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Using human head lice to unravel neglect and cause of death

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Using human head lice to unravel neglect and cause of death

SHORT TITLE:
Head lice, neglect and death

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1   ABSTRACT
2
3   Despite the common association of human lice with abandoned or neglected people, no
4   procedure to assess pediculosis, aimed to detect signs of neglect, exists. Investigating the
5   two most common forms of head louse infestation, regular and severe, we define lice-
6   markers of neglect and develop a protocol and survey form to record and assess
7   pediculosis. The study of head lice from a deceased victim of neglect helped unravel time-
8   length since death, frequency of exposure to neglect and the cause and circumstances
9   related to the death.
10   Nit-clusters are markers of neglect, indicating length and frequency of neglect episodes.
11   In the case study used here that culminated in the death of the victim, sustained
12   abandonment started circa 2 years before discovery. The lice suggested that death was
13   caused by overconsumption of a powerful calcium channel blocker (CCB), an
14   antihypertensive, an excess of which in lice food supply (blood) stops oogenesis. Despite
15   hosting thousands of adult females on the hair, lice reproduction stopped and nits were no
16   longer developed or deposited on the hairs at the root end. This short distance of the shaft
17   with no nits provided a time estimation of overdosing of almost 2 months before death.
18
19
20
21   KEYWORDS
22   Phthiraptera; *Pediculus capitis*; louse; lice; nifedipin; nit; neglect; abandonment;
23   pediculosis; plica polonica
24

25   KEY FINDINGS

26

27   • A new method of assessment of head louse infestations is proposed.

28

29   • A survey form is provided, to be used by any practitioner, nurse, teacher or family
30   member.

31

32   • Nit-cluster formation is defined as a clear marker of neglect.

33

34   • Nit-clusters allow time-length estimations of length/frequency of neglect episodes.

35

36   • Nit numbers in severe cases follow a normal distribution while nit accumulation
37   reaches a plateau.

38

39   • In the case study, it was estimated that sustained neglect started circa 2 years
40   previously.

41

42   • Frequent episodes of short-term neglect predated the sustained abandonment in the
43   case study victim.

44

45   • In the case study, lice biology suggested overconsumption of medication.

46

47   • Lice were feeding off blood with an excess of a calcium channel blocker (CCB), an
48   antihypertensive.

49

50   • Excess of a CCB in the host's circulating blood stopped lice oogenesis just 2
51   months before the host's death.

52

53   • The victim in the case study may have combined family abandonment with self-
54   inflicted neglect (overdosing of CCB).

55

56

57

58 INTRODUCTION
59
60 Head lice, *Pediculus humanus capitis* are obligatory bloodsucking ecto-parasites that live
61 on humans' scalps. Their long-term association with the human host has also become the
62 focus of recent primate and human evolution investigations, because ecto-parasites such as
63 Pediculidae lice closely mirror the evolution of their hosts (Boutellis *et al.*, 2013; Reed *et*
64 *al.*, 2004). Head lice have accompanied humans since the split of the human lineage from
65 their close cousins, the chimpanzees. Despite jumping from head to head (horizontal
66 transmission) they do not vector human pathogens. According to the circumstances their
67 reproduction can be out of control on some heads, leading to supernumerary infestations
68 (Alexander, 1984). Recent worldwide surveys confirm that both developing and developed
69 countries are equal victims of pediculosis capitis, with the rate of prevalence in school
70 children increasing rapidly and independently of their socioeconomic status (Leung *et al.*,
71 2005). Although most statistics and surveys have been conducted on school children
72 (Devore & Schutze, 2015; Jahnke *et al.*, 2009), older women are also prone to head lice
73 infestations (Maunder, 1983).
74
75 Human lice need to complete their whole life cycle on hairy human scalps. The cycle
76 involves different stages, starting with the egg, also known as a nit, followed by the larva
77 or first mobile instar, two nymphal stages and then adults (female and male). The head lice
78 full life cycle takes approximately 24 to 28 days (Buxton, 1947); very close to a calendar
79 month, giving a total of 12 to 13 generations a year (on a head with continuous
80 pediculosis). The length of the life cycle is highly stable due to the steady environmental
81 conditions on the human scalp. If the infestation becomes severe there is an overlapping of
82 generations or cycles, which indirectly affects the ability of females to oviposit. Very little
83 is known about the changes in the biology of lice due to overcrowding (Lang, 1975).
84 Mobile stages are adapted to hold the hair shaft using the claws on the tarsi of their legs.
85 Body lice (*P. h. humanus*), a subspecies continuously splitting from head lice populations
86 (Li *et al.*, 2010; Veracx & Raoult, 2012; Veracx *et al.*, 2012), attaches firmly to the fibres
87 of clothes instead of using human hair. Both subspecies can be found on people sustaining
88 severe infestations, with the body lice a direct consequence of a long term head louse
89 infestation (Alexander, 1984). Head and body lice can be separated by their morphology
90 (Ferris, 1951). The two subspecies also differ in their reproductive behaviour. Female body
91 lice deposit their eggs forming groups or clusters, while head lice do so rarely, and only in
92 severe infestations (Lang, 1975; Maunder, 1983). The characteristic pattern of nit
93 distribution in the hair of school children, with regular or occasional infestations, involves
94 a few nits attached to some isolated hairs (Alexander, 1984; Lang, 1975; Maunder, 1983).
95 Therefore, formation of nit clusters uniquely happens when female head lice are exposed
96 to crowded conditions. The physical space available to the females for oviposition between
97 nits is compromised in severe infestations, resulting in the clustering of nits and in the
98 monthly overlapping of generations (Alexander, 1984).
99
100 Human lice are considered trace evidence in a number of forensic investigations and cases
101 of neglect. Because they are bloodsucking parasites and feed very often (every ~ 2 hours
102 (Feldmeier, 2017)), their last blood-meal (i.e. the host's blood, rich in mtDNA) becomes
103 reliable evidence in cases of rape or murder. The presence of human lice at a crime scene
104 helps to identify culprits or victims, by matching the human haplotypes in their blood-meal
105 (from inside their gut) with those of the suspects or victims (Davey *et al.*, 2007; Lord *et al.*,
106 1998). The association of lice with neglect is long known, although not a topic explored in
107 recent years. Neglect is associated with severe infestations in children, elderly and the
108 homeless (Alexander, 1984; Beagley & Hann, 2016; Bennett & Kingston, 1993). In most

109   cases, it is a clinician or nurse caring for a patient who discovers and reports the heavy
110   infestation, otherwise it may remain un-reported (Beagley & Hann, 2016; Bennett &
111   Kingston, 1993; Durand *et al.*, 2018). The only existing, in-use definition and protocol for
112   diagnosing the level of pediculosis dates back to 1977. It was proposed that for a severe
113   pediculosis, a minimum of circa 200 mobile forms is expected, 10% of which have to be
114   females (Maunder, 1977). The latter is not difficult to observe because the sex ratio of head
115   lice is female biased, independently of the level of infestation (Perotti *et al.*, 2004). There
116   have been just a handful of attempts to change the method of assessment of pediculosis.
117   For example, by counting all *Pediculus* specimens (either nits, adults or nymphs) recorded
118   within a sample area of 2×2 cm and assigning a rank to the level of pediculosis, such as
119   low, moderate or high (Gazmuri *et al.*, 2014). The number of lice will, however, only be
120   informative regarding the level of the infestation and will not provide insights into the
121   time-length of the infestation, either with respect to the history of the pediculosis capitis, or
122   the circumstances surrounding the initial and later stages of infestation. The more data or
123   information collected, the better the chances of detecting and analysing frequent forms of
124   neglect.
125
126   The majority of previous studies on neglect did not consider the biology, physiology or
127   behaviour of lice. Infestation rates are generally underestimated (Chosidow, 2000), mainly
128   due to the lack of a common survey protocol. In this work, a new method for pediculosis
129   capitis diagnosis is proposed to professionals, for consideration of its feasibility and utility
130   in assessment of head lice infestations for medical or forensic analyses. The protocol
131   includes a single page form (Supplementary Materials) to be used when assessing
132   infestations in order to facilitate and speed up diagnosis, to routinely record cases of
133   pediculosis capitis, to build databases and to simultaneously assess the occurrence of
134   neglect. With the aim of defining the most critical parameters to diagnose neglect using
135   head lice infestations, a series of comparative numerical analyses of the two most frequent
136   degrees of pediculosis, regular or moderate (e.g. as typically found in school-children) and
137   severe, were performed. Data analysis especially used nit (egg) numbers, nit-clustering
138   and nit spatial distribution. For the severe infestation, data of *Pediculus h. capitis* from a
139   case study (Pilli *et al.*, 2016) involving serious neglect followed by death were analysed.
140   Both the results of the numerical analyses and of the cause of death in the case study were
141   interpreted in the light of lice biology, reproduction and oviposition behaviour.
142
143
144   MATERIALS AND METHODS
145
146   *Summary of the case study, a severe infestation followed by death*
147   The case of a severe infestation which was followed by the death of the victim has been
148   described elsewhere (Pilli *et al.*, 2016). In brief, an elderly woman in a critical condition
149   who was sustaining a massive head louse infestation was received in the emergency ward
150   of a local hospital. The patient died hours after being admitted. She had very long hair,
151   which allowed analyses based on hair growth and nit accumulation over time. An
152   investigation was initiated to establish the level of neglect and the time it had lasted (Pilli
153   *et al.*, 2016). Forensic entomologist SL collected insects and data from hair samples (Pilli
154   *et al.*, 2016), and noted that the medication consumed by the patient was nifedipine, an
155   antihypertensive, which is freely available (not restricted by prescription) in Italy where
156   the victim lived.
157
158   *Numerical analyses of hairs, nits and clusters from the case study*

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

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

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

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

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

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

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

209   the comparison of the two most common levels of infestation. It includes a new survey
210   form, supplied in Supplementary Materials.
211
212   *Availability of data and material*
213   All data used in the numerical analysis of lice and nits is provided in this manuscript (main
214   text and Supplementary Materials); a few lice specimens of the case studied are deposited
215   in the collection of one of the authors, Dr. Perotti's Laboratory, University of Reading. No
216   human hair was saved (Pilli *et al.*, 2016).
217
218
219   RESULTS
220
221   *Case study's louse population*
222   All the hairs of the victim of neglect followed by death were entangled and the majority
223   glued to each other, particularly at the occipital area of the scalp, showing a *plica polonica*
224   formation.
225   Lice collected during the autopsy were all dead at the time of sampling. From a collection
226   of 200 mobile specimens, over 40 % were adults ($N_{Adults} = 79$) and of this, > 50 % females
227   ($N_{Females} = 41$), indicating a high level of infestation and confirming the expected female
228   bias. Skin bite-marks as well as dead specimens were numerous on the upper parts of the
229   thorax of the victim presenting a gradient of infestation (bite marks) decreasing
230   downwards to the waist. There was no possibility of examination or search for nits laid or
231   attached inside the garments the victim was wearing, therefore, it is not possible to rule out
232   an ongoing transition towards body lice development.
233
234   There was not a single hair without attached nits, resulting in a 100% prevalence of
235   infestation from hairs extracted from different parts of the scalp ($N_{Hairs} = 80$). All observed
236   hairs showed a pattern of chained nit attachment arranged in clusters formed of a varied
237   number of nits.
238
239   The average length of hair that was totally covered with nits (or clusters) was 69 mm
240   (± 19.5) and the intensity of attached nits resulted in a median of 65, varying between a
241   minimum of 33 to a maximum of 104 nits/hair (Table S1). This approximates to one nit for
242   every millimetre of hair, although the main observed pattern was that of clustering of the
243   nits.
244
245   There is some evidence of correlation between the (total) length of the hair and the number
246   of clusters ($N_{Hairs} = 10$) (Fig. 1). Nit-clusters were formed by 2 to 5 nits that were
247   overlapping on their cemented-attachment site over the length of the hair (Fig. 1, inset)
248
249   Cluster intensity followed a normal distribution over the hairs ($W = 0.963$, $P = 0.199$),
250   averaging 47 clusters/hair (± 19.5) (with the same median value, of 47 clusters/hair). For
251   the nit content of the clusters, the minimum median value was 2 and the maximum 3
252   nits/cluster ($N_{Clusters} = 200$). However, 10 clusters contained 5, 49 contained only 1, while
253   the vast majority contained 2, followed in abundance by 3 and 4 nits (Fig. 1, inset).
254
255   Despite their homogeneous appearance on the hairs, the distance between nit-clusters did
256   not follow a normal distribution ($W = 0.908$, $P < 0.003$), with an average separation of 1.66
257   mm (± 0.55) between attachment sites.
258

259   Female lice averaged 2.23 mm in length ($N_{Females}$ = 18), and the 'biological distance'
260   between tarsi and gonopods averaged approximately half of the total body length, 1.27 mm
261   (Fig. 2). Each cluster covered a linear distance of 2 to 4 mm, depending on the extension of
262   the cement/cluster and on the number of nits clustered (Fig. 2, inset).
263
264   *Comparisons between severe (case) and regular (school children) infestations*
265   Analysis of population growth of the nits allowed a more comprehensive characterisation
266   and assessment of severe vs occasional infestations.
267   For the severe infestation, the monthly-accumulated number of nits and clusters showed a
268   uniform growth pattern (normally distributed), reaching a plateau of intensity. This pattern
269   significantly contrasted with the population growth from the occasional infestations, where
270   there was no population or only small deme formation ($Median_{Severe}$ = 163 and
271    $Median_{Regular}$ = 1 (W = 78, P < 0.01)) (Fig. 3).
272
273   *Case study: hair growth, nits and consumption of medicine*
274   It was not possible to know the exact hair growth rate of the host, therefore, it is not
275   advisable to estimate duration of infestation solely by measuring the length of hair carrying
276   nit-clusters. However, it was possible to estimate time of continuous infestation using nit-
277   cluster coverage and number of generations. The maximum nit-cluster linear distance
278   recorded on a hair shaft reached 113 mm (N_{Hairs} = 41). Based on an analysis of nit-cluster
279   distribution on hairs using the minimum 'biological space' and including a minimum
280   monthly overlap of 2 generations (2 clusters = 2 generations/month), a suggested time of
281   continuous infestation of 24 (± 4) months was estimated. Taking into account rather that 1
282   generation involves approximately 24-28 days, a continuous severe infestation dating back
283   between 20 and 28 months, approximately 2 years from the time of death, is suggested. In
284   addition to the reduced biological distance found between clusters, a few adult females or
285   their reproductive organs were found glued to the hair shafts. The presence of sporadic or
286   repeated infestations (of a few isolated clusters) in the oldest (distal) part of the hair shafts
287   was also noted.
288
289   A highlight of the analysis of full-length hairs was the 'unexpected' gap of clear shaft,
290   carrying no nits, for a short distance at the shafts' root. The clear length of shaft between
291   the scalp and the 1st nit, starting from the hair root, showed an average length of 1.45 mm   **cm**
292   (± 0.47) (Fig. 4 and Table S2) with a borderline normal distribution (W = 0.969, P =
293   0.0503). This could be the result of a sampling artefact, due to the number of hairs used
294   (either too many or too few) but may also suggest that, despite a massive record of living
295   adults on the hairs, there was a total lack of oviposition for a period of up to 2 months.
296
297   Observations of the reproductive system of gravid females indicated the presence of
298   interrupted, degenerated or terminated oogenesis. In general, either there were big but
299   deteriorated eggs already shrinking inside their mothers, or there was an absence of mature
300   oocytes (= ready formed nits). In addition to the inspected adults, there were a small
301   number of larvae carcasses disintegrating in the samples examined and a similar number of
302   1st nymphal stages. This is further evidence of reproductive failure, where females were
303   not able to lay their eggs and the eggs died inside the mothers; this seems to have occurred
304   for a period of up to 2 months, right before the host's death.
305
306   *New assessment method of pediculosis capitis*
307   The 'Pediculosis-capitis Survey Form' (Supplementary Materials) incorporates a few, new,
308   but rigorous criteria for facilitating decision making. The criteria are described in
309   Supplementary Materials and are summarised in the assessment form.

310   The criteria for assessment include: estimated number of infested hairs; location or
311   position of nits/clusters on the head; number of nit attachment sites; nit-cluster formation;
312   and distance between clusters. Other features to consider include, for example, the origin
313   of the louse infestations. This criterion includes a literature review of body lice and their
314   vector capacity, highlighting cases of re-emerging infectious diseases, especially from the
315   recent arrival of immigrants in Europe.
316
317
318
319   DISCUSSION
320
321   The use of just the number of mobile lice at a particular stage, e.g. adults, as a sole element
322   to analyse pediculosis, does not provide detailed information on its history, on neglect, on
323   medical condition or on the cause of death. This work emphasises the importance of nit-
324   clustering for unravelling details about the circumstances of neglect that otherwise may be
325   overlooked. Therefore, to characterise the level of a head louse infestation, the distribution
326   pattern of nit clusters on a few sampled hairs must be considered.
327
328   From the interpretation of the results for nit-clustering and their arrangement on the hair-
329   shaft it is clear that for a certain period there was a lack of space for female oviposition
330   manoeuvres. This implies highly crowded conditions typical of a severe infestation. In a
331   severe infestation, females struggle to find the space required to manoeuvre oviposition as
332   they can only grab and hold empty hair shafts and not nits (there is no
333   morphology/adaptation for this). Even if the intense infestation only covers 2 cm of the
334   hair shaft, and there is ~ 1 mm of space between small nit clusters covering these 2 cm, this
335   confirms that the patient was neglected for a period of time (2 cm approximates to 2
336   months). Using hair growth (when possible) to estimate the time when the patient was
337   exposed to neglect can help to interpret the circumstances and time of neglect, e.g.
338   indicating when it happened, or when it started. For example, a patient might be a victim of
339   neglect for only 2 - 3 months, and this might have started 6 months before its discovery. If
340   his/her hair grows at a rate of 1 cm/month, the cluster formation should be found ~ 3·5 cm
341   from the scalp and should cover at least 2 cm of hair-shaft. Over these 2-3 cm of nit-
342   attachments, the clusters should be separated by at least 1 mm, as that is the female's
343   minimum required space for grabbing the hair to oviposit. The use of hair growth has to
344   consider the age of the patient/victim, the older the person the slower the growth.
345
346   Nit-clusters were slightly and unevenly distributed on the shafts (non-normally distributed),
347   which could be due to the expected intra-population body size variation of gravid females.
348   The average distance of 1.66 mm (± 0.55) between attachment sites clearly suggests,
349   however, that there was no room for extra manoeuvres. The immediate consequence of
350   crowdedness is the overlapping of generations in ovipositing sites (here called clusters), a
351   feature already observed by early researchers (Buxton, 1947), but not considered important
352   until now. The predominance of 2-nit clusters followed by 3-nit clusters (showing a
353   Poisson distribution, Fig. 1 inset) might also indicate overlapping of a number of
354   generations or group oviposition or even a few demes becoming body lice. Females of
355   body lice are gregarious and nits are deposited in groups in the same area of cloth shared
356   by several females (Veracx et al., 2012).
357
358   Based solely on the oviposition behaviour of lice, the estimated length of time of the
359   continuous severe infestation suggests a sustained neglect of approximately 2 years in
360   duration. This represents a clear case of neglect of an elder (Bennett & Kingston, 1993).

361 Furthermore, the presence of intermittent infestations, of a few consecutive clusters in the
362 distal or older parts of the hair shafts, suggests that neglect was likely experienced
363 repeatedly, even before the 2 year period of intense and out of control infestation built up.
364
365 The victim of neglect presented a *plica polonica*, a characteristic feature of gross head lice
366 infestations, confirming neglect (Alexander, 1984). In this condition, the hairs are glued
367 into a sticky secretion and emanate a particular spoiled vinegar smell. *Plica polonica* was
368 common in Poland in the 1200s, when it was described: then it was already a sign of poor
369 hygiene (Brzezinski *et al.*, 2016). In this case study, the doctors and nurses treating the
370 patient noticed the *plica* formation, as well as the huge louse population extending to the
371 torso (Connor *et al.*, 2016).
372
373 Unfortunately, it was not possible to sample lice from the torso area or its clothes to
374 confirm the presence of the two *Pediculus humanus* subspecies. Finding body lice together
375 with a gross head lice infestation on one human host is not novel. Head lice evolving into
376 body lice have been documented on the same individual, particularly homeless people
377 (Veracx *et al.*, 2012). The adaptation to nest in clothing can only occur in head lice of
378 mtDNA Clade A (Li *et al.*, 2010). At present, however, there is no phenotype or
379 morphotype available to identify mitochondrial clades. Clade A has a worldwide
380 distribution and is predominant in European countries and it is likely that it was carried by
381 the patient. The transition from head to body lice on a person can only be achieved
382 following a very long term exposure and continuous infestation (Veracx *et al.*, 2012) and
383 this is another clear sign of a patient being a victim of neglect and of having suffered a
384 long-term, severe pediculosis capitis.
385
386 Of particular interest was the finding of a gap at the root of all the hair shafts examined,
387 characterised by the absence of nits from the root-end of the shaft to the last nit oviposited
388 onto it. With an average length of 1.45 mm (±0.47) of nit absence, this represents an   **cm**
389 abnormal situation for lice biology. Head lice gravid females oviposit circa 5 mm from the
390 host scalp (Buxton, 1947; Lang, 1975; Lapeere *et al.*, 2005; Roy & Ghosh, 1944). This
391 distance cannot be modified, it is a physiological requirement for the proper development
392 of the embryo. Nits depend on a temperature gradient determined by the distance, also
393 measured in time (as the hair grows), from the scalp producing heat (Alexander, 1984;
394 Buxton, 1940; Buxton, 1947). The estimated length of the gap with no nits suggests a time
395 lapse of ~ 2 months and it was found despite many thousands of mobiles, particularly
396 females, crawling on the head. Lice larvae and 1st instar nymphs (the youngest stages)
397 were very rare and gravid females' internal or not yet laid oocytes were found to be
398 degenerating or terminated (shrunk) (numbers not collected). This was a clear indication
399 that extrinsic factors were affecting lice reproduction.
400
401 The medication consumed by the patient, nifedipine, is used for the treatment of high
402 blood pressure. This substance actively blocks the movement of calcium through calcium
403 channels, being a well-known calcium channel blocker (CCB). The active ingredient is
404 almost fully attached to proteins in plasma, and metabolised to inactive compounds with a
405 half-life of up 4 hours (Hilal-Dantan & Brunton, 2014). This makes the drug available in
406 blood for several hours, providing a plentiful supply at variable doses in lice blood meals.
407 This drug can be purchased without prescription in Italy, and the patient was able to buy
408 and consume it as desired.
409
410 In most sexually reproducing animals, egg activation is induced by the process of
411 fertilisation. The sperm mediates a continuous release of intra-oocyte calcium which

412 allows the completion of meiosis and the development of the vitelline membrane to
413 prevent access by more sperm. In this context, arthropods are more flexible than other
414 animals, with sperm being no longer the exclusive trigger of egg activation. Insects, such
415 as lice, use intra-oocyte calcium waves occurring while the eggs or oocytes move through
416 the female's oviducts (Horner & Wolfner, 2008; Kaneuchi *et al.*, 2015; Perotti *et al.*,
417 2007).

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

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

444 Conflicts of interest
445 None

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

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553
554
555
556

557   FIGURE Legends

558

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

565

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

571

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

577

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

587

INSET

Frequency observed

Number of nits/cluster (N$_{Clusters}$ = 200)

Hair Length (mm)

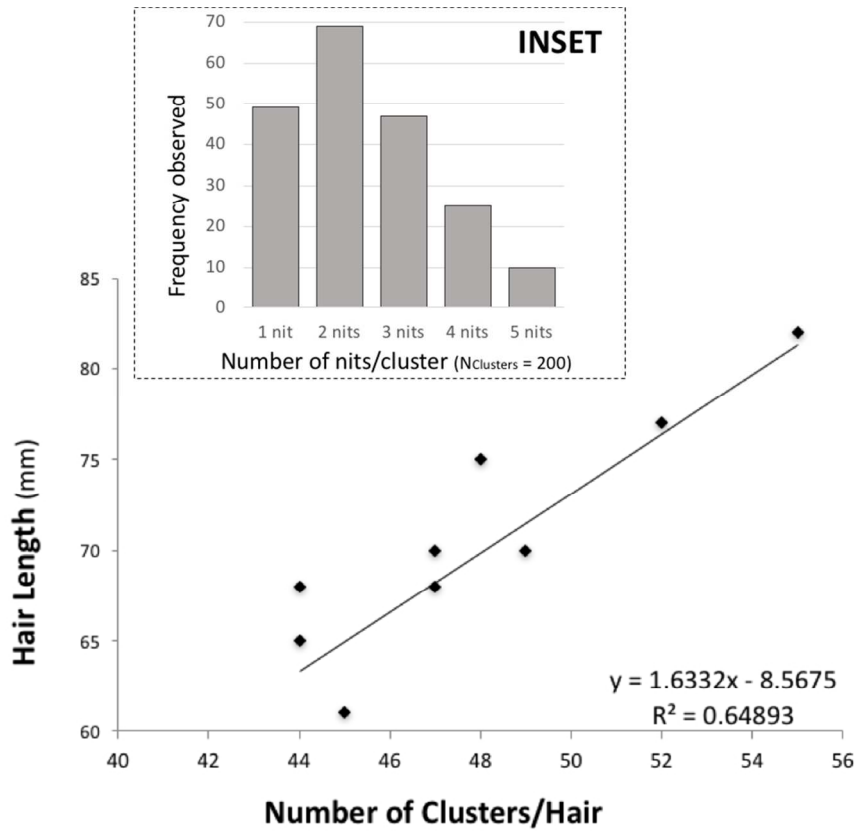$y = 1.6332x - 8.5675$
$R^2 = 0.64893$

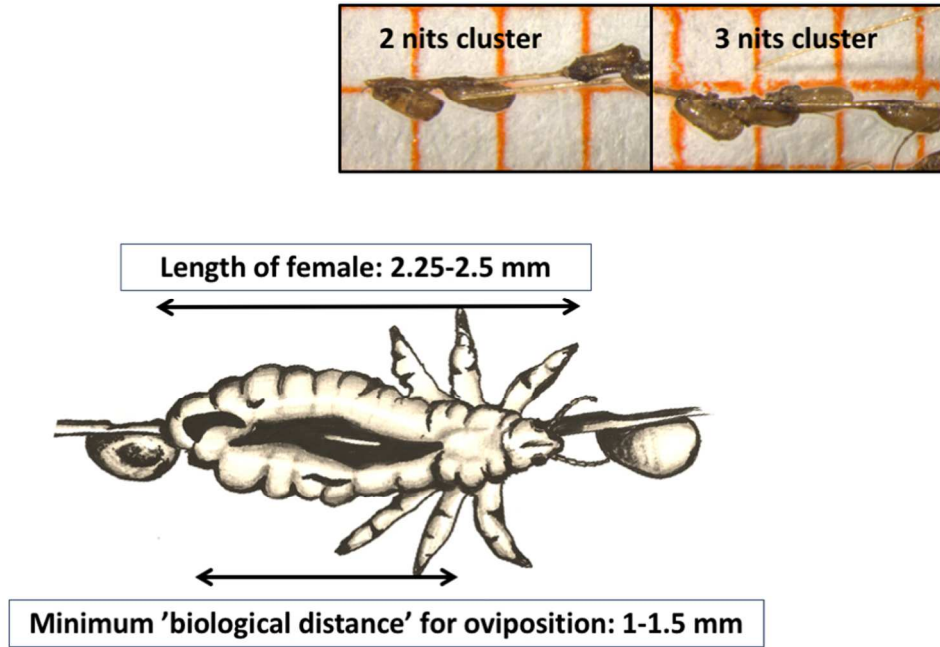**Number of Clusters/Hair**

Figure 1

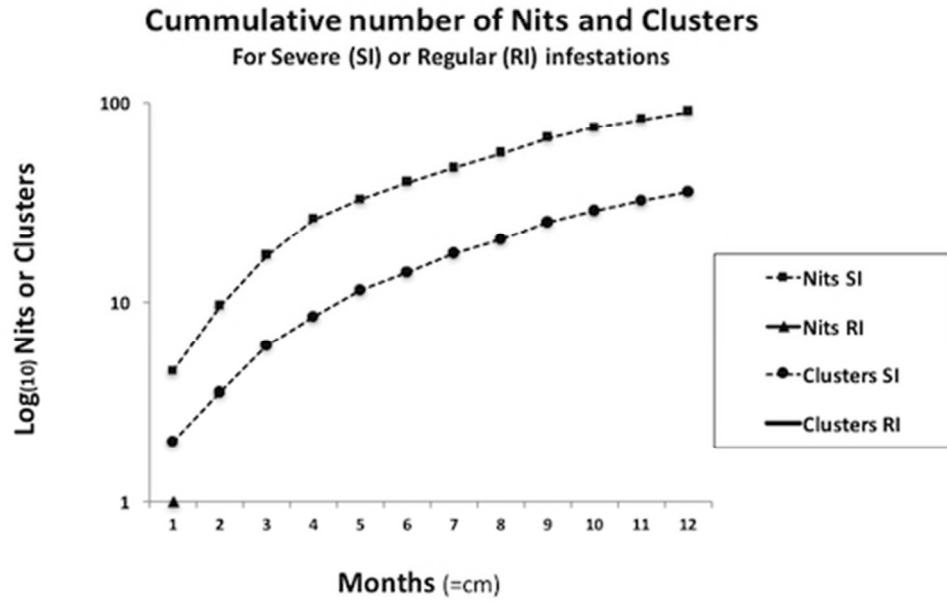200x175mm (127 x 127 DPI)

Figure 2

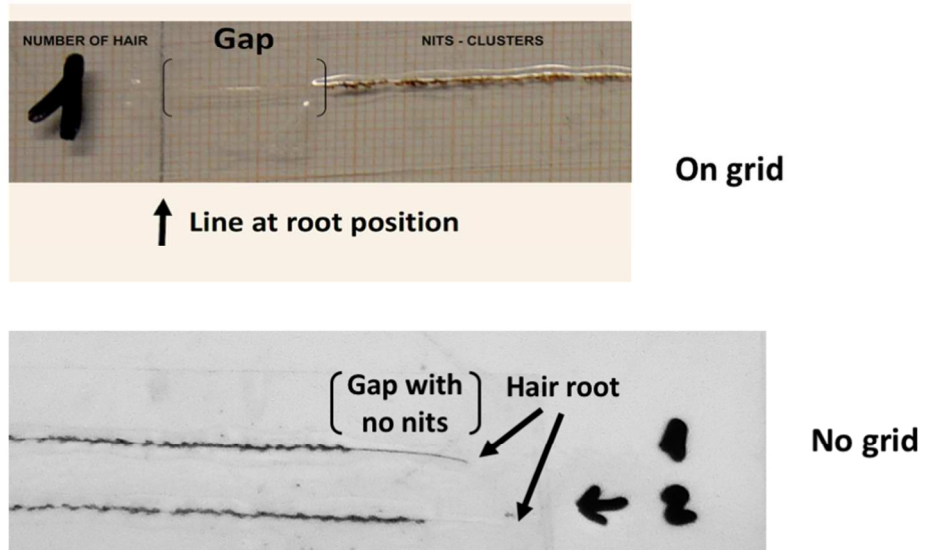276x191mm (92 x 92 DPI)

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 **Other**	-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		

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

6

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