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Absence of *Mycobacterium bovis* infection in dogs and cats residing on infected cattle farms: Michigan, 2002

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SUMMARY

A cross-sectional field study was performed to evaluate infection in dogs and cats living on farms with *Mycobacterium bovis*-infected cattle. The purpose was to determine pet infection status and assess their risk to farm families and/or tuberculosis-free livestock. Data and specimens were collected from 18 cats and five dogs from nine participating farms. ELISA testing for *M. bovis* and *M. avium* was conducted. Fifty-one biological samples were cultured; all were negative for *M. bovis*, although other *Mycobacterium* species were recovered. No radiographic, serological or skin test evidence of mycobacterial infection was found. These negative results may be due to the low level of *M. bovis* infection in the cattle and the limited duration of exposure of pets to infected cattle residing on the same farm. No evidence was found to indicate that pets residing on *M. bovis*-infected Michigan cattle farms pose a risk to humans or *M. bovis*-free livestock; however, precautionary advice for farm owners was provided.

INTRODUCTION

Causing disease in a wide range of mammals, *Mycobacterium bovis* has the broadest host range of the members of the *Mycobacterium tuberculosis* complex [1] and is well established as a zoonotic disease. Historically, milk-borne transmission has been responsible for most human *M. bovis* infections. In developed countries, this route of transmission was virtually eliminated following the widespread adoption of milk pasteurization. *M. bovis* has recently become established in the wild white-tailed deer population of the northeast portion of Michigan's lower peninsula. Identified in 1994 in a hunter-harvested white-tailed deer, *M. bovis* has been found in 449 deer (out of 105885 tested) up to 2002. From 1996 to 2002, several additional species have been tested and found positive for *M. bovis* infection in Michigan including: 19 coyotes, eight raccoons, seven black bear, four bobcat, three red fox, two opossum, two elk and one semi-feral domestic cat [2, 3]. At the time of this study, 23 infected cattle herds have been found [4]. The wide diversity of infected species suggests several potential new routes of transmission for *M. bovis* from animals to humans.

Pet to cattle, cattle to pet

Scientific literature describing the role of domestic pets in the transmission of *M. bovis* on the farm (livestock to pet, pet to livestock) is fairly limited and quite dated. While uncommon in both dogs and cats, historical data suggests that dogs were more likely to be infected with *M. tuberculosis* following exposure to

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infected humans, while cats were more likely to be infected with *M. bovis* with exposure assumed to be related to the consumption of contaminated animal products [5]. Historically, farm cats and dogs were at very high risk of acquiring *M. bovis* from infected cattle; 4/9 dogs and 24/52 cats were affected after exposure to positive cattle in a Pennsylvania study [6]. It is therefore feasible that pets could play a role in the maintenance of *M. bovis* on a farm [7], however, literature describing pet transmission to cattle is hypothetical [8] or limited to references from Eastern Europe in the 1950s and 1960s [9–12].

Transmission from pets to humans

Literature describing the role of pets in transmission of *M. bovis* to humans is also very limited, although transmission would again be biologically plausible. Early necropsy studies (1930–1965) revealed a tuberculosis (TB) prevalence ranging from 2·0% to 13% in cats and 0·4% to 2·0% in dogs [13]. There is no evidence that dogs and cats have transmitted *M. bovis* infection to humans; only one inconclusive cat-to-human reference was found [14]. In Michigan, wild carnivores and omnivores are considered dead-end hosts for *M. bovis*. These animals are most typically exposed to infection via the consumption of infected deer carcasses, thus resulting in a gastrointestinal clinical presentation with limited potential for transmission to people or other animals [15].

Clinical presentation

Clinical findings in dogs infected with *M. tuberculosis* include: anorexia, loss of body weight, lethargy, vomiting and leukocytosis; radiography revealed pleural and pericardial effusion, ascites, and hepatomegaly [16]. In cats, the most common clinical sign associated with *M. bovis* infection was a moist skin lesion [17]. Additional clinical signs included lymphadenopathy (primarily the head and mesenteric lymph nodes) and liver, spleen and lung lesions in generalized cases [3, 17]. It is also notable that in the Pennsylvania study, TB infection frequently occurred without apparent clinical signs in the pets [6].

Pets and the control of *M. bovis*

The regulatory response following the detection of *M. bovis* infection in a herd of cattle is to place the farm under quarantine. The herd is then either scheduled for depopulation, or placed on a rigorous testing schedule to remove TB responders from the herd [18]. United States federal recommendations include removing other susceptible livestock and pets from the farm during the cattle depopulation phase [19]. However, regulatory officials in Michigan have not included pets in their depopulation efforts.

This study assesses the potential role that dogs and cats may play in the transmission of *M. bovis* to livestock and humans, and evaluates their possible role in the epidemiology of the current Michigan outbreak. To accomplish this objective, the exposure and *M. bovis*-infection status of the dogs and cats living on farms where cattle had recently been diagnosed with *M. bovis* was evaluated. Pet owners on these farms were also offered advice on how to prevent pet exposure to *M. bovis* and how to minimize human exposure to potentially infected dogs and cats.

METHODS

Farm enrolment

From October 1997 until August 2002, 23 Michigan cattle farms were found to be infected with *M. bovis* and were placed on a control programme by the Michigan Department of Agriculture. This study took place in June and August 2002 and attempted to include all recently or currently infected farms. All farms were located in the northern portion of the lower peninsula of Michigan. Repeated attempts were made to contact the owners of all 23 farms to invite participation in the study. Phone calls were attempted initially, followed by several on-farm visits if phone contact was unsuccessful.

Pet enrolment

A pet was considered eligible for inclusion in the study if it was >6 month of age and resided on the farm when infected cattle were present. All cats including 'barn', 'feral', and 'indoor only' were eligible for the study. Written informed consent was obtained from the pet owner for each specific pet and each clinical procedure. Historical information obtained for each pet included: age, gender, physical description, range (cats: indoor only, indoor/outdoor, outdoor only; dogs: tied, loose, free to wander off the farm), diet, raw milk exposure, length of time living on farm, exposure to cattle (sharing barn), vaccination status and medical history. Live traps were used if necessary to

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capture outdoor cats. If the owner desired, the pets were spayed or neutered and returned to the farm within 2 days. If the owner did not wish to have the dog or cat returned to the farm, consent for euthanasia was obtained.

Clinical examination and specimen collection

The clinical examination and sample collection took place at a local veterinary clinic. A single veterinarian was used to perform all procedures, to ensure consistency. Sedation was used at the clinician's discretion. For both cats and dogs, the protocol included a physical examination, radiographs of the chest and abdomen, fine-needle aspirate of any enlarged superficial lymph nodes, the collection of rectal and oral swabs and a 5-ml blood sample. For cats, a combined feline leukemia (FeLV) and feline immunodeficiency virus (FIV) ELISA test was done. The remaining serum was frozen and sent to Dr C. Thoen's Laboratory (College of Veterinary Medicine, Iowa State University, Ames, IA, USA) for comparative ELISA (*M. bovis* and *M. avium*) [20] testing. For dogs, 0·1 ml of 250 TU PPD (tuberculin units of purified protein derivative) was placed intradermally in the inner surface of the pinna, with interpretation of the skin test made by the researcher within 48–72 h [7]. If the owner consented to euthanasia of unwanted feral animals, the above protocol was followed with the exception of the collection of the fine-needle aspirate and the faecal and oral swabs. The animals were euthanized at the local clinic and transported on ice the next day to the Michigan State University, Diagnostic Center for Population and Animal Health (DCPAH), East Lansing, MI, USA.

Necropsy protocol

Necropsies were performed at DCPAH, the day following euthanasia at the local clinic. The necropsy included gross examination of all tissues, and the collection of the following tissue pools for mycobacterial culture: cranial (parotid, submandibular and retropharyngeal) and thoracic (mediastinal and tracheo-bronchial) lymph nodes and lungs, abdominal lymph nodes (mesenteric and ileo-caecal), abdominal viscera (spleen, liver, kidney), small and large intestine. The following tissues were fixed in formalin and examined histologically: brain, cranial lymph nodes, tonsil, trachea, lung, thoracic lymph nodes, heart, spleen, kidney, liver, pancreas, adrenal gland, abdominal lymph nodes, small and large intestine. Ziehl–Neelsen acid-fast staining was applied only to slides exhibiting lesions suggestive of mycobacteriosis on the histological examination.

Radiological examination

The radiographs were reviewed by a board-certified radiologist in the College of Veterinary Medicine (Michigan State University, East Lansing, MI, USA) for evidence of mycobacterial infection. The radiologist was given only the animal's age and study identification number. The thoracic and abdominal radiographs were evaluated for evidence of lymphadenopathy and the lungs evaluated for abnormal parenchymal changes.

Microbiology and strain typing

Mycobacterial culture and identification was performed at the Michigan Department of Community Health, Bureau of Laboratories, Mycobacterial Laboratory, Lansing, MI, USA. Recommended procedures were followed for specimen digestion, concentration and examination [21]. Sediment of concentrated specimens was examined microscopically for acid-fast bacilli. Sediment of the specimens was then re-suspended by the addition of 1·5 ml PBS solution, and aliquots were inoculated onto a slant that contained Lowenstein–Jensen medium (Lowenstein–Jensen BB20909, Becton Dickinson, Sparks, MD, USA), onto a slant that contained a Middlebrook-based medium (Middlebrook 7H11S, Becton Dickinson) and into a vial that contained broth for microbial culture (Bactec 12B broth vial, Becton Dickinson). Media were examined for growth at least weekly for 8 weeks. Acid-fast bacteria were tested by use of a genetic probe (Accuprobe, Gen-Probe, San Diego, CA, USA) [22] to determine whether the bacteria were members of the *M. tuberculosis* complex. Biochemical testing and high- performance liquid chromatography were used to differentiate *M. bovis* from other members of the *M. tuberculosis* complex and to speciate other mycobacteria [21, 23, 24].

Determination of minimum exposure period

We estimated the minimum period during which each dog or cat could have been exposed to infected cattle on the farm. To do this, we first determined the

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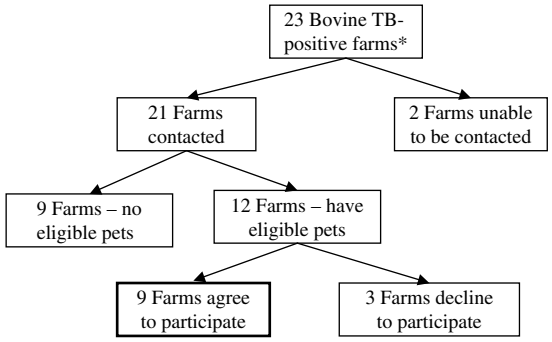

Fig. 1. Flow diagram showing how participating farms were selected. * Number of positive farms at 8 August 2002.

minimum period that infected cattle were present on the farm (the minimum infection period) by calculating the difference between the date when cattle on the farm tested positive to *M. bovis* (based on the caudal fold skin test results) and the date when all infected cattle were subsequently removed from the farm. We then determined the length of time that each pet was present on the farm during the above-mentioned minimum infection period. This estimate is considered the *minimum* exposure period, because the cattle may have been positive for months to years prior to being tested and found to be positive for *M. bovis* infection.

RESULTS

At the end of the study period, 23 farms had been identified as *M. bovis* infected. Twenty-one (91%) farm owners were successfully contacted and invited to participate in the study. Nine (43%) of the 21 contacted farm owners had no dogs or cats eligible for inclusion. Twelve (57%) had eligible pets, of which nine (75%) agreed to participate (Fig. 1). Eighteen cats and five dogs were enrolled in the study from these nine farms (seven beef, two dairy). Characteristics including age, gender, diet, housing and exposure for enrolled dogs and cats are summarized in Table 1.

Cats

Only four cats (22%) had ever been vaccinated (for any disease) and for only one cat was vaccination status up to date. All cats were FIV negative; two (11%) cats were FeLV positive. The two FeLV-positive cats were euthanized and a full necropsy performed. The 18 cats experienced a wide range of expected barn cat ailments: ear mites/otitis externa ($n=5$, 28%), bloated abdomen ($n=3$, 17%), missing hair/poor hair coat/scabby skin ($n=3$, 17%), tracheitis ($n=2$, 11%), enlarged submandibular lymph nodes ($n=2$, 11%), conjunctivitis ($n=2$, 11%), runny eyes, dental disease, congestion of the lungs, and diarrhoea (one each). Sixteen oral swabs and 16 faecal swabs were submitted for mycobacterial culture (two per live cat). In addition, eight pooled organ samples were submitted for culture (four for each of the two euthanized cats). All culture results were negative for *M. bovis*; one faecal culture was positive for *M. avium* complex. Radiographs were found to be unremarkable for all 18 cats; no signs of lymphadenopathy in the abdomen or thorax were noted. Gross pathological and histological examination of the two euthanized cats revealed no evidence of mycobacterial infection. Three cats tested ELISA positive for *M. avium* at the 1:160 dilution. Two of these cats also tested positive for *M. bovis* but at $<1:160$ dilution; these results were determined to be cross-reactions with *M. avium*.

Dogs

All dogs were allowed to run loose on the farm and presumably had substantial contact with the cattle. No dogs showed noticeable or measurable TB skin test response at 48–72 h. An oral swab and a faecal swab from each of the five dogs were submitted for mycobacterial culture (two per dog). All culture results were negative for *M. bovis*. Two faecal cultures were positive for *Mycobacterium* spp. (group IV unclassified). Radiographs of the thorax and abdomen were found to be unremarkable for all five dogs, comments included: mild right loss of cranial waist ($n=1$) and widened mediastinum due to fat, loss of cranial cardiac waist, mild right heart enlargement ($n=1$). No signs of lymphadenopathy in the thorax or abdomen were noted. Negative responses were detected on the ELISA test for all five dogs for the *M. bovis* and *M. avium* antigens.

DISCUSSION

The herds on the participating farms had a low prevalence of cattle infected with *M. bovis*. Only 14/869 (1·6%) cattle tested were either suspect or positive using the comparative cervical test. Following necropsy of all comparative cervical suspect and reactor cattle, an average of less than one gross lesion per bovine (11/14) was found in the cattle

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Table 1. *Summarized characteristics of study participants by species*

Characteristic	Cats ($n=18$)	Dogs ($n=5$)
Gender	11 Males (61%)	2 Males (40%)
Average age (yr)	4·1 (range 1·0–12·5)	6·1 (range 2·0–11·5)
Routinely fed	Yes, $n=15$ (83%)	Yes, $n=5$ (100%)
Fed raw milk	Yes, $n=5$ (28%)	Yes, $n=0$ (0%)
Outdoor only	Yes, $n=12$ (67%)	Yes, $n=4$ (80%)
Known or likely to have shared barn with infected cattle	18 (100%)	0 (0%)
Average exposure period* (months)	2·3 (range 1·0–7·0)	4·0 (range 2·0–8·0)

* Calculated as the minimum possible exposure period.

on the participating farms, indicating the absence of advanced, heavily diseased cattle with the potential to shed high numbers of infectious organisms. The low prevalence of infection in the herd and low severity (limited progression) of infection in individual animals may explain the apparent lack of transmission to the dogs and cats.

During the course of the outbreak in Michigan, the control and eradication efforts by state and federal agriculture officials intensified. Positive farms were quickly identified by contact tracing or by area testing, and the time between diagnosis and depopulation of infected cattle was shortened. Thus, the minimum exposure period became progressively shorter as the bovine TB eradication efforts in Michigan became more efficient at early detection of infection in cattle. The detection and control efforts also decreased the likelihood of infected cattle progressing to a clinical stage of disease where *M. bovis* would be transmitted via shedding into the milk.

The historical method of infection for cats with *M. bovis* has been through the consumption of infected raw milk. Five of the study cats came from the two dairy farms. Although these cats (5/18, 28%) did routinely consume raw milk, it is highly unlikely that any of the milk cows had infection that had progressed to the point of shedding *M. bovis* in their milk. Only one animal was found positive by comparative cervical testing per dairy farm and only one gross lesion was detected in each of these positive cows. Furthermore, none of the positive cows had lesions in the supra-mammary lymph nodes, further reducing the likelihood of shedding of organisms into the milk. All of our study cats were likely to have slept in the barn with the infected cattle. However, because the cattle were not heavily infected, exposure via aerosolized droplets was unlikely.

Wild carnivores that have acquired *M. bovis* in Michigan have presented as gastrointestinal infection, presumably as the result of consuming gut piles left from hunted infected deer, or by scavenging or hunting infected animals [15]. In our study population, all five dogs were routinely fed by their owners, decreasing the likelihood that they would consume deer gut piles. According to the owners, the dogs were routinely allowed to run loose on the farm, but did not often leave the farm premise, so exposure to gut piles would expectedly have been rare. The study dogs did not sleep in the barns with the infected cattle so aerosol transmission is also unlikely.

Transmission of *Mycobacterium* spp. from an infected dog has never been documented. Carnivores are most likely to be infected via consumption of infected milk or meat and present with gastrointestinal infection. Thus cats and dogs are generally felt to be less likely to transmit the disease unless the disease progresses to a systemic infection due to suppression of the immune system. Four out of five of our study dogs were strictly outdoor dogs and all were in fair to good health, making them a very low risk for clinical disease and shedding, even if they had become infected.

Cats pose a higher transmission risk to both humans and cattle than dogs for several reasons: they appear to be less resistant to infection than dogs, they have a closer relationship with both cattle (sharing the barn, consuming raw milk) and with humans (more likely to be indoor/outdoor and sleep in same bed as humans), they have recently been proven scavengers [25] and they are susceptible to common viruses, FeLV and FIV, which specifically compromise their immune system. An immunocompromised cat is more susceptible to infection in general, and a correlation between FIV, *M. bovis* infection and clinical disease

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has been recently hypothesized [26]. Thus, cats infected with FeLV or FIV and exposed to *M. bovis* could pose a much higher risk to human owners (and cattle) than an immunocompetent cat, as the disease is more likely to progress clinically, increasing the likelihood of transmission to others.

Diagnosis of *M. bovis* in live dogs or cats is very difficult, and our study protocol included all non-invasive procedures available at the time. Only two cats were offered for post-mortem examination; both were positive for FeLV, perhaps making them the best candidates for *M. bovis* isolation if it were present.

CONCLUSION

In the final analysis, no evidence was found to indicate the transmission of *M. bovis* from infected cattle to farm dogs or cats. The likelihood of dog and cat infection was judged to be minimal due to a low risk of cattle exposure, a low expected exposure dosage and a relatively short duration of exposure to the infected cattle. In Michigan, even if a farm dog or cat were to become infected, its potential to transmit infection to humans or cattle is estimated to be very low.

Recommendations

Despite the low risk of infection of pets and transmission from pets, the following prevention recommendations were made to pet owners on the farms infected with *M. bovis*:

- Do not feed pets raw milk.
- Keep house cats strictly in the house, and barn cats out of the house.
- If barn cats are allowed into the house, keep them away from your face, especially if they are ill.
- Do not allow dogs to roam freely.
- Keep pets healthy (fed and vaccinated) because an ill or weak animal is more susceptible to infection with *M. bovis* and more likely to progress to clinical disease if infected.

In addition, each farm owner was strongly encouraged to have family members and employees evaluated for possible *M. tuberculosis* complex exposure on an annual basis.

Regulatory veterinarians should carefully assess the health status of pets on infected cattle farms and seriously consider following the federal recommendations to depopulate pets that have been heavily exposed to infected cattle. Cattle owners should clearly understand that pets do pose a health threat, albeit remote, to their family and to TB-free livestock purchased to re-populate the farm. Because skin testing in both dogs and cats is unreliable, and infected pets may be asymptomatic, the development and use of reliable ante-mortem tests should be considered as an *in-vivo* testing alternative for domestic pets on *M. bovis*-infected farms. In fact, a study by members of our group is currently under way for the evaluation of several different ante-mortem assays for bovine TB detection in cats.

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DECLARATION OF INTEREST

None.

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