



Effect of a New Head Lice Treatment, Abametapir Lotion, 0.74%, on Louse Eggs: A Randomized, Double-Blind Study

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Abstract

Few head lice treatments have demonstrated effectiveness against louse eggs. Abametapir, a metalloproteinase inhibitor, is able to target metalloproteinases critical to egg hatching and louse development. In this double-blind, phase 2 study, 50 subjects aged ≥ 3 years with active head lice infestation were randomized to receive a single treatment of abametapir lotion, 0.74%, or vehicle (control), applied to scalp and hair for 10 minutes. Ovicidal efficacy was measured by recording the hatch rate of eggs collected from each subject's hair before and after treatment and incubated for 14 days. With abametapir, 100% of treated eggs remained unhatched compared with 64.0% for vehicle. Accounting for pretreatment hatch rates, the absolute reduction in egg hatching was 92.9% for abametapir versus 42.3% for vehicle ($P < .0001$). The most frequently reported adverse event was rash (16%). Abametapir lotion, 0.74%, demonstrated significant ovicidal activity against head lice eggs with a single application.

Keywords

clinical, head lice, louse eggs, ovicidal, abametapir

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Introduction

Head lice (*Pediculus capitis*) are common human ectoparasites, typically observed in school-aged children.¹ The current standard of care is to treat hair with a topical pediculicide. To effectively treat a head lice infestation, it is important to control all stages of the ~30-day life-cycle, including the egg, nymph, and adult stages. Louse eggs are laid on the hair shaft, typically less than 1.5 cm from the scalp,² and hatch 7 to 12 days later.³ The nymph stage lasts 6 to 12 days, followed by adult lice that are capable of reproducing. An adult female louse lays 4 or 5 eggs per day over the next 16 days.⁴

Few products directly target the egg stage of the life cycle, and those that do claim to kill lice eggs have not demonstrated 100% ovicidal activity; hence, the majority recommend a second treatment administered 7 to 14 days after the first in order to eliminate any lice that hatched from eggs present during the initial treatment.⁵ These include the most commonly used over-the-counter products, containing synergized pyrethrin or synthetic

pyrethroid (ie, permethrin) insecticide. Of the currently available prescription treatments, there is little published evidence of direct ovicidal activity with the exception of malathion lotion,⁶ although its product information recommends a second treatment 7 to 9 days after the first if lice are still present.⁷ In addition, spinosad is reported to have ovicidal activity⁸; however, this product is also recommended for a second treatment if live lice are seen 7 days after the first treatment.⁹ To date, topical ivermectin (0.5%) is the only product that states it is a “single-use” product for the treatment of head lice infestations.^{10,11} Of note, ivermectin lotion is not ovicidal but acts as a post-occlusion nymphicide that detrimentally affects the

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newly emerged nymphs' ability to feed, resulting in mortality.¹² Though not sold as a prescription product in the United States, it should be noted that dimethicone is also registered as a single-use treatment.

The process of louse egg hatching is considered to involve multiple proteinases, including metalloproteinases.¹³ It has been demonstrated that metal-chelating agents can inhibit this proteinase activity and significantly reduce egg hatching *in vitro*.¹³ Abametapir is a metalloproteinase inhibitor able to target metalloproteinases critical to louse development and the hatching process.¹⁴ It has been shown to be effective in eliminating head louse infestations in 2 phase 3 trials.¹⁵ In these trials, abametapir was administered in a lotion at a concentration of 0.74% to dry hair for 10 minutes. This single application resulted in elimination of lice in more than 80% of study participants.

To assess clinical ovicidal activity, head lice eggs are commonly treated while on a subject's head, then collected and incubated under conditions to facilitate egg hatching.¹⁶⁻¹⁹ Comparing eggs before and after treatment allows accurate assessment of the hatch rate of treated and untreated eggs from the same head. This approach to assessing ovicidal efficacy has been termed "ex vivo" because although treatment occurs on the human head, egg incubation takes place off the head. This experimental design was employed in the current study to assess the ovicidal efficacy of abametapir lotion.

Subjects and Methods

Ethical Approval and Informed Consent

This study was conducted in Melbourne, Australia, between May and September 2014, under the Therapeutic Goods Administration Clinical Trials Notification scheme, and in accordance with the Declaration of Helsinki and the National Health and Medical Research Council. Study conduct was in accordance with the International Council for Harmonisation guidelines for Good Clinical Practice. The study protocol was approved by an independent human research ethics committee (#HREC2014-03-138). Written informed consent was obtained from each subject or legal representative (parent or legal guardian). The study is registered at ClinicalTrials.gov (identifier NCT02097485).

Study Participants

Study participants were healthy male and female subjects aged 3 years and older, with active head louse infestation (defined as ≥ 3 live head lice and ≥ 10 undamaged and unhatched head louse eggs).

Study Design

This phase 2 study was a double-blind, randomized, vehicle-controlled, parallel-group study in subjects aged 3 years and older with an active head lice infestation. The study was designed to assess the ovicidal efficacy of a single application of abametapir lotion compared with a vehicle control, when applied to the scalp and hair for 10 minutes at the study site. Fifty subjects were randomized 1:1 to receive either abametapir lotion or vehicle lotion.

All subjects completed a screening visit (days -7 to 0) in which trained evaluators systematically examined the scalps of the subjects for up to 15 minutes to detect any live lice and eggs. Subjects were randomized to either abametapir lotion or vehicle lotion. On day 0, before application of the investigational product, at least 5 undamaged eggs located on hair shafts <1 cm from the scalp were randomly selected as untreated controls and removed from each subject's head by hair clipping. Study drug (abametapir lotion or vehicle lotion) was applied to the dry scalp and hair, left for 10 minutes, and then rinsed with warm water and towel dried. Immediately after treatment, the random egg collection process was repeated. Hair shafts collected at the site both before and after treatment were microscopically examined to assess egg viability; nonviable eggs (non-ellipsoid, squashed, flattened, or crushed) were discarded. Viable eggs were incubated at 30°C ($\pm 1^\circ\text{C}$) and ~60% relative humidity for 14 days. All eggs were then examined by an independent assessor to determine whether eggs were hatched, partially hatched, or unhatched. The assessor was blinded to the treatment assignments and the time of collection of the egg samples. The proportion of pretreatment versus posttreatment hatched eggs was compared across treatment groups following incubation. Subjects returned to the site on day 1 (+1) and day 7 (+2) to assess for the presence of live lice.

Endpoints

The primary efficacy endpoint was the proportion of hatched eggs following a 14-day incubation period, comparing those collected before treatment with those collected after treatment with abametapir lotion or vehicle.

The primary safety endpoints were defined as changes in the irritation scores of scalp and eye assessments and the proportion of subjects reporting treatment-emergent adverse events (TEAEs) at day 1. Scalp and eye examinations were performed at screening, baseline, and day 1. Scalp irritation was graded on 4-point scales for erythema and edema, pruritus, and excoriation and pyoderma (0 = none, 1 = mild, 2 = moderate, and 3 = severe).

Adverse Events

Adverse events (AEs) were collected from days 0 to 7 (+2) and coded according to MedDRA (March 2014, v17.0). AEs were either spontaneously reported by participants or in response to questioning or observations by the investigator. TEAEs were summarized by system organ class, preferred term, and treatment group.

Statistical Analysis

The primary efficacy analysis compared the difference in proportion of hatched eggs (posttreatment minus pretreatment) between abametapir lotion and vehicle based on a generalized estimating equation (GEE). The GEE model used the binary egg status (hatched/unhatched) as the response variable with the logit link function. Treatment group and time point (pretreatment/posttreatment) were fixed factors in the model, and subject was included as a random factor. For the model to accommodate estimates under boundary conditions (when all outcomes are the same), if all eggs were unhatched, the response was imputed as $0.50/N$, and if all eggs were hatched, the response was imputed by $(1-0.50)/N$, where N is the number of viable and incubated eggs for that treatment and time combination. Compound symmetry was assumed for all eggs within a subject with an allowance for separate variances for each time point. Statistical analyses of the efficacy and safety data were performed by Array Biostatistics LLC using SAS software for Windows, v9.3 (2011, SAS Institute, Inc, Cary, NC).

Results

Enrollment and Follow-up

Of 50 subjects enrolled at a single site, 25 were treated with abametapir lotion and 25 with vehicle lotion. All 50 completed the study as planned (Figure 1).

Subject Demographics

Demographic characteristics were similar between the treatment groups, with most subjects being female (84.0% and 96.0% in the abametapir and vehicle groups, respectively). All subjects were white. Mean subject age was 8.5 years (range = 3-17 years in the abametapir group and 3-12 years in the vehicle group).

Efficacy

After the 14-day incubation period, 100% of eggs treated with abametapir lotion remained unhatched, versus 64% of eggs treated with vehicle lotion. The hatch rate in the

abametapir group was reduced from 93.3% before treatment to 0% after treatment, compared with the vehicle group in which the hatch rate was reduced from 79.5% to 36.0% (Figure 2). Using the GEE model to account for the correlation of eggs within subjects, the absolute reduction in hatch rate for the abametapir group was 92.9% (95% confidence interval [CI], 86.5-99.4) versus 42.3% (95% CI, 30.2-54.4) for the vehicle group. The difference in absolute reduction of hatch rates was 50.6% (95% CI, 36.9-64.3; $P < .0001$; Table 1).

In the abametapir group, the proportion of louse-free subjects was 92.0% (23 of 25 subjects) on day 1 and 88.0% (22 of 25 subjects) on day 7. In the vehicle group, 64.0% of subjects were louse free on day 1 and 32.0% on day 7 (16 of 25 subjects, and 8 of 25 subjects, respectively). Overall, more subjects treated with abametapir lotion were free of head lice at both follow-up visits than those treated with vehicle lotion (88.0% vs 32.0%).

Safety

Scalp irritation assessment showed that 96% of subjects experienced pruritus at baseline (grades 1 [mild] to 3 [severe]). On day 1, 64% of the abametapir lotion group had grade 0 pruritus compared with 28% of vehicle-treated subjects. Erythema and/or edema was only recorded in 1 subject at baseline (grade 1) and 2 subjects on day 1 (both grade 1, vehicle-treated). Scalp excoriation and pyoderma was assessed as grade 1 for 1 (4.0%) subject in the abametapir lotion group and for 2 (8.0%) subjects in the vehicle group at both baseline and day 1. No eye irritation was reported for either group.

The most frequently reported TEAEs for both treatment groups were skin and subcutaneous tissue disorders. The most commonly reported treatment-related TEAE was rash (16% in the abametapir lotion group; 8% in the vehicle lotion group). All TEAEs were mild in severity, with 1 moderate TEAE (rash) in the abametapir lotion group that resolved by day 4. Additionally, there were no serious adverse events reported in this study and no subjects discontinued due to AEs. All TEAEs had resolved by day 5 (Table 2).

There were no clinically significant changes in vital signs or general appearance following application of either abametapir lotion or vehicle lotion.

Discussion

Head lice infestation involves the presence of live lice and viable louse eggs on scalp and hair; therefore, pediculicides should ideally target both life stages to deliver effective control.³ However, the majority of available treatments require a second treatment due to their lack of direct ovicidal activity²⁰; poor compliance with the

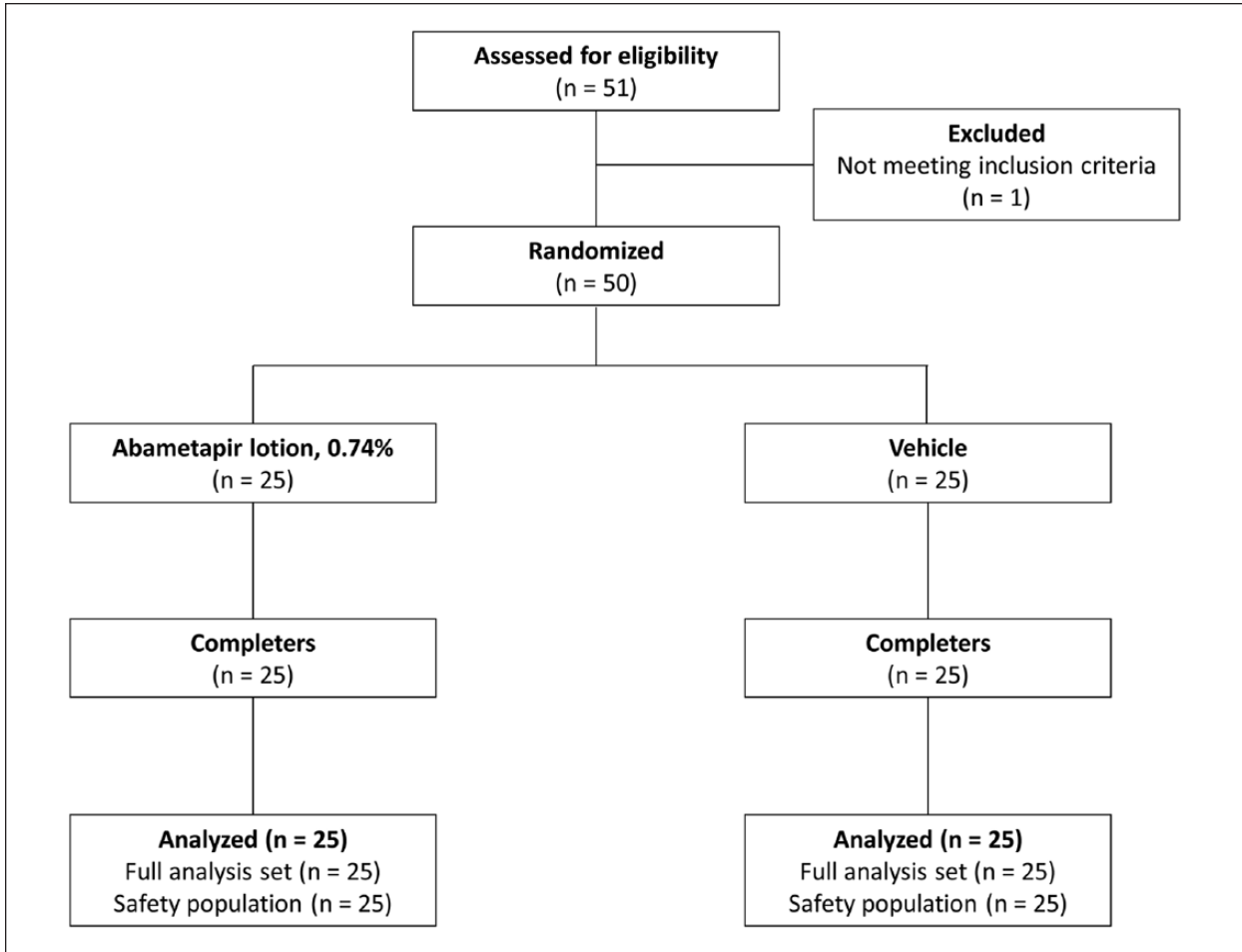


Figure 1. Flow diagram of subject disposition.

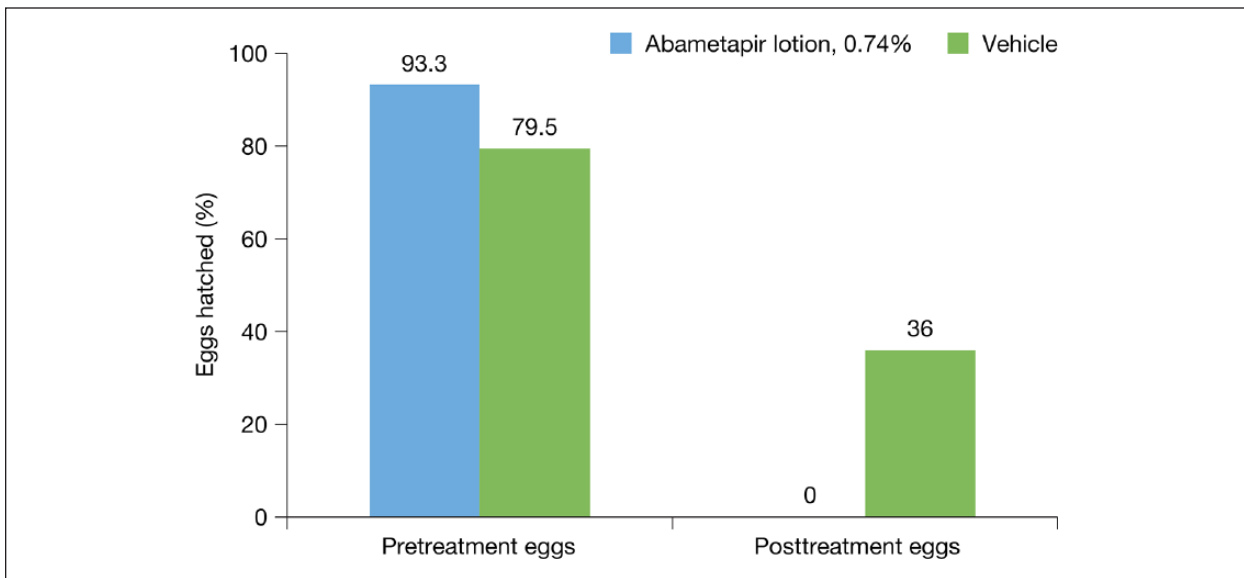


Figure 2. Hatch rate of eggs collected before and after treatment with abametapir lotion or vehicle, after a 14-day incubation period.

Table 1. Summary of Hatched Eggs by Time, Treatment Arm, and Overall Full Analysis Set.

Egg Hatch Status	Abametapir (N = 25)	Vehicle (N = 25)	Treatment Difference ^a	P
Pretreatment				
Eggs incubated ^b , n	119	117		
Hatched	111 (93.3%)	93 (79.5%)		
Unhatched	8 (6.7%)	24 (20.5%)		
Posttreatment				
Eggs incubated ^b , n	130	136		
Hatched	0	49 (36.0%)		
Unhatched	130 (100.0%)	87 (64.0%)		
Percentage of hatched eggs from GEE model^{c,d} (95% CI)				
Pretreatment	93.3 (82.8, 97.5)	79.4 (69.4, 86.7)	13.9 (3.3, 24.5)	
Posttreatment	0.3 (0.3, 0.4)	37.1 (27.9, 47.3)	-36.7 (-46.4, -27.1)	
Change (posttreatment minus pretreatment)	-92.9 (-99.4, -86.5)	-42.3 (-54.4, -30.2)	-50.6 (-64.3, -36.9)	<.0001

Abbreviations: CI, confidence interval; GEE, generalized estimating equation.

^aTreatment difference = abametapir proportion minus vehicle proportion.

^bn = number of pretreatment and posttreatment eggs incubated.

^cPercentage of hatched eggs was estimated using a GEE model with fixed effects for time, treatment, and a time-by-treatment interaction. The response is the binary outcome of egg hatch status with the logit link function. Subject is a random factor. Compound symmetry is assumed for all eggs within a subject with an allowance for separate variances for each time point.

^dIf a treatment-by-time combination has all unhatched eggs, the response is imputed 0.50/N, where N is the number of viable and incubated eggs in that treatment-by-time interaction.

Table 2. Summary of Treatment-Emergent Adverse Events^a.

System Organ Class, Preferred Term	Abametapir 0.74% (N = 25), n (%) e	Vehicle (N = 25), n (%) e
Number of subjects with ≥ 1 TEAE	6 (24.0%), 7	2 (8.0%), 2
Skin and subcutaneous tissue disorders		
Rash	4 (16.0%), 4	2 (8.0%), 2
Pruritus	1 (4.0%), 1	0
General disorders and administration site conditions		
Pain	1 (4.0%), 1	0
Nervous system disorders		
Headache	1 (4.0%), 1	0

Abbreviations: e, number of events; n, number of subjects with TEAE; TEAE, treatment-emergent adverse event.

^aSubjects with multiple events were only counted once per level of summarization.

administration and/or timing of this second treatment can result in reinfestation from newly hatched eggs, creating a challenge for both practitioners and parents. Here, we demonstrated that treatment of louse eggs with abametapir lotion prevented louse egg hatching for 100% of treated eggs.

Previous in vitro studies have evaluated the ovicidal efficacy of abametapir lotion on a resistant strain of head lice. In these studies, both the abametapir compound (0.74% in isopropanol) and the abametapir lotion, 0.74%, formulation demonstrated 100% ovicidal activity against eggs from a DDT (dichlorodiphenyltrichloroethane)-resistant and permethrin-resistant laboratory-based SF-HL strain of the human head louse (*Pediculus humanus capitis*, De Geer, Anoplura: Pediculidae).¹⁴ Of note, these studies assessed the efficacy of abametapir

treatment at different stages of egg development (0-2, 3-5, and 6-8 days). Regardless of egg stage, abametapir treatment eliminated the viability of the eggs, with 100% of eggs remaining unhatched.

Demonstrating that the 0.74% abametapir lotion formulation was ovicidal in the clinical setting was a key challenge in designing this study. Historically, demonstrating ovicidal activity involved using laboratory-based body lice (*Pediculus humanus humanus*) colonies as surrogates for head lice,²¹ while other studies involved collecting head lice eggs from subjects in the field, and then treating and incubating them.^{5,22} More recently, field-derived head lice were used to establish a laboratory-based colony that could be used to evaluate ovicidal activity of compounds.²³ However, to demonstrate clinical ovicidal activity, the test compound must be applied

directly to the eggs while on the subject's head. Conducting an *in vivo* study would involve identifying and treating some eggs, and monitoring their posttreatment hatching on the head, while other eggs on the same subject's head remain untreated. This methodology is impractical given that untreated lice can continue laying eggs, or reinfestation may occur with new eggs being laid after treatment, making it impossible to accurately track and assess the origin and outcome of specific eggs.

The design of the current study enabled the assessment of ovicidal activity using eggs treated on the head. This study combined *in vivo* treatment (thereby replicating real-world treatment) with *ex vivo* evaluation of eggs and hatch rates. Using this methodology, a single 10-minute application of abametapir lotion was shown to be 100% effective in preventing egg hatching, with an absolute reduction in hatch rate of 92.9%, thus demonstrating significant ovicidal activity. Notably, the vehicle did not significantly inhibit hatching, indicating that it is the abametapir compound that provides the ovicidal activity in the formulation. The hatch rate of the untreated eggs was comparable to previously reported rates, further validating this approach.^{12,17,18,23-26}

Ovicidal activity for other head lice treatments have been reported for malathion 0.5%. Eggs were removed from infested children and immersed in malathion 0.5% lotion for 10 minutes and then incubated for 14 days. This study had a 0% hatch rate (recorded as the combination of stillborn and nonviable eggs). Several over-the-counter formulations were similarly tested, demonstrating limited ovicidal activity compared with untreated hatch rates of 91% to 93%.⁵ Among prescription medications, there are some suggestions that spinosad 0.9% suspension has ovicidal activity; however, this is based on the proportion of subjects in their phase 3 studies that did not require a second treatment, rather than a direct analysis of egg hatching rates,^{8,20} while benzyl alcohol has no ovicidal activity²⁰ and ivermectin works as a nymphicide.¹²

In this study, head lice were eradicated in 88% of subjects, demonstrating that abametapir lotion has the potential to eliminate both adult lice and eggs with a single treatment. The lousicidal efficacy of abametapir lotion has been investigated in 2 large phase 3 studies,¹⁵ resulting in high rates of lice elimination (>80%) with a single 10-minute treatment. This result strongly implied that abametapir killed both unhatched eggs and crawling lice. However, the evidence for ovicidal activity was indirect since the measurement for efficacy was the detection of no live lice in the hair of subjects 14 days after treatment. Any eggs detected at 14 days would have been considered not viable given the well-established time these eggs take to hatch (7-12 days). The study

design used here enabled a clear assessment of the ovicidal efficacy of abametapir and therefore provided evidence for the ability of this compound to act as an effective ovicide in a clinical setting.

The hatch rates seen in the pretreatment groups of this study were 93.3% and 79.5%. Inherent hatching rates of head lice eggs *in vitro* have been reported as being between 70% and 95%, in line with the hatch rates observed in this study and indicating that the incubator conditions were suitably conducive to head lice eggs hatching.^{12,18,19,23-26} It should be noted that the GEE model was designed to allow for the difference recorded between groups in pretreatment hatch rates.

Abametapir lotion was well tolerated in subjects aged 3 years and older, with the TEAEs reported being skin related, as expected. No subjects discontinued the study due to AEs and no serious adverse events, or other notable events, were reported.

Conclusion

Abametapir lotion 0.74% has been developed as a single 10-minute, topical treatment for head lice infestation. Two large phase 3 studies demonstrated efficacy in treating louse infestations in subjects aged 6 months and older.¹⁵ The current study demonstrated that abametapir lotion is effective in killing louse eggs.

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Author Contributions

VMB was involved in the study design and drafted the initial manuscript.

SH was involved in the study design, and critically reviewed and revised the manuscript.

TA was involved in the study design, study management and monitoring, and critically reviewed and revised the manuscript.

SS conducted retrospective due diligence on the study design, conduct and interpretation of the results on behalf of Dr. Reddy's Laboratories Inc., and critically reviewed and revised the manuscript.

KA conducted retrospective due diligence on the study design, conduct and interpretation of the results on behalf of Dr. Reddy's Laboratories Inc., and critically reviewed and revised the manuscript.

HA conceptualized the study design, and critically reviewed and revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: VB and HA are stockholders in Hatchtech Pty Ltd. KA was an employee and SS is an employee of Dr. Reddy's Laboratories Inc.; both own stock in the company. The other authors have no potential conflicts of interest relevant to this article to disclose.

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