Parasitol Res (2014) 113:3439–3444
DOI 10.1007/s00436-014-4012-8

ORIGINAL PAPER

Influence of the formulations in removing eggs of *Pediculus humanus capitis* (Phthiraptera: Pediculidae)

Isabel Ortega-Insaurralde · Ariel Ceferino Toloza · María Inés Picollo · Claudia Vassena

Received: 2 June 2014 / Accepted: 26 June 2014 / Published online: 19 July 2014

Abstract Head lice lay eggs in human head hairs in order to reproduce. There is a difficulty associated to the process of detaching these eggs: they are tightly gripped to the hair by a secretion produced by female head lice. The physical removal of eggs has become an important part of treatment of louse infestations. The finding of new products to loosen the eggs is necessary to avoid mistaken diagnosis or reinfestations. This work aimed to compare different kinds of pediculicide formulations in order to find if their presentations represented differences in the egg remover effect. We also wanted to present a new device to test the efficacy of the egg remover formulations. Products with creamy presentations (Bio infant lice and egg remover® and hair conditioner) and one containing dimethicone (Nyda®) showed the lower mean forces compared with the control (lower mean forces represented best removal activity). Whereas, the Biferdil egg remover® (gel) and Nopucid Tribit® (hydroalcoholic lotion) had no egg removal effect, presenting the highest mean forces (177.82 and 189.99 mN, respectively) compared with the control. Additionally, we proposed a removal index (RI) to compare the efficacy of different products on the egg removal activity (RI>0, good performance). The higher index values were for Bio infant lice and egg remover® (0.72) and Biferdil hair conditioner® (0.58). The lowest index values were for Biferdil egg remover® (−0.26) and Nopucid Tribit® (−0.35).The formulation of over the counter pediculicides in the egg remover effect was discussed.

I. Ortega-Insaurralde (✉) · A. C. Toloza · M. I. Picollo · C. Vassena
Centro de Investigaciones de Plagas e Insecticidas (CONICET-CITEDEF), Juan Bautista de La Salle 4397, B1603ALO Villa Martelli, Buenos Aires, Argentina
e-mail: iortega@citedef.gob.ar

C. Vassena
Universidad Nacional de San Martín, General San Martín, Buenos Aires, Argentina

Keywords *Pediculus humanus capitis* · Eggs · Removal products · Pediculicide

Introduction

Head lice (*Pediculus humanus capitis* De Geer) are insect parasites, feeding exclusively on the blood from their hosts. Man is the only known host of this parasite. Although any part of the scalp may be colonized, lice favour the nape of the neck and the area behind the ears, where the eggs are usually laid. During its lifespan of 4 weeks, a female louse can lay 50–150 eggs (Mumcuoglu et al. 2009).

The eggs of lice are attached to the hair by a female secretion called cement. The cement is produced by collateral glands situated near the female reproductive organ system (Burkhart and Burkhart 2005). The amount of cement used in attaching an egg varies greatly according to the species, in the case of *P.h. capitis*, the cement surrounds almost the entire egg (except for the operculum) and it also comprehends a cylinder sheath that covers a portion of hair. The egg cylinder also varies its length among eggs (Lapeere et al. 2005).

Recently, it was found that the egg sheath or the cement functions as a tight grip, holding the sheath to the shaft of hair, rather than a sticky glue attachment (Burkhart and Burkhart 2005). Studies on the composition of the nit sheaths indicate that they are of proteinaceous nature, possibly cross-linked to aliphatic components. The protein composition is similar to that of hair keratin, although it is structurally more rigid, possibly because of the large amount of tyrosine and phenylalanine residues, which could allow hardening of the egg sheath (Burkhart et al. 1999 and Burkhart and Burkhart 2005).

There is a sustainable quantity of previous work on in vitro ovicidal assays with a high spectrum of compounds and high efficacy (Abdel-Ghaffar and Semmler 2006 and Abdel-Ghaffar et al. 2011; Mehlhorn et al. 2011; Gallardo et al.

2012); nonetheless, there is little about egg remover compounds.

The physical removal of eggs has become an important part of treatment of louse infestations. In a study performed in schools from Buenos Aires (Argentina), the head lice infestation rate was 29.7 % (Toloza et al. 2009).The presence of eggs alone is not an accurate indicator of an active head louse infestation. Examination of over 16,000 children revealed that 11–19 % of them were infested with living lice and eggs, while another 22–30 % had dead eggs only (Mumcuoglu et al. 1990). When it comes to live eggs, their presence represents a new generation of lice ready to recolonize the head. Thus, finding new products to loosen the eggs is necessary to avoid mistaken diagnosis or reinfestations. However, there are a lot of over-the-counter (OTC) products with no egg remover effect due to the use of the incorrect active ingredients based on false theoretical basis. For instance, there are egg removers using enzymes which are supposed to attack the chitin in the egg sheath, ignoring that there is no chitin in its composition (Burgess 2010).

There is one previous work that described a method to measure egg removal parameters (Lapeere et al. 2005). In few words, a hair with an egg was caused to move by an electrical motor. They measured the forces during the procedure of hair sliding through the egg cylinder with a force transducer. Burgess (2010) and Lapeere et al. (2014) tested the efficacy of egg removal formulations using similar methodology. These authors found that several OTC products were not effective in vitro.

This work aims to compare different pediculicide formulations in the egg remover effect. More specifically, the goals were to study the efficacy to remove eggs of three commercial Argentinean products (Biferdil egg remover®, Bio infant lice and egg remover® and Nopucid Tribit®) in comparison to Nyda®. We also used traditional products like hair conditioner and vinegar. In addition, we developed a simpler device to test the efficacy of egg removers.

Materials and methods

Biological samples Hairs with head louse eggs (*P.h. capitis*) were collected from infested children of 6 to 12 years old (Fig. 1). These children regularly attend elementary schools in Buenos Aires where health educational programmes for head lice infestation are performed. Only pupils whose parents had given informed consent for participation were examined. The protocol of hair and egg removal is described in Gallardo et al. 2013. Briefly, the hair was divided in bilateral halves, and each half was combed ten times. After the hairs were detached gently from every kid scalp by using a metal comb, they were stored in our laboratory. The protocol for egg collection was approved by the ad hoc committee of the Centro de Investigaciones de Plagas e Insecticidas (Research Center of Pests and Insecticides), Buenos Aires, Argentina.

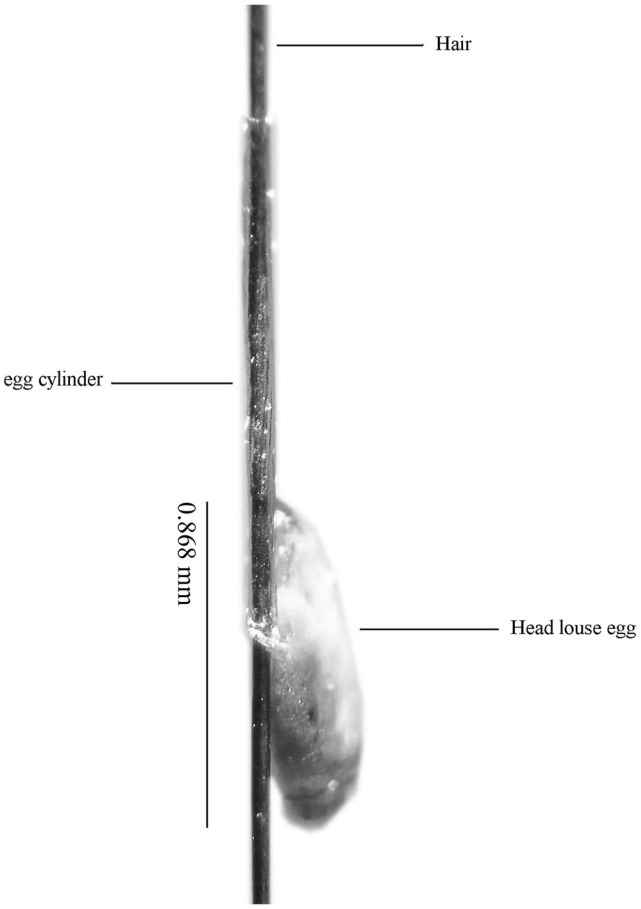

Fig. 1 Microphotograph of an egg attached to a hair. Detail of egg cylinder attached to the hair observed by stereoscopic microscope (×63)

Commercial products Details of the OTC products tested are shown in Table 1. We assessed the efficacy of three available commercial products in Argentina, (1) Biferdil egg remover with nanospheres® developed by Biferdil (www.biferdil.com/), Buenos Aires, Argentina. (2) Bio infant lice and egg remover® by Laboratorios Bioesencia (www.bioesencia.com/), Buenos Aires, Argentina. (3) Nopucid Tribit® by ELEA, Buenos Aires, Argentina. In addition, we tested the efficacy of a dimethicone-based lotion called Nyda® presented by Casen Fleet laboratories purchased in Sitges, Spain. Besides, we used vinegar (7 % of acetic acid, Menoyo®, Buenos Aires, Argentina) because of its well-known use in treatments at home and a Biferdil common hair conditioner® (Biferdil, Buenos Aires, Argentina).

Measurement of egg removal force The method here presented was developed as a simpler alternative to the methods reported by Lapeere et al. (2005) and Burgess (2010). The device consisted of a weighting scale that allowed us to

Table 1 Tested products for their egg removal activity

Trade name	Producer	Presentation	Active compound
Bio infant®	Laboratorios Bioesencia, Argentina	Cream	Hydrolysed bitter wood (*Quassia amara* L.), hydrolysed cedar (*Juniperus virginiana* L.) and hydrolysed common rue (*Ruta graveolens* L.)
Biferdil egg remover®	Biferdil, Argentina	Gel	Vegetal-derived enzymes with nanospheres
Nopucid Tribit®	Laboratorio ELEA, Argentina	Lotion	Ciclopentaxiloxane (33 %)
Nyda®	G. Pohl-Boskamp GmbH &Co., Germany	Lotion	Dimethicone (92 %)

measure the weight in grams required to cause eggs to slide along hair shafts. The set-up included a small metal plate (35 mm) with a 0.1-mm diameter hole through which a single hair could be beaded; an aluminium container; several little scales between 50 and 2,500 mg; a stand and a holder (Fig. 2).The force values in milliNewton (mN) were obtained by multiplying the data (weights) with the acceleration of gravity (9.8 m/s^2).

The test consisted in the immersion of every hair in 5 ml of each product allowing that the egg was totally immersed. Once the exposure period finished, the hair (without post-wash) was beaded through the small metal hole plate in the measurement device. We used dry hairs as controls.

The scalp end of the hair with a single attached egg remained above the metal plate. The distal end of the hair that passed through the hole was stuck to the container by silicon (Suprabond®). Increased weights were added to the container until the applied force exceeded the inertial grip of the cement on the hair and the egg started to slide. The force required to initiate the hair displacement was recorded for each treatment. Eggs were observed under a stereoscopic microscope (Olympus S261) after the measurement. No egg was damaged during treatment. Force measurements on untreated hairs, wet hairs and hair treated with vinegar were compared with measurements on hairs treated with the commercial products. A minimum of 20 eggs on hairs per treatment was evaluated.

Statistical analysis Data analysis was performed using Infostat software (www.infostat.com.ar/). The removal index (RI) was calculated according to $RI=[-(N_t-N_c)/N_c]\times 100$, where N_c is the percentage of removed eggs of the control, and N_t is the percentage of removed eggs of the treatment. The

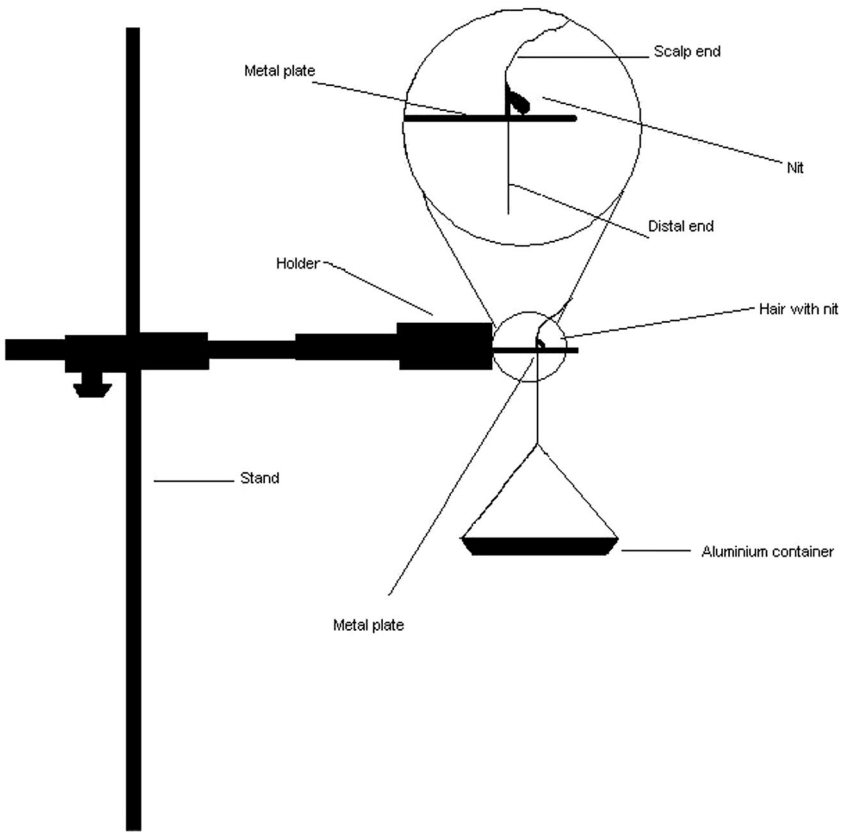

Fig. 2 Measurement device. Simple and feasible device to measure the force required to initiate hair displacement

treatment means were analysed by ANOVA. They were transformed with log10 and then were separated using the Duncan test. The threshold for statistical significance was at $p<0.05$. The RI showed those treatments that are more likely to remove eggs. RI values over zero showed efficacy in the egg removal effect.

Results

Mean egg removal forces and their respective standard errors are shown in Table 2. The experimental error (due to the measurement method) was of 0.49 mN. The differences among treatments are also shown in Table 2 due to the Duncan test ($p<0.05$). The most effective product was the Bio infant lice and egg remover® with a value of 39.68 mN. The next lower mean force was the one for the hair conditioner (59.15 mN) followed by vinegar (76 mN) and Nyda® (79.1 mN). The Biferdil egg remover® (gel) and Nopucid Tribit® (hydroalcoholic lotion) showed higher mean forces (177.82 mN and 189.99 mN, respectively) than the control. The removal index revealed that almost all the treatments, including wet hair treatment, were effective in egg removal effect (Fig. 3). The higher index values were for Bio infant lice and egg remover® (0.72) and Biferdil hair conditioner® (0.58). The lowest index values were for Biferdil egg remover® (−0.26) and Nopucid Tribit® (−0.35).

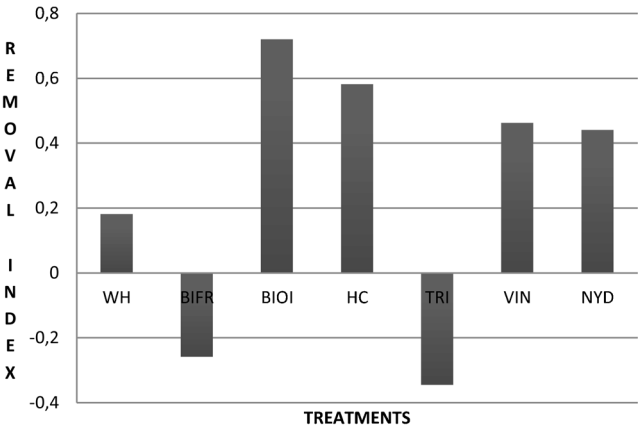

Fig. 3 Removal index (RI). RI>0=removal activity; RI<0=no removal activity. The Bio infant remover® (BIOI) and Biferdil hair conditioner® (HC) reveals the best performance in removing the eggs. *WH* wet hair, *BIFR* Biferdil egg remover®, *TRI* Nopucid Tribit®, *VIN* vinegar, *NYD* Nyda®

Discussion

In order to ensure more efficient control of louse infestations on a community level, health authorities in several countries suggested the "no egg" policy, i.e. the immediate dismissal of a child from a school, camp or child-care setting until all head lice, eggs have been removed, before the infested individual could be readmitted to the institution (Mumcuoglu et al. 2006). The presence of successful OTC pediculicides with ovicidal effect does not guarantee the accomplishment of this policy if no egg removal effect is obtained by their application. Therefore, the detachment of dead eggs is, nowadays, an important component in itself of the head louse control programmes. Health authorities should provide updated basic head lice information. Checking frequency, thoroughness and open communication should be emphasized (Rukke et al. 2014).

The strength of the head louse cement represents a real challenge for parents and health personnel who want to get rid of eggs. The presence of live eggs closely to the scalp is a highly probable evidence of active infestation that might produce reinfestation; however, the presence of dead eggs often causes unnecessary treatments. Despite the case, the importance of removal products is decisive.

Table 2 Mean forces required to remove eggs after different treatments. Each hair with a single egg attached was immersed for 2 min in 5 ml of the tested product. The measured forces were estimated as the weights (in grams multiplied by the acceleration of gravity) applied to the scalp end of the hair when the egg started to slide

Hair treatment	Mean force (mN)	SEM	Type of formulation	Multiple comparison[a]
Bio infant remover®	39.68	5.45	Cream	A
Biferdil hair conditioner®	59.15	6.58	Cream	B
Vinegar	76.01	6.92	Hydro alcoholic solution	C
Nyda®	79.11	7.58	Lotion	C
Wet hair	115.72	8.04	None	D
Dry hair	141.23	12.59	None	D, E
Biferdil egg remover®	177.82	9.97	Gel	E
Nopucid Tribit®	189.99	14.79	Lotion	F

SEM standard error of the mean, *mN* milliNewton

[a] Means in same column followed by different letters are significantly different by Duncan test ($p<0.05$)

Here, we wanted to show the situation of this issue in Argentina. We tested three types of formulations: a gel, a cream and a hydro alcoholic lotion. Besides, we used vinegar, hair conditioner and Nyda® (an international pediculicide based on silicones). Our hypothesis was that the removal activity to a large degree depends on the type of formulation rather than the active ingredient itself. The products of creamy formulations would be the ones with better removing effect. Our removal index has shown that creamy products had the best performance in loosen the eggs (Bio infant lice and egg remover® (RI=0.72) and Biferdil hair conditioner® (RI= 0.58). It is similar to what was found by Lapeere et al. (2014), the tested hair conditioner showed high removing activity of eggs. This could be explained by the fact that lubricant compounds can penetrate the pores between the cement and the hair, helping to loosen the egg cylinder during combing or in a stricter way the conditioner coats the hair by Van der Waals attraction, making it moist and soft (Lapeere et al. 2014). There is not a real chemical reaction acting on a certain component of the cement, only lubricating effect. In addition, lubricating materials may also show relatively low surface tension, making surfaces smoother (Burgess 2010).

In second place, the vinegar and Nyda also had removal effect. The vinegar is a solution of acetic acid 7 %. Here, it showed a significant removal activity in contrast to Burgess' results that showed that soaking hairs in vinegar was no more effective to facilitate egg removal than wet hair (Burgess 2010). Moreover, Mumcuoglu (1999) reported that 5 % acetic acid is effective in detaching eggs from the hair. Nyda® beholds 92 % of dimethicone. The dimethicone is a widely used silicone with emollient properties thus it is not surprising its good performance. Lapeere et al. (2014) reported a formulation containing cyclomethicone (another silicone) with egg removal activity in vitro. Meanwhile, Nopucid Tribit® (lotion) and Biferdil egg remover® (gel) showed no performance as egg removal agents.

One of our objectives was to present a simple, homemade, affordable dispositive to measure the necessary force to make the eggs slide. It resumes the benefits of the measure device shown in Lapeere et al. (2005). The force values were higher than those founded by Lapeere et al. (2005) and Burgess (2010) and because our method shows less sensibility due to its characteristics (the perception of the moment when the egg becomes detached from the hair relies on the eye of the researcher, the measurement device error, etc.) but it is still reliable since it was possible to differentiate the removal effect of each tested product.

Lapeere et al. (2014) used the method described in Lapeere et al. (2005) to test the effect of deionized water, ordinary conditioner and a couple of egg removal products. They take into account the maximum force and the average force, while we recorded the necessary force to release the egg and a different method to record this. We share with them the fact that using hairs from different persons introduced certain variability to the tests and also that performing the assays over one hair did not portray the reality of an average egg remover treatment and combing which involves more hairs and their eggs pulling the comb all at once. Additionally, we proposed a removal index that represents a feasible and acute statistical indicator to compare the effectiveness of different products on the egg removal activity.

The value of our work is to make awareness of the vehicles of over-the-counter products in the removal activity. Before promoting products of this nature, their efficacy should be proven by in vitro tests before clinical studies.

Acknowledgments The authors wish to thank all the authorities of the elementary schools where hairs with nits were collected. This investigation received financial support from Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina) and ELEA® laboratory (Argentina). We also want to thank Monica Germano for helping with statistical analysis and Gonzalo Roca Acevedo for helping with the figures.

Conflict of interest The authors declare no competing financial interests with any of the evaluated products.

Ethical standards The experiments in this work comply with the current laws of Argentina.

References

Abdel-Ghaffar F, Semmler M (2006) Efficacy of neem seed extract shampoo on head lice of naturally infected humans in Egypt. Parasitol Res 100:329–332. doi:10.1007/s00436-006-0264-2

Abdel-Ghaffar F, Al-Quraishy S, Al-Rasheid K, Mehlhorn H (2011) Efficacy of a single treatment of head lice with a neem seed extract: an in vivo and in vitro study on nits and motile stages. Parasitol Res. doi:10.1007/s00436-011-2484-3

Burgess I (2010) Do nit removal formulations and other treatments loosen head louse eggs and nits from hairs? Med Vet Entomol 24:55–61. doi:10.1111/j.1365-2915.2009.00845.x

Burkhart CN, Burkhart CG (2005) Head lice: Scientific assessment of the nit sheath with clinical ramifications and therapeutic options. J Am Acad Dermatol 53:129–33. doi:10.1016/j.jaad.2005.01.134

Burkhart CN, Burkhart CG, Gunning WT, Arbogast J (1999) Scanning electron microscopy of the egg of the human head louse nit (Anoplura: *Pediculus humanus capitis*) with clinical ramifications. J Med Entomol 36:530–2. doi:10.1016/j.jaad.2005.01.134

Gallardo A, Mougabure-Cueto G, Vassena C, Picollo MI, Toloza AC (2012) Comparative efficacy of new comercial pediculicides against adults and eggs of *Pediculus humanus capitis* (head lice). Parasitol Res 110:1601–1606. doi:10.1007/s00436-011-2668-x

Gallardo A, Toloza A, Vassena C, Picollo MI, Mougabure-Cueto G (2013) Comparative efficacy of commercial combs in removing head lice (*Pediculus humanus capitis*) (Phthiraptera: Pediculidae). Parasitol Res 12(3):1363–1366. doi:10.1007/s00436-012-3208-z

Lapeere H, Brochez L, Vander Haeghen Y, Mabilde C, Vander Stichele R, Leybaert L, Naeyaert JM (2005) Method to measure force required to remove *Pediculus humanus capitis* (Phthiraptera: Pediculidae) eggs from human hair. J Med Entomol 42(1):89–93. doi:10.1603/0022-2585(2005)042[0089:MTMFRT]2.0.CO;2

Lapeere H, Brochez L, Verhaegue E, Vander Stichele RH, Remon JP, Lambert J, Leybaert L (2014) Efficacy of products to remove eggs *of Pediculus humanus capitis* (Phthiraptera: Pediculidae) from the human hair. J Med Entomol 51(2): 400–407. doi:10.1603/ME13106

Mehlhorn H, Abdel-Ghaffar F, Al-Rasheid K, Schmidt J, Semmler M (2011) Ovicidal effects of a neem seed extract preparation on eggs of body and head lice. Parasitol Res 109:1299–1302. doi:10.1007/s00436-011-2374-8

Mumcuoglu KY (1999) Prevention and treatment of head lice in children. Paediatr Drugs 1:211–218. doi:10.2165/00128072-199901030-00005

Mumcuoglu KY, Miller J, Gofin R et al (1990) Epidemiological studies on head lice infestation in Israel. I. Parasitological examination of children. Int J Dermatol 29:502–506. doi:10.1111/j.1365-4362.1990.tb04845.x

Mumcuoglu KY, Meinking TA, Burkhart CN, Burkhart CG (2006) Head louse infestations: the "no nit" policy and its consequences. Int J Dermatol 45(8):891–896. doi:10.1111/j.1365-4632.2006.02827.x

Mumcuoglu KY, Gilead L, Ingber A (2009) New insights in pediculosis and scabies. Exp Rev Dermatol 4:285–302. doi:10.1586/edm.09.18

Rukke BA, Soleng A, Heggen Lindstedt H, Ottesen P, Birkemoe T (2014) Socioeconomic status, family background and other key factors influence the management of head lice in Norway. Parasitol Res 113:1847–1861. doi:10.1007/s00436-014-3833-9

Toloza A, Vassena C, Gallardo A, González-Audino P, Picollo MI (2009) Epidemiology of *Pediculus capitis* in elementary schools of Buenos Aires, Argentina. Parasitol Res 104:1295–1298. doi:10.1007/s00436-008-1324-6