A New Insecticide Delivery Method for Control of Fur Mite Infestations in Laboratory Mice

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THE FUR MITES, *Myobia musculi, Radfordia affinis* and *Myocoptes musculinus* (Acari: Myobiidae), are important ectoparasites of laboratory mice. These mites can infest whole colonies of laboratory rodents and may adversely affect the general health of mice[1]. Mite infestations often cause hypersensitivity-induced skin lesions[2] that can promote secondary bacterial infection. Infested mice may have altered immune statuses[3], and epidermis and hair regrowth may be affected in some mouse strains[4]. A mouse experiencing a severe or prolonged mite infestation may permanently disfigure itself by vigorous scratching.

The toxicity of mitacidal agents, both to animals and caretakers, and low treatment cost-effectiveness have hampered the control of fur mites in laboratory mice. Though scientists have assessed many methods of fur mite control[2,5-7], most methods do not work in the long-run. *M. musculi* infestations in laboratory mouse colonies can be effectively curbed with the permethrin, an acaricide, as recent evaluations have shown[8]. Permethrin, the investigators discovered, provided satisfactory short-term reductions in mite infestations. When the investigators applied permethrin directly onto the mice or mixed it in powder form with wood shavings, it did not produce any noticeable effect in the mice. Their histopathologic findings in the mouse livers, lungs and kidneys were normal. Presumably, scientists would use permethrin as an acaricide unless it was difficult to administer, or if it was expensive and ineffective.

Investigators found that permethrin-impregnated cotton, intended as rodent nest material, effectively kept immature ticks from infesting natural populations of the white-footed mouse, *Peromyscus leucopus*[9]. The investigators distributed treated cotton in dispensing tubes and placed them at regular intervals throughout the wooded and brush-covered habitat frequented by this mouse. Cotton disappeared from the dispensing tubes (presumably into mouse nests)-as it did, the investigators captured increasing numbers of tick-free mice. When the investigators placed treated cotton in captured *Peromyscus leucopus* cages, they found the animals readily accepted the cotton and would create fluffy nests with the material (unpublished).

We thought that permethrin might also kill mites infesting laboratory mice. To test the effectiveness of permethrin for that purpose, we supplied laboratory mice with cotton balls impregnated with three concentrations of permethrin.

Materials and Methods

We conducted the study in a colony containing over 1200 transgenic mice (including about 20 strains). All the mice were backcrossed to CD-1 or Balb/C mice. The colony had been in existence for about 24 months and was infested with fur mites. We housed all the mice in standard shoebox cages, each holding 1-5 mice. We kept the cages on five open stainless steel racks in one animal room. We provided the mice with water and food *ad libitum*. An animal technologist cleaned the cages weekly and lined the cage bottoms with fresh wood chip bedding (Alphachip, Northeast Wood Products).

To begin the study, we randomly selected and marked eight cages from each rack. We looked for mites and mite eggs on the heads and backs of 50% of the mice from each of the marked cages. To perform this step, we used the scotch tape impression method with microscopic interpretation: we thoroughly rubbed each mouse sampled with clear adhesive tape, affixed the hair-covered tapes to glass microscope slides, and examined them at 40X magnification. We have found this method to be as reliable as scraping or post-mortem examination for diagnosing fur mite infestations. We examined mice from each marked cage using the procedure described above both before, and at one-, two-, and four-week intervals after the permethrin treatment.

Following the initial examination, we gave the animal technologist bags of cotton balls. We had saturated some cotton balls with a range of permethrin and left some untreated. We then instructed the technologist to provide all the cages on a rack either with cotton balls containing the same concentration of permethrin or untreated cotton. To prepare the cotton balls, we soaked them in aqueous solutions[9] containing 0.16, 1.6 and 2.4% of commercially formulated permethrin (Permanone 40 Mfg-Fairfield

American Corp., Frenchtown, NJ) to produce treated cotton containing 0.5, 5.0 or 7.4% active permethrin (w/w) when dried. The volume of liquid we used just saturated the cotton mass (about 10 times the weight of the cotton). One must take into account the absorbent properties of the dosing material to arrive at the permethrin concentrations noted above.

After soaking the cotton, we air-dried it. We confirmed the concentration of permethrin in the various batches of cotton by examining acetone extracts from treated cotton balls using reverse phase high pressure liquid chromatography. For this step, we used a C18 column with the sample diluted in 80% methanol in water. We monitored the effluent from the column at 280 nm and used a computer to calculate the area of each peak.

The technologist placed up to 25 cotton balls in each cage (about five cotton balls per mouse) and put fresh cotton in all the cages at cleaning time. The animal technologist, alone, knew the concentration of acaricide used for each rack. We performed all sampling, examinations and evaluations without that knowledge.

Results

Eighty-eight percent of the mice we initially examined (n = 76) were infested with mite eggs and 40% were infested with nymphal or adult mites: Ninety percent of the mice we originally examined were infested with either eggs or mites. We identified mites of the *M. musculi*, *R. affinis*, and *M. musculinus* species, but made no attempt to separate these species in the actual counts. Heavy infestations more than 5 adult or nymphal mites or more than 15 eggs per slide-occurred on about 20% of the mice. Only about 10% of the mice we sampled suffered skin lesions or other signs of severe mite infestation.

The mice readily accepted the treated and untreated cotton and used it to construct nests. After the first treatment, the number of mice infested with either adult or nymphal mites decreased in cages with treated cotton (*See* **Table 1**), although we observed no reduction in the number of mite egg-infested mice. After two weeks of treatment, nearly all the mice nesting in cotton balls containing either 5.0 or 7.4% permethrin were free of adult and nymphal mites. Additionally, the number of animals infested with mite eggs decreased. After four weeks of treatment, all mice nesting in cotton balls containing either 5.0 or 7.4% permethrin were completely free of adult and nymphal mites, and less than half the original number were infested with mite eggs.

In contrast, fewer of the mice treated with 0.5% permethrin-soaked cotton were mite-free. After 4 weeks of this treatment, the mite infestations persisted. The number of mite-infested mice after four weeks was close to the number of infested mice in cages receiving untreated cotton.

Discussion

Investigators have found that several formulations of permethrin are effective acaricides against ectoparasites of rodents and other animals, in the wild[9], or in laboratory[8] and commercial settings[10,11]. Many of the features of this acaricide, including its low degree of toxicity to the animals and their caretakers, make it more advantageous than other products and methods for controlling fur mites.

Acute, short-term, and long-term studies show that dermal or oral exposure of up to 2000-5000 mg permethrin per kg body weight (that is up to 125 mg per 25 g mouse) is not toxic to mice[12]. We calculate that effective application rates using our method – five cotton balls containing either 5% or 7.4% permethrin per 25 g mouse – would deliver just 1200-1800 mg permethrin per kg body weight, or a maximum exposure to about 46 mg per 25 g mouse. Furthermore, impregnating cotton with permethrin binds much of the toxicant (up to 80%) to the cotton fiber so it is not readily available for absorption (B. McNally, personal communication). Most toxicological studies use application methods intended to maximally absorb solvents into the application material (i.e. oral and dermal applications formulated in corn oil or solvents).

Even considering the focused method of our permethrin application, we apparently needed a relatively high concentration of this acaricide to completely control fur mites infesting mice in our study. Reportedly, other investigators have successfully controlled *M. musculi* when directly applying as little as 0.5 mg permethrin per mouse either as a dust or liquid emulsion[8]. At our lowest treatment level (cotton containing 0.5% permethrin, or about 120 mg per mouse) we failed to completely control adult and nymphal mites, although fewer of the mice were mite-infested after 4 weeks of treatment than before treatment began.

Our treatment might require more permethrin due to the actual exposure degree of mites to the active ingredient impregnated onto the treated fiber, the nature of the impregnation process, or it may be due to different susceptibilities of mite species or strains between studies. It may be that a combination of these three possibilities accounted for the seemingly higher concentration required for successful treatment.

Though our method seems to require more chemical

than others, it has several advantages: treated nest material is odor-free; since the toxicant is contained on the cotton fiber, problems such as overdosing, spillage or toxic dusts and fumes, are reduced; animal technologists can routinely add nest material to cages when they clean them; both the cotton and the permethrin are biodegradable so technologists can dispose of them safely with other refuse; the natural nest-building behavior of mice is satisfied by the cotton supply, perhaps making them more secure and less prone to other stresses.

In our study, mice in cages with 5%- and 7.4%-permethrin-soaked cotton balls were mite-free after four weeks, although a few embryonated and empty mite eggs remained on the fur of some mice. We observed that eggs present prior to the initial treatment and eggs on control mice could be distributed anywhere along the entire hair shaft. Most of the eggs we found on treated mice later in the study were usually nearer the distal end of the hair. Eggs that we observed close to the base of the hair shaft were more likely embryonated, while eggs we observed near the distal end were most often empty. Additionally, after three and four weeks of treatment, we found eggs on fewer of the mice treated with 5% and 7.4% permethrin-soaked cotton than at the beginning of the study. Taken together, these observations suggest that new oviposition ceased near the time we first applied the permethrin.

Given the 23-day natural life-cycle of these mites[13], the fact that egg hatching is nonsynchronous and continuous, and that permethrin treatment is not ovicidal, a minimum of four to five treatment weeks for an infested mouse colony is apparently necessary to prevent reinfestation. Furthermore, supplying mice colonies with treated nest material over an extended period of time can prevent new infestations in settings when one introduces outside mice into the colony, or if feral animals could contaminate the colony[14].

TABLE 1

Summary of actual counts of mite eggs, nymphs and adults infesting mice treated with permethrin-impregnated cotton.

Rack #	Treatment	Mite stage	Proportion of mice infested on Week 0	Week 1	Week 2	Week 4
	5%	mites	5/16	1/15	1/15	0/15
		eggs	11/16	9/15	3/15	5/15
II	7.4%	mites	5/15	0/16	0/15	0/15
		eggs	11/15	11/16	2/15	3/15
III	0.5%	mites	10/16	1/15	7/15	2/15
		eggs	16/16	15/15	7/15	8/15
IV	Control	mites	6/15	13/15	9/13	4/13
		eggs	15/15	14/15	10/13	12/13
V	7.4%	mites	4/14	1/14	0/11	0/12
		eggs	14/14	14/14	4/11	6/12

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