NOTE


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We propose to replace the species designation *Mycobacterium tuberculosis* subsp. *caprae* (Aranaz et al. 1999) by *Mycobacterium bovis* subsp. *caprae* comb. nov., since isolates of this subspecies share their main growth, biochemical and genetic characteristics with *M. bovis* and not with *M. tuberculosis*. These include negative biochemical test results for niacin accumulation and nitrate reduction as well as genetic features like the presence of an *M. bovis*-specific mutation in the *oxyR* locus, absence of the *mtp40* sequence and a specific mutation in the *gyrB* gene, all of which have been described as characteristics for the differentiation of *M. bovis*. The only obvious biochemical character that differentiates the *caprae* subtype from other *M. bovis* isolates is susceptibility to pyrazinamide (PZA), which is due to the lack of a single point mutation in the *pncA* gene. However, susceptibility to PZA among clinical isolates of *M. bovis* isolates has been reported previously and, thus, may now been explained by a PZA-susceptible subspecies of *M. bovis*. We conclude that the species designation *M. tuberculosis* subsp. *caprae* is misleading and not correct in light of the biochemical and genetic characteristics and propose that the accurate designation of isolates of this subtype is *M. bovis* subsp. *caprae*.

**Keywords:** *Mycobacterium tuberculosis* complex, *Mycobacterium bovis*, *M. tuberculosis*, subsp. *caprae*

*Mycobacterium bovis* is a member of the *Mycobacterium tuberculosis* complex (MTBC), which is composed of the closely related species *M. tuberculosis*, *M. bovis*, *Mycobacterium africanum* and *Mycobacterium microti* (Wayne & Kubica, 1986). The close relationship has been confirmed by DNA–DNA hybridization, multilocus enzyme electrophoresis and sequencing of the 16S rDNA and the 16S-to-23S rDNA internal transcribed spacer (Feizabadi et al., 1996; Frothingham et al., 1994; Kirschner et al., 1993; Niemann et al., 2000a; Wayne & Kubica, 1986). However, the members of the MTBC differ in their host range and pathogenicity (Wayne & Kubica, 1986), as *M. bovis* can cause disease in a wide range of domestic or wild animals like cattle or goats as well as in humans, whereas *M. tuberculosis* is the major cause of human tuberculosis (Wayne & Kubica, 1986). Hence, the accurate species differentiation of clinical isolates is necessary for epidemiological and public health purposes.

Routine identification of MTBC isolates can be performed easily by using commercially available gene probes (Shinnick & Good, 1994), whereas differentiation of *M. tuberculosis* and *M. bovis* is generally carried out by a number of biochemical tests (Wayne & Kubica, 1986). For example, *M. bovis* shows dysgonic growth and is negative for nitrate reduction and niacin accumulation, whereas *M. tuberculosis* is easily iden-
Table 1. Biochemical and genetic characteristics of *M. tuberculosis*, *M. bovis* and the caprine genotype isolates

Data are summarized from our previous studies (Niemann *et al.*, 2000a, b).

<table>
<thead>
<tr>
<th>Character</th>
<th><em>M. tuberculosis</em></th>
<th><em>M. bovis</em></th>
<th>Caprine genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony morphology</td>
<td>Eugonic</td>
<td>Dysgonic</td>
<td>Dysgonic</td>
</tr>
<tr>
<td>Niacin accumulation</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PZA sensitivity*</td>
<td>s</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Change of colour of bromcresol medium</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth on Lebek medium</td>
<td>Aerophilic</td>
<td>Microaerophilic</td>
<td>Microaerophilic</td>
</tr>
<tr>
<td>Growth in presence of 1 µg TCH ml⁻¹</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Presence of <em>mtb</em>40</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Presence of <em>oxy</em>R mutation (G → A, position 285)</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Presence of <em>gyr</em>B mutation (G → A, position 756)</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Presence of <em>pnc</em>A mutation (C → G, position 169)</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Spoligotype (characteristic features)</td>
<td>At least one of spacers 39–43 present</td>
<td>Spacers 39–43 absent</td>
<td>Spacers 39–43 and 3–16 absent</td>
</tr>
</tbody>
</table>

*Testing was performed in BACTEC 460TB (Becton Dickinson Microbiology Systems) using 100 µg PZA ml⁻¹. s, Susceptible; r, resistant.

Fig. 1. Spoligotype patterns of 10 randomly chosen clinical isolates of each of *M. bovis* (PZA-resistant), the caprine genotype (PZA-susceptible) and *M. tuberculosis*. *M. bovis* and the caprine genotype can be separated from *M. tuberculosis* by the lack of spacers 39–43. The caprine genotype additionally lacks spacers 3–16. Spoligotyping, which is based on the detection of various non-repetitive spacer sequences located between small repetitive units (direct repeats) in the DR locus of MTBC strains by a reverse line-blotting technique, was performed as described previously (Kamerbeek *et al.*, 1997) by using a commercially available membrane (Isogen).

tified by its special colony morphology (eugonic) and by positive test results for nitrate reduction and niacin accumulation (Wayne & Kubica, 1986) (Table 1). As a further criterion for the differentiation of *M. bovis*, intrinsic resistance to pyrazinamide (PZA) has been described (Wayne & Kubica, 1986). However, some studies also report susceptibility to PZA among *M. bovis* isolates (Collins *et al.*, 1981; Wayne *et al.*, 1991) and we have noticed in recent years in our laboratory that a proportion of at least 5% *M. bovis* strains are susceptible to PZA.

Recently, Aranaz *et al.* (1999) proposed a new subspecies of *M. tuberculosis*, *M. tuberculosis* subsp. caprae (caprine genotype), that differed from *M. bovis* in susceptibility to PZA and resistance to 1 and 2 µg thiophen-2-carboxylic acid hydrazide (TCH) ml⁻¹ (susceptible to 5 and 10 µg TCH ml⁻¹). On a molecular
level, isolates of this subtype can be identified easily by a characteristic spoligotype pattern (lack of spoligotype spacers 3–16). Previously, MTBC isolates showed a characteristic spoligotype pattern (lack of spoligotype spacers 39–43, all characters described for the main test criteria (except PZA resistance) for $M$. tuberculosis. Hence, there is no evidence for their denomination as $M$. tuberculosis subsp. caprae and so doing would be misleading for both epidemiological and routine purposes. Considering the data presented, we propose to transfer $M$. tuberculosis subsp. caprae to the species $M$. bovis, and consequently to differentiate these two subspecies, $M$. bovis subsp. bovis subsp. nov., showing resistance to PZA, and $M$. bovis subsp. caprae comb. nov., being sensitive to PZA.

In our work, we analysed a randomly chosen collection of clinical $M$. bovis isolates with respect to their biochemical and genetic features. We found that susceptibility to PZA in clinical $M$. bovis isolates from Germany is linked with a $pncA$ wild-type sequence, the caprine genotype characteristic spoligotype pattern (Fig. 1) and a specific single-nucleotide polymorphism in the $gyrB$ gene (Niemann et al., 2000a, b). With respect to other biochemical characters analysed, the isolates completely fulfilled the criteria for identification as $M$. bovis, as they were negative for niacin accumulation and nitrate reduction, showed no change of colour of bromcresol medium and showed dysgonic growth and microaerophilic growth on Lebek medium, hence, clearly discriminating them from $M$. tuberculosis.

In our study, we have confirmed the clinical importance of the caprine genotype of $M$. bovis in humans and further demonstrated that strains of this subtype can also cause disease in cattle (Niemann et al., 2000a). Hence, the predominant isolation from sheep and goats might be specific for Spain.

In conclusion, isolates of the caprine genotype fulfil all the main test criteria (except PZA resistance) for $M$. bovis, but not for $M$. tuberculosis. Hence, there is no evidence for their denomination as $M$. tuberculosis subsp. caprae and so doing would be misleading for both epidemiological and routine purposes. Considering all the data presented, we propose to transfer $M$. tuberculosis subsp. caprae to the species $M$. bovis, and consequently to differentiate these two subspecies, $M$. bovis subsp. bovis subsp. nov., showing resistance to PZA, and $M$. bovis subsp. caprae comb. nov., being sensitive to PZA.

**Description of Mycobacterium bovis subsp. caprae comb. nov.**

_Basonym_: Mycobacterium tuberculosis subsp. caprae (Aranaz et al., 1999).

The description is the same as that given for _Mycobacterium bovis_ by Wayne & Kubica (1986) with the addition that isolates are susceptible to PZA.

**References**


