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Mycobacterium avium Subsp. *paratuberculosis* from Free-Ranging Deer and Rabbits Surrounding Minnesota Dairy Herds

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Abstract

The objectives of this study were to estimate the prevalence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) among deer and rabbits surrounding infected and noninfected Minnesota dairy farms using fecal culture, and to describe the frequency that farm management practices were used that could potentially lead to transmission of infection between these species. Fecal samples from cows and the cow environment were collected from 108 Minnesota dairy herds, and fecal pellets from free-ranging white-tailed deer and eastern cottontail rabbits were collected from locations surrounding 114 farms; all samples were tested using bacterial culture. In addition, a questionnaire was administered to 114 herd owners. Sixty-two percent of the dairy herds had at least 1 positive fecal pool or environmental sample. A total of 218 rabbit samples were collected from 90% of the herds, and 309 deer samples were collected from 47% of the herds. On 2 (4%) of the farms sampled, 1 deer fecal sample was MAP positive. Both farms had samples from the cow fecal pool and cow environment that were positive by culture. On 2 (2%) other farms, 1 rabbit fecal sample was positive by culture to MAP, with one of these farms having positive cow fecal pools and cow environmental samples. Pasture was used on 79% of the study farms as a grazing area for cattle, mainly for dry cows (75%) and bred or prebred heifers (87%). Of the 114 farms, 88 (77%) provided access to drylot for their cattle, mainly for milking cows (77/88; 88%) and bred heifers (87%). Of all study farms, 90 (79%) used some solid manure broadcasting on their crop fields. Of all 114 farms, the estimated probability of daily physical contact between cattle manure and deer or rabbits was 20% and 25%, respectively. Possible contact between cattle manure and deer or rabbits was estimated to occur primarily from March through December. The frequency of pasture or drylot use and manure spreading on crop fields may be important risk factors for transmission of MAP among dairy cattle, deer, and rabbits. Although the MAP prevalence among rabbits and deer is low, their role as MAP reservoirs should be considered.

Résumé

Les objectifs de cette étude étaient d'estimer la prévalence de Mycobacterium avium *ssp.* paratuberculosis *(MAP) parmi les populations de cerfs et de lapins en périphérie de fermes laitières infectées et non-infectées du Minnesota à l'aide de la culture de fèces et de décrire la fréquence avec laquelle les pratiques de régie de ferme utilisées pouvaient potentiellement conduire à la transmission de l'infection entre les espèces. Des échantillons de fèces provenant des vaches et de leur environnement ont été amassés sur 108 fermes laitières du Minnesota, et des échantillons de fèces de cerf de Virginie et de lapins à queue blanche ont été ramassés de l'entourage de 114 fermes; tous les échantillons ont été testés par culture bactérienne. De plus, un questionnaire a été distribué au propriétaire de 114 troupeaux. Au moins 1 échantillon positif a été trouvé dans 62 % des troupeaux laitiers, soit à partir du pool de matières fécales soit à partir d'un échantillon de l'environnement. Un total de 218 échantillons de lapin ont été amassés à partir de 90 % des troupeaux, et 309 échantillons provenant de cerf ont été amassés à partir de 47 % des troupeaux. À partir de 2 (4 %) des fermes échantillonnées, 1 échantillon de fèces de cerf s'est avéré positif. Des échantillons provenant du pool de fèces de vaches et de l'environnement de ces deux fermes se sont révélés positifs en culture. À partir de 2 autres fermes, 1 échantillon de fèces de lapin s'est avéré positif. Des échantillons provenant du pool de fèces de vaches et de l'environnement de ces deux fermes se sont révélés positifs en culture. Du pâturage était utilisé par 79 % des fermes dans l'étude comme pacage pour les animaux, principalement les vaches taries (75 %) et les taures saillies ou non (87 %). Parmi les 114 fermes, 88 (77 %) laissaient accès aux animaux à un enclos, principalement les vaches en lactation (77/88; 88 %) et les taures saillies (87 %). De toutes les fermes étudiées, 90 (79 %) utilisaient de l'épandage partiel de fumier solide sur leurs champs de récolte. Parmi toutes les fermes, la probabilité estimée d'un contact physique quotidien entre du fumier de bovin et les cerfs ou les lapins étaient respectivement de 20 % et 25 %. Des contacts possibles entre le fumier de bovin et les cerfs ou les lapins étaient estimés se produirent plus souvent entre les mois de mars et décembre. La fréquence d'utilisation de pâturage ou d'enclos et l'épandage de fumier sur les champs de culture pourraient être des facteurs de risque importants pour la transmission de MAP parmi les bovins laitiers, les cerfs et les lapins. Bien que la prévalence de MAP parmi les lapins et les cerfs soit faible, leur rôle comme réservoir de MAP doit être considéré.*

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Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP), the causal agent of Johne's disease (JD) or paratuberculosis, is a facultative intracellular bacterium that infects both wild and domestic ruminants. In cattle, the usual route of infection is fecal-oral, with young cattle primarily becoming infected by exposure to manure from infected adult cattle or their environment (1). The disease manifests in adult cows and results in economic losses due to reduced milk production, loss of body weight, and premature culling for slaughter.

Clinical paratuberculosis has been diagnosed in a number of free-ranging wild ruminant species, such as Rocky Mountain bighorn sheep (*Ovis canadensis*) (2), red deer (*Cervus elaphus hippelaphus*) in Switzerland (3), key deer (*Odocoileus virginianus clavium*) in southern Florida, and tule elk (*Cervus elaphus nannodes*) in California (4–6). In both cattle and wild ruminants, the gastrointestinal tract (predominantly the small intestine) is the primary site of infection. Clinical signs of paratuberculosis are manifested by emaciation, which is accompanied by diarrhea in cattle (7). Among wild ruminants, diarrhea has been observed in infected red deer (8,9).

The introduction of MAP into an animal population can occur when infected animals contaminate the grazing area with their feces. Because of the chronic pattern of JD, animals can continue to contaminate large areas over a long period of time (7). *Mycobacterium avium* subsp. *paratuberculosis* is highly resistant to extreme weather conditions and can survive in various substances for many months (10,11). Therefore, the potential for MAP to be transmitted to other grazing wildlife and domestic animals is high.

Limited information is available about relationships between JD in cattle and deer populations. In the United States (USA), infected cattle herds have been considered to be the source of infection for wild axis (*Axis axis*) and fallow deer (*Dama dama*) that were using common pastures (12). Soil from cattle pastures contaminated by MAP was considered the source of infection for wild ruminants in Ireland (13). In Connecticut, USA, MAP was isolated from 2 white-tailed deer shot on a cattle farm with history of JD (14). Pavlik et al (15) isolated the same MAP strains from free-ranging deer and cattle occupying sympatric ranges in the Czech Republic, and suggested an association between infections in both species.

Other wildlife have also been suggested as possible reservoirs for MAP. In Scotland, MAP was found in rabbits (*Oryctolagus cuniculus*) that shared the same pasture as infected cattle (16) and also in red fox (*Vulpes vulpes*) and stoats (*Mustela erminea*) on farms with infected cattle (17). Greig et al (18) found a significant association between the presence of MAP in rabbit mesenteric lymph nodes and cattle on farms with a previous or current history of JD, and suggested that the same strain infected both cattle and rabbits. In addition, Daniels et al (19) found that grazing beef cattle did not avoid pasture contaminated with rabbit feces in different concentrations, indicating a potential risk for JD transmission from rabbits to cattle.

Despite a proposed JD link between wild ruminants or rabbits and domestic ruminants, especially dairy cows, no study has evaluated the potential risk factors associated with transmission between these animal groups. The objectives of this study were to assess the frequency of farm practices that can lead to MAP transmission among dairy cattle, deer, and rabbits and to estimate the prevalence of MAP, using fecal culture, in deer and rabbits adjacent to Minnesota farms with or without infected dairy cows.

Material and methods

Dairy farm sampling

One hundred and fourteen dairy herds were selected from a database available for 2 JD programs in Minnesota. Of the 114 herds, cattle and environmental samples were collected from 108 herds, including 80 herds known to be infected from previous testing in the Johne's Disease Control Program (JDCP) of the Minnesota Board of Animal Health (MBAH) and 28 herds known to be noninfected based on previous testing (between the years 2001 and 2002) in the Voluntary Johne's Disease Herd Status Program (VJDHSP) of the MBAH. Samples of cattle feces and farm environment (manure or manure with soil or bedding) were collected during the summer of 2002. To assess herd infection status, fecal samples were obtained from up to 100 cows in each herd and were tested using bacterial culture in pools made up of 5 cows (20). Up to 2 environmental samples were obtained from selected locations on each farm including cow alleyways, dry cow area, manure storage, fields near cow area, and other areas (21).

Farm questionnaire

A questionnaire was administered to all 114 herd owners selected during farms visits. The objective of the questionnaire was to describe the prevalence of the following risk factors for possible MAP transmission between cattle and wildlife: the use by cattle of pasture or drylot (dirt lot or fields with limited grazing), manure spreading on pasture or crop fields, frequency and location of wildlife being seen, and the possibility of physical contact with feces (stepping or laying on, sniffing, ingestion) between cattle and rabbits or deer (questionnaire available upon request).

Wildlife sampling around dairy farms

Fecal pellets of free-ranging white-tailed deer (*Odocoileus virginianus*) and eastern cottontail rabbits (*Sylvilagus floridanus*) were collected from the areas surrounding the 114 farms, from February through March 2002 (n = 60) and November 2002 through March 2003 (n = 54). Winter sampling was selected to facilitate the collection of fecal pellets. In some areas, fecal pellets of snowshoe hare (*Lepus americanus*) and white-tailed jackrabbit (*Lepus townsendii*) may have also been collected near crop fields (22); no attempt was made to distinguish between fecal pellets from different rabbit species. From the area surrounding each farm (up to 1500 m for deer pellets and 200 m for rabbit pellets), up to 10 deer pellet piles ($>$ 20 g each), and approximately 100 g of rabbit pellets were collected. Each deer fecal sample was obtained from 1 pile with a minimum distance of approximately 10 m between piles. Rabbit pellets were collected from an extensive area of the farm, in order to maximize the probability of sampling different individuals without a minimal distance between sampled pellets. Since a very small amount of feces (2 g) is used for the bacterial culture procedure, when the amount of pellets collected was $>$ 80 g, the number of cultures was increased by dividing the sample into 2 equal parts. Rabbit pellets were collected

Table I. The use of pasture and drylot by cattle on 114 Minnesota dairy farms

Outside area	Number of farms (%)	Average size (acres)	Animal use: number of farms (% Farms use outside area) Cows	Heifers	Months of use/ % Farms use outside area Jan–Dec	Apr–Nov	Oct–Apr	Other
Pasture	90 (79%)	34	68 (75%)	78 (87%)	9%	65%	0	26%
Drylot	88 (77%)	2.4	74 (84%)	67 (76%)	56%	14%	13%	17%

Table II. Frequency and coverage distribution (% of farms within crop) of manure spreading on crop fields on Minnesota dairy farms

Crop	Number of farms used the crop	Frequency of manure spreading (times a year) Year-round	2/y	1/y	Every other year	Every $\geq$ 3 y	Percent of crop fields covered with manure during year 1% to 24%	25% to 49%	50% to 74%	75% to 100%
Corn	112	4%	28%	54%	6%	8%	4%	13%	15%	68%
Alfalfa Hay	61	0%	8%	71%	8%	13%	47%	16%	11%	26%
Other Crops	45	9%	7%	70%	7%	7%	23%	15%	24%	38%
Pasture	15	33%	27%	40%			73%	20%	0%	7%

primarily around the farmyard (near housing, farm buildings, or cattle area) and the adjacent wooded area, on average 50 m from the cow barn or calves pen. Occasionally, rabbit feces were also collected on the edge of a corn or hay field of the premise, or near an adjacent wooded area, on average 190 m from cows barn. Deer feces were collected from corn and hay fields on the premise, wooded areas adjacent to crop fields, and occasionally from the farm backyard if available, on average 530 m from the cow barn.

Wildlife sampling in areas with no dairy farms — "negative control group"

Fecal samples of deer and rabbits were collected from 4 areas where contact with livestock was unlikely, including: a) 12 fecal samples of rabbits from urban/suburban locations within Minneapolis and St. Paul; b) feces and up to 10 g of mesenteric lymph nodes from 23 deer that were shot by Minnesota Department of Natural Resources (DNR) personnel at a Minneapolis airport, including 6 fawns, 8 adult males, and 9 does; c) fecal samples (50 g) and 10 mL of jugular blood for MAP antibody-testing using serum enzyme-linked immunosorbent assay (ELISA) from 12 adult deer in north central Minnesota captured by the DNR; and d) 12 piles of fecal samples collected at a state park in southeast Minnesota, approximately 20 km from the nearest dairy farm.

Laboratory testing

Fecal samples, environmental samples, and mesenteric lymph nodes were tested at the Minnesota Veterinary Diagnostic Laboratory (MVDL) using bacterial culture for MAP, method previously described elsewhere (23). Briefly, a sedimentation culture procedure was used (24) with 72 h of sedimentation prior to inoculation of 4 tubes containing Herrold's egg yolk medium. Colony counts were recorded weekly for 16 wk for cattle feces and environment cultures and for 24 wk for deer and rabbit pellet cultures. The degree of fecal-shedding was categorized as negative, light (mean of 1 to 10 colonies per tube [CPT]), moderate (mean of 11 to 50 CPT), or heavy (mean of > 51 CPT). Herd, farm environment, or wildlife was defined as infected if at least 1 sample was positive to MAP. Deer serum samples were tested for MAP antibodies using a commercially available serum ELISA (IDEXX Laboratories, Westbrook, Maine, USA) for cattle.

Statistical analysis

Descriptive analysis was performed using computer software (Microsoft Excel; Microsoft Corporation, Bloomington, Minnesota, USA). All statistical analysis was performed using a commercial statistical software program (SAS/STAT User's guide, Release 8.02; SAS Institute, Cary, North Carolina, USA). The Chi-square Fisher's exact test (PROC FREQ; SAS Institute) with 95% CI, was used to determine association between the use of pasture or drylot and contact between wildlife and cattle manure. A one-sample Student's t-test was used to determine differences between average study herds fecal pool prevalence and fecal pool prevalence of individual herds with positive wildlife. In both tests, $P < 0.05$ was considered significant.

Results

Dairy farms

The results from cattle and environmental testing are presented in detail elsewhere (21). In brief, 64 (80%) of the 80 JDCP herds had at least 1 positive pool and 2 (7%) of the 28 VJDHSP herds had 1 positive pool each. The farm environment was determined to be positive in 94% of the 66 herds with positive pools.

Descriptive analysis of prevalence of potential risk factors for transmission between cattle and wildlife

Pasture and drylot access — Of the 114 study farms, 90 (79%) used pasture for grazing cattle (Table I). Of the 90 farms that used pasture for cattle, deer and rabbit feces were found on 46% and 90%,

Table III. Frequency of observing deer and rabbits by farmers and number of dairy farms where fecal samples were collected

Frequency of observing	Farms observed deer (%) n = 114	Farms deer fecal samples collected (%) n = 54	Farms observed rabbits (%) n = 114	Farms rabbit fecal samples collected (%) n = 102
Daily	23%	50%	42%	45%
Weekly	26%	26%	27%	26%
Monthly	14%	11%	15%	14%
Seasonally (Oct–Dec)	24%	13%	10%	12%
Rarely/never	13%	0%	6%	5%

Table IV. Frequency of contact (% of farms) between deer/rabbits and cattle around Minnesota dairy farms (n = 114 farms)

Wildlife/Frequency	Daily	Weekly	Monthly	$<$ Monthly	Almost never
Deer/cows	18%^{b}	13%^{b}	9%^{b}	11%^{b}	49%^{a}
Deer/heifers	20%^{b}	14%^{b}	11%^{b}	11%^{b}	44%^{a}
Rabbits/cows	22%^{b}	16%^{b}	12%^{b}	18%^{b}	32%^{a}
Rabbits/heifers	28%^{a}	18%^{b}	15%^{b}	17%^{b}	22%^{b}

^{ab} Different subscripts in a row indicate statistical significance ($P < 0.05$)

respectively. On 69% of the 90 farms, cow fecal pools were positive. On 50 (56%) of the 90 farms, the pasture used by dry cows was sampled and 5 (10%) of the farms were positive by culture.

Of the 114 study farms, 88 (77%) provided access to drylots for their cattle (Table I). Dry cow drylots were sampled on 40 (45%) farms and 8 (20%) were positive by culture. Deer and rabbit fecal samples were found on 45% and 89% of the 88 farms, respectively.

Manure application — Of the 114 study farms, 79% used solid manure broadcasting on their crop fields, and 47% and 41% used slurry surface application and slurry subsurface (injected) application, respectively. On average, 64% and 45% of the total manure applied on fields was from broadcasting and slurry application (surface and subsurface), respectively. Of 90 farms that used pasture for cattle, 15 (17%) had spread manure on pasture during the previous 3 y (Table II). The manure sources for the pasture area were from both cows and heifers. On 68%, 26%, and 38% of the 114 farms, manure was spread on 75% to 100% of the total area of corn, alfalfa hay, and other crop fields area (soybeans, oats), respectively (Table II). For all types of crops, manure was primarily spread once a year and often during the winter. While the main source of manure for all field crops was from both heifers and cows, 16% of farms also spread manure from steers or bulls.

Interaction between cattle and wildlife — During the 2 y before the questionnaire was administered, deer were seen by the farmers around the farm a minimum of once per week year-round on 26% of the 114 farms. On 24% of the farms, deer were occasionally seen during October through December (Table III). On most of the farms where farmers reported observing deer, group size usually ranged between 1 and 3; however, on 27 farms, deer group size ranged between 10 and $>$ 50. Deer were mainly seen around crop fields (especially alfalfa) or in adjacent wooded areas near cattle pastures and occasionally near the farmyard. Two farms reported deer approaching the farmyard during winter to eat stored silage and hay. Rabbits were seen by farmers at least daily or weekly on 69% of the 114 farms (Table III). Rabbits were most often seen in the farmyard, in the wooded areas adjacent to the farmyard or crop fields or at the edge of crop fields.

On 49% of the 114 farms, farmers estimated the probability of physical contact between cattle and deer feces to be at least "monthly" (Table IV). The probability of contact between cattle and deer and their respective feces was more likely to occur between the months of April and December (in cows on 79% of the farms and in heifers on 70% of the farms), the main period when cattle are in pasture. The estimated probability of daily contact between rabbits and cattle feces was 22% and 28% for cow and heifers, respectively (Table IV). The contact between rabbits and cattle and their feces was estimated to occur year-round (in cows on 52% of the farms and in heifers on 50% of the farms) or during the months of March to December (in cows on 40% of the farms and in heifers on 50% of the farms).

There was an association (OR = 5.4, 95% CI = 2.1 to 14.2) between the use of pasture and contact between deer and cow and heifer manure (Table V). There was also an association between the use of pasture and contact between rabbit and cow or heifer manure (OR = 3.6, 95% CI = 1.3 to 9.5).

There was no statistically significant association between the use of drylot and contact between cows or heifers and deer (Table V). There was an association between the use of drylot by heifers and contact between rabbits and heifer manure, though no statistically significant association was found between the use of drylot by cows and contact between cow manure and rabbits (Table V).

Results from wildlife sampling

All deer and rabbit fecal samples collected in areas where exposure to cattle was highly unlikely (no dairy farms) were negative by culture for MAP, as were all mesenteric lymph nodes extracted from deer. All 12 deer serum samples tested negative for antibodies for MAP.

Table V. Association (odds ratio [OR], 95% CI) between the use of pasture or drylot by cows or heifers and contact between deer or rabbits and cattle manure around Minnesota dairy farms (*n* = 114)

	Use of pasture		Use of drylot	
Contact between	Deer	Rabbits	Deer	Rabbits
Cows	9.8 (3.9 to 24.6)	4.0 (1.7 to 9.1)	0.9 (0.4 to 1.9)	1.9 (0.9 to 4.5)
Heifers	5.8 (2.3 to 14.5)	4.1 (1.7 to 10.3)	1.6 (0.7 to 3.5)	4.0 (1.61 to 10.0)

One hundred and fourteen farms were sampled for feces from deer, rabbits, or both. Rabbit samples (*n* = 218) were collected from 90% of the 114 farms and 309 deer samples were collected from 47% of the farms. On 2 (4%) of farms where deer feces were collected, 50 km apart in northern Minnesota, 1 deer fecal sample was positive for MAP. Both samples were defined as low shedders (1 to 10 CPT). On one farm, samples were collected approximately 300 m from the cow barn in a wooded area near an alfalfa field. On the second farm, feces were collected near a cornfield (adjacent to a wooded area) approximately 400 m from the cow barn and 200 m from a cow pasture. On both farms, the cattle and environmental samples were positive to MAP. On one of these farms, the potential for physical contact between deer and manure of dry cows and heifers on pasture was estimated as daily between October and May. The farmers reported seeing deer weekly during spring with a usual group size of 2 to 3 and maximum of 6. On the second farm, the frequency of possible contact between cattle (dry cows and bred heifers) and deer feces was estimated as less than monthly and only between April and October. On this farm, deer were seen weekly only during spring and fall ("seasonally"), and the usual group size was 3 to 10 with a maximum of 20. The cattle fecal pool prevalence in those 2 farms was 45% and 19%, compared to an average 26% fecal pool prevalence among all 66 herds with positive pools (no statistical difference between groups).

On 2 (2%) of farms where rabbit pellets were collected, 1 rabbit fecal sample was positive by culture for MAP. These pellets were collected near the cow area on one farm and near the heifer area on the other. On one of the farms, pellets were collected approximately 20 m from the cow barn or heifer area. The sample shedding level was low (mean of 1 to 10 CPT); however, cows and environmental samples were negative by culture to MAP. Nevertheless, in the year 2000, the farm had a cow with a positive fecal culture. The farmer estimated that rabbits were observed "daily" around the farm and estimated the potential contact between cows and rabbit feces as "never," but the potential contact between heifers and rabbits was estimated as "monthly" during winter and "daily" during summer. On the second farm, pellets were collected approximately 50 m from the cow barn and 5 m from the heifer area. In rabbit feces, the level of shedding was moderate (10 to 49 CPT), and cows and environmental samples were MAP positive. The cattle fecal pool prevalence for this farm was 25%, which did not statistically differ ($P > 0.05$) from the average fecal pool prevalence; the maximum CPT in the cow and environmental samples was heavy (> 100 CPT). The farmer estimated the frequency that rabbits were seen around the farm as "monthly," and the potential for contact between rabbits and cow or heifer feces as "never" and "less than monthly," respectively. Probable season of contact with heifers was estimated as between May and October, when cattle are in pasture.

Discussion

This is the first study to describe the use of farm management risk factors related to MAP transmission among cattle, deer, and rabbits. Furthermore, this is the first US study to assess the prevalence of MAP infection in rabbits around dairy farms. A strength of this study was the large sample sizes of herds, cows, environment, and wildlife. Previous reports have indicated MAP existence in several wild ruminant species, but the relationship between livestock and these species is unclear. Riemann et al (12) suggested a common source for infection rather than an association between the prevalence of MAP in 10 dairy herds and deer at Point Reyes, California. In Scotland, an association was found between herds with a JD history and infected rabbits (16). These 2 studies did not relate farm management practices with MAP infection in deer and rabbits.

The current study population included deer and rabbits across Minnesota both in areas where dairy herds did and did not exist, as well as from various wildlife habitats where deer density varies. A limitation was that deer and rabbit fecal samples were not associated with known individuals. Consequently, it is possible that several samples from the same individuals were collected, especially in the case of deer. Another limitation of this study was that sampling of fecal pellets did not permit us to evaluate whether the presence of MAP in the feces was due to active infection or simply to bacteria passing through the digestive system after ingestion of infected feces (cattle or wildlife). This positive shedding has been previously described for cows (25). However, one of the positive rabbit fecal samples was a moderate shedder (11 to 49 CPT), which increases the probability of primary MAP infection. A further assessment of MAP infection would require the collection of animals and bacterial culture, and histopathological examination of intestinal tissue and lymph nodes. On the other hand, it is possible that some deer and rabbits were shedding below the limits of detection for the current fecal culture method. It is the first time that the current method, different from that described previously (18), was used to detect MAP in rabbits. The current method had been previously used at the MVDL to detect MAP in non-domestic ruminants with positive cultures, such as white-tailed deer and elk. Another limitation of the current study was that due to the low number of positive wildlife fecal samples, it was not possible to detect statistical associations between MAP in wildlife and infected herds, nor to detect associations between MAP in wildlife and farm management practices.

Modes of transmission of MAP within the herds of dairy cattle are well described in the literature (1), and are related to farm management practices, such as cleanliness of calving barns and contact between calves and infected manure through feed, farm utensils, and farm employees. Two studies (12,26) have suggested that manure spread on pasture and sharing pasture between wildlife and cattle are possible sources of common infection for deer and rabbits. Nevertheless, risk factors for MAP transmission between cattle and wildlife or vice versa have not been previously described.

Our study shows that while MAP can be found among rabbit and deer feces around dairy farms, the apparent fecal prevalence is low (about 2% and 4% of sampled dairy farms, respectively). This suggests that the primary source for MAP transmission does not involve wildlife, but instead farm practices such as use of a common calving pen and feeding pooled colostrum to young heifers. Although the objective of this study was not to determine the direction of MAP transmission, whether from cattle to rabbits or deer or vice versa, it is reasonable to assume, based on the low fecal prevalence of MAP among deer and rabbits and the high prevalence among cattle and their environment, that the primary direction (or risk) of MAP transmission is from cattle to deer or rabbits.

Our questionnaire results suggest that one risk factor for transmission of infection between cattle and wildlife is direct contact with feces of infected animals or the ingestion of contaminated vegetation through pastures and drylot. The use of pasture or drylots by milking or dry cows likely involves a higher risk for cattle to wildlife transmission than use of pasture by heifers, especially among farms with a high frequency (daily or weekly) of possible contact between cattle feces and wildlife, because MAP shedding occurs through feces primarily of adult cows and less often among heifers.

Potential transmission from rabbits to cattle has been the subject of recent studies (27). Beard et al (27) showed experimentally that MAP isolates from naturally infected rabbits (*Oryctolagus cuniculus*) are capable of infecting young calves and causing early JD histological lesions. Daniels et al (19) found that during grazing, cattle do not avoid contact with experimentally deposited contaminated rabbit feces. Linking these findings to our study, and given that heifers are most susceptible to MAP infection, the use of pasture or drylots for prebred heifers is a possible risk for MAP transmission from wildlife to young cattle.

Another potential risk factor for MAP transmission from cattle to deer or rabbits is the farm practice of manure spreading on crop fields and pasture. Although there are no data available regarding the survival of MAP on crop fields, there are some data available about the survival of MAP in slurry (11), which is the main source of manure spreading in the current study. In the current study, samples of stored manure on 60% of the MAP positive farms were positive to MAP (21). Since most of the farms spread manure on crop fields during the winter months, when only corn residuals are available in the fields, it is possible that deer or rabbits can ingest contaminated manure while searching for waste corn.

Because of the presence of MAP in wildlife, their role as MAP reservoirs should be considered, particularly where contact with cattle is possible (in pasture or drylot). It is also important to consider the potential risk that deer pose for between-herd transmission given the fact that they move large distances (28). Rabbits move shorter distances, although they can also come into contact with cattle of neighboring herds. Furthermore, it is possible that the infected deer or rabbits did not necessarily acquire the bacteria from the study farms, but from other infected farms. In this case, wildlife can serve as a vehicle to transmit the bacteria between dairy herds. This risk is especially important to noninfected herds, where deer and rabbits could contribute to MAP introduction into the herd

In summary, it is important to reinforce that domestic species pose a higher risk of MAP transmission to wildlife than wildlife pose to domestic species. First, MAP prevalence in cows was much higher than among wildlife feces. In addition, cattle and cattle manure are transported farther distances than deer can travel, especially when considering the low prevalence in wildlife.

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