**Supplementary Materials**

**Using human head lice to unravel neglect and cause of death**

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|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **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/AN/AN/AN/AN/A |  |  |
| **Evaluator2:** | **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].

**LITERATURE**

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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 |