

Investigation on prevalence, risk factors, and genetic diversity of *Pediculus humanus capitis* among primary school children

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ARTICLE INFO

Original paper

Article history:

Received: August 09, 2021

Accepted: November 29, 2021

Published: December 01, 2021

Keywords:

Prevalence; Primary School; Children; *Pediculus humanus capitis*; Erbil; Cox1 gene

ABSTRACT

Pediculosis is an integumentary disease caused by the ecto-parasite *Pediculus humanus capitis*, which infests human hair. It is a common public health problem that is most prominent worldwide in elementary school children. The current study aimed to investigate the prevalence, risk factors, and genetic diversity of *P. humanus capitis* among primary school children in the Erbil province. For this purpose, this study was conducted from October 2019 to December 2019 among 1100 randomly selected elementary school children aged 6-12. Data collection was performed via a regular questionnaire and physical hair examination. For the genetic diversity part, after collecting one louse randomly from each individual, DNA was extracted. The mitochondrial Cox1 gene was then amplified by universal primer and PCR. Gene sequencing was performed by ABI (BioNEER, South Korea). Data analysis was done by Chi-Square and T-test using the SPSS ver. 23. The overall infestation rate was 21.27%, and the rate was significantly higher among females (34.93%) compared to males (7.91%). Some variables had found the prevalence rate to be strongly affected. This included age; the rate was not significant (26.87%) in the age group 8-9 years compared to other age groups. According to hair length, the rate was significantly increased (36.52%) among children with tall hair. In terms of hair type, the incidence of curly-haired children was significantly higher (31.54%); in terms of hair color, there were not significant differences among blonde children (25.90%) and others. According to the results of Cox1 gene sequencing, of 234 infested children to lice, 86 (36.75%) of them were exposed to clade A, 38 (16.24%) were exposed to clade B, clade C has not been seen among any children (0%), 105 students (44.87%) were exposed to clade D, and 5 of them exposed to clade E (2.14%). Eventually, a significantly higher incidence (33.78%) was reported in rural primary school children. The infection rate of human head lice in Erbil province is still high, which is one of the health problems of children in public schools.

DOI: <http://dx.doi.org/10.14715/cmb/2021.67.4.44> Copyright: © 2021 by the C.M.B. Association. All rights reserved.



Introduction

Pediculus humanus capitis is an ectoparasite that causes infestation of the human scalp by pediculosis. Feed on the human blood; an infestation usually occurs in the pediatric population in the 6-12 year age group (1). *P. humanus capitis* is known to live throughout the hair, since it depends on the host's blood, tends to live on the hair or even clothes of the person, crawls around the clothes, making sure they stay close to the body of their host (2).

Pediculus humanus capitis lives on the human scalp, where it is attached to the hair's shafts, nourishes blood and completes all stages of its life

cycle: egg, nymph, and adult (3). The adult head louse is 2 to 4 mm long (sesame seed size), has six legs, and is usually tanned in color to grayish-white. The adult louse will appear darker in persons with dark hair. The female lives for up to 3 to 4 weeks and may lay up to 10 eggs per day once she is mature (4). The eggs are called nits; they are oval-shaped flask and usually yellow to white; these tiny eggs are firmly attached to the base of the hair shaft within approximately 4 mm of the scalp with a glue-like substance produced by the louse. The eggs are incubated by body heat, and the hatching may vary from 7 to 12 days depending on whether the ambient climate is hot or cold (5). After

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three molts (instar), the nymphs mature and become adults about 7-10 days after hatching. The female louse may mate and start laying viable eggs approximately 1.5 days after becoming an adult. Unless treated, the cycle repeats itself roughly every three weeks (6).

Head lice symptoms are not imminently harmful to your health; they can cause significant discomfort and disrupt daily life. Scalp pruritus: Pruritus is the medical term for "itchiness." Itchiness of the scalp results from the irritative properties of lice saliva that comes into contact with your skin while feeding your blood (7). Normally head lice infest new hosts only by close contact between individuals, the most common route of lice transmission is head-to-head contact. The number of children per family, the sharing of beds and wardrobes, and health care in particular areas such as school and socioeconomic status were important factors in head lice infestation. Girls are two –to four times as often infested as boys (8).

Diagnosing the condition is by finding live lice in the hair (7). Special attention should be paid to the area near the ears and the neck nape to detect the lice. If lice are detected, the entire family (particularly children under the age of 13) needs to be checked (9).

It is difficult to prevent head lice, especially in young children. The surest way to prevent transmission is to avoid head-to-head contact. Children should be taught not to share personal belongings like combs, brushes, and hats. Prompt treatment of persons infested can help in prevention (10). To prevent lice from returning, you should wash all bed linen, towels, and clothing in at least (54° C) very hot water, separate hair care products and items such as ponytail holders, combs, hairbrush and soak them in alcohol rubbing or a medicated shampoo, you can also wash them in very hot water (11).

Assessing genetic diversity allows us to have more accurate and better information about this parasite. Past molecular studies on *Pediculus humanus capitis* showed five genetically distinct lineages (clade A to clade E lice) based on two mitochondrial genes (*cox1* and *cytb*) (12). Clade A, D, and E lice consist of head and body lice, whereas clade B and C lice comprise only head lice. Clade A has a global distribution; Clade B has been reported in Europe, Australia, North, and Central America, South Africa,

and Algeria; Clade C is found in Thailand, Nepal, and Africa (Ethiopia, Senegal); Clade D has been reported in Pakistan, Nepal, and Ethiopia; Clade E is found in Mali, Ethiopia, and Algeria. Contrary to numerous reports, researchers rarely investigated the genetic diversity of head lice and the molecular studies of their potential associated pathogens (13, 14).

Our study aimed to study the prevalence of *P. humanus capitis* infestation among children in primary school and study the effect of certain demographic parameters on *P. humanus capitis* prevalence. Also, genetic diversity was considered for further evaluation.

Materials and methods

The study was conducted between October to end of December 2019; the samples were collected from 1100 (556 boys/ 544 girls) students aged (6-12 years old) from various primary schools in Erbil Governorate, Iraq.

Sample collection and Examination

The infestation of the head lice was documented via visual Examination, where the heads of the school children were inspected using both visual inspection of the scalp and fine-tooth combing to detect infestation as they were sensitive methods for identification of the head lice, as well as cheap and simple methods (15). The whole head was examined visually, although special attention was paid to the nape of the head and behind the ears, in ample daylight, and by using olive oil combing of the hair with fine-toothed on white paper for about 3-5 minutes. The children would be considered as positive if there were any evidence of head lice (detected head lice adults or nymphs, and live or dead eggs). With each student, the following details were recorded: age, gender, and length of hair, type of hair, bathing frequency, mother education, and several family members to detect the cause of head lice infestation.

Genetic evaluations

For molecular evaluations of each individual, a louse was randomly selected and kept in 70% alcohol. Before DNA extraction, the lice were first rinsed with distilled water and dried on filter paper. NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany) was used for DNA extraction. Agarose gel electrophoresis

was used to determine the quantity and quality of extracted DNA. GelRed was used for gel staining. The DNA bands of each sample with the least smear indicate the desired quality of the extracted DNA for the sample. In order to study the molecular structure, the COX1 locus of the mitochondrial genome was used. For this purpose, the following universal primer was used:

Forward Primer:

5'-GCCGGGATAGTCGGAACAGC-3'

Reverse Primer:

5'-CCTCCTGCAGGGTCGAAAAA-3'

PCR cycles for this experiment included the initial denaturation step of 5 minutes at 95°C. Then, in 35 cycles, the denaturation step was performed for 45 seconds at 95°C. The annealing step was performed at 50°C in 40 seconds. The initial extension step was performed for 60 seconds at 72°C. The final extension step was performed at 72°C for 7 minutes. After PCR, gene samples were taken on the agarose gel to ensure the correct reaction and the formed bands were examined for quality and molecular weight. Depending on the type of gel, the ethidium bromide staining method was used to expose the alleles. Then, the stained gel was scanned by GEL/Doc (BIORAD, Italy) and the Quantity One program. Samples were sequenced by ABI (BioNEER, South Korea). Nucleotide sequence analysis of the studied genes was determined using MEGA 6 software.

Statistical analysis

Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS v.23) program, with a statistical significance *P-value* of <0.05 and a non-significance *P-value* of >0.05. The data were analyzed using “Independent Samples T-test” and “Chi-square.

Results and discussion

As shown in Table (1), the overall infestation incidence with *P. humanus capitis* among 1100 children was 21.27% (234/1100), and the infestation rate among females was higher than among males at 34.93% (190/544) and 7.91% (44/556) respectively. Concerning *P. humanus capitis*, a statistically significant difference (*P-value* = 0.00) was found between the sex.

The highest infestation rate was recorded in the age group (8-9 years) at 26.87% (90/335), and the lowest rate was 13% (13/100) in children twelve years of age and above Table (1). Concerning *P. humanus capitis*, a statistically non-significant difference (*P. value* = 0.19) was observed among age groups.

According to hair length, the highest infestation rate among children with tall hair was 36.52% (130/356), and the lowest infestation rate was 13.98% (104/744) with short hair (Table 1). A statistically significant difference (*P-value* = 0.00) in relation to hair length was reported among infested children.

As seen in Table 1, the highest infestation rate among children with curly hair was 31.54% (41/130), and the lowest infestation rate among children with straight hair was 19.90% (193/970). A statistically significant difference (*P-value* = 0.002) in relation to *P. humanus capitis* was observed among children according to hair type.

The highest rate of infestation was recorded among children with yellow hair color, which was 25.90% (36/139), and the lowest rate among children with black hair color, which was 20.10% (126/627) (Table 1). Statistically, a non-significant difference (*P-value* = 0.3) was recorded according to hair color among children.

A significant correlation (*P-value* = 0.02) was reported in pediculosis (Table 1) depending on the mother's education. The highest infestation rate among children with an illiterate mother was 23.8% (168/706), and the lowest infestation rate among children with a university-educated mother was 16.55% (24/145).

The highest infestation rate among children who bathed one time/week was 28.03% (44/157), and the lowest infestation rate among children who bathed two times/week was 18.75% (72/384). Statistically non-significant difference (*P-value* = 0.25) in relation to *Pediculus humanus capitis*.

Finally, the highest infestation rate among primary school children was 33.78% (100/296) in rural areas, while the lowest infestation rate was 13.62% (32/235) in urban private schools (Table 1). A statistically significant difference (*P-value* = 0.00) was observed among children from different primary school locations.

Table 1. Distribution of *P. humanus capitis* infestation among children concerning different variables

Variables	Total no. of examination	No. +ve (%)	No.-ve (%)	P-value
Gender				
Male	556	44 (7.91)	512 (92.09)	0.00
Female	544	190 (34.93)	354 (65.07)	
Total	1100	234 (21.27)	866 (78.73)	
Age group				
6-7	379	75 (19.79)	304 (80.21)	0.19
8-9	335	90 (26.87)	245 (73.13)	
10-11	286	56 (19.58)	230 (80.42)	
≥12	100	13 (13)	87 (87)	
Total	1100	234 (21.27)	866 (78.73)	
Length of hair				
Short	744	104 (13.98)	640 (86.02)	0.00
Tall	356	130 (36.52)	226 (63.48)	
Total	1100	234 (21.27)	866 (78.73)	
Type of hair				
Straight	970	193 (19.90)	777 (80.10)	0.002
Curly	130	41 (31.54)	89 (68.46)	
Total	1100	234 (21.27)	866 (78.73)	
Color of hair				
Yellow	139	36 (25.90)	103 (74.10)	0.3
Brown	334	72 (21.56)	262 (78.44)	
Black	627	126 (20.10)	501 (79.90)	
Total	1100	234 (21.27)	866 (78.73)	
Mother education				
Illiterate	706	168 (23.80)	538 (76.20)	0.02
Initial education	249	42 (16.87)	207 (83.13)	
University education	145	24 (16.55)	121 (83.45)	
Total	1100	234 (21.27)	866 (78.73)	
Frequency of bathing				
One time	157	44 (28.03)	113 (71.97)	0.25
Two times	384	72 (18.75)	312 (81.25)	
Three times	559	118 (21.11)	441 (78.89)	
Total	1100	234 (21.27)	866 (78.73)	
Primary schools location				
Rural	296	100 (33.78)	196 (66.22)	0.00
Urban Private	235	32 (13.62)	203 (86.38)	
Urban government	569	102 (17.93)	467 (82.07)	
Total	1100	234 (21.27)	866 (78.73)	

Prevalence of *P. humanus capitis* infestation among children in relation to number of family

In relation to the size of the family (Figure 1), the highest rate was 60% (3/5) recorded in children involved in the family containing 12 persons, and the lowest rate was 11.49% (24/201) recorded in children with four persons in the family. Statistically, significant relation (P-value = 0.00) was observed among family size with *P. humanus capitis* infestation.

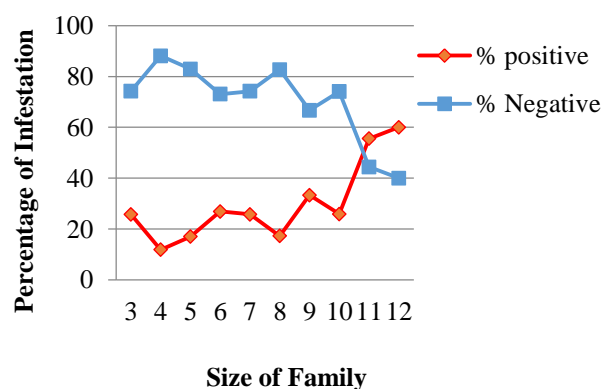


Figure 1. Prevalence of *P. humanus capitis* infestation among children in relation to size of family

Genetic Diversity Evaluations

The results of gel electrophoresis for the COX1 gene showed a 373 bp fragment for all samples (Figure 2). The sequencing of this fragment for the four clade A, clade B, clade D, and clade E are shown in Figure 3. Because clade C was not present in the samples, no sequencing was performed.



Figure 2. Agarose gel electrophoresis of PCR reaction for COX1 gene

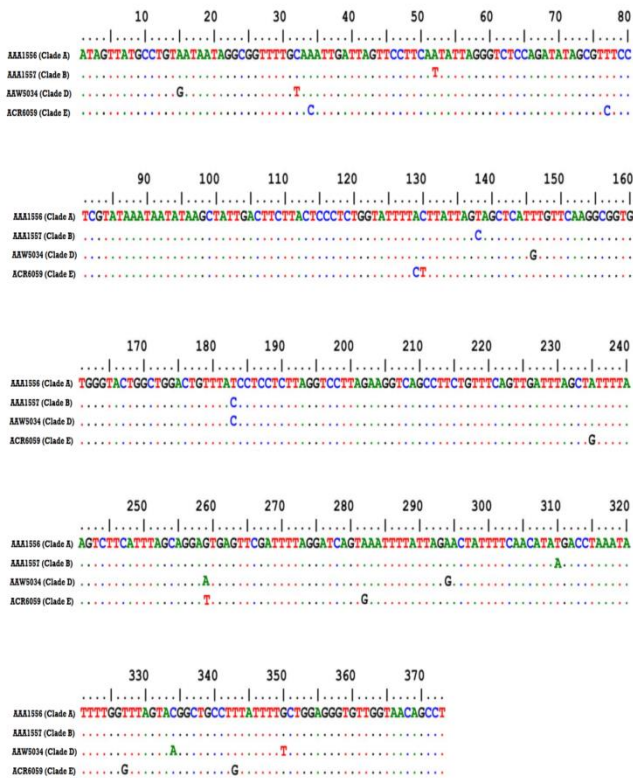


Figure 3. Alignment of the nucleotide sequences of COX1 gene in *Pediculus humanus capitis* among primary school children

According to the results of COX1 gene sequencing, among 234 infested children to lice, 86 (36.75%) of them were exposed to clade A, 38 (16.24%) were exposed to clade B, clade C has been seen among none of them (0%), 105 students (44.87%) were exposed to clade D, and 5 of them exposed to clade E (2.14%) (Figure 4).

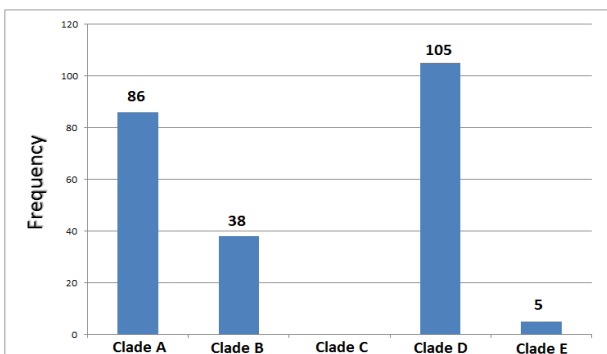


Figure 4. Distribution of clades A, B, C, D, and E head lice in Primary School Children in Erbil province, North of Iraq; Clade C lice was not detected among these school children

This study was conducted to elucidate the rate of pediculosis prevalence among school children in the

Erbil Governorate and the impact of some potential risk factors on such incidence.

The overall prevalence of pediculosis among school children in this study was 21.27% (Table 1). This result was very close to another study done in Erbil, which detected 21.25% (16). In Jordan, was 26.6% (17). But higher than those reported in Baghdad 13.5% (18), in Kalar 14.43% (19), in France 3.3% among schoolchildren (20). The cause of this variation in the prevalence of head lice infestation may be due to several factors, including the number of head-to-head contacts, diagnostic techniques, eradication methods, pesticide resistance, and knowledge of the head lice and perception of pediculosis as a health problem. Many factors also contribute to an increase in head lice prevalence, such as poor hygiene and socioeconomic status, lack of medical treatment, and resistance of the parasite to the treatment (21, 22).

Our result shows a significant difference (P-value = 0.00) of infestation concerning sex, which appeared in females 34.93% (190/544) higher than males 7.91% (45/556) (Table 1). So this result was in agreement with a study by Tappeh *et al.* (23), while in disagreement with Kassiri and Esteghali results (24). The higher infestation rate among females can be explained by the difference in hair length, which has shown to be significantly associated with an infestation, longer hair increases the likelihood of infestation, girls usually have longer hair than boys and also have different behaviors for boys. Boys have brief contact during daily play and sports activities. In contrast, girls tend to have closer, prolonged head contact in small groups (25).

We found that the highest infestation rate in the age group (8-9 years) was 26.87% (90/335) (Table 1), and the lowest rate was 13% (13/100) recorded among children 12 years of age and above. Statistical non-significant difference (P-value= 0.1) in relation to *P. humanus capitis* infestation was observed between age groups. Our result was in agreement with Khidhir *et al.* (26) and Kalari *et al.* (27), while in disagreement with Obaid results (28). The most infested age group (8-9 years) was also the most abundant participating school-aged children. This is a crucial period during which changes in behavior could occur quickly.

Before this time, children in school are usually nurtured by their mothers or older siblings. They'll be gradually left to become independent after it. These and other causes could partly explain why this age group is vulnerable and, in various studies, mostly found to be infested with head lice (29).

Infestation ratio of *P. humanus capitis* concerning the hair length in which it reached in overall 36.52% (130/356) in children with tall hair while 13.98% (104/744) in children with short hair as shown in (Table 1). About *P. humanus capitis* infestation, a statistically significant difference (P-value = 0.00) was observed between short and tall hair. Our outcome was consistent with Omidi *et al.* (30) results. The high rate of infestation of *P. humanus capitis* among children with tall hair is harder to comb and keep clean than short hair (31).

The highest infestation rate among children with curly hair was 31.54% (41/130), as illustrated in (Table 1), and the lowest infestation rate among children with straight hair was 19.90% (193/970). A statistically significant difference was observed among children by type of hair (P-value = 0.002). Our result was consistent with results of Khidhir *et al.* (26), while in disagreement with Kassiri and Esteghali results (24). Generally, those with curly hair have been heavily infested, which could be related to the lack of consciousness. Curly hair is harder to comb and keep clean when compared to straight hair (31).

The prevalence of *P. humanus capitis* among children with hair color (Table 1) shows that the highest infestation rate was 25.90% (36/139) among children with yellow hairs. In contrast, the lowest rate was 20.10% (126/627) among children with black hair affected by *P. humanus capitis*. Statistically, there was no significant difference (P-value = 0.3) in pediculosis to hair color. This difference can be because the egg's color is usually white to yellow, which can be difficult to detect on yellow hair. Our result was disagreed with the results of Gharsan *et al.* (32).

The infestation was more common in children with low-educated parents, 23.80 % (168/706), while less common was 16.55% (25/145) in students with high-educated parents, as shown in (Table 1). Statistically, according to maternal education, a significant difference (P-value = 0.02) was observed between

infested children. This finding was in agreement with earlier study results (33). Because of their awareness and social communication, educated mothers have more information about head lice infestation and its prevention (33).

Prevalence of *P. humanus capitis* infestation by bathing frequency (Table 1) shows that the highest infestation rate was 28.03% (44/157) in children who bathed once a week, and the lowest infestation rate was 18.75% (72/384) in children who bathed twice a week. Despite this, there was no significant difference between Pediculosis (P-value = 0.25) in relation to the bathing frequency in children. Our result was in agreement with results of Mohammed (17). At the same time, it was in disagreeing with Rashmi *et al.* findings (34). Better personal hygiene is defined as an important component for Pediculosis prevention (17).

Prevalence of *P. humanus capitis* infestation concerning residence (Table 1) shows the highest infestation rate in a rural state school in kawrgosk and daratoo 33.78% (100/296) and the lowest rate among private schools in the urban state in Erbil city 13.62% (32/235). A statistically significant difference (P-value = 0.00) was recorded among children in different primary school locations. Factors that may explain the higher prevalence in rural areas could be attributed to poor personal hygiene among family members due to scarcity of water resources and semi-nomadic lifestyle, relative difficulty accessing treatment health services, and lack of basic hair care knowledge. Some researchers maintain that lice infestation occurs in all classes of socioeconomic (34). Others have reported that infestation of lice among lesser socioeconomic classes is more common in rural areas (26). Extreme poverty and overcrowded dwellings were reported in the literature to be closely linked to poor hygiene practices and less concern for head lice infestation (32).

Finally, the relationship of *P. humanus Capitis* infestation prevalence about the family size (Figure 1) shows that the highest rate of infestation 60% (3/5) recorded in a family with 12 children. In comparison, the lowest infestation rate was 11.49% (24/201) recorded in a family with four children. We observed a significant difference (P-value = 0.00) between pediculosis in relation to the family's size; our result

was in agreement with Moosazadeh *et al.* findings (35). This difference seems that if the size of the household population is high, parents will be less able to address their children's health status. As a result, crowded households are expected to have close contact between individuals, and therefore there will be higher prevalence rates. Or they may sleep together in the same bed in large family size and wear the same blanket or the same clothes and comb (31).

To consider more about *P. humanus Capitis*, evaluating genetic diversity could be helpful. Different researchers showed that Cox1 DNA sequencing, as a mitochondrial gene, could reveal the genetic diversity of head lice (36). The results of our study showed that the most common type of lice among Iraqi students is clade D (44.87%). After that, clade A (36.75%) was in the second place. The frequencies of clade B and clade E were 16.24% and 2.14%, respectively. No clade C was observed among any of the students which is inferred that clade C is probably non-existent in the Iraqi student community.

Conclusion

According to the results of the current study, the prevalence of the infestation with *P. humanus capitis* was high among children, and there was a significant difference in *P. humanus capitis* infestation with gender, mother education, length of hair, type of hair, and residency. The result of genetic diversity showed that the most common type of lice among Iraqi students is clade D (44.87%). Then clade A (36.75%) was in the second place. The frequencies of clade B and clade E were 16.24% and 2.14%, respectively. No clade C was observed among any of the students. It showed that clade C is probably non-existent in the Iraqi student community.

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