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Isolation of *Mycobacterium avium* subsp *paratuberculosis* (Map) from Feral Cats on a Dairy Farm with Map-infected Cattle

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ABSTRACT: Paratuberculosis is an economically important disease of dairy cattle caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map). The role of nonruminant, nondomestic animals in the epidemiology of paratuberculosis in cattle is unclear. To examine nonruminant, nondomestic animals for the presence of Map, 25 feral cats, nine mice (species unknown), eight rabbits (*Sylvilagus floridanus*), six raccoons (*Procyon lotor*), and three opossums (*Didelphis virginiana*) were collected from a midwestern dairy with known Map-infected cattle. *Mycobacterium avium* subsp. *paratuberculosis* was isolated from the mesenteric lymph node from seven of 25 (28%) feral cats. Ileum was culture-positive for three of these seven cats, and an isolation of Map was also made from the ileum of one of nine (11%) mice. Tissue samples from other species were negative as determined by Map culture; microscopic lesions consistent with paratuberculosis were not seen in any animal. Restriction fragment polymorphism analysis of isolates from cats and dairy cattle suggest interspecies transmission. The means by which interspecies transmission occurred may be through ingestion of Map-contaminated feces or waste milk or through ingestion of Map-infected prey. Shedding of Map from infected cats was not evaluated. The epidemiologic role of Map-infected feral cats on dairy farms requires further investigation.

Key words: Feral cats, Johne's disease, *Mycobacterium avium* subsp. *paratuberculosis*, paratuberculosis.

Mycobacterium avium subsp. *paratuberculosis* (Map) is the causative agent of paratuberculosis (Johne's disease) in ruminants. Ruminant paratuberculosis manifests as chronic diarrhea and weight loss after a prolonged incubation period that often exceeds 2 to 5 yr. Because of the potential economic losses associated with this disease, producers have attempted to eliminate paratuberculosis from herds through extensive testing and removal of infected animals. Until recently, paratuberculosis was only associated with domestic ruminants such as cattle, sheep, and goats with sporadic occurrence in free-ranging ruminants such as white-tailed deer (*Odocoileus virginianus*; Libke and Walton, 1975; Chiodini and Van Kruingen, 1983), Key deer (*Odocoileus virginianus clavium*; Quist et al., 2002), bighorn sheep (*Ovis canadenis*), Rocky Mountain goats (*Oreamnos americanus*; Williams et al., 1983), tule elk (*Cervus nannodes*; Jessup et al., 1981), and bison (*Bison bison*; Buergelt et al., 2000).

In Scotland, Map infection has been identified in several species of nonruminant wildlife that were sampled around dairy farms on which paratuberculosis was present in cattle. Map was identified from intestine or mesenteric lymph nodes in foxes (*Vulpes vulpes*), stoats (*Mustela erminea*), hares (*Lepus europaeus*), badgers (*Meles meles*), rats (*Ratus norvegicus*), wood mice (*Apodemus sylvaticus*), crows (*Corvus corone*), rooks (*Corvus frugilegus*), and jackdaw (*Corvus monedula*) (Beard et al., 2001a). Additionally, although Map was not isolated, histopathologic changes consistent with paratuberculosis were noted in intestines or mesenteric lymph nodes of weasels (*Mustela nivalis*), and acid-fast bacteria were seen in one bank vole (*Clethrionomys glareolus*; Beard et al., 2001a). In rabbits, microscopic pathologic changes in tissues similar to those seen in infected cattle (i.e., granulomatous enteritis) were observed (Beard et al., 2001b). The potential effects of Map on wildlife health and population dynamics and the potential reservoir status of such Map-infected populations are unknown. This information is critical to the

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design and implementation of effective strategies to control or eradicate paratuberculosis.

Although Map has been reported sporadically in the US in free-ranging ruminants, the status of nonruminant wildlife is unknown. This study was conducted to examine various species of nondomestic, nonruminant animals for the presence of Map around a midwestern dairy farm with known paratuberculosis in cattle.

Nonruminant, nondomestic animals were collected from a midwestern dairy with a known history of paratuberculosis. Animals were trapped in live traps (Havahart®, Woodstream Co., Lititz, Pennsylvania, USA) baited for raccoons (*Procyon lotor*), opossum (*Didelphis virginiana*), rabbits (*Sylvilagus floridanus*), rodents, and feral cats. Traps were monitored every morning and rebaited if needed. Trapped animals were anesthetized with a mixture of ketamine (Ketaset®, Fort Dodge Animal Health, Ft. Dodge, Iowa, USA) and xylazine (Xyla-ject®, Phoenix Pharmaceutical Inc., St. Joseph, Missouri, USA) at recommended dosages (Kreeger, 1999). Anesthetized animals were euthanatized by intracardiac injection of sodium pentobarbital and transported to the laboratory for immediate necropsy.

All animals were trapped and collected on the premises of a 1,200-cow dairy in the midwestern US. Traps were placed in and around feed storage buildings, calf rearing facilities, milking parlor, and loafing sheds. The dairy has been operational for 35 yr, and in 1994, an expansion from approximately 100 cows to the current level of 1,200 adult cows began. Paratuberculosis was initially diagnosed on this dairy in 1996 after the purchase of replacement heifers as part of this expansion. Serologic prevalence of paratuberculosis in 1999, as determined by enzyme-linked immunosorbent assay (ELISA; IDEXX, Westbrook, Maine, USA), was approximately 10%. An active paratuberculosis monitoring and management program has been in place for 5 yr and the current serologic prevalence is 2.9%. All cows are tested by ELISA at the beginning of their dry period. Positive cows are identified and marked, and colostrum and milk for calves is pasteurized before feeding.

From findings of previous studies of Map infection in nonruminant animals, terminal ileum and mesenteric lymph node were collected from all sampled animals and bacteriologic isolation of Map was done as described previously (Stabel et al., 2003). Confirmation of Map colonies on agar slants was done by polymerase chain reaction (PCR) to amplify the Map-specific genetic element *IS900* as described (Stabel et al., 2003). Samples of tonsil (except for rodents), esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph node, liver, and spleen were collected and processed for microscopic analysis and visualization of acid-fast bacteria as described previously (Stabel et al., 2003).

DNA was isolated from bacteria and processed for restriction fragment length polymorphism analysis (RFLP) with the restriction endonucleases *Bst*EII and *Pst*I according to methods previously described (Pavlik et al., 1999). Two additional restriction endonucleases (*Bcl*I and *Pvu*II) were used in a similar manner. The *IS900* probe was prepared by PCR amplification of DNA from the Map strain 19698 as described previously (Pavlik et al., 1999). In addition to samples from trapped animals, isolates from feces and mesenteric lymph nodes of cows from the farm in the study were also examined by RFLP.

A total of 25 feral cats, eight rabbits, six raccoons, three opossums, and nine mice (species unknown) were examined. Neither macroscopic nor microscopic lesions consistent with paratuberculosis were observed. Similarly, acid-fast bacilli were not observed in any sample examined. *Mycobacterium avium* subsp. *paratuberculosis* was isolated from seven of 25 cats (28%) and one of nine mice (11%). Map was isolated from the mesenteric lymph nodes of all seven positive cats and from the ileum

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TABLE 1. Isolation of *Mycobacterium avium* subsp. *paratuberculosis* (Map) from mesenteric lymph node (LN) and ileum of feral cats collected from a dairy containing Map-infected cattle.

	Map (CFU/g tissue)[a]	
Cat no.	Mesenteric LN	Ileum
1	6	3
3	3	1
6	83	2
7	55	0
9	1	0
14	2	0
15	7	0

[a] Mean number of colonies on four different agar slants per gram of tissue processed for bacteriological culture.

in three of these positive animals. A Map isolate also was obtained from the ileum of one mouse. At least one of four agar slants from each of the positive animals contained Map colonies. The colony-forming units (CFU) per gram of tissue from cats (Table 1) varied from 1 to 83, with higher counts obtained from the mesenteric lymph node (range 3–83 CFU/g) than from the ileum (range 1–3 CFU/g). *Mycobacterium avium* subsp. *paratuberculosis* was not isolated from any tissues obtained from raccoons, opossums, or rabbits.

Sufficient bacterial growth was obtained from three of the seven Map-positive cats to conduct RFLP analysis. The *Bst*EII RFLP banding pattern observed for the cat isolates was identical to that observed for all bovine isolates (fecal or mesenteric lymph node) from the same herd, and all isolates were classified as C1 according to the RFLP classification system previously described (Pavlik et al., 1999). When DNA was digested with *Pst*I and probed for the *IS900* sequence, a similar banding pattern was noted for all cat isolates (Fig. 1A). On examination of other isolates ($n = 5$) obtained from mesenteric lymph nodes from cows of this herd, one cow had the same banding pattern as the bovine fecal isolate (Fig. 1B) while all other bovine isolates resembled those obtained from feral cats.

Digestion of DNA with *Pvu*II did not result in any useful data after probing with the 229-bp *IS900* sequence (data not shown). However, *Bcl*I digestion of DNA demonstrated distinct banding patterns between the cat isolates (Fig. 2). Two of the three cats shared similar banding patterns with the bovine isolates and the lab strain *M. avium* subsp. *paratuberculosis* 19698. In contrast, one cat had a dissimilar banding pattern with numerous additional bands.

Although this report represents findings around a single dairy in a limited number of species, it does represent the first documented association of Map-infected cattle with Map-infected feral cats and the first report of naturally occurring Map infection in cats. The similarity of the RFLP patterns of Map cultures from cats and those found in feces or tissues from cattle on this dairy suggests interspecies transmission; possibly spillover from infected cattle to cats. It is unknown whether these animals were shedding Map in feces or whether they developed a detectable immune response. With the exception of rabbits, lesions described in nonruminant wildlife have generally been mild (Beard et al., 2001b; Daniels et al., 2003). Although lesions consistent with paratuberculosis were not seen in any of the Map-infected cats, the isolation of Map from mesenteric lymph nodes suggests that cats were truly infected and not transiently colonized by Map passing through the gastrointestinal tract.

The means by which cats were infected is unclear. Discussions with the herd owner confirmed that cats had free access to feed storage areas, loafing areas, and calf-rearing facilities. Colostrum and milk from even subclinically infected cows can contain up to 7 CFU per 50 ml of milk (Sweeney et al., 1992b). Although milk was commonly made available to the cats in the calf-rearing facility, pasteurized milk rather than nonpasteurized waste milk was fed. However, direct transmission to cats through the unintended consumption of raw nonpasteurized waste milk at other lo-

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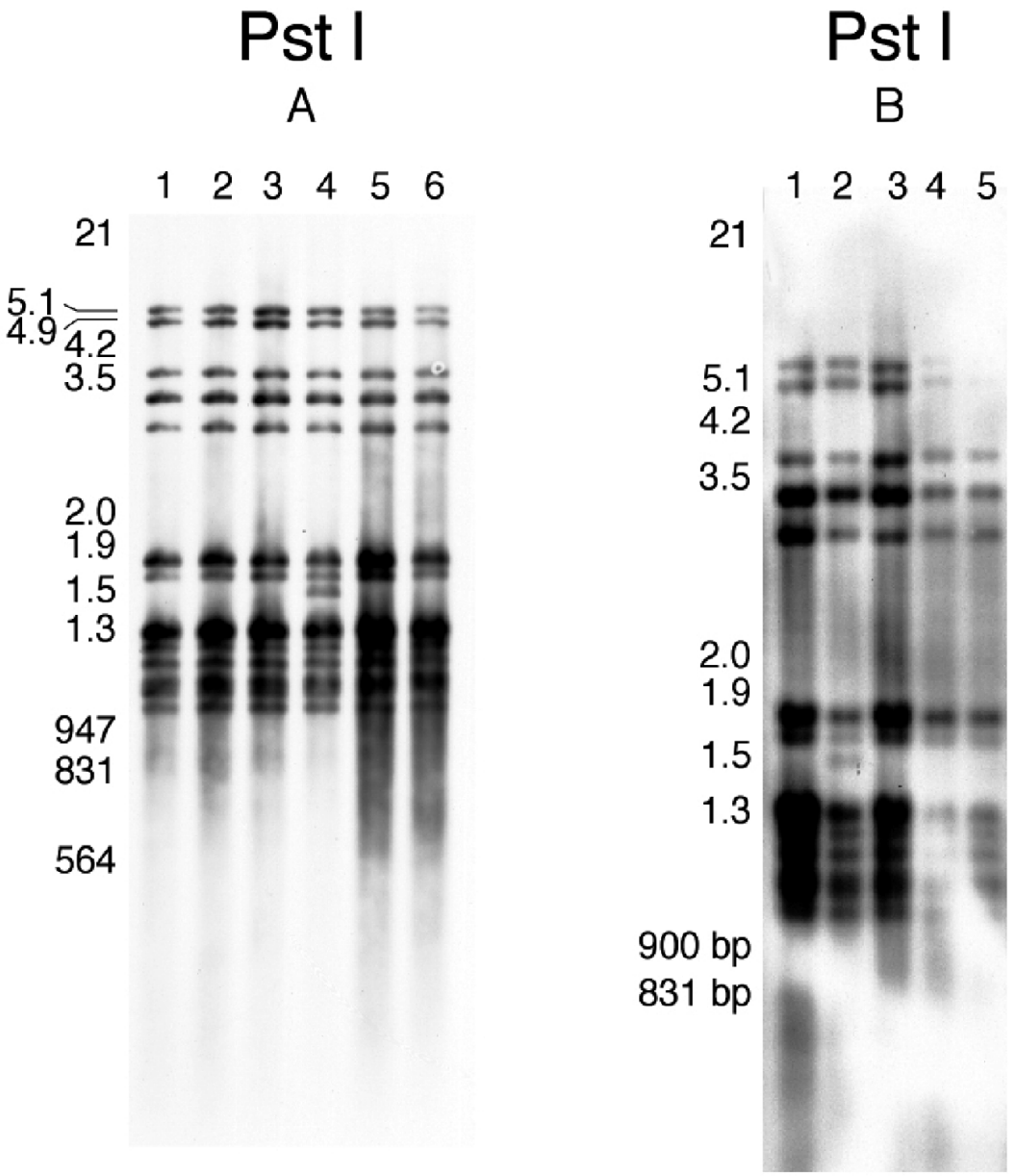


FIGURE 1. (A) RFLP types of *Mycobacterium avium* subsp. *paratuberculosis* isolated from three feral cats (lanes 1–3), feces from a dairy cow (lane 4), and reference strains K10 and 19698 (lanes 5–6) after digestion with the restriction endonuclease *Pst*I. Note additional band from the bovine fecal isolate at approximately 1.5 kb. (B) RFLP types of *M. avium* subsp. *paratuberculosis* isolated from mesenteric lymph nodes from five dairy cattle from the study site after digestion with the restriction endonuclease *Pst*I. Isolates from bovine mesenteric lymph nodes have banding patterns similar to those seen from feral cats. Lanes 1, 3, 4, and 5 have a pattern similar to those seen in the cats from Figure 1A. Note that lane 2 has the same banding pattern as the bovine fecal isolate from Figure 1A.

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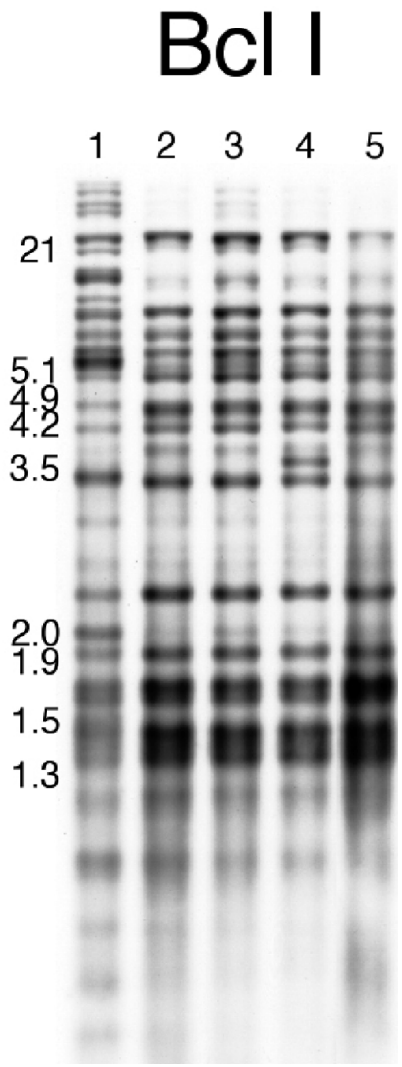


FIGURE 2. RFLP types of *Mycobacterium avium* subsp. *paratuberculosis* isolated from three feral cats (lanes 1–3), feces from a dairy cow (lane 4), and reference strain 19698 (lane 5) after digestion with restriction endonuclease *Bcl*I. Note the distinct banding pattern of the isolate from a cat in lane 1.

cations cannot be discounted. Clinically affected cattle can shed as much as 2×10^5 CFU of Map per gram of feces (Sweeney et al., 1992a). Direct fecal-oral transmission to cats might also have occurred as a result of self-grooming, and experimentally, oral infection of cats with Map has been suggested. In a study by Johnson and Pratt (1944), two of 14 cats orally inoculated with pure Map cultures or intestinal scrapings from cattle with clinical paratuberculosis developed transient diarrhea and gross lesions suggestive of paratuberculosis. Microscopic examination of intestines from these cats, however, revealed minimal inflammation and few acid-fast bacteria.

Studies in Scotland have isolated Map from species that could be considered prey for feral cats (e.g., mice, rats, rabbits, voles, etc.). Additionally, Map was isolated from the ileum of one of nine mice sampled in this study. Indirect transmission from cattle to feral cats through ingestion of Map-infected prey is also a plausible means of infection. In Scotland, predation as a mode of transmission of Map is suggested by the higher prevalence of Map in prey rather than predator species around dairies (Daniels et al., 2003).

Cats are susceptible to infection with various mycobacteria. Documented cases of *Mycobacterium* infections in cats include *M. bovis, M. tuberculosis, M. avium* subsp. *avium, M. lepraemurium, M. microti, M. smegmatis, M. fortuitum, M. chelonae, M. phlei,* and *M. xenopi* (Snider, 1971; Tomasovic and Purcell, 1976; Wilkinson et al., 1982; Scheiffer and Middleton, 1983; White et al., 1983; Drolet, 1986; Studdert and Hughes, 1992; deLisle, 1986; Gunn-Moore et al., 1996). Generally, infections with these mycobacterial agents result in cutaneous lesions with regional lymphadenopathy. Lesions were not seen in any of the cats from which Map was isolated, and PCR to amplify the *IS900* fragment was used to differentiate the Map isolates from other species of tuberculous and nontuberculous mycobacteria.

RFLP analysis has been used previously to document transmission of Map between rabbits and cattle, finding that rabbit and cattle isolates around selected farms in Scotland were indistinguishable (Greig et al., 1999). The RFLP patterns from all cats and the bovine feces were most similar to the most frequent Map RFLP type identified by the *Bst*EII restriction endonuclease (type C1; Pavlik et al., 1999). Minor

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differences seen in the banding patterns with *Pst*I and *Bcl*I indicate that the cat and bovine isolates are similar. The significance of the minor differences in banding patterns between the cat isolates is unclear.

The epidemiologic role of Map-infected feral cats remains to be investigated. Cats might be true spillover hosts and represent an epidemiologic dead end. Alternatively, cats might be true maintenance hosts and serve as a source of infection to other susceptible hosts. However, the lack of lesions, combined with the low number of Map colonies isolated from the mesenteric lymph node and ileum, suggests that shedding is likely to be minimal. Populations of feral cats exist worldwide, and numbers in the US were estimated at 73 million in 2000 (Levy et al., 2003). Feral cats can be found in both urban and rural environments and are common around dairies, where they are often used as a means of rodent control. Conclusions regarding the true role of Map-infected feral cats in the epidemiology of paratuberculosis in domestic livestock will require further investigation.

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