EXPERIMENTAL INOCULATION OF MEADOW VOLES (MICROTUS PENNSYLVANICUS), HOUSE MICE (MUS MUSCULUS), AND NORWAY RATS (RATTUS NORVEGICUS) WITH MYCOBACTERIUM BOVIS

Authors: Clarke, Kathy-Anne R., Fitzgerald, Scott D., Zwick, Laura S., Church, Steven V., Kaneene, John B., et al.

Source: Journal of Wildlife Diseases, 43(3) : 353-365

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-43.3.353

Journal of Wildlife Diseases, 43(3), 2007, pp. 353–365

EXPERIMENTAL INOCULATION OF MEADOW VOLES (*MICROTUS PENNSYLVANICUS*), HOUSE MICE (*MUS MUSCULUS*), AND NORWAY RATS (*RATTUS NORVEGICUS*) WITH *MYCOBACTERIUM BOVIS*

Kathy-Anne R. Clarke,[1,2] Scott D. Fitzgerald,[1,2,7] Laura S. Zwick,[1] Steven V. Church,[3] John B. Kaneene,[4] Ann R. Wismer,[2] Carole A. Bolin,[1,2] Joseph A. Hattey,[2] and Vilma Yuzbasiyan-Gurkan[5,6]

[1] Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, Lansing, Michigan 48910–8107, USA
[2] Diagnostic Center for Population and Animal Health, College of Veterinary Medicine, Michigan State University, Lansing, Michigan 48910–8107, USA
[3] Michigan Department of Community Health, Tuberculosis Laboratory, Lansing, Michigan 48909, USA
[4] Population Medicine Center, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824, USA
[5] Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan 48824, USA
[6] Small Animal Clinical Sciences, Michigan State University, East Lansing, Michigan 48824, USA
[7] Corresponding author (email: fitzgerald@dcpah.msu.edu)

ABSTRACT: *Mycobacterium bovis* has a wide host range that includes several wildlife species, and this can hamper attempts to eradicate bovine tuberculosis from livestock. The purpose of this study was to determine if common rodent species, namely meadow voles (*Microtus pennsylvanicus*), house mice (*Mus musculus*), and Norway rats (*Rattus norvegicus*), that inhabit the bovine tuberculosis endemic area of Michigan, can be experimentally infected with *M. bovis*. The objectives of the study were: 1) to determine if these rodent species can be infected, and if so, to document attendant pathologic processes/pathogenesis; 2) to detect any fecal shedding of *M. bovis*; and 3) to evaluate the relative susceptibility of the three species to *M. bovis* infection. For each species ($n=36$) there were two treatment ($n=12$/group) and one or two control groups depending on species ($n=6$–12/group); the maximum study duration was 60 days. The meadow vole treatments consisted of high dose inocula that were given by oral or intranasal routes, whereas the house mice and Norway rats were given only oral inocula at either a high or low dose. Of the three species, meadow voles were most susceptible to *M. bovis* infection. Upon intranasal inoculation, all 12 voles were infected as determined by gross and microscopic lesions and culture of *M. bovis* from tissue and feces. Seven of the 12 meadow voles inoculated orally were infected. House mice also were susceptible; *M. bovis* was isolated from 14 of 24 animals. Only one Norway rat in the high dose treatment group was positive by culture and this was the only animal from which minimal attendant lesions were observed. Results of this study indicate that meadow voles and house mice can be infected with *M. bovis* and might serve as spillover hosts. Concerted efforts should, therefore, be made to reduce or eliminate these rodents on premises where *M. bovis*-infected livestock are present.

Key words: Bovine tuberculosis, *Microtus pennsylvanicus*, *Mus musculus*, mycobacteriosis, *Mycobacterium bovis*, rodents, *Rattus norvegicus*.

INTRODUCTION

Mycobacterium bovis can infect a wide range of species including humans (Grange and Yates, 1994; O'Reilly and Daborn, 1995), and there is a need to understand the potential for cross-species infection and to identify reservoir hosts. To date, recognized wildlife reservoirs for *M. bovis* include Eurasian badgers (*Meles meles*) in Great Britain, brush-tail possum (*Trichosurus vulpecula*) and ferrets (*Mustela furo*) in New Zealand, and white-tailed deer (*Odocoileus virginianus*; WTD) in Michigan, USA (Morris et al., 1994; de Lisle et al., 2001, 2002).

Subsequent to discovering in 1994 that WTD in Michigan could serve as a reservoir for *M. bovis*, there has been ongoing surveillance of WTD and other potential reservoir hosts in this state. Several carnivorous and omnivorous species such as coyote (*Canis latrans*), raccoon (*Procyon lotor*), bobcat (*Lynx rufus*), Virginia opossum (*Didelphis virginiana*), and red fox (*Vulpes vulpes*) have tested positive

and have been identified as spillover hosts. In spillover hosts, infections occur sporadically but do not persist in the population (Morris et al., 1994; O'Reilly and Daborn, 1995; deLisle et al., 2001). To date, WTD are the only known reservoir host associated with the Michigan outbreak (Schmitt et al, 1997; Bruning-Fann et al., 2001). The presence of these infected wildlife species can represents an obstacle in the control or eradication of *M. bovis* in livestock that are in contact with infected wildlife; there also is a potential public health risk associated with human contact with infected wildlife (O'Reilly and Daborn, 1995; Nelson, 1999; Cousins, 2001; Bengis et al., 2002; Wedlock et al., 2002).

Experimental *M. bovis* studies to determine the susceptibility of wildlife species that are commonly associated with livestock production areas in Michigan have been completed for Virginia opossum, American crow (*Corvus brachyrhynchos*), European starling (*Sturnus vulgaris*), rock pigeon (*Columba livia*), and mallard (*Anas platyrhynchos*; Butler et al., 2001; Diegel et al.; 2002; Fitzgerald et al.; 2003a, b, 2005). At present, there are no reported studies of *M. bovis* infection and attendant risk factors in three wild rodent species that might also be common on Michigan farms. These include the meadow vole (*Microtus pennsylvanicus*), house mouse (*Mus musculus*), and Norway rat (*Rattus norvegicus*).

The objectives of the study were: 1) to determine if the meadow vole, house mouse, or Norway rat can be infected with *M. bovis* by experimental inoculation, and if so, to document attendant pathologic processes/pathogenesis; 2) to detect any fecal shedding of *M. bovis*; and 3) to evaluate the relative susceptibility of the three species to *M. bovis* infection.

MATERIALS AND METHODS

Inocula preparation

Inocula were prepared by the Mycobacteria/Mycology Laboratory Unit of the Michigan Department of Community Health (MDCH), Lansing, Michigan, USA. The *M. bovis* isolate originated from a positive WTD identified by annual surveillance and was confirmed as the Michigan strain by restriction fragment length polymorphism (RFLP; Whipple et al, 1997; Bruning-Fann et al., 1998). Aliquots of 7-day growth in Middlebrook 7H9 broth were frozen at −70 C. As needed, aliquots were thawed, diluted to 1:100 with sterile water, and colony-forming units (CFU) per unit of volume were determined by plate counts. Aliquots were diluted to attain the desired 1×10^5 dose; the final titer was confirmed by plate count at the time of inoculation. Meadow voles and house mice were anesthetized prior to inoculation with isoflurane (IsoFlo®, Abbott Animal Health, Chicago, Illinois, USA) administered in an inhalation chamber attached to a gas anesthesia machine with a precision vaporizer. Animals were dosed orally via a tomcat catheter gavage. Voles were inoculated intranasally (IN) in each nostril via a micropipette.

Study design

Each animal was weighed prior to inoculation (postinoculation day 0) and at weekly intervals thereafter. They were evaluated daily for signs of respiratory distress, weight loss or other signs of ill health (bristled hair, hunched posture, reluctance to move). When necessary for humane reasons or at the end of the experiment, animals were euthanized with an overdose of isoflurane (IsoFlo®, Abbott Animal Health). All experimental procedures were approved by the All-University Committee on Animal Use and Care at Michigan State University (MSU).

Meadow voles

Thirty-six meadow voles (19 male, 17 female) were sourced from the MSU meadow vole colony. The colony was negative for a standard profile of common pathogens (Sendai virus, pneumonia virus of mice, mouse hepatitis virus, reovirus type 3, lymphocytic choriomeningitis virus). Although previously positive for Helicobacter (*Helicobacter* spp., not *bilis* or *hepaticus*) the colony also tested negative for *Helicobacter* at the time the animals were sourced. To detect fecal shedding, fecal samples for mycobacterial culture and isolation were obtained from each vole at 1 day preinoculation, 1 day postinoculation (PI), and from surviving animals on day 30 PI. Voles were randomly assigned to one of four groups: 12 received 5×10^3 CFU of *M. bovis* orally in a total volume of 0.5 ml, six oral

sham inoculates were given a similar volume of sterile water, 12 were given 1×10^5 CFU intranasally, a total volume of 20 µl in each nostril, and six IN sham inoculates were given a similar volume of sterile water. The voles were housed in a secure BSL-3 facility in rodent cages placed in Horsfal units. Rodent chow (Teklad 22/5 rodent diet [W] 8640, Harlan Teklad, Troy, Illinois, USA) and water were supplied ad libitum. Voles were euthanized at 30 and 60 days PI or earlier if they exhibited marked weight loss or signs of illness.

At necropsy, total body weight (TBW) in grams (g) was obtained for each animal. The weights of the lung, liver, and spleen also were recorded. Tissues harvested at necropsy were preserved in 10% neutral buffered formalin (NBF) and included brain, nasal turbinates, trachea, lung, heart, liver, kidney, spleen, gonad, adrenal gland, small intestine (SI), large intestine (LI), and cranial, thoracic, and abdominal lymph nodes. Tissues were routinely processed and sectioned (5 µm) for staining with hematoxylin and eosin (H and E). All tissues were also stained with Ziehl-Neelsen (acid-fast).

Tissues for mycobacterial culture were collected using sterile instruments and were grouped into three pools: pool A (lung, tracheobronchial lymph nodes, and cranial lymph nodes); pool B (liver, kidney, spleen); and pool C (SI, LI, and mesenteric lymph nodes).

House mice

Thirty-six house mice (18 male, 18 female) were sourced from the Geriatrics Center, University of Michigan, Ann Arbor, Michigan, USA. All mice tested negative for a standard profile of common pathogens prior to inoculation. Fecal samples were procured from each mouse as described for the voles on day 0 and day 1 PI, and from surviving animals on days 20 and 40 PI. The mice were randomly assigned to one of three groups: 12 received a high dose of *M. bovis* (1×10^4 CFU), 12 received a lower dose (1×10^2 CFU), and 12 were sham-inoculated controls. Each mouse received 0.25 ml total volume orally. The mice were housed and fed in a manner identical to the voles. Mice were euthanized at 20, 40, and 60 days PI or earlier if they exhibited marked weight loss or signs of illness.

The necropsy protocol for the mice was identical to that previously described for the voles. However, nasal turbinates were not included in the tissues harvested from voles. Histopathologic processing and staining in addition to the tissues harvested and pooled for mycobacterial culture were identical to that in the voles.

Norway rats

Thirty-six male Norway rats were obtained from Charles River Laboratories, Portage, Michigan, USA. All rats tested negative for a standard profile of common pathogens prior to inoculation. Fecal shedding was assessed in each rat as previously described for house mice. The rats were randomly assigned to one of three groups: 12 received a high dose of *M. bovis* (5×10^3 CFU), 12 a low dose (1×10^2 CFU), and 12 were sham-inoculated controls. The rats were housed and fed in a manner identical to the voles. Rats were euthanized at 20, 40, and 60 days PI.

The necropsy protocol, histopathologic processing, and staining as well as mycobacterial culture were identical to that in the mice.

Mycobacterial isolation and identification

Mycobacterial cultures were performed at the Tuberculosis Laboratory, MDCH. Tissue specimens were homogenized, digested, and concentrated (Kent and Kubica, 1985); fecal samples did not require homogenization and were cultured after digestion and concentration. For each sample, a Lowenstein-Jensen medium slant, a Middlebrook 7H11S medium slant (Remel, Lenexa, Kansas, USA), and a BACTEC 12B broth vial (Becton-Dickinson, Sparks, Maryland, USA) were inoculated. Media were examined weekly for mycobacterial growth for up to 8 wk. Cultures determined to contain acid-fast organisms by slide examination (Kent and Kubica, 1985) were tested by nucleic acid probe (Accuprobe®, Gen-Probe®, San Diego, California, USA) to ascertain if they were members of the *M. tuberculosis* complex (Reisner et al., 1994). Biochemical testing and high-performance liquid chromatography was performed to identify species and to differentiate *M. bovis* from other members of the *M. tuberculosis* complex (Kent and Kubica, 1985; Butler et al., 1991; Reisner et al., 1994).

Statistical analysis

The SAS version 9.1.3 statistical software was used for all analyses (SAS Institute, Inc., Cary, North Carolina, USA). The Wilcoxon rank-sum test was used to determine whether there were significant ($P<0.05$) changes in total body weight and organ weights within and between treatment groups. The two-tailed Fisher's exact test was used to determine if the

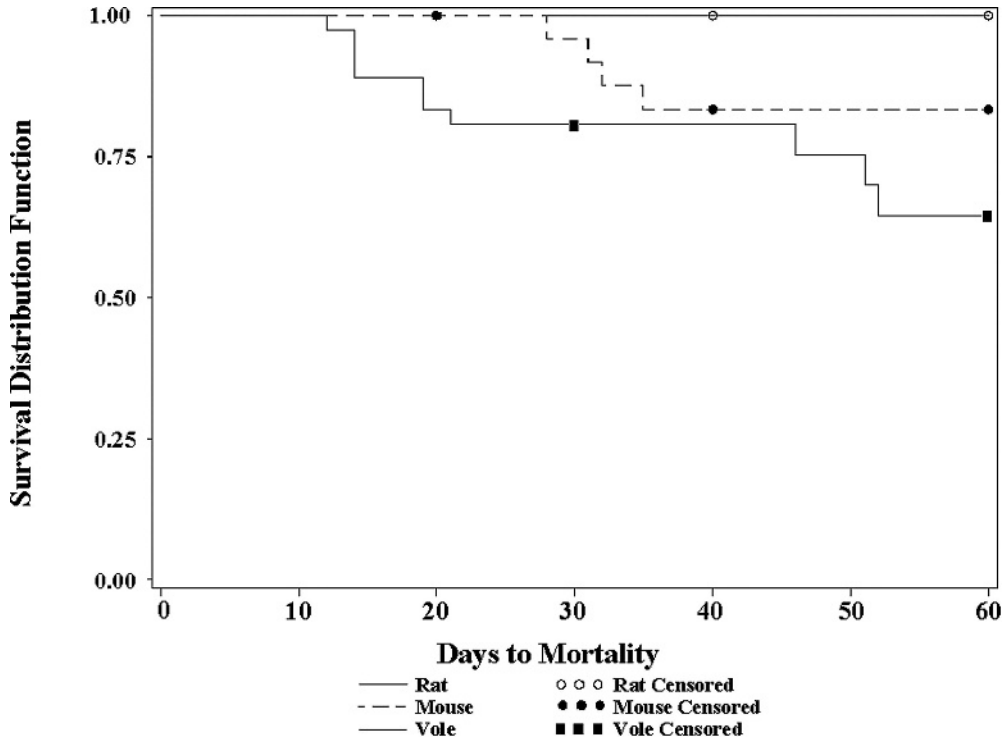

FIGURE 1. Results of the nonparametric Kaplan-Meier survival analysis to determine whether duration of survival was significantly influenced by the animal used. The rate of mortality was significantly higher in meadow voles and house mice compared to the Norway rats.

presence or absence of gross and microscopic lesions or positive mycobacterial cultures were dependent on route of inoculation. A Kaplan-Meier test was used to determine whether treatment and species significantly influenced survival.

RESULTS

Clinical response

Ten of the infected meadow voles exhibited respiratory distress and either died or were euthanized prior to the end of the experiment. This included six voles that were inoculated orally and four that were inoculated by the IN route. Four of the 12 house mice in the HD group died prematurely as a result of infection. After adjusting for dose (mice and rats) and route of inoculation (voles), the mortality rate was highest in voles (28%) as compared to house mice (11%) and Norway rats (0%; Cochran-Mantel-Haenszel $\chi^2=12.36$, $P=0.0021$) and voles died earlier than mice; the mean time to death was 26.8 days for voles and 31.5 days for mice (Fig 1).

Although *M. bovis*-infected voles were clinically affected (moribund, lack of appetite, marked decrease in activity, respiratory distress/ abdominal breathing) differences in weights of infected animals and controls were not detected (Wilcoxon rank-sum $\chi^2=5.15$; $P=0.0795$). Lung weights for *M. bovis*-infected voles (IN and oral inoculates) were higher than observed in control animals (Wilcoxon rank-sum $\chi^2=11.85$; $P=0.0027$); however, there were no differences in the liver and spleen weights of the inoculated and control voles. Four of the 12 mice in the HD group lost weight (1.7–5.4 g) during the first 30 days of the study (two of these were euthanized and two died), but after this time period, remaining mice were able

TABLE 1. Gross lesions (granulomas, caseous necrosis, mottling and consolidation of the lungs) and microscopic lesions (granulomatous inflammation, acid-fast bacteria, and multinucleated giant cells) consistent with mycobacteriosis detected at necropsy in the rodents experimentally inoculated with *Mycobacterium bovis*.

				Tissue No. with gross lesions (no. with microscopic lesions)				
Species	Route or dose	Day	*n*	Lung	LN[a]	Liver	Turbinates	Spleen
Meadow vole	IN[b]	30	7	5 (4)	6 (5)	2 (7)	(6)	1 (6)
	Oral	30	7	7 (7)	3 (3)	0 (7)	(1)	2 (7)
	IN	60	5	3 (4)	5 (5)	1 (5)	(5)	5 (5)
	Oral	60	5	0 (0)	0 (0)	0 (0)	(0)	0 (0)
House mouse	HD[c]	20	4	3 (3)	1 (1)	0 (0)	NA[e]	0 (1)
	LD[d]	20	4	1 (1)	0 (0)	0 (0)	NA	0 (0)
	HD	40	4	4 (4)	2 (1)	0 (1)	NA	0 (1)
	LD	40	4	1 (1)	0 (0)	0 (0)	NA	0 (1)
	HD	60	4	0 (0)	1 (0)	0 (0)	NA	1 (0)
	LD	60	4	0 (3)	0 (2)	0 (0)	NA	1 (1)
Norway rat	HD	20	4	0 (0)	0 (1)	0 (0)	NA	0 (0)
	LD	20	4	0 (0)	0 (0)	0 (0)	NA	0 (0)
	HD	40	4	0 (0)	0 (0)	0 (0)	NA	0 (0)
	LD	40	4	0 (0)	0 (0)	0 (0)	NA	0 (0)
	HD	60	4	0 (0)	0 (0)	0 (0)	NA	0 (0)
	LD	60	4	0 (0)	0 (0)	0 (0)	NA	0 (0)

[a] Lymph node.

[b] IN=Intranasal.

[c] HD=High dose.

[d] LD=Low dose.

[e] NA=not applicable.

to regain and maintain body weight and weights did not differ between inoculated and control mice (Wilcoxon rank-sum $\chi^2=0.39$; $P=0.8243$). As with voles, lung weights were significantly higher in inoculated mice (HD and LD) when compared with control mice (Wilcoxon rank-sum $\chi^2=9.50$; $P=0.0087$). Inoculated Norway rats (HD and LD) had a lower mean total body weight than sham-inoculated controls (Wilcoxon rank-sum $\chi^2=22.38$; $P=<0.0001$) but there were no observable differences in any of the organ weights between inoculated and control rats.

Gross lesions

Gross lesions suggestive of mycobacteriosis were detected in 19 of the 24 inoculated voles. In the voles with mycobacterial pneumonia, the lungs failed to collapse fully. Multifocal granulomatous to pyogranulomatous pneumonia was characterized by several pale tan foci ranging from pinpoint up to 5 mm in diameter randomly disseminated in the pulmonary parenchyma. The tan foci were friable and soft to gritty. Some animals had hepatomegaly and splenomegaly. Large numbers of granulomas were disseminated in the hepatic and splenic parenchyma (moderate to severe multifocal granulomatous hepatitis and splenitis). Lymphadenitis was evident in submandibular, parotid, cervical, tracheobronchial, and mesenteric lymph nodes. The affected lymph nodes were enlarged with multifocal to coalescing caseogranulomas that ranged from pinpoint up to 3 mm in diameter (Table 1 and Figs. 2 and 3). Nonmycobacteriosis-associated lesions were detected in two voles; severe cecal dilation and an ovarian mass were observed in one vole and a severe urogenital infection in another (cystitis with enteritis in the adjoining intestine, both transmural). In addition,

FIGURE 2. Bilaterally enlarged submandibular and superficial cervical lymph nodes in an intranasally-inoculated meadow vole at 51 days postinoculation.

two of the voles had unidentified cutaneous mites.

Twelve of the 24 inoculated mice had gross lesions suggestive of mycobacteriosis. The lungs, which were diffusely firm, had multiple pale tan foci that were soft and friable to gritty. Foci ranged from 1 mm up to 5 mm in diameter. The extent of the pulmonary lesions varied from mild to severe and lesions were evident in eight of the mice (all animals were in the HD group; days 20 and 40 PI; Table 1). One mouse in the LD group (day 40 PI) had congested lungs which were mottled red and tan. Enlarged lymph nodes were detected in four mice (superficial cervical, tracheobronchial, mesenteric, inguinal, lumbar; one mouse in the HD group on day 20 PI; two mice in the HD group on day 40 PI; one mouse in the LD group on day 60 PI). Splenomegaly was present in two mice on day 60 PI (one each in HD and one LD groups). A nonmycobacteria-associated lesion was present in one mouse; a 4-mm-diameter focal pulmonary neoplasm (a mouse in the HD group on day 20 PI).

There were no gross lesions suggestive of *M. bovis* infection in any of the rats on post-mortem evaluation.

Microscopic lesions

Histologic lesions were most extensive and severe in voles (Table 1 and Fig. 4). In each of the affected organs, lesions were moderate to severe, multifocal to coalescing, caseogranulomatous, and granulomatous to pyogranulomatous. Numbers of acid-fast bacilli ranged from abundant to rare. Of note in the microscopic lesions seen in the voles was mineralization in some of the lymph nodes with caseogranulomatous lymphadenitis. Moderate to severe granulomatous and necrotizing rhinitis were prominent features in the IN inoculates (11 of 12) and in a single oral inoculate (Fig. 5). Nonmycobacteriosis-associated lesions detected in meadow voles included colonic nematodiasis in five

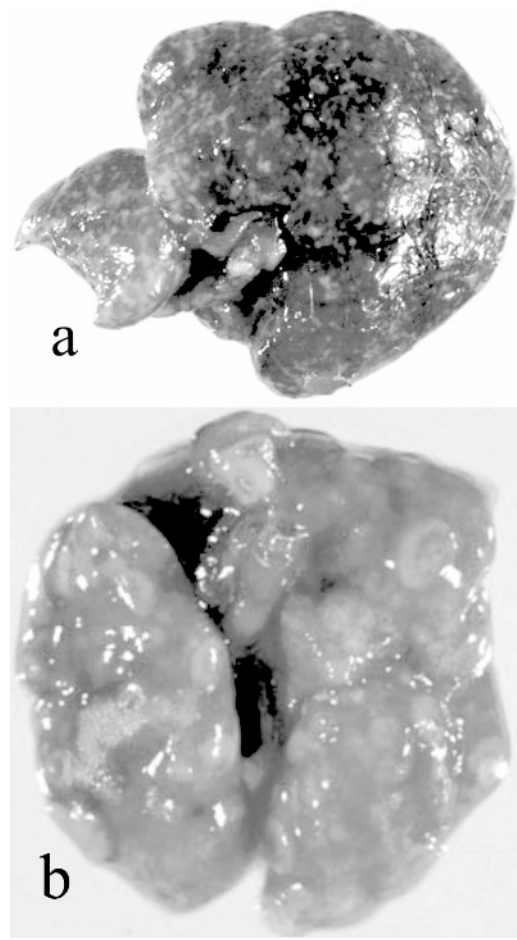

FIGURE 3. (a) Liver from an intranasally-inoculated meadow vole at 52 days postinoculation. Granulomas, ranging from 1 to 2 mm in diameter are randomly disseminated in the parenchyma. (b) The lungs from the same vole were diffusely firm, noncollapsing and contained similar granulomas.

TABLE 2. Mycobacterial tissue culture results for the three rodent species.

Species	Route or dose[a]	Tissue pools[b] Number positive/number tested		
		A	B	C
Meadow vole	IN	12/12	12/12	11/12
	Oral	7/12	7/12	7/12
House mouse	HD	8/12	9/12	5/12
	LD	5/12	2/12	1/12
Norway rat	HD	1/12	0/12	0/12
	LD	0/12	0/12	0/12

[a] IN = Intranasal, HD = High dose, LD = Low dose.

[b] The tissues were pooled for culture: A = lung, tracheobronchial lymph nodes, cranial lymph nodes; B = liver, kidney, spleen; C = small intestine, large intestine, mesenteric lymph node.

animals, an ovarian teratoma in one vole, cystitis (transmural chronic fibrosing, pyogranulomatous, and necrotizing with intralesional bacteria), and adjacent transmural enteritis in one vole.

Histologic lesions in house mice were consistent with mycobacteriosis; lesions were observed in 11 mice (seven HD and four LD) and were present most frequently in the lungs (Table 1). Acute to subacute lesions were seen in mice that were euthanized on days 20 and 40 PI and presented as severe coagulative necrosis (sequestrum) with moderate to massive numbers of acid-fast bacilli (also macrophages on day 40 PI). Chronic granulomatous pneumonia with infiltrates of macrophages, epithelioid cells, and rare multinucleated giant cells were evident in mice euthanized on day 60 PI. Acid-fast bacilli were present in small numbers in macrophages in small numbers or were rare. Other organs affected were lymph nodes (three tracheobrochial and one mesenteric) and spleen in four mice, and liver in one animal. In each of these tissues acid-fast bacilli were present in macro-

FIGURE 4. Photomicrograph of the lung from meadow vole at 19 days postinoculaton. Severe, diffuse granulomatous and necrotizing pneumonia is evident. Bar = 200 μm.

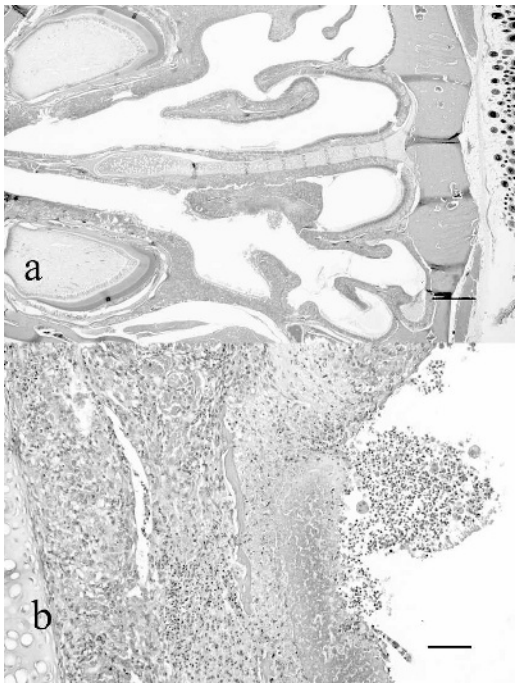

FIGURE 5. (a) Photomicrograph of the nasal turbinate from an intranasally-inoculated meadow vole at 60 days postinoculatin with moderately severe, focally extensive granulomatous rhinitis. Bar = 400 μm. (b) At higher magnification a mixed inflammatory exudate (necrotic cellular debris, degenerate neutrophils, multinucleated giant cells) is adhered to the mucosal surface of the nasal turbinate. Bar = 50 μm.

phages, but tissue architecture was otherwise unaffected. The only nonmycobacteriosis lesion detected microscopically was a focal pulmonary bronchiogenic adenocarcinoma in one mouse day 20 PI.

In a single Norway rat (HD group, 20 days PI) there was focal aggregation of small numbers of multinucleate giant cells which contained a few acid-fast bacilli within a tracheobronchial lymph node. In another rat (LD group, day 60 PI), sections of cerebrum and a single blood vessel (vein) contained a few acid-fast bacilli. Nonmycobacteriosis-associated lesions seen in the Norway rat included a meningeal granular cell tumor (control rat day 40 PI) and a focal sperm granuloma in the testis in another control rat (day 40 PI).

Mycobacterial isolation and identification

In the voles all fecal cultures were negative prior to inoculation; sham-inoculated controls remained negative on days 1 and 30 PI. Eight of the 12 IN inoculated voles and nine of 12 oral inoculates had positive fecal cultures on day 1 PI. Two of the five surviving IN inoculates had positive fecal cultures on day 30 PI, whereas fecal cultures from the five surviving oral inoculates sampled at 30 days PI were negative. All fecal cultures taken prior to inoculation and on days 1, 20, and 40 PI for the house mice and rats were negative.

Positive tissue cultures were obtained in all 12 of the IN inoculated voles and in seven of the oral inoculates. Tissues from control voles were negative. Tissues from 14 of 24 (nine HD and five LD) inoculated mice were positive by culture. All tissues from sham-inoculated mice were negative. A single HD rat had a positive culture for tissues in pool A on day 20 PI. Tissues in pools B and C were negative. Cultures from tissues of all other rats were negative (Table 2).

Statistical analysis

A difference in the frequency of *M. bovis* culture-positive animals was observed between species (Fisher's exact test, $P<0.0001$); meadow voles and mouse mice were most susceptible. Meadow voles were infected by both inoculation routes, and there was a significant difference in the number of gross lesions present in intranasal versus orally-inoculated animals (Fisher's exact test, $P<0.05$). Voles also had a higher prevalence of histologic lesions (Fisher's exact test, $P<0.05$).

With house mice, the frequency of gross lesions differed between the high dose low dose treatments (Fisher's exact test, $P=0.04$). However, a difference in the frequency of microscopic lesions observed in these treatment groups was not detected (Fisher's exact test, $P>0.05$).

DISCUSSION

The results of this study indicate that meadow voles are very susceptible to infection with *M. bovis* via oral and intranasal routes, and that house mice also are susceptible to infection via the oral route. Conversely, Norway rats appear to be very resistant to experimental infection with high doses of *M. bovis* given via the oral route. Respiratory/ inhalation and oral routes of experimental infection were chosen because they represent the routes of infection known to occur in established wildlife reservoirs (brush tail possums, Eurasian badgers, and WTD) and in spillover hosts of *M. bovis* (O'Reilly and Daborn, 1995; de Lisle et al., 2001). The doses selected were chosen to provide sufficient challenge and accelerate any expected pathologic responses in these species. Similar doses have been used in other inoculation animal studies both within this laboratory and by other researchers (Corner and Presidente, 1980, 1981; Buddle et al., 1994; Diegel et al., 2002).

Evidence in support of meadow voles being more susceptible than house mice and Norway rats to experimental inoculation with *M. bovis* include lethal infections in 10 of the 24 inoculated animals, extensive gross and histologic lesions (19 animals), and positive mycobacterial cultures (19 animals). Sensitivity of voles to *M. bovis* seen in this study supports the work of Jespersen (1974, 1975, 1976, 1977a, b) and Jespersen et al. (1977) in common voles (*Microtus arvalis*), field voles (*Microtus agrestis*), bank voles (*Clethrionomys glareolus*), and the vole rat (*Arvicola terrestris*), all of which developed lethal infections following experimental inoculation of *M. bovis*. Similar results were obtained earlier by Griffith (1937, 1939) and Wells (1938). Gross lesions including caseous lymphadenitis as well as granulomatous pneumonia, splenomegaly, and hepatitis were observed in these earlier studies and are consistent with results from this study.

The results of our study demonstrate that infected voles are capable of disseminating *M. bovis* via their feces. The isolation of *M. bovis* in feces from 17 voles on day 1 PI most likely resulted from passive transit through the gastrointestinal tract. Although the majority of these (nine) positive results came from voles that were inoculated orally, eight positive fecal cultures were obtained from IN inoculated voles. This suggests that the inoculum was either swallowed during administration or was coughed up from the respiratory tract and subsequently swallowed. Two of the IN inoculates had positive fecal cultures at 30 days postinoculation, which indicates both dissemination of *M. bovis* and the potential for longer-term fecal shedding.. Fecal shedding does occur with the closely related *M. microti*, or vole bacillus, which like *M. bovis* is a member of the *M. tuberculosis* complex. *Mycobacterium microti* can be spread from vole to vole via ingestion of the excreta from infected voles (Rankin and McDiarmid, 1968) and acid-fast bacilli have been reported in the feces of field voles experimentally inoculated with *M. microti* (Griffith, 1939); however, these acid-fast bacilli were not identified as *M. microti* through culture.

Meadow voles were more susceptible to infection via the oral route than the IN route. Six of the animals in the oral treatment group died or were euthanized in less than 21 days PI as compared to loss of a single IN inoculate on day 19 PI and three other animals between days 46 to 52 PI.

Positive fecal cultures and the ability to survive *M. bovis* infections, suggest that meadow voles could represent a potential reservoir host. However, this potential is not supported by field data and it is not known if *M. bovis* infections can persist in meadow vole populations.

The results of this study are the first to document oral infection of a wild-type house mice with *M. bovis*. Although Lefford (1984) states that adult mice

cannot be infected by virulent tubercle bacilli via the gastrointestinal tract, Pierce et al. (1947) did demonstrate that immature house mice (4–6 wk old) were susceptible to oral infection with *M. tuberculosis*. Extensive gross and microscopic lesions in orally-infected mice as well as positive mycobacterial cultures from the lungs, liver, and spleen are indicative of *M. bovis* dissemination from the gastrointestinal tract. Gross and microscopic lesions in these mice were most severe in the lung, which is similar to previously published studies of *M. bovis*-infected laboratory mouse strains (Griffith, 1907b; Glover, 1944; Ratcliffe 1952; Ratcliffe and Palladino, 1953; Gunn, Nungester and Hougen, 1933–34, cited in Darzins 1958). Similar to what has previously been reported in mice experimentally infected with *M. tuberculosis* (Cosma et al., 2003), caseation or calcification of pulmonary granulomas was not evident in the lungs of infected mice in this study. Although experimental infections are possible, results from field studies provide no evidence that house mice are naturally infected with *M. bovis* (Little et al., 1982; Wilesmith et al., 1986; Fischer et al., 2000; Pillali et al., 2000).

Infections were more severe in mice that received a HD inoculum. A total of eight mice (67%) in the HD group had gross and microscopic lesions consistent with mycobacteriosis compared to four (33%) mice in the LD group. Results from house mice also suggest that those animals receiving an HD oral inoculum of *M. bovis* might be able to control infection. Adverse effects in this group occurred up to day 40 PI. By this time, severe pulmonary disease had occurred in seven of eight animals in this group. All eight animals were culture positive and four deaths due to mycobacteriosis occurred between days 28 and 35 postinoculation. After day 40, only one of four surviving mice tested positive by culture. The converse was seen in mice receiving the LD oral inoculum; chronic pulmonary disease was detected at day 60 PI and tissues from three of the four animals in this group were culture positive. These differences might relate to dose dependent variation in stimulating a cell-mediated immune response. House mice might serve as an ideal animal model for tuberculosis in humans infected with *M. tuberculosis* and *M. bovis* because there is evidence that some of these mice were able to recover from their infection.

Although fecal cultures in the house mice were consistently negative, *M. bovis* was cultured from the intestinal tissue pools of six mice. This confirms infection or retention of *M. bovis* following oral exposure, and suggests that some fecal shedding might have occurred despite the negative culture results from fecal samples. These negative results might have resulted from the sampling methods used in our study.

The lack of gross lesions and negative fecal cultures in Norway rats indicates resistance to oral infection with *M. bovis*. However, there was one positive tissue culture (lung, tracheobronchial, and cranial lymph nodes) in a single rat in the HD group on day 20 PI. This rat also had histologic lesions consistent with mycobacterial persistence/colonization as multinucleated giant cells with acid-fast bacilli were present in the tracheobronchial lymph node. This is indicative of dissemination of the mycobacteria from the site of infection within this rat. Lack of gross and microscopic lesions and negative culture attempts from Norway rats in this study beyond 20 days PI is consistent with prior reports of resistance in rats; this is related to the cell-mediated immune response to *Mycobacterium* spp. infection (Thorns et al., 1982). In a recent study with female Lewis rats, Sugawara and associates (2004) report that animals infected by aerosol with *M. tuberculosis* developed granulomatous lesions in the lungs, spleen, lymph nodes, and liver. Although rats might be susceptible to *Mycobacterium* spp. via a respiratory route, it is unlikely that wild type rats

would be naturally exposed to such a large dose (2×10^6 CFU) of *M. tuberculosis* by aerosol.

It is unclear why a decreased total body weight in inoculated rats was observed when none of these rats exhibited any other adverse clinical signs (respiratory distress, bristled hair, reluctance to move, etc.). A similar lack of clinical signs in rats infected with *M. bovis* following experimental inoculation is reported by Griffith (1907a) with intraperitoneal (IP) and subcutaneous (SQ) routes as well as by Wessels (1941) who utilized the IV route. In these studies, *M. bovis* was detected in several tissues (lungs, liver, spleen, lymph nodes, kidneys, omentum, and bone marrow) which was not observed in our study. Accordingly, one can conclude that Norway rats are essentially resistant to infection with *M. bovis* at high oral doses and are incapable of disseminating this organism laterally via fecal shedding. Support for rats being dead-end hosts for *M. bovis* is gleaned from previously reported environmental survey studies in which rats were negative on culture (Wilesmith et al., 1986; Pillai et al., 2000) or lacked lesions in the face of positive cultures (four animals total) (Bosworth, 1940; Little et al., 1982).

In conclusion, the results of this study indicate that meadow voles are highly susceptible to infection with *M. bovis* via both oral and intranasal routes and can shed *M. bovis* in their feces. In voles, lesions consistent with mycobacteriosis occurred in several tissues, including the lungs, liver, and spleen, various lymph nodes, and in the intranasal inoculates and nasal turbinates. House mice are also susceptible to infection with *M. bovis* via the oral route but are apparently less efficient in transmitting mycobacteria via fecal shedding when compared to the vole. Most lesions in affected mice were associated with the lungs. Of the three rodent species tested in this study, the Norway rat is the most resistant to oral infection with *M. bovis*.

Although meadow voles and house mice are unlikely to encounter *M. bovis* in their natural environment at doses equivalent to the HD inocula used in this study, it is recommended that appropriate measures be taken to eliminate these animals or at least control their numbers on premises where bovine tuberculosis-positive animals are present. Further, it would be strongly advised that access of voles and mice to food and water sources of domestic animals be restricted wherever possible.

ACKNOWLEDGMENTS

We would like to thank R. Miller and R. Dysko as well as S. Durkee from the University of Michigan's Unit for Lab Animal Medicine who were instrumental in providing us with the house mice used in this study. We would also thank R. Common, Divison of Human Pathology and S. Thon, at the Diagnostic Center for Population and Animal Health, Michigan State University, for their assistance with photography and the preparation of photomicrographs. This study was funded in part by a grant from the United States Department of Agriculture, National Research Initiative, Cooperative State Research, Education, and Extension Service.

LITERATURE CITED

Bengis, R. G., R. A. Kock, and J. Fischer. 2002. Infectious animal diseases: The wildlife/livestock interface. Revue Scientifique et Technique. Office International des Epizooties 21: 53–65.

Bosworth, T. J. 1940. Further observations of the wild rat as a carrier of *Brucella abortus*. Journal of Comparative Pathology and Therapy 53: 42–49.

Bruning-Fann, C. S., S. M. Schmitt, S. D. Fitzgerald, J. B. Payeur, D. L. Whipple, T. M. Cooley, T. Carlson, and P. Friedrich. 1998. *Mycobacterium bovis* in coyotes from Michigan. Journal of Wildlife Diseases 34: 632–636.

———, ———, ———, J. S. Fierke, P. D. Friedrich, J. B. Kaneene, K. A. Clarke, K. L. Butler, J. B. Payeur, D. L. Whipple, T. M. Cooley, J. M. Miller, and D. P. Muzo. 2001. Bovine tuberculosis in free-ranging carnivores from Michigan. Journal of Wildlife Diseases 37: 58–64.

Buddle, B. M., F. E. Aldwell, A. Pfeffer, and G. W. de Lisle. 1994. Experimental *Mycobacterium bovis* infection in brushtail possum (*Trichosurus vulpecula*): Pathology, haematology and

lymphocyte stimulation responses. Veterinary Microbiology 38: 241–254.

BUTLER, K. L., S. D. FITZGERALD, D. E. BERRY, S. V. CHURCH, W. M. REED, AND J. B. KANEENE. 2001. Experimental inoculation of European starlings (*Sturnus vulgaris*) and American crows (*Corvus brachrhynchos*) with *Mycobacterium bovis*. Avian Diseases 45: 709–718.

BUTLER, W. R., K. C. JOST, AND J. O. KILBURN. 1991. Identification of mycobacteria by high-performance liquid chromatography. Journal of Clinical Microbiology 29: 2468–2472.

CORNER, L. A., AND P. J. A. PRESIDENTE. 1980. *Mycobacterium bovis* infection in the brush-tailed possum (*Trichosurus vulpecula*): I. Preliminary observations of experimental infection. Veterinary Microbiology 5: 309–321.

———, AND ———. 1981. *Mycobacterium bovis* infection in the brush-tailed possum (*Trichosurus vulpecula*): II. Comparison of experimental infections with an Australian cattle strain and a New Zealand possum strain. Veterinary Microbiology 6: 351–366.

COSMA, C. L., D. R. SHERMAN, AND L. RAMAKRISHNAN. 2003. The secret lives of pathogenic mycobacteria. Annual Review of Microbiology 57: 641–676.

COUSINS, D. V. 2001. *Mycobacterium bovis* infection and control in domestic livestock. Revue Scientifique et Technique. Office International des Epizooties 20: 71–85.

DARZINS, E. 1958. The mouse in experimental tuberculosis. *In* The bacteriology of tuberculosis. University of Minnesota Press, Minneapolis, Minnesota, pp. 340–356.

DE LISLE, C. W., C. G. MACKINTOSH, AND R. G. BENGIS. 2001. *Mycobacterium bovis* in free-living and captive wildlife, including farmed deer. Revue Scientifique et Technique. Office International des Epizooties 20: 86–111.

———, R. G. BENGIS, S. M. SCHMITT, AND D. J. O'BRIEN. 2002. Tuberculosis in free-ranging wildlife: detection, diagnosis and management. Revue Scientifique et Technique. Office International des Epizooties 21: 317–334.

DIEGEL, K. L., S. D. FITZGERALD, D. E. BERRY, S. V. CHURCH, W. M. REED, J. G. SIKARSKIE, AND J. B. KANEENE. 2002. Experimental inoculation of North American opossums (*Didelphis virginiana*) with *Mycobacterium bovis*. Journal of Wildlife Diseases 38: 275–281.

FISCHER, O., L. MATLOVA, J. BARTL, L. DVORSKA, I. MELICHAREK, AND I. PAVLIK. 2000. Findings of mycobacteria in insectivores and small rodents. Folia Micobiologica 45: 147–152.

FITZGERALD, S. D., L. S. ZWICK, K. L. DIEGEL, D. E. BERRY, S. V. CHURCH, J. G. SIKARSKIE, AND J. B. KANEENE. 2003a. Experimental aerosol inoculation of *Mycobacterium bovis* in North American opossums (*Didelphis virginiana*). Journal of Wildlife Diseases 39: 418–423.

———, ———, D. E. BERRY, S. V. CHURCH, J. B. KANEENE, AND W. M. REED. 2003b. Experimental inoculation of Pigeons (*Columba livia*) with *Mycobacterium bovis*. Avian Diseases 47: 470–475.

———, K. G. BOLAND, K. R. CLARKE, A. WISMER, ———, D. E. BERRY, S. V. CHURCH, J. A. HATTEY, AND C. A. BOLIN. 2005. Resistance of Mallard ducks (*Anas platyrhynchos*) to inoculation with *Mycobacterium bovis*. Avian Diseases 49: 144–146.

GLOVER, R. E. 1944. Infection of mice with *Mycobact. tuberculosis* (bovis) by the respiratory route. British Journal of Experimental Pathology 25: 141–149.

GRANGE, J. M., AND M. D. YATES. 1994. Zoonotic aspects of *Mycobacterium bovis* infection. Veterinary Microbiology 40: 137–151.

GRIFFITH, A. S. 1907a. Inoculation experiments on rats with tuberculous material of bovine origin. Royal Commission on Tuberculosis Second Interim Report Part II, Appendix. Vol. I, pp. 481–490, 633, 692–693.

———. 1907b. Inoculation experiments on mice with tuberculous material of bovine origin. Royal Commission on Tuberculosis Second Interim Report Part II, Appendix. Vol I, pp. 491–496.

———. 1937. Experimental tuberculosis in field voles and mice. Veterinary Record 49: 982–984.

———. 1939. The relative susceptibility of the field vole to the bovine, human and avian types of tubercle bacilli and to the vole strain of acid-fact bacillus (Wells, 1937). Journal of Hygiene (London) 39: 244–259.

JESPERSEN, A. 1974. Infection of *Arvicola terrestris* (vole rat) with *M. tuberculosis* and *M. bovis*. Acta Pathologica et Microbiologica Scandinavica Sect. B 82: 667–675.

———. 1975. Infection of *Microtus arvalis* (common vole) with *Mycobacterium tuberculosis* and *Mycobacterium bovis*. Acta Pathologica et Microbiologica Scandinavica Sect. B 83: 201–210.

———. 1976. Multiplication of *Mycobacterium tuberculosis* and *Mycobacterium bovis* in *Microtus agrestis* (field vole). Acta Pathologica et Microbiologica Scandinavica Sect. B 84: 57–60.

———. 1977a. Infection of *Clethrionomys g. glareolus Schreb.* (red mice) with *Mycobacterium tuberculosis* and *Mycobacterium bovis* injected subcutaneously. Acta Pathologica et Microbiologica Scandinavica Sect. B 85: 329–333.

———. 1977b. Infection of *Clethrionomys g. glareolus Schreb.* (red mice) with *Mycobacterium tuberculosis* and *Mycobacterium bovis* injected intraperitoneally. Acta Pathologica et Microbiologica Scandinavica Sect. B 85: 397–402.

———, M. WEIS BENTZON, AND S. MOLLER. 1977. Infection of *Clethrionomys g. glareolus Schreb.*

(red mice) with *Mycobacterium bovis* injected intravenously. Acta Pathologica et Microbiologica Scandinavica Sect. B 85: 415–426.

KENT, P. T., AND G. P. KUBICA. 1985. Public health mycobacteriology. A guide for the level III laboratory. US Department of Health and Human Services, Atlanta, Georgia, pp. 207.

LEFFORD, M. J. 1984. Diseases in mice and rats. *In* The Mycobacteria. A sourcebook. Part B, G. P. Kubica and L. G. Wayne (eds.). Marcel Dekker, Inc., New York, New York, pp. 947–977.

LITTLE, T. W. A., C. SWAN, H. V. THOMPSON, AND J. W. WILESMITH. 1982. Bovine tuberculosis in domestic and wild mammals in an area of Dorset III. The prevalence of tuberculosis in mammals other than badgers and cattle. Journal of Hygiene (London) 89: 225–234.

MORRIS, R. S., D. U. PFEIFFER, AND R. JACKSON. 1994. The epidemiology of *Mycobacterium bovis* infections. Veterinary Microbiology 40: 153–177.

NELSON, A. M. 1999. The cost of disease eradication. Smallpox and bovine tuberculosis. Annals of the New York Academy of Sciences 894: 83–91.

O'REILLY, L. M., AND C. J. DABORN. 1995. The epidemiology of *Mycobacterium bovis* infections in animals and man: A review. Tubercule and Lung Disease 76 Supplement 1: 1–46.

PIERCE, C. H., R. J. DUBOS, AND G. MIDDLEBROOK. 1947. Infection of mice with mammalian tubercle bacilli grown in tween-albumin liquid medium. Journal of Experimental Medicine 86: 159–174.

PILLAI, S. D., K. W. WIDMER, L. J. IVEY, K. C. COKER, E. NEWMAN, S. LINGSWEILER, D. BACA, M. KELLEY, D. S. DAVIS, N. SILVY, AND L. G. ADAMS. 2000. Failure to identify non-bovine reservoirs of *Mycobacterium bovis* in a region with a history of infected dairy-cattle herds. Preventative Veterinary Medicine 43: 53–62.

RANKIN, J. D., AND A. MCDIARMID. 1968. Mycobacterial infections in free-living wild animals. Symposia of the Zoological Society of London 24: 119–131.

RATCLIFFE, H. L. 1952. Tuberculosis induced by droplet nuclei infection. Pulmonary tuberculosis of predetermined initial intensity in mammals. American Journal of Hygiene 55: 36–48.

———, AND V. S. PALLADINO. 1953. Tuberculosis induced by droplet nuclei infection. Initial homogeneous response of small mammals (rats, mice, guinea pigs and hamsters) to human and bovine bacilli and the rate and pattern of tubercle development. Journal of Experimental Medicine 97: 61–68.

REISNER, B. S., A. M. GASTON, AND G. L. WOODS. 1994. Use of Gen-Probe AccuProbes to identify *Mycobacterium avium* complex, *Mycobacterium tuberculosis* complex, *Mycobacterium kansasii*, and *Mycobacterium gordonae* directly from BACTEC TB broth cultures. Journal of Clinical Microbiology 32: 2995–2998.

SCHMITT, S. M., S. D. FITZGERALD, T. M. COOLEY, C. S. BRUNING-FANN, L. SULLIVAN, D. BERRY, T. CARLSON, R. B. MINNIS, J. B. PAYEUR, AND J. SIKARSKIE. 1997. Bovine tuberculosis in free-ranging white-tailed deer from Michigan. Journal of Wildlife Diseases 38: 275–281.

SUGAWARA, I., H. YAMADA, AND S. MIZUNO. 2004. Pathological and immunological profiles of rat tuberculosis. International Journal of Experimental Pathology 85: 125–134.

THORNS, C. J., J. A. MORRIS, AND T. W. A. LITTLE. 1982. A spectrum of immune response and pathological conditions between certain animal species to experimental *Mycobacterium bovis* infection. British Journal of Experimental Pathology 63: 562–572.

WEDLOCK, D. N., M. A. SKINNER, G. W. DE LISLE, AND B. M. BUDDLE. 2002. Control of *Mycobacterium bovis* infections and the risk to human populations. Microbes and Infection 4: 471–480.

WELLS, A. Q. 1938. The susceptibility of voles to human and bovine strains of tubercle bacilli. British Journal of Experimental Pathology. 19: 324–328.

WESSELS, C. C. 1941. Tuberculosis in the rat. I. Gross organ changes and tuberculin sensitivity in rats infected with tubercle bacilli. The American Review of Tuberculosis 43: 449–458.

WHIPPLE, D. L., P. R. CLARKE, J. L. JARNAQUIN, AND J. B. PAYEUR. 1997. Restriction fragment length polymorphism analysis of *Mycobacterium bovis* isolates from captive and free-ranging animals. Journal of Veterinary Diagnostic Investigation 9: 381–386.

WILESMITH, J. W., P. E. SAYERS, T. W. LITTLE, J. I. BREWER, R. BODE, G. D. HILLMAN, D. G. PRITCHARD, AND F. A. STUART. 1986. Tuberculosis in East Sussex. IV. A systematic examination of wild mammals other than badgers for tuberculosis. Journal of Hygiene (London) 97: 37–48.

Received for publication 1 February 2006.