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Article

Accepted Version

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Journal:	*Parasitology*
Manuscript ID	PAR-2018-0239.R1
Manuscript Type:	Research Article
Date Submitted by the Author:	n/a
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Key Words:	Phthiraptera, Pediculus capitis, louse, lice, nifedipin, nit, neglect, abandonment, pediculosis, plica polonica

Using human head lice to unravel neglect and cause of death

SHORT TITLE:
Head lice, neglect and death

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ABSTRACT

Despite the common association of human lice with abandoned or neglected people, no procedure to assess pediculosis, aimed to detect signs of neglect, exists. Investigating the two most common forms of head louse infestation, regular and severe, we define lice-markers of neglect and develop a protocol and survey form to record and assess pediculosis. The study of head lice from a deceased victim of neglect helped unravel time-length since death, frequency of exposure to neglect and the cause and circumstances related to the death.

Nit-clusters are markers of neglect, indicating length and frequency of neglect episodes. In the case study used here that culminated in the death of the victim, sustained abandonment started circa 2 years before discovery. The lice suggested that death was caused by overconsumption of a powerful calcium channel blocker (CCB), an antihypertensive, an excess of which in lice food supply (blood) stops oogenesis. Despite hosting thousands of adult females on the hair, lice reproduction stopped and nits were no longer developed or deposited on the hairs at the root end. This short distance of the shaft with no nits provided a time estimation of overdosing of almost 2 months before death.

KEYWORDS

Phthiraptera; *Pediculus capitis*; louse; lice; nifedipin; nit; neglect; abandonment; pediculosis; plica polonica

KEY FINDINGS

- A new method of assessment of head louse infestations is proposed.
- A survey form is provided, to be used by any practitioner, nurse, teacher or family member.
- Nit-cluster formation is defined as a clear marker of neglect.
- Nit-clusters allow time-length estimations of length/frequency of neglect episodes.
- Nit numbers in severe cases follow a normal distribution while nit accumulation reaches a plateau.
- In the case study, it was estimated that sustained neglect started circa 2 years previously.
- Frequent episodes of short-term neglect predated the sustained abandonment in the case study victim.
- In the case study, lice biology suggested overconsumption of medication.
- Lice were feeding off blood with an excess of a calcium channel blocker (CCB), an antihypertensive.
- Excess of a CCB in the host's circulating blood stopped lice oogenesis just 2 months before the host's death.
- The victim in the case study may have combined family abandonment with self-inflicted neglect (overdosing of CCB).

INTRODUCTION

Head lice, *Pediculus humanus capitis* are obligatory bloodsucking ecto-parasites that live on humans' scalps. Their long-term association with the human host has also become the focus of recent primate and human evolution investigations, because ecto-parasites such as Pediculidae lice closely mirror the evolution of their hosts (Boutellis *et al.*, 2013; Reed *et al.*, 2004). Head lice have accompanied humans since the split of the human lineage from their close cousins, the chimpanzees. Despite jumping from head to head (horizontal transmission) they do not vector human pathogens. According to the circumstances their reproduction can be out of control on some heads, leading to supernumerary infestations (Alexander, 1984). Recent worldwide surveys confirm that both developing and developed countries are equal victims of pediculosis capitis, with the rate of prevalence in school children increasing rapidly and independently of their socioeconomic status (Leung *et al.*, 2005). Although most statistics and surveys have been conducted on school children (Devore & Schutze, 2015; Jahnke *et al.*, 2009), older women are also prone to head lice infestations (Maunder, 1983).

Human lice need to complete their whole life cycle on hairy human scalps. The cycle involves different stages, starting with the egg, also known as a nit, followed by the larva or first mobile instar, two nymphal stages and then adults (female and male). The head lice full life cycle takes approximately 24 to 28 days (Buxton, 1947); very close to a calendar month, giving a total of 12 to 13 generations a year (on a head with continuous pediculosis). The length of the life cycle is highly stable due to the steady environmental conditions on the human scalp. If the infestation becomes severe there is an overlapping of generations or cycles, which indirectly affects the ability of females to oviposit. Very little is known about the changes in the biology of lice due to overcrowding (Lang, 1975). Mobile stages are adapted to hold the hair shaft using the claws on the tarsi of their legs. Body lice (*P. h. humanus*), a subspecies continuously splitting from head lice populations (Li *et al.*, 2010; Veracx & Raoult, 2012; Veracx *et al.*, 2012), attaches firmly to the fibres of clothes instead of using human hair. Both subspecies can be found on people sustaining severe infestations, with the body lice a direct consequence of a long term head louse infestation (Alexander, 1984). Head and body lice can be separated by their morphology (Ferris, 1951). The two subspecies also differ in their reproductive behaviour. Female body lice deposit their eggs forming groups or clusters, while head lice do so rarely, and only in severe infestations (Lang, 1975; Maunder, 1983). The characteristic pattern of nit distribution in the hair of school children, with regular or occasional infestations, involves a few nits attached to some isolated hairs (Alexander, 1984; Lang, 1975; Maunder, 1983). Therefore, formation of nit clusters uniquely happens when female head lice are exposed to crowded conditions. The physical space available to the females for oviposition between nits is compromised in severe infestations, resulting in the clustering of nits and in the monthly overlapping of generations (Alexander, 1984).

Human lice are considered trace evidence in a number of forensic investigations and cases of neglect. Because they are bloodsucking parasites and feed very often (every ~ 2 hours (Feldmeier, 2017)), their last blood-meal (i.e. the host's blood, rich in mtDNA) becomes reliable evidence in cases of rape or murder. The presence of human lice at a crime scene helps to identify culprits or victims, by matching the human haplotypes in their blood-meal (from inside their gut) with those of the suspects or victims (Davey *et al.*, 2007; Lord *et al.*, 1998). The association of lice with neglect is long known, although not a topic explored in recent years. Neglect is associated with severe infestations in children, elderly and the homeless (Alexander, 1984; Beagley & Hann, 2016; Bennett & Kingston, 1993). In most

cases, it is a clinician or nurse caring for a patient who discovers and reports the heavy infestation, otherwise it may remain un-reported (Beagley & Hann, 2016; Bennett & Kingston, 1993; Durand *et al.*, 2018). The only existing, in-use definition and protocol for diagnosing the level of pediculosis dates back to 1977. It was proposed that for a severe pediculosis, a minimum of circa 200 mobile forms is expected, 10% of which have to be females (Maunder, 1977). The latter is not difficult to observe because the sex ratio of head lice is female biased, independently of the level of infestation (Perotti *et al.*, 2004). There have been just a handful of attempts to change the method of assessment of pediculosis. For example, by counting all *Pediculus* specimens (either nits, adults or nymphs) recorded within a sample area of 2 × 2 cm and assigning a rank to the level of pediculosis, such as low, moderate or high (Gazmuri *et al.*, 2014). The number of lice will, however, only be informative regarding the level of the infestation and will not provide insights into the time-length of the infestation, either with respect to the history of the pediculosis capitis, or the circumstances surrounding the initial and later stages of infestation. The more data or information collected, the better the chances of detecting and analysing frequent forms of neglect.

The majority of previous studies on neglect did not consider the biology, physiology or behaviour of lice. Infestation rates are generally underestimated (Chosidow, 2000), mainly due to the lack of a common survey protocol. In this work, a new method for pediculosis capitis diagnosis is proposed to professionals, for consideration of its feasibility and utility in assessment of head lice infestations for medical or forensic analyses. The protocol includes a single page form (Supplementary Materials) to be used when assessing infestations in order to facilitate and speed up diagnosis, to routinely record cases of pediculosis capitis, to build databases and to simultaneously assess the occurrence of neglect. With the aim of defining the most critical parameters to diagnose neglect using head lice infestations, a series of comparative numerical analyses of the two most frequent degrees of pediculosis, regular or moderate (e.g. as typically found in school-children) and severe, were performed. Data analysis especially used nit (egg) numbers, nit-clustering and nit spatial distribution. For the severe infestation, data of *Pediculus h. capitis* from a case study (Pilli *et al.*, 2016) involving serious neglect followed by death were analysed. Both the results of the numerical analyses and of the cause of death in the case study were interpreted in the light of lice biology, reproduction and oviposition behaviour.

MATERIALS AND METHODS

Summary of the case study, a severe infestation followed by death

The case of a severe infestation which was followed by the death of the victim has been described elsewhere (Pilli *et al.*, 2016). In brief, an elderly woman in a critical condition who was sustaining a massive head louse infestation was received in the emergency ward of a local hospital. The patient died hours after being admitted. She had very long hair, which allowed analyses based on hair growth and nit accumulation over time. An investigation was initiated to establish the level of neglect and the time it had lasted (Pilli *et al.*, 2016). Forensic entomologist SL collected insects and data from hair samples (Pilli *et al.*, 2016), and noted that the medication consumed by the patient was nifedipine, an antihypertensive, which is freely available (not restricted by prescription) in Italy where the victim lived.

Numerical analyses of hairs, nits and clusters from the case study

SL collected lice adults and full-length hairs extracted from all parts of the scalp. The hairs were used for further lice investigation, including estimation of the number of nits as well as clusters of nits, distances between nits and the number of nits/cluster.

Eighty full hairs were collected. To determine total nits/hair, 41 hairs were analysed. Nit-cluster analyses were undertaken on 10 of these 41 hairs, which presented up to 20 continuous and crowded nit clusters. A total of four hairs (of the 41) were also used to count the total number of nits and clusters for an estimated period of 12 months, with a month considered equal to 1 centimetre of hair length (Lapeere *et al.*, 2005).

Numerical analyses of regular infestations and comparison of infestations

Nits counted on 20 consecutive attachment sites in the severe infestation and whole hairs in regular infestations, were used to compare attachment distances and formation of clusters. Data of regular infestations (occasional, typical of school children) were provided from previous projects (Perotti *et al.*, 2004). For comparison purposes, four hairs of regular infestations were included (Perotti *et al.*, 2004). Growth curves of monthly accumulated values were built with the Log_{10} of nit or cluster numbers. The use of Log_{10} allowed better visualisation of extreme values, such as occurred in severe infestations.

Nits within a cluster all belong to one generation, they are oviposited by a close cohort of females (Lang, 1975). There is a minimum physical space needed by females to manoeuvre to properly deliver and attach eggs to the hair shaft. In this study, this biological space is defined as the spatial distance required by a gravid female to hold the hair during oviposition using both the tarsal claws and gonopods for attachment. If a female louse is not provided with this minimum 'biological space' for oviposition, it would likely glue itself to the hair shaft, together with the egg and die *in situ*. Both parts of the female body, tarsi and gonopods, therefore, need access to an unoccupied, specific length of hair-shaft.

Statistical analyses

Statistical analyses used W (Shapiro-W) and Wilcoxon for normality tests in PAST3 (Hammer *et al.*, 2001) and Microsoft Excel 2013 for descriptive parameters and correlations.

Microscopic analysis of female lice reproductive organs

Oviducts and ovaries of ten gravid females were dissected using a stereo microscope (Leica M125) and inspected for their quality and state of development using a phase contrast microscope (Leica DMLB). The females, as well as a few removed developing nits, were kept in 75 % (v/v) ethanol. Before inspection, they were rehydrated in PBS (Phosphate basic (Na) solution) (Perotti *et al.*, 2007), as rehydration smooths the tissues and restores a 'living' appearance. Despite the hydration treatment, ovaries and nits were very damaged and fragile.

Development of a new protocol for assessing pediculosis capitis

A comprehensive literature review, addressing early methods of evaluation of head lice infestations, such as counts of eggs or mobile stages guided the layout of the new method of assessment presented here (Alexander, 1984; Beagley & Hann, 2016; Buxton, 1947; Devore & Schutze, 2015; Lang, 1975; Leung *et al.*, 2005; Maunder, 1977; Maunder, 1983; Perotti *et al.*, 2004; Roy & Ghosh, 1944). The new method incorporates information generated from lice biology and from the analysis of the case study described above, plus

the comparison of the two most common levels of infestation. It includes a new survey form, supplied in Supplementary Materials.

Availability of data and material

All data used in the numerical analysis of lice and nits is provided in this manuscript (main text and Supplementary Materials); a few lice specimens of the case studied are deposited in the collection of one of the authors, Dr. Perotti's Laboratory, University of Reading. No human hair was saved (Pilli *et al.*, 2016).

RESULTS

Case study's louse population

All the hairs of the victim of neglect followed by death were entangled and the majority glued to each other, particularly at the occipital area of the scalp, showing a *plica polonica* formation.

Lice collected during the autopsy were all dead at the time of sampling. From a collection of 200 mobile specimens, over 40 % were adults ($N_{Adults} = 79$) and of this, > 50 % females ($N_{Females} = 41$), indicating a high level of infestation and confirming the expected female bias. Skin bite-marks as well as dead specimens were numerous on the upper parts of the thorax of the victim presenting a gradient of infestation (bite marks) decreasing downwards to the waist. There was no possibility of examination or search for nits laid or attached inside the garments the victim was wearing, therefore, it is not possible to rule out an ongoing transition towards body lice development.

There was not a single hair without attached nits, resulting in a 100% prevalence of infestation from hairs extracted from different parts of the scalp ($N_{Hairs} = 80$). All observed hairs showed a pattern of chained nit attachment arranged in clusters formed of a varied number of nits.

The average length of hair that was totally covered with nits (or clusters) was 69 mm (± 19.5) and the intensity of attached nits resulted in a median of 65, varying between a minimum of 33 to a maximum of 104 nits/hair (Table S1). This approximates to one nit for every millimetre of hair, although the main observed pattern was that of clustering of the nits.

There is some evidence of correlation between the (total) length of the hair and the number of clusters ($N_{Hairs} = 10$) (Fig. 1). Nit-clusters were formed by 2 to 5 nits that were overlapping on their cemented-attachment site over the length of the hair (Fig. 1, inset)

Cluster intensity followed a normal distribution over the hairs ($W = 0.963$, $P = 0.199$), averaging 47 clusters/hair (± 19.5) (with the same median value, of 47 clusters/hair). For the nit content of the clusters, the minimum median value was 2 and the maximum 3 nits/cluster ($N_{Clusters} = 200$). However, 10 clusters contained 5, 49 contained only 1, while the vast majority contained 2, followed in abundance by 3 and 4 nits (Fig. 1, inset).

Despite their homogeneous appearance on the hairs, the distance between nit-clusters did not follow a normal distribution ($W = 0.908$, $P < 0.003$), with an average separation of 1.66 mm (± 0.55) between attachment sites.

Female lice averaged 2.23 mm in length ($N_{Females}$ = 18), and the 'biological distance' between tarsi and gonopods averaged approximately half of the total body length, 1.27 mm (Fig. 2). Each cluster covered a linear distance of 2 to 4 mm, depending on the extension of the cement/cluster and on the number of nits clustered (Fig. 2, inset).

Comparisons between severe (case) and regular (school children) infestations

Analysis of population growth of the nits allowed a more comprehensive characterisation and assessment of severe vs occasional infestations.

For the severe infestation, the monthly-accumulated number of nits and clusters showed a uniform growth pattern (normally distributed), reaching a plateau of intensity. This pattern significantly contrasted with the population growth from the occasional infestations, where there was no population or only small deme formation ($Median_{Severe}$ = 163 and $Median_{Regular}$ = 1 ($W = 78$, $P < 0.01$)) (Fig. 3).

Case study: hair growth, nits and consumption of medicine

It was not possible to know the exact hair growth rate of the host, therefore, it is not advisable to estimate duration of infestation solely by measuring the length of hair carrying nit-clusters. However, it was possible to estimate time of continuous infestation using nit-cluster coverage and number of generations. The maximum nit-cluster linear distance recorded on a hair shaft reached 113 mm (N_{Hairs} = 41). Based on an analysis of nit-cluster distribution on hairs using the minimum 'biological space' and including a minimum monthly overlap of 2 generations (2 clusters = 2 generations/month), a suggested time of continuous infestation of 24 (± 4) months was estimated. Taking into account rather that 1 generation involves approximately 24-28 days, a continuous severe infestation dating back between 20 and 28 months, approximately 2 years from the time of death, is suggested. In addition to the reduced biological distance found between clusters, a few adult females or their reproductive organs were found glued to the hair shafts. The presence of sporadic or repeated infestations (of a few isolated clusters) in the oldest (distal) part of the hair shafts was also noted.

A highlight of the analysis of full-length hairs was the 'unexpected' gap of clear shaft, carrying no nits, for a short distance at the shafts' root. The clear length of shaft between the scalp and the 1st nit, starting from the hair root, showed an average length of 1.45 mm (± 0.47) (Fig. 4 and Table S2) with a borderline normal distribution ($W = 0.969$, $P = 0.0503$). This could be the result of a sampling artefact, due to the number of hairs used (either too many or too few) but may also suggest that, despite a massive record of living adults on the hairs, there was a total lack of oviposition for a period of up to 2 months.

cm

Observations of the reproductive system of gravid females indicated the presence of interrupted, degenerated or terminated oogenesis. In general, either there were big but deteriorated eggs already shrinking inside their mothers, or there was an absence of mature oocytes (= ready formed nits). In addition to the inspected adults, there were a small number of larvae carcasses disintegrating in the samples examined and a similar number of 1st nymphal stages. This is further evidence of reproductive failure, where females were not able to lay their eggs and the eggs died inside the mothers; this seems to have occurred for a period of up to 2 months, right before the host's death.

New assessment method of pediculosis capitis

The 'Pediculosis-capitis Survey Form' (Supplementary Materials) incorporates a few, new, but rigorous criteria for facilitating decision making. The criteria are described in Supplementary Materials and are summarised in the assessment form.

The criteria for assessment include: estimated number of infested hairs; location or position of nits/clusters on the head; number of nit attachment sites; nit-cluster formation; and distance between clusters. Other features to consider include, for example, the origin of the louse infestations. This criterion includes a literature review of body lice and their vector capacity, highlighting cases of re-emerging infectious diseases, especially from the recent arrival of immigrants in Europe.

DISCUSSION

The use of just the number of mobile lice at a particular stage, e.g. adults, as a sole element to analyse pediculosis, does not provide detailed information on its history, on neglect, on medical condition or on the cause of death. This work emphasises the importance of nit-clustering for unravelling details about the circumstances of neglect that otherwise may be overlooked. Therefore, to characterise the level of a head louse infestation, the distribution pattern of nit clusters on a few sampled hairs must be considered.

From the interpretation of the results for nit-clustering and their arrangement on the hair-shaft it is clear that for a certain period there was a lack of space for female oviposition manoeuvres. This implies highly crowded conditions typical of a severe infestation. In a severe infestation, females struggle to find the space required to manoeuvre oviposition as they can only grab and hold empty hair shafts and not nits (there is no morphology/adaptation for this). Even if the intense infestation only covers 2 cm of the hair shaft, and there is ~ 1 mm of space between small nit clusters covering these 2 cm, this confirms that the patient was neglected for a period of time (2 cm approximates to 2 months). Using hair growth (when possible) to estimate the time when the patient was exposed to neglect can help to interpret the circumstances and time of neglect, e.g. indicating when it happened, or when it started. For example, a patient might be a victim of neglect for only 2 - 3 months, and this might have started 6 months before its discovery. If his/her hair grows at a rate of 1 cm/month, the cluster formation should be found ~ 3·5 cm from the scalp and should cover at least 2 cm of hair-shaft. Over these 2-3 cm of nit-attachments, the clusters should be separated by at least 1 mm, as that is the female's minimum required space for grabbing the hair to oviposit. The use of hair growth has to consider the age of the patient/victim, the older the person the slower the growth.

Nit-clusters were slightly and unevenly distributed on the shafts (non-normally distributed), which could be due to the expected intra-population body size variation of gravid females. The average distance of 1.66 mm (± 0.55) between attachment sites clearly suggests, however, that there was no room for extra manoeuvres. The immediate consequence of crowdedness is the overlapping of generations in ovipositing sites (here called clusters), a feature already observed by early researchers (Buxton, 1947), but not considered important until now. The predominance of 2-nit clusters followed by 3-nit clusters (showing a Poisson distribution, Fig. 1 inset) might also indicate overlapping of a number of generations or group oviposition or even a few demes becoming body lice. Females of body lice are gregarious and nits are deposited in groups in the same area of cloth shared by several females (Veracx *et al.*, 2012).

Based solely on the oviposition behaviour of lice, the estimated length of time of the continuous severe infestation suggests a sustained neglect of approximately 2 years in duration. This represents a clear case of neglect of an elder (Bennett & Kingston, 1993).

Furthermore, the presence of intermittent infestations, of a few consecutive clusters in the distal or older parts of the hair shafts, suggests that neglect was likely experienced repeatedly, even before the 2 year period of intense and out of control infestation built up.

The victim of neglect presented a *plica polonica*, a characteristic feature of gross head lice infestations, confirming neglect (Alexander, 1984). In this condition, the hairs are glued into a sticky secretion and emanate a particular spoiled vinegar smell. *Plica polonica* was common in Poland in the 1200s, when it was described: then it was already a sign of poor hygiene (Brzezinski *et al.*, 2016). In this case study, the doctors and nurses treating the patient noticed the *plica* formation, as well as the huge louse population extending to the torso (Connor *et al.*, 2016).

Unfortunately, it was not possible to sample lice from the torso area or its clothes to confirm the presence of the two *Pediculus humanus* subspecies. Finding body lice together with a gross head lice infestation on one human host is not novel. Head lice evolving into body lice have been documented on the same individual, particularly homeless people (Veracx *et al.*, 2012). The adaptation to nest in clothing can only occur in head lice of mtDNA Clade A (Li *et al.*, 2010). At present, however, there is no phenotype or morphotype available to identify mitochondrial clades. Clade A has a worldwide distribution and is predominant in European countries and it is likely that it was carried by the patient. The transition from head to body lice on a person can only be achieved following a very long term exposure and continuous infestation (Veracx *et al.*, 2012) and this is another clear sign of a patient being a victim of neglect and of having suffered a long-term, severe pediculosis capitis.

Of particular interest was the finding of a gap at the root of all the hair shafts examined, characterised by the absence of nits from the root-end of the shaft to the last nit oviposited onto it. With an average length of 1.45 mm (±0.47) of nit absence, this represents an abnormal situation for lice biology. Head lice gravid females oviposit circa 5 mm from the host scalp (Buxton, 1947; Lang, 1975; Lapeere *et al.*, 2005; Roy & Ghosh, 1944). This distance cannot be modified, it is a physiological requirement for the proper development of the embryo. Nits depend on a temperature gradient determined by the distance, also measured in time (as the hair grows), from the scalp producing heat (Alexander, 1984; Buxton, 1940; Buxton, 1947). The estimated length of the gap with no nits suggests a time lapse of ~ 2 months and it was found despite many thousands of mobiles, particularly females, crawling on the head. Lice larvae and 1st instar nymphs (the youngest stages) were very rare and gravid females' internal or not yet laid oocytes were found to be degenerating or terminated (shrunk) (numbers not collected). This was a clear indication that extrinsic factors were affecting lice reproduction.

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The medication consumed by the patient, nifedipine, is used for the treatment of high blood pressure. This substance actively blocks the movement of calcium through calcium channels, being a well-known calcium channel blocker (CCB). The active ingredient is almost fully attached to proteins in plasma, and metabolised to inactive compounds with a half-life of up 4 hours (Hilal-Dantan & Brunton, 2014). This makes the drug available in blood for several hours, providing a plentiful supply at variable doses in lice blood meals. This drug can be purchased without prescription in Italy, and the patient was able to buy and consume it as desired.

In most sexually reproducing animals, egg activation is induced by the process of fertilisation. The sperm mediates a continuous release of intra-oocyte calcium which

allows the completion of meiosis and the development of the vitelline membrane to prevent access by more sperm. In this context, arthropods are more flexible than other animals, with sperm being no longer the exclusive trigger of egg activation. Insects, such as lice, use intra-oocyte calcium waves occurring while the eggs or oocytes move through the female's oviducts (Horner & Wolfner, 2008; Kaneuchi *et al.*, 2015; Perotti *et al.*, 2007).

In the discussed case of severe neglect, lice were feeding for years off a host who was on a regular daily consumption of nifedipine, but blood with these moderate daily doses of nifedipine seems not to have affected lice reproduction for about two years. However, approximately two months before the patient was admitted to hospital and died, lice reproduction had become seriously compromised and stopped. A massive population of lice unable to reproduce suggests an overdose of nifedipine by the victim, powerful enough to stop egg activation and lice development. Lice reproductive behaviour enables us to estimate about 45 - 60 days prior to death as the time when their neglected host started to exceed safe or recommended doses of nifedipine.

Lice biology indicates a complex case of neglect, initially involving family abandonment (Pilli *et al.*, 2016). The sustained parasitosis lasted for about 2 years and was followed by self-inflicted neglect in the form of self-overdosing of medication, a well known reaction in abandoned elders (Bennett & Kingston, 1993; Burnett *et al.*, 2018; O'Connor, 2018).

Acknowledgements
We thank MSc student Jasdeep Rai for the sketches of a louse and nits (from a photograph provided by MAP) in Figure 2; and Dr. Henk Braig for help translating German literature. We would like to thank the Erasmus Staff Mobility scheme between the Universities of Reading (UK) and Pavia (Italy) that allowed MAP and SL to meet and discuss this work.

Funding
MAP research is supported by the BBSRC, Project Reference BB/N001443/1

Conflicts of interest
None

Author's contributions
SL collected the data, including photographs, contributed with the design of the study and revised a final version of the manuscript. MAP designed the study, performed the data analysis, graphs, interpretation of the results and has written the manuscript.

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FIGURE Legends

Figure 1. Main Graph: distance between nit-clusters over a length of hair covered with nits. Nit-cluster coverage with a maximum length between 60 and 85 is shown, the longer the hair shaft, the more clusters there are. A positive correlation is shown, confirming the maintenance of a minimum distance between nits, needed by females to manoeuvre for oviposition. Inset: frequency of nits in the clusters, the largest clusters containing 5 nits are the least frequent.

Figure 2. Position of the female louse over the hair shaft at oviposition. A clear shaft length of 2.25 - 2.5 mm allows the female to position its body for the correct attachment of a nit. In crowded conditions this space reaches a minimum 'biological space'. Inset (top right): close up of nit-clusters in the crowded habitat of the severe infestation, clusters of 2 and 3 nits are shown.

Figure 3. Comparison of the progressive accumulation of nits and clusters between severe and regular infestations. The two curves shown belong to the severe infestation, presenting a sustained growth reaching almost a plateau of saturation, or maximum growth, due to limited space. In terms of clusters, regular infestations neither persist, reproduce nor grow, hence no data is shown (no curves, full lines or triangles).

Figure 4. Hair shaft gap lacking nits, above the root of the hair samples. The absence of nits is a consequence of the interruption in lice reproduction due to the highly concentrated calcium channel blocker in the blood of the host, which arose from the victim's over consumption or overdosing.
On grid (above). One hair, the root is indicated as "Line at root position" and the nit-free "Gap" is shown between the brackets. The shaft at the root is not visible due to its transparency.
No grid (below). Two hairs, numbered 1 and 2. The roots are indicated by arrows and the nit-free "Gap" corresponds to the position of the brackets.

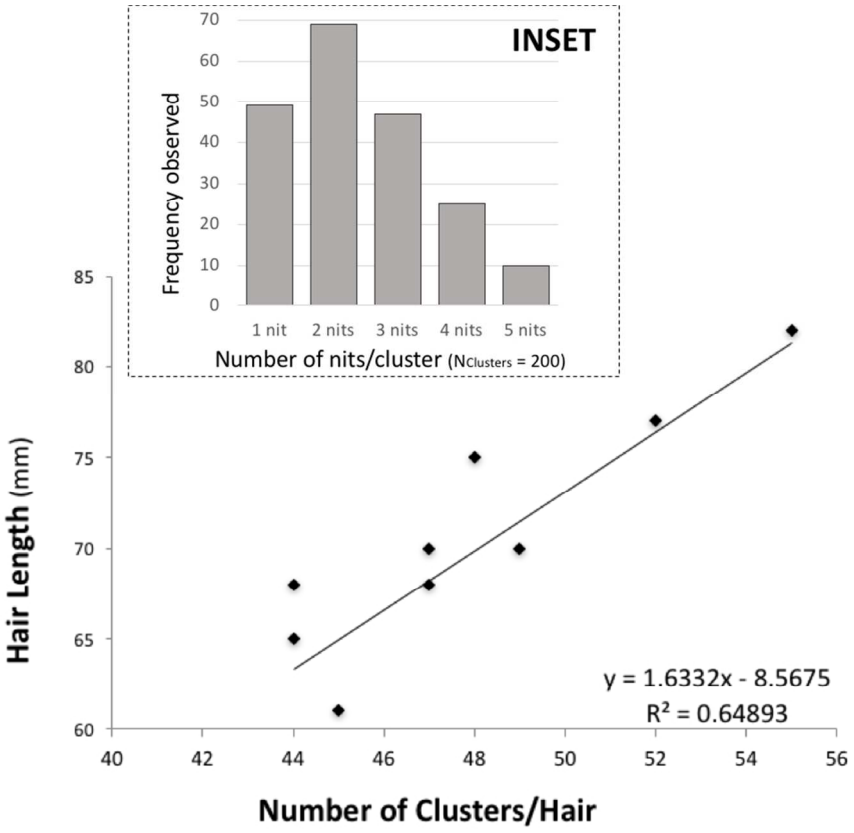

Figure 1

200x175mm (127 x 127 DPI)

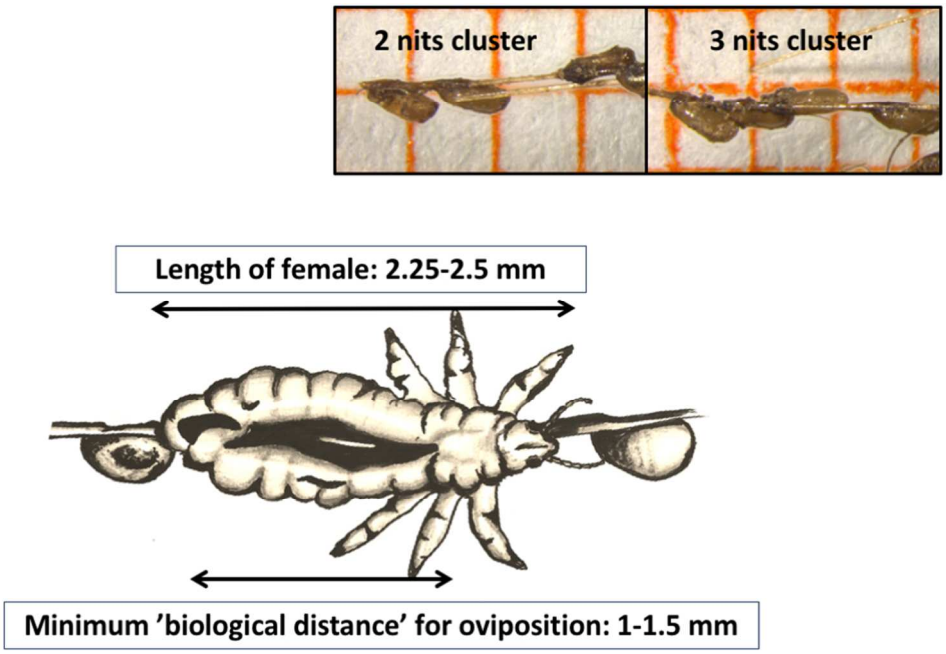

Figure 2

276x191mm (92 x 92 DPI)

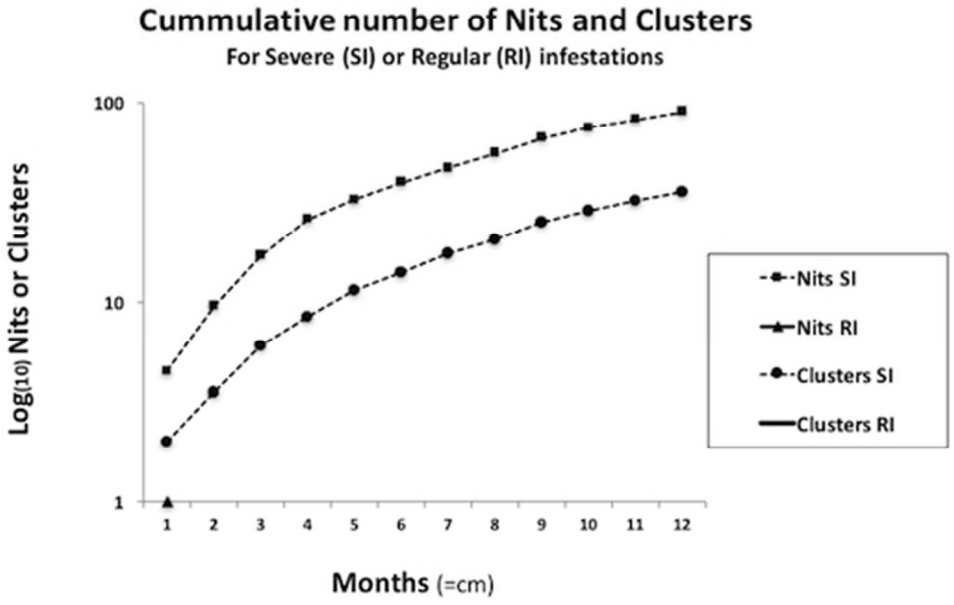

Figure 3

190x142mm (72 x 72 DPI)

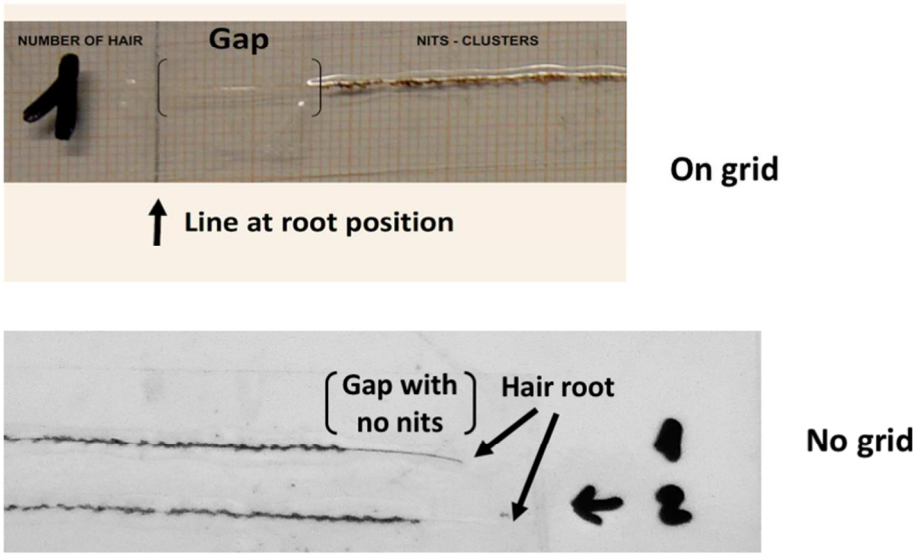

Figure 4

276x191mm (92 x 92 DPI)

Supplementary Materials

Using human head lice to unravel neglect and cause of death

Simonetta Lambiase and M. Alejandra Perotti

TABLE OF CONTENTS

FEATURES	SEVERE INFESTATION	tick [X] accordingly	REGULAR INFESTATION	tick [X] accordingly	INTERMEDIATE tick [X] accordingly
Number of infested hairs (showing attached nits)	95-100% of the hair-cover		1-10% of hairs carrying nits. Therefore, the majority of hairs do not have nits.		
Location of nits on head	Any part of the scalp has been colonized		Nits found mainly behind ears, occipital area and at the temples		
Number of nit attachment-sites	Several attachments of nits per infested-hair.[1]		1 attachment site per infested-hair (rarely a hair with 2 can be found)		
Cluster formation (nits in groups or clusters)	Nits in groups of 2 or more, glued on the same cement coverage on the hair shaft.[1]		N/A		
Distance between clusters/and gaps from last oviposited nit to root	-Clusters separated by a minimum biological distance [the space required by a gravid female to securely hold the hair shaft to glue the egg]. The very minimum distance between tarsi and gonopods approx. 1mm. -Overlapping of generations = clustering of nits and crowed arrangement of clusters. [Head louse life cycle ≈ 1 month = 1 cm of hair] -Due to lack of space to oviposit the distance between clusters (biological space) is reduced to a minimum. -Transition towards body lice (although this can happen from mtDNA Clade A only) -Gaps length		N/A N/A N/A N/A N/A		
Other					

Evaluator[2]:	**Patient:**
Evaluator's Affiliation:	**Place:**
Signature, date and place	**Consent: YES NO (if ethics review/consent exist, add it)** **Observations:**

[1] Taking a photo with a mobile phone and then zoom it will allow a better *in situ* observation
[2] Evaluators are any persons performing the test: practitioners, nurses, teachers, and even family members.

CRITERIA FOR SURVEY, DEFINITIONS AND BACKGROUND

This work proposes a modern approach for assessing the level of pediculosis capitis. A survey-form based on research (this work and historical accounts, literature review) is presented here (above). Gathering lice data in future assessments enable to predict: i) the level of an infestation, all recorded on the hair, because lice leave nits behind which last attached to the shaft up to several years; ii) time estimation of the duration of neglect, based on exposure to head lice and their activity on the host; iii) a comprehensive description of the history and nature of the infestation; and, iv) based on changes in the physiology and development of lice and their nits, a proposed interpretation of the medical condition of a patient or, if occurring, of the cause of death.

Estimated number of infested hairs: This has been previously used in assessments, specially by physicians[1, 2] and gives an overall intensity of the infestation. Regular infestations will carry nits only on a few hairs.

Location or position of nits/clusters on the head: In severe infestations hairs should be examined from all parts or areas of the scalp, as all areas are colonised. However, in regular infestations, hairs carrying nits are rare but can be particularly concentrated behind ears, occipital area and at the temples[2].

Number of nit attachment-sites: This criterion has never been used before and it is proposed here for the first time; it does not require the counting of attachment sites of many hairs. If the infestation is severe, there are more than 2 attachment sites in any one hair taken randomly from the head; while from a regular infestation finding just 1 attached nit will be difficult from a random sampled hair. Number of attachments/hair is informative of the intensity and time length of infestation, in that it can indicate either highly repetitive or long-term exposure to head lice.

Cluster formation: Nits joining the cement or attachment site, forming clusters are only present in severe or gross infestations[1, 3, 4]. This characterisation of the level of infestation using clusters is proposed here for the 1st time, as it allows the most accurate diagnosis. Therefore, cluster formation is a unique feature of severe or heavy infestations, and should be always considered as a powerful element of assessment in investigations of neglect, this is a clear indicator or sign that neglect took place[5, 6].

Distance between clusters and from last nit to scalp: Estimating the average separation between clusters (Fig. 2), from a heavily infested hair informs of the oviposition behaviour of the females. The minimum (average) distance of 1 mm between clusters is indicative of a prolonged severe pediculosis capitis, independently of the length of hair covered by the nits. Gaps at the root end of severe and prolonged infestations have to be considered, as they inform of changes in the health condition of the victim of neglect. The case study investigated here, of a severe infestation due to neglect and followed by death explains the value of this information.

Other features to consider: Head lice belonging to mtDNA Clade A have a worldwide distribution and are particularly prevalent in Europe. As pointed above, this clade is able to evolve into body lice given the opportunity. Body lice are vectors of several pathogens.

Europeans suffering of heavy infestations likely carry Clade A, therefore, have a high chance of becoming infested also with body lice. This is of particular importance in management and control of re-emerging infectious diseases. Body lice transmit human pathogens while head lice do not. The Proteobacteria *Rickettsia prowazekii* and *Bartonella quintana*, and the spirochete *Borrelia recurrentis*[7, 8] are becoming the focus of attention in relation with the ongoing humanitarian crisis experienced in Europe due to the daily or weekly intake of thousands of immigrants[9, 10]. Therefore, in Europe, a victim of neglect can be hit twice by in addition to suffering of lice, falling victim of one of the bacterial pathogens transmitted by body lice. *Borrelia recurrentis* has being already detected in 2016 in refugee patients in Italy[7].

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Table S1. A total of 41 Hairs examined for number of nits and length covered with nits

ID Hair	Nits	Length w/Nits (cm)	Rate Nits/mm
1	79	9.5	0.83
2	104	8.3	1.25
3	102	7.5	1.36
4	87	10	0.87
5	65	7.1	0.92
6	86	8.5	1.01
7	72	6.4	1.13
8	75	6.5	1.15
9	47	5.8	0.81
10	75	7.6	0.99
11	72	9.6	0.75
12	93	11.3	0.82
13	68	5.8	1.17
14	62	7.1	0.87
15	49	7.4	0.66
16	50	9	0.56
17	65	6.7	0.97
18	63	6.2	1.02
19	93	9	1.03
20	64	6.5	0.98
21	48	4	1.20
22	40	4.2	0.95
23	52	5.1	1.02
24	33	4.3	0.77
25	67	7.4	0.91
26	67	7.4	0.91
27	62	5.7	1.09
28	46	6.3	0.73
29	41	3.5	1.17
30	60	4.4	1.36
31	38	3.3	1.15
32	55	4.3	1.28
33	50	6.4	0.78
34	71	10	0.71
35	72	8.8	0.82
36	81	7.7	1.05
37	100	9.1	1.10
38	63	5.2	1.21
39	62	6.1	1.02
40	96	6.9	1.39
41	99	8.7	1.14

Hair sample	Root gap cm
1	1.7
2	0.8
3	0.6
4	2.2
5	1.9
6	2.1
7	1.3
8	1.5
9	1
10	0.8
11	1.5
12	2.2
13	0.7
14	0.9
15	0.5
16	1.2
17	0.9
18	0.8
19	1.7
20	2.2
21	2.1
22	1.8
23	1.7
24	1.9
25	1.4
26	1.8
27	1.2
28	0.7
29	1.9
30	1.4
31	1.5
32	0.5
33	1.5
34	0.7
35	1.1
36	0.8
37	1.6
38	1.4

39	2.3
40	1.7
41	1.9
42	1.8
43	1.4
44	1.7
45	2.1
46	1
47	0.8
48	1.3
49	1.6
50	1.2
51	1.2
52	1.8
53	1.1
54	0.9
55	2.3
56	2
57	1.7
58	1.4
59	1.5
60	1.2
61	1.5
62	1.3
63	1.9
64	2.1
65	2
66	1.8
67	1.6
68	1.6
69	1.9
70	1.7
71	1.4
72	1.5
73	1.2
74	1.9
75	0.5
76	1.1
77	1.8
78	1.6

79	0.9
80	1.3
AVER	1.45
STD	0.475
Median	1.5