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CASE REPORT

Transmission of *Mycobacterium orygis* (*M. tuberculosis* Complex Species) from a Tuberculosis Patient to a Dairy Cow in New Zealand

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***Mycobacterium orygis*, previously called the oryx bacillus, is a member of the *Mycobacterium tuberculosis* complex and has been reported only recently as a cause of human tuberculosis in patients of South Asian origin. We present the first case documenting the transmission of this organism from a human to a cow.**

CASE REPORTS

In 2011, an 11-year-old dairy cow from the Waikato area of New Zealand, case A, was sent to slaughter at the end of its working life. All cows age 12 months and older in the herd had been subjected to a bovine tuberculin intradermal skin test annually as part of surveillance requirements for bovine tuberculosis under the management of the Animal Health Board, the New Zealand agency responsible for managing the eradication scheme for this disease. All skin testing in the herd was negative since herd establishment in 1986, apart from one cow with a positive skin test in 1999, which was subsequently determined to be negative by ancillary comparative tuberculin testing. Case A was tested nine times during its life and had not reacted to tuberculin at any of these tests.

At meat inspection, case A was identified as having a single 2- to 3-cm encapsulated granulomatous lesion in a mediastinal lymph node. Histopathology of fixed tissue revealed granulomatous inflammation with central caseation and mineralization with a mixture of epithelioid cells, lymphoid cells, and occasional Langhan's giant cells surrounding the necrotic tissue. Acid-fast organisms were identified in the sample. Liquid culture (Bactec 460; Becton, Dickinson) of a fresh sample of the lymph node was positive for mycobacteria, and the isolate was identified as a member of the *Mycobacterium tuberculosis* complex (ACCUPROBE; bioMérieux). The isolate was initially assumed to be *Mycobacterium bovis*, which is endemic in wildlife in some parts of New Zealand, and the herd was placed under infected movement control in accordance with the New Zealand Biosecurity Act (1993).

On DNA subtyping, the organism had an unusual restriction endonuclease analysis pattern (3) and a unique variable number tandem repeat/direct repeat (VNTR/DR) profile (9). An organism with this genetic profile had never been seen in New Zealand during 30 years of subtyping 3,800 animal isolates of the *M. tuberculosis* complex, 99.8% of which were *M. bovis*, and the remainder being *Mycobacterium pinnipedii*. Because of its unusual features, the organism was further characterized by a molecular biological approach. PCR tests for five regions of difference that distinguish between species of the *M. tuberculosis* complex (B, G, H, I, and J in Fig. 1) excluded the possibility that the organism was *M. tuberculosis*, *M. bovis*, *M. pinnipedii*, *Mycobacterium microti*, or *Mycobacterium caprae*, and it was provisionally identified as *Mycobacterium africanum*, an obligate human pathogen. Since this species had not been previously found in any animal in New Zealand, an epidemiological investigation of the farm and its practices was carried out, which identified case B.

Case B was a 38-year-old woman who had been born and lived in India until emigrating to New Zealand in 1990. In 2002, after a prolonged period of increasing ill health lasting several years, case B was diagnosed with tuberculosis and treated with isoniazid, rifampin, and pyrazinamide over a period of 6 months; ethambutol was started but discontinued after 3 weeks once the isolate was found to be fully sensitive to the first three drugs. Case B made a complete recovery and has remained symptom free ever since. She had no known household contact with any other tuberculosis case either in New Zealand or in India; however, she recalled being told as a child that an elderly gentleman who she had known and whose cows grazed with her family's cows had died of tuberculosis. During her childhood in India, case B did have contact with cattle and buffalo in a rural setting. At the time of diagnosis, acid-fast organisms were isolated from sputum and identified as belonging to the *M. tuberculosis* complex, but the isolate was not further characterized at that time.

Additional investigation in 2011 revealed that case B had been working on the farm at the time when she was unwell but had not yet been diagnosed with tuberculosis and that she had been responsible for rearing the calves, including case A, in the year 2000. With this knowledge, case B's isolate was tested further using a genotype test (Hain Lifescience) which gave a result consistent with *M. africanum*. Subsequently, further molecular biological tests of the organism from both case A and case B were performed to include all the genomic markers shown in Fig. 1, and this revealed that the true identity of both isolates was *M. orygis*.

The species identification of both the human (case B) and cow (case A) isolates was performed by testing for the presence or absence of 10 large sequence polymorphisms called regions of difference (RD) and by DNA sequencing the informative regions of three genes. DNA extracts from the case A and case B isolates as well as from reference mycobacterial strains of *M. tuberculosis*, *M.*

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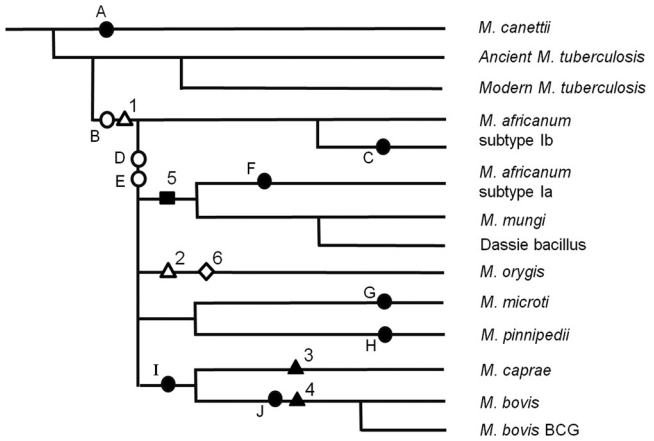

FIG 1 Summary diagram and phylogenetic positions of the genomic markers tested on the isolates from case A and case B as well as on one reference strain each of *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. microti*, and *M. pinnipedii*. Ten RDs are represented by circles and denoted A to J; four different alleles of *gyrB* are denoted 1 to 4, an allele of Rv1510 is denoted 5, and an allele of Rv2042c is denoted 6. Presence or absence of RDs was determined by PCR using primers reported from previous studies, as shown in Table 1.

africanum, *M. pinnipedii*, *M. microti*, and *M. bovis* were amplified by PCR using the primers in Table 1. In the case of DNA sequencing to identify gene alleles, the same primers were used for both amplification and sequencing. All reference strains had the expected genotypes. The isolates from case A and case B had an identical genotype that was characteristic of *M. orygis*, with deletion of three RDs represented by the unfilled shapes for RDs B, D, and E and the gene alleles 1, 2, and 6 and no deletion of other RDs or other gene sequence changes (filled shapes) compared to *M. tuberculosis* (Fig. 1).

Cattle in New Zealand are frequently infected with *M. bovis*, principally from infected wildlife (12), so the isolation of a strain of the *M. tuberculosis* complex after slaughter of a cow from a previously uninfected herd was not unusual. However, the subsequent discovery that the isolate was *M. orygis*, an organism that had not been previously reported in cattle or humans in New Zealand and that had not at that time been reported as a cause of human tuberculosis, was a particular surprise. The finding of genotypically identical organisms in the human patient and the cow, the circumstances surrounding these infections, and the time difference between them was clear epidemiological evidence that the dairy cow was infected as a calf during rearing in 2000 from the farm worker, who at the time had untreated infectious tuberculosis due to *M. orygis*. There was no evidence found that the farm worker had infected members of her household or other calves. Previously, *M. orygis* was regarded as an animal pathogen of the oryx and other animals and has only recently been reported in humans (13).

The clear distinction of *M. orygis* from *M. bovis* and other species of the *M. tuberculosis* complex was first described only 7 years ago (8), although unusual features of this organism were noted much earlier at the time when it was regarded as a strain of *M. bovis* (14). Incorrect identification of the organism as *M. africanum* by Hain's genotype test occurs because both *M. orygis* and *M. africanum* share the $gyrB^{1450}$ (G→T) mutation on which the test is based (13). Until the recent report of its finding in humans from South Asia, it had been isolated from a range of animals in eastern Africa, the Arabian Peninsula, and South Asia, and a conclusion in the report of its human isolation was that the cases were due to animal-to-human transmission and that humans may be accidental dead-end hosts (13). The findings in this present report indicate that transmission from humans to animals can occur and that careful investigation of other cases of *M. orygis* is required to determine whether humans or animals were the primary source.

The only report of human-to-animal tuberculosis transmission in New Zealand is that of a dairy farmer with pulmonary *M. bovis* infection reinfecting his dairy herd in 1972, resulting in the ongoing detection of lesioned animals despite an effective test and slaughter policy (1). Cattle have historically been considered resistant to *M. tuberculosis* (7), but there have been several reports recently of *M. tuberculosis* infection of cattle (2, 4, 11). This appears to be frequent in some herds in Ethiopia (4) and China (2), suggesting that in certain countries, humans are a significant source of tuberculosis infection for cattle.

These findings illustrate that although rare, human-to-cattle spread of tuberculosis is capable of occurring even in developed countries but that this could be overlooked if diagnostic testing identifies the causal organism only to the level of the *M. tuberculosis* complex. Cases in the literature reported as *M. tuberculosis*, *M. bovis*, or *M. africanum* may in some cases have been caused by *M. orygis*, and the organism may therefore be more common than previously thought. Further typing of archived organisms from historical cases may be warranted. This study confirms that *M. orygis* is capable of causing clinical disease in humans and provides the first conclusive evidence that humans are not necessarily dead-end hosts but are capable of transmitting the organism to animals. This raises the possibility that *M. orygis* might also be spread from human to human and emphasizes the need for further study of the epidemiology of this organism and its role in animal and human tuberculosis.

The study's primary purpose was fulfilled, as the identification of the organism allowed staff from the Animal Health Board to determine the risk that the organism could be transmitted within herd or potentially to local wildlife and also enabled them to develop appropriate ongoing surveillance. From an understanding of the potential public health risks, the Ministry of Health was notified and became fully involved in the investigation, and ex-

TABLE 1 Source of primers

RD or allele	RD or gene name	Reference
A	RD12	16
B	RD9	16
C	RD8	10
D	RD10	15
E	RD711	15
F	RD702	5
G	$RD1^{mic}$	16
H	$RD2^{seal}$	16
I	RD12	16
J	RD4	16
1, 2, 3, 4	*gyrB*	6
5	Rv1510	5
6	Rv2042c	13

perts were consulted in other countries. This case study substantiates the value of collaboration between national and international members of Public Health and Veterinary Institutions and provides an excellent example of a successful One Health approach.

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