Journal of the American Association for Laboratory Animal Science
Copyright 2009
by the American Association for Laboratory Animal Science

Vol 48, No 6
November 2009
Pages 754–756

Helicobacter spp. in Wild Mice (*Peromyscus leucopus*) Found in Laboratory Animal Facilities

Melissa C Dyson,* Kathryn A Eaton, and Cherie Chang

Wild rodents are a potential source for pathogen introduction into laboratory animal research facilities. A study was designed to assess wild mice found at our institution by infectious disease surveillance. Wild white-footed mice (*Peromyscus leucopus*) were captured with live capture traps placed in areas in which wild mice had been reported in several animal facilities. Captured animals were euthanized by inhalation of CO_2, blood was collected by cardiocentesis (n = 10), and necropsy was performed (n = 8). Serum samples were negative for antibodies to mouse parvovirus (types 1 and 2), mouse minute virus, Sendai virus, pneumonia virus of mice, mouse hepatitis virus, Theiler murine encephalomyelitis virus, reovirus, rotavirus, lymphocytic choriomeningitis virus, mouse adenovirus, ectromelia virus, K virus, cilia-associated respiratory bacillus, and *Mycoplasma pulmonis*. Of the 8 animals that were necropsied, pelt and cecal examinations were negative for ectoparasites and pinworms, respectively. Histopathologic examination of brain, heart, lungs, liver, kidney, spleen, stomach, and small intestine revealed bacteria morphologically compatible with *Helicobacter* spp. in the cecal and colonic glands and occasionally in the gastric lumen and pits. Mesenteric lymph nodes and feces from 8 of the animals were submitted for PCR analysis for the detection of mouse parvovirus, mouse minute virus, mouse hepatitis virus, and *Helicobacter* spp.; 7 of the samples were PCR-positive for *Helicobacter* spp. At this time, wild mice found in our animal facilities do not appear to be a significant source of common laboratory mouse viral pathogens. However, they are a potential source of *Helicobacter* infections.

Despite careful efforts to prevent invasion of wild mice into research facilities, these pests sometimes gain access to areas that house or use laboratory animals. This risk is increased during the building of new animal facilities, when portions of the structure are open to the environment during construction. In addition to perpetuating physical damage to facilities by chewing, feeding, nest building, and contamination with urine and feces, these wild rodents represent a potential source of infectious agents to laboratory rodents. When laboratory rodents are housed in microisolation caging or ventilated caging systems, agents of particular concern would be those that are persistent in the environment, such as the murine parvoviruses. These types of organisms can be present on surfaces and materials that may come into contact with laboratory rodents, including work and cage surfaces. Rodents housed in conventional caging (with open, wire cage tops) have an increased chance of exposure to any infectious agent that wild rodent pests may carry because the laboratory and wild animals could interact directly with one another and because laboratory rodents could be exposed to feces and urine from wild rodents.

Published reports regarding assessment of wild mice for the presence of infectious diseases of concern to laboratory rodents are sporadic. Deer mice (*Peromyscus maniculatus*) found in New Mexico were free of bacterial, viral, and parasitic pathogens.[3] Wild house mice (*Mus musculus*) captured in Idaho and 2 tropical pacific islands and imported to start breeding colonies were reported to have serum antibodies to murine cytomegalovirus, mouse hepatitis virus, and lymphocytic choriomeningitis virus.[7] Wild *Mus musculus* captured around the London zoo were reported to be infected with the pinworm species *Aspiculuris tetraptera*,[2] and other studies have found *Helicobacter* species in wild mice captured in forests in Brazil[3] and the around an urban university in the United States.[9]

In response to reports of wild mice in some of our animal facilities, a program of live trapping, necropsy, and infectious-agent screening was instituted. Wild white-footed mice (*Peromyscus leucopus*; also known as the wood mouse) were captured in live traps and euthanized for assessment. This species is a grayish or brownish rodent found over a large geographic area, ranging from Canada to Central America, is semiarboreal and omnivorous, and inhabits brushy and woody habitats. In addition, *P. leucopus* can also be found year-round in human-occupied buildings.[5]

To date only 10 wild mice have been captured in our facilities, 6 of which were captured in a new facility that was in the final stages of construction. Captured mice were assessed for evidence of common rodent pathogens according to the protocols used for routine rodent health surveillance in our institution.

Materials and Methods

All procedures performed on live animals described in this report were approved by the Institutional Animal Care and Use Committee of the University of Michigan.

Trapping and animal collection. Sherman-style aluminum folding live traps (23 cm × 9 cm × 8 cm) were placed in 4 separate animal facilities in which wild mice had been noted. Locations within these facilities included hallways, cagewash areas, garages, loading docks, and feed and bedding rooms. Traps were baited with peanut butter and an apple slice to provide a source of food and water. Traps were set Monday morning and taken up on Friday by noon. Cage placement was documented on a checklist, and facility husbandry staff evaluated traps twice daily for mice, which were euthanized by inhalation of CO_2 within 24 h of entrapment.

Received: 20 Feb 2009. Revision requested: 16 Mar 2009. Accepted: 03 Jun 2009.
Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, Michigan.
**Corresponding author. Email: mcdyson@med.umich.edu*

Necropsy and sample collection. Immediately after euthanasia, blood was collected through cardiocentesis ($n = 10$), and necropsy was performed ($n = 8$); 2 animals inadvertently were not necropsied at the time of blood sampling. Complete gross examination was performed, and brain, heart, lungs, liver, kidney, spleen, stomach, small intestine, cecum, colon, salivary glands, lymph nodes, and skin were examined histologically (including Steiner silver stain). The pelt was examined for ectoparasites by allowing it to cool in a culture dish and subsequently examining it under a dissecting microscope. Cecal contents were collected and examined under a dissecting scope for the presence of pinworms.

Diagnostic testing. Serum samples were submitted to a commercial diagnostic laboratory (Charles River Diagnostic Laboratories Research Animal Diagnostic Services, Wilmington, MA) and evaluated for antibodies to laboratory rodent pathogens in the assessment profile for mice by using the multiplexed fluorometric immunoassay serology testing platform. Presence of serum antibodies to the following agents was evaluated: mouse parvovirus (types 1 and 2), mouse minute virus, Sendai virus, pneumonia virus of mice, mouse hepatitis virus, Theiler murine encephalomyelitis virus, reovirus, rotavirus, lymphocytic choriomeningitis virus, mouse adenovirus, ectromelia virus, K virus, cilia-associated repiratory bacillus, and *Mycoplasma pulmonis*. Both feces and mesenteric lymph nodes were collected and submitted for PCR analysis at a commercial diagnostic laboratory (Charles River Diagnostic Laboratories Research Animal Diagnostic Services) for mouse parvovirus, mouse minute virus, and mouse hepatitis virus; only feces were tested for *Helicobacter* spp. The laboratory's parvovirus PCR panel consists of a primary general test for parvovirus, followed (if positive) by specific tests for mouse parvovirus and mouse minute virus; the mouse hepatitis virus PCR assay is a single test. The *Helicobacter* PCR assay is run as simultaneous single tests that include a genus-specific *Helicobacter* assay and 2 species-specific tests, 1 for *H. bilis* and 1 for *H. hepaticus*.[6]

Fecal samples positive on the *Helicobacter* PCR panel were sent to another commercial diagnostic laboratory (Research Animal Diagnostic Laboratories, Columbia, MO) for further differentiation. At this laboratory, the *Helicobacter* PCR tests comprise a genus-specific *Helicobacter* reaction that, if positive, is followed by a multiplex PCR assay that can differentiate between *H. bilis*, *H. hepaticus*, *H. rodentium*, *H. trogontum*, *H. typhlonius*, and nonspeciated *Helicobacter* species. The genus-specific *Helicobacter* PCR test is based on previously described primers;[1] information regarding the species-specific assays is proprietary.

Results

All 10 animals were free of antibodies to mouse parvovirus, mouse minute virus, Sendai virus, pneumonia virus of mice, mouse hepatitis virus, Theiler murine encephalomyelitis virus, reovirus, rotavirus, lymphocytic choriomeningitis virus, mouse adenovirus, ectromelia virus, K virus, cilia-associated repiratory bacillus, and *Mycoplasma pulmonis*. Among the 8 animals that were necropsied, no significant lesions were detected on gross examination. Histopathologic examination with Steiner silver stain revealed large, tightly coiled bacteria, morphologically compatible with *Helicobacter* species in the cecal and colonic glands and occasionally in the gastric lumen and pits; no bacteria were detected in the liver. Pelt and cecal examinations were negative for ectoparasites and pinworms, respectively. Of the 8 mice tested, 6 were fecal PCR-positive for *H. rodentium* and 1 was fecal PCR-positive for both *H. rodentium* and *H hepaticus*; the remaining mouse was negative for both *Helicobacter* species.

Discussion

At this time, the wild *P. leucopus* found in our animal facilities do not appear to be a significant source of common laboratory mouse viral pathogens, cilia-associated respiratory bacillus, *Mycoplasma pulmonis*, pinworms, or ectoparasites. However, these mice are a potential source of *Helicobacter* infections. In the present study, 6 mice were fecal PCR-positive for *H. rodentium*, and 1 mouse was positive for both *H. hepaticus* and *H. rodentium.* In addition, histologic examination revealed bacteria morphologically compatible with *Helicobacter* species in the cecal and colonic glands and occasionally in the gastric lumen and pits. No colitis or hepatitis was present. As indicated by the PCR results, the lower bowel likely was colonized, but *Helicobacter* organisms were a minor component of the microbiota in these mice and did not cause disease. This result is expected, because *Helicobacter* infections of the lower bowel do not cause disease in immunocompetent mice.[11] However, the presence of these organisms in wild mice suggests that they could be a source of *Helicobacter* species that are either potentially pathogenic in some laboratory mouse strains or that could interfere with experimental results. Even low numbers of *Helicobacter* organisms in wild mice could be a reservoir for infection of susceptible laboratory mouse strains. The significance of the *Helicobacter* organisms visualized in the stomach is not known. These organisms were rare and may have been transient. These organisms were not cultured and speciated, because doing so is not a part of our routine disease surveillance procedures. However, culturing and speciating the organisms in the stomach would be a valuable future pursuit to confirm and further investigate the potential risk of *Helicobacter* organisms carried by wild rodents.

In rodents, *Helicobacter* organisms have been found in the cecum, colon, and liver.[10] *Helicobacter* species typically are not considered pathogenic in immunocompetent mice. However, in some mouse strains and in immunodeficient or immunologically modified animals, some *Helicobacter* species can cause disease. For example, *H. hepaticus* and *H. bilis* have been associated with typhlitis, colitis, hepatitis in susceptible mice, and *H. hepaticus* has been associated with hepatocellular carcinoma.[10] *Helicobacter rodentium* has not been shown to cause disease alone, but it has been implicated in confounding disease during coinfection with *H. bilis* and *H. hepaticus*.[10,8] Although these organisms are not normally pathogenic and did not cause disease in the wild mice we evaluated, they represent a potential source of infection for laboratory mice.

Because of the potential for these organisms to compromise the health of animals and confound research results, many laboratory animal institutions and vendors strive to maintain their rodent populations free from *Helicobacter* infection. Because *Helicobacter* organisms are mainly transmitted through the fecal–oral route, transmission of *Helicobacter* organisms from wild or feral rodents can be maintained principally by pest control and prevention of contact between wild mice and research rodents. If wild animals do gain access to animal facilities, biocontainment housing, including filter tops and ventilated caging, provides protection against direct interaction of wild rodents and their feces with research animals. However, surfaces including bedding, food, caging, transfer stations, flow hoods, and transport devices represent potential fomites that could carry *Helicobacter* organisms from wild mice to research animals. This possibility emphasizes the need for careful husbandry techniques and appropriate sanitization of objects that come into contact with research animals. In a 2000 study, microisolator caging, transfer of mice by using forceps dipped in disinfectant, and adherence

to a strict clean to dirty cage-changing order prevented horizontal transmission of *H. hepaticus* infection.[10,11]

The absence of pathogens of concern other than *Helicobacter* spp. in these wild mice is surprising, because *P. leucopus* can be infected with and shed many of murine pathogens.[4,7] The absence of serologic evidence of many laboratory animal pathogens in this study could be attributable to several factors, including the short lifespan of rodents in the wild and the decreased population density of these animals when not housed in laboratory environments. On the rare occasion that wild mice are found in or around a rodent animal facility, screening these animals for potential laboratory rodent pathogens would be valuable.

Acknowledgments

We would like to thank the University of Michigan Unit for Laboratory Animal Medicine (ULAM) for support of this project and Ms Paula Arrowsmith for help with sample preparation.

References

1. **Beckwith CS, Franklin CL, Hook RR Jr, Besch-Williford CL, Riley LK.** 1997. Fecal PCR assay for diagnosis of *Helicobacter* infection in laboratory rodents. J Clin Microbiol 35:1620–1623.
2. **Behnke JM.** 1975. *Aspiculuris tetraptera* in wild *Mus musculus*. The prevalence of infection in male and female mice. J Helminthol **49:**85–90.
3. **Botten J, Ricci R, Hjelle B.** 2001. Establishment of a deer mouse (*Peromyscus maniculatus rufinus*) breeding colony from wild-caught founders: comparison of reproductive performance of wild-caught and laboratory-reared pairs. Comp Med **51:**314–318.
4. **Comunian L, Moura SB, Paglia AP, Nicoli JR, Guerra JB, Rocha GA, Queiroz DMM.** 2006. Detection of Helicobacter species in the gastrointestinal tract of wild rodents from Brazil current microbiology Current Microbiology **53:**370–373.
5. **Hollingsworth CS**. Integrated pest management for Northeast schools. Ithaca (NY): National Research, Agriculture and Engineering Service.
6. **Livingstron R.** 2009. Personal communication.
7. **Miller R, Dysko R, Chrisp C, Seguin R, Linsalata L, Guehner G, Harper FM, Austad S.** 2000. Mouse (*Mus musculus*) stocks derived from tropical islands: new models for genetic analysis of life history traits. J Zoo **250:**95–104.
8. **Myles MH, Livingston RS, Franklin CL.** 2004. Pathogenicity of *Helicobacter rodentium* in A/JCr and SCID mice. Comp Med **54:**549–557.
9. **Parker SE, Malone S, Butne RM, Smith A.** 2009. Infectious diseases in wild mice (*Mus Musculus*) collected on and around the University of Pennsylvania (Philadephia) campus. Comp Med. **59:**424–430.
10. **Whary MT, Cline JH, King AE, Corcoran CA, Xu S, Fox JG.** 2000. Containment of *Helicobacter hepaticus* by use of husbandry practices. Comp Med **50:**78–81.
11. **Whary MT, Fox JG.** 2004. Natural and experimental *Helicobacter* infections. Comp Med **54:**128–158.