Epidemiologic investigation of Mycobacterium bovis in a population of cats

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Objective—To determine whether cats exposed at a residence were infected with Mycobacterium bovis, whether the tuberculin skin test can identify cats infected with M bovis, and whether an ELISA could identify tuberculosis-infected cats.

Animals—20 domestic cats exposed to a cat with laboratory-confirmed disseminated M bovis infection.

Procedure—Cats were administered a tuberculin skin test and monitored for 72 hours. Blood and fecal samples were collected. Cats were then euthanized, and postmortem examinations were performed. Tissues were examined grossly and histologically for signs of mycobacteriosis. Pooled tissue samples and fecal samples were submitted for mycobacterial culture. Blood samples were examined for evidence of tuberculosis by use of a comparative ELISA.

Results—4 cats had positive responses for the ELISA, and 2 cats had suspicious responses. All tuberculin skin tests yielded negative results. No gross or histologic lesions of tuberculosis were detected in any tissues, and mycobacteria were not isolated from tissues or feces obtained from the 20 cats.

Conclusions and Clinical Relevance—All cats that had positive or suspicious responses for the ELISA were offspring of the cat with tuberculosis. Evidence of tuberculosis was not seen in other cats at the residence, the owner, or the attending veterinarian. The most likely source of tuberculosis for the infected cat was through the consumption of M bovis-infected wildlife carcasses or offal. Because M bovis is endemic in wildlife in northeastern Michigan, there is a risk of exposure to tuberculosis in companion animals, their owners, and attending veterinarians. (Am J Vet Res 2002;63:1507–1511)

Materials and Methods

Study population—Twenty cats from the premises that were at the highest risk of having tuberculosis were included in the study. Cats were included in the study if they were exposed to the tuberculous cat (n = 12; the 7 offspring of the M bovis-infected cat and 5 cats that were housed with the offspring), were clinically ill or had a history of illness (3), or were cats that roamed freely on the premises. This included 7 offspring of the tuberculous cat (6 from a litter in 1997 and 1 from a litter in 1999) that were kept in cages on the owner’s property.

The discovery of disseminated tuberculosis in the cat suggested the possibility that the remaining cats, the owner, and the attending veterinarian could have been exposed to M bovis. Because of the lack of an antemortem test for tuberculosis in domestic cats, this situation provided a unique research opportunity. The objectives of the study reported here were to determine whether additional cats at the facility were infected with M bovis, whether the tuberculin skin test could be used to identify tuberculosis infection in cats, and whether an ELISA could be used to identify tuberculosis-infected cats.

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M. bovis purified protein derivative (PPD) was injected intradermally into the measured area by use of a 26-gauge needle and tuberculin syringe, and a waterproof marker was used to make a circle around the injection site. At 48 and 72 hours after injection, each site was visually examined and palpated, and thickness of the folded skin at each injection site was measured and recorded.

Collection of blood and fecal samples—Five milliliters of blood was collected from each cat during the time they were sedated for administration of the tuberculin skin test. Blood samples were transported to Michigan State University where they were centrifuged. Serum was harvested and stored in an ultracold freezer prior to shipment to Iowa State University for analysis by use of an ELISA. Fresh fecal samples were collected from cats at the time of the examinations and stored in refrigerated storage. Prior to necropsy at a diagnostic laboratory, the PPD injection site was removed from each cat to ensure that pathologists remained unaware of results for the tuberculin skin test. Necropsies were performed on the day after cats were euthanatized. Gross examinations were performed, and histologic examination of slides stained with H&E and with acid-fast stains was conducted. Tissue samples (lungs, liver, spleen, kidneys, uterus, and cranial, thoracic, and abdominal lymph nodes) were pooled for each cat and sent to a laboratory for mycobacterial culture. Also, any suspect lesions found during examination were submitted for mycobacterial culture.

ELISA for serum samples—An ELISA was conducted by use of modified procedures described elsewhere. Bovine PPD and M. avium PPD were diluted 1:200 and placed in separate wells of a microtiter plate, and serial dilutions (1:20, 1:40, 1:80, and 1:160) of each serum sample from each cat were prepared. Fifty microliters of protein A labeled with horseradish peroxidase (dilution, 1:2,000) was added to each well, and plates were placed on a shaker for 30 minutes. Plates were then allowed to dry for 15 minutes. After the addition of 100 µL of 2,2’-azino-di(3-ethyl-benzthiazoline-6-sulphonate) substrate solution, the plates were incubated at 22°C for 30 minutes. The color that developed as a result of the reaction was measured by use of an ELISA reader at a wavelength of 405 nm. An ELISA value of > 1.0 for a serum dilution of 1:80 was considered a positive response, whereas an ELISA value of > 1.0 for a serum dilution of 1:40 and < 1.0 for a serum dilution of 1:80 was considered a suspicious response.

Mycobacterial isolation and strain typing—Culturing and identification of mycobacteria was performed at the same laboratory. Specimens were digested, concentrated, and examined in accordance with recommended procedures.

Results
Tuberculosis was disseminated and resulted in the death of the infected cat (ie, the index case). Grossly, the cat was thin and had multiple yellow foci throughout the liver and lungs. Histologically, the mesenteric lymph nodes had severe multifocal and coalescing granulomatous lymphadenitis. There was severe and
diffuse granulomatous pneumonia in the lungs as well as mild to moderate multifocal granulomatous splenitis and hepatitis, and the heart contained multiple small foci of myocardial fibrosis with mild inflammation. Lesions were characterized by variable-sized areas of caseous necrosis surrounded by zones of histiocytic cells and a few multinucleated giant cells (Fig 1). In tissue sections stained with Ziehl-Neelsen stain, many acid-fast bacilli were found in areas of caseous necrosis (Fig 2).

Of the 20 cats enrolled in the study, there were 9 males (3 sexually intact, 6 neutered) and 11 females (9 sexually intact, 1 neutered, 1 unknown), and 10 were > 6 years old. All responses for the tuberculin skin test were negative at 48 and 72 hours after injection. We did not detect lesions compatible with tuberculosis in the 20 cats, although 7 cats had gross or histologic lesions associated with conditions other than tuberculosis (eg, interstitial nephritis, adenocarcinoma). Mycobacteria were not isolated from any fecal samples obtained from the cats. Examination of tissues stained with acid-fast stain did not reveal organisms.

Positive responses for the ELISA were detected for sera of 4 of the offspring of the M bovis-infected cat for the M bovis PPD antigen. Suspicious responses were detected for sera of 2 other cats (1 was an offspring of the infected cat, and the other was a cat housed with offspring of the infected cat). Similar responses were observed for serum of these 6 cats when tested by use of the ELISA for the M avium PPD antigen. We detected only negative responses for the ELISA for the sera of the other 14 cats for the M bovis or M avium PPD antigens.

Comparisons were made between the PGRS RFLP patterns of M bovis isolated from the tuberculous cat and PGRS RFLP patterns of isolates from other species of animals in Michigan (Fig 3). The analysis indicated that the M bovis isolate from the tuberculous cat was genetically identical to the M bovis strain endemic in the wildlife population in Michigan.

**Discussion**

*Mycobacterium bovis* was not isolated from any of the 20 cats tested in this study, as determined on the basis of mycobacterial culture. Although various tests have been investigated, there is not an effective ante-mortem test for detecting tuberculosis in domestic cats. In other studies, results of tuberculin skin tests suggested that the tuberculin skin test had poor sensitivity when used for cats, and because *M bovis* was not isolated from any cats in the study reported here, we were unable to estimate the sensitivity of the tuberculin skin test. Positive responses for the ELISA were seen for 4 of the 6 offspring born in 1997 to the infected cat, and suspicious responses were seen for 1 of the 6 offspring born in 1997 as well as a cat housed with these offspring. We have only limited information on the importance of false-positive results; however, analysis of data obtained to date indicates that this does not seem to be a problem when examining a population in which *M bovis* has been diagnosed.
Because all cats with positive or suspicious responses for the ELISA were offspring of the infected cat or housed with those offspring, there was evidence for horizontal transmission from the infected cat to her kittens and from exposed offspring to other cats that shared housing facilities. In addition, there may have been vertical transmission from the affected cat to her kittens. In 1 report, 3 cats had tuberculous lesions in the uterus, indicating the possibility of a congenital route of infection. Furthermore, mammary gland infection in cattle and the transmission of tuberculosis via milk has been clearly documented. Although the uterus and mammary glands of the tuberculous cat of the study reported here were not examined, it is possible that these organs were infected and could have served as a route of exposure for her offspring. The cat's solitary lifestyle and limited interaction with the other cats on the premises appeared to have limited the spread of tuberculosis for the other cats in this facility.

Historically, most cases of M bovis in cats have been associated with the disease in livestock through drinking unpasteurized milk and the consumption of raw meat or offal. With the success of the tuberculosis eradication program in cattle, the number of cases of tuberculosis in cats has greatly decreased.

Recently, the spread of tuberculosis from wildlife to cats is believed to have been responsible for several cases of tuberculosis in domestic cats. Tuberculosis attributable to M bovis is endemic in white-tailed deer located in the northeastern portion of the lower peninsula of Michigan, and consumption of tissues of tuberculous deer is the most likely source of this disease in a number of carnivore and omnivore species. The finding of identical PGRS RFLP patterns between the infected cat and white-tailed deer, combined with the scavenging behavior of domestic cats, suggests that the infected cat contracted tuberculosis in the same manner as other infected carnivores in the area. Analysis of RFLP patterns has proven useful in determining the epidemiologic relationships among isolates of M bovis obtained from various animals. According to 1 of the authors (DB), the laboratory used in the study reported here has performed PGRS RFLP on 163 M bovis isolates from animals (white-tailed deer, other wildlife, and cattle) and 5 M bovis isolates from humans since 1994. Of the 163 M bovis isolates recovered from wildlife during that period, 150 had an identical or closely related PGRS pattern, which indicated clonal expansion of this strain among white-tailed deer in the wild. The isolate from the infected cat reported here and the predominant pattern identified in wildlife had identical PGRS fingerprint patterns. Additionally, PGRS RFLP patterns of M bovis isolates from humans that were tested at the laboratory did not match patterns of isolates obtained from wildlife, which agreed with the finding that none of the cases of tuberculosis in humans in Michigan were epidemiologically linked to wildlife.

On the basis of the history for the M bovis-infected cat, its proximity to tuberculous deer, and the RFLP pattern of the isolate, the most likely source of the infection in the cat reported here is the consumption of a carcass of a tuberculous wild deer. Because M bovis can be transmitted from wild and domestic animals to humans, this suggests that infected companion animals in northeastern Michigan could serve as a source of tuberculosis for humans. Fortunately, the owner and attending veterinarian of our report had negative results for tuberculin skin tests conducted by public health officials.

The current recommendation is to euthanatize cats that have contact with M bovis-infected animals. The finding of 6 cats (5 of which were known to have a close association with the M bovis-infected cat) that had positive or suspicious responses for the ELISA suggested that an antemortem test for detecting tuberculosis in cats may be possible. A test capable of detecting M bovis-infected cats, especially cats that have been sufficiently exposed to M bovis to mount an immune response, would enable the removal of only those cats that are at high risk of developing tuberculosis. With a wildlife reservoir of tuberculosis and the recent increase in the number of tuberculosis-infected livestock in Michigan, an effective antemortem test for detection of tuberculosis in companion animals would be of tremendous value.

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