Managing Mouse Fur Mites at Emory

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**Executive Summary**

**The problem:**
Over the course of 2010 and 2011, mice infested with fur mites were identified in six rooms in the Whitehead Biomedical Research Building. Evidence suggested fur mites entered the facility on mice from unapproved vendors that were quarantined. Standard quarantine practices involved both treatment and diagnostics for fur mites. However, diagnostics for mite detection have at best an 85% sensitivity, and treatment programs which were considered to be efficacious ultimately turned out not to be. Thus the quarantine program was ineffective in preventing the infiltration of facilities with fur mites. Compounding the problem, mice are transported daily from the Whitehead building to other buildings on campus, thus it appears reasonable to presume that mites are present elsewhere on campus. The potential adverse effects of fur mites include dermatitis, allergic-type responses, abnormal behavior (e.g. scratching or excessive grooming) and immune dysfunction. Management of the problem requires two approaches: 1) decrease the probability of future importation of mites and 2) manage the fur mites already on campus.

**Actions taken to date:**
1) **Mite positive rooms have been quarantined.** Rooms with significant infestations were treated, with mite populations believed to have been knocked back. Degree of sterilization is unknown. Rooms with minimal evidence of infestation have been quarantined with treatment plans pending global decisions.
2) **Mouse quarantine practices have been changed.** Effective October, 2011, the DAR changed its quarantine practices to no longer accept live breeding pairs into quarantine. This change dramatically reduces the chance of exposing the existing census to new infectious diseases.
3) **Options for rederivation have been increased.** In addition to Emory’s Transgenic Mouse Facility (TMF), the DAR now offers sperm rederivation and cryopreservation services as a mechanism for mouse importation. Plans are underway to train DAR technicians in oviduct harvest to add the option of rederiving lines through fresh embryo transfer.
4) **An informal survey has been sent to sister institutions to assess their experience with mouse fur mites, treatment thereof, quarantine practices, movement of mice and availability of campus services for rederivation.** Responses to date are variable and reflect the complexity of the issues.

**Moving forward:**
The change in the mouse quarantine program dramatically reduces the possibility of future infestation. It does nothing to address the problem of the existing mite infestation, however. Additionally, the change has resulted in a nontrivial level of concern on the part of investigators. Some concerns result from misinformation and simple conversation has addressed them. Other concerns involve length of time needed for rederivation and strain background. We believe enhancing the ability to perform fresh embryo rederivation will address these concerns. A valid concern is that rederivation yields mite-free animals which must, at the moment, be housed in facilities which may be contaminated by fur mites.
To address the latter problem, there are several possibilities. First, we propose strong consideration be given to designating the Health Sciences Research Building as a “clean” facility, allowing investigators the option of rederiving mice into it and maintaining them free of select pathogens. Creation of the clean facility would allow Emory investigators the benefits of pathogen-free mice and facilitate collaboration with outside institutions. A more thorny solution was discussed previously and involves treatment of mice with ivermectin-impregnated feed. This technique was described and used enterprise-wide by a respected colleague who has remained mite-free for 4+ years [1]. Unfortunately, while this method is among the least labor intensive (compared to topical treatment of individual mice), it is still costly, time-consuming, and ivermectin has real potential detrimental effects on research. An alternative to enterprise-wide treatment is spot-treatment, which has the advantages of lower cost and less disruption to potentially innocent bystanders, but has the disadvantage of possibly allowing a low-level fur mite infestation to continue.

Our recommendations: Our first recommendation has already been enacted: to change the quarantine program. We suggest staying the course here as this action dramatically reduces the chance of importing more fur mites. Secondly, we strongly recommend the designation of the HSRB as a clean facility. This will meet investigator needs for clean mice, both on campus and for collaboration. Thirdly and in the short term, we recommend spot-treatment with ivermectin-compounded feed and monitoring of the existing mite infestation. Admittedly, the chance of successful eradication may be lower with this approach. But the enterprise-wide treatment with ivermectin, while possibly necessary, carries with it the risk of lethality and altered physiology enterprise-wide. The more “piecemeal” approach allows the institution to address the problem systematically, with less immediate expense and large-scale risk to research, and in a way that best meets investigator needs. However, this latter approach carries with it the risk of eradication failure. We will assess the impact of the changes we have made and will make, and acknowledge the possibility that further action may be needed in the future.
Further information
Fur mites
Fur mites are one of the four most common pathogens found in laboratory mice (the others being pinworms, paroviruses and noroviruses). The last two comprehensive surveys, conducted 10 years apart (1996 and 2006), assessed the prevalence of common pathogens in major US institutions, and indicated that fur mites were present in 30 to 40% of institutions responding [2, 3]. Additionally, consultations with colleagues show that our situation is not unique and that fur mites are frustrating animal resources programs across the country. Mouse fur mites dwell on the skin surface, feeding on epidermal tissue. Transmission of adults is by direct contact, although transmission via eggs on fomites such as bedding or nestlets is also possible. The mites are species-specific, and cannot be transmitted to people or pets. There are three common species of mouse fur mites (Myocoptes musculus, Myobia musculi and Radfordia affinis), and evidence suggests that DAR mice may be infested with all species. Both Myocoptes and Myobia have been visualized by direct examination of pelage samples, and Radfordia was diagnosed on PCR testing.

The impact of fur mites (and other infectious diseases should they be encountered) is compounded by the culture at Emory of free-flow of animals to and from labs and between buildings. This type of movement facilitates research, allowing animals to breed in one building, for example, but be used in another. It allows for the development of core services such as those for behavioral phenotyping or imaging, saving valuable research dollars by decreasing the redundancy of equipment and use of space. Movement of animals to facilitate research and collaboration has always been the culture at Emory, and in fact is architecturally unavoidable in some of both the older and the newer facilities. Older facilities simply do not have procedural space available (e.g. the Woodruff Memorial Research Building) and some of the newer facilities have large mega-labs shared by multiple investigators (e.g. the Pediatrics and Whitehead Buildings). From an infectious disease perspective, however, free movement of animals adds to the risk of cross-contamination.

The potential adverse effects of fur mites include dermatitis, allergic-type responses, abnormal behavior and immune dysfunction. Clinical signs in infested mice include pruritus, lymphadenopathy and weight loss. Immunocompetent mice mount a robust immune response characterized by elevated IgE levels, lymphocytopenia, eosinophilia and increases in inflammatory cytokines [4]. Inter-institutional collaborative research is also impacted by fur mites, with some institutions refusing to receive mice from institutions positive for fur mites and others requiring additional testing (and expense) prior to shipment. Appendix 1 is a list of some institutions and Emory investigators impacted in this way. Safe and effective treatment is therefore imperative both for the sake of the animal and for the research enterprise.

Ivermectin
Ivermectin was the first of the avermectins to be used in veterinary medicine [5]. It was first registered in 1981 in France for use in cattle and has been widely used as an endectocide in a variety of species for 30 years [5]. It is also the preferred treatment for
human filarial infections, such as *Wuchereria bancrofti* [6]. A macrolide antibiotic in the avermectin group originally derived from a Japanese soil fungus, *Streptomyces avermitilis*, it is an agonist for the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and can be administered orally, topically or by injection [7]. The drug acts by binding GABA-gated chloride and invertebrate-specific glutamate-gated anion channels in peripheral neuromuscular synapses, suppressing nerve impulse conduction [1]. Its usefulness as an anthelminthic results from differences in the distribution of GABA receptors between mammals and arthropods or nematodes: GABA receptors in mammals are mostly in the central nervous system (CNS) protected by the blood brain barrier, whereas in arthropods and nematodes they are found in the peripheral nervous system at the neuromuscular junction. Stimulation of GABA receptors in endo- and ecto-parasites causes flaccid paralysis and inhibits feeding of the parasite [6, 8].

**Potential adverse effects of ivermectin**

Although ivermectin has a wide margin of safety in most mammalian species, some animals are particularly sensitive to toxicity.  Ivermectin toxicity can appear in the form of severe central nervous system side effects such as depression, coma, and death [6]. Animals exhibiting these symptoms are believed to have an absence or functional deficiency of P-glycoprotein in nervous system capillary endothelium [6]. P-glycoprotein plays a role in the blood brain barrier, acting as an efflux pump to prevent the entry of specific drugs into the nervous system [9]. Ivermectin is highly lipophilic and thus usually has very poor penetration of the blood brain barrier, due to the action of the drug efflux transporters. Deficiency or disruption of this gene leads to enhanced absorption and exposure of the brain to a number of drugs, including ivermectin. Ivermectin treatment has also been reported to cause subtle effects on behavioral testing [10] and on immune function [4, 11].

Severe central nervous system side effects of ivermectin been reported particularly in collie dogs and CF-1 mice. Sensitive collies show reactions at 1/200th of the dose required to show toxicity in other dogs [6]. These collies have a genetic deletion that generates a frame shift, resulting in premature termination of the synthesis of p-glycoprotein [6]. Mice with abnormal P-glycoprotein, and thus ivermectin sensitivity, include a subpopulation of about 25% of CF-1 mice [5, 12] and other mice homozygous for disruption of the Abcb1a (previously known as mdr1a) P-glycoprotein gene [5, 13]. These mice are phenotypically the same, and show enhanced central absorption of ivermectin, while blood and hepatic levels are the same as wild-type mice, showing that differences in ivermectin disposition arise through a deficiency in p-glycoprotein rather than through an alteration in drug metabolism [6]. Ivermectin-sensitive CF-1 mice and P-glycoprotein-deficient mice evidence the severe neurologic side effects of coma and rapid death. In addition to side effects related to genotype, reports have described ivermectin toxicity in neonatal rodents [14], perhaps because P-glycoprotein protein expression in brain capillary cells is incomplete until postnatal day 21 [15]. Surprisingly, ivermectin did not alter seizure responses in either seizure-prone or seizure-resistant mice [16].

Reports of toxicity in other species include humans being co-treated for Onchocerciasis and *Loa loa*, particularly those with high microfilarial counts. In this instance, drug
interactions combined with a massive die-off of microfilaria are presumed to be the culprit [6]. Horses, cattle, pigs and rabbits show signs of neurotoxicity including depression, ataxia, rigidity, and impaired vision when given doses in excess of 4-8 times the recommended dose [5].

Aside from toxicity, treatment with ivermectin has been reported to cause subtle effects on behavioral testing and immune function in mice. In one study, treated mice were normal with regard to body weight, motor behaviors and the performance of a spatial memory task. However, ivermectin produced real changes in other behaviors. Mice were significantly more active in the open field exploration test during ivermectin treatment than before treatment, had a greater acoustic startle amplitude than control mice, and had variably lower prepulse inhibition, depending on mouse strain [10]. These results showed that ivermectin affected the behavioral responses to certain stimuli and not others, potentially stemming from a hyperreactivity to environmental stimuli in certain circumstances. In terms of immune function, ivermectin was shown to have anti-inflammatory properties, significantly diminishing the recruitment of immune cells and cytokines in a mouse model of asthma [17]. Other studies have showed an immunomodulatory effect on T-helper cells [11] and T-cell related genetic deletion in transgenic mice administered ivermectin [18].

Published report on use of ivermectin-compounded feed [19]
An institution focused on cancer research consisting of 3 vivaria, 30,000 mouse cages and approximately 120,000 mice experienced an outbreak of mouse fur mites (Myobia musculi and Myocoptes musculinus). Due to extensive trafficking of mice, the outbreak was extensive, involving approximately 40% of rooms. Because of the scale of the outbreak, the decision was made to treat all mice, regardless of apparent infestation status. Ivermectin-compounded feed was chosen as the treatment modality due to the effectiveness of the drug and the labor savings associated with a feed-based treatment mechanism (versus individual treatments). Investigators were informed of the plan by email and flyer 2 months beforehand. Because some strains and stocks are sensitive to ivermectin toxicity, investigators were requested to identify 1-2 cages for each unique strain so they could be pre-treated with the feed for 1 week and monitored for any adverse effects. If they suffered adverse effects, their cohorts were treated with an alternative treatment. Ninety percent of the mice were treated for 8 weeks with ivermectin-compounded feed. The other 10% were exempted, either because they were on special diet or suffered adverse effects. These cages were identified by the placement of a special card and treated individually with topical selamectin. On the first day of treatment, all feed was removed from hoppers and from the room. All feed was replaced with medicated feed (dyed blue for identification). At the end of the 8th week, all replacement feed in room was replaced with unmedicated feed, but feed hoppers were not emptied – they were topped off with unmedicated feed. Thus some mice received medicated feed for up to 12 weeks. Daily monitoring was done by animal care staff with adverse effects reported to veterinary staff. Additionally, breeding performance of select strains in the breeding colony was monitored. At the end of the treatment period, all sentinels were replaced due to the possibility of false-positive results due to remnants of mite eggs being present. During the treatment period, only one investigative group
reported adverse effects in their colony; these were genetically engineered strains which received intracranial injections and suffered a decreased life span seemingly as a result of treatment.

Personal communication with the reporting investigator has shown the institution to be mite-free after 4+ years.

**Costs**
The costs associated with enterprise-wide treatment with ivermectin-compounded feed are non-trivial. Quotes provided for the feed alone are $131K. Additional costs include the costs of the feed which must be discarded initially to be replaced with the medicated diet and the labor to empty and refill hoppers, to conduct a week of pre-treatment, and to monitor. The entire sentinel population also should be replaced due to the possibility for false positive results. None of these costs address the possible costs of research disruption or inadvertent adverse consequences. The vast majority of the mouse population would likely show no adverse effects, but it can only be presumed that some strains will be sensitive to the treatment.

**Informal survey of sister institutions**
On 2/16/12, at the suggestion of the SOM RAC, an email was distributed to the University of Pittsburgh, Vanderbilt University, Duke, Yale, University of Washington, University of Michigan, and the University of Alabama. The email requested response to questions relating to mouse quarantine, fur mites, availability of campus services for rederivation and movement of mice around campus. Responses received are provided in full as an appendix and reflect the complexity of the issues and the fact that there is no “correct” formula to address the issues we face. As of 2/22/12, 5 of 8 institutions responded. Responses are summarized here:

- **Fur mites**: 3/5 are currently battling fur mites and 4/5 have done so within the past year. Rooms were quarantined and treated with various compounds, including ivermectin (both topical and oral), moxidectin, and fipronil. The perception of treatment efficacy was wishy-washy, with one institution citing “few” post-treatment failures.
- **Quarantine**: 5/5 institutions have a quarantine program, one with isolators. 2/5 do not allow breeding in quarantine and 1/5 allowed it only after an unspecified treatment period.
- **“Clean” facilities**: 3/5 have facilities which only accept mice from approved vendors or via rederivation. 1/5 has also been discussing it but has no space to do so. The other 1/5 claimed all facilities are clean, but they allow mice from quarantine into the general population.
- **“Dirty” facilities**: 2/5 designate varying health status of their rooms, from clean to dirty.
- **Mouse movement**: 4/5 restrict mouse movement back into “barrier” facilities. Thus mice can move one way (out). 2/5 treat or quarantine mice prior to moves between buildings.
• Availability of campus services for rederivation: 2/5 indicated that such is an important part of their pathogen eradication program. 3/5 either do not have such services or the services are not available for rederiving infected mice.

Conclusion
The issues we face are complex. All parties agree that there is a problem that should be addressed (fur mites). Views diverge, however, on how best to manage the problem. Each strategy meets some needs and creates concerns for others. Our first recommendation has already been enacted: to change the quarantine program. We suggest staying the course here. This change in practice dramatically reduces the chance of bringing in many infectious agents. We believe investigator concerns can be addressed by working with individual investigators and also through greater collaboration between the DAR and the TMF, a process which has already begun. Secondly, we recommend providing Emory University investigators with a facility that is pathogen-free for enzootic agents on campus which tend to be issues for collaboration: the HSRB, due to open April, 2013. We further recommend spot-treatment with ivermectin-compounded feed, thereby addressing the existing identified problem and providing us with expertise in the use of the product. We will continue to monitor the population, assessing for as-yet-unidentified infestations and the impact of the changes to our quarantine practices (changes which, as an aside, lead to an estimated savings of 69K in 5 months). This approach allows the institution to address the problem systematically, with less immediate expense and large-scale risk to research, and in a way that best meets investigator needs. We will assess the impact of the changes we have made and will make, and acknowledge the possibility that further action may be needed in the future.

References


