Detection of *Mycobacterium bovis* and *Mycobacterium tuberculosis* from Cattle: Possible Public Health Relevance

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Received: 15 October 2010 / Accepted: 23 November 2010 / Published online: 19 August 2011
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Abstract *Mycobacterium bovis* and *Mycobacterium tuberculosis* infect both animals and humans. The disease epidemiology by these agents differs in developed and developing countries due to the differences in the implementation of the prevention and control strategies. The present study describes the detection of *M. bovis* and *M. tuberculosis* from specimens of lungs and pulmonary lymph nodes of four cattle died in an organized herd of 183 cattle in the state of Himachal Pradesh, India, with inconclusive skin test results. Identification and distinction of these closely related mycobacterial species was done by PCR-RFLP targeting *hsp65* gene followed by spacer oligonucleotide typing. Mixed infection of *M. bovis* and *M. tuberculosis* was detected in one cattle.

Keywords *M. bovis* · *M. tuberculosis* · Cattle · Public health · Spoligotyping

Bovine tuberculosis is a significant zoonosis that can spread to humans through aerosols produced by diseased farm animals and by ingestion of raw unpasteurized milk. Bovine tuberculosis is still common in less developed countries and economic losses can occur in cattle and farm animals from deaths, chronic disease and trade restrictions.

In developing countries bovine tuberculosis also potentially represents a significant zoonosis, the extent of which is currently unknown [1]. Several cases of human tuberculosis caused by *Mycobacterium bovis* have been described in recent years from various parts of the world [2]. Besides *M. bovis*, transmission of *Mycobacterium tuberculosis* from infected humans to animals and vice versa has been reported (reverse zoonosis) [3]. Among domestic animals, infection with *M. tuberculosis* has been most frequently identified in cattle.

We communicate the detection of *M. bovis* and *M. tuberculosis* from an organized herd of 183 cattle in Northern India and confirmation through PCR Restriction Analysis (PCR-RFLP) and Spoligotyping. In November 2006, four cows died in the Dairy Farm (with 183 cows) of Agricultural University, Palampur in the state of Himachal Pradesh in Northern India. In the routine tuberculin testing of the herd in the year 2005, all four cows were negative. However, during May 2006 these four cows reacted inconclusively to bovine tuberculin (2,000 U/animal; Indian Veterinary Research Institute IVRI, Izatnagar). It was found that overall there were 63 positive, 39 doubtful and 81 negative cattle in the farm.

In the month of July 2006, all the animals in the farm were retested using a comparative skin test with bovine and avian tuberculin (2,000 U/animal; IVRI). All the skin tests were performed as per Office International des Epizooties (OIE) manual of standards. 15 cattle exhibited positive reactions with bovine tuberculin and the four cows under study were among them. All of the 15 tuberculin-reactive animals were clinically examined in detail during the same month and were declared healthy. Among four dead cattle, two cows were of Jersey breed 9 years (cow A) and 14 years of age (cow B), respectively, one Jersey cross 15 years (cow C) and one Holstein–Friesian 14 years (cow D).

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Fig. 1 *hsp65* gene amplification restriction analysis of mycobacterial isolates amplicons. Lane M 100 bp ladder; *M. tuberculosis* reference strain; *M. bovis* reference strain; Lane D2 lung cow C; Lane D1 bronchomediastinal LN cow A; Lane 7 prescapular LN cow B; Lane 6 bronchomediastinal LN cow B; Lane 5 lung cow B; Lane 4 prescapular LN cow D; Lane 3 bronchomediastinal LN cow D; Lane 2 lung cow D; Lane 1 lung cow A

Lungs, bronchomediastinal and prescapular lymph nodes were collected aseptically at necropsy and processed for bacteriological examination. On gross examination small tuberculous nodules were found in lungs of Cow A and C but no visible growth in other two. Histopathological examination revealed characteristic tuberculous granuloma in cow A and C with non-specific findings in other two cows.

After 28-day incubation at 37°C, small moist colonies appeared on L-J slants inoculated with the specimens of lungs of cow A and C. Standard biochemical tests identified the isolate as *M. bovis*. From the mixed specimen of bronchomediastinal and prescapular lymph nodes collected from the cow B the growth was confirmed to be *M. tuberculosis*. However, no growth was found in the lungs of the same animal. Samples from the others animals showed no growth.

PCR-RFLP assay targeting 439-bp portion of the *hsp65* gene confirmed the isolates as members of *M. tuberculosis* complex [4] (Fig. 1). This restriction assay has been used earlier for the identification of *M. tuberculosis* complex organisms [7]. In addition, we employed spoligotyping which has been used worldwide for the mycobacterial strain identification [8]. Spoligotyping confirmed infection with *M. bovis* (Cow C) and *M. tuberculosis* (Cow B, D) with mixed infection in one cow (Cow A) (Fig. 2). No *M. tuberculosis* complex was detected from the immediate environment where the cattle lived, and from other tuberculin positive cows. The farm personnel and milkers could not be included in the study for want of permission from competent authority.

The detection of *M. bovis* and *M. tuberculosis* among the cattle of the same farm is an important finding. Although there are few reports of mixed infection in the clinical specimens from the cattle in India [5, 6] however, the actual prevalence of co-occurrence is still largely unknown. *M. tuberculosis* in cattle most frequently produces an acute infection and the infected animals show short term sensitization to tuberculin [4]. The possibility of transmission of infection by inhalation of bacilli from grass contaminated with infected human urine, faeces, or sputum or an animal attendant or milker with active pulmonary tuberculosis, cannot however be ruled out. Thus, thorough microbiological investigation of specimens from animals and humans is imperative from public health perspective, to confirm and differentiate the causative mycobacteria, in the places where *M. bovis* and *M. tuberculosis* coexist.

Acknowledgments Authors are thankful to Dr. V.M. Katoh, Secretary, Department of Health Research, Govt. of India and Director-General ICMR (Ex-Director NJIL & OMD, Agra, India) for support and giving access to BSL-3 facility at the institute.

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Fig. 2 Spoligotype pattern of specimens. Lane D2 *M. bovis* from lung cow C; Lane D1 *M. bovis* from bronchomediastinal LN cow A; Lane 1 *M. tuberculosis* from lung cow A; Lane 2 *M. tuberculosis* from lung cow D; Lane 3 *M. tuberculosis* from bronchomediastinal LN

cow D; Lane 4 *M. tuberculosis* from prescapular LN cow D; Lane 5 *M. tuberculosis* from lung cow B; Lane 6 *M. tuberculosis* from bronchomediastinal LN cow B; Lane 7 *M. tuberculosis* from prescapular LN cow B

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