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Comparison of Selamectin and Imidacloprid plus Permethrin in Eliminating *Leporacarus gibbus* Infestation in Laboratory Rabbits (*Oryctolagus cuniculus*)

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A shipment of New Zealand white rabbits was infested with *Leporacarus gibbus*, a rabbit fur mite. This study compared the effectiveness of selamectin with that of imidocloprid plus permethrin in eliminating the mite infestation. Rabbits were divided into 2 groups, and either selamectin or imidocloprid plus permethrin was applied topically. Visual and microscopic examinations were performed on days 0, 1, 2, 3, 4, 5, 6, 13, and 27 for 5 sites (the left and right gluteal areas, neck, ventral tail, and abdomen). Mean percentage effectiveness for each treatment was calculated for each time point. Positive and negative predictive value, sensitivity, and specificity of visual examination were determined relative to microscopic assessment. In addition, location prevalence for the mites was determined. Both treatments were 100% effective by day 13, but selamectin was 100% effective by day 3. The positive predictive value of visual examination was 96%, its negative predictive value was 86%, sensitivity was 75%, and specificity was 98%. Parasite burden was most prevalent on the right and left gluteal areas. We conclude that although both imidocloprid plus permethrin and selamectin were effective against *L. gibbus*, treatment with selamectin more rapidly eliminated the infestation.

Leporacarus gibbus (formerly *Listorphorus gibbus*) is a rabbit fur mite rarely reported to occur in laboratory rabbits.[5,14,15] *L. gibbus* belongs to the family Listrophoridae, division Psoroptida, and order Astigmata. The life cycle of this mite has not yet been described fully, but it is known that all life stages (including 2 nymphal stages) occur on the rabbit.[11]

There is 1 report of *L. gibbus* causing alopecia, moist dermatitis, and pruritus in rabbits.[18] The mite tends to populate the distal 1/3 of the hair shaft of the dorsal lumbar area and ventral tail of the rabbit and feed on sebaceous secretions and epithelial scale.[11,15] Infestation with *L. gibbus* may be underdiagnosed, because it can be missed on a tape test.[9] There is 1 report of *L. gibbbus* causing papular dermatitis in a child.[4]

Several treatment methods have been described for elimination of *L. gibbus*. These include carbaryl powder, selamectin, and imidacloprid plus permethrin.[6,17,18] Carbaryl is a reversible acetylcholinesterase inhibitor.[13] Selamectin is a macrocyclic lactone and is thought to work by increasing cell membrane permeability of the calcium channels in the peripheral nerves of the arthropod, thereby causing paralysis.[2,16] After application, selamectin is absorbed dermally and concentrates in the sebaceous glands.[3] Permethrin inactivates neuronal action potential depolarization in arthropods, causing repeated firing of peripheral nerves and leading to the insecticidal activity of the drug.[5] Selamectin is labeled for dogs and cats, and imidacloprid plus permethrin is labeled for dogs exclusively. Both are spot-on insecticides. In addition, permethrin, selamectin, and other avermectin compounds have been used successfully for the treatment of fur mites in rodents.[1,3,8,12,19] To our knowledge, the effectiveness of the aforementioned treatment methods have not been compared systematically against *L. gibbus* in rabbits. Thus, in this study, the effectiveness of selamectin and imidacloprid plus permethrin for eliminating *L. gibbus* infestation in conventionally housed rabbits in a laboratory setting was compared.

Materials and Methods

Animals. One rabbit in a previous shipment from a local conventional vendor was found with patchy alopecia. Skin scrape and tape tests were negative; a fur pluck test revealed *Leporacarus gibbus.* All subsequent shipments of rabbits obtained from this vendor were infested with *L. gibbus.* Subjects in this study comprised 30 New Zealand white rabbits (*Oryctolagus cuniculus*; weight, 1.8 to 2.27 kg) that were infested with *L. gibbus* on arrival despite the vendor's attempt to treat them with 5% carbaryl powder 3 wk prior to arrival. The animals were divided randomly into 2 groups. Their care and usage was approved by the Louisiana State University Health Sciences Center Institutional Animal Care and Use Committee and was consistent with the *Guide for the Care and Use of Laboratory Animals*[10] and the Animal Welfare Act. The Louisiana State University Health Sciences Center is fully AAALAC-accredited. All 30 rabbits belonged to the same shipment of 40 animals. All 40 animals underwent mite counts and treatment, but the 5 rabbits from each group that had the lowest initial mite counts were excluded from the study.

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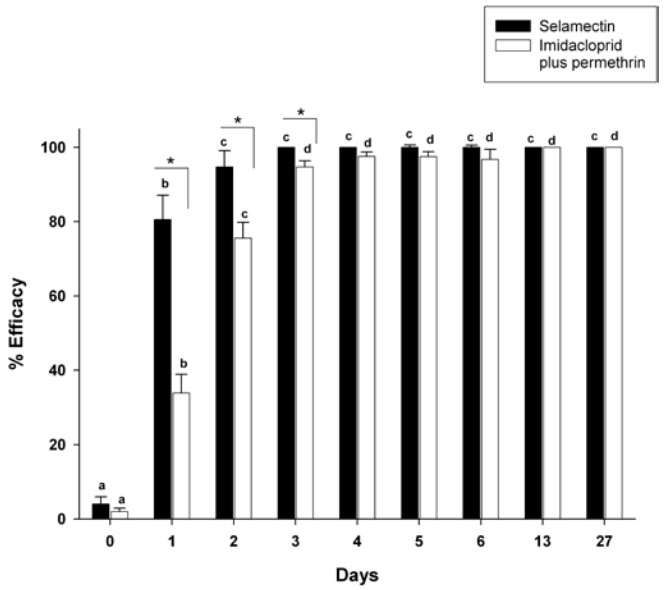

Figure 1. Mean percentage effectiveness (± SEM) per day of treatment with selamectin and imidacloprid plus permethrin. Values with different lowercase letters are significantly different ($P < 0.05$) from each other. Bars and asterisks indicate significant differences between treatments for each day.

Rabbits were singly housed in stainless steel rabbit cages (Suburban Surgical, Wheeling, IL) and were given 235 g food (Harlan Global 2031, Harlan, Indianapolis, IN) daily. Water was provided ad libitum by using water bottles. The rabbit housing room was maintained at negative air pressure, with 10 to 15 air changes per hour, at 19.4 to 21.1 °C (67 to70 °F) and 30% to 70% humidity, and on a 12:12 light:dark cycle. After arrival, the rabbits were acclimated to the facility for 7 d. During this time, they were tested (by microscopic and gross visual examination) and treated for mites while on a holding protocol. After this time, they were transferred onto research protocols.

		Microscopic Exam: Positive	Microscopic Exam: Negative	
Visual Exam	Positive	TP = 433	FP = 15	**Positive Predictive Value** = TP / (TP + FP) = 433/433 + 15 **= 97%**
	Negative	FN = 140	TN = 889	**Negative Predictive Value** = TN / (TN + FN) = 889/889 + 140 **= 86%**
		Sensitivity = TP/TP + FN =433/433 + 140 **= 76%**	**Specificity** = TN/TN + FP = 889/889 + 15 **= 98%**	

Figure 2. Sensitivity and specificity chart for visual examination versus microscopic examination for the diagnosis of *L. gibbus.* FN, false negative; TP, true negative; FP, false positive; TP, true positive.

Rabbits were assigned randomly to cages on opposite walls in the same room. All rabbits on 1 wall received selamectin, and all rabbits on the opposite wall received imidacloprid plus permethrin. One technician serviced the room; waste collection pans were scraped and rinsed daily, and the cages were washed in a Basil 4600 cage washer (Steris, Mentor, OH) every 2 wk. All animals occupied the same cage throughout the treatment period, and no cage equipment was shared between cages. During the treatment and testing periods, personnel wore disposable gowns, shoe covers, hair bonnets, masks, gloves, and they were required to change gloves between rabbits. Disposable gowns, shoe covers, hair bonnets, masks, and gloves were changed between treatment groups.

Experimental design. All rabbits had been treated with 5% carbaryl powder 3 wk prior to arrival, but both gross visual and microscopic examination revealed them to be infested with *L. gibbus* on arrival (day 0). The rabbits were treated once topically with either 0.25 mL (11 to16.6 mg/kg) selamectin (Revolution, Pfizer, New York, NY) or 0.4 mL (imidocloprid plus permethrin; 14.8 to 22.2 mg/kg and 74.1 to 111.1 mg/kg, respectively; Advantix, Bayer AG, Leverkusen, Germany) on the dorsal cervical area. Hair was parted during application to ensure contact of the insecticide with the skin.

Sample collection and mite counts. Hair was collected from 5 sites on each rabbit: dorsal neck, right lumbar area, left lumbar area, ventral tail, and ventral abdomen. Hair was grasped with 2.2-cm tipped mosquito hemostats and clipped approximately 1 mm from the skin by using iris scissors with 2.3-cm tipped blades. Enough hair was collected to completely cover the nonfrosted portion of a microscope slide (5.77 cm × 25 mm) 1 to 5 hairs thick. Separate slides were made for each of the 5 areas. Instruments were disinfected with 70% ethanol and wiped dry between sample collection of each site and gloves were changed between rabbits. Mineral oil was used to keep the hair in place on the slide and allowed for the use of cover slips. The slides were examined with high-power (×20) magnification under a light microscope. Either a veterinarian or LAT counted the mites on each slide, determining live versus dead mites and nymphs versus adults and eggs. Vitality of the mite was determined by both lack of movement and the appearance of the chitin. Exoskeleton chitin color lightened considerably on dead mites. Male and female mites were collected and placed in glycerol and submitted for confocal microscopy.

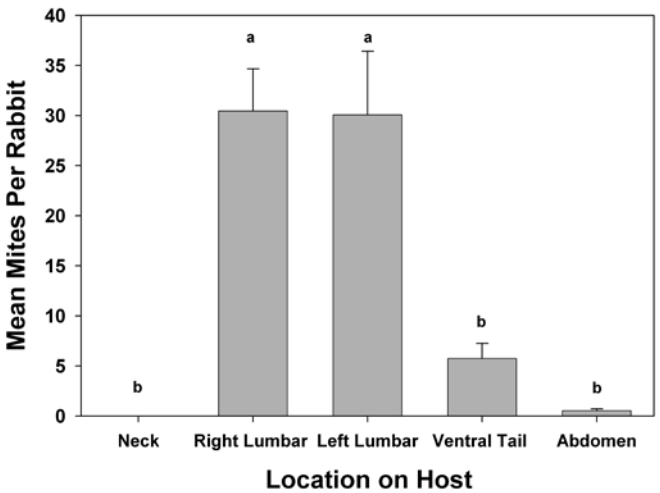

Figure 3. Mite counts (mean ± SEM) for each of the 5 locations on hosts. Values with different lowercase letters are significantly different ($P < 0.05$) from each other.

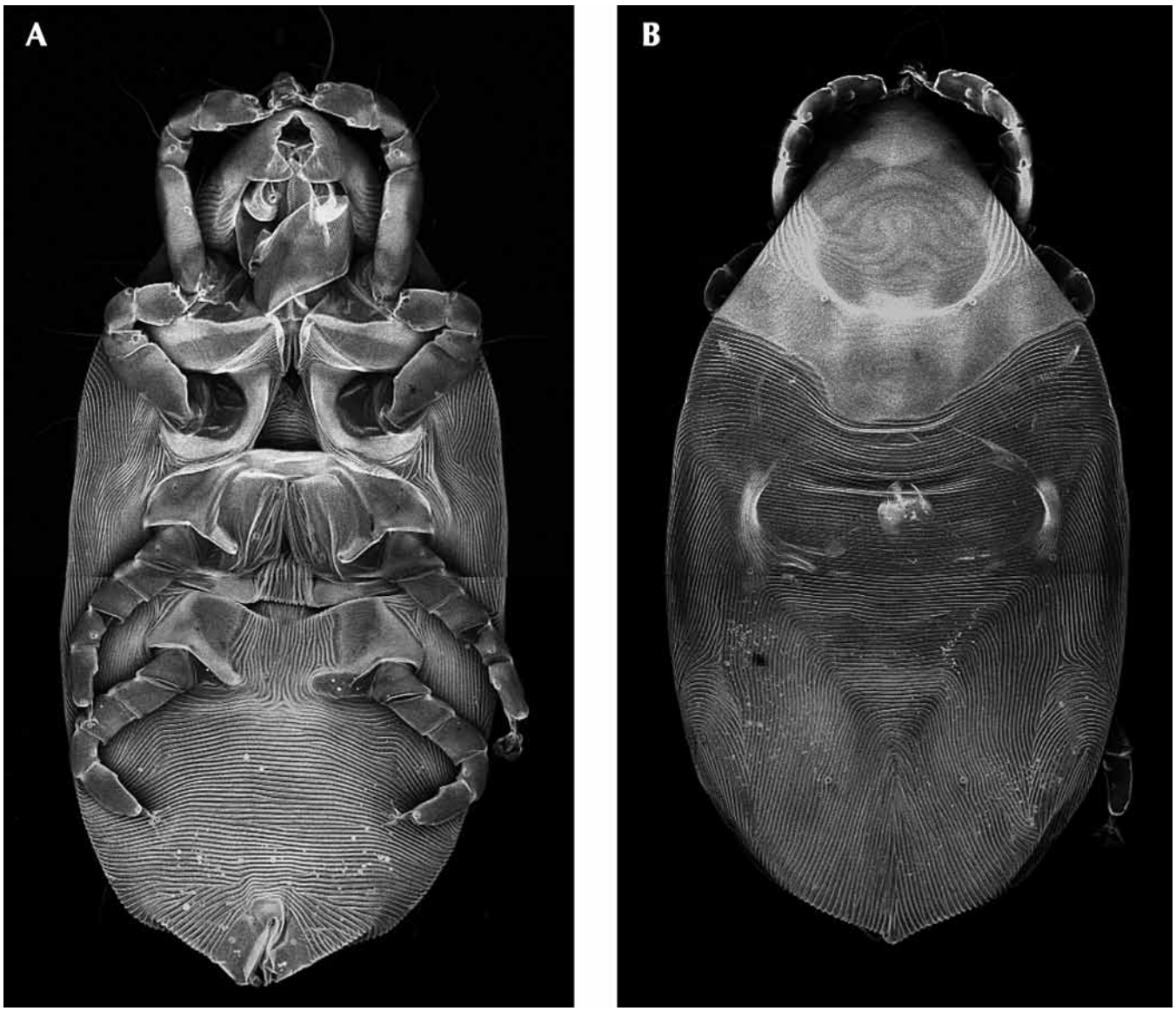

Figure 4. Female *L. gibbus*. (A) Ventral view. (B) Dorsal view.

Confocal microscopy. Representative male and female mites were collected, oriented dorsoventrally, and mounted in glycerol on a glass-bottomed tissue culture dish, and cover slips were placed over the specimens. Inherent exoskeletal autofluorescence was used to image both cranial and caudal aspects of each mite at high resolution for subsequent compiling into a montage of the entire animal. Either dorsal or ventral views were photomicrographed with a laser scanning confocal system (Radiance 2100, BioRad, Hercules, CA) built around an inverted platform (TE300, Nikon, Tokyo, Japan) and equipped with a dual Kr–Ar gas laser with corresponding photomultiplier tubes. Sample optical sections were obtained through a 40× oil objective (NA, 1.3) at 0.591-µm steps and an 8-bit resolution of 512 × 512. Cranioventral and caudodorsal aspects were compiled (Photoshop, Adobe, San Jose, CA) by using specific pixel landmarks. The same process was used to establish the caudal view.

Time points. All 30 rabbits were examined visually (gross examination) and microscopically (fur clip) daily for 6 d. Careful attention was taken to clip and observe from the same site each day. In addition, 5 rabbits still harboring live mites on day 5 were examined daily until live mites were no longer found at any site (day 10). All 30 subjects were examined again at day 13. On day 27, the remaining rabbits were examined again and hair samples collected, with the exception of 2 rabbits from the imidacloprid plus permethrin treatment group, which were euthanized at the completion of their protocol.

Data analyses. Percentage effectiveness was calculated as (number of dead mites / total number of mites) × 100%. The mean percentage effectiveness for each group were calculated and compared statistically for treatment effects by using 2-way ANOVA with day and drug as the main factors. A Dunnett test was used to compare treatment with percentage effectiveness (SigmaStat Statistical Software, SYSTAT Software, Point Richmond, CA). In addition, the number of mites per location for each rabbit was determined and group means compared by using 1-way ANOVA. Multiple comparisons were conducted with Tukey post hoc tests after each ANOVA. Significance was accepted at an α level of 0.05 or less for all statistical tests.

Results

Overall mite counts (mean ± SEM) on day 0 confirmed similar infestations between the 2 groups of rabbits. The day 0 mite count was 60.8 ± 66.4 mites per rabbit for the selamectin

group and 67.7 ± 36.9 mites per rabbit for the imidacloprid plus permethrin group.

Figure 1 depicts percentage effectiveness of selamectin and imidacloprid plus permethrin at each time point. The 2-way ANOVA revealed significant main effects of day ($F = 324.542$, $P \leq 0.001$) and drug ($F = 38.17$, $P \leq 0.001$) and a significant interaction between day and drug ($F = 17.827$, $P \leq 0.001$). Therefore, separate 1-way ANOVA were completed on data from each group. These tests revealed a significant main effect across day ($F = 129.06$, $P \leq 0.001$) indicating a difference in effectiveness between the 2 treatments. Tukey pairwise comparison indicated that selamectin reached 100% effectiveness across fewer days than did imidacloprid plus permethrin (as indicated by letters in Figure 1). More specifically, selamectin reached 100% effectiveness by day 3 after treatment, whereas imidacloprid plus permethrin was not 100% effective until day 13 after treatment. Separate *t* tests were used to determine differences between treatments for each day. The 2 insecticide treatments differed significantly ($P < 0.05$) on days 1, 2, and 3 (as indicated in Figure 1 by asterisks and bars).

No live mites were found on any of the rabbits in either treatment group on days 14 and 28 (Figure 1), when dead adult mites were seen. Eggs were found until day 8 after treatment in the imidacloprid plus permethrin group of rabbits, and live nymphs were seen until day 10 after treatment. Although eggs were found until day 7 in the selamectin group, no live nymphs were seen after day 1 after treatment.

Sensitivity and specificity of visual examination for detecting the presence of mites. The prevalence of *L. gibbus* may actually be higher than reported because this mite is easily overlooked.[7,9] To test this suggestion, we calculated the sensitivity, specificity, positive predictive value, and negative predictive value of gross

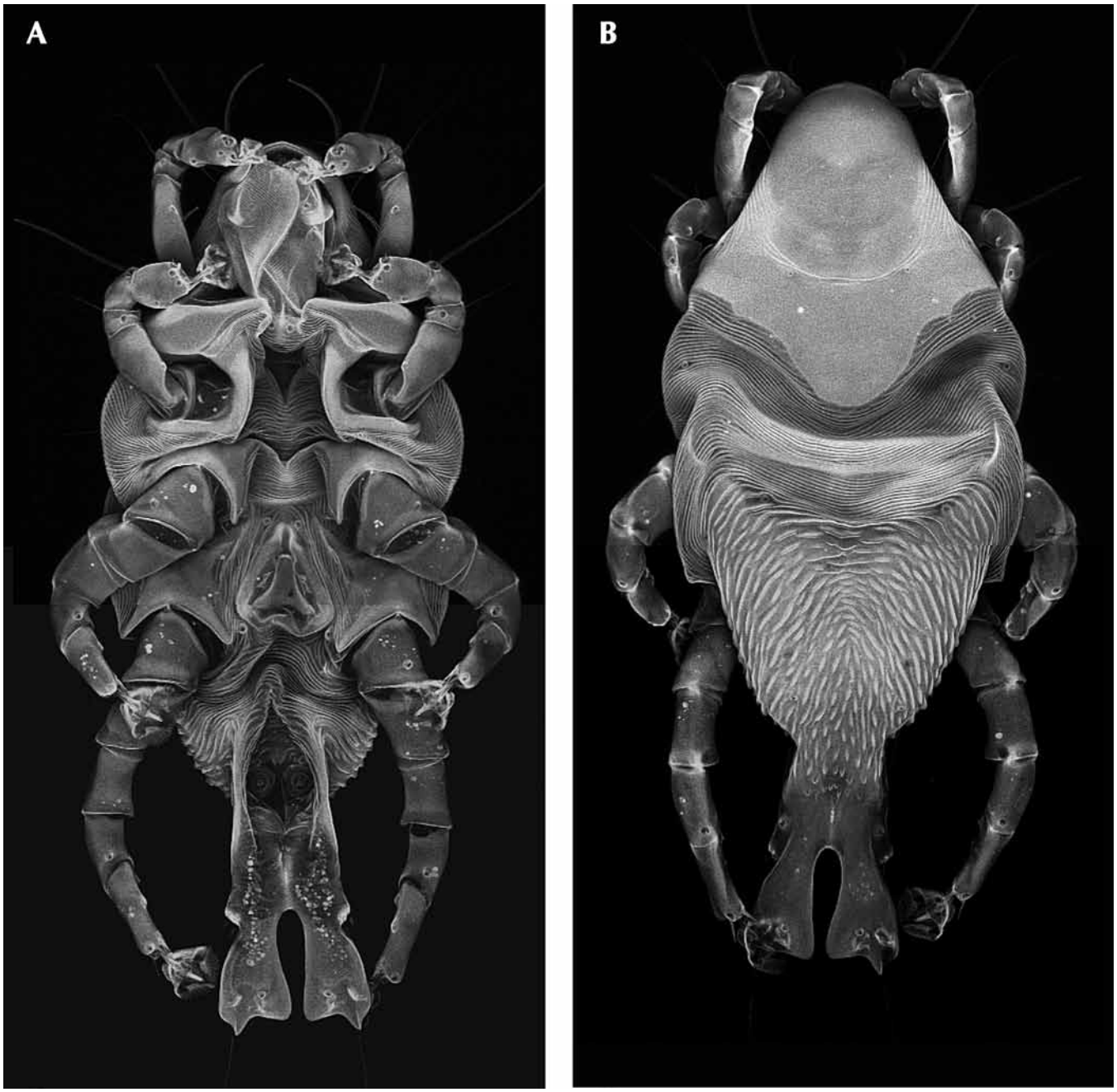

Figure 5. Male *L. gibbus.* (A) Ventral view. (B) Dorsal view.

visual examination compared with microscopic examination (considered the 'gold standard') for detecting mites (Figure 2). On gross visual exam, the rabbits were evaluated for the presence of a characteristic "salt and pepper" appearance of the mites in the fur.[11]

Parasite burden by location on host. Figure 3 shows the number of mites (mean ± SEM) per treatment group (calculated from day 0) for the 5 sample locations. Statistically significant differences of parasite burden among locations are indicated by letters a and b. While the ventral tail has been reported to be the most prevalently mite populated area,[9] we found the right and left lumbar areas were the most prevalently mite populated areas on the rabbits.

Subset of imidacloprid plus permethrin group. Five (as indicated by letters in Figure 1) rabbits in the imidacloprid plus permethrin group did not have 100% mite elimination by day 6. This subset of the group was followed until 100% miticidal effectiveness was reached. For the individual rabbits, this occurred on days 6, 7, 8, 10, and 11 (mean, 8.4 d).

Confocal microscopy. Because *L. gibbus* is reported only rarely and because of the paucity of information available, we performed confocal microscopy to confirm the identity of the mite infesting our rabbits. To our knowledge, this study is the first use of confocal microscopy with *L. gibbus*. Figures 4 and 5 reveal a remarkable sexual dimorphism in this species. Both male and female mites are laterally compressed, but the male mites have 2 adanal processes and adanal suckers.[7] The male mite is also considerably smaller than the female mite.

Discussion

In this study, the miticidal effectiveness of selamectin and imidacloprid plus permethrin against an infestation of *L. gibbus* in laboratory rabbits was compared. When faced with an infestation of *L. gibbus* (in research or clinical practice), knowledge of which drug will more rapidly eliminate the infestation is advantageous. Mite counts performed on day 0 confirmed that infestation levels were similar between groups in this study. In addition, the percentage efficacy on day 0 was not statistically significant between the 2 treatment groups.

Results demonstrated that selamectin was a more effective miticide against *L. gibbus* than was imidacloprid plus permethrin. In the selamectin group, efficacy reached 100% by day 3, whereas 100% efficacy was not achieved in the imidacloprid plus permethrin group until day 13. Our findings of eggs on the imidacloprid plus permethrin group until day 8 and nymphs until day 10 compared with eggs found until day 7 but no nymphs found past day 1 on the selamectin group suggest that selamectin may be a more potent ovicide for *L. gibbus* than is imidacloprid plus permethrin.

Infestations with *L. gibbus* are considered rare, although some investigators have suggested that the mite may actually be underreported or misdiagnosed.[7,11,15,20] Therefore, knowledge of optimal sampling sites and diagnostic methods is important. Our results indicated that the right and left lumbar areas had the highest mite counts, and mites were rarely present on the neck and ventral abdomen. Several diagnostic methods (tape test, fur pluck, skin scraping, and hair combing) have been described in the literature for testing for this parasite.[5,9,12] Because the rabbits in this project were scheduled to be sampled multiple times and because we wished to obtain a consistent sample size, we chose to refine the fur pluck method by using hemostats and scissors to clip the hair 1 mm from the skin. This method gave a consistently reproducible sample size and eliminated the pain that is caused during the fur pluck method. Because *L. gibbus* populates the middle to distal 1/3 of the hair shaft, this refinement likely yields results equivalent to the fur pluck method.[15]

Gross examination of the fur was compared with microscopic examination, and sensitivity, specificity, positive predictive value, and negative predictive value of the visual method were determined. The 98% specificity and 96% positive predictive value of the gross examination demonstrate that visual examination is likely to correctly identify animals that do not have mite infestations. Moreover, rabbits that test positive through gross examination are likely to have mite infestations. Therefore, although microscopic examination remains the 'gold standard,' gross examination of the fur may be a suitably reliable method of screening incoming shipments of rabbits for *L. gibbus*.

A negative control group was not included in this study because of space constraints and concerns about persistence of the mites in the environment, subsequent reinfestations, and potential zoonosis. However, the likelihood is small that the lack of a negative control group compromised the validity of the study. Another limitation was the previous treatment of the rabbits with carbaryl powder 3 wk before arrival. However, the action of carbaryl is relatively short-lived,[14] and there likely were no residual effects after 3 wk. The rabbits still had high mite counts on arrival. The dose of selamectin administered in this study was 15 mg per rabbit (11 to 16.6 mg/kg), whereas the dose of permethrin used was 200 mg per rabbit (74.1 to 111.1 mg/kg). These doses were recommended in the literature for the treatment of *L. gibbus* in rabbits[4,12] and were used to replicate the methods recommended in the literature to determine their effectiveness in treating incoming shipments of rabbits for *L. gibbus*.

In conclusion, our results demonstrate that both selamectin and imidacloprid plus permethrin were effective at eliminating an infestation of *L. gibbus* in conventionally housed laboratory rabbits. However, treatment with selamectin eliminated the parasite more rapidly.

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References

1. **Bean-Knudsen DE, Wagner JE, Hall RD.** 1986. Evaluation of the control of *Myobia musculi* infestations on laboratory mice with permethrin. Lab Anim Sci **36:**268–270.
2. **Bishop BF, Bruce CI, Evans NA, Goudie AC, Gration KA, Gibson SP, Pacey MS, Perry DA, Walshe ND, Witty MJ.** 2000. Selamectin: a novel broad-spectrum endectocide for dogs and cats. Vet Parasitol **91:**163–176.
3. **Bornstein DA, Scola J, Rath A, Warren HB.** 2006. Multimodal approach to treatment for control of fur mites. J Am Assoc Lab Anim Sci **45:**29–32.
4. **Burns DA.** 1987. Papular urticaria produced by the mite *Listrophorus gibbus*. Clin Exp Dermatol **12:**200–201.
5. **Dymond NL, Swift IM.** 2008. Permethrin toxicity in cats: a retrospective study of 20 cases. Aust Vet J **86:**219–223.
6. **Fisher M, Beck W, Hutchinson MJ.** 2007. Efficacy and safety of selamectin (Stronghold/Revolution) used off-label in exotic pets. International Journal of Applied Research in Veterinary Medicine **5:**87–96.
7. **Flatt RE, Wiemers J.** 1976. A survey of fur mites in domestic rabbits. Lab Anim Sci **26:**758–761.

8. **Gonenc B, Sarimehmetoglu HO, Ica A, Kozan E.** 2006. Efficacy of selamectin against mites (*Myobia musculi*, *Mycoptes musculinus*, and *Radfordia ensifera*) and nematodes (*Aspiculuris tetraptera* and *Syphacia obvelata*) in mice. Lab Anim **40:**210–213.
9. **Hughs JE.** 2004. Diagnosis and treatment of selected rabbit dermatologic disorders. Exotic DVM **5.6:**18–20.
10. **Institute for Laboratory Animal Research.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press.
11. **Kirwan AP, Middleton B, McGarry JW.** 1998. Diagnosis and prevalence of *Leporacarus gibbus* in the fur of domestic rabbits in the UK. Vet Rec **142:**20–21.
12. **Kondo S, Taylor A, Chun S.** 1998. Elimination of an infestation of rat fur mites (*Radfordia ensifera*) from a colony of Long Evans rats, using the microdot technique for topical administration of 1% ivermectin. Contemp Top Lab Anim Sci **37:**58–61.
13. **Mercurio-Zappala M, Hack JB, Salvador A, Hoffman RS.** 2007. Pralidoxime in carbaryl poisoning: an animal model. Hum Exp Toxicol **26:**125–129.
14. **Mount ME, Dayton AD, Oehme FW.** 1981. Carbaryl residues in tissues and cholinesterase activities in brain and blood of rats receiving carbaryl. Toxicol Appl Pharmacol **58:**282–296.
15. **Niekrasz MA, Curl JL, Curl JS.** 1998. Rabbit fur mite (*Listrophorus gibbus*) infestation of New Zealand White Rabbits. Contemp Top Lab Anim Sci **37:**73–75.
16. **Novotny MJ, Krautmann MJ, Ehrhart JC, Godin CS, Evans EI, McCall JW, Sun F, Rowan TG, Jernigan AD.** 2000. Safety of selamectin in dogs. Vet Parasitol **91:**377–391.
17. **Olaf Hansen NMKPWB.** 2006. Efficacy of a formulation containing imidacloprid and permethrin against naturally acquired ectoparasite infestations (*Ctenocephalides felis*, *Cheyletiella parasitovorax*, and *Listrophorus gibbus*) in rabbits. Intern J Appl Res Vet Med **4:**320–325.
18. **Patel A, Robinson KJE.** 1993. Dermatosis associated with *Listrophorus gibbus* in the rabbit. J Small Anim Pract **34:**409–411.
19. **Pullium JK, Brooks WJ, Langley AD, Huerkamp M.** 2005. A single dose of topical moxidectin as an effective treatment for murine acariasis due to *Myocoptes musculinus*. Contemp Top Lab Anim Sci **44:**26–28.**44:**56.
20. **Weisbroth SH, Scher S.** 1971. *Listrophorus gibbus* (Acarina: listrophoridae). An unusual parasitic mite from laboratory rabbits (*Oryctolagus cuniculus*) in the United States. J Parasitol **57:**438–440.