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Original Article

Prevalence of *Pediculus capitis* in schoolchildren in Battambang, Cambodia

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Abstract *Background/Purpose:* *Pediculus capitis* is the most common human ectoparasite. When it feeds on the blood through the scalp of its host, the anticoagulant in its saliva causes scalp inflammation and itching, and consequent scratching by the host causes further inflammation from bacterial infection. *P. capitis* infestation is currently a common parasitic dermatosis and a critical public health concern in underdeveloped countries.
Methods: Through naked eye inspection of *P. capitis* on or in the hair from 323 school children in Cambodia.
Results: A total of 143 children (44.3%) were found to have *P. capitis* infestation. Univariate analysis revealed that girls had a significantly higher infection rate than boys. Overall, young aged schoolchildren (10 yrs old $\leq$) showed significantly higher infection rate than old aged schoolchildren ($>$10 yrs old). Groups stratified by time revealed that schoolchildren studied at the afternoon classes than morning classes in Tuol Prum Muoy Primary School had a significantly higher risk in acquisition of *P. capitis* infestation. Multivariate analysis results indicated that relative to the boys, the girls were at a significantly higher risk of contracting *P. capitis* infection. When stratified by inspection time with the Tuol Prum Muoy Primary School morning classes as the reference, the Tuol Prum Muoy Primary School afternoon classes exhibited a significantly higher risk of *P. capitis* infection.

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Conclusion: Primary school children in Cambodia have a high *P. capitis* infection rate and thus require effective treatment and prevention measures to treat symptoms and lower the infection rate.

Introduction

Pediculus capitis is a type of hematophagous louse that has existed for more than 10,000 years, and is the most common human ectoparasite.[1] It has been a parasite on human bodies for thousands of years, with the oldest *P. capitis* specimen being found on an 8000-year-old human hair at an archaeological site in a Northeastern Brazil.[2] *P. capitis* generally lives on human hair and the scalp and is common among children. It can live on the human body for up to 30 days but cannot survive for more than 48 h after leaving its human host.[3] A single person can be infected with thousands of *P. capitis*, whereby each louse feeds up to five times daily,[2] consuming <10 μL of blood per feed. Head lice (*P. capitis*) infestation is currently a common parasitic dermatosis[4] and a crucial public health concern, especially in underdeveloped countries.[5] Studies have shown that when *P. capitis* feeds on blood from the scalp of its host, the anticoagulant in its saliva causes scalp inflammation and itching, and scratching by the host can lead to further inflammation due to secondary bacterial infection.[6] *P. capitis* can also cause cervical lymphadenopathy and conjunctivitis,[7,8] and repeated infection can lead to allergic reactions such as nasal obstruction, rhinorrhea, and nightly whistles.[9] Furthermore, *P. capitis* can carry bacteria such as *Rickettsia prowazekii*, *Bartonella quintana*, *Acinetobacter baumannii*, *Borrelia recurrentis*, and *Yersinia pestis* which can be life-threatening to the host.[5,10]

Studies conducted on the mitochondrial DNA of *Pediculus humanus* have identified five distinct mitochondrial clades that can be traced back to 2 million years ago.[11] The specific geographical distribution of each clade has been identified as follows: Clade A includes *P. capitis* and *P. humanus* (body lice) and are distributed globally[12]; Clade B constitutes only *P. capitis* and is distributed in the Americas, Europe, Australia,[13] North and South Africa,[14] and Middle Eastern region[11]; Clade C constitutes only *P. capitis* and is limited to Nepal, Thailand, Ethiopia, Senegal and Mali[14,15]; Clade D includes *P. capitis* and *P. humanus* (body lice) and is distributed in Ethiopia and Democratic Republic of the Congo[10,11,14]; Clade E constitutes only *P. capitis* and is distributed in Senegal and Mali.[11]

The Kingdom of Cambodia is one of the countries with the most unfavorable sanitary conditions in the South East Asian region and has a high malnutrition rate. In addition, less than one-third of the population has access to suitable health facilities. Rainwater serves as the main water source for most families.[16] Because of the scarcity of clean water, personal hair hygiene is difficult to maintain, especially in the case of school children aged 5–11 years.[17] A review of the literature revealed few studies on the prevalence of *P. capitis* among Cambodian school children. Therefore, the present study was aimed at investigating the conditions of the 323 students with *P. capitis* infection from two primary schools, Tuol Prum Muoy and Poupir Primary Schools in Moung Russey District, Battambang Province, Northwestern Cambodia. As the first study on the rate of *P. capitis* infection among primary school children in Cambodia, the findings of this study may assist in clarifying the current status of *P. capitis* infection among school children in the country.

Methods

Ethical considerations

This study was approved by the Battambang Provincial Health Department. All participating school children were informed of the research objective, and their parents or legal guardians signed letters of informed consent prior to the study. The final results were provided in written information to the nursing staff at the Kokoh Health Center, who were requested to treat the infected children following the standard protocol for treating lice in accordance with Cambodian Health Department guidelines.

Study setting, sampling and population

The sampling location of this study was Moung Russey District, which is in the southeast of Battambang Province, North Western Cambodia (12°42′56″N, 103°46′30″E) (Fig. 1). It is the second largest city in the country, with a total area of 11,545 km^2 and a population of 147,349. Annually, the city has a mean annual high and low temperature of 32.3 °C and 22.7 °C, respectively, and 1368 mm of precipitation. Agriculture is the main source of economic activity in this region. Health care institutions in the district include the Moung Hospital and several health centers, although not all health centers are located near primary schools. Regarding the two primary schools investigated in this study, Tuol Prum Muoy Primary School is near the Kokoh Health Center (15 Km), whereas Poupir Primary School is near Moung Hospital (10 Km). This study was conducted in January–February 2016, which is the dry season. Because no preliminary research was conducted, the general formula $n = z^2 p\ (1 - p)/d^2$ was used to determine the sample size,[18] whereby n represented the sample size, z (1.96) is the standard deviation of the 95% confidence interval (CI), p is the estimated prevalence rate (30%), and d is the relative error permitted (0.05). The minimum sample size was found to be 322.

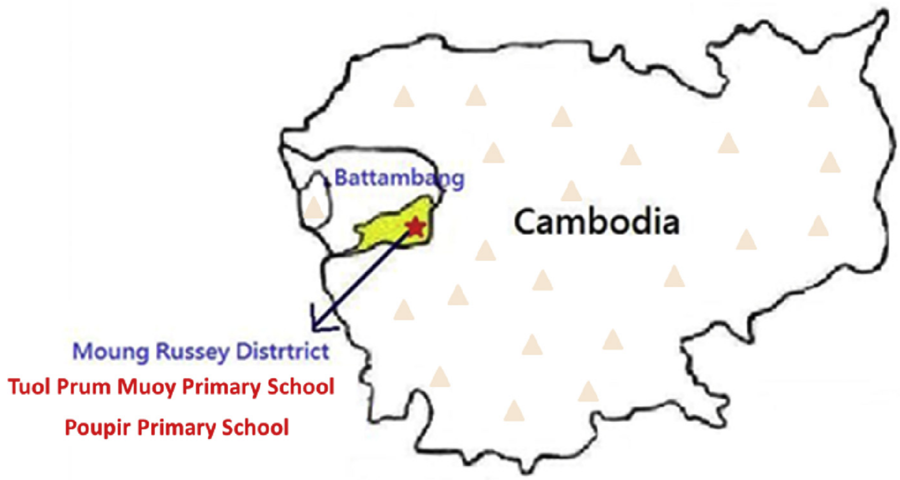


Figure 1. The Moung Russey district of Battambang province, northwestern Cambodia.

Field procedures

On the first day, the participants received detailed health education concerning *P. capitis*, which also emphasized the importance of prevention and treatment. The children were also taught other important basic health care knowledge, after which they were given letters of informed consent for their parents or legal guardians; the letters also explained the purpose of the *P. capitis* examination. The children underwent *P. capitis* examinations after the letters were signed and returned. The principal method for examining and identifying *P. capitis* was observing behind the children's left and right ears, the scalp near the neck, or the hair 1 cm above the scalp. The children were confirmed as having *P. capitis* infection if adults, nymphs, or eggs were discovered (Fig. 2).

Statistical analyses

A total of 323 Cambodian elementary school children were recruited for this study, which was aimed at stratifying the risk factors of the *P. capitis* infection rate by sex and age. The risk of *P. capitis* infection for sex, age, and different student groups was investigated using a univariate logistic regression model to calculate the odds ratios (ORs). Multivariate logistic regression was then employed to calculate the adjusted ORs for these three risk factors. SAS version 9.3 (SAS Institute, Cary, NC) was employed to conduct the statistical analysis. Results with $p < 0.05$ were considered statistically significant.

Finally, to establish a strategy for large-scale *P. capitis* screening and control, this preliminary study was conducted to construct a prediction model of *P. capitis* infection on the basis of sex and age. The predictive ability of the model was then determined using the area under the curve (AUC) of the receiver operating characteristic (ROC) to serve as a basis for future *P. capitis* screening and for questionnaire design.

Figure 2. Heavily infected hair with *Pediculus humanus capitis* (white arrow).

Results

Descriptive analysis

Because dry season and rainy season is clear in Cambodia as resulting in insufficient water source, most children take a bath and hair once per 2–3 days. Thus, it appears a big problematic issue for hair cleanness; particularly of female schoolchildren are uneasy to keep hair clean due to their long hair (personal observation).

A total of 323 school children from two primary schools were examined: 284 from Tuol Prum Muoy Primary School (87.9%) and 39 from Poupir Primary School (12.1%). Among them, 148 were boys (46.0%) and the average age was 11.0 ± 2.0 years. The children were divided into three groups according to the examination time: Tuol Prum Muoy morning classes ($n = 164$, 50.8%), Tuol Prum Muoy afternoon classes ($n = 120$, 37.2%), and Poupir Primary School ($n = 39$, 12.1%). A total of 143 school children (44.3%) were found to be infected with *P. capitis* (Table 1). When the students were stratified by age, older students (>10 yrs old) displayed a downward trend in the rate of *P. capitis* infection (Fig. 3). Stratification by sex revealed that the girls exhibited significantly higher infection rates in most age groups (total infection rate: 56.9% vs. 29.9%) (Fig. 4).

Table 1 Descriptive analysis (N = 323).

Characteristics	Missing value	n	%
Gender	1		
Male		148	46.0
Female		174	54.0
Age	1	322	11.0±2.0[a]
School			
Tuol Prum Muoy		284	87.9
Poupir		39	12.1
Examination time (by school)			
Tuol Prum Muoy- morning		164	50.8
Tuol Prum Mccoy- afternoon		120	37.2
Poupir		39	12.1
Infection of head lice (by school)			
Tuol Prum Muoy- morning		54	32.9
Tuol Prum Mccoy- afternoon		76	63.3
Poupir		13	33.3

[a] Mean ± SD.

Univariate analysis

Next, sex, age, and student group were subjected to univariate logistic regression, which revealed that the rate of *P. capitis* infection was significantly higher among girls (OR = 3.12, 95% CI = 1.964–4.957, $p < 0.0001$). Age was found to be a protective factor against *P. capitis* infection (OR = 0.843, 95% CI = 0.753–0.944, $p = 0.0030$), as evidenced by the rate of infection being lower among the older students. When students from the Tuol Prum Muoy morning classes were used as the reference group, those from the afternoon classes exhibited a higher risk of *P. capitis* infection (OR = 3.518, 95% CI = 2.147–5.765, $p < 0.0001$), although the overall difference compared with students from Poupir Primary School was nonsignificant (OR = 1.019, 95% CI = 0.485–2.137, $p = 0.0919$) (Table 2).

Multivariate analysis

After, sex, age, and student group were adjusted for with the multivariate logistic regression model, higher rates of *P. capitis* infection were observed in the girls (OR = 4.208, 95% CI = 2.49–7.112, $p < 0.0001$) and the students from Tuol Prum Muoy afternoon classes (OR = 4.275, 95% CI = 2.119–8.623, $p < 0.0001$). However, the relationship between age and *P. capitis* infection became nonsignificant (OR = 0.934, 95% CI = 0.79–1.105, $p = 0.4275$). In-depth statistical analysis revealed an interaction effect between age and student group, which resulted from the different age distributions among the different student groups (Table 2).

Receiver operating characteristic curve (ROC curve)

The ROC curve was plotted using sensitivity and 1-specificity and employed to compare the predictive ability of age and sex for *P. capitis* infection. The univariate results demonstrated that sex and age yielded AUCs of 0.6355 and 0.6100, respectively, indicating poor prediction ability. However, the AUC increased to 0.6982 when sex and age were included in the multivariate model, and the result was significantly higher than those obtained from the univariate model. An AUC of ≥0.7 is generally considered to indicate acceptable predictive ability (Fig. 5)

Discussion

According to the current literature, the mean infection rates for *P. capitis* were 15.1% ± 12.8% in Asia, 13.3% ± 17.0% in Europe, and 44.1% ± 28.0% in South America.[19] In the present study, the *P. capitis* infection rate among the Cambodian primary school children was 44.3% (143/323), which is three times higher than the average infection rates in Asia and Europe and similar to that in

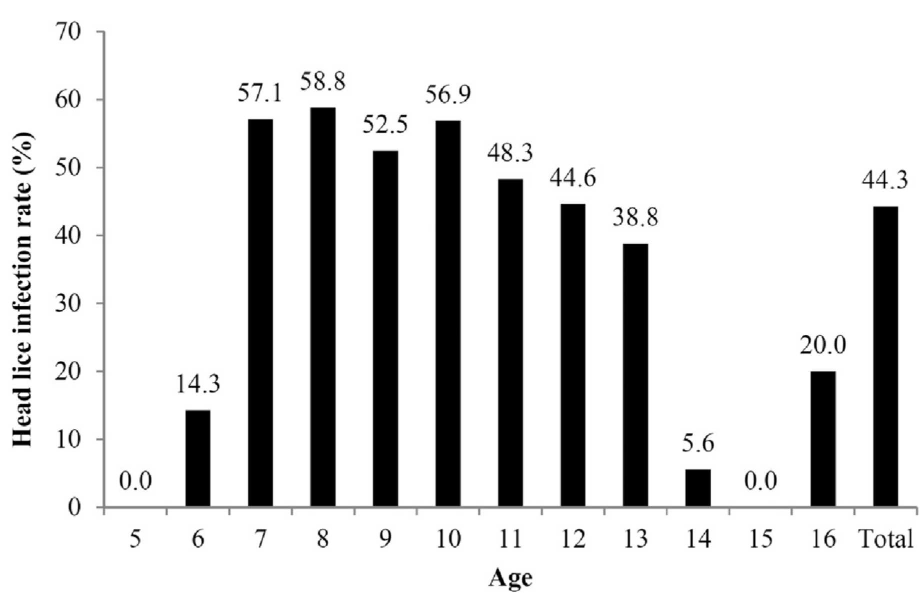


Figure 3. Infection rate of head lice stratified by age.

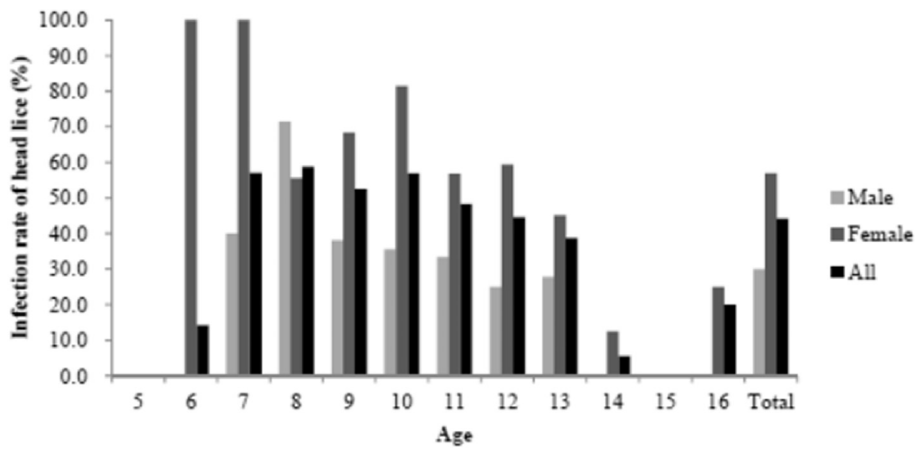


Figure 4. Infection rate of head lice stratified by age and gender (N = 323).

Table 2 Logistic regression, Univariate and multivariate analysis (N = 323).

Variable	Head lice infection			
	Crude OR (95% CI)	Crude p	Adjusted OR (95% I)	Adjusted p
Gender				
Male	ref		ref	
Female	3.12 (1.964—4.957)	**<0.0001***	4.208 (2.49—7.112)	**<0.0001***
Age	0.843 (0.753—0.944)	**0.0030***	0.934 (0.79—1.105)	0.4275
Examination time (by school)				
Tuol Prum Muoy- morning	ref		ref	
Tuol Prum Muoy- afternoon	3.518 (2.147—5.765)	**<0.0001***	4.275 (2.119—8.623)	**<0.0001***
Poupir	1.019 (0.485—2.137)	0.0919	1.151 (0.44—3.008)	0.1620

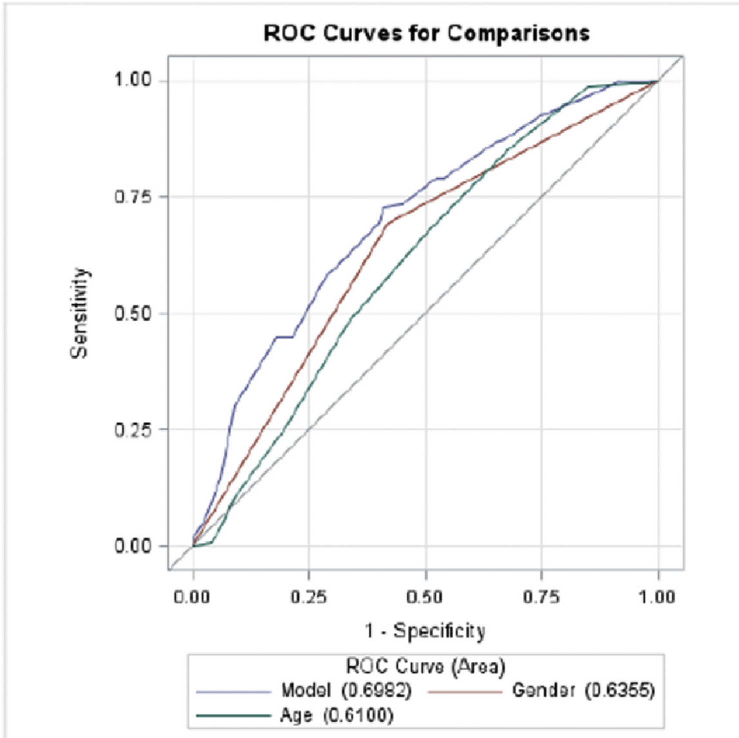
*p value < 0.05.

South America. This indicates that *P. capitis* is another public health concern in Cambodia.

The univariate analysis results show that the infection rate was significantly higher for girls than for boys (56.9% vs. 29.7%, $p < 0.0001$). This result is similar to those reported by Çetinkaya et al. (21.4% vs. 1.4%), Çetinkaya et al. (16.9% vs. 3.4%), and Kokturk et al. (13.3% vs. 1.1%),[17] with girls exhibiting significantly higher infection rates in all three studies. In addition, the age stratification revealed a downward trend in the *P. capitis* infection rate for older students, indicating that younger school children are more likely to overlook hair hygiene. Primary schools at Moung Russey District are facing a severe shortage of classrooms; hence, the division of students into morning and afternoon classes. When the groups were stratified by examination time, the children in the Tuol Prum Muoy afternoon classes (63.3%) exhibited a significantly higher infection rate compared with those in Poupir Primary School (33.3%) and those in the Tuol Prum Muoy Primary School evening classes (63.3% vs. 33.3% and 32.9%, respectively; $p < 0.0001$). The reason why children from the Tuol Prum Muoy afternoon classes had such a high infection rate requires further analysis of the involved risk factors.

The results from the multivariate analysis indicate that relative to the boys, the girls exhibited a significantly higher risk of infection (OR = 4.208, 95% CI = 2.490—7.112, $p < 0.0001$). This result is similar to those reported in previous studies, such as the female: male infection ratio of 12:1 in Turkey and 2:1 in Australia.[4] The reason for the higher infection rate in girls compared with boys is possibly related to regional and cultural factors. Such a discrepancy does not stem from the preference of *P. capitis* for females as hosts; rather, it is caused by periods of physical contact between girls (boys tend to physically contact each other less frequently and for shorter periods, such when fighting or playing sports). Throughout the present study, we also found that the school girls often formed tight groups while chatting and playing. Girls and younger students were generally considered to be at a higher risk of *P. capitis* infection, and their infection control should thus be emphasized. However, regarding the higher risk of *P. capitis* infection among the students from Tuol Prum Mccoy afternoon classes, other environmental or behavioral risk factors should be investigated to determine the cause. Because of the low number of relevant variables collected in this study, it was difficult to control the effects of other possible risk factors, which may have led to potential overestimation of the OR of these risk factors. The effects, however, should not be underestimated; further investigation of potential risk factors is thus warranted.

The ROC curve has been extensively applied in research on epidemiology, medicine, radiation technology, and social sciences.[20] The present study used the ROC curve to compare the predictive ability of sex and age for *P. capitis* infection. The results show that including sex and age in a multivariate model yields an acceptable predictive ability



ROC Model	Area under the ROC curve	Difference of estimation (95%CI)	p
Gender	0.6355	-0.0627(-0.0992 - -0.0263)	0.0007*
Age	0.6100	-0.0882(-0.1504 - -0.026)	0.0054*
Model[1]	0.6982	ref	

*$p < 0.05$. [1]Gender and age were included in the prediction model.

Figure 5. Comparison in the predictive ability between sex and age for *P. capitis* infection using ROC curve.

(AUC = 0.6982). Many rural elementary schools in Cambodia have similar environments, and it is possible that the situation of *P. capitis* infection is similar at other schools. The AUC established in the present study may facilitate predicting *P. capitis* infection in other rural elementary school students, and this may improve *P. capitis* screening and prevention measures. Nevertheless, more factors related to *P. capitis* infection were collected in future models may yield more accurate prediction results for *P. capitis* infection.

Before 2015, the mitochondrial gene analysis of *P. humanus* revealed three Clades (A, B, and C), with *P. capitis* and *Pediculus humanus humanus* belonging to Clade A, and *P. capitis* belonging to Clades B and C. Clade A is distributed globally, Clade B appears to have originated from the United States, and Clade C is currently distributed only in Africa and Southeast Asia; in 2015, a new Clade (Clade D)[10,11] containing *P. capitis* and *P. humanus humanus* was discovered in the in Ethiopia and the Democratic Republic of the Congo, and the *P. capitis* species discovered in the Democratic Republic of the Congo was the first of this clade to be found to carry *Borrelia theileri* and several species of *Acinetobacter* [14]; in addition, a fifth Clade (Clade E)[11] was discovered in Senegal and Mali, although the Clade to which the Cambodian *P. capitis* belongs to requires further research.

A. baumannii was first discovered in *P. humanus humanus* on the homeless in Marseilles (France), and has since been found in *P. humanus humanus* on the homeless from various countries. In addition, *A. baumannii* DNA is also found in the Clade A *P. capitis* in Paris elementary school students, as well as in the *P. capitis* and *P. humanus humanus* species in Ethiopia; in 2015, *A. baumannii* was detected in Clade A and Clade C *P. capitis* in Thai elementary students, although the clinical significance of the series of *A. baumannii* in *P. capitis* remains unclear.[11] Other pathogens such as *B. Quintana* are known to exist in Clades B, C, and D *P. capitis*; *B. recurrentis* is known to exist in Clade C *P. capitis*; and *Y. pestis* is known to exist in Clade A *P. capitis*.[10] Despite numerous studies having noted that *P. capitis* carries some pathogens, there is currently no direct evidence that these pathogens can be transmitted through *P. capitis*. Therefore, we argue that the pathogens discovered in *P. capitis* can be considered to only indirectly assess the risk of louse-borne diseases in humans.

This study employed naked eye observation to inspect school children's left and right ears, the scalp near their neck, and the hair 1 cm above the scalp. Children were determined to be infected if *P. capitis* adults, nymphs, or eggs were discovered. Kurt et al.[21] indicated that the detection rate when using precision detection comb is four times higher than that of naked eye observation; however, in the present study, an infection rate of 44.3% was attained through naked eye observation alone. Many of the children were severely infected (Fig. 2), with clear signs of *P. capitis* eggs on their hair, especially among the girls. This indicated a high prevalence of *P. capitis* among Cambodian school children, which requires immediate attention and a solution from related health care units.

Nevertheless, *P. capitis* does not merely affect the hosts' bodies, it can also cause psychological distress. Infected individuals are usually misunderstood as having inadequate personal hygiene, which leads to negative perceptions from others and subsequent psychological stress.[1] The fastest means for treating *P. capitis* is to shave the hair completely, in which case *P. capitis* will be unable to survive or spread through hair contact. This method might be more acceptable for boys than their female counterparts. Coupled with differences in customs, culture, and religion in different areas, the method might be infeasible in certain regions. Suggested methods for treating *P. capitis* in the literature include using dry or wet combs, pediculicide, or oral medication.[17] It is recommended that two different methods be used separately for 8—10 days, and infected persons should be checked for *P. capitis* again 14 days after treatment.[4] Other methods for controlling *P. capitis* infection suggested in literature include regular use of dry or wet combs, treating infected individuals and those who are close to them simultaneously, and medication that is effective against *P. capitis* adults, nymphs, and eggs. However, these three methods may be difficult to implement in Moung Russey District because of the scarcity of clean water and general goods and supplies. To effectively reduce the prevalence of *P. capitis* among school children in this district might require local health centers to work closely with the research team in order to propose a more systematic means of curing and preventing *P. capitis*.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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