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Possible Airborne Person-to-Person Transmission of *Mycobacterium bovis* — Nebraska 2014–2015

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Possible Airborne Person-to-Person Transmission of *Mycobacterium bovis* — Nebraska 2014–2015

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Mycobacterium bovis, one of several mycobacteria of the *M. tuberculosis* complex, is a global zoonotic pathogen that primarily infects cattle. Humans become infected by consuming unpasteurized dairy products from infected cows (1,2); possible person-to-person airborne transmission has also been reported (3). In April 2014, a man in Nebraska who was born in Mexico was determined to have extensive pulmonary tuberculosis (TB) caused by *M. bovis* after experiencing approximately 3 months of cough and fever. Four months later, a U.S.-born Hispanic girl from a nearby town who had been ill for 4–5 months was also determined to have pulmonary TB caused by *M. bovis*. The only social connection between the two patients was attendance at the same church, and no common dietary exposure was identified. Both patients had pulmonary cavities on radiography and acid-fast bacilli (AFB) on sputum-smear microscopy, indicators of being contagious (4). Whole-genome sequencing results of the isolates were nearly indistinguishable. Initial examination of 181 contacts determined that 39 (22%) had latent infection: 10 (42%) of 24 who had close exposure to either patient, 28 (28%) of 100 who were exposed to one or both patients in church, and one (2%) of 57 exposed to the second patient at a school. Latent infection was diagnosed in six contacts on follow-up examination, 2 months after an initial negative test result (4), for an overall latent infection rate of 25%. No infected contacts recalled consuming unpasteurized dairy products, and none had active TB disease at the initial or secondary examination. Persons who have *M. bovis* TB should be asked about consumption of unpasteurized dairy products (2), and contact investigations should follow the same guidance as for *M. tuberculosis* TB (4).

In April 2014, patient A, a man aged 42 years who was born in Mexico sought care for cough, fever, weight loss, and progressive debilitation over approximately 3 months. He had

arrived in Nebraska from Mexico in 2010, and initially he worked on a dairy farm* and later in construction. No information was collected regarding his prior employment in Mexico, but he did report frequent consumption of raw milk. Chest radiography was consistent with advanced TB with cavities; numerous AFB were reported from sputum-smear microscopy. The result from nucleic acid amplification testing of sputum was positive for *M. tuberculosis* complex. The isolate was resistant both to pyrazinamide (PZA), which suggested that the

*Nebraska dairies have been free of *M. bovis* infections since 1978. In recent years, *M. bovis* infections have been detected sporadically among Nebraska beef herds (late 1990s, 2005, 2009, and 2013) and an elk herd (2009) (Nebraska Department of Agriculture, unpublished data, June 29, 2015). No persons in this investigation were thought to have had contact with these sources. The whole-genome sequencing results for all veterinary *M. bovis* isolates from Nebraska are distinct from those of both patients' isolates.

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infection was caused by *M. bovis*, and to low-concentration isoniazid (INH) (5). His treatment regimen was adjusted in consultation with national experts in drug-resistant TB. He recovered slowly and remained isolated at home until results from sputum smears were negative for AFB in August.

In June 2014, patient B, a Hispanic girl aged 16 years, who was born in Nebraska to Mexican parents, sought medical care after 2–3 months of cough. She initially received treatment for presumed bronchitis and allergies, without chest radiography. She remained ill through late July, when radiography revealed a pulmonary cavity and her sputum smear had numerous AFB. *M. bovis* resistant to PZA was identified after culture confirmation. She had never traveled outside the United States and was unaware of having consumed any dairy products from Mexico. She recovered quickly and remained in isolation at home until late September. The only social connection between patients A and B was regular attendance at the same church. The patients knew one another but their interactions were reported to be minimal.

Contact investigations were conducted in accordance with published guidelines (4) focusing on household contacts of both patients, community contacts at both the church and patient B's school, coworkers of patient A, and persons who spent extended periods in a vehicle with him. Potentially exposed health care workers were notified for follow-up at their respective facilities with a request to report infections to the health department if identified. Tuberculin skin tests (TST)

and interferon gamma release assays (IGRA) were used for testing U.S.-born and foreign-born contacts, respectively; IGRA was used for all members of the church, where the majority of contacts were foreign born. Contacts whose initial results were negative, but whose exposure to either patient had ended <2 months before testing, were retested after 8–12 weeks, because immune sensitivity might not be detectable during this period after new infection (4). Persons who had positive test results indicating infection had chest radiographs to exclude active TB disease and thus establish latent infection (4).† All contacts were asked about their country of birth except those at a school attended by patient B where all were assumed to have been born in the United States. Midway through the investigation, after *M. bovis* was recognized as the causative agent, contacts who had positive test results were also asked about travel abroad and consumption of unpasteurized dairy products from Mexico.

† Tuberculosis infection is “a condition in which microorganisms [i.e., *M. tuberculosis* complex] have entered the body and typically have elicited immune responses” and “includes both latent infection and TB disease.” Latent infection “is an asymptomatic condition that follows the initial infection; the infection is still present but is dormant (and believed not to be currently progressive or invasive)” and “might progress to TB disease.” “[Active] TB disease is determined by finding anatomic changes caused by advancing infection (e.g., shadows from infiltrates on a chest radiograph) or by noting symptoms (e.g., malaise, feverishness, or cough), and typically by both.” <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5415a2.htm>.

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Twenty-four persons had extended close exposure to either patient and were regarded as high-priority contacts (4); among these, 10 (42%) had positive results at initial testing (Table 1). Two were non-Hispanic U.S.-born contacts of patient A who did not attend the church. Among 11 high-priority contacts of patient B, seven were family members who were also potentially exposed to patient A at the church. Among patient B's five siblings, four were born in the United States, had never traveled abroad, and did not recall consuming dairy products from Mexico; three of these four siblings were infected. Patient B's eldest sibling and mother, both of whom were born in Mexico, were also infected but neither had active TB disease and thus would not have been infectious. Among 100 church members (excluding patient B and high-priority contacts of either patient), 28 (28%) had latent infection, including five U.S.-born children. Among 57 school contacts, one U.S.-born child was infected. No infections among exposed health care workers were reported.

Among 77 persons for whom retesting was indicated, 56 (73%) were retested, and six (11%) were determined to have latent infection (Table 2). During the interval between the first and second tests, none had traveled abroad or recalled eating unpasteurized dairy products from Mexico. No school contacts were retested, because their exposure had ended the previous May. No infected contacts had active TB disease, and all were offered a 4-month rifampin preventive regimen.[§]

Patient A's bacterial isolate, grown at a private hospital laboratory from a sputum sample collected April 24, 2014, was sent to the Nebraska Public Health Laboratory to facilitate genotyping at the Michigan state laboratory and first-line drug susceptibility testing at Associated Regional and University Pathologists, Inc.; second-line drug susceptibility testing was conducted at CDC. Patient B's isolate was cultured by Nebraska Public Health Laboratory from a sputum sample collected during early August, and it was similarly sent for first- and second-line drug susceptibility testing and genotyping. Routine genotyping results of both patients' isolates were indistinguishable. In late September 2014, both patients' isolates were sent to the United States Department of Agriculture National Veterinary Services Laboratories (NVSL) where whole-genome sequencing was performed. Results suggested that the two patients' isolates were closely related; phylogenetic comparisons differed by only three single nucleotide polymorphisms (SNPs). The sequences

did not match others in NVSL's library, but the isolates shared a common ancestor with isolates from five cattle in Mexico.[¶]

Discussion

M. bovis primarily causes disease in cattle but also infects deer and other mammals (1). The human diseases caused by *M. bovis* and *M. tuberculosis* (i.e., the human variant) are clinically indistinguishable, and cases caused by both are reported in U.S. TB surveillance (1,2,6,7).^{**} Treatment differs, however, because *M. bovis* is inherently resistant to PZA, which is part of the routine initial TB treatment regimen (5). Bovine tuberculosis eradication programs and routine pasteurization of milk products have led to marked declines in *M. bovis* TB in humans (1), which accounted for 1.6% of U.S. TB cases in 2014 (6), with regional differences (2,6–8).

Human *M. bovis* disease is typically attributed to consumption of unpasteurized milk (or dairy products made from unpasteurized milk) in or imported from countries with affected cattle herds (1,2,7,8). Person-to-person airborne transmission of *M. bovis* has been reported infrequently, with uncertainty remaining about dietary exposures (3). Findings from contact investigations and a population study regarding infectiousness of *M. bovis* compared with *M. tuberculosis* are inconclusive (4,9,10).^{††}

Standard nucleic acid amplification test methods detect the *M. tuberculosis* complex without distinguishing between *M. tuberculosis* and *M. bovis*. Although these species can be distinguished by routine genotyping, biochemical characterization and drug susceptibility testing, which generally provide results earlier, have been historically used and can increase the index of suspicion for *M. bovis*. Whole-genome sequencing can be used to identify species and investigate transmission. NVSL sequences genomes for all U.S. *M. bovis* animal isolates, a convenience sample of cattle isolates from Mexico, and human isolates upon request.^{§§}

[¶] These cattle included four dairy cows in Nuevo León and one steer in Durango, Mexico. On the basis of whole-genome sequencing of 15 less related cattle isolates in Group 13, this *M. bovis* strain appears to be disseminated throughout Mexico but has not been identified in U.S.-origin cattle. Isolates in this group of the SB0121 family are believed to have evolved on the Iberian Peninsula.

^{**} Genotyping results from CDC's National TB Genotyping Service for the isolates from 96 TB patients with culture-confirmed disease in Nebraska during 2006–2013 indicated that all were *M. tuberculosis*.

^{††} Infectious TB “refers either to TB disease of the lungs or throat, which has the potential to cause transmission to other persons, or to the patient who has TB disease.” <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5415a2.htm>.

^{§§} NVSL has sequenced 97% of *M. bovis* isolates from affected U.S. cattle herds since 1997 and 90% from feeder and feedlot cattle (both imported and domestic) since 2000. The oldest isolate sequenced is from 1991, but isolates were not consistently archived from affected herds until 1997. NVSL sequences the *M. bovis* isolates from all animal species; the size of the database is approximately 2,500 sequences.

[§] In August 2015, a U.S.-born 11-year-old niece of patient A, who reported exposure to him only at the church, became ill with shortness of breath and cough. During the church contact investigation, she and her parents were determined to have latent infection, but they had stopped taking rifampin after only 2 months. A presumptive diagnosis of TB disease caused by *M. bovis* was made, and treatment was started in September 2015, based on clinical findings, including a new pulmonary infiltrate (4,6). Results from sputum-smear microscopy, nucleic acid amplification test, and culture were negative.

TABLE 1. Investigation setting and results of initial testing* of contacts (N = 181) exposed to one or both of two *Mycobacterium bovis* tuberculosis patients, by United States versus foreign birth — Nebraska, 2014

Investigation setting [†]	Test results negative			Test results positive			Total tested
	Foreign-born	U.S.-born	Total negative	Foreign-born	U.S.-born	Total positive	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No.
High-priority contacts of patient A	3 (23)	5 (38)	8 (62)	3 (23)	2 [§] (15)	5 (38)	13
High-priority contacts of patient B	2 (18)	4 (36)	6 (55)	2 (18)	3 [¶] (27)	5 (45)	11
Church	43 (43)	29 (29)	72 (72)	23 (23)	5 ^{**} (5)	28 (28)	100
Patient B's school	NA	NA	56 (98)	0 (0)	1 ^{**} (2)	1 (2)	57
Total	NA	NA	142 (78)	28^{††} (15)	11 (6)	39^{§§} (22)	181

Abbreviation: NA = not available.

* Tuberculin skin tests and interferon gamma release assays (IGRA) were used for testing U.S.-born and foreign-born contacts, respectively; IGRA was used for all members of the church where the majority of contacts were foreign born.

[†] The counts in the four categories of settings are mutually exclusive. Among the 13 high-priority contacts of patient A, 11 did not attend the church. Among the remaining two who were also potentially exposed to patient B at the church, one tested positive (patient B's grandfather). Among the 11 high-priority contacts of patient B, all were also potentially exposed to patient A at the church. Patients A and B were not counted as contacts for any setting.

[§] Non-Hispanic U.S.-born adults exposed at patient A's residence.

[¶] Three Hispanic siblings of patient B (aged 7, 9, and 10 years).

^{**} Six U.S.-born children. Unknown travel and dietary history.

^{††} Countries of birth are Mexico (n = 26), Guatemala (n = 1), and Philippines (n = 1).

^{§§} All 39 persons testing positive reported no knowledge of a prior positive TB test result, but information was lacking to verify the accuracy of their recall.

TABLE 2. Investigation setting and results of follow-up testing* performed 8–12 weeks after last potential exposure and after an initial negative result for contacts (N = 56) of one or both of two *Mycobacterium bovis* tuberculosis patients — Nebraska, October 2014

Investigation setting [†]	Test results negative	Test results positive	Total
	No. (%)	No. (%)	No.
High-priority contacts of patient A	0 (0)	1 [§] (100)	1
High-priority contacts of patient B	1 (25)	3 [¶] (75)	4
Church	49 (96)	2 (4)	51
Total	50 (89)	6^{**} (11)	56

* An interferon-gamma release assay was used for testing at the church where foreign-born persons predominated and for other foreign-born contacts. Tuberculin skin tests were used for U.S.-born contacts.

[†] Follow-up testing was not necessary for contacts at patient B's school because the end of their exposure was >2 months before the investigation.

[§] Non-Hispanic U.S.-born individual exposed at patient A's residence who reported no international travel at any time and no consumption of Mexico-origin unpasteurized dairy products. This person had no affiliation with the church or the school and reported no contact with patient B, who resided in a different town.

[¶] All three were patient B's family members who were also potentially exposed to patient A at the church.

^{**} All six denied both international travel and consumption of Mexico-origin unpasteurized dairy products in the interim. Among these, three were foreign born (two high-priority contacts of patient B and one church member).

Patient A might have been infected from consuming unpasteurized dairy products originating in Mexico. The timing of the illnesses, relatedness of the *M. bovis* isolates, and common church attendance suggest that patient B might have acquired infection from patient A. Findings from the contact investigations suggest possible airborne transmission, because approximately one third of the infections could not be explained by potential exposure in countries where *M. tuberculosis* complex infections are common. Consumption of imported

contaminated dairy products could not be excluded, but locally produced dairy products were unlikely to be contaminated with *M. bovis*.

The findings in this report are subject to at least four limitations. This investigation illustrates typical challenges of investigating human *M. bovis* infections. First, the incubation period has not been well studied, but it potentially ranges from months to years and might obscure ascertainment of time and nature of exposure. Second, dietary history details could be forgotten during the interim, or consumers might be unaware of the origin or pasteurization status of dairy products they consumed. Third, TST and IGRA are based on cellular immune response and cannot distinguish between old or recent infections, or whether the cause is *M. tuberculosis* or *M. bovis*. Persons from countries where both types of infection are prevalent could be infected by either species. The variable incubation period for *M. bovis* notwithstanding, the six persons whose results changed from negative to positive were probably infected only in the weeks before being examined. Finally, despite not documenting conversion in the first five U.S.-born high-priority contacts who were infected, this observed proportion of latent infections (29% [five of 17]) upon initial testing exceeds the expected background prevalence of latent infection of <2% for persons born in the United States.

An evidence base to aid epidemiologic interpretation of whole-genome sequencing results from isolates with few differences in SNPs has not been established for *M. bovis* or *M. tuberculosis*. Maintenance of patient A's isolate in culture for approximately 5 months could have provided opportunity for accrual of the additional SNPs. Airborne transmission from either patient was plausible based on disease characteristics

References

Summary

What is already known about this topic?

Mycobacterium bovis, a zoonotic pathogen of cattle, causes tuberculosis in persons who consume unpasteurized contaminated dairy products. Airborne person-to-person transmission has been suspected but is difficult to confirm.

What is added by this report?

A large contact investigation around two patients with *M. bovis* pulmonary tuberculosis and the findings from molecular epidemiology strengthen the evidence for person-to-person transmission of *M. bovis* infection.

What are the implications for public health practice?

The persistence of *M. bovis* in cattle internationally and the failure to pasteurize dairy products in many locations means that further infections in humans should be anticipated. Persons with *M. bovis* infections should be asked about foodborne exposures. Contact investigations for *M. bovis* disease should be conducted using the same methods as for *M. tuberculosis* disease.

(i.e., pulmonary cavities and AFB on sputum smears) and contact findings.

This report adds to the evidence for airborne person-to-person spread of *M. bovis* (3,9,10). Whole-genome sequencing is an emerging tool for investigating transmission. Public health responses to *M. bovis* pulmonary TB should be the same as those for *M. tuberculosis* TB, with additional inquiries about consumption of unpasteurized dairy products. The ongoing incidence of *M. bovis* TB in humans substantiates the need to control bovine tuberculosis globally and to pasteurize all milk and dairy products.

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