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Multimodal Approach to Treatment for Control of Fur Mites

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Ectoparasites pose numerous research, health, and management problems for researchers and institutions. Our facility management experience was complicated by recurrence of murine fur mite (*Radfordia affinis*) infestation after several rounds of single-mode fur mite treatment with dichlorvos in the cage bedding. Subsequently, we successfully eradicated the fur mites using a multidrug therapeutic protocol. Over an 8-wk treatment period, 2 applications of topical selamectin were administered in conjunction with amitraz- and fipronil-treated nestlets changed weekly. Mice tolerated the therapy well with no side effects noted, and to date there has been no recrudescence. To our knowledge, this report is the first to describe combined use of these specific therapeutic agents to control fur mite infestation in laboratory mice.

Several species of acarids (fur mites) infest laboratory mice; these include *Myobia musculi*, *Radfordia affinis*, *Mycoptes musculinus*, and *Psorogates simplex* (which is much less common than the other 3 species).[6] Fur mites have been reported to be present in up to 15% of barrier facilities and 30% to 40% of conventional colonies.[10,21] Despite the high incidence, fur mites are still considered an exclusionary pathogen and constitute a potential obstacle to data interpretation and to interinstitutional transfer of rodents.

Historically the treatment of fur mite infestations in laboratory rodents has proven difficult, and fur mite infestations have plagued research institutions for years. Despite the numerous therapeutic agents available to treat affected animals, outbreaks continue to occur, often related to recrudescence of a past infestation, or to a new outbreak associated with recent rodent importation from another institution and failure to detect positive animals during the quarantine period. Ectoparasites pose numerous problems for researchers and institutions alike, preventing the import or export of infected mice to and from facilities and impeding the sharing of animal models and the propagation of unique mice lines. In addition, numerous problems, such as decreased breeding rates, weight loss, skin lesions associated with ulcerative dermatitis, amyloidosis, and host immune system alterations, can confound research data.[1,9] Consequently, fur mites remain an excluded pathogen and the basis for denying transfer to many research facilities.

Previous studies indicate that a variety of treatment options have been attempted with varying degrees of success.[3,11,21] Recently a single application of moxidectin was reported to be an effective treatment for fur mites of the species *Myocoptes musculinus*.[21] Another agent, ivermectin, which has a wide margin of safety and is easy to apply topically, has had adverse effects such as impaired breeding, increased cannibalism of neonates, increased seizure activity, and death when given orally or parenterally.[3,6,22] Although ivermectin appears to be relatively safe for use in adult mice, with little reported effect on general health or body conditions and coordination, one study reported that ivermectin altered performance in some specific and sensitive behavioral tasks in mice.[9] Ivermectin use has also been linked to toxicity in young mice. Upon treatment with a 1:10 dilution of ivermectin, C57BL mice under 3 wk of age exhibited whole body seizures and tremors.[22] Similarly, Lankas and colleagues[12] demonstrated that newborn rats show increased sensitivity to the CNS toxicity of ivermectin.

Many reports claim efficacy for single-treatment regimens in treating fur mite infestations in a laboratory setting. However, our facility recently experienced recurrence of mite infestation after 4 rounds of single-mode fur mite treatment using dichlorvos (Atgard C, Boehringer Ingelheim, Ingelheim, Germany), provided as 1/2 teaspoon in the bedding of pine shavings and changed weekly for 3-wk cycles during a 2-y period. Potential reasons for this failure include insufficient contact with the insecticide, insufficient concentration of product used, and decreased sensitivity of the mites to the insecticide. The facility was a barrier facility that required scrubs, shoe covers, hair bonnets, and gloves for entry. Approximately 1500 cages of more than 10,000 cages were affected. Traffic to affected rooms was controlled closely: rooms were entered last for husbandry, veterinary care, and research activities, and no other rodent room was entered afterward.

Amitraz (Mitaban, Pharmacia & Upjohn, Kalamazoo, MI) has been used clinically in the treatment of generalized demodecosis (*Demodex canis*) in dogs in veterinary practice settings. However, to the best of our knowledge, no published studies document the use of amitraz for the treatment of fur mites in laboratory mice. As such, its use in mice for the treatment of fur mites was considered an off-label treatment. Treatment failure with amitraz could possibly occur, due to development of resistance. One study reported that amitraz resistance developed in the tick *Boophilus microplus* after several years of use in cattle populations in Caledonia.[8] Although the mechanism of action of amitraz is presently unknown, the current data, according to the manufacturer, suggest that amitraz may act upon the central nervous system of target organisms. The product insert[18] states that in vitro tests indicated that amitraz does not have noteworthy cholinesterase inhibitory action.

Recently published studies have documented instances of treatment failure and resistance of *Sarcoptes scabei* to treatment with such agents including ivermectin, lindane, crotamiton, and benzyl benzoate and resistance to 5% permethrin.[24] In

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one report, 2 cases of recurrent scabies infections in aboriginal people were found to have developed resistance both in vitro and in vivo to multiple doses of ivermectin despite previous success with such treatment.[4] The study reports that treatment failure or early recrudescence is common with single doses of ivermectin and that past infections had been curable with multiple doses of ivermectin.[4] This report is one of the 1st to document the emergence of resistance of ectoparasites to readily available treatments and highlights the need to develop novel drugs or novel strategies of treating with multiple agents in combination.

Recently, with the availability of fipronil (Frontline Plus, Merial, Duluth, GA), and selamectin (Revolution, Pfizer, Exton, PA), new potential topical treatments for fur mites have arisen. These compounds are marketed and available for topical treatment and control of infestations of common ectoparasites such as fleas, ticks, ear mites and sarcoptic mange in dogs and cats. One product we opted to use, Frontline Plus, contains the active ingredients fipronil and (S)-methoprene. Fipronil is a neurotoxin gamma-aminobutyric acid channel agonist with specificity for the insect cellular target. A study by Zhao and others[26] concluded that fipronil also uses glutamate-gated chloride channels to exert toxic effects on insects utilizing this target, because these channels exist in invertebrates but not vertebrate species. It was also reported that the sensitivity of insects to fipronil is 700- to 1300-fold higher than that of rats, a difference that could account for its high insecticide activity and low toxicity to mammals.

(S)-methoprene, the other active ingredient in Frontline Plus, is an insect growth regulator that kills developing flea eggs and larvae. Fipronil collects in the oils of the skin and hair follicles and is released onto the coat and skin, and (S)-methoprene translocates on the coat as well. A 2003 report by Curtis[5] indicated that the use of fipronil as either the 0.25% spray or the 10% concentrated solution could be used as an alternative treatment to lime sulfur dips for cheyletiellosis in cats. In light of the modes of action, the use of Frontline Plus may be an effective treatment for fur mites in rodents.

Selamectin, the active ingredient in the product Revolution, also a neurotoxin, binds to insect glutamate-gated chloride channels, causing them to open. The drug is absorbed through the skin and distributed through the blood; it concentrates in the sebaceous glands. Once selamectin binds to the receptor, the channels remain open, and chloride flows into the nerve cell, which causes damage to the nerves and muscles and results in neuromuscular paralysis in targeted parasites.[19] According to the material safety data sheet[17], selamectin is licensed for use in dogs to control flea infestation (*Ctenocephalides felis*), heartworm disease (*Dirofilaria immitis*), and ticks (*Dermacentor variables*) and for the treatment and control of sarcoptic mange (*Sarcoptes scabei*). It is licensed for use in cats to control flea infestations and heartworm disease and for treatment and control of ear mites (*Otodectes cynotis*), roundworms (*Toxocara cati*), and intestinal hookworms (*Ancylostoma tubaeforme*). A 2002 report by Chailleux[2] illustrated the efficacy of selamectin in the treatment of cheyletiellosis in cats. According to the organisms targeted, Revolution might be an effective product for treating fur mites in rodents.

Since their development and widespread availability, both selamectin and fipronil have been reported to successfully treat ectoparasites in guinea pigs, rabbits, wild mice, and other species. In 2002, Pritt[20] described the use of fipronil and selamectin individually to eradicate lice infestations in guinea pigs. McTier and others[13] published the results of a study using selamectin topically to treat ear mite infestations in rabbits which can eradicate infestations with *Psoroptes cuniculi*. Fipronil was reported to be useful to control immature *Ixodes scapularis* in *Peromyscus leucopus*[7] and was found to effectively kill 100% of adult fleas for at least 10 wk when used on California ground squirrels.[14]

Few adverse side effects have been reported with relatively low doses of fipronil. However, at higher doses, some negative effects have been reported. One such study indicated a decrease in the pregnancy index in Wistar rats that were given topical applications of fipronil at a dose of 280 mg/kg, which resulted in a 67% reduction in pregnancies among treated rats.[16] In addition, the United States Environmental Protection Agency Fact Sheet states that decreased litter size, decreased weights of litters, decreased fertility, and fetal death had been observed in rats tested at higher doses.[23] In comparison, the doses we used in the current study were much lower (8.28 mg/kg) than doses reported to have negative effects.

Therefore we hypothesized that the use of new insecticides in combination would be effective in the eradication of a persistent infestation at a large academic institution. Here we report on a fur mite outbreak at our facility that was successfully treated with low doses of multiple agents in conjunction after repeated attempts with a single agent failed to control a persistent infestation.

Materials and Methods

Humane care and use of animals. All animals were handled according to approved protocols from the Institutional Animal Care and Use Committee at Harvard University and were housed in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, and in compliance with the *Guide for the Care and Use of Laboratory Animals*.[15]

Mice. The mice in this study were of the FVB, CBA/J, and C57BL/6J strains, including some knockout mice on C57BL/6J background. Additional strains may have been present, but because investigators were not required to list strains on cage cards, some strains treated may not have been accounted for. Animals treated were of various ages, ranging from 10-d-old neonates to adult. All mice entered the facility from one of several approved commercial vendors or from academic institutions after successful completion of an 8-wk quarantine evaluation, during which the mice were subjected to dichlorvos treatment in the bedding for the initial 3 wk, and sentinels were not treated.

Housing and husbandry. All mice were housed in rodent rooms in a 'shower-in' barrier facility with autoclaved bedding, water, and cages. Mice were housed at 4 adults per cage or dam and litter in ventilated caging systems (Techniplast, Exton, PA), with 75 air changes per hour under positive pressure. Animal rooms were kept on a 12:12-h light:dark photoperiod, with temperature maintained at 22.8 to 23.8 °C and relative humidity of 30% to 70%. Cages were changed every 2 wk, and water and food were changed every week. All animals were fed rodent chow (Purina 5058, PMI Nutrition International, St Louis, MO) and had access to ad libitum autoclaved water.

The sentinel program consisted of testing sentinel mice every 3 mo. The sentinels were exposed to dirty bedding of cages on the same rack for a minimum of 6 wk. Sentinel testing included testing for ectoparasites (fur mites) via pelage plucks, endoparasites (pinworms) via tape tests, and fecal floatation. Serology was done to screen for Sendai virus, pneumonia virus of mice, mouse hepatitis virus, Theiler murine encephalomyelitis virus, reovirus, *Mycoplasma pulmonis*, mouse parvoviruses, and rotavi-

rus. Once a year serology also included testing for lymphocytic choriomeningitis virus and ectromelia virus. A gross necropsy was performed on all sentinels.

Additional personal protective equipment (2nd set of shoe covers, hair bonnet, disposable lab coat) was required for entry into rooms affected by the fur mite outbreak, in addition to gear (scrubs, shoe covers, hair bonnet, and gloves) mandatory for the rest of the barrier facility. Prior to exiting a fur mite-positive room, the extra protective equipment was removed and disposed of in the room.

Treatment and testing. Approximately 1500 cages, involving 2 rooms in 1 facility building, were treated overall during the 8-wk study. Mice were housed with a maximum density of 4 adult mice per cage; however, only a small sampling of cages were directly tested with pelage plucks over the course of the treatment period, due to time and labor constraints. Prior to treating all mice in both rooms, 2 pelage plucks from the back of the neck and over the shoulders were collected per animal from a subset (n = 48) of mice that had previously tested positive and had been treated previously with Atgard. At the start of the treatment program, all 12 cages tested (4 mice per cage) were positive based on pelage plucks. Hair was placed on a drop of oil on a microscope slide and examined for the presence of mites and eggs. These cages were identified as positive during a previous outbreak and despite treatment remained positive for mites.

The topical treatment was prepared by diluting 1 ml of 120 mg/ml selamectin in 70% ethanol to yield a treatment dose of 290 mg/100 ml, as described by Winchester and others.[25] All furred mice receive an initial topical dose of 0.1 ml of the selamectin solution. Furred mice included any neonate older than 10 d of age and all adult mice including pregnant and nursing females. A 2nd topical application was applied 30 d after the initial dose. At the time of the 2nd application, any neonate that was too young (not furred) at the time of the initial dose received a single topical dose of selamectin. To supplement the topical treatment, all cages received nestlets (Ancare Corporation, Bellmore, NY),—one infused with fipronil (0.29%, 3 ml total) and one infused with amitraz (19%, approximately 4 ml)—at the start of topical treatment. To infuse the nestlets, sheets of nestlets were placed in large sanitized plastic containers, and then the solution was distributed equally over the sheet to ensure all nestlets had coverage. Nestlets were allowed to absorb the solution and then were placed in stacks in plastic bags, labeled, and brought to the animal facility for use. Nestlets were replaced with freshly treated nestlets weekly for 8 wk.

Mice from the 12 known-positive cages were tested at 18, 48, 65, and 75 d after the 1st topical treatment. Mice were monitored for any signs of toxicity or illness, including neurologic signs, seizures, tremors, muscle weakness, and morbidity.

Results

Radfordia affinis mites and eggs were observed on all slides prior to the start of treatment. During the treatment period, mice did not exhibit any adverse clinical signs or any neurologic signs. The initial pelage test revealed the presence of live adults and viable eggs on all mice. However, by 18 d after the initial topical treatment, pelage tests from cages that had originally tested positive were negative for live adult mites, although egg casings and eggs were still observed in some cages (Table 1). By day 48, no adult mites were seen, only desiccated eggs (presumably nonviable) and egg casings were present. Nestlet changes proceeded for the entire 8-wk period. At the conclusion of the 2-mo treatment period, negative results were verified with pelage plucks on days 65 and 75. The results confirmed the absence of live adults and desiccated eggs in all treated mice. All treated rooms continued to be negative according to both sentinel reports and random pelage plucks of previously positive cages 5 mo after cessation of treatment. In light of the negative pelage plucks on previously positive cages and negative sentinel tests, the additional protective equipment requirements were lifted and rooms returned to normal health status. In addition, animals exported to other institutions underwent fur plucks, and no adult mites or evidence of eggs have been detected (data not shown).

Table 1. Results of cages with findings from pelage plucks during treatment period

No. of days post-treatment	No. of adult mites	No. of eggs	No. of casings and nonviable eggs
0	12	6	0
18	0	1	3
48	0	0	5
65	0	0	1
75	0	0	0

Discussion

An outbreak of fur mites in a barrier facility is problematic for veterinary staff and researchers alike. Detection can be challenging, with a high probability of false negatives. Successful treatment is difficult to achieve and maintain because of the risks of reinfestation or recrudescence, possibly in part due to parasiticide resistance. To reduce the possibility of resistance or recrudescence, we hypothesized that a multidrug protocol would be effective at controlling a fur mite infestation at our institution.

A 2-mo cycle of topical treatment was chosen to ensure that multiple life cycles of mites were overlapped. The exact lifecycle of the *Radfordia affinis* mite is not well described, but transmission is known to be via direct contact. The addition of fipronil- and amitraz-treated nestlets provided treatment coverage for neonates that were too young to receive topical treatment. Cages with litters not receiving topical treatment were noted so that they could be treated during the 2nd round. This aggressive treatment strategy was implemented to combat the outbreak, which typically is difficult to eradicate for a variety of reasons. First, there is difficulty in accurately testing for the presence of fur mites, due to the high rate of false-negative tests. Second, it is challenging to distinguish between recrudescence of an outbreak versus reinfestation. Therefore, to help minimize these risks, we chose to treat with 2 doses of topical selamectin 30 d apart in conjunction with 2 different types of treated nestlets, and this regimen was effective in controlling fur mite infestations at our facility.

In general, adult mice of various strains tolerated the multiagent approach, with some mice showing increased activity after topical application, but no other adverse effects were noted. It is possible that there may be increased incidence of adverse effects in neonates, such as death or neurologic signs such as muscle twitching or seizures, although these signs were not noted during this study. One reason for this lack of adverse effects may have been that neonates younger than 10 d were not treated topically but only were exposed to treated nestlets. Strain variability may represent a potential source of adverse effects, but during our study period this pattern was not observed with the limited number of strains of mice housed in affected rooms. It

is reasonable to assume that there exists strain variation with respect to susceptibility to 1 of the compounds used, but we did not note this pattern, and there are no documented cases in the literature. This multimodal to treating a fur mite outbreak at our facility has been successful, with no recrudescence noted at 5 mo after the cessation of the last nestlet change and more than 6 mo after the last topical application.

We recognize that this treatment plan is bold and may be cost-prohibitive for some institutions. However, based on an average caging capacity of 4 adult mice per cage, the cost in our study was $3.17 per cage or $0.79 per mouse for a 2-mo treatment period. The cost of the labor was not figured into the treatment cost, as nestlets were added at regular cage changes by the animal care staff. The application of the topical treatment required approximately 12 h time of 2 people. The costs associated with this treatment regimen were determined to be reasonable at our institution, as compared with those of other possible actions that were unpopular among research investigators. Such possibilities included rederiving colonies and depopulating and repopulating. This treatment regimen was considered the most feasible in terms of time and cost of lost time and animals to researchers with affected animals. A recent publication by Pullium and colleagues[21] indicates effective treatment of fur mites with a single dose of moxidectin, and institutions may decide to use this method and find success. A multimodal approach still may be needed in situations in which eradication with a single agent has repeatedly failed or in which recrudescence is a chronic problem, such as was encountered at our institution. Currently there are a variety of methods, and this multimodal approach was effective in treating a chronic problem that had plagued our institution for several years.

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