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Insights About Head Lice Transmission From Field Data and Mathematical Modeling

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Abstract

Head lice infest millions of school-age children every year, both in developed and developing countries. However, little is known about the number of lice transferred among children during school activities, because direct methods to study this are almost impossible to implement. This issue has been addressed following an indirect method, which consist in collecting data of real infestation from several children groups and using a mathematical model of lice colonies to infer how the infestation observed might have evolved. By determining the events that would most likely lead to infestations as those observed, we find that severe infestations are most likely initiated by a relatively large number of lice transferred at the same moment or within relatively short time spans. In turn, analysis of the data obtained from screenings of the same groups of children a few days apart shows evidence of such transmission events. Interestingly, only children with severe infestations could harbor the lice necessary for this type of transmission. Thus, they play the same role as 'superspreaders' in epidemiology. As part of our experimental study it is also shown that a simple procedure of combing can be very effective to remove all mobile lice, and thus could be used as an effective preventive measure against those severe infestations that are responsible for the spread of pediculosis.

Key words: mathematical model, epidemiology, *Pediculus humanus capitis*, Argentina

Pediculus humanus capitis (De Geer; Phthiraptera: Pediculidae), commonly known as the human head louse, is an obligate parasite found worldwide. Prevalence varies among human populations mainly depending on gender, age, and control methods (Heukelbach 2010), but it is particularly high in school age children (Burgess 2009). In the United States alone, every year more than 10 million people (mostly children 6–12 yr old) are newly infested with head lice (West 2004). In Argentina, a study over 1,800 children (5–13 yr old) revealed an overall head lice infestation of 30% (Toloza et al. 2009). Head lice cause emotional and social distress because pediculosis is often associated with poor personal hygiene and poverty, even though this association has been shown to be unfounded (Burgess 2004). In addition, in recent years head lice have been found to carry bacteria associated with typhus (Robinson et al. 2003), trench fever (Sasaki et al. 2006), and hospital-acquired infections (Bouvresse et al. 2011).

The two main strategies against the spread of pediculosis are individual head lice control and prevention of head lice transmission. Traditionally, the main treatment to control head lice has been based in a wide variety of neurotoxic synthetic insecticides (Burgess 2009). However, there is ample evidence that resistance to insecticides is developing in head lice populations (Hodgdon et al. 2010, Toloza et al. 2014). This is probably one of the main reasons why head lice infestations have been increasing worldwide (Falagas et al. 2008).

Given that resistance seems to have developed against a wide range of insecticides (Heukelbach 2010), a promising alternative to the development of new products could be to act at the level of head lice transmission. Transmission of head lice can occur through direct physical contact, especially head-to-head contact and also via inanimate objects, also known as fomites (Burkhart and Burkhart 2007). However, evidence has shown that fomites play a minor role in head lice transmission (Takano-Lee et al. 2005, Canyon and Speare 2010). For this reason, it is very important to understand the details of the head-to-head transmission process. Unfortunately, a direct study of this in schoolchildren is virtually impossible because it would require following not only the movements of each child, but also the movement (or at least the transfer) of each louse in each head. Head lice reared in vitro have been used to understand the mechanics of lice transfer between single hairs or hair tufts (Canyon et al. 2002, Takano-Lee et al. 2005). It was reported that adult lice were much more likely to disperse and this tendency was independent of the population density or hunger of the exposed insects. However, it is not clear how pertinent this can be for head lice transmission taking place in a real setting.

Mathematical models have long been used as tools in integrated pest managements programs (Ruesink 1976, Worner 1991). As far as we know, there have been only two attempts at using mathematical models to understand the spread of pediculosis. Stone et al. (2008) considered pediculosis as an infectious disease and used a classical epidemiological model. They consider neither the life cycle of the head louse nor the infestation intensity of each individual (i.e., the actual number of lice present). On the other hand, Laguna and Risau-Gusman (2011) focused on colonies of head lice (each colony corresponds to one infested head) and their interactions. With this model it is possible to simulate computationally the growth of individual and collective infestations, in order to assess the impact of lice control strategies.

In this article, we obtain information about the process of head lice transmission using a twofold approach. First, data collected from several schools and a children's home are combined with the predictions of a mathematical model (Laguna and Risau-Gusman 2011) in order to obtain the most likely events that can give rise to the infestations found. Then, a simple probabilistic argument is used to show that these events do seem to have taken place in some of the groups screened.

Subjects and Methods

Field Studies

The field studies were conducted in groups of school age children from Buenos Aires, Argentina. Only children whose parents had given informed consent for participation were examined. In the children`s home consent was given by the head of the institution, The freedom to refuse to participate in the research was clearly established in each case. As the present research was not an interventional study as stated by the Argentinean regulations, acceptance of the protocol by an ethical commission was not required at the time of this research work.

A total of 237 children (123 girls and 114 boys) aged 4–10 yr old were examined for head lice. Infestation intensities were classified as: light when less than 10 mobile lice were collected, mild when this number was between 10 and 20, and severe when more than 20 live mobile head lice were found.

Study 1

This study was conducted during May–June 2012, in three elementary schools located in Buenos Aires. Children between 6 and 9 yr old of six school classes were examined twice, with 7 d between visits for four of these classes, and 10 and 15 d for the remaining classes. Because of the internal dynamics of the school and of each class, it was not possible to have exactly the same time between visits for the six classes. In the two visits we screened 102 different children (52 girls: 50 boys), comprising 85% of the children in the six classes. In the first visit 79 children (40:39) were examined, whereas in the second visit 85 (42:43) children were examined. Of all these children, 62 (30:32) were present in both visits. The aim of this study was to obtain data of head lice transmission in schools. Full details can be found in Supplementary Table S1.

Study 2

This study was conducted during March–May 2013, in eight classes in two of the schools previously visited in study 1. Only one visit to each class was performed. All the children screened were 6–11 yr old. The classes included here were not the same as in study 1. We examined 114 children (61 girls: 53 boys), comprising 65% of the children in the eight classes. The purpose of this study was to obtain more data in order to improve our statistics of head lice infestations in schools and also our statistics about the efficacy of the lice removal procedure. Full details can be found in Supplementary Table S2.

Study 3

This study was conducted at a children's home (i.e., a residential institution devoted to the care of children whose biological parents are deceased or otherwise unable or unwilling to take care of them) located in Villa Martelli, Buenos Aires, during February–May 2013. The site was visited four times separated by 12, 48, and 36 d, respectively. We examined 22 children in the four visits (11 girls: 11 boys). Only eight children (6:2) were present in all the visits. The aim of this study was to get an insight on head lice transmission in a place that is clearly different from a school. Full details can be found in Supplementary Table S3.

Lice Removal Procedure

In this work, all the combings were conducted by the same person in order to avoid individual variations that might skew the results about the infestation intensity of the screened children. The comb used was a metal comb ASSY that was purchased in the market in Buenos Aires, Argentina. The ASSY metal comb had teeth of 37 mm of length, with 0.09 mm between teeth, and a metal straight grip (Gallardo et al. 2013).

The lice removal procedure (or *combing procedure*) consisted of two consecutive steps. In the first step, the head of each child was combed with a regular hairbrush in order to disentangle the hair. The scalp was divided in six areas: first it was split in two bilateral halves from the forehead backwards to the nape, and then each half was divided into three areas, one near the forehead, one in the middle and another one near the nape. Each area was combed with one pull of the metal comb through the hair belonging to that area. This was repeated twice for every head. The lice collected in this step were stored in Petri dishes. The second step consisted of the complete removal of mobile lice (not eggs) by combing the head for at least 10 additional minutes, or until no lice were found.

Head lice collected within a 2-h period were transported to the laboratory according to the protocol developed by Picollo et al. (1998). The protocol for lice collection was approved by the ad hoc committee of the Centro de Investigaciones de Plagas e Insecticidas (CIPEIN-UNIDEF, Buenos Aires, Argentina), and archived in our laboratory (# BA20061995ARG, June 1995) (Picollo et al. 1998). Lice were transferred to an environmental chamber (Lab-Line instruments, Melrose Park, IL) at 18 ± 0.5°C, 70–80 ± 1% relative humidity (RH) in the dark until they were counted. The number of eggs, nymphs and adult male and female lice was recorded under a stereoscopic zoom microscope (NIKON SMZ 10).

The combing procedure, followed by complete removal, were performed in all the visits of the three studies, except in the first visit of study 1, when only the combing procedure was performed.

Mathematical Model

The mathematical model used here is based on the model developed by Laguna and Risau-Gusman (2011). In this discrete time model, the state of a head lice colony at each time step (which represents 1 d) is calculated from the state of the colony at a previous step using the formalism of Leslie matrices (Leslie 1945, Lefkovitch 1965). The population of the colony is divided into three stages: eggs, nymphs, and adults. Each of these classes is in turn subdivided into age substages. Thus, there are adults of 1 d, adults of 2 d, etc. (i.e., adults that have molted from the nymph stage 1 d ago, 2 d ago, etc.). At each time step, individuals have three possibilities: move to the next substage of the same stage, move to the next stage (more specifically, to the first substage of the next stage) or die. For example, nymphs that are 7 d old can either move to the subclass nymph 8 d old or to the class adult 1 d old.

The parameters of the model are the probabilities of moving between stages, and they were estimated from the detailed data available on the life cycle of the head louse (Takano-Lee et al. 2003). More details are given in the Supplementary Material.

It has been assumed that the colony is closed except for the introduction of some females (representing lice transmission). This is modeled by incrementing in one unit the number of members of the subgroup of that age at that given day.

It is also assumed that in the colony there are no interactions between individuals. To compensate for the absence of mating interactions, it has been assumed that the females that start the colony can lay fertile eggs during 8 d (Bacot 1917, Takano-Lee et al. 2003) and that the females of the colony can lay eggs during their completely adult life. This models the fact that males may not be available at first, but they should be readily available as the colony grows. For the sake of simplicity it has also been assumed that lice do not leave the colony. In real infestations, the number of lice that leave the colony is probably a very small fraction of the total number of lice, and therefore their removal should not affect the dynamics of the colony. More details about the model are given in Section II of the Supplementary Material.

Analysis of Data Using the Mathematical Model

Some of the questions regarding head lice transmission in a particular setting can be posed in terms of the relationship between the state of a colony at a given time and the state of the same colony some time before. For example, the question: What were the events that have driven the infestation in the head of a child in a certain period? can be rephrased as: What is the initial state of a colony and how many (and when) external lice have to be added in order to have a colony with the same composition as that on the child's head?

If each colony is characterized only by the number of nymphs and adult lice present, then the state of a colony can be represented as point in a XY diagram. There are many states that are represented by the same point in such a diagram (e.g., two groups with the same number of nymphs and adult lice could be composed by individuals of different ages). However, this representation is convenient because when an infested head is screened it is virtually impossible to determine the exact age of each removed louse, but the total number of nymphs and adult lice present can be determined with a reasonable degree of accuracy. The growth of the colony from a given initial state can be simulated using the mathematical model and can be represented as a curve in the XY diagram. If a point corresponding to a real infestation (obtained from a screening) is sufficiently close to the curve, it can be inferred that this particular infestation is likely to have been caused by the same initial state and external lice that characterize the curve. Conversely, if the point is far from the curve, it can be inferred that those conditions cannot have yielded the infestation found. In the next subsection some of the curves that can be obtained from the mathematical model are presented.

Growth of a Lice Colony Initiated by One, Two or Three Females

As an illustrative example, we describe in detail the growth of a colony that starts with the youngest female capable of laying eggs, which in our model occurs after 4 d of adulthood. This corresponds to the curve with circles in Fig. 1A, where the number of nymphs is plotted against the number of adult lice during the first 7 wk of the colony. In the first weeks of the colony, growth proceeds in a step-like manner. It takes approximately 1 wk until the first eggs laid by the founder female start to hatch (in the inset, this corresponds

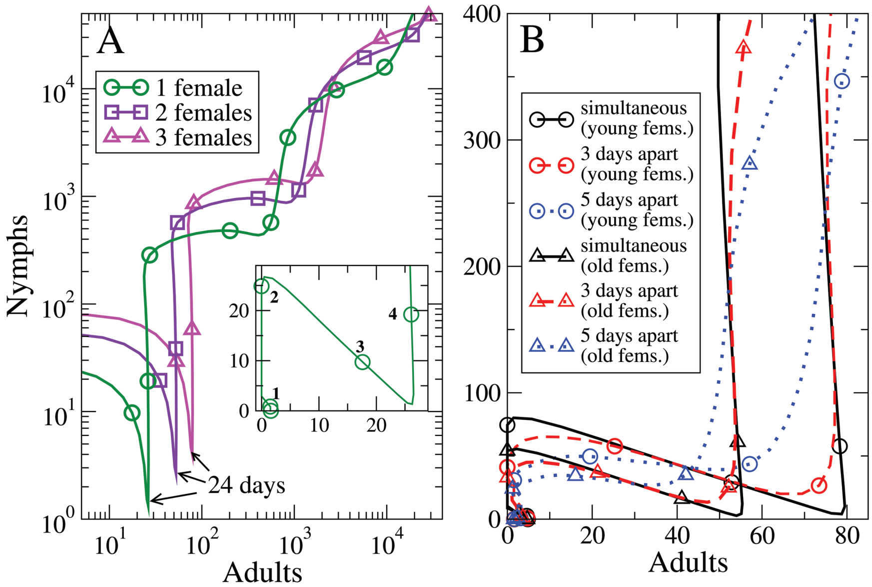

Fig. 1. Mathematical model of colony growth. The curves represent the growth of lice colonies initiated by different sets of females. To avoid cluttering the curves, symbols only mark the state of colonies at a whole number of weeks after the introduction of the first female. Panel A: 12 wk of the colonies initiated by one, two, or three females transferred on the same day (in log-log scale). Inset: Detail of the first 4 wk for the colony initiated with one female, in linear scale. Panel B: 5 wk of colonies initiated by three females introduced with three different schedules: the same day, 3 d apart and 5 d apart. Females are classified as 'young' or 'old', when they have molted 2 or 10 d, respectively, before founding the colony. Note the linear scale in this panel.

to the point marked '1'). After that point, the number of nymphs increases steadily during the following 7 d, without any increase in the number of adults (first vertical sector of the curve in the inset). At day 15, the first nymphs start to molt into adults, thus increasing the number of adults and decreasing the number of nymphs. During this stage the adults lay eggs that will only start to hatch after day 24, reaching what we call a *turning point*. This is followed by a rapid increase in the number of nymphs with almost no increase in the number of adults, giving a new vertical growth that lasts approximately 2 wk. At this point, the first nymphs start to molt into adults and are replaced by new nymphs that emerge from eggs. The steps become less steep as time progresses because the various lice generations start to mix, and the growth becomes exponential. This is the reason why the plot of the main panel of Fig. 1A is better presented in logarithmic scale.

When the colony is founded by more than one female, the growth of the colony is qualitatively similar (Fig. 1A). In this case, the times at which the new females are transferred into the colony should also be considered. Figure 1B shows the growth of colonies initiated by three females of different ages transferred at different time points. It can be seen that when females are introduced three or less days apart, the differences between the colonies become rather small. However, females introduced separated by more than 3 d give curves that deviate appreciably from the previous ones.

Results

Data Collected and Statistics of Infestations

As shown in Table 1, pediculosis was observed in all the studied sites. The number of examined and infested boys and girls are detailed for each visit. The overall prevalence varied from 26.4 to 69%. In the schools, prevalence was similar with an average value of 28.2%, while in the children´s home was of 69%. There were no significant differences between the infestation intensities of girls and boys (χ^2 = 0.42, $P > 0.05$). A summary of the head lice collected is shown in Table 2. We have found in all the studies a significantly female-biased sex ratio with values of 65, 61, and 65%, respectively.

The combination of the data presented in Tables 1 and 2 allow us to calculate the average intensities of infestation in each case. In study 2 infestation had an average of 16.5 lice (of which 5.1 were adults), whereas in the second visit of study 1 infestations had an average of 20.7 lice (9.1 adults). It is important to note that only the combing procedure was performed (and thus only a partial removal of lice) in the first visit of study 1, i.e., a few days before the second visit. The average of lice removed in the first visit was 36.9 (15.9 adults), but this must be considered only as a lower bound to the real values for the infestations.

In studies 1 and 2, the distribution of head lice was heavily skewed by a few very severe infestations. Therefore, the median is an indicator that gives a better characterization of the distribution. The median values obtained were 12 lice (3 for adult lice) in the first visit of study 1, 5 (3 for adults) in the second visit of study 1, and 7 (2 for adults) in study 2.

In study 3 not only the prevalence was higher in the four visits than it was in the schools, but the infestation was also more severe in three of the four visits (visits 1, 3, and 4). For these three visits, the average infestation was 37.1 mobile lice (13.3 adults), with a median of 16.5 lice (8 adults). In the second visit the prevalence was relatively low, probably because a complete lice removal was performed from the heads of 15 of the 22 children of the home in the previous visit, which took place only 12 d before.

Full details of the data collected in the three studies are provided in the first section of the Supplementary Material.

Efficacy of the Combing Procedure

For each of the heads examined, the number of lice collected by means of the combing procedure ('removed lice'), versus the number of lice removed afterwards, when total removal was completed ('remaining lice') is shown in Fig. 2. In the figure, each point corresponds to a single head and the same group of data is shown in three different panels in order to show separately the effectiveness of the combing procedure for adults and nymphs. Points very close to the horizontal axis represent heads for which combing was very effective (they have very few remaining lice) whereas points with a high value in the vertical axis correspond to heads for which the combing procedure has had a low efficacy.

In light infestations, combing was very effective and there were no significant differences in either boys or girls. More specifically, when the total number of mobile lice was ≤10, all insects were removed in 100% of the cases for boys (n = 20) and 97% of the cases for girls (n = 33) (χ^2 = 0.6, $P > 0.05$).

Interestingly, in light infestations there were also no significant differences in the effectiveness of combing for removing adult lice or nymphs (Fig. 2B and C). When the number of adult lice was ≤10, all of them were removed in 85% of the cases for girls (n = 39), and in 81% of the cases for boys (n = 21). Similarly, when the number of nymphs was ≤10, all were removed in 86% of the cases for girls (n = 37) ($\chi^2_G = 0.015$, $P > 0.05$) and 84% for boys (n = 24) ($\chi^2_B = 0.069$, $P > 0.05$). This is somewhat surprising given that nymphs can be much smaller than adult lice.

Table 1. Summary of children examined and infestations found

Study	Visit	Examined			Infested (%)		
		Children	Girls	Boys	Children	Girls	Boys
1	1	79	40	39	20 (25.3)	12 (30)	8 (20.5)
	2	85	42	43	23 (27.0)	14 (33.3)	9 (20.9)
2	1	113	60	53	34 (30)	23 (38.3)	11 (20.7)
3	1	15	8	7	8 (53.3)	4 (50)	4 (57.1)
	2	15	10	5	9 (60)	6 (60)	3 (60)
	3	12	8	4	11 (91.7)	7 (87.5)	4 (100)
	4	16	9	7	12 (75)	8 (88.9)	4 (57.1)

Columns indicate the number of examined and infested children, discriminating girls and boys. The resulting prevalences are indicated between brackets.

On the other hand, when the infestation was severe (>20 mobile lice), the combing procedure was significantly less effective for girls than for boys. For severe infestations, the combing procedure removed 53% of all mobile lice for girls, and 80% of all mobile lice for boys (Mann–Whitney U =21, n_B = 6, n_G = 17, P < 0.05, two-tailed).

Comparison Between the Predictions of the Model and the Collected Data

In order to extrapolate the insights obtained by comparing the predictions of the mathematical model with the collected data, it is necessary that the data are as representative as possible. We do not include the data collected in the second visit of study 1, nor the second visit to the children's home, because a few days before these visits lice removal had been performed (either partial or complete), and thus the infestations recorded in the following visit cannot be considered as typical of the schools nor of the children's home. The remaining data are plotted in Fig. 3.

The first case analyzed corresponds to infestations caused by the transfer of a single female head louse of different ages. In Fig. 4, the curves representing the simulation of the growth of the corresponding lice colonies are plotted along with the points representing the data collected. For practical reasons, only three curves are shown, corresponding to colonies initiated by founders who have molted 2, 11, and 21 d before being transferred to the non-infested head. Older females have not been considered because it is known that both their fecundity and the viability of the eggs are very low (Bacot 1917, Takano-Lee 2003). Twenty-four days after its foundation, the colony reaches a point at which all the lice of the first generation (i.e., the children of the female founder) have become adults. From now on, this article this point will be called a *turning point*. From this, the number of nymphs grows very rapidly and the number of adults does not increase again until the number of nymphs is very large, indeed much larger than in all but one of the infestations found (in the figure, the almost vertical part of the three curves grows vertically until at least the value of 250 nymphs). This means that none of the data points of Fig. 4A which have more than 30 adults can be reached by the curves because they are placed to the right of the turning point. In Fig. 4B, the growth of the curves for much longer times than in Fig. 4A is shown. Note that the curve of the older female is close to one data point, corresponding to a girl of the study 1 that has a severe infestation (identified as S1s1c2g3 in Supplementary Table S1). Besides, Fig. 4B confirms that the other six points corresponding to infestations with >30 adults are not reached by these curves even for very long times. This implies that from the infestations found, those with the largest number of adult lice are unlikely to have been initiated by a single female (except in the case previously commented).

When infestations are initiated by more than one female, the corresponding curves (shown in Fig. 5) are qualitatively similar to the

Table 2. Summary of lice collected in the three studies

Study	Visit	Nymphs	Adults ♀	Adults ♂	Adults ♀ +♂	Total
1	1	418	181	137	318	736
	2	265	137	73	210	475
	(1 + 2)*	673	318	210	528	1,211
2	1	388	104	68	172	560
3	1	75	69	59	128	203
	2	34	11	5	16	50
	3	542	90	36	126	668
	4	122	108	50	158	280
	(1 + 2+3 + 4)*	773	278	150	428	1,201

Columns indicate the number of nymphs and adult lice, discriminating sex. Rows marked with * indicate the number of collected lice in the corresponding study, including all the visits.

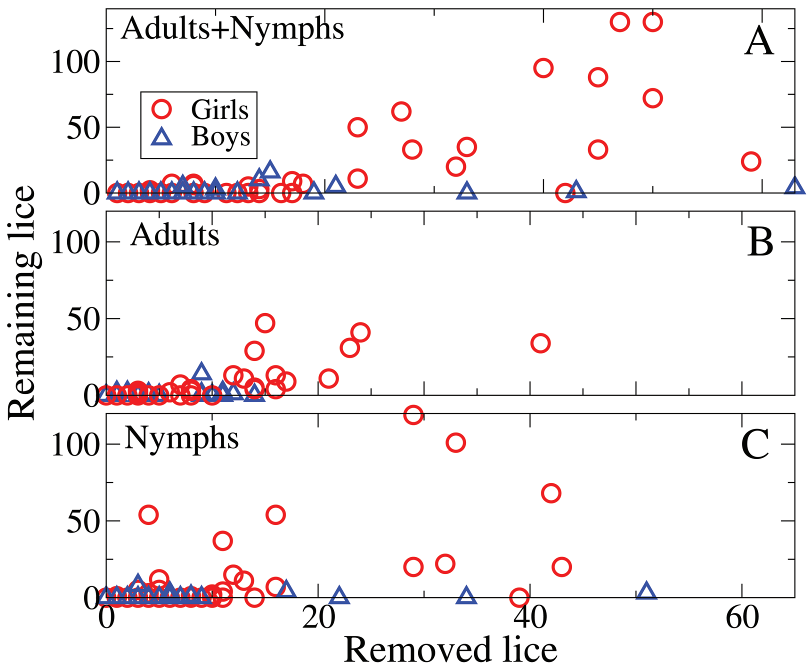

Fig. 2. Efficacy of the combing procedure. Each symbol represents the number of lice collected from one head following the combing procedure ('removed lice') versus the lice that were collected afterwards, when total removal was performed ('remaining lice'). Circles and triangles indicate heads of girls and boys, respectively. Symbols in panel A correspond to total number of lice (adults plus nymphs), whereas in panels B and C they correspond to only adult lice and nymphs, respectively.

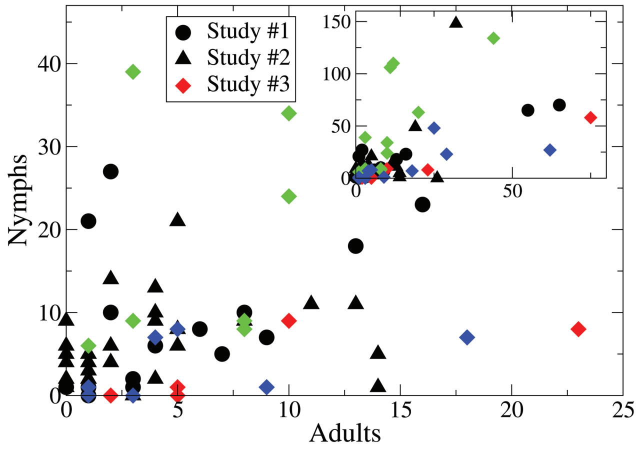

Fig. 3. Infestations found in the three studies. Each symbol represents the number of nymphs and adult lice collected from one head. In the inset, the same information is plotted but for a wider range of values. Black circles correspond to heads examined in the first visit of study 1. Black triangles are data of the study 2. Red, green, and blue diamonds represent heads examined in visits 1, 3, and 4 of study 3, respectively (colors visible in the online version).

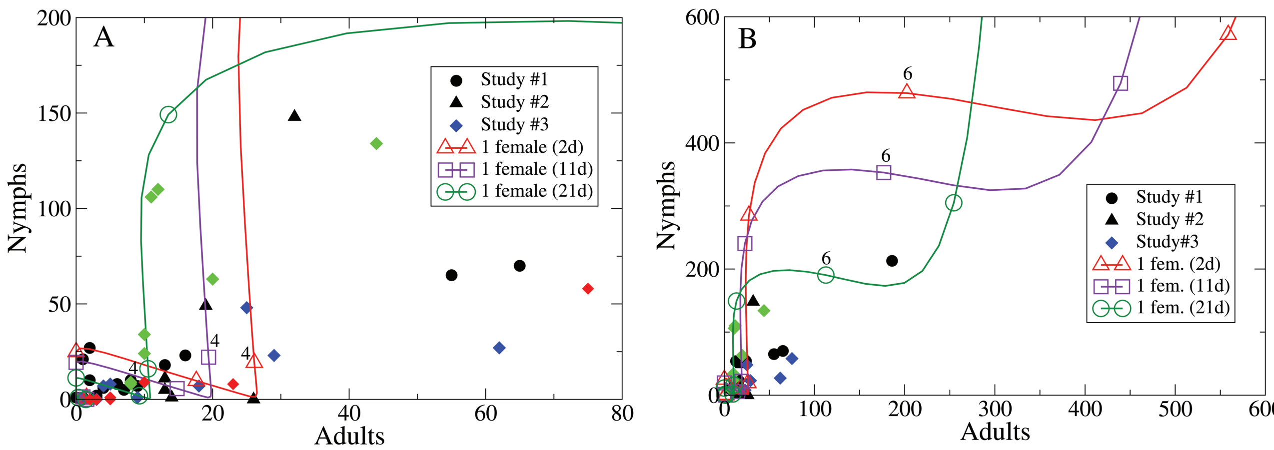

Fig. 4. Data and predictions of a mathematical model of infestations originated by a single female. Full symbols represent the number of nymphs and adult lice collected from a single head in studies 1, 2, and 3. Lines indicate the growth of a colony obtained with the mathematical model: curves with open triangles, squares and circles correspond to the state of a colony started by a single female that has molted 2, 11, and 21 d before the start of the colony, respectively. Big open symbols represent the state of colonies a whole number of weeks after the introduction of the first female (the number printed close to some symbols mark the corresponding week). Curves in both panels differ only in the length of the simulation of colony growth. A: 0–5 wk. Panel B: 0–7 wk.

case analyzed above. Even though the curves for two female founders (full curves in Fig. 5) are closer to the points corresponding to severe infestations than the curves of one female, there are at least four points, representing the most severe infestations found (>50 adult lice), that are far from these curves. Such points might, however, be reached by the dashed curves of Fig. 5, representing colonies founded by three females.

The mathematical model allows us not only to predict the growth a colony for different number of founders, but also to understand the influence of the *timing* of their transfer to the colony. In the simulations mentioned above for three female founders, they are transferred the same day. Fig. 6 shows what occurs when the transfer of the three females are separated by different time intervals. The curves are closest to the points representing severe infestations only when the time between transfers is less than 5 d.

Indirect Evidence of Lice Transmission in the Collected Data

It has been shown above that severe infestations (>50 adult lice) are most likely to be caused by the transfer within a short time (<5 d) of at least three females. A direct confirmation of this kind of transfer is almost impossible because it would imply monitoring very closely all the heads in a group of children. However, we were able to detect in the data some indirect evidence of such transfers. Furthermore, using a probabilistic argument we can also suggest what was the source of these infestations. We focused on study 1 because there is a group of children that was screened twice, with visits separated by 7–15 d.

First, the possibility that the adult lice found in the second visit were already present in the first visit, either as nymphs or as eggs has to be ruled out. It is known that it takes more than 8 d for a nymph to become adult (third instar molt), implying that eggs would become adult lice after >9 d (Bacot 1917, Takano-Lee 2003). Therefore, if the second visit was performed <8 d after the first one, the lice found cannot have come from eggs that remained in the first visit. For visits separated by more than 8 d, we have only considered the infestations of children for which no eggs were found in the first visit. The possibility that some nymphs, or even young adults, could have escaped the combing procedure performed on the first visit is minimized by considering in the following only children with very mild infestations (or no infestation at all) on the first visit.

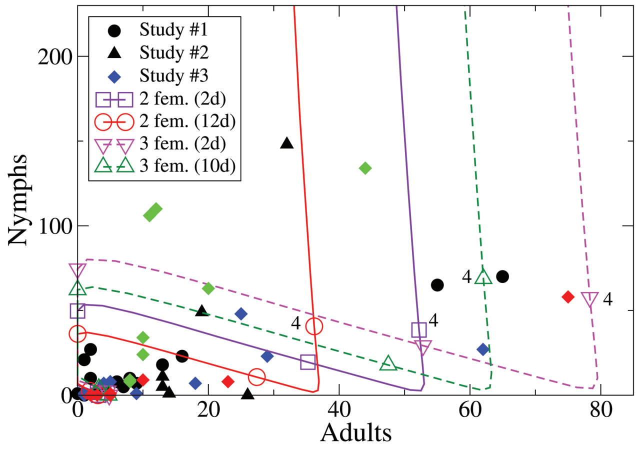

Fig. 5. Data and mathematical model of infestations initiated by two and three females. Full symbols represent the number of nymphs and adult lice collected from a single head in studies 1, 2, and 3. Full lines with open squares and circles were obtained with the mathematical model and represent the growth of colonies initiated by two females molted 2 and 12 d before the start of the colony, respectively. Dashed lines with open triangles up and down are infestations initiated by three females molted 2 and 10 d before the start of the colony, respectively. In all the curves, big open symbols represent the state of the colonies a whole number of weeks after the introduction of the females (the numbers printed close to some symbols mark the corresponding week).

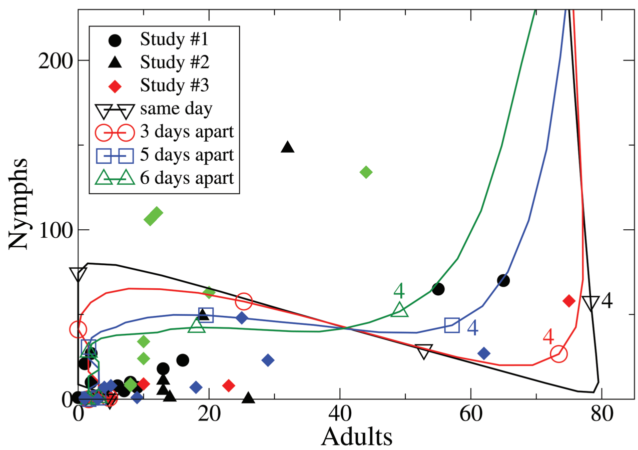

Fig. 6. Data and mathematical model of infestations initiated by three females with different schedules. Full symbols represent the number of nymphs and adult lice collected from a single head in studies 1, 2, and 3. Curves correspond to the state of a lice colony started by three females, transferred to the head with four different schedules. Black curve: females transferred the same day. Dashed curve with open circles: females transferred separately on days 0, 3, and 5 (i.e., 2 d apart). Dotted line with open squares: females transferred 3 d apart. Dashed line with open triangles up: females transferred 5 d apart. Full line with open triangles down: females transferred 6 d apart). In all the curves, big open mark the state of the colonies at a whole number of weeks after the introduction of the first female (the number printed close to some symbols mark the corresponding week).

In study 1, six school classes were examined twice with 7 d between visits for four of these classes, and 10 and 15 d for the remaining classes (Supplementary Table S1). Of the 61 children that were present in the two visits, we can assume that 55 children (25 girls and 30 boys) were free of lice after having been screened in the first visit, because 48 were not infested at all, and 7 had only light infestations (i.e., less than five mobile lice). We can assume that after the combing procedure these seven children were free of lice because the effectiveness of the combing procedure for such light infestations was almost 100%, as shown above (see also Fig. 2). In the second visit, 11 of these 55 children were found to be infested. Of these infested children, five had between three and seven adult lice. According to what was shown in the previous section, this is the kind of lice transfer that could cause a severe infestation. But some of these infestations were 'clustered' in the same classes, which makes it likely that the lice may have come from a head in the same class as each cluster, as the following probabilistic argument shows.

In principle, the children could have acquired their infestations at school or at home. In the first case the infestations are independent events if there is no kinship between the children infested. If acquired at school, the infestations may not be independent if their origin is the head of the same child (note that in our first visit we could not screen every child in each class and we did not perform a complete removal, making it likely that, outside of the 55 children considered, other children in each class could have remained infested). In the following we show that the probability that the infestations are independent is very low, which implies that they must have originated in lice transferred from the same head. Considering that transmission between girls might be different from transmission between boys, these two cases are addressed separately.

Out of 25 girls that were free of lice after the first visit of study 1, we found seven infested girls (S1s1c2g4, S1s2c1g3, S1s3c2g1, S1s3c3g3, S1s3c3g4, S1s3c3g6, and S1s3c3g7) in the second visit. Thus, if it is assumed that the observed infestations have had independent origins (i.e., each infestation was caused by a different children, either at school or at home), the probability of having an infestation in the period between visits would be $P_G = 7/25 = 0.28$. But four of the seven infested girls belonged to the same class. Within this class, six girls were free of lice after our first visit. If the infestations are independent, the probability that four out of six girls are infested between visits is $P = 0.048$ ($P = C(6,4)\ P_G^4\ (1-P_G)^2$, where C(6,4) is the binomial coefficient for 6 and 4). In other words, if the infestations are independent events the probability that four out of six girls of the same class become infested is only $P = 0.048$.

A similar result for boys was obtained. Out of 30 boys that were free of lice after the first visit, we found four infested boys (S1s1c1b2, S1s1c1b3, S1s1c1b4, and S1s3c1b1). The probability of independent infestations is thus $P_B = 4/30 = 0.133$. Three of these four infested boys belonged to the same class, where seven boys had been free of lice after our first visit. The probability that three out of seven boys get infested between visits if the infestations are independent is $P = 0.047$ ($P = C(7,3)\ P_B^3\ (1-P_B)^4$, where C(7,3) is the binomial coefficient for 7 and 3).

The low *P* values obtained show that it is very likely that the infestations found were not independent and that they can be a consequence of lice transfer from the *same* sources. Because there was no kinship between the infested children (data not shown), it is likely that the source of the infestations belonged to the same class. From the data, we can even hint at the source of some of the infestations. In the class to which the four newly infested girls belong (S1s3c3), there was a girl (S1s3c3g5) with a very severe infestation who was not screened in the first visit and may have acted as a superspreader among girls in the week between visits. A similar situation arises for the newly infested boys, since in their class (identified as S1s1c1) there was a boy (S1s1c1b1) who had a severe infestation in the first visit (and therefore the combing procedure probably did not remove every lice in his head), who also might have acted as a superspreader among boys.

Discussion

Even though pediculosis is a worldwide problem that has accompanied humanity for centuries, very little is known about the dynamics of head lice infestation and reinfestation in human groups, and particularly in children. For example, it is still not known whether the severe infestations found in some children are simply regular infestations that have been left untreated for many weeks, or whether they are infestations whose development is significantly different from light infestations. A direct study of the dynamics of lice transfer in a group of children is extremely difficult because it should record not only the movements of each child but also the transfer of every louse. Here, this issue was addressed following a more indirect route. We have collected data of real infestations from several children groups, and we have used a mathematical model of lice colonies and simple probabilistic arguments to infer how the infestation observed in each head might have evolved.

The prevalences found in our field study are relatively high, as compared to prevalences in other parts of the world (Falagas 2008), but they are similar to the prevalences found in other studies conducted in Argentina (Chouela et al. 1997, Perotti et al. 2004, Toloza et al. 2009). The prevalence in study 3, which took place in a children's home where 22 children live together, was much higher than in studies 1 and 2 (carried out in schools).

Our estimation of the lice that remained after the first visit, together with the number of lice removed by combing in both visits, allow us to give a quantitative estimate on the efficacy of combing. For infestations of less than 10 mobile lice (50% of the infestations found at schools), the simple combing procedure had an effectiveness of >85%, whereas for infestations with less than 15 mobile lice (70% of the infestations found) the effectiveness was >90%.

The results from the mathematical model suggest that the most severe infestations found (>50 adult lice) must have originated from the transfer to each head of at least three females at the same moment or within a few days. Notice that, since male and female lice are equally able to disperse (Takano-Lee et al. 2005), this implies that it is likely that some males would also be transferred with the females. This suggests that the transfer of more than three lice may not be uncommon. By analyzing data coming from screenings of the same children groups, separated by a few days, indirect evidence of such transfers was found. Furthermore, the clustering of the infestations associated to those transfers makes it very likely that these infestations could be caused by the same sources. Our approach quantifies the idea that the clustering of infestations in the same school class is probably evidence from head-lice transmission between children in that class (Speare and Buettner 2000). Further inspection of the data shows that in each of the classes where these clusters were found, there is a severely infested child that could have acted as the source of the infestations observed, playing the role of a 'superspreader' (Galvani and May 2005).

If the inferences from the indirect approach used in this article are correct, they would imply that superspreaders are essential in the spread of pediculosis because severe infestations would be caused by head lice transfers from superspreaders. Furthermore, the suggestion that these transfers probably happen at the same time or within short periods of time would imply that head lice spread at a rather large rate. This does not necessarily contradict the finding of Canyon et al. (2002) that only a small fraction of lice was able to move between hairs in in vitro bioassays, since in a real environment the frequency and duration of head to head contacts should also be taken into account. Additionally, it is probably yet another evidence that fomites do not play a significant role in the spreading of pediculosis (Canyon and Speare 2010).

The approach used here is indirect and relies on a number of assumptions, especially regarding the mathematical model used. Even though the model was built using the most detailed data available about the biology of the head louse, it must be remembered that the data come from head lice reared in artificial environments. However, given the difficulties of studying the biology of *P. humanus capitis* in its natural environment, this sort of data is generally used in those references where the life cycle of the louse is described. Such data have even been used to develop more effective treatments against head lice infestations (Mumcuoglu 2006, Lebwohl et al. 2007). Our aims here were much more modest: to provide a first step towards a qualitative understanding of how head lice transmission takes place and its effect for the development of an infestation.

It can be argued that the high prevalence of pediculosis in the population we have studied limits the validity of our conclusions. While this may be true, and it certainly would be interesting to understand head lice transmission in places where pediculosis prevalence is much lower, it is important to point out that high prevalences are not only to be found in developing countries. Prevalences similar to the ones found in our studies have been found in some settings in such developed countries as Israel (Mumcuoglu et al. 1990), Australia (Speare and Buettner 1999), South Korea (Huh et al. 1993), and the United Kingdom (Downs et al. 2007).

Given the difficulties of performing a direct study of pediculosis in children, the use of mathematical models, even with their evident limitations, can give useful insights that would be almost impossible to obtain by other means. One way to improve the predictions would be to supplement the model to include interactions between lice colonies, but this would necessitate data about the movement of children at school. Interestingly, the recently developed wearable proximity sensors have led to many studies on the temporal patterns of contacts between schoolchildren (Stehlé et al. 2011, Barrat et al. 2014). Future work will focus in the incorporation of this feature in our model to make it more realistic, in order to be able to predict collective behaviors and propose efficient control strategies.

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Supplementary Material

Supplementary data are available at *Journal of Medical Entomology* online.

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