Veterinary Microbiology 176 (2015) 274–281

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Extent of *Mycobacterium bovis* transmission among animals of dairy and beef cattle and deer farms in South Korea determined by variable-number tandem repeats typing

Sungmo Je [a], Bok Kyung Ku [b], Bo-Young Jeon [a], Jae-Myoung Kim [b], Suk-Chan Jung [b], Sang-Nae Cho [a,*]

[a] Department of Microbiology and Institute for Immunology and Immunological Diseases, Brain Korea 21 Plus Project for the Medical Sciences, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea
[b] Bacteriology and Parasitology Division, Animal and Plant Quarantine Agency, Anyang 430-757, Gyeonggi-do, Republic of Korea

ARTICLE INFO

Article history:
Received 11 February 2014
Received in revised form 28 December 2014
Accepted 5 January 2015

Keywords:
Mycobacterium bovis
Bovine tuberculosis
VNTR typing
Korea

ABSTRACT

Identifying sources of *Mycobacterium bovis* transmission would be essential for establishing effective control programs of bovine tuberculosis (TB), a major zoonosis threatening human health worldwide. As an effort to determine the extent of *M. bovis* transmission among dairy and beef cattle and deer populations, a mycobacterial interspersed repetitive units (MIRU)-variable-number tandem repeats (VNTR) typing method was employed for analysis of 131 *M. bovis* isolates from 59 Holstein dairy cattle, 39 Korean beef cattle, and 33 deer. Of 31 MIRU-VNTR markers, 15 showed allelic diversity. The most discriminatory locus for *M. bovis* isolates was VNTR 3336 ($h = 0.59$) followed by QUB 26, MIRU 31, VNTR 2401, and VNTR 3171 which showed high discriminatory power ($h = 0.43$). The combined VNTR loci had an allelic diversity of 0.83. On the basis of the VNTR profiles of 30 VNTR loci, 24 genotypes were identified, and two genotypes were highly prevalent among all *M. bovis* isolates (33.6% and 19.1%, respectively), thus indicating that more than 50% of the isolates shared common molecular characteristics. Six additional genotypes were common in 2 of the 3 animal species, suggesting a wide interspecies transmission of *M. bovis*. This study thus demonstrates that MIRU-VNTR typing is useful in differentiation of *M. bovis* isolates and that *M. bovis* transmission occurs frequently among farmed animal species, highlighting the importance of bovine TB control programs in different animal species which are often raised in the same villages.

1. Introduction

Mycobacterium bovis is the causative agent of bovine tuberculosis (TB) which is an infectious and chronic wasting disease that causes significant problems for agricultural economies (Morris et al., 1994). *M. bovis* can infect cattle, deer, wildlife, as well as humans, and bovine TB is thus classified as a major zoonosis that threatens human public health worldwide. *M. bovis* is responsible for 5–10% of human TB in developing countries where unpasteurized milk is widely consumed in the absence of proper bovine TB control programs (Cosivi et al., 1998). In developed countries, the proportion of infection caused by *M. bovis* in human TB cases is very low, ranging from 0.3% to 1.5%. Recently, however, *M. bovis* infection in humans increased due to spread of human immunodeficiency virus infection not only in developing countries but also in developed countries (Grange, 2001).

* Corresponding author. Tel.: +822 2228 1819; fax: +822 392 7088.
E-mail addresses: raycho@yuhs.ac, raycho@yonsei.ac.kr (S.-N. Cho).

http://dx.doi.org/10.1016/j.vetmic.2015.01.002
0378-1135/

For establishment of effective bovine TB control programs, identifying sources of *M. bovis* infection is of great value. In the countries where *M. bovis* infection rate is low among dairy cattle, wild animals such as possum, badger, and deer are the major sources of its transmission (Mathews et al., 2006; Tweddle and Livingstone, 1994). Therefore, *M. bovis* control programs include an eradication of *M. bovis* infection in such wild animals either by segregation, vaccination or killing the animals. In other countries including South Korea, however, little is known about reservoirs other than dairy cattle, so bovine TB control programs have been focused only on dairy cattle leaving farmed beef cattle and farmed deer untouched despite many of their farms are located in the same villages.

In South Korea, deer had been imported since 1986, and there are approximately 50,000 farmed deer throughout the country which consist mainly of reindeer, elk and red deer. There are little wild deer in the country, so that contact between wild and farmed deer would rarely occur. Since deer comprise a small portion of domestic animals in South Korea, *M. bovis*-infected deer were not considered a significant source of bovine TB infection to dairy cattle, and annual bovine TB screening test has not been performed on deer until recent random screening tests in the 2010s.

Bovine TB control programs include test and culling of *M. bovis*-infected animals or depopulation of entire animals in the *M. bovis*-infected herds. Due to economic reasons, however, only partial culling of *M. bovis*-infected animals has been more widely adopted leaving some infected animals in the affected herds. In addition, bovine TB control programs are often implemented only to dairy cattle leaving other farmed animals such as beef cattle and deer uncontrolled despite their farms are located in the same villages in certain countries including South Korea. If there are *M. bovis*-infected beef cattle or deer in the villages where dairy cattle farms are located, those animals will be easily sources of *M. bovis* transmission to the dairy cattle. However, there has been no evidence yet of *M. bovis* transmission among dairy cattle, beef cattle, and deer in South Korea.

Recently, molecular typing methods have been developed and employed to compare relatedness of various pathogens including *M. bovis*. Among various molecular typing methods, variable-number tandem repeats (VNTR) typing based on the copy numbers of various repeating units in the genome including mycobacterial interspersed repetitive units (MIRU), exact tandem repeats (ETR), and Queen's University Belfast (QUB), gave most discriminatory power for *M. bovis* (Haddad et al., 2004). In addition, the MIRU-VNTR method which employs a polymerase chain reaction (PCR) has advantages of simplicity and digitalization of the results (Haddad et al., 2004). Since the repeating units are highly stable in the genome of *M. bovis*, the VNTR typing method also gave reproducible results (Allix et al., 2006). In this study, therefore, MIRU-VNTR typing method was employed to compare *M. bovis* isolates from Holstein dairy cattle, Korean beef cattle, and deer to determine the extent of *M. bovis* transmission among those animals in South Korea.

2. Materials and methods

2.1. M. bovis isolates

A total of 131 *M. bovis* isolates which were cultured from 59 Holstein dairy cattle, 39 Korean beef cattle (*Hanwoo*), and 33 deer (elk and reindeer) showing TB-like lesions at the Veterinary Service Laboratory and Quarantine Agency, Anyang, were included in this study. Tuberculous specimens were obtained from the animals showing positive tuberculin test with induration of $\geq$5 mm or clinical signs of TB in the farms of Gyeonggi, Chungcheong, and Jeolla provinces, South Korea. These *M. bovis* isolates were collected consecutively during the period of seven years from 2003 to 2010. The isolates from Holstein dairy cattle ($n = 59$) were collected from 2003 to 2006, and the isolates from Korean beef cattle ($n = 39$) and deer ($n = 33$) were collected from 2007 to 2010.

Hilar lymph node samples from animals suspected positive for bovine TB were collected, homogenized in sterile saline solution, and decontaminated with 2% NaOH for 15 min at room temperature. After centrifugation at 4,000 rpm for 15 min, the pellet was incoculated on Lowenstein–Jensen (LJ) media (Difco, Detroit, MI, USA) containing 0.05% pyruvate and incubated for 6 to 8 weeks at 37 °C. *M. tuberculosis* H37Rv was used as a reference strain whose the genomic sequence information is available.

2.2. DNA preparation

Genomic DNA was extracted from *M. bovis* isolates as described below (Roring et al., 2002). In brief, *M. bovis* isolates obtained from LJ media were grown in Middlebrook 7H9 liquid medium (Difco, Detroit, MI, USA) supplemented with oleic acid-albumin-dextrose-catalase and Tween 80 for 3 to 4 weeks at 37 °C. Cultures were collected by micro-centrifugation at 12,000 rpm for 10 min and re-suspended in 250 μL of sterile distilled water. The suspended bacteria were boiled in a water bath for 5 min, and supernatants were collected after removing the cellular debris by centrifugation. DNA concentration was measured at 260 nm with a spectrophotometer (Pharmacia Biotech, Piscataway, NY, USA) and stored at −20 °C until use in PCR reactions.

2.3. MIRU-VNTR analysis

MIRU-VNTR analysis was performed by PCR amplification of 12 MIRUs, 3 ETRs (A to C), 4 QUBs (11a, 15, 18, and 26), and 11 VNTR loci (0424, 1895, 1955, 2347, 2401, 2990, 3171, 3232, 3336, 3690, and 4156) with previously reported primer pairs (Supplementary Table 1) (Frothingham, 1998; Le Flèche et al., 2002; Skuce et al., 2002; Cowan et al., 2002; Supply et al., 2006). Smart *Taq* Pre-Mix (Solgent, Daejeon, South Korea) was used for PCR amplification, which was performed with a 20-μL PCR pre-mixture containing each primer set (forward and reverse) at a final concentration of 0.5 μM, and an amount of thermolyate sample corresponding to an absorbance of 20 ng of DNA as a template. PCR reaction was carried out by