 4 (2.6%) due to lack of assistance, 4 (2.6%) due to cancellation of services and 13 (8.6%) due to high volume of snake rescue calls.

Mode rescuers use their own transport and complete the rescues in less than an hour

The most used mode of transport to attend snake rescue calls was personal motorbikes [131 (86.2%)]. 12 (7.9%) rescuers used their cars, three (2%) participants took public transport, two (1.3%) walked and one (0.7%) used a bicycle to reach the location of snake rescue. Most rescuers [92 (60.5%)] stated that it took them on average between 16-30 minutes to travel to the location of the snake rescue, 28 (18.4%) stated it took under 15 minutes, 25 (16.4%) took between 31-60 minutes and four (2.6%) took between one-two hours. For non-venomous snakes, 81 (53.3%) rescuers took on average under 10 minutes, 50 (32.9%) took between 10-20 minutes, 18 (11.8%) took 21-30 minutes and 1 (0.7%) took 31-40 minutes to complete the rescues. For venomous snakes, 49 (32.2%) took under 10 minutes, 69 (45.4%) participants took 10 to 20 minutes, 23 (15.1%) took 21-30 minutes, 6 (3.9%) took 31 to 40 minutes, and 1 (0.7%) took 41 to 50 minutes. In other cases, it took longer than the time highlighted here. As venomous snake removals require more due care and attention, it took slightly longer than non-venomous snake removals. Following the successful removal of snakes, 128 (84.2%) rescuers stated that they did not provide the snakes (dead or alive) to the callers as they may kill them. However, 19 (12.5%) rescuers had to hand over the snakes to those people due to their pressure.

Snakes are mostly released in safe habitats

Most rescuers [104 (68.4%)] had rescued over 50 snakes, 8 (5.3%) 41-50 snakes, 5 (3.3%) rescued 31-40 snakes, 11 (7.2%) rescued, 21-30 snakes, 14 (9.2%) rescued between 11-20 snakes, and 10 (6.6%) rescued 1-10 snakes in the last year. 61 (40.1%) rescuers mostly rescued venomous snakes, while 56 (36.8%) rescued mostly non-venomous snakes and 35 (23%) stated there was no difference between the number of venomous and non-venomous snake rescues that they performed. 18 (11.8%) rescuers rescued the most snakes in January, 11 (7.2%) in February, 39 (25.7%) in March, 67 (44.1%) in April, 69 (45.4%) in May, 24 (15.8%) in June, 23 (15.1%) in July, 17 (11.2%) in August, 29 (19.1%) in September, 41 (27%) in October, 68 (44.7%) in November and 55 (36.2%) in December (**Figure 2**). Most rescuers [145 (95.4%)] released the snakes into safe habitats. One rescuer killed the snake and seven handed over the snakes to the forest department. 88 (57.9%) rescuers reported that they maintain accurate records of snake removals, 63 (41.4%) stated they did not and three stated that they either passed the information to their seniors or the forest department.

Snake rescuers take various measures to ensure their safety

117 (77%) stated they avoid distraction and stay focused during the rescues to alleviate any potential harm to the snakes and themselves. Moreover, 93 (61.2%) rescuers said that they were aware of spectators and kept their distance, 82 (53.9%) were able to read the snake's body language including stress levels, 75 (49.3%) kept a snake bag/container ready, 63 (41.4%) knew where to grab the snake, 57 (37.5%) used the right tools e.g. boots and tongs and 39 (25.7%) made their presence known to the snakes. Notably, 39 (25.7%) rescuers did not display the snakes to the public once captured. If indoors, 37 (24.3%) rescuers vacated the people first, and 19 (12.5%) moved large objects or furniture out of the room. If outdoors, 33 (21.7%) cleared the stockpiles, woods and bricks that are kept near the houses. 81 (53.3%) rescuers kept an emergency number to call in case of being bitten, whilst others did not have any emergency contact details. 94 (61.8%) stated they know how to provide first aid in the event of snakebites. Notably, 133 (81.1%) rescuers also educate people during the rescue on the dos and don'ts of snakebites if they are bitten.

Snake rescuers often experience snakebites

76 (50%) participants never had a snakebite incident during their rescues, however, 41 (27%) had non-venomous snakebites only, 20 (13.2%) stated they had venomous snakebites only and 13 (8.6%) had been bitten by both venomous and non-venomous snakes. For non-venomous bites, 11 (7.2%) had been bitten only once, 3 (2%) bitten twice, 9 (6%) bitten 3 times, 3 (2%) bitten 4 times, 2 (1.3%) 5 times, 3 (2%) 6 times, 1

(0.7%) bitten 7 times, 1 bitten (0.7%) 8 times and 9 (6%) over 10 times. All of them spent between INR 1000-5000 for their treatments following non-venomous snakebites. For venomous snakebites, 10 (6.6%) have been bitten once, 2 (1.3%) bitten two times, 1 (0.7%) bitten three times, 3 (2%) bitten four times, 1 (0.7%) bitten five times and 1 (0.7%) bitten more than 10 times. They spent between INR 1000-40,000 for their treatments. 101 (66.4%) rescuers incurred injuries other than snakebites, including cuts, scrapes, strains, fractures, being bitten by a cat, a road traffic accident, head injury and dehydration. The number of bites was classified into zero bites, 1-4 bites and 5+ bites to analyse the correlation between rescuers and their bites and compared with various parameters. The results demonstrate a statistically significant association between the time involved in snake rescues and the number of bites indicating that the longer they are involved in rescues, the more chance of getting bitten by snakes. An association with the forest department, the ability to identify between venomous and non-venomous snakes and undergoing education on snake rescues were not significantly associated with the number of bites (**Table 1**). The results also suggest that there is no strong evidence to demonstrate that the type of training received was associated with the number of snakebites of any type (**Table 2**).

Snake rescuers believe snakebite public awareness activities will mitigate the snakebite burden

141 (92.8%) rescuers would be interested in learning more about snakebites and their public awareness. Moreover, 147 (96.7%) participants would like to have a snakebite awareness programme in their villages to reduce snakebites in their areas and they would attend should there be one. Most rescuers [130 (85.5%)] agreed that village awareness programmes would be useful, 126 (82.9%) participants thought that teaching at schools and colleges would be useful, 51 (33.6%) stated leaflets, 28 (18.4%) posters, 30 (19.7%) TV advertisements, 19 (12.5%) newspaper advertisements, 48 (31.6%) recommended using social media and 13 (8.6%) stated other methods such as street theatre plays and social gatherings.

Snake rescuers face several challenges and need significant support

37 (24.3%) rescuers spent over INR 5000 to provide their services, 12 (7.9%) spent between INR 4000-5000, 24 (15.8%) spent between INR 2000-4000, 21 (13.8%) spent between INR 1000-2000, and 12 (7.9%) spent between INR 500-1000 per year from their pocket to continue to provide their services. 64 (42.1%) rescuers stated that the lack of government support, travelling long distances [62 (40.8%)], transport costs [54 (35.5%)], lack of family support [26 (17.1%)], lack of life insurance [26 (17.1%)], lack of community support [21 (13.8%)], lack of proper equipment [18 (11.8%)], risk of snakebites [12 (7.9%)], lack of education on snakes [8 (5.2%)] and inability to afford treatment if bitten [4 (2.6%)] as the most important challenges they face to provide this service. 120 (78.9%) stated they require assistance with purchasing appropriate handling equipment. 97 (63.8%) stated they needed snake hooks, 75 (49.3%) required boots, 75 (49.3%) needed gloves, 72 (47.4%) needed snake tongs, 71 (46.7%) needed snake bags, and 55 (36.2%) required shin guards. Overall, 83 (54.6%) rescuers stated better equipment, 125 (82.2%) better training, 44 (28.9%) felt more snake rescuers were needed, 34 (22.4%) free/reduced treatment costs in case of injury, 29 (19.1%) liability insurance cover for oneself and dependants considering the risky nature of their role, and 24 (15.7%) access to medical care. 135 (88.8%) stated they would like more snake rescuers to be available closer to their village/town city. 137 (90.1%) stated they would like the government, local politicians, and health services to recognise snake rescuers by providing licensing and identity cards. 10 (6.6%) would like to receive monetary benefits, 4 (2.6%) suggested reimbursement of transportation costs, 7 (4.6%) stated simply gratitude and to be treated with respect, and 3 (2%) stated being issued with appropriate safety equipment.

Snake rescuers are mostly not using appropriate equipment to handle snakes

123 (80.9%) rescuers use snake hooks, 81 (53.3%) use snake bags, 56 (63.8%) wear boots, 35 (23%) use tongs, 30 (20%) wear gloves, and 9 (5.9%) stated they wear shin guards. In some cases, they borrow these tools from others when going for rescues.

Several rescuers stated that snake hooks [35 (87.5%)], snake bags [26 (65%)], boots [17 (42.5%)], snake tongs [12 (30%)], gloves [10 (25%)] and shin guards [4 (10%)] were mainly provided by their employers or NGOs. 20 (69%) participants were given snake hooks, 13 (44.8%) were given boots, 10 (34.5%) were given

gloves, 9 (31%) were given snake bags, 7 (24.1%) were given snake tongs, and 4 (13.8%) were given shin guards by their friends. Personally, 66 (84.6%) rescuers had purchased their snake hooks, 47 (60.3%) bought snake bags, 33 (42.3%) bought boots, 22 (28.2%) purchased snake tongs, 14 (17.9%) bought gloves and 8 (10.3%) bought shin guards (**Table 3**). The results for non-venomous snakebites suggested a statistically significant difference between those who did and did not use a snake bag. The number of non-venomous snakebites was lower for those using a snake bag. This group had a mean of 1.1 non-venomous snakebites per person, compared with a mean of 1.8 bites for those not using a snake bag. None of the other types of equipment were significantly associated with the number of non-venomous snakebites (**Table 4**).

Discussion

India's climatic variability with high temperatures and humidity provides the perfect environment for herpetofauna to thrive and develop abundantly. Historically snakes would live far from human civilisation in dense forests and near water sources. As the country's population continues to grow at an advanced pace, so too does its demand for space and resources. This has resulted in anthropogenic expansion into rural areas and the conversion of forests into arable land. This deforestation, habitat degradation and loss has displaced snakes and induced a decline in herpetofauna not just in India alone but in developing nations globally [5]. This displacement is causing a shift in the habitat ranges of snakes bringing them into closer proximity to humans and finding themselves inside human dwellings. Snakes are drawn to human structures due to the abundance of prey (rodents and amphibians) and favourable hiding spots and shelter. Historical fear of snakes and lack of public awareness and knowledge on being able to distinguish between venomous and non-venomous species leads to increasing human-snake conflicts. The rate of population growth shows no signs of slowing down, especially with India's burgeoning population which drives development into more and more diverse wildlife habitats. This not only serves to lead to an increase in human-wildlife conflicts but also to the development of human-snake conflict. Snake rescuers play a vital role in mitigating human-snake conflict, and calls for snake removals usually come from towns, villages, and fringes of forest areas where snakes commonly dwell [6].

India has vast biological diversity as well as reptile and snake diversity in line with its vast geographical size and features; with over ~300 snake species, ~60 of which being venomous inhabiting various habitats, it is no surprise that human encroachment threatens their survival and increases the risk of SBE. The Indian Wildlife Protection Act of 1972 legislates the safeguarding of all animals which means no snake species should be handled without permission from the relevant forestry department. Habitats have their distinct ecosystems and human movement often damages and can destroy these delicate balances, causing further issues for other wildlife as well as snakes. The lack of developed infrastructure and lack of snakebite awareness campaigns and public health measures to deal with snakebite envenomation compounds the mortality and disability rates reported. Community snake rescuers therefore play an incredibly important and overlooked role in not only rescuing snakes but also venomous snakes' potentially preventing SBE. It is important to recognise that snake rescue and snake handling is a highly skilled vocation and poses significant challenges to the rescuer especially if the snake concerned is venomous. There are great social, religious, and superstitious beliefs when it comes to snakes and this fear is more pronounced in rural communities. Fear and phobia of snakes can result in their indiscriminate killing along with it being viewed as an effective SBE preventative measure.

Currently, snake rescuers are local volunteers who take it upon themselves to help their local community and save snakes. Very few have received formal training and use inappropriate or no equipment that puts them at higher risk of being bitten. While most rescuers are sensible and act out of generosity and care to the community, some are behaving inappropriately, using their status as snake rescuers to gain fame and recognition on social media. These people post videos of themselves dangerously handling snakes to gain views and notoriety, risking their lives and the lives of the public for views and likes. This kind of behaviour is dangerous, not only to those nearby but also to those who watch the videos and try to mimic this behaviour afterwards. The influence of this dangerous behaviour on impressionable minds can be profound, as it teaches

inappropriate handling techniques that can be fatal, but also influences the wider public and youngsters into believing that it is ok to play with snakes. This type of behaviour has resulted in numerous envenomation and hospitalisations, which are putting an unnecessary demand and a financial burden on the health service, as well as hindering vital care for those that need it most. Snakebites can be avoided during snake rescue if snake handling is properly controlled and regulated.

Our study highlights some of the key challenges faced by snake rescuers. The main underlying motivation which drives their role is their keen interest in snakes, conservation and helping their respective communities. However, to do this communities are relying on the goodwill of snake rescuer to be able to avert their professional commitments if this is not their primary work and potentially travel far distances incurring out-of-pocket expenses and with a lack of suitable equipment to fulfil the communities' request. This is not an easy task, especially when it comes to venomous snakes and the stakes are much higher in case they are bitten. To encourage and ensure communities have close access to well-trained snake rescuers more well-trained snake rescuers are needed. Fear of unfair stigmatisation and discrimination is also a prevalent thought for many snake rescuers as sometimes snake rescue is considered a 'lowly' occupation. Having endorsement by local government for their essential and valuable role in society is much needed as snake rescuers yearn to be officially recognised for the work that they do. The ability of communities to be able to call upon snake rescuers highlights an opportunity for snake rescuers to not only provide a service-saving snakes but also to impart valuable SBE awareness to afflicted communities. The ability to recognise and identify non-venomous and venomous snakes can reduce their indiscriminate killing. Moreover, if communities were aware of the important role of snakes and reptiles as predators this would aid in greater awareness of their conservation. Therefore, encompassing snake rescuers in valuable awareness activities can serve as a ground-up approach to reducing the SBE burden. Moreover, uneducated low-income agricultural workers are most at risk of snakebite envenomation, with up to 72% of bites occurring while working in the field [7]. Educating more agricultural workers about snakes and training them about safe handling procedures would help to reduce the rate of incidences, these workers could also act as ambassadors for the protection and safe handling of snakes.

Snake rescuers require appropriate training on not just snake identification and rescue but also appropriate translocation as the ability to identify suitable habitats avoids any further damage to India's reptile fauna. Thus, snake rescuers must be trained in snake handling as well as snake identification and relocation, by meeting an agreed-set minimum set of requirements to be able to provide this service. Our study has shown that in the majority of cases, the snakes are released back into the wild after being rescued. However, it is imperative to identify if the new habitat of release is also suitable to support the growth and development of captured snakes, otherwise, the unfortunate outcome will also be death [8] [9] [10]. To avoid unnecessary reptile destruction, it is important that snake rescuers can identify this, allowing high-risk species to remain protected.

As wildlife species are protected by legislative measures the volunteers conducting this altruistic work must be also appropriately regarded in terms of their training, identification with members of the public and noted for the service they provide. Appropriate alignment with the government forestry departments has been conducted in other Indian states, such as Maharashtra. Alignment with the local government forestry departments can provide snake rescuers with the foundation and courage to continue their vital work without any societal ramifications. Also, as they are involved in a high-risk profession, they need to be provided with the right equipment for the right job and access to free medical care in case of SBE. Having equipment needs standardised and provided free of charge for use can result in their increased uptake as well as any unnecessary accidents to ensure all safety protocols are adhered to. This can ensure snake rescuers are appropriately qualified to conduct the task at hand with minimal risk to themselves and the communities they work for.

India is run by a central government, with local state governments in charge of their state, this results in different regulations being in place between states. Currently, in Tamil Nadu, there is no protocol in place for snake removals or reporting incidences of snakes in the home. Kerela a neighbouring state has implemented its regulations surrounding snake removals and rescuers. They have brought in legislation controlling who is

permitted to perform snake removals, these snake rescuers are awarded licenses that permit them to perform routine snake removals and relocations. Stipulations for the license include a clear background check and mandatory attendance of snake handling training. Licenses can be withdrawn if snake rescuers are reported to be behaving inappropriately, putting the lives of the snake, themselves, or the public at risk with their actions or if they are no longer deemed as being medically fit or competent to perform the task safely. Licensed rescuers will receive professional training to handle and remove snakes safely, proper safety and handling equipment for removals and health insurance that covers medical expenses associated with snakebites.

Snake rescue is a high-risk occupation and is conducted unwaveringly by small communities of volunteers' location and distance, other occupational requirements and lack of equipment and medical coverage severely hinder their ability to conduct this work. Our study recommends appropriate training and authorisation to perform this work, insurance to be covered in case of snakebites and the ability to recruit more volunteers so communities can easily access a snake rescuer once needed. As snake rescuers are the first person of authority on the ground, utilising this ability to impart SBE awareness and conservation knowledge would also be critical to mitigating SBE in rural communities.

Conclusions

Snake rescuers are vital to conserving snakes as well as mitigating SBE. National protocols on snake rescue considering regional species specificity and venomous and nonvenomous snakes are needed along with appropriate training and accreditation. This is already being implemented in some states in India, however, national coverage can serve to standardise training and help mitigate the SBE burden as well as protect snakes in line with the Indian Wildlife Protection Act of 1972. Appropriate tools, their use along record keeping are important to track activities along with recording vital ecological data on snakes, which could also highlight areas of concern where more snake rescuers may be needed. Snake rescuers play an important public service role, utilising this to impart vital public health measures on SBE prevention and basic first aid would serve a dual purpose in reducing SBE mortality.

Materials and Methods

Data collection

Data was collected from 154 snake rescuers using an online survey distributed across Tamil Nadu, India. 152 consented to take part and 2 withdrew consent. The information was collected between April 2022 to June 2022.

Statistical Methods

All statistical analyses were performed using SPSS (IBM, USA) and R (Lucent Technologies, USA) to evaluate the association between the snake rescuers and snakebites.

The analyses focussed on the association between the number of characteristics of the rescuers and the number of snakebites received. The data suggested that the number of snakebites had a very skewed distribution, with a large group receiving no snakebites. As a result, this outcome was categorised for analysis into three groups: 0, 1– 4, and 5 or more snakebites. All characteristics of the snake rescuers were categorical. As a result, the association with the number of snakebites (on a categorical scale) was assessed using the Chi-square test. The exception was for the ability to recognise venomous snakes, where Fisher's exact test was preferred due to the small numbers in one of the groups.

Figure 1: A) A geographical heatmap displaying participant responses from the 38 districts in Tamil Nadu, India. Districts with the highest response rate are highlighted in 'red' as they received over 21 responses. Districts that had between 11-20 responses are coloured in 'orange', and districts that had between 6-10 responses are highlighted in 'yellow. The districts with the lowest response rates between 1-5 are highlighted in 'green'. Districts with no responses are coloured in 'grey'. The gender (**B**) and age breakdown (**C**) of snake rescuers.

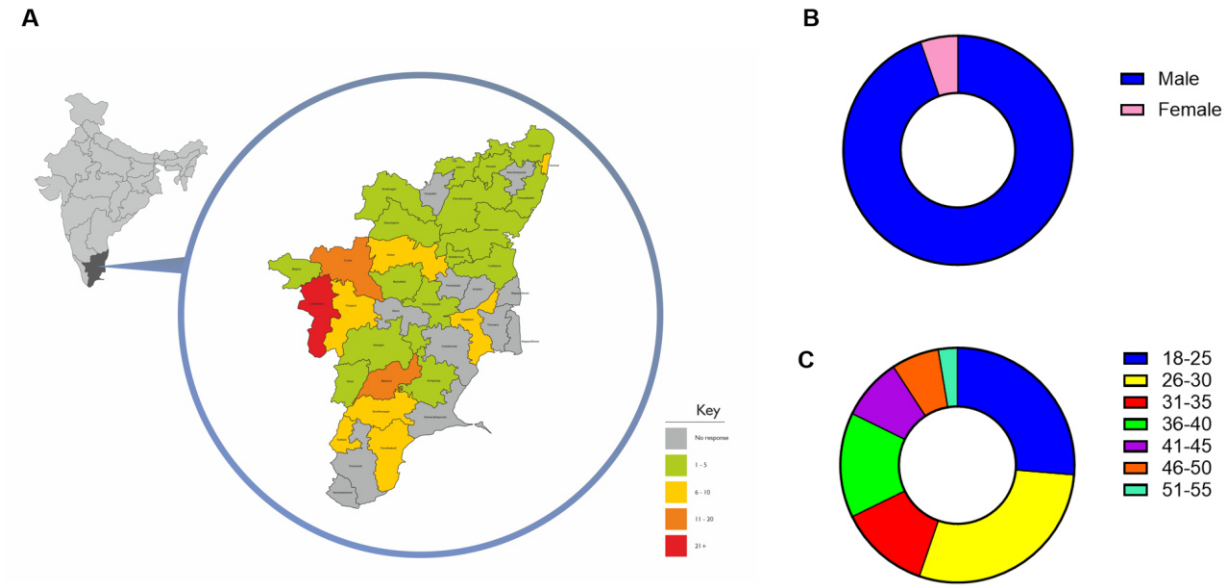


Figure 2: Months of the year in which most snakes were rescued by the snake rescuers.

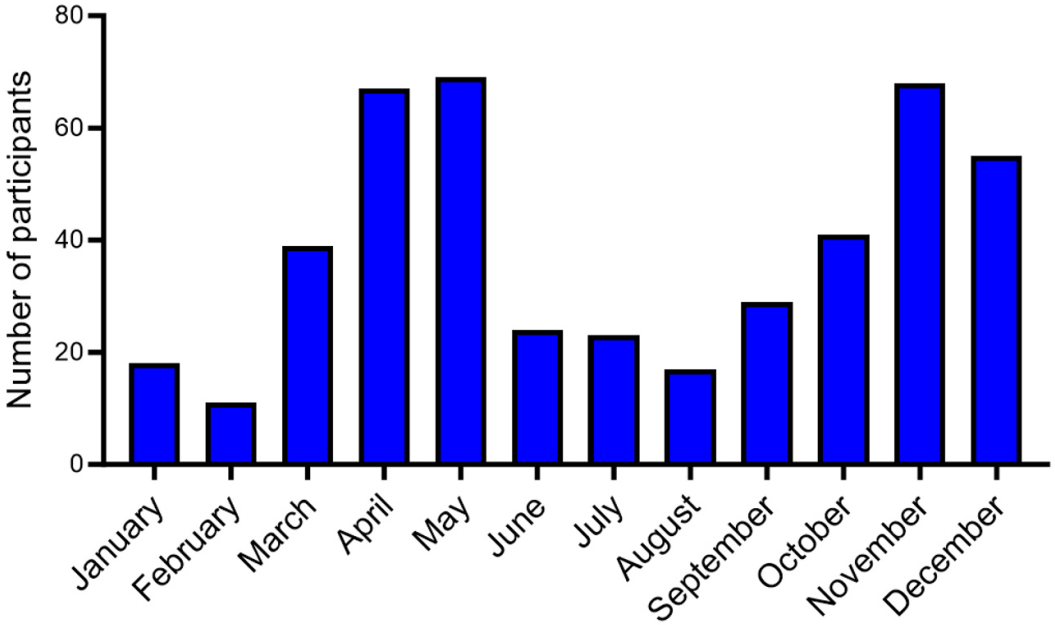


Table 1: The association between time involved in snake rescue, snakebites and the association with the forestry department, ability to identify venomous snakes and education on snakes.

Group	Category	0 bites n (%)	1 – 4 bites n (%)	5+ bites n (%)	P-value
Time involved in snake rescue	<4 years	35 (76%)	7 (15%)	4 (9%)	**0.004**
	4-10 years	24 (43%)	21 (38%)	11 (20%)	
	>10 years	21 (42%)	22 (44%)	7 (14%)	
Associated with forest department	No	37 (55%)	21 (31%)	9 (13%)	0.94
	Yes	43 (52%)	27 (33%)	12 (15%)	
Able to identify venomous snakes	No	4 (80%)	1 (20%)	0 (0%)	0.40
	Yes	74 (51%)	49 (34%)	22 (15%)	
Education on snake rescue	No	23 (44%)	21 (31%)	8 (15%)	0.27
	Yes	58 (57%)	29 (33%)	14 (14%)	

Table 2: Associations between type of training and number of snakebites. Types of training were either practical, theoretical or both.

Training type	Outcome	Specific Training	n	Number bites Mean ± SD	P-value
Non-venomous snakes	Non-venomous bites	No training	57	1.2 ± 2.6	0.57
		One type	26	1.5 ± 3.0	
		Both types	69	1.6 ± 3.1	
Venomous snakes	Venomous bites	No training	59	0.7 ± 1.8	0.06
		One type	29	0.1 ± 0.3	
		Both types	63	0.3 ± 1.0	

Table 3: Equipment used by snake rescuers and their respective ownership.

	Boots	Shin guards	Snake hook	Snake tongs	Gloves	Snake bag
Equipment used	56 (37.6%)	9 (6%)	123 (82.6%)	35 (23.5%)	30 (20.1%)	81 (54.4%)
Provided by employer or NGO	17 (42.5%)	4 (10%)	35 (87.5%)	12 (30%)	10 (25%)	26 (65%)
Provided by personal friends	13 (44.8%)	4 (13.8%)	20 (69%)	7 (24.1%)	10 (34.5%)	9 (31%)
Purchased personally	33 (42.3%)	8 (10.3%)	66 (84.6%)	22 (28.2%)	14 (17.9%)	47 (60.3%)
Borrow when needed	4 (57.1%)	2 (28.6%)	3 (42.9%)	2 (28.6%)	3 (42.9%)	4 (57.1%)
Not applicable – I do not use these tools	6 (85.7%)	5 (71.4%)	4 (57.1%)	6 (85.7%)	4 (57.1%)	3 (42.9%)

Table 4: Associations between equipment used and number of snakebites.

Equipment type	Number		Number		P-value
	n	Mean ± SD	n	Mean ± SD	
Non-venomous bites					
Boots	96	1.6 ± 2.9	56	1.2 ± 2.9	0.07
Shin guards	143	1.4 ± 2.8	9	2.4 ± 3.6	0.15
Snake hook	30	1.3 ± 3.0	122	1.5 ± 2.9	0.59
Snake tongs	117	1.5 ± 2.9	35	1.3 ± 2.8	0.76
Gloves	122	1.2 ± 2.6	30	2.5 ± 3.8	0.05
Snake bag	72	1.8 ± 3.2	80	1.1 ± 2.6	**0.04**
Venomous bites					
Boots	95	0.4 ± 1.1	56	0.4 ± 1.6	0.12
Shin guards	142	0.5 ± 1.3	9	0.0 ± 0.0	0.13
Snake hook	30	0.2 ± 0.8	121	0.5 ± 1.4	0.16
Snake tongs	116	0.4 ± 1.0	35	0.7 ± 2.0	0.51
Gloves	121	0.4 ± 1.3	30	0.4 ± 1.3	0.41
Snake bag	71	0.6 ± 1.8	80	0.2 ± 0.6	0.26

References

1. Chippaux, J.P., *Snakebite envenomation turns again into a neglected tropical disease!* J Venom Anim Toxins Incl Trop Dis, 2017. **23**: p. 38.
2. Kasturiratne A, W.A., de Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, Savioli L, Lalloo DG, de Silva HJ, *The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths.* PLoS Med, 2008. **5**(11): p. e218.
3. Suraweera, W., et al., *Trends in snakebite deaths in India from 2000 to 2019 in a nationally representative mortality study.* Elife, 2020. **9**.
4. Mohapatra, B., et al., *Snakebite mortality in India: a nationally representative mortality survey.* PLoS Negl Trop Dis, 2011. **5**(4): p. e1018.
5. Gibbons, J.W., D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. Metts, J. L. Greene, T. M. Mills, Y. Leiden, S. M. Poppy, and C. T. Winne. , *The global decline of reptiles, Deja vu amphibians.* BioScience, 2000. **50**: p. 653-666.
6. R, R.J., Dusi., *A Review on Wildlife Rescue Activities in North Kerala, India. 143. 1004-1010.* . Indian Forester, 2017. **143**.
7. Vaiyapuri, S., et al., *Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India.* PLoS One, 2013. **8**(11): p. e80090.
8. Cornelis, J., *Killing them softly: a review on snake translocation and an Australian case study.* Herpetological Journal, 2021(Volume 31, Number 3): p. 118-131.
9. Devan-Song, A., et al., *Is long-distance translocation an effective mitigation tool for white-lipped pit vipers (Trimeresurus albolabris) in South China?* Biological Conservation, 2016. **204**: p. 212-220.
10. Reinert, H.K., & Rupert, R. R. , *Impacts of Translocation on Behavior and Survival of Timber Rattlesnakes, Crotalus horridus.* Journal of Herpetology, 1999. **33**(1): p. 45–61. .

General conclusions

Snakebite envenomation (SBE) is a neglected tropical disease affecting the rural impoverished in many tropical and subtropical countries. Millions of people in these countries are deeply affected globally with upwards of 2.7 million cases of snakebite envenoming, resulting in approximately 140,000 deaths. Southeast Asia bears one of the largest snakebite burdens and India is estimated to account for half of the resultant global deaths and disabilities [1]. It is widely agreed that the current statistics despite serving to baseline the epidemiology of the disease are greatly underestimated due to the lack of sufficient reporting, data acquisition and health infrastructure of these countries, very much akin to other neglected tropical diseases in the same orbit.

As with many developing countries, the human-wildlife conflict in India has been steadily increasing as the country continues to develop. The cost of this conflict is always at the expense of humans and principally the agro-pastoral communities across India; from elephants, (*Elephas maximus*) to tigers (*Panthera tigris*) and rhinoceros (*Rhinoceros unicornis*), the venomous snakes of medical importance in India now take centre stage and have ecological implications especially on the conservation of India's diverse reptile fauna [2] [3].

India is one of the most populous countries in the world with an estimated 1.4 billion people calling the country home. According to recent World Bank surveys, the agricultural sector is the largest source of livelihood for Indians, and so most rural households depend on this as their primary source of income. The 'big four' venomous snakes of India the common krait (*Bungarus caeruleus*), Indian cobra (*Naja naja*), Russell's viper (*Daboia russelii*) and saw-scaled viper (*Echis carinatus*) are of the most medical importance as they contribute greatly to the fatalities, resulting injuries, trauma, and disability.

There are distinct and varied multisectoral issues that must be addressed to counter SBE numbers in India from the lack of field data, level of public health importance, especially since it is not a notifiable indication, public health infrastructure, government spending and policy development. When it comes to treating SBE there is a lack of standardised treatment protocols across the country, ASV effectivity and availability, a lack of trained professionals, systemic illiteracy, and a lack of training for healthcare providers. Moreover, treatment for patients can be further complicated by the venom variability in Indian snakes and the multitude of clinical manifestations observed following SBE. The patient's pathway to care is limited due to a lack of patient awareness of access to care and health-seeking behaviour, reliance on traditional healers, and fear of extreme treatment costs at tertiary healthcare facilities.

This thesis aimed to dissect some of these variables and dive deeper into some of the compounding issues to elucidate how to affect positive change, the essential factors with high contributary value to the SBE issue, and how to effectively mitigate these to save lives. Our study was built upon foundational work that highlighted working-age adults are most at risk of SBE and as a result, have negative and pronounced socioeconomic impacts on families and their agricultural communities [4] [5].

The ability to detect the offending snake from an SBE event is critical for healthcare professionals when it comes to correctly diagnosing the patient as well as determining the antivenom needed to save their life. It is well regarded that providing appropriate treatment as soon as possible after the bite is crucial for patients, and lengthy delays result in a much worse prognosis for patients and a more complex clinical burden for healthcare professionals attempting to treat the patient. At present India does not have any diagnostic tests available on the market that can be used to effectively detect which snake patients have been bitten by. Instead, clinicians rely on patient symptomatology and clinical signs such as vital envenomation effects e.g. coagulopathies in Russell's viper patients, ptosis because of cobra envenomation etc. The various pathologies associated with the hemotoxic, neurotoxic, and neuromuscular paralyses effects of venom can overlap across the offending snake due to the molecular complexities of venom and their multitude of biological consequences. This complicates the process of treatment for clinicians as they are forced to conduct multiple medical tests increasing the time to treatment for patients as well as contributing to increased final costs to the patient. In the absence of a regulatory-approved diagnostic test novel biomarkers require investigation that can be utilised in this setting.

In our study, we wanted to investigate which early biomarkers can be utilised for AKI as a serious medical complication which greatly affects Russell's viper bite patients. Here we identified NGAL as a suitable early diagnostic biomarker of AKI in an SBE event. Our work highlighted and recommended (where possible) the use of NGAL at point-of-care testing from plasma samples from SBE patients to initiate RRT as early as clinically possible to reduce AKI and further renal damage. This also reduces complex renal care for patients, which would significantly reduce the treatment costs. So far, the outcomes of this work have been instrumental to healthcare providers in India with them already initiating NGAL testing, and the outcomes of this study must be promoted to save more lives. Future work on a larger scale with a larger sample size and whether NGAL can be used as a biomarker in patients bitten by other snakes requires development.

The socioeconomic ramifications of a single SBE event on rural populations are poorly understood and previous studies suggest the fear of high treatment costs prevents patients from seeking access to healthcare to treat SBE [4]. To determine the key factors contributing to treatment costs for patients we investigated various facets of SBE treatment and their associated costs across several private

tertiary healthcare settings in Tamil Nadu, India. It was determined that on average most patients (~80%) are left with a hospital bill of ~INR100,000 or less, which was equivalent to ~$1000 US dollars. Delays in seeking care create medical complications which in turn increases the end costs to the patient, as it can result in more involved medical care such as surgical interventions, dialysis, ventilation support, extended hospitalisation, and administering more complex lifesaving medication. The notion of avoiding immediate care by SBE victims and their families to save on final treatment costs is a destructive fallacy. This study served to highlight that communities require better awareness regarding the risks and costs associated with delayed care. Appropriate medical insurance as well as better regulation of costs in private healthcare settings need to be developed further so that patients avoid financial destruction because of a single SBE event.

To mitigate the financial burden of high treatment costs we aimed to understand the implications of antibiotic interventions as wound infections are usually secondary to SBE. Moreover, delayed healing and chronic wounds can be life-threatening leading to surgical debridement, sepsis, and even multiple organ failure all of which would eventually serve to exacerbate the final treatment costs to the patient. Deploying the most cost-effective antibiotic therapy to the patient for the specific bacterial strain they are infected by aids clinicians in making the right choice for patients from not only an economic standpoint but also when considering the threat and rise of widespread global antibiotic resistance. Our study therefore aimed to provide a greater understanding of snakebite wound infections and their associated costs, with most of our data derived from Russell's viper bite patients. It was evident that the treatment costs can vary depending on the antibiotics administered and our work confirms that antibiotics should only be used in SBE victims when the clinical diagnostic criteria for a wound infection have been met rather than administered as a precautionary measure. Antibiotic resistance is a global issue, and any unnecessary administration hastens this process which would only be even more detrimental to rural agricultural communities. Our work here highlights the need for healthcare providers and clinicians to be aware of diverse and clinically effective treatment strategies as well as providing affordable treatment to patients.

Health systems require healthcare workers, from community support workers to technicians and clinicians as they all play a vital role in caring for millions of people. Our research shows that despite the high burden of SBE in India, there is no standardised training or curriculum on SBE for healthcare professionals across India. Knowledge of snakebites amongst all healthcare professionals and clinical management and treatment guidelines vary across India. To determine change and or detect improvement in this discipline we were first required to baseline the level of snakebite education and awareness of all healthcare professionals across India.

Our work highlighted that awareness of snakebites along with the ability to identify and name the 'big four' snakes was limited along with basic first aid application and grasping the importance of various

environmental and ecological measures to prevent SBE all of which were poorly understood. Healthcare professionals across India are neither confident in treating snakebites nor in their clinical management, they also self-classify their current knowledge as being limited. Importantly, the level of exposure to snakebite patients or years of experience acquired in their profession has no bearing on their SBE knowledge. Our study identifies some key gaps and illustrates that greater training and education are needed for healthcare professionals to improve their clinical management skills relating to SBE. Most importantly our study demonstrated that healthcare professionals are engaged, open and motivated to learn more and acquire such clinical skills. A developed curriculum on SBE management in the Indian context is needed for every professional from community healthcare workers to clinical consultants who require improved basic first aid knowledge to help save patients' lives as well as reduce the clinical manifestations seen later which contribute to escalated costs for the patient. This must also include the ecological and environmental measures patients at risk can take to prevent further bites in their communities. The curriculum must be inclusive for all in the healthcare chain and compulsory training modules should be introduced. This is also in line with the WHO's goals to strengthen the Indian healthcare system. Training should be well integrated from a local to national level. Continued professional development and skills that are relevant to snakebite management must be taught. The development and implementation of learning tools and regional guidelines are needed to improve the administration of first aid, clinical diagnosis, and treatment and must also consider the mental health toll of an SBE event, especially in cases where long-term physical rehabilitation is needed. Overall, these would serve to improve treatment outcomes for patients and foster the development of trust in the healthcare service.

At present we do not have a comparable model country with effective SBE prevention strategies, that endemic countries such as India can follow; and to date, no widescale SBE prevention campaign in any SBE-affected country has occurred (not limited to smaller campaigns by NGOs). In fact, since the million-death study published in 2011, we have no further defined data on whether the situation in the country has deteriorated remained the same or improved [6] [7]. The WHO wishes to halve the deaths due to SBE by 2030, to meet the sustainability goals, however, the Indian SBE problem has many nuances.

To prevent SBE, we had a dual focus, firstly to create engaging awareness materials to drive public awareness by including relevant elements to ensure our health messaging was effective and secondly to affect change and health behaviours in the younger generations to instil unprompted health responses to effectively educate children, the country’s future on SBE awareness. We targeted to conceptualise a campaign in Tamil Nadu to increase SBE awareness building on previous foundational research by stakeholders which indicated what was needed and wanted as well as to normalise conversations on SBE prevention using multimedia, digital, print and television to reach wider audiences. The awareness campaign aimed to use a creative approach to help individuals access

preventable actions they can undertake as well as better understand actions to take in case of snakebite. Having a two-pronged approach looking at prevention to empower communities and information dissemination aided communities to be more informed when it came to better care for patients and to encourage seeking medical treatment promptly.

Accessing public health information is challenging in developing countries with high illiteracy and low literacy rates and educational strategies and public health information must utilise and consider these target populations, for example, individuals that live in more developed urban areas with access to education have access to health information easier. This requires a wide range of educational tools as well as innovative approaches to convey useful information to help change lives. Thus, we wanted to design a campaign that was accessible to everyone and considering our target population, we wanted to introduce our messaging in the simplest yet engaging manner.

We created easily identifiable culturally relevant graphics and fictional cartoon 'characters' to be used for health information campaigns for snakebites. To ensure consistency in messaging these were developed into a wide range of materials such as posters, leaflets, books, colouring books as well as animations.

The Indian film industry, 'Bollywood', is a central part of Indian culture and its popularity is wide-reaching and cinematic productions have been shown to impact social and public health awareness [8]. Thus, it was critical for us, despite limited funding to also produce animations as a tool for public awareness. Creating and delivering animations with appropriate formatting was important as it allows anyone with mobile smartphone access to view the animation and it can be played at any time even when a device does not have access to the internet [9]. In this way, local health professionals as well as individuals can view the animation on any given portable device to disseminate the information widely. Animations therefore serve as an innovative way of capturing the public with low levels of education as well as children who will not yet have been exposed to formal learning or who may not be, due to their socio-economic situation. The visual impact of graphics is understood to enhance learning goals and outcomes as well as greater learning gains overall [10] [11] [12].

It is important to note that there is currently no government funding to create or conduct SBE campaigns and when one reviews the budget set forth by the WHO in their strategy for control and prevention it is clear that campaigns of this nature require a heavy investment [13]. Scientifically and medically relevant public health campaigns require important information to be distilled to the key messaging most needed by the communities affected. This requires various trained individuals such as animators' illustrators and focus groups and is challenging to ensure relevancy. Funding for neglected tropical diseases is limited and so funding for campaigns such as this is also limited.

The comprehensive range of tools developed can now be disseminated in multiple formats and are suitable for both adults and children alike and help us achieve our aims in educating SBE endemic

communities. A multimedia approach despite high initial investment is considered to also be the most cost-effective way of disseminating information [14]. The success of these tools meant that they eventually achieved local government backing and endorsement by the Government of Tamil Nadu, India.

Considering India's complex historical and religious relationship with snakes along with snakebites being an endemic issue, it was important to determine what role this may play within the societal framework and what impact this has. Anecdotal reports from our fieldwork suggested that the fear and or phobia of snakes was a contributory factor in people killing snakes and the culling of such was conducted to protect their households from SBE. This facet of the human-snakebite conflict greatly impacts the diversity of the reptile and snake fauna in India. Moreover, it was important to underpin the psychological characteristics SBE can have on communities as current mental health work on SBE is developing and research suggests that the impact of SBE has a far more lasting psychological impact on patients. To understand this better, we first sought to identify the level of snake phobia present in a snakebite-endemic country and our study utilised the SNAQ12, a clinically reputable psychometric questionnaire that can easily determine if a patient has a snake phobia or not. Our study was the first to be conducted in a snakebite endemic country and contrary to previous work highlighted unique gender differences whereby males are more snake phobic than females. Various hypotheses have been proposed for this principally it could be a result of more negative exposure due to more males conducting agricultural work. This study allows us to focus our efforts when it comes to awareness campaigns as well as mitigate the unnecessary killing of snakes and encourage wider ecological awareness. Additionally, the results of this work help us to apply them to a clinical setting to reduce the mental health burden of developing phobias in SBE patients subsequently.

India is a developing country with an expanding population amid this rapid development loss of natural habitats is becoming more pronounced and is a cause for concern. This is especially important when one considers the human snakebite conflict as more snakes enter homes searching for food sources. This results in communities relying on snake rescuers to retrieve them and release them in habitats further away from humans. Saving snakes is a risky business and the young men and women who take up this service are often in harm's way and unfortunately facing deadly consequences because of SBE. To mitigate the human snakebite conflict and increase greater awareness of snake ecology and conservation we needed to determine the challenges faced by such snake rescuers. Our work highlighted that effective training on venomous and non-venomous snakes is needed from a reputable source and that this training must be accredited so that snake rescuers do not face discrimination as a result of their important work and also to provide them with recognition for their public service. We must acknowledge the risks they face in being able to perform their duty and we must ensure that they have access to the necessary equipment, insurance as well as access to care in case of an envenomation event. Furthermore, having appropriate accreditation and training means that

snake rescuers can serve a dual purpose in serving their communities by imparting valuable knowledge and advice to afflicted communities in a ground-up approach, thus saving lives.

Our work here highlights the promising outcomes of utilising a one-health approach across the various facets of the snakebite issue and how there is no single lone solution. We would like to encourage various sectors of society to unify aims and objectives in achieving these milestones. This thesis marks the start of a journey towards increasing awareness of SBE in communities in India as well as empowering them with the tools to prevent snakebites, access care and achieve our mission of reducing snakebite deaths and disabilities and serving as part of a wider programme of change.

Action points

Recommendations for the public

- We must ensure that general members of the public have appropriate access to and awareness of SBE prevention measures.
- The pathway to seeking care is unobstructed and awareness of the importance of seeking treatment signified to prevent unnecessary loss of limbs and disability.
- Adequate insurance coverage is provided to the people most at risk of SBE and healthcare bureaucracy is minimised to ensure uptake to prevent the socioeconomic impact of SBE.

Recommendations for healthcare professionals

- Healthcare professionals are adequately trained on the basics of treating SBE especially frontline community workers who will often see patients first before any clinical staff.
- Healthcare providers need to be aware of the mental health burden post-recovery for SBE patients.
- Healthcare providers should also be able to advise patients on awareness and prevention of future snakebites.
- Clinicians are aware of appropriate biomarkers and clinical testing criteria as well as ASV administration to improve patient outcomes and reduce the financial burden on patients.
- Healthcare professionals must be mindful of the socioeconomic impact of SBE on patients so improving clinical treatment along with increased patient awareness should serve to reduce this.

Recommendations for the Government

- Improved public health policy and involvement are needed by the government in SBE is imperative, to not only capture SBE data but to instil it to be a notifiable disease.
- The development of a compulsory medical curriculum for all healthcare professionals and continued professional development is required.
- Authorization and accreditation of snake rescuers are needed to ensure snake species are conserved and to avoid untrained individuals becoming harmed.
- Support awareness activities by NGOs and partner organisations.
- Support insurance schemes for some of the poorest communities to alleviate the socioeconomic burden for patients.
- Better regulation and alignment of tertiary healthcare centres and hospitals to ensure there is no regional variation in costs for patients.

References

1. Suraweera, W., et al., *Trends in snakebite deaths in India from 2000 to 2019 in a nationally representative mortality study.* Elife, 2020. **9**.
2. Shaffer, L.J., et al., *Human-Elephant Conflict: A Review of Current Management Strategies and Future Directions.* Frontiers in Ecology and Evolution, 2019. **6**.
3. Gulati, S., et al., *Human casualties are the dominant cost of human-wildlife conflict in India.* Proc Natl Acad Sci U S A, 2021. **118**(8).
4. Vaiyapuri, S., et al., *Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India.* PLoS One, 2013. **8**(11): p. e80090.
5. Williams, H.F., et al., *Challenges in diagnosing and treating snakebites in a rural population of Tamil Nadu, India: The views of clinicians.* Toxicon, 2017. **130**: p. 44-46.
6. Mohapatra, B., et al., *Snakebite mortality in India: a nationally representative mortality survey.* PLoS Negl Trop Dis, 2011. **5**(4): p. e1018.
7. Jha, P., et al., *Prospective study of one million deaths in India: rationale, design, and validation results.* PLoS Med, 2006. **3**(2): p. e18.
8. Khattri, N.a.S., Arshita, *Role of Bollywood Cinema in Shaping Youngersters for Social Awareness.* PSYCHOLOGY AND EDUCATION 2021. **58**(2): p. 6243-6247.
9. Bohonos, J., et al., *Program Planning and Animated Videos as Learning Tools in Sub-Saharan Africa.* International Journal of Adult Education and Technology, 2022. **13**(1): p. 1-20.
10. Bello-Bravo, J., et al., *An assessment of learning gains from educational animated videos versus traditional extension presentations among farmers in Benin.* Information Technology for Development, 2017. **24**(2): p. 224-244.
11. Julia Bello-Bravo, I.B., *Animated Videos as a Learning Tool in Developing Nations: A Pilot Study of Three Animations in Maradi and Surrounding Areas in Niger.* EJISDC The Electronic Journal of Information Systems In Developing Countries, 2017. **55**(1).
12. Pate, J.W., et al., *Creating online animated videos to reach and engage youth: Lessons learned from pain science education and a call to action.* Paediatr Neonatal Pain, 2020. **2**(4): p. 131-138.
13. WHO, *World Health Organization: Snakebite envenoming: a strategy for prevention and control* 2019.
14. Allom, V., et al., *Comparing the Cost-Effectiveness of Campaigns Delivered via Various Combinations of Television and Online Media.* Front Public Health, 2018. **6**: p. 83.

Appendices

toxins

MDPI

Article

Repurposing Cancer Drugs Batimastat and Marimastat to Inhibit the Activity of a Group I Metalloprotease from the Venom of the Western Diamondback Rattlesnake, *Crotalus atrox*

Harry J. Layfield [1,†], Harry F. Williams [1,2,†], Divyashree Ravishankar [1], Amita Mehmi [1], Medha Sonavane [1], Anika Salim [1], Rajendran Vaiyapuri [2], Karthik Lakshminarayanan [2], Thomas M. Vallance [1], Andrew B. Bicknell [3], Steven A. Trim [4], Ketan Patel [3] and Sakthivel Vaiyapuri [1,*]

1 School of Pharmacy, University of Reading, Reading RG6 6UB, UK; harrylayfield@gmail.com (H.J.L.); harry@toxiven.com (H.F.W.); divyasri.april86@gmail.com (D.R.); A.Mehmi@student.reading.ac.uk (A.M.); m.sonavane@pgr.reading.ac.uk (M.S.); anika.salim@pgr.reading.ac.uk (A.S.); T.M.Vallance@pgr.reading.ac.uk (T.M.V.)
2 Toxiven Biotech Private Limited, Coimbatore, Tamil Nadu 641042, India; raj@toxiven.com (R.V.); karthik@toxiven.com (K.L.)
3 School of Biological Sciences, University of Reading, Reading RG6 6UB, UK; a.b.bicknell@reading.ac.uk (A.B.B.); ketan.patel@reading.ac.uk (K.P.)
4 Venomtech Limited, Sandwich, Kent CT13 9ND, UK; s.trim@venomtech.co.uk
* Correspondence: s.vaiyapuri@reading.ac.uk
† These authors contributed equally to this paper.

Received: 16 April 2020; Accepted: 7 May 2020; Published: 9 May 2020

Abstract: Snakebite envenomation causes over 140,000 deaths every year, predominantly in developing countries. As a result, it is one of the most lethal neglected tropical diseases. It is associated with incredibly complex pathophysiology due to the vast number of unique toxins/proteins present in the venoms of diverse snake species found worldwide. Here, we report the purification and functional characteristics of a Group I (PI) metalloprotease (CAMP-2) from the venom of the western diamondback rattlesnake, *Crotalus atrox*. Its sensitivity to matrix metalloprotease inhibitors (batimastat and marimastat) was established using specific in vitro experiments and in silico molecular docking analysis. CAMP-2 shows high sequence homology to atroxase from the venom of *Crotalus atrox* and exhibits collagenolytic, fibrinogenolytic and mild haemolytic activities. It exerts a mild inhibitory effect on agonist-induced platelet aggregation in the absence of plasma proteins. Its collagenolytic activity is completely inhibited by batimastat and marimastat. Zinc chloride also inhibits the collagenolytic activity of CAMP-2 by around 75% at 50 µM, while it is partially potentiated by calcium chloride. Molecular docking studies have demonstrated that batimastat and marimastat are able to bind strongly to the active site residues of CAMP-2. This study demonstrates the impact of matrix metalloprotease inhibitors in the modulation of a purified, Group I metalloprotease activities in comparison to the whole venom. By improving our understanding of snake venom metalloproteases and their sensitivity to small molecule inhibitors, we can begin to develop novel and improved treatment strategies for snakebites.

Keywords: *Crotalus atrox*; metalloprotease; snake venom; neglected tropical disease; rattlesnake; batimastat; marimastat; antivenom

Key Contribution: This study details the purification of a Group I (PI) snake venom metalloprotease (SVMP) from the venom of *Crotalus atrox* venom and the efficacy of various proposed inhibitors on this isolated SVMP in comparison to the whole venom. Both batimastat and marimastat, commercially

available matrix metalloprotease inhibitors, effectively abrogate the metalloprotease activity of the isolated SVMP and whole venom.

1. Introduction

In 2017, snakebite envenomation (SBE) was reinstated to the list of neglected tropical diseases by the World Health Organisation [1,2]. SBE is estimated to occur in at least 1.8–2.7 million people, resulting in around 80,000–137,000 deaths and over 400,000 amputations worldwide per year [3]. The distribution of fatalities is primarily concentrated in rural tropical areas that are some of the world's poorest and most healthcare deprived communities [1,4,5]. Prompt access to antivenom therapy and appropriate medical facilities is crucial in order to protect victims from death, potential extensive limb injuries and the possibility of subsequent, long-term disabilities [4]. The current antivenoms are considered to be sub-optimal in preventing venom-induced tissue damage due to their inability to access the affected local tissues [4,5]. Hence, the development of small molecules that are able to neutralise the locally acting venom components would be highly beneficial in treating SBE, specifically SBE-induced muscle damage and/or tissue necrosis.

Snake venoms are a complex mixture of bioactive proteins and peptides that have evolved over time to assist in subduing and killing prey as quickly as possible, as well as having a secondary role in prey digestion and defence [5]. The clinical effects of SBE range from mild local reactions to more serious life-threatening conditions depending on a variety of variables including the size, species and locality of the snake [6,7], ontogeny [8,9], body mass and health of the victim and the total volume of venom injected [1,10]. Snake venoms are composed of both enzymatic and non-enzymatic components. The enzymatic components of viper venoms primarily include the snake venom metalloproteases (SVMPs), serine proteases and phospholipase A_2 (PLA_2), whereas non-enzymatic venom components include three-finger toxins, C-type lectins and disintegrins amongst many others [5]. The western diamondback rattlesnake, *Crotalus atrox* (*C. atrox*) is likely to be responsible for the majority of SBE-induced fatalities in Northern Mexico [11]. *C. atrox* venom has an abundance of two major protein families, SVMPs and serine proteases, which together account for approximately 70% of the total protein found within the venom [12].

SVMPs are zinc-dependent enzymes that vary in molecular mass from approximately 20 to 100 kDa and are responsible for the haemorrhagic effects and local tissue damage frequently seen upon viper envenomation [13,14]. SVMPs are classified into PI to PIV depending on the presence of additional domains [15]: PI—only a metalloprotease domain; PII—a metalloprotease and a disintegrin domain; PIII—a metalloprotease domain, a disintegrin-like and a cysteine-rich domain; PIV—two C-type lectin domains in addition to all the domains present in PIII. SVMPs are involved in a wide range of toxic activities, including the degradation of collagen and other basement membrane components, fibrinogen and a range of other proteins [13]. The peptidomimetic molecules, batimastat and marimastat are broad-spectrum matrix metalloprotease (MMPs) inhibitors [16] that have been proposed as next generation treatment options for the SVMP-induced effects of SBE [17]. This inhibition is achieved by mimicking the cleavage site of natural substrates and binding to the zinc ion found in the active site of these proteases. In this way, batimastat and the orally bioavailable and similar compound, marimastat are able to inhibit both matrix metalloproteases as well as SVMPs [5]. An improved understanding of MMPs, their inhibitors, and their relationship with SVMPs will aid in the development of improved therapeutic strategies for SBE.

SVMPs in *C. atrox* venom account for 49.7% of total venom, which breaks down further to 22.4% PI and 27.3% PIII SVMPs [12]. In order to determine the therapeutic potential of batimastat and marimastat against PI venom metalloproteases, here, we report the purification and functional characterisation of a PI metalloprotease with a molecular weight of around 23 kDa from the venom of *C. atrox*. The sensitivity of the purified protein to inhibition by batimastat and marimastat was established in comparison to the

whole *C. atrox* venom. Together, this study supports the potential beneficial effects of these molecules against the broad spectrum of pathological effects induced by SVMPs.

2. Results

2.1. Purification and Identification of CAMP-2

To purify a PI SVMP from the venom of *C. atrox*, we deployed a two-dimensional chromatography approach. Initially, 50 mg of whole *C. atrox* venom was applied to a cation-exchange (SP-HP) chromatography column (Figure 1A) followed by the analysis of collected fractions using SDS-PAGE (Figure 1B). Due to the abundance of the target protein at a molecular weight of around 23 kDa (typical for a PI SVMP [18]), the selected fractions (6–9) were further fractionated by gel filtration (Superdex 75, 1.6 × 70 cm) chromatography (Figure 1C). Following SDS-PAGE analysis (Figure 1D), selected fractions (67–72) were further run through the same gel filtration column to remove any impurities from the protein of interest (Figure 1E,F). Finally, a pure protein with a molecular weight of approximately 23 kDa was isolated, which we henceforth refer to as CAMP-2 (denoting the second SVMP that we have isolated from the venom of *C. atrox*). The molecular weight of the isolated protein was confirmed under native conditions using chymotrypsinogen A (25 kDa) as a marker in gel filtration chromatography (indicated with an arrow in Figure 1C,E) and under denaturing (reduced) conditions using SDS-PAGE (Figure 1F).

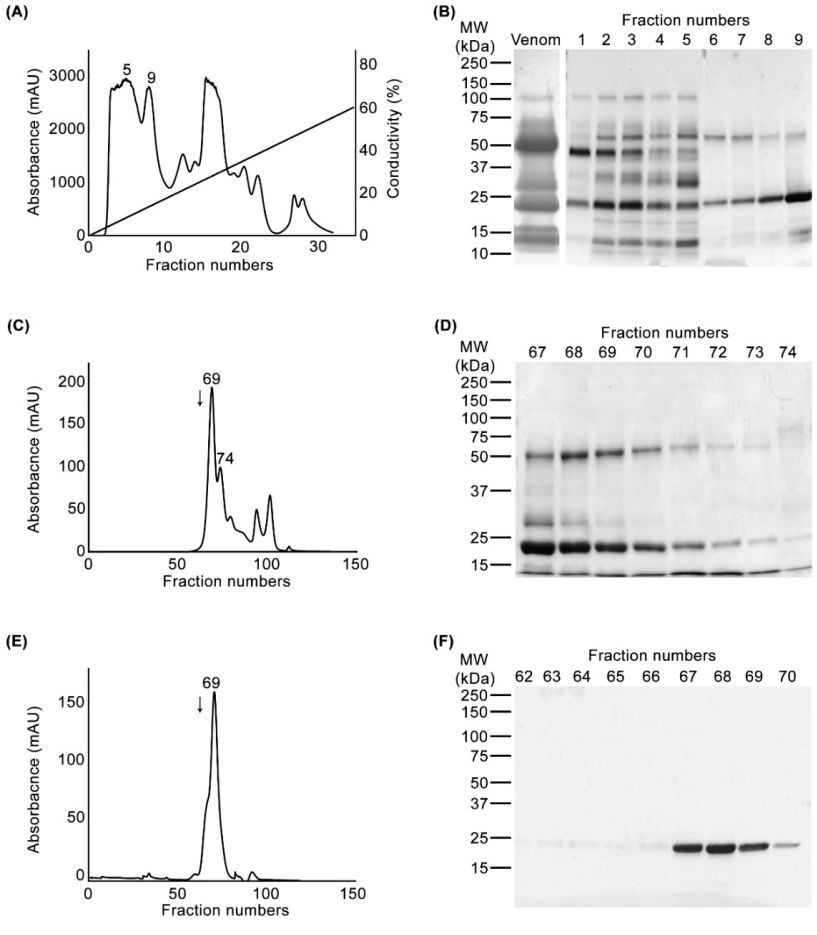


Figure 1. Purification and identification of CAMP-2. 50 mg of whole *C. atrox* venom was fractionated

using a cation exchange chromatography column (**A**) And the collected fractions were analysed by SDS-PAGE (**B**). (**C**) A chromatogram showing the gel filtration chromatography profile of fractions 6 to 9 collected from the cation exchange column. (**D**) SDS-PAGE analysis of selected fractions resulting from the gel filtration chromatography. (**E**) The chromatogram from the second run of gel filtration chromatography using fractions 67–72 from the previous run, and SDS-PAGE analysis showing the purified protein (**F**). The gels shown were stained with Coomassie brilliant blue. The arrow in (**C**) and (**E**) indicates the position of chymotrypsinogen A (25 kDa), which was used as a molecular weight marker in the same gel filtration column. (**G**) Tryptic digested peptides of the purified protein were analysed by mass spectrometry and the data show 52.2% identity to a previously sequenced PI SVMP, atroxase, from *C. atrox* venom. The mass spectrometry-identified peptide sequences of purified protein are shown in yellow on the sequence of atroxase.

To determine the identity of the purified protein, it was subjected to trypsin digestion and subsequent analysis by mass spectrometry (Figure 1G). Mascot analysis of the MS/MS data suggested that the isolated protein possesses a high sequence identity to atroxase, a 23 kDa SVMP from the venom of *C. atrox* [19,20]. The peptide sequences resulting from the mass spectrometry covered 52.2% of atroxase, suggesting that the purified protein is highly likely to be atroxase although we cannot confirm this due to the lack of complete sequencing from this study. However, these data confirm that the purified protein is a PI SVMP with a molecular weight of 23 kDa, and it is likely to be atroxase, which was previously purified and characterised as a non-haemorrhagic protease with fibrin(ogen)olytic activities [20–23].

2.2. CAMP-2 Exerts Collagenolytic, Fibrinogenolytic and Haemolytic Activities

To determine the roles of CAMP-2, various functional assays using synthetic and natural substrates were performed in comparison to the whole *C. atrox* venom. Fluorogenic substrates such as DQ-gelatin and EnzCheck^{TM} lipid-based substrate were used to assess if CAMP-2 possesses metalloprotease (collagenolytic) and PLA_2 activities, respectively. Similar to the whole *C. atrox* venom (Figure 2A), CAMP-2 (Figure 2B) exhibited strong collagenolytic activity. While the whole *C. atrox* venom showed clear PLA_2 activity, CAMP-2 did not display any PLA_2 activity (Figure 2C). These data not only suggest that CAMP-2 is an SVMP, but it is also free from any PLA_2 impurities which have a similar molecular weight. Moreover, human fibrinogen, a plasma protein which is a natural substrate for some SVMPs, was incubated with CAMP-2, before the digest was analysed by SDS-PAGE to determine its effects on fibrinogen. This analysis showed that CAMP-2 has fibrinogenolytic activity and notably, it exerts high specificity for the Aα chain of fibrinogen, as it was completely digested within 10 min (Figure 2D) although over a longer time (e.g., 12 h), it also began to degrade the Bβ chain while the γ chain of fibrinogen remained largely unaffected. Similarly, CAMP-2 showed a mild haemolytic effect compared to the whole venom when incubated with human red blood cells over 24 h (Figure 2E). These data suggest that CAMP-2 is a collagenolytic, fibrinogenolytic and mildly haemolytic enzyme.

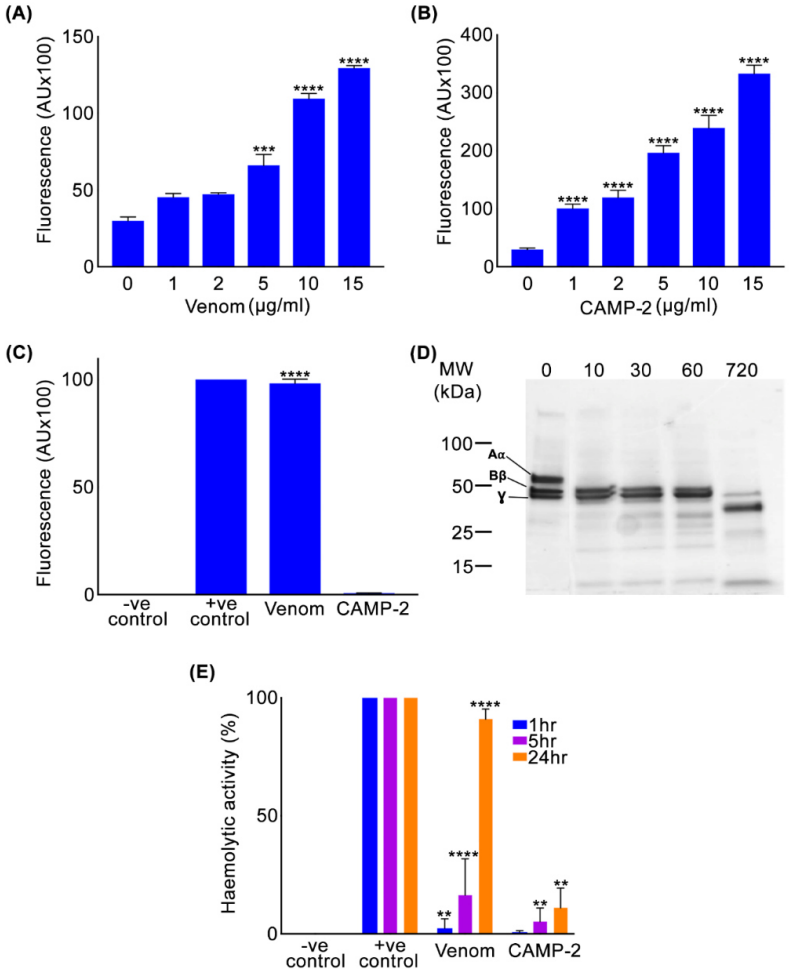


Figure 2. Functional characterisation of CAMP-2. The metalloprotease (collagenolytic) activity of different concentrations of whole *C. atrox* venom (**A**) and CAMP-2 (**B**) was assessed using DQ gelatin (a fluorogenic substrate). (**C**) the PLA_2 activity of whole *C. atrox* venom and CAMP-2 was analysed using EnzCheck™ lipid-based substrate. (**D**) The fibrinogenolytic activity of CAMP-2 was analysed by incubating it with human fibrinogen and the samples collected at different time points (0–720 min as indicated at the top) were assessed by SDS-PAGE and Coomassie staining. (**E**) Haemolytic activity of whole *C. atrox* venom and CAMP-2 was analysed by incubating them with human red blood cells and analysing the cell-free supernatant by spectrometry. The positive (+ve) control represents the complete lysis achieved using 1% (*v*/*v*) Triton-X in PBS. Data represent mean ± S.D. ($n = 3$). The *p*-values shown were calculated using one-way ANOVA followed by posthoc Tukey's test using GraphPad Prism (** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$).

2.3. CAMP-2 Inhibits Human Platelet Aggregation

To determine if CAMP-2 is able to affect human platelet function, platelet aggregation assays using platelet-rich plasma (PRP) and isolated platelets from human whole blood were used. CAMP-2 at both low (3 µg/mL) and high (10 µg/mL) concentrations did not induce platelet aggregation on its own (indicated as 0–5 min in aggregation traces shown in Figure 3). However, 10 µg/mL CAMP-2 inhibited (Figure 3A,B) platelet aggregation (by around 25%) induced by a cross-linked collagen-related peptide (CRP-XL) when isolated platelets were used, although the low concentration did not show any significant effect. This inhibitory effect was absent when PRP (i.e., in the presence of plasma proteins) was used (Figure 3C,D). When the whole *C. atrox* venom was used, at a low concentration (3 µg/mL) it displayed mild inhibitory effects on CRP-XL induced platelet aggregation, although at a higher (10 µg/mL) concentration it has possibly lysed (even in the absence of an agonist as shown in

the aggregation traces between 0 and 5 minutes) the platelets when both isolated platelets (Figure 3E,F) and PRP (Figure 3G,H) were used. These results demonstrate that although CAMP-2 is able to display a minimal inhibitory effect on platelet aggregation when isolated platelets were used, it is unable to affect their function in the presence of plasma proteins, indicating that this may not be its primary role in humans. However, the whole venom may induce the lysis of platelets based on the concentrations injected during the bite.

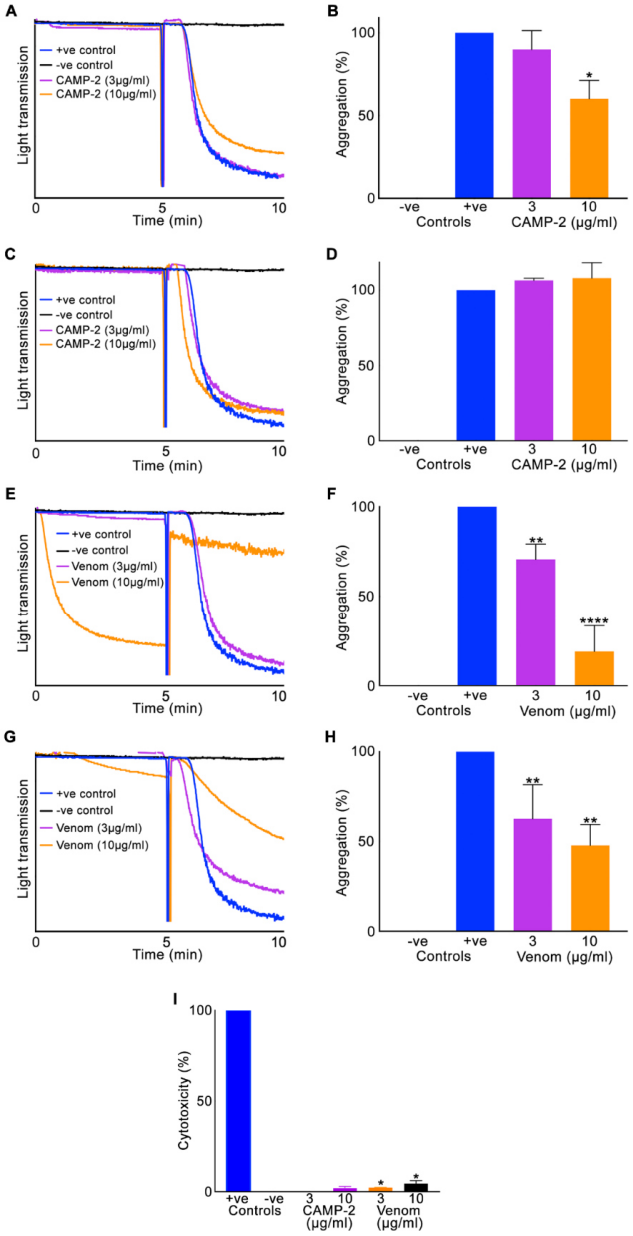


Figure 3. Effect of CAMP-2 on human platelets. The effect of CAMP-2 on human isolated platelets (**A**,**B**) and platelet-rich plasma (PRP) (**C**,**D**) was analysed in the presence and absence of a platelet agonist, cross-linked collagen-related peptide (CRP-XL) by aggregometry. Similar experiments using isolated platelets (**E**,**F**) and PRP (**G**,**H**) were performed using the whole *C. atrox* venom. The aggregation traces shown are representative of three separate experiments. (**I**) The cytotoxicity of CAMP-2 and the whole venom was determined by incubating them with human platelets for 30 min and analysing using a lactate dehydrogenase (LDH) assay kit by spectrometry. The positive (+ve) control (100% cytotoxicity) was achieved using a lysis buffer provided in the kit. Data represent mean ± S.D. (n = 3). The p-values shown were calculated using one-way ANOVA followed by a posthoc Tukey's test using GraphPad Prism (* $p \leq 0.05$, ** $p \leq 0.01$, and **** $p \leq 0.0001$).

In order to assess if CAMP-2 and the whole venom are able to exert direct cytotoxic effects on platelets, lactate dehydrogenase (LDH) assay using human isolated platelets was performed. These results indicated that CAMP-2 does not have any cytotoxic effects on platelets at the concentrations tested in this study, although the whole venom displayed a mild (around 10% at 10 µg/mL) cytotoxic effect on platelets (Figure 3I) similar to its effect on platelet aggregation (Figure 3E–H).

2.4. Chlorides and a Metal Chelator Affect Metalloprotease Activity of CAMP-2

As zinc-dependent proteases, SVMPs rely on free divalent cations such as calcium for catalysis, the impact of various metal chlorides on the metalloprotease activity of CAMP-2 was assessed. Diverse concentrations of both zinc and calcium chloride were used to assess if they would interfere with the metalloprotease (collagenolytic) activity of CAMP-2 and the whole *C. atrox* venom. The results demonstrate that while zinc chloride significantly reduced the metalloprotease activity observed with both the whole venom (Figure 4A) and CAMP-2 (Figure 4B), calcium chloride potentiated this activity of whole venom (Figure 4C) and CAMP-2 (Figure 4D).

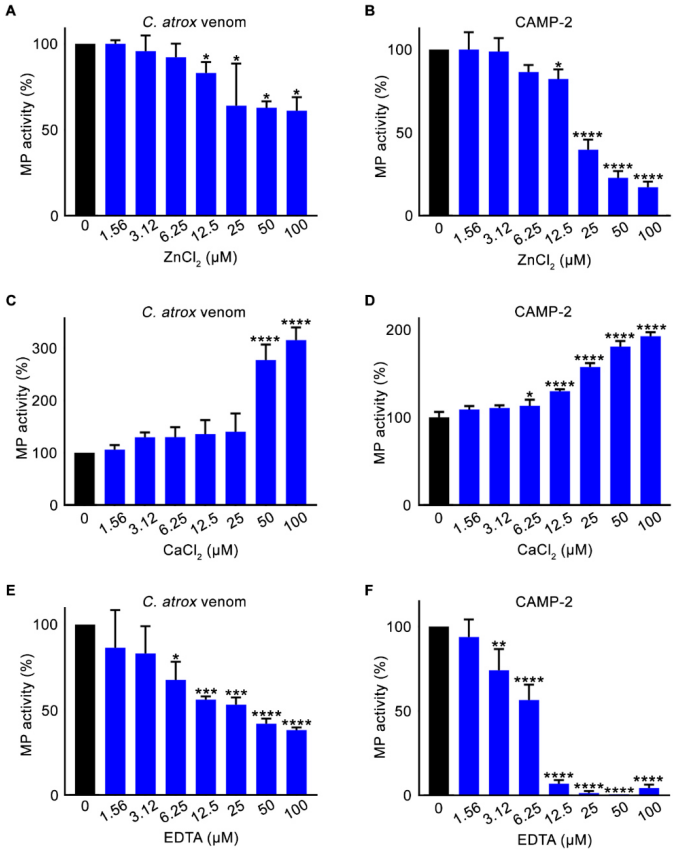


Figure 4. The effect of chlorides and a metal chelator on the metalloprotease activity of CAMP-2. The effect of different concentrations of zinc chloride on the metalloprotease (collagenolytic) activity of whole venom (**A**) and CAMP-2 (**B**) was analysed using DQ-gelatin as a substrate by spectrofluorimetry. Similarly, the effect of various concentrations of calcium chloride (**C**,**D**) and a metal chelator, EDTA (**E**,**F**) on whole *C. atrox* venom, as well as on CAMP-2, was analysed. Data represent mean ± S.D. (n = 3). The p-values shown are as calculated by one-way ANOVA followed by posthoc Tukey's test using GraphPad Prism (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$).

Similarly, the effect of a metal chelator on proteolytic activity was assessed using ethylenediaminetetraacetic acid (EDTA). The metalloprotease activity of both whole venom (Figure 4E) and CAMP-2 (Figure 4F) was strongly inhibited by different concentrations of EDTA, further corroborating that CAMP-2 is a metalloprotease and sensitive to metal chelators such as EDTA.

2.5. Marimastat and Batimastat Inhibit the Activity of CAMP-2

After confirming the biological actions of CAMP-2, the effects of MMP inhibitors, batimastat and marimastat on this protein in comparison to the whole *C. atrox* venom were analysed. Different concentrations of these inhibitors were incubated with 2 µg of CAMP-2 or whole venom for 5 min prior to analysing their metalloprotease (collagenolytic) activity using DQ-gelatin by spectrofluorimetry. The results suggest that marimastat (Figure 5A,B) and batimastat (Figure 5C,D) are able to inhibit the metalloprotease activity of both the whole venom and CAMP-2. A concentration of around 3 µM was able to completely inhibit the activity of both the whole venom and CAMP-2. Moreover, neither marimastat nor batimastat exerted any cytotoxic effects on human platelets, as analysed by an LDH assay (Figure 5E,F). These data suggest that these two MMP inhibitors are effective at inhibiting PI SVMPs such as CAMP-2, and they do not possess any cytotoxic activities.

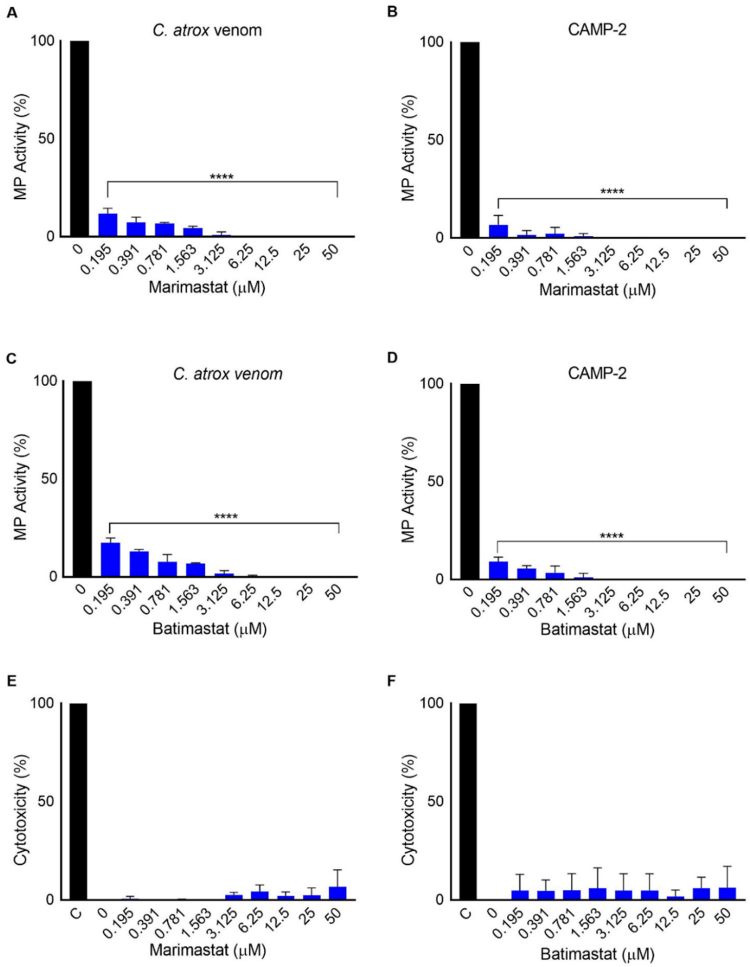


Figure 5. Inhibitory effects of marimastat and batimastat on metalloprotease activity of CAMP-2 and whole *C. atrox* venom. The inhibitory effects of various concentrations of marimastat (on whole *C. atrox* venom (**A**) or CAMP-2 (**B**)) and batimastat (on whole *C. atrox* venom (**C**) or CAMP-2 (**D**)) were quantified in vitro using a fluorogenic substrate, DQ-gelatin by spectrofluorimetry. The inhibitors were incubated with the whole venom or CAMP-2 for 5 min prior to the addition of DQ-gelatin and further incubation of 30 min prior to measuring the level of fluorescence by spectrofluorimetry. The cytotoxicity of marimastat (**E**) and batimastat (**F**) on human platelets was also assessed using a lactate dehydrogenase (LDH) assay. C represents the positive control (100% lysis) achieved using the lysis buffer provided in the kit. Data represent mean ± S.D. ($n = 3$). The p-values shown are as calculated by one-way ANOVA followed by posthoc Tukey's test using GraphPad Prism (**** $p \leq 0.0001$).

2.6. Interactions of CAMP-2 with Batimastat and Marimastat

The mass spectrometry (Figure 1G) and functional data suggested that CAMP-2 is highly likely to be a previously characterised PI SVMP, atroxase, from the venom of *C. atrox*. Hence, we have used the complete sequence of atroxase (Uniprot accession number: Q91401) as CAMP-2 in this study for further analysis. Sequence analysis confirmed that CAMP-2 displayed approximately 74% and 50% identity with other metalloproteases, atrolysin C (PI SVMP; PDB accession numbers: 1DTH and 1ATL) and catrocollastatin (PIII SVMP; PDB accession number: 2DW0), respectively, from the same *C. atrox* venom. A three-dimensional structure of CAMP-2 was generated using homology modeling in Swiss Model Server [24] based on the template of adamalysin II (PDB accession number: 4AIG) from the venom of *Crotalus adamanteus*. A pairwise sequence alignment confirmed that CAMP-2 exhibited 83% identity with adamalysin II and, therefore, this protein has been used as a template instead of atrolysin C to develop the structure of CAMP-2. The superimposing of CAMP-2 with the structure of adamalysin has displayed a minimum rmsd of 0.142 Å, which further emphasises the reliability of this model.

Following the validation of the modeled structure of CAMP-2, molecular docking analysis was performed using AutoDock 4.2 [25] with the chemical structures (obtained from PubChem) of batimastat and marimastat. Three-dimensional atomic coordinates of these compounds were generated using the Online SMILES Translator and Structure File Generator (NCI NIH server (https://cactus.nci.nih.gov/translate/)). Subsequently, the compounds were subjected to energy-minimization using the PRODRG server. The docking analysis revealed that both the inhibitors exhibited comparable binding energy and reliable hydrogen bond interactions with the active site residues of CAMP-2 (Table 1). While batimastat possessed slightly higher binding energy and inhibitory constant, marimastat formed a greater number of hydrogen bonds and hydrophobic interactions with CAMP-2 (Figure 6A–D). Notably, batimastat was found to interact with one of the catalytic triad residues, His 154, through its backbone nitrogen, while marimastat formed a bifurcated hydrogen bond interaction with the active site, Glu 145, through its sidechain oxygen (Table 1). These results are in line with the inhibitory effects observed in the metalloprotease assay (Figure 5A–D).

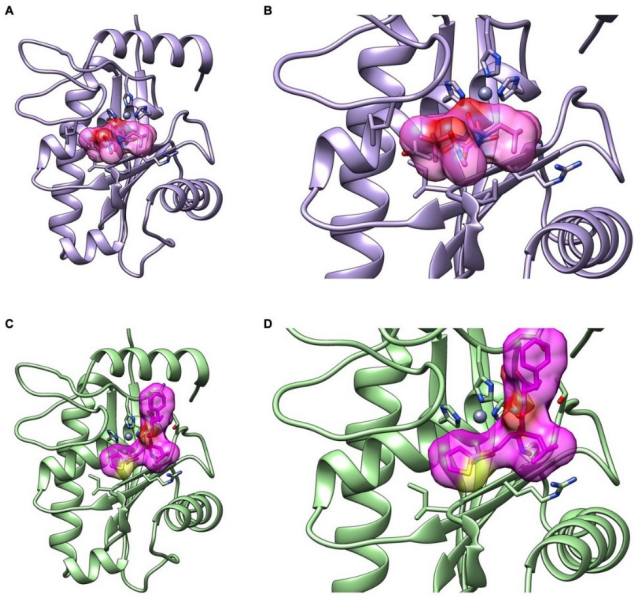


Figure 6. Interactions of marimastat and batimastat with the modeled structure of CAMP-2. The structure of CAMP-2 was modeled using the crystal structure of adamalysin II, a highly (83%) similar SVMP from the venom of *Crotalus adamanteus* as a template. This structure was used for molecular docking analysis (using AutoDock) to evaluate the interactions of marimastat (**A**,**B**) and batimastat (**C**,**D**) with CAMP-2. The structures shown in (**B**,**D**) are enlarged views of the docking complex (catalytic site with the small molecule inhibitors batimastat and marimastat).

Table 1. Results of molecular docking of CAMP-2 with the inhibitors batimastat and marimastat using AutoDock (a molecular docking tool).

Compound	Binding Energy (kcal/mol)	Ligand Efficiency (kcal/mol)	Inhibitory Constant (μM)	H Bond Interactions (D-H . . . A)	Distance (Å)
Batimastat	−5.59	−0.17	79.37	ALA 114 N-H . . . O HIS 154 N-H . . . O	2.8 2.8
Marimastat	−5.29	−0.23	132.52	N-H . . . O LYS 109 ILE 111 N-H . . . O GLY 112 N-H . . . O N-H . . . O(E2) GLU 145 O-H . . . O(E2) GLU 145	3.2 3.2 3.0 2.6 3.1

3. Discussion

Common effects of SBE caused by vipers include haemorrhage, rhabdomyolysis, oedema and severe muscle damage, and often these result in permanent disabilities [1]. These consequences can trigger serious lifestyle and socio-economic ramifications for victims, particularly those in rural areas with difficulty in accessing affordable and effective antivenom [26,27]. The most abundant proteins within viper venoms that are responsible for many of these consequences are SVMPs [13]. The currently used antivenom therapy has proven to be largely ineffective in treating local tissue damage induced by SVMPs due to their inability to access the damaged site because of the large size of antibodies and damaged/blocked blood capillaries around the bite site [5]. As a result, clinicians are forced to employ surgical procedures such as fasciotomy, debridement or limb amputation in severe cases to treat/remove the affected tissues [27]. Therefore, developing an alternative therapy is critical to prevent/treat SBE-induced muscle damage and subsequent disabilities. The use of MMP inhibitors such as batimastat and marimastat has been proposed to inhibit the activities of SVMPs from various venoms [5]. These drugs were originally developed for cancer but failed in clinical trials—batimastat due to its poor solubility and low oral bioavailability, and marimastat, although it showed much promise and reached phase II and III clinical trials, was eventually discontinued after failing to demonstrate a survival benefit [28]. Its longer-term use also leads to debilitating "musculoskeletal syndrome" [29]. Despite these failings, their applications (particularly, orally bioavailable marimastat) in treating the acute local effects induced by SBE would be greatly beneficial to prevent SBE-induced disabilities. Hence, in this study, we have evaluated the efficacy of batimastat and marimastat, specifically on a purified PI SVMP from the venom of *C. atrox*.

We have previously used the two-dimensional chromatography approach (e.g., a combination of ion exchange and gel filtration) as an effective method to purify various venom components, including a 50 kDa PIII SVMP CAMP from the venom of *C. atrox* [14,30]. Here, we have deployed a similar approach to purify a PI SVMP with a molecular weight of 23 kDa from the venom *C. atrox*. The mass spectrometry analysis of this purified protein (entitled CAMP-2) suggests that this is highly likely to be a previously sequenced protein, atroxase, from the same venom. Further functional assays confirmed that CAMP-2 is a collagenolytic, fibrinogenolytic and mildly haemolytic enzyme. At a relatively high concentration, CAMP-2 was also found to inhibit human platelet aggregation. These functions of CAMP-2 are very similar to the ones that have been reported for atroxase (a non-haemolytic protease with fibrin(ogen)olytic activity) [20,23]. Although the complete sequence of CAMP-2 was not obtained in this study, based on the mass spectrometry and functional data, this protein is highly similar, if not identical, to atroxase.

Given that SVMPs directly and indirectly mediate local tissue damage, the inhibition of these enzymes is likely to reduce SBE-induced local tissue damage [13]. Here, the sensitivity of CAMP-2 to MMP inhibitors (batimastat and marimastat) was tested in comparison to the whole *C. atrox* venom. There is a great degree of both structural and functional homology between SVMPs and human variants of MMPs [31]. This implies that substrate/inhibitor interactions between these subfamilies are likely to be similar [31]. Marimastat is a hydroxamic acid derivative which exerts broad-range metalloprotease

inhibition by mimicking the cleavage site of collagen substrates [31]. When comparing the structural differences between these two compounds, marimastat has an additional hydroxyl group, increasing its hydrophilicity and thus, improving its pharmacokinetic properties [32]. Although they are likely to inhibit the majority of SVMPs, the additional domains found in PII, PIII, and PIV SVMPs would most likely be unaffected by these inhibitors. Indeed, batimastat and marimastat have already been reported to inhibit SVMPs in various venoms under in vitro and in vivo [33–35] settings. Similar to previous studies, the metalloprotease activity of both whole venom and CAMP-2 were almost completely inhibited by MMP inhibitors at around 3 μM. Notably, as a PI SVMP, CAMP-2 possesses only the metalloprotease domain. These data emphasise that batimastat and marimastat are both likely to act as broad-spectrum inhibitors for SVMPs including the PI SVMP analysed in this study. In contrast to these inhibitors, which target only the metalloprotease domain of SVMPs, antivenoms, on the other hand, are probably capable of binding all, or many of, the domains including the metalloprotease domain in SVMPs. While the large size of antibodies affects their ability to reach the bite site in time to prevent local tissue damage, the small molecule inhibitors are likely to reach and act rapidly for SBE-induced tissue damage. Multiple injections of these inhibitors have also been suggested to treat SBE, although the consequences of inhibiting human MMPs in the body are yet to be elucidated.

In the past, EDTA has been used to treat SBE under clinical/in vivo settings [36]. As a chelating agent, it binds to divalent cations and therefore affects the catalytic activity of metalloproteases. Several studies have reported the impact of EDTA on venom and other human metalloprotease activities [36]. In this study, EDTA has inhibited the metalloprotease activity of both the venom and CAMP-2; however, its use is problematic, and being orally bioavailable, marimastat is a far better clinical approach, especially considering the passing of stage II clinical trials. Similarly, $ZnCl_2$ has proven to be a successful inhibitor of SVMPs through its ability to cause stereochemical and structural instabilities of metalloproteases when used in excess [37,38]. $ZnCl_2$ has also shown a significant inhibitory effect on the metalloprotease activity of whole venom and CAMP-2 at many of the concentrations tested. Another observation is that the plateau of the SVMP activity observed at 25, 50 and 100 μM concentrations when the whole venom was used. This may be as a result of Zn^{2+} saturation, which may have caused Zn^{2+} to bind to other ions and proteins within the whole venom, rather than just the metalloproteases [39].

As marimastat and batimastat have proven to be effective in the inhibition of SVMP activity of CAMP-2, a cytotoxicity assay was completed to establish whether they are cytotoxic to human cells, such as platelets. As the LDH cytotoxicity assay measures whether the plasma membrane is damaged, this can be correlated with the effects that the compounds would have on other cells in the human body. If they present signs of cytotoxicity, they would not be appropriate for human use. However, both marimastat and batimastat showed no significant cytotoxic effects on platelets at the numerous concentrations tested and also passed phase I safety trials in humans [28]. This highlights their potential for further development into next-generation treatments for SBE in humans. However, the long-term side effects of their use and dosage requirements are still unknown and demand extensive in vivo research before they can be fully supported as an adjunctive treatment for SBE. The use of structural biology to screen and identify small molecules that are likely to affect venom toxins is also beneficial in finding alternative treatments for SBE. In this study, the structure of CAMP-2 was modeled and used in a docking analysis with batimastat and marimastat. Batimastat possessed higher binding energy and inhibitory constant, whereas marimastat has formed multiple hydrogen bonds with the active site of CAMP-2. While batimastat was found to interact with one of the catalytic triad histidine (His 154) residues through its backbone nitrogen, marimastat was observed to form a bifurcated hydrogen bond interaction with another active site residue, glutamate (Glu 145), through its sidechain oxygen. Due to the easy access and robustness of these *in silico* approaches, they can be used to analyse the binding efficiencies of these and other similar molecules with SVMPs from diverse venoms to determine their potential in treating SBE.

SVMPs play important roles in the overall pathophysiology of viper envenoming by inducing local tissue damage and haemorrhage, which can be primarily attributed to their potential to degrade basement membrane components and affect coagulation factors [18,40]. SVMPs activate two key coagulation factors, factor X and prothrombin, to exhibit their procoagulant effects. SVMPs are also known to digest plasma fibrinogen, which may induce clotting effects in some cases, but, largely, it induces the consumption coagulopathy by reducing the functional fibrinogen levels in the plasma, which subsequently results in bleeding. CAMP-2 has also exerted its ability to degrade fibrinogen, specifically the Aα chain, with delayed/minimal effect on Bβ chain. SVMPs [18,40], as well as venom serine proteases [41], show varying specificity to cleave the Aα and/or Bβ chains of fibrinogen with rarely an effect on the γ chain. Therefore, in addition to their impact on local tissue damage, the effects of SVMPs on the blood coagulation factors, specifically fibrinogen, should also be reduced in order to combat SBE-induced pathological complications.

In conclusion, SVMPs are one of the key venom toxins that should be neutralised as quickly as possible following a snakebite, particularly in the case of vipers. The inability of antivenoms to counteract the effects of SVMPs, particularly at the local bite site, emphasises the urgent need to develop alternative, small molecule-based treatments to minimise SBE-induced tissue damage, which often results in permanent disabilities. In this study, we give a comprehensive analysis of the applications of batimastat, marimastat, EDTA and $ZnCl_2$ in inhibiting a purified PI SVMP, CAMP-2, in comparison to the whole *C. atrox* venom. The cytotoxic effects of batimastat and marimastat were also analysed. Hence, this study demonstrates a robust method to screen small molecule inhibitors for therapeutic utility against venom toxins using a spectrum of functional assays and *in silico* techniques in order to develop alternative therapies for SBE.

4. Materials and Methods

4.1. Protein Purification

Lyophilised *C. atrox* venom (50 mg) (Sigma Aldrich, Dorset, UK) was dissolved in 1 mL of 20 mM Tris.HCl (pH 7.4) (ThermoScientific, Loughborough, UK), and after centrifugation at 5000× *g* for 5 min to remove the undissolved materials, the supernatant was applied to a 5-mL HiTrap™ Sepharose (SP) HP cation exchange chromatography column (GE Healthcare, Amersham, UK) and fractionated using an Akta Purifier (GE Healthcare, Amersham, UK). Fractions were collected at a rate of 1 mL/min using 20 mM Tris.HCl pH 7.4 (Buffer A) and 1 M NaCl prepared in Buffer A (Buffer B) with a gradient reaching 60% Buffer B over 30 min. All fractions containing the target protein were pooled together, desalted and concentrated using Vivaspin ultra-centrifugal filtration tubes (Sartorius, Epsom, UK), and applied to a gel filtration column (Superdex 75) to further purify the target protein. Fractions were collected at a rate of 1 mL/min using 20 mM Tris.HCl (pH 7.4).

The fractions were stored on ice and analysed by Bradford protein assay according to the manufacturer's protocol (ThermoScientific, Loughborough, UK) and SDS-PAGE, as described previously [42]. The protein assay was run, and the intensity of the color developed was measured at 600 nm using an Emax spectrophotometer (Molecular Devices, Wokingham, UK). Bovine serum albumin (BSA) (ThermoScientific, Loughborough, UK) was used as a standard in the protein assay. For SDS-PAGE, the proteins were denatured using reducing sample treatment buffer (RSTB) (10% (*w*/*v*) SDS (ThermoScientific, Loughborough, UK), 10% (*v*/*v*) β-mercaptoethanol (Sigma Aldrich, Dorset, UK), 1% (*w*/*v*) bromophenol blue (Sigma Aldrich, Dorset, UK), 50% (*v*/*v*) glycerol (ThermoScientific, Loughborough, UK) and 20 mM Tris.HCl (pH 7.4)) and heating at 90 °C for 10 min. Samples (30 µL from each fraction) were loaded into precast gradient (4–15%) Mini-PROTEAN® TGX™ gels (Bio-Rad, Watford, UK) alongside a protein molecular weight marker (Bio-Rad, Watford, UK) and resolved using a Mini-Protean II apparatus (Biorad, Watford, UK). Gels were immersed in a staining solution (0.1% (*w*/*v*) Coomassie Brilliant blue R250 (Sigma Aldrich, Dorset, UK) dissolved in 10% (*v*/*v*) acetic acid (ThermoScientific, Loughborough, UK), 40% (*v*/*v*) methanol (ThermoScientific, Loughborough, UK)

and 50% deionized water) for 1 h on a plate shaker, washed 3x with deionised water for 5 min and destained (10% (*v*/*v*) acetic acid, 10% (*v*/*v*) methanol and 80% deionized water) for 3 h or until protein bands became clear.

4.2. Mass Spectrometry Analysis

A gel slice (from SDS-PAGE) containing CAMP-2 was subjected to tryptic digestion before undergoing mass spectrometry analysis at Alta Bioscience (Birmingham, UK), as we described previously [14]. Both MS and MS/MS scans were cross-referenced against the Uniprot protein database using the Sequest algorithm (Thermo fisher PD 1.4) in order to determine the identity of the purified protein.

4.3. Fluorogenic Assays

The metalloprotease (collagenolytic) activity of the venom or the purified protein (CAMP-2) was measured using a fluorogenic substrate, DQ™-gelatin (ThermoScientific, Loughborough, UK). Several concentrations of the purified protein or venom (with and without different concentrations of batimastat, marimastat, EDTA, $ZnCl_2$ and $CaCl_2$ (Sigma Aldrich, Dorset, UK)) were added to a black 96-well plate in triplicates along with appropriate controls. Then DQ-gelatin (10 μg/mL) was added to each well, and following mixing, the plate was incubated at 37 °C and the level of fluorescence was measured at various time points using an excitation of 485 nm and emission wavelength of 520 nm in a FLUOstar OPTIMA (BMG Labtech, Ortenberg, Germany) spectrofluorimeter. To determine PLA_2 activity, an EnzChek™ Phospholipase A_2 Assay Kit (ThermoFisher Scientific, Loughborough, UK) was used in accordance with the manufacturer's instructions.

4.4. Fibrinogenolytic Assay

CAMP-2 (1 mg/mL) was mixed with fibrinogen (10 mg/mL) (Sigma Aldrich, Dorset, UK) in PBS and incubated at 37 °C for various time points. Samples (50 μL) were taken at time intervals of 10, 30 and 60 min and then again after 12 h and immediately mixed with 25 μL of RSTB before boiling at 90 °C for 10 min. Each sample was then analysed by SDS-PAGE, as explained above.

4.5. Human Blood Collection, Platelet Preparation and Aggregation Assay

Blood samples from healthy human volunteers were obtained in accordance with the approved procedures by the University of Reading Research Ethics Committee (UREC 17/17 approved: 10 May 2017) and after obtaining written informed consent. The platelets were prepared, as described previously [42]. Blood was collected using venepuncture into vacutainers containing 3.2% (*w*/*v*) citrate. For PRP, blood samples were centrifuged at 102× *g* for 20 min at 20 °C. PRP was rested for 30 min at 30 °C in a water bath before use. For isolated platelets preparation, the PRP was mixed with 3 mL of acid citrate dextrose (ACD) and 10 ng/mL prostacyclin ($PG1_2$ dissolved in EthOH) (Sigma Aldrich, Dorset, UK) and mixed gently by inversion and centrifuged at 1413 *g*. Modified tyrodes-HEPES buffer (1 ml with 5 mM glucose) was added together with 150 μL of ACD to the platelet pellet to resuspend the pellet, and the final volume was made up to 25 mL using a pre-warmed modified tyrodes-HEPES buffer. A further 3 mL of ACD and 10 ng/mL prostacyclin was added prior to centrifuging at 1413× *g* for 10 min at 20 °C. Finally, the supernatant was discarded, and the platelets were resuspended in modified tyrodes-HEPES buffer at a density of 4×10^8 platelets/mL. The aggregation assays were performed using isolated platelets or PRP with 0.5 μg/mL CRP-XL (obtained from Professor Richard Farndale, University of Cambridge, UK) as an agonist. The level of aggregation in the presence and absence of different concentrations of venom or CAMP-2 was monitored using an optical aggregometer (model 700, Chrono-log, USA).

4.6. Haemolytic Assay

Human erythrocytes were collected from the dense red blood cells found in the bottom of vacutainers following centrifugation for the collection of PRP, as mentioned above. These erythrocytes were then washed three times by mixing with an equal volume of PBS, centrifuging at 2000× *g* for 2 min, and discarding the supernatant. The haemolytic activity was measured using these washed human erythrocytes suspended in calcified phosphate-buffered saline (PBS). The erythrocytes were treated with different concentrations of CAMP-2 or venom and incubated at 37 °C for various time points. A detergent, Triton X-100 (1%) (Sigma Aldrich, Dorset, UK), and PBS were used as a positive and negative control, respectively. Following incubation, the samples were centrifuged at 2000× *g* for two minutes and 50 µL of supernatant was pipetted into a 96-well plate and the absorbance was measured at 540 nm using a spectrophotometer.

4.7. LDH Cytotoxicity Assay

An LDH cytotoxicity assay kit (ThermoFisher, Loughborough, UK) was used in accordance with the manufacturer's instructions. Briefly, human platelets were incubated at 37 °C for 30 min prior to incubation with different concentrations of venom or CAMP-2 or small molecule inhibitors for five minutes. These results were compared with those from the positive control (100% lysis) achieved using the lysis buffer provided. The substrate mix from the kit was added to the platelets and incubated for another 30 min and subsequently stopped using the stop solution provided. The level of absorbance was read at 490 and 650 nm using a Fluostar Optima (BMG Labtech, Ortenberg, Germany) spectrofluorimeter. The assay was performed in duplicates using platelets obtained from three individual donors.

4.8. Structure Modelling and Molecular Docking

A three-dimensional structure of CAMP-2 was developed using homology modeling in the Swiss Model Server [24] based on the crystal structure of adamalysin II (PDB accession number: 4AIG) from the venom of *Crotalus adamanteus* as a template. Before proceeding to the docking simulation, protein preparation steps such as fixing charge for the Zn^{2+} metal ion, adding solvation parameters and polar hydrogens to the metalloprotease were carried out. AutoDock [25] necessitates pre-calculated grid maps for each type of atom present in the ligand molecule being docked because it stores the potential energy produced from interacting with the macromolecule. This grid surrounds the region of interest (active site) of the macromolecule. A grid box of size 50 × 50 × 50 Å with a spacing of 0.375 Å was prepared at the active site of CAMP-2 metalloprotease. The Lamarckian genetic algorithm was used to identify the best conformers. Throughout the docking process, a maximum of 15 conformers was considered per compound. AutoDock (version 4.0 SRC, California, USA) was compiled and run under Microsoft Windows XP operating system.

4.9. Statistical Analysis

All statistical analyses were performed with GraphPad Prism (version 7.0, Graphpad Prism, California, USA, 2018). For most of the data, the statistical significance was analysed using one-way ANOVA, which was followed by a posthoc Tukey's test.

Author Contributions: Conceptualization, S.V., R.V. and H.F.W.; Methodology, K.L., S.V.; Software, K.L.; Formal Analysis, K.L., H.J.L., H.F.W., S.V.; Investigation, H.J.L., M.S., D.R., A.S., H.F.W. and T.M.V.; Resources, S.A.T., A.B.B., K.P. and S.V.; Writing-Original Draft Preparation, H.J.L., H.F.W., A.M. and S.V.; Writing-Review & Editing, All authors.; Supervision, R.V., H.F.W., S.V., S.A.T., A.B.B. and K.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gutierrez, J.M.; Calvete, J.J.; Habib, A.G.; Harrison, R.A.; Williams, D.J.; Warrell, D.A. Snakebite envenoming. *Nat. Rev. Dis. Primers* **2017**, *3*, 17079. [CrossRef]
2. Chippaux, J.P. Snakebite envenomation turns again into a neglected tropical disease! *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2017**, *23*, 38. [CrossRef]
3. Kasturiratne, A.; Wickremasinghe, A.R.; de Silva, N.; Gunawardena, N.K.; Pathmeswaran, A.; Premaratna, R.; Savioli, L.; Lalloo, D.G.; de Silva, H.J. The global burden of snakebite: A literature analysis and modelling based on regional estimates of envenoming and deaths. *PLoS Med.* **2008**, *5*, e218. [CrossRef] [PubMed]
4. Williams, D.; Gutierrez, J.M.; Harrison, R.; Warrell, D.A.; White, J.; Winkel, K.D.; Gopalakrishnakone, P. The Global Snake Bite Initiative: An antidote for snake bite. *Lancet* **2010**, *375*, 89–91. [CrossRef]
5. Williams, H.F.; Layfield, H.J.; Vallance, T.; Patel, K.; Bicknell, A.B.; Trim, S.A.; Vaiyapuri, S. The Urgent Need to Develop Novel Strategies for the Diagnosis and Treatment of Snakebites. *Toxins (Basel)* **2019**, *11*, 363. [CrossRef] [PubMed]
6. Sunagar, K.; Undheim, E.A.; Scheib, H.; Gren, E.C.; Cochran, C.; Person, C.E.; Koludarov, I.; Kelln, W.; Hayes, W.K.; King, G.F.; et al. Intraspecific venom variation in the medically significant Southern Pacific Rattlesnake (Crotalus oreganus helleri): Biodiscovery, clinical and evolutionary implications. *J. Proteom.* **2014**, *99*, 68–83. [CrossRef] [PubMed]
7. Fry, B.G.; Winkel, K.D.; Wickramaratna, J.C.; Hodgson, W.C.; Wüster, W. Effectiveness of Snake Antivenom: Species and Regional Venom Variation and Its Clinical Impact. *J. Toxicol. Toxin Rev.* **2003**, *22*, 23–34. [CrossRef]
8. Pla, D.; Sanz, L.; Sasa, M.; Acevedo, M.E.; Dwyer, Q.; Durban, J.; Perez, A.; Rodriguez, Y.; Lomonte, B.; Calvete, J.J. Proteomic analysis of venom variability and ontogeny across the arboreal palm-pitvipers (genus Bothriechis). *J. Proteom.* **2017**, *152*, 1–12. [CrossRef]
9. Modahl, C.M.; Mukherjee, A.K.; Mackessy, S.P. An analysis of venom ontogeny and prey-specific toxicity in the Monocled Cobra (Naja kaouthia). *Toxicon* **2016**, *119*, 8–20. [CrossRef]
10. Harrison, R.A.; Hargreaves, A.; Wagstaff, S.C.; Faragher, B.; Lalloo, D.G. Snake envenoming: A disease of poverty. *PLoS Negl. Trop. Dis.* **2009**, *3*, e569. [CrossRef]
11. Yanez-Arenas, C.; Peterson, A.T.; Mokondoko, P.; Rojas-Soto, O.; Martinez-Meyer, E. The use of ecological niche modeling to infer potential risk areas of snakebite in the Mexican state of Veracruz. *PLoS ONE* **2014**, *9*, e100957. [CrossRef] [PubMed]
12. Calvete, J.J.; Fasoli, E.; Sanz, L.; Boschetti, E.; Righetti, P.G. Exploring the venom proteome of the western diamondback rattlesnake, Crotalus atrox, via snake venomics and combinatorial peptide ligand library approaches. *J. Proteome Res.* **2009**, *8*, 3055–3067. [CrossRef] [PubMed]
13. Gutierrez, J.M.; Rucavado, A. Snake venom metalloproteinases: Their role in the pathogenesis of local tissue damage. *Biochimie* **2000**, *82*, 841–850. [CrossRef]
14. Williams, H.F.; Mellows, B.A.; Mitchell, R.; Sfyri, P.; Layfield, H.J.; Salamah, M.; Vaiyapuri, R.; Collins-Hooper, H.; Bicknell, A.B.; Matsakas, A.; et al. Mechanisms underpinning the permanent muscle damage induced by snake venom metalloprotease. *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007041. [CrossRef]
15. Fox, J.W.; Serrano, S.M. Structural considerations of the snake venom metalloproteinases, key members of the M12 reprolysin family of metalloproteinases. *Toxicon* **2005**, *45*, 969–985. [CrossRef]
16. Rasmussen, H.S.; McCann, P.P. Matrix metalloproteinase inhibition as a novel anticancer strategy: A review with special focus on batimastat and marimastat. *Pharm. Ther.* **1997**, *75*, 69–75. [CrossRef]
17. Knudsen, C.; Laustsen, A.H. Recent Advances in Next Generation Snakebite Antivenoms. *Trop. Med. Infect. Dis.* **2018**, *3*, 42. [CrossRef]
18. Markland, F.S., Jr.; Swenson, S. Snake venom metalloproteinases. *Toxicon* **2013**, *62*, 3–18. [CrossRef]
19. Baker, B.J.; Wongvibulsin, S.; Nyborg, J.; Tu, A.T. Nucleotide sequence encoding the snake venom fibrinolytic enzyme atroxase obtained from a Crotalus atrox venom gland cDNA library. *Arch. Biochem. Biophys.* **1995**, *317*, 357–364. [CrossRef]
20. Willis, T.W.; Tu, A.T. Purification and biochemical characterization of atroxase, a nonhemorrhagic fibrinolytic protease from western diamondback rattlesnake venom. *Biochemistry* **1988**, *27*, 4769–4777. [CrossRef]

21. Tu, A.T.; Baker, B.; Wongvibulsin, S.; Willis, T. Biochemical characterization of atroxase and nucleotide sequence encoding the fibrinolytic enzyme. *Toxicon* **1996**, *34*, 1295–1300. [CrossRef]
22. Baker, B.J.; Tu, A.T. Atroxase—A fibrinolytic enzyme isolated from the venom of western diamondback rattlesnake. Isolation, characterization and cloning. *Adv. Exp. Med. Biol.* **1996**, *391*, 203–211. [PubMed]
23. Willis, T.W.; Tu, A.T.; Miller, C.W. Thrombolysis with a snake venom protease in a rat model of venous thrombosis. *Thromb. Res.* **1989**, *53*, 19–29. [CrossRef]
24. Waterhouse, A.; Bertoni, M.; Bienert, S.; Studer, G.; Tauriello, G.; Gumienny, R.; Heer, F.T.; de Beer, T.A.P.; Rempfer, C.; Bordoli, L.; et al. SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Res.* **2018**, *46*, W296–W303. [CrossRef] [PubMed]
25. Morris, G.M.; Huey, R.; Olson, A.J. Using AutoDock for ligand-receptor docking. *Curr. Protoc. Bioinform.* **2008**, *24*, 8.14.1–8.14.40. [CrossRef] [PubMed]
26. Vaiyapuri, S.; Vaiyapuri, R.; Ashokan, R.; Ramasamy, K.; Nattamaisundar, K.; Jeyaraj, A.; Chandran, V.; Gajjeraman, P.; Baksh, M.F.; Gibbins, J.M.; et al. Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India. *PLoS ONE* **2013**, *8*, e80090. [CrossRef]
27. Williams, H.F.; Vaiyapuri, R.; Gajjeraman, P.; Hutchinson, G.; Gibbins, J.M.; Bicknell, A.B.; Vaiyapuri, S. Challenges in diagnosing and treating snakebites in a rural population of Tamil Nadu, India: The views of clinicians. *Toxicon* **2017**, *130*, 44–46. [CrossRef]
28. Winer, A.; Adams, S.; Mignatti, P. Matrix Metalloproteinase Inhibitors in Cancer Therapy: Turning Past Failures Into Future Successes. *Mol. Cancer Ther.* **2018**, *17*, 1147–1155. [CrossRef]
29. Vandenbroucke, R.E.; Libert, C. Is there new hope for therapeutic matrix metalloproteinase inhibition? *Nat. Rev. Drug Discov.* **2014**, *13*, 904–927. [CrossRef]
30. Vaiyapuri, S.; Hutchinson, E.G.; Ali, M.S.; Dannoura, A.; Stanley, R.G.; Harrison, R.A.; Bicknell, A.B.; Gibbins, J.M. Rhinocetin, a venom-derived integrin-specific antagonist inhibits collagen-induced platelet and endothelial cell functions. *J. Biol. Chem.* **2012**, *287*, 26235–26244. [CrossRef]
31. Howes, J.M.; Theakston, R.D.; Laing, G.D. Neutralization of the haemorrhagic activities of viperine snake venoms and venom metalloproteinases using synthetic peptide inhibitors and chelators. *Toxicon* **2007**, *49*, 734–739. [CrossRef] [PubMed]
32. Rasmussen, H.S. Batimastat and Marimastat in Cancer. In *Antiangiogenic Agents in Cancer Therapy*; Teicher, B.A., Ed.; Humana Press: Totowa, NJ, USA, 1999; pp. 399–405.
33. Rucavado, A.; Escalante, T.; Gutiérrez, J.M.A. Effect of the metalloproteinase inhibitor batimastat in the systemic toxicity induced by Bothrops asper snake venom: Understanding the role of metalloproteinases in envenomation. *Toxicon* **2004**, *43*, 417–424. [CrossRef] [PubMed]
34. Rucavado, A.; Escalante, T.; Franceschi, A.; Chaves, F.; León, G.; Cury, Y.; Ovadia, M.; Gutiérrez, J.M. Inhibition of local hemorrhage and dermonecrosis induced by Bothrops asper snake venom: Effectiveness of early in situ administration of the peptidomimetic metalloproteinase inhibitor batimastat and the chelating agent CaNa2EDTA. *Am. J. Trop. Med. Hyg.* **2000**, *63*, 313–319. [CrossRef] [PubMed]
35. Escalante, T.; Franceschi, A.; Rucavado, A.; Gutiérrez, J.M.A. Effectiveness of batimastat, a synthetic inhibitor of matrix metalloproteinases, in neutralizing local tissue damage induced by BaP1, a hemorrhagic metalloproteinase from the venom of the snake Bothrops asper. *Biochem. Pharm.* **2000**, *60*, 269–274. [CrossRef]
36. Ainsworth, S.; Slagboom, J.; Alomran, N.; Pla, D.; Alhamdi, Y.; King, S.I.; Bolton, F.M.S.; Gutierrez, J.M.; Vonk, F.J.; Toh, C.H.; et al. The paraspecific neutralisation of snake venom induced coagulopathy by antivenoms. *Commun. Biol.* **2018**, *1*, 34. [CrossRef] [PubMed]
37. Cordeiro, F.A.; Coutinho, B.M.; Wiezel, G.A.; Bordon, K.F. Purification and enzymatic characterization of a novel metalloprotease from *Lachesis muta rhombeata* snake venom. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2018**, *24*, 32. [CrossRef] [PubMed]
38. Gomez-Ortiz, M.; Gomis-Ruth, F.X.; Huber, R.; Aviles, F.X. Inhibition of carboxypeptidase A by excess zinc: Analysis of the structural determinants by X-ray crystallography. *Febs Press* **1997**, *400*, 336–340. [CrossRef]
39. Takeda, S. ADAM and ADAMTS Family Proteins and Snake, Venom Metalloproteinases: A Structural Overview. *Toxins* **2016**, *8*, 155. [CrossRef]
40. Kini, R.M.; Koh, C.Y. Metalloproteases Affecting Blood Coagulation, Fibrinolysis and Platelet Aggregation from Snake Venoms: Definition and Nomenclature of Interaction Sites. *Toxins (Basel)* **2016**, *8*, 284. [CrossRef]

41. Yamazaki, Y.; Morita, T. Snake venom components affecting blood coagulation and the vascular system: Structural similarities and marked diversity. *Curr. Pharm. Des.* **2007**, *13*, 2872–2886. [CrossRef]
42. Vaiyapuri, S.; Sage, T.; Rana, R.H.; Schenk, M.P.; Ali, M.S.; Unsworth, A.J.; Jones, C.I.; Stainer, A.R.; Kriek, N.; Moraes, L.A.; et al. EphB2 regulates contact-dependent and contact-independent signaling to control platelet function. *Blood* **2015**, *125*, 720–730. [CrossRef] [PubMed]

Appendix 2: Case report: Russell's viper envenomation induces rectus sheath haematoma.

Toxicon 224 (2023) 107037

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Case report

Russell's viper envenomation induces rectus sheath haematoma

Subramanian Senthilkumaran [a,1], José R. Almeida [b,1], Jarred Williams [b,1], Anika Salim [b], Harry F. Williams [c], Ponniah Thirumalaikolundusubramanian [d], Ketan Patel [e], Sakthivel Vaiyapuri [b,*]

[a] Manian Medical Centre, Erode, 638001, Tamil Nadu, India
[b] School of Pharmacy, University of Reading, Reading, RG6 6UB, UK
[c] Toxiven Biotech Private Limited, Coimbatore, 641042, Tamil Nadu, India
[d] The Tamil Nadu Dr M.G.R Medical University, Chennai, 600032, Tamil Nadu, India
[e] School of Biological Sciences, University of Reading, Reading, RG6 6UB, UK

ARTICLE INFO

Handling Editor: Denise Tambourgi

Keywords:
Russell's viper
Daboia russelii
Rectus sheath haematoma
Snakebite envenomation
Computed tomographic imaging
Fothergill and carnett signs

ABSTRACT

Snakebite envenomation causes systemic and local manifestations, which result from the individual or synergistic actions of multiple venom components. The pathological hallmarks of medically important venomous snakes such as the Indian Russell's viper (*Daboia russelii*) are well known. Envenomation by Russell's viper is typically characterised by coagulopathies, muscular damage, nephrotoxicity, and neurotoxicity. However, recent reports have revealed several unusual complications that provide a better understanding of Russell's viper envenomation effects. To further strengthen this, here, we report a case of Russell's viper bite that induced acute abdominal pain, which was intensified on day two and conservatively treated under medical supervision. Both Fothergill and Carnett signs were positive for this patient. An ultrasound imaging revealed a dissimilar dense mass, and the abdominal computed tomography scan confirmed rectus sheath haematoma. The clinical management involved the administration of polyvalent antivenom, packed red blood cells, fresh frozen plasma, and platelets. The patient recovered gradually and was discharged from the hospital eight days after the bite. Overall, this case presentation shares an uncommon experience and adds new insights into the complex series of rare pathological events associated with Russell's viper bites in India. The scientific documentation of relatively infrequent entities based on an ongoing living assessment of medical experiences, for example, this rectus sheath haematoma, constitutes valuable guidance for an adequate diagnosis and timely treatment. Essential awareness among clinicians and further research on understanding the molecular relationship between Russell's viper venom and rectus sheath haematoma will improve patient outcomes and understanding of this condition, respectively.

1. Introduction

Snakebite envenomation (SBE), a high-priority neglected tropical disease, is associated with a range of clinical conditions leading to deaths or permanent disabilities (Gutiérrez et al., 2017). Successful clinical management of SBE requires prompt diagnosis, adequate medical training for healthcare professionals, and a deeper understanding of the broad spectrum of clinical manifestations including rare complications (Hamza et al., 2021). India remains the 'capital of SBE' due to a large number of incidents resulting in more than 58,000 deaths every year (Suraweera et al., 2020). Russell's viper (*Daboia russelii*) is the protagonist snake involved in the majority of SBE in India, accounting for over 40% of incidents (Vaiyapuri et al., 2013; Samuel et al., 2020; Senji Laxme et al., 2021). The classic symptoms of Russell's viper envenomation are coagulopathy, local muscle damage, nephrotoxicity, and neurotoxicity (Mahasandana et al., 1980; Kumar et al., 2018; Ratnayake et al., 2019). However, several recently published articles have highlighted that the mode of action and clinical complications caused by the Indian Russell's viper venom are much broader. For example, the Indian Russell's viper induced several rare complications such as the Wunderlich syndrome (Senthilkumaran et al., 2022a,b), pseudoaneurysm (Senthilkumaran et al., 2022a,b), priapism (Senthilkumaran et al., 2021) and salivary calculus development in submandibular gland (Arathisenthil et al., 2022). Similarly, here, we report a case of rectus

* Corresponding author.
E-mail address: s.vaiyapuri@reading.ac.uk (S. Vaiyapuri).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.toxicon.2023.107037
Received 8 December 2022; Received in revised form 7 January 2023; Accepted 20 January 2023
Available online 21 January 2023

sheath haematoma (RSH) in a female patient following Russell's viper envenomation in India.

RSH is an uncommon, and underdiagnosed disorder characterised by an accumulation of blood in the anterior rectus abdominis muscle (Paschou et al., 2014, Mahamad Arif and Syed Alwee Al'Aidrus, 2021). The pathogenesis of RSH is multifactorial with non-specific nature of clinical signs and symptoms. Its manifestations are varied depending on several factors, for example, the degree of peritoneal irritation, and the extensivity and location of the haematoma (Hatjipetrou et al., 2015). As an acute abdominal presentation, RSH is often accompanied by severe/sharp non-radiating pain, vomiting, tenderness, fever, chills, and nausea. Notably, a lower palpable abdominal mass that never crosses the midline is a typical feature of the RSH (Siu et al., 2003). RSH is usually misdiagnosed and easily confused with more prevalent intra-abdominal conditions, i.e. appendicitis, incarcerated inguinal hernia, and cholecystitis, among others (Buffone et al., 2015). Fothergill and Carnett signs are clinical clues that can guide and assist in reaching an earlier diagnosis of RSH (Yale et al., 2020). The non-invasive computed tomography scanning constitutes a gold standard method for identifying and monitoring this condition (Bello and Blanco 2019). To the best of our knowledge, the development of RSH following SBE has not been previously described in the literature. Hence, this report provides essential awareness and treatment guidelines for clinicians specifically in rural areas to tackle this issue following envenomation by Russell's viper and other snakes.

2. Case report

A 46-year-old female without any comorbidities was bitten by a snake on her left foot while she was working in a sugarcane field. The offending snake (which was brought to the hospital) was identified as Russell's viper by a trained herpetologist (Fig. 1A). The patient was taken to a local hospital immediately (within 30 minutes) after the bite, where she received 100 mL (10 vials) of polyvalent antivenom raised against the Indian 'Big Four' snakes (Russell's viper, Indian cobra, krait, and saw-scaled viper) as she demonstrated pain, local oedema and haematological [prolonged 20 minutes whole blood clotting test and international normalised ratio (INR) of blood clotting] manifestations, typical clinical features of Russell's viper envenomation. The administration of antivenom has corrected these manifestations, although, on the second day following the bite, she developed sudden sharp abdominal pain associated with nausea but there was no history of vomiting, loose stools, or fever. An ultrasound examination of the abdomen confirmed a non-mobile heterogeneous dense mass on the left upper quadrant extending along the omentum. The pain lasted for the whole day and gradually worsened with a significant drop in haemoglobin levels from 9 g/dL to 5 g/dL. Hence, she was referred (around 24 hours after the bite) to the emergency department of our hospital for further management.

Upon arrival, she was conscious but anxious and looked pale and afebrile. Her blood pressure was 80/60 mmHg with a heart rate of 122 beats/min and room air saturation of 92%. She had abdominal tenderness at the lower left quadrant of the abdomen and a palpable mass. The mass was tender on palpation, but there was no rebound tenderness and muscular rigidity. Both Fothergill and Carnett tests (clinical tests to ascertain RSH in patients with any abdominal injuries) were positive. Auscultation of the abdomen revealed a hypoactive bowel sound. Her haemoglobin level was 4.0 g/dL with a haematocrit of 12.8% and the platelet count was 86,000/μl (Table 1). The activated partial thromboplastin time (aPTT) was 41.8 seconds, prothrombin time (PT) was 20.7 seconds, and the INR of clotting was 2.1. All other biochemical tests were normal (Table 1). The ultrasound scan was performed to rule out any intra-abdominal haemorrhage, and it confirmed no solid organ injury or free fluid. Therefore, a CT scan of the abdomen was performed, and it revealed a haematoma on the rectus sheath of the abdominal wall (Fig. 1B). There was no predisposing condition in this patient including anti-coagulation therapy, constipation, coughing and family history of bleeding diathesis or haematological diseases. There were also no renal impairments in this victim. She received 10 vials (100 mL) of polyvalent antivenom raised against the Indian 'Big Four' snakes (Bharath Serums and Vaccines Limited, Mumbai, India) over 45 minutes in 250 mL of normal saline. In addition, six units each of packed red blood cells, fresh frozen plasma, and platelet concentrates were administered. The abdominal pain gradually subsided, her haemodynamics were optimised, haemoglobin values increased to 9.5 g/dL and the INR value decreased to 1.27. She was discharged eight days later (from admission to our hospital). The weekly follow-up monitoring over three months did not reveal any further effects in this patient.

3. Discussion

SBE-induced abdominal bleeding is an infrequent event that has been poorly documented in the literature. Recently, a rare case of retroperitoneal and peritoneal diffuse haematomas in a 11-year-old child

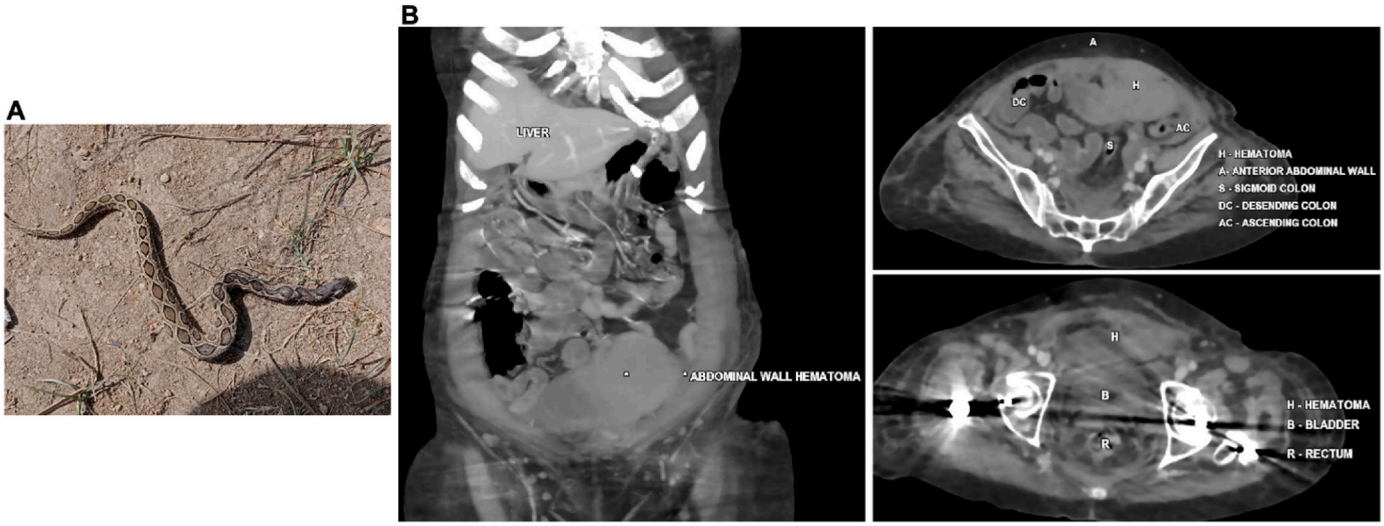


Fig. 1. The development of rectus sheath haematoma in a patient following Russell's viper bite. A, the offending snake was identified as Russell's viper by a trained herpetologist. **B**, the computed tomography scan images of different views of the abdominal region confirm the presence of a large haematoma at the abdominal wall ascertaining the diagnosis of rectus sheath haematoma.

Table 1
Laboratory results of the patient upon admission to the emergency department.

Specimen	Investigation	Results	Unit	Normal range
EDTA Whole Blood	Haemoglobin	4.0	gms%	13.0–16.0
EDTA Whole Blood	Total RBC count	3.45	Millions/μL	4.00–5.00
EDTA Whole Blood	HCT	12.8	%	41.00–50.00
EDTA Whole Blood	MCV	88.1	fl	81.10–96.00
EDTA Whole Blood	MCH	29.0	pg	27.20–33.20
EDTA Whole Blood	MCHC	32.9	%	32–36
EDTA Whole Blood	Total WBC count	16.97	$x10^3$ Cells/μL	4.00–11.00
EDTA Whole Blood	Neutrophils	14.8	$x10^3$ Cells/μL	2.0 to 7.0
EDTA Whole Blood	Lymphocytes	0.79	$x10^3$ Cells/μL	1.0 to 3.0
EDTA Whole Blood	Monocytes	1.36	$x10^3$ Cells/μL	0.1 to 0.8
EDTA Whole Blood	Eosinophils	0	$x10^3$ Cells/μL	0.02 to 0.5
EDTA Whole Blood	Basophils	0.02	$x10^3$ Cells/μL	0.02 to 0.1
EDTA Whole Blood	Neutrophils	87.2	%	55–75
EDTA Whole Blood	Lymphocytes	4.7	%	15–30
EDTA Whole Blood	Eosinophils	0	%	1–5
EDTA Whole Blood	Monocytes	8.0	%	2–10
EDTA Whole Blood	Basophils	0.1	%	Up to 1
EDTA Whole Blood	Platelet Count	86	$x10^3$ Cells/μL	150–450
EDTA Whole Blood	MPV	9.4	fl	6.5–12.0
EDTA Whole Blood	PDW	8.4	fl	9.0–13.0
Serum	Urea	33	mg/dL	15–40
Serum	Creatinine	0.98	mg/dL	0.6–1.2
Serum	Uric acid	3.5	mg/dL	2.4–6.1
Serum	Bilirubin (total)	1.10	mg/dL	0.2–1.2
Serum	Bilirubin (direct)	0.37	mg/dL	0–0.2
Serum	Bilirubin (indirect)	0.73	mg/dL	0.2–0.9
Serum	SGOT	32	U/L	5–35
Serum	SGPT	40	U/L	5–45
Citrated plasma	Prothrombin time	20.7	Seconds	11.5–16.0
Citrated plasma	aPTT	41.8	Seconds	26.0–40.0
Citrated plasma	INR	2.1	Ratio	

RBC, red blood cells; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cells; MPV, mean platelet volume; PDW, platelet distribution width; aPTT, activated partial thromboplastin time; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamate-pyruvic transaminase.

following a severe envenomation was reported (Kassegne et al., 2020). So far, RSH is an under-recognised cause of acute abdominal pain in SBE victims. To the best of our knowledge, the relationship between RSH and SBE has not been previously reported. In general, RSH occurs due to the tearing of the epigastric arteries or damage to intramuscular blood vessels because of tearing/damage to the muscle itself. Based on a retrospective analysis of the characteristics of the patients, this devastating bleeding complication is usually located infraumbilically and never crosses the midline, barring a few exceptions (Sabanis et al., 2020). Nevertheless, haematoma below the level of the arcuate line of Douglas causes an indirect irritation on the peritoneum due to the absent posterior rectus sheath in this region masquerading as acute abdomen pain (Kapan et al., 2008). Multiple etiological factors have been identified for RSH. Usually, it has been linked to trauma, abdominal surgery, trocar site injury after laparoscopic surgeries, subcutaneous drug injections, warfarin therapy, haematological diseases, uncontrolled hypertension, violent coughing, vigorous physical exercise, and pregnancy. However, in a few rare cases, it also developed spontaneously (Nizam et al., 2020). In this study, we report the development of RSH in a 46-year-old female victim following Russell's viper bite and clinical management strategies used for the diagnosis and treatment of this condition. This patient developed RSH despite prompt antivenom treatment in a local hospital following the bite.

The probable mechanism of RSH in the patient reported in this study may be due to a combination of thrombocytopenia, increased vascular fragility, dysfunctional platelets, coagulopathy, and increased fibrinolysis (Slagboom et al., 2017). From a pathogenesis standpoint, these haemodynamic disturbances are probably orchestrated by toxins that damage the endothelial layer of the vasculature (Rucavado et al., 2018), procoagulant and fibrinolytic enzymes (Mukherjee 2014), and proteins that modulate platelet function (Kalita et al., 2019). Factor X activator (RVV-X) (Takeya et al., 1992), factor V activator (RVV-V) (Tokunaga et al., 1988), daborhagin-M and daborhagin-K (Chen et al., 2008) are classical examples of toxins in Russell's viper venom related to haemostatic disturbances. Earlier studies have demonstrated that Russell's viper venom components trigger multifocal toxicity through dynamic changes in the levels of coagulation factors accompanied by consumptive coagulopathy and severe impact on clotting cascades (Isbister et al., 2015). On the other hand, snake venom metalloproteases (SVMPs) exert their haemorrhagic effects by cleaving key components of the basement membrane of capillaries with a direct impact on the mechanical stability of vessel walls (Gutiérrez et al., 2016a). The understanding of this biological phenomenon was built based on experimental results obtained using microscopic, histological, biochemical, and proteomic strategies (Gutiérrez et al., 2016b). Therefore, clinical findings, such as uncommon RSH, add a new dimension to the current views of SBE-induced bleeding complications that must be addressed through future research using integrative approaches including prospective clinical studies, omic tools and *in vitro, ex vivo* and/or *in vivo* functional assays to establish the molecular interactions between venom toxins and key factors that regulate the cardiovascular system, specifically blood coagulation.

Diagnosis of RSH can be challenging, mainly because its unspecific symptoms are highly similar and often shared with other causes of acute abdominal pain (Costello and Wright 2005). As reported in this patient, overlapping clinical manifestations must be carefully evaluated to avoid misdiagnosis or delayed diagnosis, which may hinder early and non-invasive intervention. The ability to recognise important clinical signs prevents negative exploratory laparotomies and consequently reduces morbidity and mortality risks associated with surgical procedures. Fothergill and Carnett signs were positive in this SBE victim, similar to clinical examination among patients with different aetiologies of the RSH (Yale et al., 2020). These physical findings are fundamental elements of the diagnostic process, contributing to the differentiation of RSH from other frequent intra-abdominal conditions. The basic principle behind these bedside techniques is the assessment of the position of a palpable mass and the modulation of pain intensity in response to tensing of the abdominal muscles (Yale et., 2020). The presence of an unchanged abdominal mass in terms of location (does not cross the midline), which can be palpated in both relaxed and contracted muscle conditions, characterises Fothergill sign (Bello and Blanco 2019). The other uncomplicated test of high diagnostic value called Carnett sign is associated with increased or maintained pain and tenderness during muscle movement. To illustrate this point, as a first step, clinicians delimit the region of maximum tenderness by palpation in the resting state. Then, the patient tenses the abdominal wall by lifting the head and shoulders or both legs, while the site of pain continues to be monitored. Confirmation of the sign considers the comparison of pain and

tenderness in both circumstances. In summary, this test is considered positive when the pain during contraction is equal to or more intense than the initial screening (Yale et., 2020). These methods were reported and proposed for the recognition of abdominal wall haematoma approximately a century ago and it remains useful to this day (Yale et., 2020). Hence, awareness of this rare clinical entity and its symptoms is crucial in the differential diagnosis of acute abdominal pain and in the development of an alternative and cost-effective strategy in under-resourced rural settings. These aspects must be considered due to the disproportionate impact of SBE in tropical rural areas with under-resourced health systems (Gutiérrez et al., 2017). Taken together, the simple clinical criteria discussed above may assist in better triage of patients with RSH, including those caused by SBE (Senthilkumaran et al., 2012). In the same context, the combination of diagnostic imaging techniques (e.g., ultrasonography and CT scans) will provide a significant increase in sensitivity and specificity in the diagnosis of acute abdominal pain (Pierro et al., 2018). Ultrasonography is a valuable imaging tool, however, it plays a relatively limited role in the diagnosis of RSH. This approach is non-specific with a 71% sensitivity (Moreno Gallego et al., 1997). Occasionally, it is difficult to differentiate intra-peritoneal lesions from extra-peritoneal lesions by ultrasound, as seen in our case. The limitations rely on the interpretation of the images and errors derived from the probe-induced tenderness (Costello and Wright 2005). However, CT scans produce high-quality images and show better discriminative ability in terms of localisation, size, origin, extension, and evaluation of the collection of blood outside of the blood vessels in the rectus sheath (Hamza et al., 2021). Thus, CT evaluation aids in narrowing down the correct diagnosis and it should be adopted as a first-line technique when available.

Prompt clinical management remains a cornerstone in the treatment of haemodynamically stable patients with no evidence of active bleeding or expanding haematoma (Liao and Puckett 2021). In our patient, the clinical outcomes were favourable following primary treatment with antivenom, packed cells, fresh frozen plasma, and platelet transfusion. This first-line treatment is reported as an appropriate choice for stable individuals that do not require surgical exploration. Similar combinatory therapy has been successfully employed in other RSH cases in non-SBE patients (Kapan et al., 2008; Buffone et al., 2015). Invasive control of active bleeding is considered only if conservative treatments fail and the clinical severity criteria characterised by haemodynamic instability, neurological deficit and continued bleeding are present (Pierro et al., 2018). In line with this, surgical intervention was considered unnecessary for this patient due to positive clinical evaluation and possible complications associated with this aggressive procedure. In addition, postoperative mortality after RSH surgical management is relatively high with the risk of re-bleeding (Cereda et al., 2017). Therefore, clinicians have employed endovascular coil embolisation, as an alternative in those patients who are refractory to conservative treatment that requires additional intervention (Senthilkumaran et al., 2009). This minimally invasive therapeutic modality has ensured high clinical success with recognised safety, effectiveness and low morbidity (Méndez et al., 2022).

In conclusion, the conservative diagnosis and treatment based on the recognition of simple signs and imaging modalities proved to be very useful for a successful outcome in this patient with RSH. The improvement of the clinical management for SBE requires multidisciplinary strategies, including robust training and knowledge about the unusual manifestations, their diagnosis, and therapies. The detailed description of rare clinical observations such as RSH following Russell's viper bites represents a starting point that opens avenues for further studies from basic research towards the clinic and helps to establish standard treatment protocols for SBE.

Credit author statement

Subramanian Senthilkumaran: Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Visualization, Supervision. **José R. Almeida:** Writing – original draft, Writing – review & editing, Visualization. **Jarred Williams:** Writing – original draft, Writing – review & editing, Visualization. **Anika Salim:** Writing – original draft, Writing – review & editing, Visualization. **Harry F. Williams:** Formal analysis, Writing – review & editing, Supervision. **Ponniah Thirumalaikolundusubramanian:** Formal analysis, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Ketan Patel:** Formal analysis, Resources, Writing – review & editing. **Sakthivel Vaiyapuri:** Conceptualization, Methodology, Formal analysis, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision.

Ethical statement

This research was conducted according to the Declaration of Helsinki and the ethical guidelines of the Indian Council of Medical Research. The data collection, consent form, and information sheet were approved by the Institutional Ethics Committee at Toxiven Biotech, Tamil Nadu, India (Reference number: ICMR-Toxiven Ethics, 2022/1) and University of Reading Research Ethics Committee (Reference number: UREC 23/05). A written consent was obtained before this case report was published.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

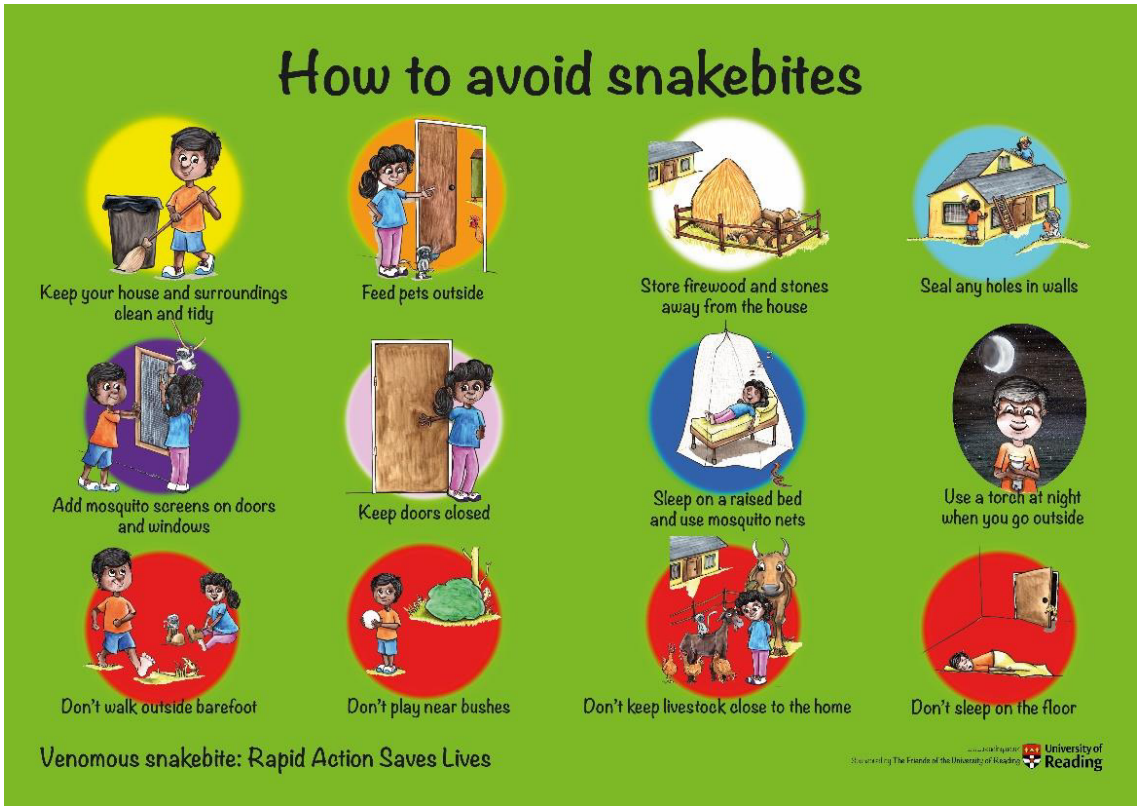
All the data associated with this article are presented within this article.

References

Arathisenthil, S.V., et al., 2022. Rapid development of a salivary calculus in submandibular gland and its potential causes in a young victim following Russell's viper bite. Toxicon 206, 85–89.
Bello, G., Blanco, P., 2019. Giant rectus sheath hematoma. The Ultrasound Journal 11 (1), 13.
Buffone, A., et al., 2015. Management of patients with rectus sheath hematoma: personal experience. J. Formos. Med. Assoc. 114 (7), 647–651.
Cereda, A., et al., 2017. Percutaneous artery embolization of bleeding rectus sheath hematomas in hemodynamically unstable patients: outcomes of 43 patients in a tertiary referral hospital. Emergency Care Journal 13 (2).
Chen, H.-S., et al., 2008. P-III hemorrhagic metalloproteinases from Russell's viper venom: cloning, characterization, phylogenetic and functional site analyses. Biochimie 90 (10), 1486–1498.
Costello, J., Wright, J., 2005. Rectus sheath haematoma: 'a diagnostic dilemma. Emerg. Med. J. 22 (7), 523.
Gutiérrez, J.M., et al., 2016a. Hemorrhage caused by snake venom metalloproteinases: a journey of discovery and understanding. Toxins 8 (4), 93.
Gutiérrez, J.M., et al., 2016b. A comprehensive view of the structural and functional alterations of extracellular matrix by snake venom metalloproteinases (SVMPs): novel perspectives on the pathophysiology of envenoming. Toxins 8 (10).
Gutiérrez, J.M., et al., 2017. Snakebite envenoming. Nat. Rev. Dis. Prim. 3 (1), 17063.
Hamza, M., et al., 2021. Clinical management of snakebite envenoming: future perspectives. Toxicon X 11, 100079.
Hatjipetrou, A., et al., 2015. Rectus sheath hematoma: a review of the literature. Int. J. Surg. 13, 267–271.
Isbister, G.K., et al., 2015. Venom concentrations and clotting factor levels in a prospective cohort of Russell's viper bites with coagulopathy. PLoS Neglected Trop. Dis. 9 (8), e0003968.
Kalita, B., et al., 2019. RGD-independent binding of Russell's Viper venom Kunitz-type protease inhibitors to platelet GPIIb/IIIa receptor. Sci. Rep. 9 (1), 8316.
Kapan, S., et al., 2008. Rectus sheath hematoma: three case reports. J. Med. Case Rep. 2, 22.
Kassegne, I., et al., 2020. Severe snakebite envenomation revealed by an acute abdomen. J. Surg. Case Rep. 2020 (6), rjaa148.

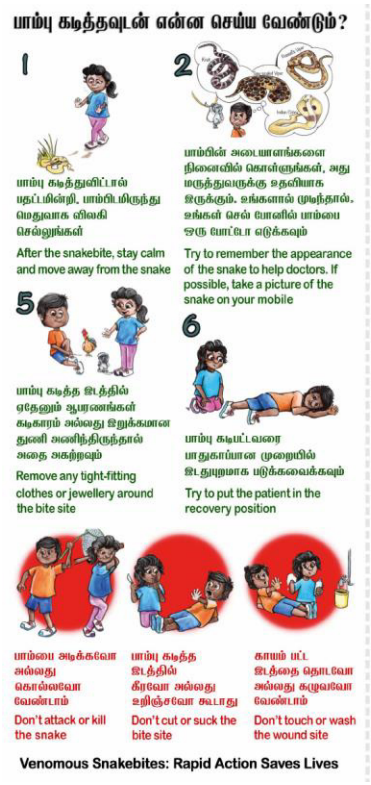
Kumar, K.S., et al., 2018. Clinical and epidemiologic profile and predictors of outcome of poisonous snake bites - an analysis of 1,500 cases from a tertiary care center in Malabar, North Kerala, India. Int. J. Gen. Med. 11, 209–216.
Liao, E.-D., Puckett, Y., 2021. A proposed algorithm on the modern management of rectus sheath hematoma: a literature review. Cureus 13 (11).
Mahamad Arif, A.N.F., Syed Alwee Al'Aidrus, S.S., 2021. Fatal rectus sheath hematoma: a rare autopsy phenomenon. Egypt. J. Food Sci. 11 (1), 23.
Mahasandana, S., et al., 1980. Clinical manifestations of bleeding following Russell's viper and Green pit viper bites in adults. Southeast Asian J. Trop. Med. Publ. Health 11 (2), 285–293.
Méndez, P., et al., 2022. Rectus Sheath Hematoma Embolization: when, Why, and for Whom? Vascular Disease Management.
Moreno Gallego, A., et al., 1997. Ultrasonography and computed tomography reduce unnecessary surgery in abdominal rectus sheath haematoma. Br. J. Surg. 84 (9), 1295–1297.
Mukherjee, A.K., 2014. The pro-coagulant fibrinogenolytic serine protease isoenzymes purified from Daboia russelii russelii venom coagulate the blood through factor V activation: role of glycosylation on enzymatic activity. PLoS One 9 (2), e86823.
Nizam, A., et al., 2020. Spontaneous rectus sheath hematoma masquerading as acute abdomen. A case report. Maedica (Bucur) 15 (3), 412–415.
Paschou, E., et al., 2014. An uncommon presentation of spontaneous rectus sheath hematoma with acute kidney injury due to obstructive uropathy and prerenal azotemia. Case Reports in Emergency Medicine, 164245, 2014.
Pierro, A., et al., 2018. Spontaneous rectus sheath hematoma: the utility of CT angiography. Radiol Case Rep 13 (2), 328–332.
Ratnayake, I., et al., 2019. Early identification of acute kidney injury in Russell's viper (Daboia russelii) envenoming using renal biomarkers. PLoS Neglected Trop. Dis. 13 (7), e0007486.
Rucavado, A., et al., 2018. Systemic vascular leakage induced in mice by Russell's viper venom from Pakistan. Sci. Rep. 8 (1), 16088.
Sabanis, N., et al., 2020. Fondaparinux-associated rectus sheath hematoma: skating on thin ice. Cureus 12 (5), e7938.
Samuel, S.P., et al., 2020. Venomous snakebites: rapid action saves lives—a multifaceted community education programme increases awareness about snakes and snakebites among the rural population of Tamil Nadu, India. PLoS Neglected Trop. Dis. 14 (12), e0008911.
Senji Laxme, R.R., et al., 2021. Biogeographic venom variation in Russell's viper (Daboia russelii) and the preclinical inefficacy of antivenom therapy in snakebite hotspots. PLoS Neglected Trop. Dis. 15 (3), e0009247.
Senthilkumaran, S., et al., 2009. Rectus sheath hematoma: a diagnostic dilemma in acute abdomen. Indian J Trauma Anaesth Crit Care 10, 34–37.
Senthilkumaran, S., et al., 2012. Rectus sheath hematoma: clinical examination is the key. Am. J. Emerg. Med. 30 (9), 2069–2070.
Senthilkumaran, S., et al., 2021. Priapism following a juvenile Russell's viper bite: an unusual case report. PLoS Neglected Trop. Dis. 15 (3), e0009242.
Senthilkumaran, S., et al., 2022a. Development of Wunderlich syndrome following a Russell's viper bite. Toxicon 215, 11–16.
Senthilkumaran, S., et al., 2022b. Ultrasound-guided compression method effectively counteracts Russell's viper bite-induced pseudoaneurysm. Toxins 14 (4).
Siu, W.T., et al., 2003. Spontaneous rectus sheath hematoma. Can. J. Surg. 46 (5), 390.
Slagboom, J., et al., 2017. Haemotoxic snake venoms: their functional activity, impact on snakebite victims and pharmaceutical promise. Br. J. Haematol. 177 (6), 947–959.
Suraweera, W., et al., 2020. Trends in snakebite deaths in India from 2000 to 2019 in a nationally representative mortality study. Elife 9, e54076.
Takeya, H., et al., 1992. Coagulation factor X activating enzyme from Russell's viper venom (RVV-X). A novel metalloproteinase with disintegrin (platelet aggregation inhibitor)-like and C-type lectin-like domains. J. Biol. Chem. 267 (20), 14109–14117.
Tokunaga, F., et al., 1988. The factor V-activating enzyme (RVV-V) from Russell's viper venom. Identification of isoproteins RVV-V alpha, -V beta, and -V gamma and their complete amino acid sequences. J. Biol. Chem. 263 (33), 17471–17481.
Vaiyapuri, S., et al., 2013. Snakebite and its socio-economic impact on the rural population of Tamil Nadu, India. PLoS One 8 (11), e80090.
Yale, S.H., et al., 2020. Fothergill and Carnett signs and rectus sheath hematoma. J. Rural Med. 15 (3), 130–131.

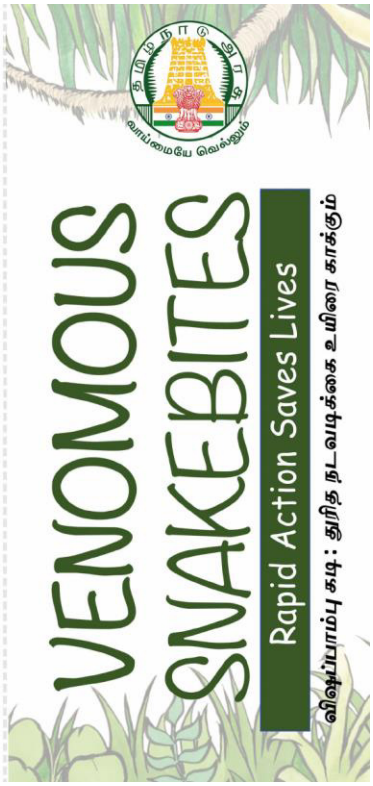
Appendix 3: Development of SBE awareness poster



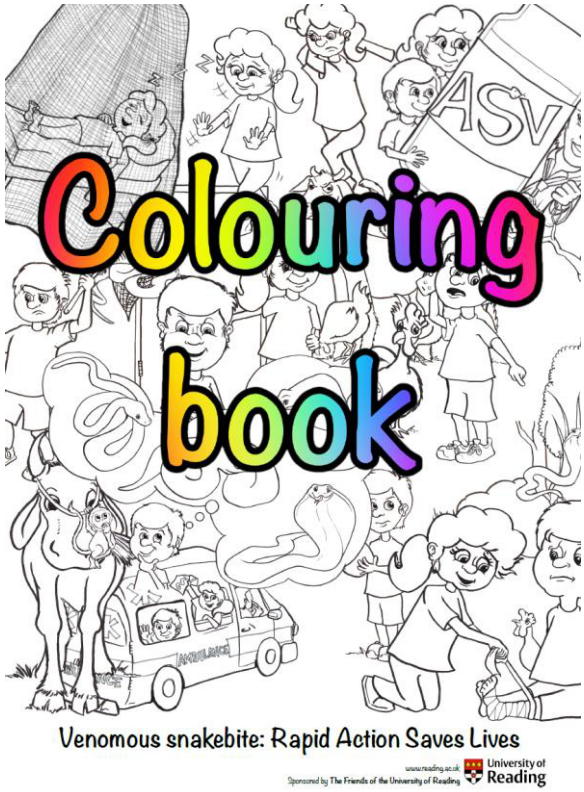
Appendix 4: SBE awareness poster approved by the Government of Tamil Nadu, India

Appendix 5: SBE awareness leaflet approved by the Government of Tamil Nadu, India





Appendix 6: SBE awareness colouring books for children available in English and Tamil language



Appendix 7: SBE awareness animations

Animation 1: How to avoid snakebites:

https://youtu.be/69Ean1ZKi3Y?si=iIGzo-KtKJ5B0n52

Animation 2: What to do if a snake bites you:

https://youtu.be/y2WaybA3_Fk?si=WdFode1mxd49EMEy