

*Rapid development of a salivary calculus  
in submandibular gland and its potential  
causes in a young victim following  
Russell's viper bite*

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1 **Rapid development of a salivary calculus in submandibular gland and its potential causes**  
2 **in a young victim following Russell's viper bite**

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18  
19 **Abstract**

20 Russell's viper bites are known to cause a range of haemotoxic, neurotoxic, myotoxic,  
21 cytotoxic and nephrotoxic complications. However, the impact of Russell's viper bites as well as bites  
22 from other venomous snakes on sialolithiasis has not been previously reported. Here, we present  
23 an interesting case where a Russell's viper bite induced the rapid development of a calculus in  
24 submandibular gland in a 10-year-old boy. Upon admission, the victim did not show any symptoms  
25 of swelling and/or pain around his oral cavity. He received antivenom treatment to normalise his  
26 coagulation parameters, however, on day three he developed swelling and extreme pain around his  
27 right mandibular region. An ultrasound investigation revealed the presence of a calculus in his  
28 submandibular gland, which was removed using a minor surgical procedure. The histopathological  
29 examination revealed this as a poorly calcified salivary calculus, which is composed of cell debris,  
30 mucopolysaccharides and lipids. The mechanisms behind its rapid development following a  
31 snakebite are unclear although this could be linked to excessive inflammation or modifications to the  
32 composition of saliva induced by venom toxins or other unknown factors. This report reveals an  
33 unusual complication induced by a Russell's viper bite and alerts clinicians who treat snakebites to  
34 be aware of such envenomation effects. Moreover, this will lead to novel research to explore the  
35 relationship between venom toxins and functions of salivary glands.

36 **Key words**

37 Snakebite envenomation; Russell's viper; salivary calculi; salivary glands; sialolithiasis; inflammation

38 **Introduction**

39 Sialolithiasis is the formation of calculi in salivary glands, primarily in ducts connecting the  
40 glands to the oral cavity (Delli et al., 2014; Hammett and Walker, 2021; Kraaij et al., 2014). This  
41 occurs predominantly in adults (mostly in males) who are aged between 30 and 60 years old with  
42 occurrence among children being rare (Kraaij et al., 2014). Between the three pairs of salivary glands  
43 (parotid, submandibular, and sublingual), calculi formation has been predominantly (over 85%  
44 cases) reported in submandibular glands (Grases et al., 2003; Kraaij et al., 2014), mostly in the  
45 excretory ducts (Sigismund et al., 2015). There are no precise mechanisms or causative factors  
46 established yet for the development of salivary calculi although they are broadly linked to various  
47 anatomical features of salivary glands and composition of calculi (Grases et al., 2003). For example,  
48 accumulation of bacteria, foreign bodies (Marchal et al., 2001), cellular debris (Harrison, 2009) and  
49 excessive inflammation, which triggers the infiltration of neutrophils (Schapher et al., 2020) in ducts

50 are likely to result in sialolithiasis. The presence of micro calculi (concrements) in the excretory duct  
51 system in submandibular glands even in many healthy, asymptomatic individuals (Sigismund et al.,  
52 2015) may act as nuclei for the deposition of various organic and inorganic molecules from saliva to  
53 ultimately form large calculi over a long period (Grases et al., 2003; Sivasubramaniam et al., 2015).  
54 Moreover, ducts in submandibular glands allow a slower salivary flow which may be affected when  
55 the salivary thickness is increased leading to the development of sludge and ultimately calculi.  
56 Similarly, increased ionic contents (specifically calcium) and modified amylase activities may induce  
57 the formation of salivary calculi (Kraaij et al., 2014; Schröder et al., 2017).

58 Snakebite envenomation (SBE) has been reported to cause diverse neurotoxic, haemotoxic,  
59 myotoxic, cytotoxic and nephrotoxic complications based on the type of offending snake and the  
60 nature of their venom (Gutiérrez et al., 2017; Williams et al., 2019). Russell's viper (*Daboia russelii*)  
61 is a key species that causes numerous bites and resulting deaths and disabilities in India (Samuel  
62 et al., 2020; Vaiyapuri et al., 2013). While viper venoms generally induce haemotoxic effects,  
63 Russell's viper venom causes both haemotoxic and neurotoxic effects (Silva et al., 2017; Silva et al.,  
64 2016). In addition, Russell's viper bites are known to induce rare complications such as priapism  
65 (Senthilkumaran et al., 2021) and bilateral blindness (Subasinghe et al., 2014). While Russell's viper  
66 bite-induced swelling in parotid glands has been reported in few case reports (Deepak et al., 2013;  
67 Sasidaran et al., 2017), the impact of SBE including bites from Russell's viper on salivary calculi  
68 formation has not been previously documented. Hence, we report this unusual case of rapid salivary  
69 calculus formation in submandibular gland in a 10-year-old boy following a Russell's viper bite. This  
70 rare case is not only reporting an unusual complication of Russell's viper bite, but it also opens a  
71 new avenue to explore the impacts of venom toxins on the pathophysiology of salivary glands.  
72 Moreover, this will create awareness among clinicians who treat SBE to closely monitor the  
73 development of this type of rare complications in SBE victims to provide better care.

#### 74 **Case presentation**

75 A 10-year-old boy with no previous medical conditions was brought to our emergency  
76 department following a snakebite on his left foot while walking in a field nearly two hours prior to the  
77 admittance. A tourniquet had been placed above the bite site using a banana tree fibre by the victim's  
78 father. They also brought the offending snake (killed), which was identified as a Russell's viper by a  
79 herpetologist (**Figure 1A**). Upon admission, the boy was conscious, well oriented, afebrile, and mildly  
80 anxious. Swelling was observed around the bite site although there was no excessive bleeding or  
81 tissue damage (**Figure 1B**). His initial vital signs were measured as; blood pressure - 116/72 mm  
82 Hg, heart rate - 129 beats/min, respiratory rate - 26 breaths/min, temperature - 36.9°C, and oxygen  
83 saturation on room air - 100%. There were no obvious signs of swelling in his lymph nodes or around  
84 the oral cavity. He exhibited typical features of Russell's viper bite-induced systemic (gum bleeding),  
85 and local (swelling, developing wound and mild bleeding at bite site) envenomation effects. The 20-  
86 minute whole blood clotting time was prolonged and therefore, he received 100 mL of polyvalent  
87 antivenom (Bharat Serums and Vaccines Limited, India) intravenously according to the standard  
88 protocols. The antivenom administration was well tolerated with no signs of adverse reactions. He  
89 received another 100 mL of antivenom and four units of fresh frozen plasma to normalise his  
90 coagulation parameters over the next twenty-four hours. His other haematologic, metabolic, and  
91 biochemical parameters were within the normal limits. He was comfortable on the second day with  
92 no obvious local inflammatory changes. However, on the third day (while staying at the hospital), he  
93 complained of sudden onset of pain and swelling on the right mandibular region. On bimanual  
94 palpation, right submandibular gland was warm and tender with swollen lymph node. Oral  
95 examination has revealed a firm, tender, inflamed and erythematous duct opening with hard swelling  
96 situated near the right premolars, which was extremely painful on palpation. There was no  
97 associated discharge or bleeding from the affected area. The occlusal radiograph indicated the  
98 absence of any salivary calculi although an ultrasound scan of his right cheek confirmed the  
99 presence of a calculus measuring around 6 mm (**Figure 1C**). His salivary analysis confirmed  
100 moderately increased thickness and threads in mucus, and a few epithelial and bacterial cells.  
101 However, no crystals or major differences in biochemical parameters (including calcium levels) were  
102 noticed in saliva (**Table 1**). Under local anaesthesia, an incision was made at the ductal orifice and  
103 the calculus together with surrounding affected tissues was exposed and removed (**Figure 1D**).  
104 Absorbable sutures were placed to close the incised area and his post-operative healing was smooth  
105 and uneventful. Histopathological examination of salivary gland tissues extracted together with the

106 calculus displayed the infiltration of inflammatory cells and ductal dilation, and the composition of  
107 calculus as glycoproteins, cellular debris, lipids and mucopolysaccharides (**Figure 1E**). The boy was  
108 discharged on day seven, and his subsequent weekly reviews did not display any further  
109 complications.

## 110 **Case discussion**

111 While SBE is known to induce a diverse spectrum of complications in humans (Williams et  
112 al., 2019), to the best of our knowledge, its impact on sialolithiasis has not been previously reported.  
113 Moreover, sialolithiasis is commonly encountered among adults who are aged between 30 and 60  
114 years old (Grases et al., 2003; Hammett and Walker, 2021). Hence, this report presents an  
115 interesting case of SBE (specifically Russell's viper bite)-induced sialolithiasis in a 10-year-old boy  
116 with no previous history of any medical conditions. Here, we discuss various potential reasons for  
117 sialolithiasis in this patient, although this could have independently developed without any impacts  
118 by SBE. Notably in this patient, the salivary calculus has appeared to be formed rapidly following the  
119 bite despite antivenom treatment. Similarly, the calculus could have formed already, but the area  
120 became very sensitive and extremely painful following the bite. Although occlusal radiography has  
121 been used to identify salivary calculi that are rich in calcium (Kim et al., 2016), it is not helpful in  
122 identifying poorly calcified calculi as in this case. Hence, it is important to use alternative diagnostic  
123 tools such as ultrasound and computed tomography (CT) scans, digital subtraction sialography and  
124 sialendoscopy to corroborate the presence of calculi (Goncalves et al., 2017; Hammett and Walker,  
125 2021; Kim et al., 2016). Salivary calculi normally form at the rate of 1 mm per year, and they are  
126 noticeable only after several years (Grases et al., 2003). However, in this case, the incidence of pain  
127 with associated swelling was discovered within three days following the bite. Therefore, further  
128 investigation on the physical and biochemical nature of the calculi that are formed acutely (within a  
129 short period of time as reported in this case) and chronically (formed over a longer time as reported  
130 generally for sialolithiasis) should be thoroughly investigated to determine the mechanisms and  
131 major differences between these two methods. While the mechanisms behind the development of  
132 salivary calculi in this case are not entirely clear, here we discuss some plausible mechanisms for  
133 this incident.

134 The production of saliva and its delivery to the oral cavity is a tightly regulated phenomenon  
135 (Delli et al., 2014). Therefore, any adverse changes to the anatomical features of salivary glands  
136 and ducts are likely to result in sialolithiasis. For example, inflammation in glands or ducts will lead  
137 to infiltration of immune cells (specifically neutrophils) (Hammett and Walker, 2021; Marchal et al.,  
138 2001; Schröder et al., 2017), stenosis or dilation and accumulation of minerals, foreign bodies, or  
139 cellular debris, which will result in sialolithiasis (Delli et al., 2014; Grases et al., 2003; Harrison, 2009;  
140 Kraaij et al., 2014; Marchal et al., 2001). Schapher et al. (Schapher et al., 2020) reported the impact  
141 of inflammation in salivary glands by increasing the sudden flow of neutrophils in the ducts leading  
142 to release of neutrophil extracellular traps (NETs), which subsequently induced the formation of  
143 calculi by acting as a scaffold. Although NETosis is an inflammatory response elicited by neutrophils  
144 mainly to remove the invading pathogens and cellular debris, it has been demonstrated to induce  
145 the deposition of various organic and inorganic materials in different organs in addition to salivary  
146 glands (Maueröder et al., 2015; Muñoz et al., 2019). In the present case, it was evident that  
147 inflammatory cells were infiltrated in the ducts of submandibular glands. Moreover, the accumulation  
148 of cellular debris, glycoproteins and mucopolysaccharides was noted during pathological  
149 examination of extracted calculi. The venom of the saw-scaled viper (*Echis carinatus*) has been  
150 reported to induce NETosis (Katkari et al., 2016), although no direct link to sialolithiasis has been  
151 established. Russell's viper bites are known to induce inflammatory responses and cytotoxicity due  
152 to the large proportion of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) present in this venom (Kalita et al., 2018). In  
153 addition, the snake venom metalloproteases (Teixeira Cde et al., 2005) and serine proteases  
154 (Menaldo et al., 2013) are able to contribute to the development of inflammation. Hence, the  
155 components of Russell's viper venom could have induced the inflammation in salivary glands  
156 resulting in infiltration of neutrophils and NETosis. The NETs released might have acted as a base  
157 for the rapid accumulation of cellular debris, phospholipids and mucopolysaccharides to develop  
158 poorly calcified calculus. However, it is unclear on why the toxic venom components have targeted  
159 only salivary glands instead of any other organs although the damage to other organs could be  
160 minimal and hence unnoticed.

161 The saliva secreted from submandibular glands contains higher concentration of calcium  
162 than from other salivary glands (Harrison et al., 1993). The submandibular calculi are formed with  
163 over 70% inorganic materials, mostly calcium phosphates in the form of hydroxylapatite (Grases et  
164 al., 2003; Hammett and Walker, 2021). The formation of salivary calculi has been demonstrated to  
165 occur due to accumulation and deterioration of calcium rich saliva in the ductal area (Grases et al.,  
166 2003). It was suggested that an organic molecule or particle forms a base micro calculus upon which  
167 other salts, organic and inorganic matters accumulate to form large calculi over a long time (Harrison,  
168 2009). Indeed, the presence of micro calculi has been reported even in many healthy individuals and  
169 they can slowly induce congestion in ducts and stasis of saliva resulting in inflammation and further  
170 growth even in asymptomatic people (Epivatianos and Harrison, 1989). In addition to calcium,  
171 phospholipids and secreted glycoproteins will contribute to the development of calculi (Boskey et al.,  
172 1981; Boskey et al., 1983; Harrison, 2009). Higher concentrations of bicarbonate in saliva are also  
173 able to alter the ionic composition of saliva and induce NETosis (Leppkes et al., 2016). Therefore,  
174 all these factors could have contributed to the rapid development of calculus in the present case  
175 although the calculus was poorly calcified. Notably, in this patient, there were no major changes in  
176 their biochemical parameters in saliva including calcium and bicarbonate levels compared to the  
177 reference levels. PLA<sub>2</sub> present in Russell's viper venom causes cytotoxic effects (Chakrabarty and  
178 Sarkar, 2015) and these may in turn induced the accumulation of cellular debris and associated  
179 components to accumulate around the pre-existing base calculi. Acinar cells and duct cells are the  
180 major cellular components of salivary glands. Acinar cells are involved in serous saliva secretion  
181 while the duct cells secrete a more mucinous saliva (Kondo et al., 2015). The acinar cells produce  
182 an isotonic fluid that is modified into a hypotonic fluid by duct cells before its release into the oral  
183 cavity (Kondo et al., 2015). It has been reported that intracellular calcium plays a significant role in  
184 the secretion of saliva (Ambudkar, 2016). Indeed, calcium acts as a regulator of various ion pathways  
185 involved in salivary secretion. CRISPs (cysteine-rich secretary proteins) that are largely found in  
186 venoms of vipers (including Russell's viper) and elapid snakes have been reported to block calcium  
187 ion channels in various cell types (Tadokoro et al., 2020). Hence, these non-enzymatic proteins  
188 could have affected calcium channels which might have affected the salivary production and its ionic  
189 concentrations resulting in increased thickness of saliva. Therefore, studying the impact of CRISPs  
190 of Russell's viper venom may demonstrate their influence on ion channels involved in salivary  
191 production and their ultimate impact on the formation of salivary calculi. Moreover, an increase in  
192 bicarbonate content in saliva precipitates more calcium and phosphate ions leading to formation of  
193 calculi (Grases et al., 2003; Hammett and Walker, 2021). Hence, investigating the effects of Russell's  
194 viper venom components in modulating bicarbonate synthesis and maintenance will also be useful.  
195 Notably, the composition of Russell's viper venom differs based on their geographical locations  
196 (Kalita et al., 2018). Therefore, it might be possible for Russell's vipers from specific regions to induce  
197 this type of unusual complications in victims especially children.

198 Since Russell's viper bites are known to cause neurological complications (Kalita et al., 2018;  
199 Silva et al., 2017), it is important to determine the impact of these venom toxins on neural networks  
200 that regulate salivary secretion. Salivary neural networks involve autonomous, parasympathetic, and  
201 sympathetic stimulations (Ekström, 1989; Proctor and Carpenter, 2007). The parasympathetic  
202 signalling system in saliva secretion involves acetylcholine and noradrenaline release (Proctor and  
203 Carpenter, 2014). Both acinar and duct cells express cholinergic receptors as acetylcholine is the  
204 major neurotransmitter secreted by parasympathetic vagal fibres. Russell's viper venom consists of  
205 pre-synaptically active neurotoxic, PLA<sub>2</sub> (Kalita et al., 2018). Therefore, further investigation into the  
206 effects of this venom on salivary neural cells might shed light on the impact of Russell's viper bite on  
207 the salivary neural systems and their association with the calculi formation. In addition, SBE including  
208 from Russell's vipers is known to induce acute pain and long-term complications in multiple regions  
209 other than the bite site (Waidyanatha et al., 2019). Therefore, it is possible that the calculus might  
210 have formed already, but the area was sensitised and became painful due to the direct and/or indirect  
211 actions of venom toxins.

212 We cannot rule out the effects of antivenom administered in this victim on sialolithiasis, as  
213 the calculi formation was discovered post antivenom treatment. Although this patient did not display  
214 any adverse reactions upon infusion of antivenom, the antigen-antibody (venom/antivenom)  
215 complexes formed might have triggered inflammatory responses specifically in salivary glands  
216 and/or ducts through inflammatory responses (de Silva et al., 2016; Morais and Massaldi, 2009).

217 The polyvalent antivenom used in this case is known to cause adverse reactions due to  
218 immunoglobulin complexes and impurities. Among the adverse reactions caused by antivenom, IgE-  
219 mediated and non-IgE mediated anaphylactic reactions are known to cause increased vascular  
220 permeability and mucous secretion (de Silva et al., 2016; Morais and Massaldi, 2009). Similarly, the  
221 fresh frozen plasma transfused in this patient could have triggered or contributed to this complication  
222 as it is known induce hypersensitivity reactions (Pandey and Vyas, 2012) although it was not evident  
223 in this case. These reactions could affect the rate of saliva production in submandibular glands and  
224 its composition. Hence, this aspect should be further investigated.

225 In conclusion, it is not entirely clear if venom components alone induced the rapid formation  
226 of poorly calcified salivary calculus in this case, or it was an aggravation of an already growing micro  
227 calculi due to excessive inflammation. There is also a potential possibility that it was due to adverse  
228 reactions to the antivenom or fresh frozen plasma. Further research into these types of unusual  
229 cases can not only help us in understanding the molecular mechanisms of venom (and antivenom)  
230 components but also enlighten us with the wide spectrum of envenomation complications which will  
231 result in revising treatment guidelines for better clinical management of SBE. This case report will  
232 assist clinicians in areas where SBE commonly occurs to be aware of such unusual clinical  
233 manifestations specifically among children.

#### 234 **Ethical statement**

235 The data collection, consent form, and information sheets were approved by the Institutional  
236 Ethics Committee at Toxiven Biotech, Tamil Nadu, India (Reference number: ICMR-Toxiven Ethics  
237 2021/1). A written consent was obtained from the patient's parents (since the patient was a minor)  
238 to collect and publish the data presented in this article.

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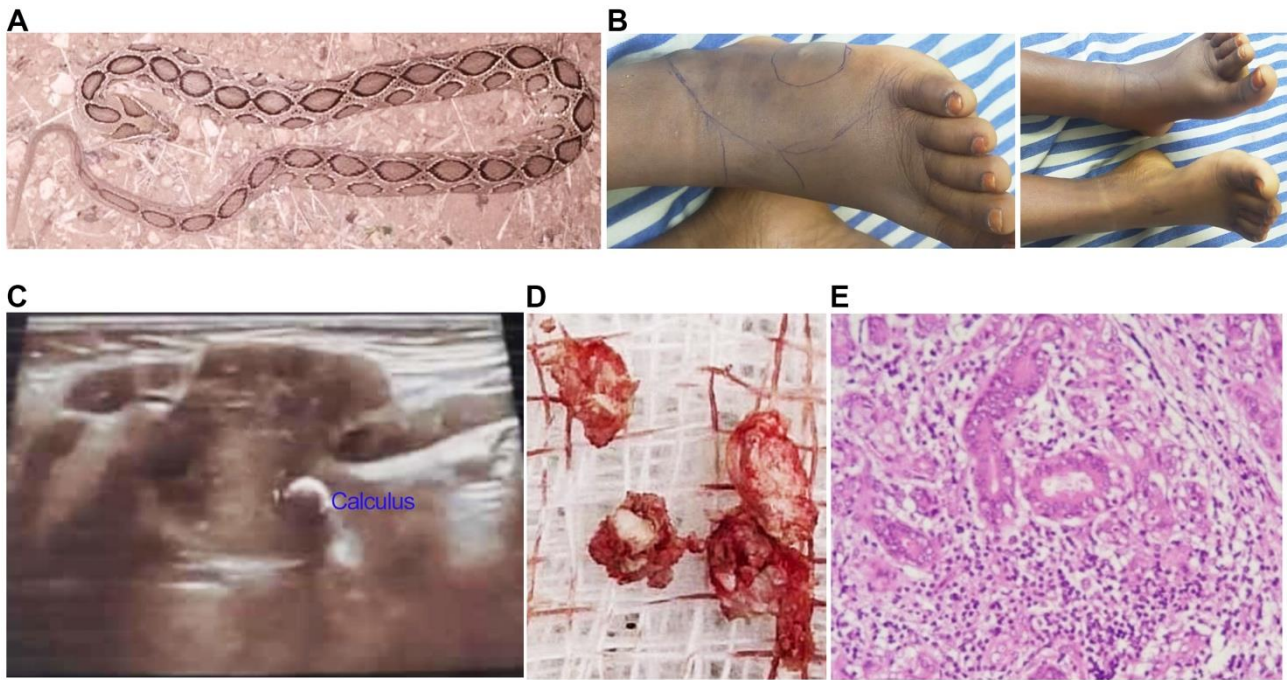
362 **Figure Legnds**

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364 **Figure 1:** **A**, the offending snake was identified as a Russell's viper. **B**, bite area shows mild swelling  
365 and developing wound. **C**, an ultrasound scan reveals the presence of a calculus at the duct of  
366 submandibular gland. **D**, extracted calculus with affected tissues using a minor surgery. **E**,  
367 haemotoxylin and eosin staining of extracted salivary tissues demonstrates the excessive infiltration  
368 of leukocytes.

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370 **Figure 1**



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389 **Table 1:** Salivary analysis report

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<b>Parameter analysed</b>	<b>Results</b>	<b>Unit</b>	<b>Reference range</b>
Colour	White	-	-
Appearance	Turbid	-	-
Reaction	Alkaline	-	-
pH	7.12	-	-
Specific gravity	1.53	-	-
Cast	Mucus ++	-	-
Crystals	None	-	-
Mucus threads	++	-	-
Others	None	-	-
Sodium	130	mmol/L	135 to 150
Potassium	4.0	mmol/L	3.5 to 5.0
Chloride	112	mmol/L	98 to 107
Bicarbonate	21	mmol/L	22 to 29
Calcium	7.1	mg/dL	8.4 to 10.2
Pus cells	0	-	0-1/HPF
Red blood cells	0	-	0-1/HPF
Epithelial cells	4-6	-	2-3/HPF
Bacteria	Present	-	-

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