

Short Communication

Isolation of *Mycobacterium tuberculosis* complex (MTBC) from dairy cows in China

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Eleven thousand five hundred and eighty non-blood samples from dairy cows were subjected to mycobacterium culture and genotyping. As a result, a total of 142 isolates of *Mycobacterium tuberculosis* complex (MTBC) were identified. Among them, 65 were *Mycobacterium tuberculosis*, while 77 *Mycobacterium bovis*. The genotype of *M. tuberculosis* strains was mainly Beijing family. In addition, the isolation rates of MTBC were 33.89% for lung lymph nodes, 2.81% for nasal swabs, and 3.95% for pharyngeal swabs from cattle positive to tuberculin skin test, respectively. This evidence implied that *M. tuberculosis* infection in cattle is a new risk to public health and should be paid more attention.

Key words: *Mycobacterium tuberculosis* complex, cows, tuberculosis, zoonosis.

INTRODUCTION

Tuberculosis (TB) is a chronic and wasting anthroponosis caused by *Mycobacterium tuberculosis* complex (MTBC) including *Mycobacterium tuberculosis*, *Mycobacterium bovis* and other members. *M. tuberculosis* mainly infects human, while *M. bovis* has a broad host range, can infect cattle, human and many other species of animals. Human can get infected with *M. bovis* from cattle through unpasteurized milk, contaminated air or contagious transmission (Thorn et al., 2010). The *M. bovis* causing TB was estimated to comprise about 10% of human

TB cases (Sunder et al., 2009; Michel et al., 2010). This transmission augments the TB burden of human, which leads to about 8 millions of TB new cases, 1.5 to 2.0 million deaths globally per year. Furthermore, about 95% of new cases and deaths exist in developing countries (Whelan et al., 2010). On the other hand, *M. tuberculosis* was recently reported to be isolated from dairy cattle in China (Chen et al., 2009; Du et al., 2011), India (Ocepek et al., 2005), Ethiopia (Ameni et al., 2011), and goats in Nigeria (Cadmus et al., 2009). Since the spillover of *M. tuberculosis* from human to domestic animals, maintenance in these animals for unknown time, and possible spillback transmission to human sometime, would likely present new risk to human health, it is urgently necessary to evaluate further, the situation of *M. tuberculosis* infection on a broader scale.

Based on our previous findings of *M. tuberculosis* infection in cattle in China (Chen et al., 2009; Du et al., 2011), we extended our investigations by taking samples

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Abbreviations: TB, Tuberculosis; MTBC, *Mycobacterium tuberculosis* complex; TST, tuberculin skin test; PPD, purified protein derivative; PCR, polymerase chain reaction.

from extensive areas in this study and aimed to determine more accurately, the status of *M. tuberculosis* infection in dairy cattle.

MATERIALS AND METHODS

Sampling collection

The nasal and pharyngeal swabs were gotten from the live animals, while the lung lymph nodes of cattle were from the slaughtering houses. A total of 11580 samples were collected from 108 dairy cattle farms in 17 provinces of China, including 8197 nasal swabs, 2563 pharyngeal swabs, and 251 milk from live animals. In addition, 549 lung lymph nodes, and 20 tracheal swabs of the cattle were collected from the slaughter houses.

Bovine TB detection and bacterial identification

Single intradermal tuberculin skin test (TST) with purified protein derivative (PPD), was used to detect the *M. bovis* infection status of the live cattle. The conventional bacterial isolation was performed by treating the samples with 4% NaOH and inoculating the samples onto Lowenstein-Jensen (L-J) media, and maintaining the culture for about one month in a 37°C incubator until the growth of typical brown, granular colonies. The standard biochemical typing was performed as previously described (Chen et al., 2009). Further genotyping of the isolates was conducted with multiplex polymerase chain reaction (PCR), and Spoligotyping by using genomic DNA. For multiplex PCR, seven sets of primers were specific to 7 genes whose names in *M. tuberculosis* H37Rv genome (GenBank NC_000962) were listed as follows: 16S rRNA, RV0577, IS1561, Rv1510, Rv1970, Rv3877/8 and Rv3120. The PCR differentiates the various sub-species of MTBC according to development of the amplified products to different sets of primers as follows: *M. tuberculosis* 1234567, *M. bovis* 1236, BCG 123, *M. africanum* subtype I 12367, *M. africanum* subtype II 123467, *M. microti* 12467, *M. caprae* 12346, and MOTT (*M. avium* subsp. *avium*) 1 (Huard et al., 2003). After determination of the MTBC subspecies, the genotyping of the isolates with standard Spoligotyping over 43 spacers was further conducted with a commercial kit (Isogen Bioscience BV, Maarssen, The Netherlands). It can determine MTBC species such as *M. tuberculosis* and *M. bovis*, and differentiate *M. tuberculosis* lineages, especially Beijing family. The amplification of the spacers with the primers specific to direct repeated sequences, hybridization with the oligonucleotide probes and signal development on the Spoligotyping membrane, were sequentially performed according to the manufacturer's protocol. The *M. tuberculosis* H37Rv and BCG Tokyo strain were used as the control. The typical Spoligotype pattern of *M. tuberculosis* Beijing family comprises only the last nine spacers among the 43 spacers. The details of these methods were previously described in our published paper (Chen et al., 2009). All the experiments dealing with materials with potential live mycobacteria were performed in the facility of bio-safety level 3 at Huazhong Agricultural University.

RESULTS

The three research groups took samples independently from the Northeast, Northwest and South areas of China, and isolated MTBC according to the unified methods. A total of 11580 samples yielded 345 mycobacterium strains. They consist 41.16% (142/345) of MTBC (genoty-

ping) and 58.84% (203/345) of non-tuberculous mycobacteria (NTM). Among the MTBC isolates, 45.77% (65/142) were *M. tuberculosis*, while the rest 54.23% (77/142) were *M. bovis*. Among the *M. tuberculosis* strains, 32.31% (21/65) showed the spoligotyping pattern of 0000-0000-0003-771, a typical genotyping of *M. tuberculosis* Beijing family. In addition, another 2 strains were defined as Beijing-like family strains because the Spoligotyping pattern (0000-0000-0002-771) was slightly different from the typical pattern of Beijing family. The rest 77 isolates (54.23%) were *M. bovis*. The MTBC isolation rates for different kinds of samples were compared. The lung lymph nodes from 59 TST positive and asymptomatic cows from one farm slaughtered in accordance with the legal requirements were subjected to mycobacterium isolation and MTBC genotyping. The results showed that MTBC isolation rate reached 33.89% (20/59). The multiple PCR and spoligotyping determined that this farm was infected by *M. bovis*. The other 490 lung lymph node samples from asymptomatic cows were randomly collected at the slaughter houses, and the MTBC culture obtained 9 *M. bovis* isolates with the isolation rate of 1.84% (9/490).

A total of 8197 nasal swabs were cultured for mycobacterium isolation, and the general isolation rate of MTBC (*M. tuberculosis* and *M. bovis*) was 1.07%. When only the TST positive cattle was considered, the isolation rate was 2.81%, 2.6 folds higher than the general isolation rate. Furthermore, when the pharyngeal swabs from the TST positive cattle were concerned, the isolation rate of MTBC was 3.95% (43/1089), which were 1.40 folds higher than that of the nasal swabs. The rest 1474 pharyngeal swabs were randomly collected with unknown TST background, and the MTBC isolation rate was 1.63% (24/1474), higher than that of nasal swabs. Therefore, although it is easier to take nasal samples than pharyngeal swabs, it would be possible to underestimate the MTBC shedding status. However, no MTBC strains were isolated from 251 milk samples and 20 tracheal swabs from TST positive cattle.

DISCUSSION

“Test-slaughter” is a common strategy all over the world to control and eradicate bovine tuberculosis. Since *M. bovis* can infect a wide range of domestic and wild animals, a potential reservoir of *M. bovis* for domestic animals, it is difficult to eradicate this disease. Up to date, only few developed countries are free of bovine tuberculosis even after implementation of “test-slaughter” policy for a long time (Everett, 2006; Thoen et al., 2010). It is much more difficult to enforce this policy in developing countries due to the following reasons. First, it is a very expensive method which puts a heavy financial burden on stakeholders' shoulders. Second, the farmers do not like to cull the positive animals because they are gene-

rally asymptomatic due to the persistence of *M. bovis* (Michel et al., 2010), and the government compensation is usually far less than the price of the cattle. Since the dairy industry is rapidly developing in recent decades in China (Fuller et al., 2006), the threat of bovine tuberculosis caused by *M. bovis* to humans has increased. Apparently, the fact revealed by this study makes the situation from bad to worse. In accordance to our previous investigation, the *M. tuberculosis* strains are not uncommon in cattle population (Chen et al., 2009; Du et al., 2011). Compared to the previous reports, this study was conducted within much broader areas, which included 17 provinces, and covered half of the country. Among the *M. tuberculosis* strains, the dominant species was Beijing family. This is in agreement with the fact that *M. tuberculosis* Beijing family causes about 80% of the TB cases in China (Pang et al., 2012). Furthermore, this genotype was more virulent than other lineages of *M. tuberculosis* (Krishnan et al., 2011). Therefore, this evidence would be of significance to improve the current strategy of bovine tuberculosis control in China and other developing countries. In addition, aerosol transmission would be a main route of transmission because the MTBC strains were solely isolated from respiratory samples such as lung lymph nodes, nasal and pharyngeal swabs in this study. Taken altogether, these findings demonstrated that *M. tuberculosis* infection in cattle exists not as a low probability extreme event, but as a new risk to public health and should be paid more attention.

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