CHARACTERISATION OF MYCOBACTERIUM BOVIS STRAINS ISOLATED FROM FARM AND WILD ANIMALS IN POLAND

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Abstract

Sixty-one isolates of M. bovis (58 from cattle and three from wild animals) from eight regions of Poland were analysed. Molecular analysis was done using HAIN and spoligotyping methods. Drug susceptibility of the isolates to streptomycin, isoniazid, rifampicin, ethambutol, and pyrazinamide was tested by proportional methods on solid and liquid media. By spoligotyping, 47 (77%) isolates were identified as M. bovis subsp. bovis and 14 (23%) isolates were identified as M. bovis subsp. caprae. Eleven animals of the domestic cattle (18%) and all wild animals were infected by M. bovis subsp. caprae. Among cattle infected by M. bovis, 12 spoligotypes were identified, most of them not registered in the SpolDB4 database. The strains isolated from 15 animals of the domestic cattle were the same spoligo pattern. In conclusion, transmission of mycobacteria among the farm and wild animals has been suspected.

Key words: cattle, tapir, bison, M. bovis, spoligotyping, genotyping test, Poland.

Mycobacterium bovis has one of the broadest host range of all known pathogens belonging to Mycobacterium tuberculosis complex (M. tbc.complex). Susceptible species include cattle, humans, non-human primates, goats, cats, dogs, pigs, buffaloes, badgers, possums, deer, seals, walruses, elephants, and others (7, 10). Each of M. tbc. complex subspecies is known to infect humans (9). Members of the M. tbc. complex are highly related mycobacteria exhibiting remarkable nucleotide sequence level homogeneity despite varied pathogenicity, geographic range, and epidemiology (5).

Many studies conducted in European countries and outside Europe indicate a growing share of tuberculosis caused by Mycobacterium bovis in humans and animals (15). Bovine tuberculosis occurs in almost all developed and developing countries of the world (6, 13). The studies of many authors show that the disease can be transmitted via respiratory and alimentary route (e.g. by infected milk) between affected people and animals in both directions (4, 11). The transmission of tuberculosis causes significant personal losses in many countries. On the other hand, euthanasia of sick animals causes great financial losses that are covered from the state budget. Evaluation of this phenomenon in Poland could help with the implementation of the tuberculosis prevention system.

This paper describes for the first time in Poland the precise identification of Mycobacterium bovis strains (two subspecies bovis and caprae ) isolated from cattle and wild animals in Poland in 2008-2009, using microbiological and modern molecular techniques.

Material and Methods

Material for the research consisted of 61 strains of mycobacteria; 58 strains from domestic cattle and three from wild animals: two from tapir anta (Tapirus terrestris) and one from bison (Bison bonasus caucasicus). The clinical samples for diagnosis were from eight Polish provinces (Fig. 1). The mycobacteria were isolated from the lymph nodes, lungs and pleura, liver, spleen, and visceral peritoneum.

Culture and identification. Tissue samples after decontamination procedure were cultivated on Loewenstein-Jensen slants containing pyruvate. Isolates were identified on the basis of their macroscopic features and standard biochemical profile (2), and with the use of BACTEC 460TB (Becton Dickinson, USA). M. tbc. complex was differentiated from other mycobacteria by NAP TB differentiation test (3). Geno Type MTBC (HAIN Lifescience GmbH, Germany) was used to differentiate M. tbc. complex species on the basis of the polymorphism of gene coding gyrase B (12).

Drug susceptibility testing (DST) to streptomycin (SM), isoniazid (INH), rifampicin (RMP), ethambutol (EMB), and pyrazinamide (PZA) was performed using the
proportion method on Loewenstein-Jensen medium and/or the modified proportion method in BACTEC 460TB (Becton Dickinson, USA), according to the manufacturer's instructions (3).

DNA extraction. DNA extracts from mycobacterial cells for spoligotyping were prepared by suspending approximately 10 mg of wet bacterial cells in 100 μl of sterile distilled water (Sigma, Germany) and subsequently heated at 100°C for 30 min to inactivate and lyse the cells. Cell debris was removed by centrifugation at 13,000 × g for 2 min. The lysates were stored at −20°C until use (14).

Spoligotyping. Spoligotyping was performed as described previously by Kamerbeek et al. (8). Clusters of isolates were defined as two or more M. bovis strains with identical spoligotypes. M. tuberculosis H37Rv (ATCC 27294) and M. bovis BCG (ATCC 27289) were included as reference strains in each spoligotype experiment.

Results and Discussion

M. bovis was the only mycobacterial species found in tissues collected from the examined cattle and wild animals. All strains tested in NAP C14 test were identified as M. tbc complex with niacin negative results. The hybridisation test (HAIN method) and spoligotyping revealed 47 (77%) strains of M. bovis subsp. bovis and 14 (23%) strains of Mycobacterium bovis subsp. caprae. Among 58 animals of the domestic cattle, 47 (77%) were infected with M. bovis subsp. bovis and 11 (18%) with M. bovis subsp. caprae. Three wild animals were infected by M. bovis subsp. caprae (Table 2). All strains were susceptible to first-line antituberculous drugs: SM, INH, RMP, and EMB (SIRE). All M. bovis subsp. bovis strains were resistant to PZA, and all M. bovis subsp. caprae strains were sensitive to PZA.

Fig. 1. M. bovis in selected provinces in Poland.
Twelve spoligotypes were identified in strains isolated from the cattle, most of the molecular patterns were not registered in SpolDB4 database. The strains isolated from 15 animals of the domestic cattle showed the same spoligo pattern. All strains were grouped in five clusters, seven strains were orphans. Out of 14 strains of M. bovis subsp. caprae, four clusters have been identified, most of them isolated from eight farm animals and two wild animals living in two different regions (IV, II) (Fig. 1, Tables 1, 2). All 47 M. bovis subsp. bovis isolates had spoligotype patterns, which showed the typical absence of spacers 39 to 43 (Fig. 2). Eight isolates (17%) did not have spacers 17 to 38. All the strains had no spacers in positions 9-10 and 14-16. All 14 M. bovis subsp. caprae isolates had spoligotype patterns with absence of spacer in positions 1, 3-16, and 39-43 (Fig. 2). Ten (71.4%) isolates had no spacers at position 28, two (14.2%) isolates at positions 25 to 34, and one (7.1%) isolate had no spacers at positions 28 and 38.

Geographical distribution of spoligotypes showed the same pattern among 19 cattle living in the region VIII (Fig. 1). Next cluster represented by strains isolated from 15 animals of the domestic cattle living in different regions (region IV - six strains, region VII - three strains, region V - two strains, region VIII - two strains, region III - one strain, region VI - one strain) (Fig. 1). Other strains of M. bovis subsp. bovis represented three clusters (by two strains each), seven strains had an orphan pattern (Table 1).

Molecular methods in diagnosis of tuberculosis have contributed significantly to a better understanding of the dynamics of the mycobacteria transmission and became an essential tool in tuberculosis epidemiology. There is a considerable public health significance of M. bovis infection in humans and animals and the disease has emerged as a major zoonotic problem in many countries (16).

The presented study describes for the first time in Poland the outbreak of tuberculosis in animals caused by two subspp. of M. bovis – bovis and caprae, and on the basis of the defined spoligotypes indicates the probability of their interspecies transmission (1). The study confirms the occurrence of M. caprae among infected bovines in Poland.

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>Clinical material</th>
<th>GenoType MTBC</th>
<th>Spoligotype</th>
<th>Molecular family by SpolDB4 database</th>
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</thead>
<tbody>
<tr>
<td>19</td>
<td>lymph nodes</td>
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<td>676727777777600</td>
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</tr>
<tr>
<td>15</td>
<td>lymph nodes (lung, pleura, peritoneum)</td>
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<td>bov</td>
</tr>
<tr>
<td>2</td>
<td>lymph nodes</td>
<td>M. bovis ssp. bovis</td>
<td>676743777777600</td>
<td>not registered</td>
</tr>
<tr>
<td>2</td>
<td>lymph nodes</td>
<td>M. bovis ssp. bovis</td>
<td>676743777777600</td>
<td>not registered</td>
</tr>
<tr>
<td>1</td>
<td>lymph nodes</td>
<td>M. bovis ssp. bovis</td>
<td>676743777777600</td>
<td>not registered</td>
</tr>
<tr>
<td>1</td>
<td>lymph nodes</td>
<td>M. bovis ssp. bovis</td>
<td>676743777777600</td>
<td>not registered</td>
</tr>
<tr>
<td>1</td>
<td>lymph nodes</td>
<td>M. bovis ssp. bovis</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>lymph nodes</td>
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<td>676743777777600</td>
<td>BOVIS1</td>
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</table>

Explanations: bov, BOVIS1, CAP – molecular family by SpolDB4 database.

<table>
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<th>Number of isolates</th>
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<th>GenoType MTBC</th>
<th>Spoligotype</th>
<th>Molecular family by SpolDB4 database</th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td>lymph nodes</td>
<td>M. bovis ssp. caprae</td>
<td>200003777777600</td>
<td>CAP</td>
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<tr>
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<td>M. bovis ssp. caprae</td>
<td>200003777777600</td>
<td>CAP</td>
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</table>


<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>Clinical material</th>
<th>GenoType MTBC</th>
<th>Spoligotype</th>
<th>Molecular family by SpolDB4 database</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>lymph nodes, lung</td>
<td>M. bovis ssp. caprae</td>
<td>200003777777600</td>
<td>CAP</td>
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<tr>
<td>1</td>
<td>lymph nodes, lung, spleen, liver</td>
<td>M. bovis ssp. caprae</td>
<td>200003777777400</td>
<td>not registered</td>
</tr>
</tbody>
</table>


Table 1
Identification of M. bovis isolates from cattle

Table 2
Identification of M. bovis isolates from wild animals
M. bovis subsp. bovis

Fig. 2. Analysis of spacers of M. bovis subsp. bovis strains and M. bovis subsp. caprae.

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References


