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Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from Free-Ranging Birds and Mammals on Livestock Premises

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Surveys for *Mycobacterium avium* subsp. *paratuberculosis* infection in free-ranging mammals and birds were conducted on nine dairy and beef cattle farms in Wisconsin and Georgia. Specimens were collected from 774 animals representing 25 mammalian and 22 avian species. Specimens of ileum, liver, intestinal lymph nodes, and feces were harvested from the larger mammals; a liver specimen and the gastrointestinal tract were harvested from birds and small mammals. Cultures were performed by using radiometric culture and acid-fast isolates were identified by 16S/IS*900*/IS*1311* PCR and mycobactin dependency characteristics. *M. avium* subsp. *paratuberculosis* was cultured from tissues and feces from 39 samples from 30 animals representing nine mammalian and three avian species. The prevalence of infected wild animals by premises ranged from 2.7 to 8.3% in Wisconsin and from 0 to 6.0% in Georgia. Shedding was documented in seven (0.9%) animals: three raccoons, two armadillos, one opossum, and one feral cat. The use of two highly polymorphic short sequence repeat loci for analysis of 29 of the 39 strains identified 10 alleles. One allelic pattern broadly shared in domestic ruminants ("7,5") appeared in approximately one-third of the wildlife *M. avium* subsp. *paratuberculosis* isolates studied. Given the few cases of shedding by free-ranging animals compared to the volume of contaminated manure produced by infected domestic ruminant livestock, contamination of the farm environment by infected wildlife was negligible. Wildlife may, however, have epidemiological significance for farms where *M. avium* subsp. *paratuberculosis* recently has been eliminated or on farms free of *M. avium* subsp. *paratuberculosis* but located in the geographic vicinity of farms with infected livestock.

Mycobacterium avium subsp. *paratuberculosis* infection can be an economically disruptive herd health problem for domestic ruminant species. The infection results in Johne's disease, a chronic granulomatous gastroenteritis leading to emaciation and death. Cases of the infection also have been reported in a number of free-ranging ruminant species (28). Elk (*Cervus elaphus*) at Point Reyes National Seashore in California (16) and a population of bighorn sheep (*Ovis canadensis*) in Colorado have remained infected over several years (13). Ongoing studies in The Netherlands and Italy suggest the involvement of roe deer (*Capreolus capreolus*) in the epidemiology of *M. avium* subsp. *paratuberculosis* in isolated areas with associated livestock infection (20, 24, 26).

There are few reports of *M. avium* subsp. *paratuberculosis* isolations from nonruminant free-ranging wildlife. A suspected isolate of *M. avium* subsp. *paratuberculosis* was reported from a European brown hare (*Lepus europaeus*) in England in 1977 (17), and lesions consistent with *M. avium* subsp. *paratuberculosis* infection were described in a rabbit (*Oryctolagus cuniculus*) in Scotland in 1990 (2). The initial confirmation of *M. avium* subsp. *paratuberculosis* in free-ranging nonruminant wildlife was from rabbits on farms with a history of *M. avium* subsp. *paratuberculosis* infection in Scotland (5, 11, 12). Subsequently, *M. avium* subsp. *paratuberculosis* was isolated from tissue samples from foxes (*Vulpes vulpes*), stoats (*Mustela erminea*) (3), weasels (*Mustela nivalis*), badgers (*Meles meles*), wood mice (*Apodemus sylvaticus*), Norway rats (*Rattus norvegicus*), European brown hares, jackdaws (*Corvus monedula*), rooks (*Corvus frugilegus*), and crows (*Corvus corone*) (4) in these same areas. A study based on rabbit and deer fecal samples collected from the ground on dairy farms with infected cattle in Minnesota yielded *M. avium* subsp. *paratuberculosis* isolates from two eastern cottontail fecal samples and two white-tailed deer fecal samples (23).

For the most effective control of this challenging disease in domestic livestock and to assess its threat, if any, to wildlife health, it is important to determine the extent to which wildlife species may serve as reservoirs for the organism. In its report on Johne's disease, the National Research Council recommended that field studies be conducted to assess natural infections of wildlife near livestock operations with endemic Johne's disease (19). The goal of the present study was to evaluate both the presence of *M. avium* subsp. *paratuberculosis* in tissue and *M. avium* subsp. *paratuberculosis* shedding by free-ranging mammals and birds sharing habitat with infected livestock in two ecologically different regions of the United States.

MATERIALS AND METHODS

Study sites. Intensive sampling of wildlife was conducted on nine livestock premises. Study sites were chosen in two regions to evaluate wildlife and wildlife-livestock interactions under the different environmental and ecological conditions. In Wisconsin, specimens were collected from four dairy farms known to contain livestock infected with *M. avium* subsp. *paratuberculosis* (Farm W1, Monroe County; W2, Outagamie County; W3, Sheboygan County; W4, Jackson County). In Georgia, specimens were collected from two dairy farms (G2, Brooks County; G3, Jefferson County) and three beef cattle farms (G1, Early County;

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G4, Oglethorpe County; G5, Grady County) with histories of *M. avium* subsp. *paratuberculosis*-infected livestock. All samples collected were from free-ranging animals captured on the selected premises and, in the case of the dairy farms, in the immediate vicinity of the dairy barns.

The infection prevalence in cattle for Wisconsin premises was determined by consecutive years of fecal culture and serologic testing. Prevalence data for G1 came from its long-term participation in a U.S. Department of Agriculture, Agricultural Research Service project on *M. avium* subsp. *paratuberculosis*. Fecal cultures were used to determine the prevalence for Georgia premises G2-G5. Farms W4 and G5 initially were considered to be negative controls: all cows on the farms had tested negative by enzyme-linked immunosorbent assay or fecal culture prior to our survey. However, one W4 cow and six G5 cows were confirmed to be infected with *M. avium* subsp. *paratuberculosis* after the wildlife sampling had been conducted. These farms were therefore recategorized as infected premises.

Wildlife species evaluated. Wild mammal and bird species sampled were those with the highest potential for exposure to contaminated materials and/or that posed the highest risk for contamination of livestock feed or forage on the selected farms. Targeted species included mammals of the orders *Artiodactyla*, *Carnivora*, *Didelphimorphia*, *Lagomorpha*, *Rodentia*, and *Xenarthra*. Targeted avian species included members of the order *Passeriformes* (*Columbidae*, *Corvidae*, *Emberizidae*, *Fringillidae*, *Passeridae*, and *Sturnidae*). Wild animals sampled represented 25 species of mammals and 22 species of birds and were collected by using authorized, species-appropriate methods (live-capture traps and shooting). Methods for capture of birds included mist nets and shooting.

Samples. Four samples (ileum, liver, intestinal lymph node, and feces) were harvested from the larger mammals. Additional sections of these samples from the larger mammals were fixed in 10% buffered formalin and stored for future histopathological evaluation if *M. avium* subsp. *paratuberculosis* was isolated from tissue or feces. Two samples (liver and the entire gastrointestinal tract) were collected from smaller mammals and birds: the gastrointestinal tract was homogenized, and three aliquots were taken from the homogenate for culture. The samples were chilled and shipped overnight to the Johne's Testing Center, University of Wisconsin for culture.

Study size. Samples were collected from free-ranging wildlife associated with nine cattle premises in Wisconsin and Georgia from September 2003 to January 2004. Attempts were made to acquire samples from the same species at each site to facilitate comparison without introducing species-specific epidemiological variables. The actual number of animals sampled per species was affected by species abundance at the study sites. Observations and capture success data gathered while collections were in progress were used to determine the numbers of individuals to sample per species.

Assays. Cultures and isolate identification were performed at the Johne's Testing Center by using the radiometric (BACTEC) culture method (6). Acid-fast isolates were identified as *M. avium* subsp. *paratuberculosis* using 16S/IS*900*/IS*1311* plus L1/L9 integration site PCRs, amplification of a *M. avium* subsp. *paratuberculosis* unique sequence (locus 251) plus mycobactin dependency growth characteristics. Twenty-nine isolates confirmed as *M. avium* subsp. *paratuberculosis* were further genotyped by PCR-sequencing of two short sequence repeat (SSR) loci. Cluster analysis and genotype association dendrogram development based on these SSR (G and GGT residue repeats) alleles was performed (1, 15).

Histopathology. Tissues from culture-positive animals were embedded in paraffin and sectioned at 3 to 4 μm. Individual sections were stained with hematoxylin and eosin for routine examination and with Ziehl-Neelsen acid-fast stain to search for acid-fast bacteria. Histopathology analyses were conducted at the Southeastern Cooperative Wildlife Disease Study.

TABLE 1. Thirty-nine *M. avium* subsp. *paratuberculosis* wildlife isolates by site, species, and sample type

Site	Species	Sample type(s)[a]				
		Ileum	MLN	Liver	Fecal	GI tract
G1	Raccoon	+	−	−	−	*na*
	Cottontail	+	−	−	−	*na*
	Armadillo	+	+	+	−	*na*
	Cotton rat	*na*	*na*	*na*	*na*	+
	Opossum	−	+	−	−	*na*
	Opossum	−	−	−	+	*na*
G2	Raccoon	−	+	−	−	*na*
G5	Armadillo	−	−	−	+	*na*
	Armadillo	−	−	−	+	*na*
W1	Shrew	−	−	+	−	*na*
	Sparrow	*na*	*na*	*na*	*na*	+
	Feral cat[b]	+	+	−	+	*na*
	Snipe	*na*	*na*	*na*	*na*	+
W2	Norway rat	*na*	*na*	*na*	*na*	+
	Starling	*na*	*na*	−	*na*	+
	Starling	*na*	*na*	+	*na*	−
	Starling	*na*	*na*	+	*na*	−
	Starling	*na*	*na*	−	*na*	+
	Feral cat	+	+	−	−	*na*
	Raccoon	−	+	−	−	*na*
	Raccoon	+	+	−	−	*na*
	Raccoon	−	−	−	+	*na*
	Raccoon	−	+	−	−	*na*
W3	Raccoon	−	+	−	+	*na*
	Starling	*na*	*na*	−	*na*	+
	Starling	*na*	*na*	−	*na*	+
	Starling	*na*	*na*	−	*na*	+
W4	Raccoon	+	+	−	+	*na*
	Pocket gopher	*na*	*na*	*na*	*na*	+
	Skunk	−	+	−	−	*na*
	Study totals	7	11	4	7	10

[a] The full gastrointestinal (GI) tract was homogenized, and three aliquots were incubated for small mammals or birds. In some cases, separate liver samples were collected (e.g., starling). Specimens were collected either as pooled gastrointestinal tract (ileum, mesenteric lymph node [MLN], liver, and feces not collected separately [*na*]) or as the individual tissues and feces (gastrointestinal tract not pooled [*na*]).

[b] Two lymph node samples were incubated, and isolates were obtained from both.

RESULTS

M. avium subsp. *paratuberculosis* was isolated from 39 of 2,752 samples (1.4%) collected from 30 of 774 free-ranging animals (3.4%) (Table 1). Culture-positive samples came from 12 free-ranging species, including nine mammalian species (raccoon [*Procyon lotor*], $n = 8$; nine-banded armadillo [*Dasypus novemcinctus*], $n = 4$; feral cat [*Felis catus*], $n = 2$; Eastern cottontail [*Sylvilagus floridanus*], $n = 1$; opossum [*Didelphis virginiana*], $n = 1$; hispid cotton rat [*Sigmodon hispidus*], $n = 1$; Norway rat, $n = 1$; northern short-tailed shrew [*Blarina brevicauda*], $n = 1$; striped skunk [*Mephitis mephitis*], $n = 1$) and three avian species (European starling [*Sturnus vulgaris*], $n = 7$; house sparrow [*Passer domesticus*], $n = 1$; common snipe [*Gallinago gallinago*], $n = 1$).

Shedding, as indicated by the presence of *M. avium* subsp. *paratuberculosis* in fecal samples, was detected in seven (0.9%) animals: three raccoons (WI), two armadillos (GA), one opossum (GA), and one feral cat (WI) (Table 2). Isolations of *M. avium* subsp. *paratuberculosis* were made from tissue samples from two of these raccoons and the feral cat, but for one raccoon, the two armadillos and the opossum, feces was the sole culture-positive sample. Isolations of *M. avium* subsp. *paratuberculosis* were made at least four times from each of the tissue types selected for testing in this survey (Table 2).

Acid-fast and hematoxylin-and-eosin-stained tissues from culture-positive raccoons ($n = 10$), opossums ($n = 3$), armadillos ($n = 3$), feral cats ($n = 2$), and skunk ($n = 1$) were

TABLE 2. Total number of infected animals by species

Species	No. of animals		Prevalence (%)
	Infected	Sampled	
Armadillo	4	23	17.4
Feral cat	2	18	11.1
Hispid cotton rat	1	41	2.4
Eastern cottontail	1	56	1.8
Opossum	2	54	3.7
Raccoon	8	42	19.0
Norway rat	1	4	25.0
Southeastern shrew	1	4	25.0
Striped skunk	1	10	10.0
Common snipe	1	1	100
House sparrow	1	60	1.7
European starling	7	40	17.5

examined. Neither acid-fast bacteria nor granulomatous infiltrates consistent with *M. avium* subsp. *paratuberculosis* infection in ruminants were apparent in any of the tissues examined.

The prevalence of *M. avium* subsp. *paratuberculosis*-infected wildlife (number of animals from which *M. avium* subsp. *paratuberculosis* was isolated/number animals sampled) ranged from 2.7 to 8.3% on the premises in Wisconsin (Table 3) and from 0 to 6.0% in Georgia (Table 4). Overgrowth of nonmycobacterial organisms in tissue samples may have hindered isolation of mycobacteria at some farms (premises sample contamination ranged from 0.7 to 11%). In the majority of cases, the contaminated sample was the homogenized gastrointestinal tract.

There was no statistical correlation between the test-prevalence of *M. avium* subsp. *paratuberculosis* in livestock versus wildlife on the farms ($r = 0.31$). There was no difference in prevalence of *M. avium* subsp. *paratuberculosis* infection in wildlife among all farms in general ($\chi^2 = 14.43$, df = 8, $P > 0.10$), but test-positive wildlife were slightly more prevalent on farms in Wisconsin than in Georgia ($\chi^2 = 3.10$, df = 1, $P < 0.10$). Also, there was no difference in prevalence in wildlife on dairy farms versus beef cattle farms ($\chi^2 = 0.32$, df = 1, $P > 0.10$). At least one *M. avium* subsp. *paratuberculosis*-infected free-ranging animal was found on all four Wisconsin sites and on three of five Georgia sites. Of the two farms in Georgia where *M. avium* subsp. *paratuberculosis* was not isolated from wildlife, one was a dairy with a prior history of Johne's disease at a low level but with test-negative cattle at the time of this survey, the other was a beef operation with a cattle test-positive prevalence of 9.1%.

Twenty-nine of the 39 *M. avium* subsp. *paratuberculosis* wildlife isolates were subjected to genotyping based on a recently described set of SSR markers (1, 10, 18). This analysis revealed a total of 10 distinct alleles (Table 5) in 11 animal species. Cluster analysis identified one major allele defined by the G-residue and GGT-residue repeat numbers as "7,5". This allele was identified in 29% of the 29 *M. avium* subsp. *paratuberculosis* isolates studied and was one of two alleles shared among isolates from both geographic locations (Table 5). Georgia isolates had five alleles: "7,5", "7,3", "7,4", "12,5"; and "8,4". Isolates from Wisconsin carried 9 distinct alleles (Table 5). The raccoon isolates (all from Wisconsin) carried 4 alleles: "14,5", "7,4", "7,5", and "13,5". Isolates from starlings showed five alleles. Unusually, one *M. avium* subsp. *paratuberculosis* isolate from a skunk mesenteric lymph node carried a genotype typical for *Mycobacterium avium* subsp. *avium* (identified as 2GC4G, 3GGT). Three isolates from an armadillo carried three different alleles and were distinct from all other *M. avium* subsp. *paratuberculosis* isolates in that they carried a unique

TABLE 3. *M. avium* subsp. *paratuberculosis* isolated from free-ranging wildlife in Wisconsin

Parameter	Farm			
	W1	W2	W3	W4
Farm type/herd size	Dairy/70	Dairy/1,400	Dairy/396	Dairy/50
Herd prevalence[a] (%)	17.7	10.3	13.5	2.0
No. of wild animals sampled	77	120	101	109
No. of samples collected	323	462	370	367
% Contaminated[b]	5.3	8.7	8.1	0.8
No. of positive samples[d]	6	12	5	5
No. of positive animals[d]	4	10	4	3
Infection prevalence (%)	5.2	8.3	4.0	2.7
No. of animals shedding[c]	1	1	1	1

[a] By ELISA.
[b] Lost due to overgrowth of nonmycobacterial organisms.
[c] *M. avium* subsp. *paratuberculosis* isolated from fecal samples.
[d] Positive, that is, for *M. avium* subsp. *paratuberculosis*.

TABLE 4. *M. avium* subsp. *paratuberculosis* isolated from free-ranging wildlife in Georgia

Parameter	Farm				
	G1	G2	G3	G4	G5
Farm type/herd size	Beef/740	Dairy/430	Dairy/140	Beef/21	Beef/69
Herd prevalence[a] (%)	1.6	7.4	0.0	9.1	10.1
No. of wild animals sampled	100	48	63	56	100
No. of samples collected	268	198	240	142	382
% Contaminated[b]	0.7	6.1	4.6	3.5	11.0
No. of samples positive[d]	8	1	0	0	2
No. of animals positive[d]	6	1	0	0	2
Infection prevalence (%)	6.0	2.1	0	0	2.0
No. of animals shedding[c]	1	0	0	0	2

[a] G1 values were determined by ELISA; G3 to G5 values were determined by individual fecal culture; G2 values were determined by fecal pools of five. A value of 7.4% represents eight positive pools of five cows from a total of 108 pools and assumes one positive cow per pool.
[b] Lost due to overgrowth of nonmycobacterial organisms.
[c] *M. avium* subsp. *paratuberculosis* isolated from fecal samples.
[d] See Table 3, footnote *d*.

TABLE 5. Wildlife *M. avium* subsp. *paratuberculosis* isolates by short-sequence repeat genotype

G-GGT pattern	Species (farm of origin)
13G-5GGT	Starling (W2), raccoon (W4)
12G-5GGT	Norway rat (W2)[a], starling (W3)
15G-5GGT	Shrew (W1), starling (W2 and W2)
14G-5GGT	Cat (W3, W3, and W3), raccoon (W2), starling (W3), snipe (W1)
7G-5GGT/10G	Cat (W2), raccoon (W2, W3, and W4), starling (W3), opossum (G1), cotton rat (G1), cow (G2)
14G-3GGT	Cat (W2)
2GC4G-3GGT	Skunk (W4)
7G-3GGT	Armadillo (G1)
7G-6GGT	Starling (W2)
7G-4GGT	Cat (W2), raccoon (G2), armadillo (G1)
8G-4GGT	Armadillo (G1)

[a] No GGT data for this isolate.

IS*1311*-PCR-RFLP profile (data not shown). Similarly, two isolates from a cat showed 2 distinct alleles ("7,5" and "14,3").

DISCUSSION

This report provides evidence of *M. avium* subsp. *paratuberculosis* infection in a wide range of nonruminant wildlife species in North America and confirms that the nonruminant infections reported in Scotland (3, 4) were not an isolated occurrence. In both of these studies *M. avium* subsp. *paratuberculosis* was isolated from tissue and feces from mammals and birds, and infections were detected on numerous premises. However, both the number of isolates and species infection prevalences were lower in our surveys than reported in Scotland (4, 11, 12).

It is now clear that *M. avium* subsp. *paratuberculosis* may infect animals in multiple taxonomic groups, but the pathogenic capacity of the organism for these species is still an open question. In Scotland, histological lesions were seen in rabbits (11, 12), foxes, weasels, a stoat, wood mouse, and crow (4). The absence of histological lesions in tissues from infected animals in our study may be related to the phase of infection in the culture-positive animals examined, to a lack of pathology associated with *M. avium* subsp. *paratuberculosis* infection in these species, or to the *M. avium* subsp. *paratuberculosis* strain present on these farms. The impact on wildlife health of this organism has yet to be determined.

The isolation of *M. avium* subsp. *paratuberculosis* from both tissue and fecal samples in our study is evidence of true infection, not "pass-through" of recently ingested contaminated material. Shedding of *M. avium* subsp. *paratuberculosis* may result in wildlife to wildlife, wildlife to livestock, and/or livestock to wildlife transmission. It has been postulated that livestock could become infected by ingesting forage contaminated by infected rabbits as they may excrete up to 4×10^6 CFU per g of feces (7, 8, 9). Although we did not quantify *M. avium* subsp. *paratuberculosis* in feces, our detection of shedding in raccoons, opossums, an armadillo and a feral cat is in line with surveys in Scotland in which *M. avium* subsp. *paratuberculosis* was found in the feces of foxes (3 of 27), a stoat (1 of 6), wood mice (2 of 2), crows (4 of 12), and a rook (1 of 1) (4).

Fecal contamination of the environment on dairy farms by *M. avium* subsp. *paratuberculosis*-infected cows is extensive (22). Given the number of infected cattle in a herd, typical farm animal density and the volume of contaminated feces produced by infected cows, the potential for farm contamination by cattle is many orders of magnitude greater than contamination that would be produced by infected wildlife at the prevalences shown in the present study.

Strain typing using two highly polymorphic SSR loci identified 10 alleles with a predominance of one strain appearing in approximately one-third of the wildlife *M. avium* subsp. *paratuberculosis* isolates studied. Although there are a few "host-associated" *M. avium* subsp. *paratuberculosis* alleles, this "7,5" allele is present in cattle, sheep, and goat populations (10). In fact, the"7,5" allele represents ca. 10% of the strains analyzed on a nationwide collection of strains (N. B. Harris, unpublished data). Taken together, the distribution of a major set of alleles and multiple unique genotypes are suggestive of interspecies transmission. The observation that multiple strains were isolated from an armadillo and a feral cat is consistent with several ongoing investigations that have identified multiple alleles in cattle from infected herds (Harris, unpublished). It is likely that a scavenging wildlife species or even cattle in infected herds may carry *M. avium* subsp. *paratuberculosis* with different alleles if they are exposed to multiple strains at multiple time points. The significance of this observation in the transmission of *M. avium* subsp. *paratuberculosis* within and between herds and the ability of specific *M. avium* subsp. *paratuberculosis* clones to predominate in the transmission cycle across species needs to be elucidated.

Spillover of infectious organisms from closely held domestic herds to wildlife is of concern to wildlife managers and livestock producers both because it affects wildlife health and because wild animals may serve as potential reservoirs or disseminators of infection to livestock. A recent study reported that verocytotoxin-producing *Escherichia coli* isolates obtained from a starling and a Norway rat were identical to cattle isolates on surrounding farms (21). Ruminant and nonruminant wildlife may become exposed to *M. avium* subsp. *paratuberculosis* via feeding on contaminated grain, forage in pastures, feces, or on infected prey (8). Given the higher prevalence of *M. avium* subsp. *paratuberculosis*-infected cattle on the farms in Wisconsin, the higher prevalence of *M. avium* subsp. *paratuberculosis* in wildlife in Wisconsin was predictable. However, there was no correlation between infection prevalence in cattle and wildlife among the nine farms. Factors affecting wildlife exposure to *M. avium* subsp. *paratuberculosis* may include microhabitat ecology, organism survival, water and soil conditions, herd size, husbandry methods, and variations in species interactions, plus grooming and feeding habits. No conclusions could be drawn as to a premise's domestic agriculture infection prevalence and surrounding wildlife infection rates in the current study.

Potential routes of transmission of *M. avium* subsp. *paratuberculosis* from wildlife back to livestock include fecal contamination of feed and forage, both in farm buildings and in pastures (8). Individuals of some wildlife species may live for several years with home ranges that cover areas large enough to include more than one farm. Raccoon home ranges average 40 to 100 ha, and movements may be made to areas outside the home range to visit temporary food sources (14). Infected wildlife may shed organisms such as *M. avium* subsp. *paratuberculosis* over a period of time, and this could include some degree of shedding on the farm where the infection originated, as well as on nearby farms. Recovery of *M. avium* subsp. *paratuberculosis* from environmental samples on sheep and goat farms in New Zealand was very low 5 months after the infected stock were removed, but infected wildlife with longer life spans may continue shedding at some level beyond this period of time (27). Environmental contamination may be further amplified by protozoa ubiquitous in soil, water and plants. Studies have noted that *M. avium* subspecies *avium* can infect *Acanthamoeba castellani* and be found in both protozoal life stages (trophozoite and cyst) where they are resistant to inactivation by chemical disinfectants and UV light (25). If also true for *M. avium* subsp. *paratuberculosis*, control of manure contamination of the environment should become an even higher priority in Johne's disease control programs.

Infected wildlife may have the greatest effect on the epide-

miology of *M. avium* subsp. *paratuberculosis* infection on farms that have eliminated all infected livestock from the premises or on *M. avium* subsp. *paratuberculosis*-free farms in the same geographic area as infected farms. If wildlife become infected and shed the organism in a sufficient volume and in accessible locales frequented by susceptible livestock (i.e., young animals), it is possible that domestic ruminant species could become reinfected.

Studies on the pathogenesis and shedding of *M. avium* subsp. *paratuberculosis* in wildlife are needed to assess the impact of the infection on wildlife populations and the risk that infected wildlife may pose to livestock. Direct or indirect transmission among wildlife species, independent of continual contact with infected domestic livestock, might result in the establishment of carriers or local wildlife reservoirs. Subsequent transmission of *M. avium* subsp. *paratuberculosis* from wildlife back to livestock could interfere with state or national Johne's disease control programs. Comprehensive information on the duration and volume of shedding in infected wildlife could be used to calculate contamination levels under different environmental conditions and clarify the impact of this important animal infection for both captive and free-ranging species.

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