Use of Permethrin Eradicated the Tropical Rat Mite (*Ornithonyssus bacoti*) from a Colony of Mutagenized and Transgenic Mice

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The tropical rat mite, *Ornithonyssus bacoti*, was identified in a colony of mutagenized and transgenic mice at a large academic institution. *O. bacoti* is an obligate, blood-feeding ectoparasite with an extensive host range. Although the source of the infestation was likely feral rodents, none were found in the room housing infested mice. We hypothesize that construction on the floor above the vivarium and compromised ceiling integrity within the animal room provided for vermin entry and subsequent *O. bacoti* infestation. *O. bacoti* infestation was eliminated by environmental decontamination with synthetic pyrethroids and weekly application of 7.4% permethrin-impregnated cotton balls to mouse caging for five consecutive weeks. Visual examination of the macroenvironment, microenvironment, and colony for 38 days confirmed the efficacy of treatment. We noted no treatment-related toxicities or effects on colony production.

An effective, comprehensive pest management program is crucial to the operation of an animal resource program and the conduct of quality animal research. Ideal pest control programs preclude the entry of vermin and eliminate their harborage within the facility (16). Despite vigilance, vermin infestations occasionally occur and require prompt eradication to prevent compromise to animals, personnel, and research.

The tropical rat mite, *Ornithonyssus bacoti* (Fig. 1), is an obligate, blood-feeding parasite with world-wide distribution. *O. bacoti* outbreaks involving laboratory rodents and personnel have previously been reported (9-12). Natural hosts of *O. bacoti* include several species of rats and mice, hamsters, gerbils, voles, and other wild rodents. Nonrodent hosts include cats and other wild and domestic carnivores, some birds, opossums, and humans (1, 7).

In laboratory mice, severe *O. bacoti* infestations have been associated with decreased litter production (12), anemia, and death (11). In most instances, outbreaks of *O. bacoti* are recognized only when humans are attacked in the absence of an animal host (8). The nonspecific dermatitis caused by the mite's attack commonly is referred to as "rat-mite dermatitis" and results from an inflammatory reaction to the mite's saliva as it takes a blood meal (4). Several infectious diseases including murine typhus, rickettsialpox, Q fever, tularemia, eastern equine encephalitis, hemorrhagic fever with renal syndrome, coxsackievirus, Langat virus, and plague have been experimentally transmitted by *O. bacoti* (1, 7), raising additional public health concerns.

Methods to eliminate *O. bacoti* from laboratory facilities can be confounded by the parasite's 13-day life cycle (1, 6) which leads to a rapid increase in the number of mites, difficulty in detection and identification, large numbers of eggs released per female (12), harborage of ova in the environment, ability to live 2 to 3 weeks off the host (4), and failure to eradicate feral rodents (21). This report describes the eradication of *O. bacoti* from a colony of mutagenized and transgenic mice. Whereas previous reports have described the use of various insecticides to eradicate *O. bacoti* from laboratory mice (10-12), ours is the first to describe a multipronged approach using permethrin.

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Materials and Methods

Animals. We were confronted with the task of eradicating *O. bacoti* from a colony of 2200 mice housed in a barrier area within a large animal facility. The mice were part of an N-ethyl-n-nitrosourea mutagenesis program studying the role of the genome in nervous system development and function. The barrier area was located in a corner of the animal facility and consisted of a central corridor, four animal rooms, and a storage room. Entry into this area was by card access and required removal of personal clothing and donning commercially laundered scrubs, disposable gowns, masks, gloves, hair bonnets, and shoe covers. The colony was contained in one room and was derived from one commercial source and five in-house breeding programs.

Mice were housed in static, polycarbonate isolator cages (Allentown Caging Equipment, Co, Inc., Allentown, N.J.), on autoclaved, contact hardwood bedding (Northeastern Products, Warrensburg, N.Y.), with four to five mice per cage and 80 to 93 cages per rack on five racks, and maintained on a 12:12-h light:dark cycle at 72 ± 1°F (ca. 22.2 ± 0.6°C). Cage bottoms were changed twice weekly, and complete change-out of isolator lids, wire-bar grills, water bottles, and sipper tubes was performed biweekly. Change-outs were performed observing strict, aseptic isolator technique within a horizontal flow animal transfer station (NuAire, Plymouth, Minn.).

All cages and implements were washed in a mechanical washer with final rinse at 180°F (ca. 82°C) and were autoclaved at 250°F (ca. 121°C) for 15 min prior to entry into the area. A temperature-recording label (Temp-Tape 180, Pharmacal Research Laboratories, Inc., Naugatuck, Conn.) was used daily to ensure final rinse temperature. Steam chemical integrators (SteriGage LR, 3M, St. Paul, Minn.) were used with each autoclave cycle to ensure sterility; quality control was further assessed monthly by using a biological indicator (Verify, Steris Corp., Mentor, Ohio). Mice received acidified water and irradiated pelleted rodent chow (Harlan, Indianapolis, Ind.) ad libitum.

Colony health surveillance was monitored quarterly with serology and biannually with gross necropsies and evaluation of cecal content and cooled sample pelage by using a dissecting microscope. Mice were antibody-negative for the following viral and bacterial pathogens: mouse hepatitis virus, Sendai virus, pneumonia virus of mice, reovirus-3, Theiler's murine encephalomyelitis virus, ectromelia virus, *Mycoplasma pulmonis*, mouse parvovirus, mice minute virus, mouse rotavirus, and lymphocytic choriomeningitis virus. The colony was

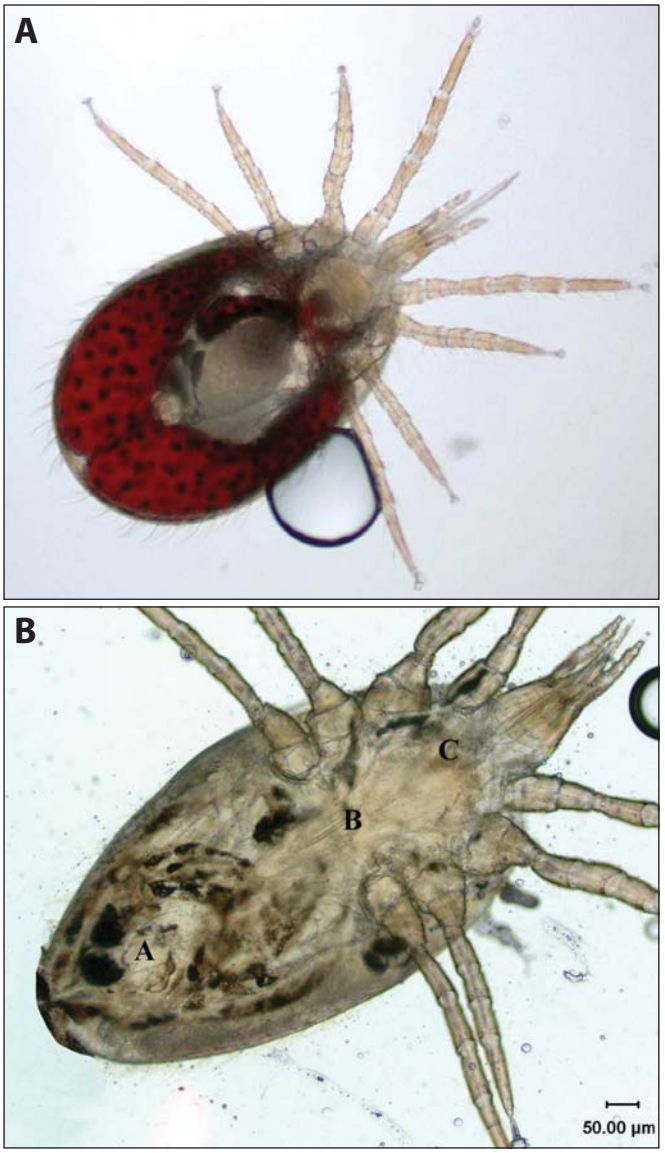

Figure 1. The tropical rat mite, *Ornithonyssus bacoti*. (A) Dorsal view of a blood-engorged mite. (B) Ventral view of mite with anal [A], genital [B], and sternal [C] shields labeled.

historically free of endo- and ectoparasites. Vermin entry into the area was monitored by trap surveillance. All mice were housed, cared for, and used in compliance with the *Guide for the Care and Use of Laboratory Animals* (16) in a program accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

Parasite identification. On 13 December 2004, a research technician responsible for colony management observed an arthropod moving across a workstation within the animal room. The technician had recently complained of intense pruritus, acute pain, dermatitis localized to the neck, and suspected insect bites (Fig. 2). On 21 December 2004, additional arthropods were collected and identified with assistance from a veterinary pathologist and two veterinary parasitologists as *O. bacoti* on the basis of characteristic microscopic morphology. Because of the propensity of *O. bacoti* to hide in crevices between feedings, infested cages were identified by tapping isolator lids on a solid surface and observing for mites. *O. bacoti* infestation was seen on all racks, with the heaviest infestation observed on racks two and three. The investigator was notified of plans to eradicate the mite. Movement of animals and equipment into and out of the room was halted immediately. In addition, traffic patterns were redirected so

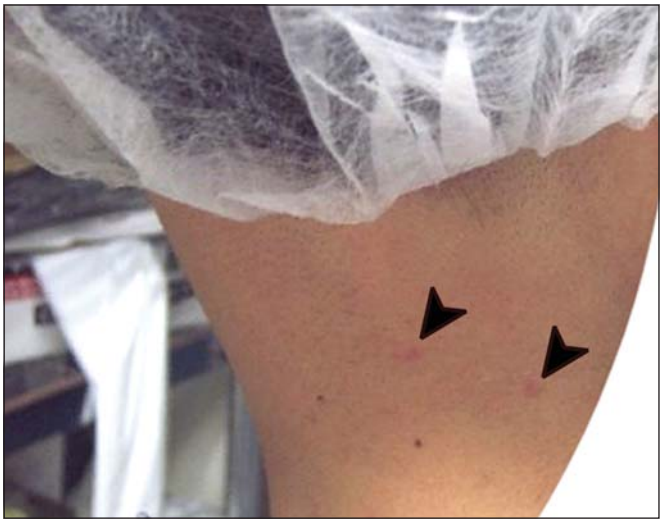

Figure 2. Papular lesions on the neck of the research technician.

that the infested room was entered last. Animal care and research staff were briefed on the public health implications of *O. bacoti* infestation, detection methods, and their role in the eradication program. All staff examined the mite grossly and microscopically.

Treatment protocol. Prior to initiation of treatment, a live, dirty-bedding contact sentinel and a colony mouse were shipped to a diagnostic laboratory for comprehensive necropsy and serological evaluation. Treatment began on 4 January 2005. All mice were changed into clean, autoclaved cages. Two to three 7.4% permethrin-impregnated cotton balls (MiteArrest, EcoHealth, Boston, Mass.) were added to each cage, depending on cage density. Cotton balls were replaced weekly for 5 weeks. Dirty caging and bulk trucks leaving the room were sprayed with a 2.5% permethrin concentrate (Enforcer Outdoor Insect Killer, Enforcer Products, Inc., Cartersville, Ga.). A 0.15% pyrethrin spray (Adams Flea and Tick Spray, Farnam Companies, Inc., Phoenix, Ariz.) was misted on the horizontal flow transfer station, other workstations, and small equipment.

The following day, mice were placed onto clean, autoclaved racks and moved into an adjacent holding area, leaving the infected racks in their initial room. The room was cleaned and sanitized with a quaternary ammonia (Quatracide, Pharmacal Research Laboratories) and allowed to dry for 2 h. Air vents were sealed, and the room fogged with a 0.05% pyrethrin fogger (Indoor Insect Fogger, The Ortho Group, Columbus, Ohio).

On the morning of the third day, all remaining disposable items (e.g., rodent feed, paper products, writing utensils) were double-bagged and removed for incineration. The racks were removed and taken to dirty cage wash for sanitation. All permanent fixtures including lights were opened and sprayed with the 2.5% permethrin concentrate. The floor drain was flushed and sprayed with the concentrate. Cracks around light fixtures were repaired by caulking. A ceiling access panel was opened and mouse feces were observed on the panel and in the surrounding ceiling space. The panel was disinfected with a quaternary ammonia, and permethrin concentrate was sprayed in the ceiling space. A No-Pest Strip (United Industries Corp., St. Louis, Mo.) containing 18.6% dichlorvos was placed in the ceiling space, the access panel closed, and the edges sealed with duct tape. Additional No-Pest Strips were placed in the floor drain and behind the filter of the horizontal flow transfer station. Lastly, boric acid powder (PIC Corporation, Linaen, N.J.) was placed around the periphery of the room. The animals were returned to the room.

Another complete cage change-out was performed 3 weeks after treatment began (on 24 and 25 January 2005), with continued surveillance. The safety and effectiveness of the eradication program

Table 1. Questions posed to people who entered the *O. bacoti*-infested room between 1 July 2004 and 13 December 2004

1. In the past 6 months, did you take equipment or supplies into the infested room? *If yes, what was brought in and how was it processed before entry?*
2. Do you work with animals in any other facilities or in other areas of the vivarium? *If yes, indicate which facilities, areas, or rooms.*
3. Do you have exposure to pet rodents at home?
4. Have you observed wild rodents at home?
5. Do you have exposure to other animals at home? *If yes, indicate species.*
6. Have you noticed any loose or trapped rodents or rodent feces in the infested room?
7. Have any cages of mice entered the infested room directly or through quarantine in the past year? *If yes, where did they come from?*
8. Were mice taken in or out of the infested room since 1 July 2004? *If yes, where were they taken*?

were monitored by animal husbandry and investigator staff through daily visual observation of mice, cages, racks, and work-stations for the presence of *O. bacoti*. Isolator lids were assessed biweekly at the time of cage change, as previously described. The veterinary staff conducted weekly rounds in the area to check for mite re-infestation and neurological signs associated with treatment toxicity.

Outbreak investigation. We interviewed 21 of the 22 people who entered the infested room since 1 July 2004; one staff member was no longer employed by the institution and could not be contacted. The interview questions (Table 1) were modeled after a general outbreak investigation questionnaire proposed by Koszdin and colleagues (13) and were designed to determine the source and extent of *O. bacoti* infestation.

Results

Thorough sanitation and treatment of the animal room, total cage and rack changes, and weekly application of permethrin-impregnated cotton balls to all cages for five consecutive weeks resulted in eradication of *O. bacoti* from the colony. The macroenvironment, microenvironment, and colony were assessed daily for 38 days after initiation of treatment, and no mites were found. The mice accepted the treated cotton as nesting material and exhibited no adverse reactions to permethrin. In addition, colony breeding was not affected adversely by permethrin treatment.

Necropsy and serological results. At necropsy, no gross or histopathological lesions were detected. Both mice were antibody-negative for common viral and bacterial pathogens. In addition, no endo- or ectoparasites were observed.

Interview results. Of the 21 people interviewed, 3 had experienced episodes of pruritus and biting sensations after being inside the infested animal room. Only one of the three individuals reported having skin lesions. None of these people sought medical treatment, and all dermatological problems resolved after the treatment of the colony was begun. No subsequent dermatological problems were reported. In addition, eight people had been into other areas of the vivarium before entering the infested room on the same day; these areas were assessed and were not infected with *O. bacoti*. A total of 10 people reported bringing equipment into the room; of those, five stated that the equipment was either autoclaved or disinfected prior to entry. None of the people surveyed reported seeing loose or trapped rodents or loose rodent feces in the room. Although no one noted exposure to pet rodents at home, three people reported exposure to wild rodents at home: one woman found a dead rat in her garage, and the other two people stated that their pets had brought home feral rodents. Of the 21 people who responded, 13 had one or more pets at home; 12 of these people owned dogs or cats, and the remaining person owned an iguana.

Discussion

Various insecticides, such as malathion (12), methyl carbonate (9), lindane (11), and Vapona (10) have been successful in eradicating *O. bacoti* infestations in laboratory mice. The effectiveness of permethrin against *O. bacoti* has not been reported previously, but the insecticide has been used with success in treating other murine external parasites (2, 14). Synthetic pyrethroids, such as permethrin, are derived from chrysanthemums (3) and are among the least-toxic insecticides to mammals (15). In mice, the concentration of permethrin that killed 50% of the test population when given dermally as a single dose was ≥ 2500 mg/kg (3, 15). The total permethrin exposure per mouse in our study ranged from 177.6 mg/kg to 296 mg/kg. Although this exposure level is below that suggested by the manufacturer, this concentration range was effective in eliminating *Myobia musculi* from transgenic mice (20).

The literature contains a limited number of references describing the immunomodulatory effects of topical permethrin exposure; however, in those studies, the compound was applied directly to the animal to achieve maximum absorption (17-19). We believed the benefit of rapid elimination of *O. bacoti* outweighed any potential risk of using permethrin. Anecdotal information suggests cotton fibers bind as much as 80% of the permethrin toxicant so that it is not readily available for absorption (14). Our method of insecticide delivery has several advantages. Permethrin-impregnated cotton balls eliminate or reduce toxin aerosolization, overdosage, and spillage. In addition, the preparation is easily administered, and both the cotton and insecticide are biodegradable. Finally, the cotton supply makes use of the nest-building instinct of mice.

O. bacoti only resides on the host to feed and returns to its hiding area between host feedings (10). Therefore, successful eradication must involve decontamination of the environment. Ideally, animals should have been changed into nonparasitized cages and racks at the same time, but we were limited by the number of racks in our facility. Interestingly, *O. bacoti* was observed in one room only. Animal husbandry and research staff performed tasks among all rooms within the barrier area without complete changes of personal protective equipment (PPE). Staff also stored and reused disposable gowns. Ironically, these practices did not contribute to the spread of the infestation to other rooms, but the reuse of gowns may have contributed to the research technician being bitten, as the mites may have harbored in the gowns. In response to *O. bacoti* infestation, we issued guidelines requiring complete changes of PPE between animal rooms; PPE also has been designated for single use only.

Our change-out and room sanitation schedule likely contributed to the magnitude of the infestation. Because the isolator lids were changed only biweekly, the lids provided a suitable hiding and breeding environment for the mites. In addition, the room had not been evacuated and sanitized since the colony was started in September 2003.

Identification and elimination of the source of *O. bacoti* is essential in eradication and prevention of re-infestation. *O. bacoti* most likely gained entrance to our facility via feral rodents. Mouse feces were identified inside an unsecured ceiling access panel and the surrounding ceiling space. The access panel was located above racks two and three; incidentally, these racks were the most heavily parasitized. There was recent construction and drilling on the floor above the vivarium, as part of a laboratory renovation. It is likely that holes created by floor–ceiling penetration provided for vermin entry into the ceiling

space. In addition, construction vibrations may have disturbed rodent nests, causing distribution of mites throughout the ceiling space. We hypothesize mites or eggs entered through cracks in the access panel, were harbored or hatched on isolator lids, and parasitized the mice for blood meals. Our institution is located in Tennessee, and *O. bacoti* has been reported to infest feral white footed mice (*Peromyscus leucopus*) within the state (5). Although we did not identify active evidence of feral rodent infestation in the ceiling space, our hypothesis is supported by the heavy infestation of racks directly under the access door and by the results of our epidemiological survey. None of the three people who reported seeing wild rodents at their home had experienced any dermatological problems and had limited access to the room, thereby lowering the possibility that they were the source of the infestation. Although some items (i.e., a computer, iris scissors, and glass pipettes) brought into the room were not sanitized prior to entry, we do not believe this was the source of our infestation. These items were brought directly from investigator laboratories and had not been exposed to animals.

The epidemiology of this infestation was a great learning opportunity for our staff. In response to this outbreak, the institution has contracted with a professional vermin expert to eliminate vermin from crawl spaces and other areas throughout the facility. We attribute the success of the eradication of *O. bacoti* from the colony to strict quarantine; thorough environmental decontamination; reassessment of standard operating procedures for cage and rack changes and total room sanitation; effectiveness of permethrin treatment; and the cooperation of animal husbandry, veterinary, and investigator staff. Equally important were the prompt speciation of the mite, identification of potential sources of infestation, and elimination of sources of re-infestation. Maintenance of colonies free of *O. bacoti* is primarily dependent on husbandry and facility management practices to exclude feral rodents and their parasites from the animal facility.

Acknowledgments

The authors gratefully acknowledge James Flowers, PhD, and Bruce Hammerberg, DVM, PhD (North Carolina State University, College of Veterinary Medicine, Raleigh) for assistance in speciation of *O. bacoti*. We also thank Georgette Hill, DVM, PhD (National Toxicology Program, National Institute of Environment Health Sciences, Research Triangle Park, N.C.) and Jerold Rehg, DVM, DACLAM, DACVP (St. Jude Children's Research Hospital, Memphis, Tenn.), for their review of preliminary versions of this manuscript. Wendell Franklin, Sherry Frazier, RLATG, Lou Jackson and W. Stewart McCloy are commended also; without their commitment to animal care, it is unlikely that this protocol would have been successful.

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