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SHORT COMMUNICATIONS

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Shared Feed as a Means of Deer-to-Deer Transmission of *Mycobacterium bovis*

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ABSTRACT: To determine the ability of experimentally inoculated white-tailed deer (*Odocoileus virginianus*) to transmit *Mycobacterium bovis* to naïve deer through the sharing of feed, four deer were intratonsillarly inoculated with 4×10^5 colony-forming units of *M. bovis*. On a daily basis, feed not consumed by inoculated deer after approximately 8 hr was offered to four naïve deer maintained in a separate pen, where direct contact, aerosol transmission, or transmission through personnel were prevented. After 150 days, naïve deer were euthanized and examined. All naïve deer had lesions consistent with tuberculosis and *M. bovis* was isolated from various tissues. The most commonly affected tissues were lung, tracheobronchial lymph nodes, and mediastinal lymph nodes. This study demonstrates the potential for indirect transmission of *M. bovis* through the sharing of feed. Intentional or unintentional feeding of deer by wildlife or agricultural interests in regions where *M. bovis* infection is endemic should be avoided because both direct and indirect transmission through sharing of feed are enhanced.

Key words: Deer, feeding, *Mycobacterium bovis*, *Odocoileus virginianus*, transmission, tuberculosis.

In 1994, a free-ranging white-tailed deer (*Odocoileus virginianus*) in Michigan (USA) was diagnosed with tuberculosis due to *Mycobacterium bovis* infection (Schmitt et al., 1997). Subsequent surveys conducted by the Michigan Department of Natural Resources and Michigan State University Animal Health Diagnostic Laboratory (Ann Arbor, Michigan) identified a focus of *M. bovis* infection in free-ranging white-tailed deer in northeast Michigan. This focus represents the first known reservoir of *M. bovis* in free-ranging wildlife in the United States. Several factors are thought to have contributed to establishment and persistence of *M. bovis* in this wildlife reservoir. These factors include the large number of cattle infected with *M. bovis* in Michigan during the late 1950s (Frye, 1995), when it is likely that *M. bovis* was transmitted from cattle to free-ranging deer; a deer population that has grown steadily beyond normal habitat carrying capacity to focal concentrations of 19 to 23 deer/km^2 (Schmitt et al., 1997); and long-term winter feeding of large volumes of sugar beets, carrots, corn, apples, pumpkins, and pelleted feed to deer to prevent migration and decrease winter mortality in order to keep deer numbers high for hunting purposes (Schmitt et al., 1997). The resulting increased population, combined with prolonged crowding of deer around feeding sites, provided increased opportunity for deer-to-deer contact and enhanced transmission of tuberculosis. DNA analysis of *M. bovis* isolates from Michigan white-tailed deer showed that the majority of deer were infected with a single strain, suggesting a single source of infection (Whipple et al., 1997). The presence of *M. bovis* in wildlife also represents a serious threat to domestic livestock. As of this writing, 30 cattle herds infected with *M. bovis* have been identified in Michigan since the identification of tuberculosis in white-tailed deer. Restriction fragment length polymorphism (RFLP) analysis of *M. bovis* isolates from deer and cattle show that they are identical, suggesting that cattle were infected through contact with free-ranging white-tailed deer (Whipple et al., 1999).

It has been assumed that supplemental

feeding of deer leads to prolonged crowding of deer, with greater potential for deer-to-deer transmission of *M. bovis* and maintenance of infection in this susceptible wildlife population. Previous work has shown that deer in close contact can transmit *M. bovis* to penmates (Palmer et al., 2001). This study was conducted to determine the ability of experimentally infected white-tailed deer to transmit *M. bovis* to uninfected deer through indirect contact by the sharing of feed.

Deer used in this study were from a research herd maintained at the National Animal Disease Center, Ames, Iowa (USA) (42°3′N, 93°63′W). The foundation animals for this herd, started in 1998, were tested by tuberculin skin testing and found negative for exposure to *M. bovis.* Since 1998, all animals euthanized because of injuries or unforeseen, untreatable illness have received thorough postmortem examinations, and no evidence of *M. bovis* infection has been found. Eight 12-mo-old white-tailed deer (castrated males and females) were divided into two groups identified as inoculated ($n=4$) and naïve ($n=4$). Four deer were experimentally inoculated by intratonsillar instillation of 4×10^5 colony-forming units of *M. bovis* strain 1315 as previously described (Palmer et al., 1999). Strain 1315 was originally isolated from a white-tailed deer in Michigan in 1995. Four naïve deer were housed in a separate pen with no direct contact with inoculated deer. Each pen was approximately 16 m^2 and located inside a biosecurity level 3 (BL-3) building with directional airflow and high-efficiency particulate air (HEPA) filtration to prevent room-to-room transfer of air. Airflow velocity was controlled to provide 10.4 air changes/hr in each animal pen, and air was passed through HEPA filters before exiting the building. Protocols designed for feeding and cleaning of pens prevented transfer of *M. bovis* between rooms by personnel. Deer in each pen had access to a circulating watering device and were fed a pelleted feed (Deer and Elk Complete Feed 55P3, Purina Mills, St. Louis, Missouri, USA). Inoculated deer were offered an excess of feed in the morning and beginning 14 days after inoculation, uneaten feed was transferred from the pen of inoculated deer to the pen of naïve deer at the end of the day. Such transfer of feed continued for the duration of the study.

Previous studies have shown that consistent gross and microscopic lesions are present in experimentally infected animals 90–180 days after inoculation (Palmer et al., 2002); therefore, 120 days after inoculation, all experimentally inoculated animals were euthanized by intravenous administration of sodium pentobarbital. Naïve deer were similarly euthanized 150 days after the inoculation date of inoculated animals (136 days after the beginning of feed sharing).

Specimens collected for bacteriologic culture and microscopic examination from inoculated and naïve deer included tonsil; lung; liver; and mandibular, parotid, medial retropharyngeal, tracheobronchial, mediastinal, mesenteric, and hepatic lymph nodes. Additional specimens collected from naïve deer included spleen, kidney, brain, and nasal turbinate. Specimens for bacteriologic culture were placed individually in sterile bags and stored at −80 C until processing. Processing of specimens was as previously described (Palmer et al., 1999). Results were considered positive if *M. bovis* was isolated.

Samples for microscopic examination were fixed in 10% neutral buffered formalin and processed by routine paraffin embedment techniques. Sections were cut 5 μm thick, stained with hematoxylin and eosin (H&E), and examined by light microscopy. Adjacent 5-μm sections were cut from specimens with lesions suggestive of tuberculosis (caseonecrotic granulomata) and stained by the Ziehl-Neelsen technique for visualization of acid-fast bacteria (Sheehan and Hrapchak, 1980). Microscopic findings were considered positive when lesions consistent with tuberculosis contained acid-fast bacilli.

TABLE 1. Distribution of gross (G) or microscopic (M) lesions or isolation of *M. bovis* (B) in tissues from deer exposed to feed previously offered to deer intratonsillarly inoculated with 4×10^5 colony forming units of *M. bovis*.

Tissue	Deer number			
	436	448	544	561
Medial retropharyngeal LN[a]	—[b]	B	—	—
Tracheobronchial LN	G,M,B	G,M,B	G,B	G,M
Mediastinal LN	G,M,B	G,M	G,M,B	—
Lung	G,M,B	G,M,B	G,B	B
Liver	M	—	—	—
Hepatic LN	M	B	—	—
Nasal turbinates	B	—	—	—

[a] LN = lymph node.
[b] — = no gross or microscopic lesions and no isolation of *M. bovis*.

At necropsy, all experimentally inoculated deer had developed disseminated tuberculosis, with the tonsil; lung; and medial retropharyngeal, tracheobronchial, mediastinal, and hepatic lymph nodes most commonly containing tuberculous lesions. In contrast, the most common tissues containing tuberculous lesions in naïve deer were the lung and tracheobronchial and mediastinal lymph nodes (Table 1). The medial retropharyngeal lymph node was not as frequently affected as in experimentally inoculated deer. *Mycobacterium bovis* was isolated from the nasal turbinates of one naïve deer. No gross or microscopic lesions were seen, and *M. bovis* was not isolated from tonsil; spleen; kidney; brain; or mandibular, parotid, or mesenteric lymph nodes from naïve deer.

This study demonstrates that experimentally inoculated white-tailed deer efficiently transmit *M. bovis* to naïve deer through sharing of feed. Previous studies have shown that experimentally infected deer shed *M. bovis* in nasal secretions and saliva and that feed can become contaminated with *M. bovis* by such fluids (Palmer et al., 2001). This study demonstrates that such contaminated feed can serve as a means of indirect transmission of *M. bovis* between deer.

Lesions in experimentally inoculated deer were similar to those previously described in experimentally inoculated and naturally infected deer, in which the medial retropharyngeal lymph nodes, lungs, and tracheobronchial and mediastinal lymph nodes are the most common sites for tuberculous lesions (Schmitt et al., 1997; Palmer et al., 1999, 2000). However in naïve deer, although the lungs and tracheobronchial and mediastinal lymph nodes were commonly involved, no lesions were seen in the medial retropharyngeal lymph nodes, and *M. bovis* was isolated from the medial retropharyngeal lymph node of only one of four animals. The reason for this difference in lesion distribution is unclear but could be a result of differences in route of inoculation, dosage of inoculum, or duration of infection. Pelleted feed used in this study might have contained fine feed particles that were inhaled during feeding, resulting in more prevalent lesions in lungs and associated lymph nodes. The effect of different feed types on the efficiency of indirect transmission through shared feed requires investigation. Moreover, in this study, although feed sharing began 14 days after inoculation of experimentally infected deer, the precise date that naïve deer became infected is unknown, and lesion distribution could have been affected by the duration of the infection. Finally, because all four naïve deer were housed together, the possibility of transmission of *M. bovis* between naïve deer cannot be excluded. The focus of lesion development in the lungs and tracheobronchial and mediastinal lymph

nodes suggests either an aerosol route of infection or a strong predilection for colonization of *M. bovis* to these tissues regardless of the route of infection. Although BL-3 building construction and ventilation systems are designed to prevent room-to-room spread of infectious agents and feeding and cleaning protocols were also designed to prevent room-to-room transfer of *M. bovis,* the remote possibility of another unknown route of transmission in this study cannot be entirely excluded.

In previous studies, feeding of shelled corn spiked with *M. bovis* to cattle resulted in lesions centered on the lungs and tracheobronchial and mediastinal lymph nodes, with less common involvement of the medial retropharyngeal and mesenteric lymph nodes, as in the lesion distribution seen in this study (Whipple, unpubl. data). Transmission studies with experimentally inoculated or naturally infected calves produced a similar pattern of gross lesions in in-contact calves to that seen in this study (Neill et al., 1989; Costello et al., 1998). However, only one third to one fourth of in-contact calves developed tuberculosis, in contrast to the 100% transmission of *M. bovis* seen in this study.

Although shedding of *M. bovis* by experimentally infected deer has been shown to be variable and intermittent (Palmer et al., 2001), transmission in this study might have been artificially enhanced by the dose of inoculum administered to experimentally inoculated deer and the high probability of multiple exposures of naïve deer to *M. bovis*–contaminated feed. Intermittent shedding of *M. bovis,* especially during the later stages of infection, has also been documented in cattle after experimental intranasal inoculation (Neill et al., 1989; Costello et al., 1998). In free-ranging deer, the dose of *M. bovis* received during natural infection is not known but is likely highly variable and probably involves multiple exposures, especially in areas where disease prevalence is high.

Under appropriate conditions, *M. bovis* can persist in the environment for weeks or months (Dufield and Young, 1985; Jackson et al., 1995; Tanner and Michel, 1999). *Mycobacterium bovis* survived 5–14 days in infected tissues during seasons other than winter; however, during winter, *M. bovis* persisted in infected tissues for up to 6 wk (Tanner and Michel, 1999). Another study has shown persistence on feedstuffs (corn, carrots, apples, hay, sugar beets) at 0 C for up to 16 wk (Whipple, unpubl. data).

In Switzerland, naturally infected roe deer (*Capreolus capreolus*) are suspected to have infected domestic cattle with *M. bovis* through contaminated feed (Bischofgerger and Nobholz, 1964). Cattle have also been shown to become infected by contact with pastures contaminated with feces or urine from *M. bovis*–infected badgers (*Meles meles*; Little et al., 1982). In northern Michigan, where *M. bovis* infection is endemic in free-ranging white-tailed deer, large numbers of deer around feeding sites provide opportunity for close contact and direct transmission of *M. bovis,* as well as a common area where infected deer can contaminate feed used by large numbers of deer, creating the opportunity for indirect transmission. This study demonstrates that the possibility of transmission through indirect means such as shared feed must be considered. Wildlife managers in endemic areas should discourage practices that promote gathering and crowding of deer because it enhances both direct and indirect transmission of tuberculosis. Similarly, agricultural agencies and producers should consider whether agricultural practices that make livestock feeds available to free-ranging deer might act as a source of indirect transmission of tuberculosis between deer, from cattle to deer, and from deer to cattle. Regardless of the cause, human activity that results in crowding and gathering of deer in areas of endemic tuberculosis will make disease control more difficult and eradication unlikely in both wildlife and domestic livestock.

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