**Short Communication**

# Transmission of tuberculosis caused by *Mycobacterium caprae* between dairy sheep and goats

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**Abstract**

Increasing number of caprine tuberculosis (TB) reports grant the consideration of this zoonosis as an emerging disease that can play a role in the epidemiology of bovine TB in endemic areas. An outbreak of TB was detected in a caprine/ovine dairy mixed herd. Tuberculin skin test positive goats and ewes were euthanized and subsequent postmortem investigations were performed. *Mycobacterium caprae* (spoligotype profile SB0157) was isolated from tuberculous lesions detected in both sheep and goats. Our findings evidenced the direct transmission of the infection between both species elucidating that not only goats but also sheep may act as domestic reservoirs of TB compromising the eradication of TB in cattle. The results have implications for animal TB epidemiology and public health risk management.

**Keywords:** Tuberculosis, Goats, Sheep, Domestic reservoir, *Mycobacterium caprae*, Diagnosis.

## 1. Introduction

Emergence of caprine TB, caused either by *Mycobacterium bovis* or *Mycobacterium caprae*, has been recently highlighted in a number of reported cases in European countries that conduct ongoing bovine TB eradication campaigns such as Portugal, UK, Spain, Ireland or Italy (Cunha et al., 2011; Daniel et al., 2009; Rodriguez-Campos et al., 2012; Shanahan et al., 2011; Zanardi et al., 2013). Goats are very susceptible to TB infection (Pérez de Val et al., 2013, 2011) and caprine TB causes economic losses in endemic areas (Daniel et al., 2009; Seva et al., 2002). In addition, infected goats in contact with cattle can act as domestic reservoirs of bovine TB (Guta et al., 2014; Napp et al., 2013; Zanardi et al., 2013).

On the other hand, sheep have largely been considered as less susceptible to TB than other ruminant species such as cattle or goats (Caswell and Williams, 2016). Indeed, only a few cases of ovine TB have been reported in the Iberian Peninsula (Aranaz et al., 1996; Cunha et al., 2011; Gutierrez et al., 1997; Muñoz Mendoza et al., 2012). Recent studies conducted in sheep cohabiting with infected cattle or/and wildlife indicate that they were related with bovine TB outbreaks in certain epidemiological situations (Broughan et al., 2013; Malone et al., 2003; Muñoz-Mendoza et al., 2016; Pesciaroli et al., 2014; van der Burgt et al., 2013).

Even though caprine and ovine TB still remain absent from the world organization for animal health (OIE) noticeable disease list, EU regulations enforce national TB control plans for milking non-bovine species (Regulation 853/2004 of the European Council).

The present work was aimed to investigate to the transmission of the infection between sheep and goats from a mixed infected herd.

## 2. Materials and Methods

### Study herd and tuberculin skin test

In May 2015, an outbreak of caprine and ovine TB in a mixed dairy herd of *Murciano-Granadina* goats (N = 170) and *Ripollesa* sheep (N = 340) was detected after testing goats and ewes with the single intradermal comparative cervical tuberculin (SICCT) test. Briefly, bovine and avian tuberculin batches (CZV, Porriño, Spain), previously verified by the Spanish Reference Laboratory for bovine TB (Santa Fe, Spain) in order to ensure a potency of ≥ 20,000 IU, were stored at 4-8 °C. Bovine and avian tuberculin were inoculated, using a Dermojet® syringe, on the right and the left side of the neck, respectively. The skin-fold thickness was recorded before and 72 ± 4 hours after the tuberculin injection. The severe criteria for results interpretation was used (Bezos et al., 2012). Animals were considered positive if the increase of skin-fold thickness at the bovine tuberculin inoculation site was ≥ 1 mm and thicker than the increase at the avian tuberculin inoculation site, or if the presence of clinical signs at the injection site of the bovine tuberculin were observed.

**Post-mortem examination**

All SICCT test-positive animals were slaughtered and gross pathology examination was conducted to confirm the disease in 11 goats and 3 ewes.  At necropsy, the pulmonary lymph nodes (mediastinal and tracheobronchial) of all animals, as well as other tissues with macroscopic TB-like lesions, were collected for bacteriological culture. In addition, a section of tissues with gross lesions was fixed with 10%-buffered formalin for histological examination. Sections of 4 µm were stained with haematoxylin and eosin and were examined using light microscopy. Ziehl-Neelsen staining was also performed to support the presumptive diagnosis of the lesions (Table 1).

**Bacteriology**

Once having the histopathological presumptive diagnosis, the harvested fresh samples were processed for bacteriological culture accordingly. First of all, tissues were homogenized in 10 ml sterile water. The homogenates were decontaminated for 30 min. with a final concentration of 0.35 % w/v hexadecylpyridinium chloride. Afterwards, the decontaminated homogenates were centrifuged at 2471 × g for 30 min. Supernatants were discarded and pellets were cultured in selective media using swabs. TB-suspected samples were cultured in Löwenstein-Jensen (LJ) with pyruvate and Coletsos media (BD Difco™, Sparks, MD, USA). All cultures were incubated at 37° C. Cultures were read every week up to 3 months. Colonies were primarily identified as MTBC by PCR (Wilton and Cousins, 1992). Then, *M. tuberculosis complex* isolates were identified by DVR-spoligotyping (Kamerbeek et al., 1997).

## 3. Results

Seventeen out of 170 goats (10.0 %) and 11 out of 340 ewes (3.2 %) were positive to SICCT test.

Necropsy was conducted in 11 and 3 SICCT test positive goats and ewes, respectively. Seven goats and 2 ewes showed caseous-necrozinting TB-like lesions (Table 1). All of them showed gross lesions in the thoracic cavity (lungs and/or pulmonary lymph nodes, Fig 1A), that were subsequently confirmed as TB-like by histopathology (Fig. 1C). One of the ewes also showed extra-pulmonary TB-like lesions in mesenteric lymph nodes (Fig. 1B) and spleen (Fig. 1D) in addition to extensive pulmonary tuberculosis.

Positive cultures were obtained from TB granulomatous lesions of all animals with gross lesions. All isolates were confirmed as MTBC by multiplex PCR and in all cases the spoligotype profile *M. caprae SB0157* (Mbovis.org) was obtained by DVR-spoligotyping (Table 1).

## 4. Discussion

Even though relationships between MTBC strains isolated from sheep, goats and cattle were previously suggested in other Spanish regions using an spatiotemporal epidemiological approach (Muñoz-Mendoza et al., 2016; Rodriguez-Campos et al., 2012), to our knowledge, we report herein the first evidence of TB direct transmission between dairy sheep and goats in a mixed flock.

Since experimental infections have elucidated that goats are highly susceptible to MTBC infection (Pérez de Val et al., 2011), rapid detection of caprine TB outbreaks is crucial to eradicate the infection in positive herds and to prevent the spread of the disease to cattle herds and wildlife. Indeed, infected goats in contact with cattle can act as domestic reservoirs of bovine TB (Napp et al., 2013; Zanardi et al., 2013). Recently, an experimental infection of lambs with *M. caprae* (Balseiro et al., 2017) showed that the progress of the infection (monitored by clinical signs and immune responses), as well as postmortem findings (pathological extension and bacterial load) were similar to those previously found in experimentally infected goats (Pérez de Val et al., 2011).

Furthermore, recent studies in sheep cohabiting with infected cattle indicate that they may be also involved in bovine TB outbreaks in certain epidemiological situations (Broughan et al., 2013; Malone et al., 2003; Muñoz-Mendoza et al., 2016) . Our results support the fact that sheep is a susceptible species to TB infection in an outbreak scenario and may also represent a potential TB domestic reservoir to goats. Therefore, sheep testing for TB status is strongly recommended when cohabiting with other TB-positive susceptible species such as cattle or goats. Also, *M. caprae* has already been reported to cause tuberculosis in human patients in Spain (Rodríguez et al., 2009). Thus, public health risks need to be considered, particularly since unpasteurized milk from goats and ewes may be consumed or used to manufacture dairy products.

Most caprine and ovine Spanish herds are not subjected to routine TB diagnostic tests. The present outbreak was detected because the farmer joined a voluntary program aimed to qualify the Catalan goat herds as TB-free. Thus there is no recorded TB historical data of the herd. No epidemiological relationship of this herd with infected cattle (such as shared pastures) was reported, and the contact with potential wildlife reservoirs was deemed minimal. Moreover, taking into account that Catalonia is a low-prevalence area of TB in cattle (0.32 % in 2015, according to the Spanish Ministry of Agriculture and Fisheries, Food and Environment data), the entry of an undiagnosed goat or sheep from a TB infected herd was established as the most likely source of infection.

Accordingly, a single *M. caprae* strain (SB0157) was confirmed in the investigated herd. To date, this spoligotype profile is the most frequent *M. caprae* isolate in Spain and the fourth most frequent spoligotype among all the MTBC isolated from animals in Catalonia, including cattle (mycoDB.es, MAPAMA-UCM, Spain).

Cross-reactions caused by other mycobacteria cannot be excluded in the four skin test positive reactors (3 goats and one ewe) that did not show TB gross lesions and were negative to bacteriological culture. However, a high specificity of the SICCT test has been previously reported for goats under different epidemiological situations (Bezos et al., 2012) and, in addition, there were no confirmed cases of paratuberculosis in the herd. Therefore, the most likely explanation is that these animals were still in an early stage of TB infection and, thus visible lesions had not yet developed.

Animal TB is a multi-host disease which requires a holistic approach. Infected sheep and goats may hinder bovine TB eradication programs. This paper highlights the critical role of a thorough laboratory diagnosis in both the management of multispecies TB outbreaks and the elucidation of their epidemiological relationships.

## Acknowledgments

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## FIGURES

**FIGURE 1**. **Tuberculosis lesions in a sheep. A:** Caseous-necrotizing lesions in the lungs (arrowheads point to the granulomas). **B:** Granulomatous lymphadenitis in mesenteric lymph nodes. **C:** Hematoxylin and eosin staining micrograph showing granulomatous pneumonia lesions in A: central mineralized necrotic core surrounded by epithelioid macrophages and a few multinucleated giant cells (arrowhead) and fibrosis in the outer layers. **D:** Spleen with TB granulomatous lesions. Hematoxylin and eosin stain. Necrotic area surrounded by macrophages and the occasional Langhans cell (arrowhead).

## TABLES

**TABLE 1. Pathological and bacteriological findings.**

| Animal | Species | Location of TB compatible lesions | ZN[a] | Culture | Spoligotype |
|---|---|---|---|---|---|
| **1** | Goat | Mediastinal and tracheobronchial LNs[b] | + | *M. caprae* | SB0157 |
| **2** | Goat | Lung | + | *M. caprae* | SB0157 |
| **3** | Goat | Mediastinal and tracheobronchial LNs | + | *M. caprae* | SB0157 |
| **4** | Goat | Tracheobronchial LN | + | *M. caprae* | SB0157 |
| **5** | Goat | - | - | - | N/D |
| **6** | Goat | Lung, Tracheobronchial LN | + | *M. caprae* | SB0157 |
| **7** | Goat | Lung, Tracheobronchial LN | + | *M. caprae* | SB0157 |
| **8** | Goat | Lung | + | *M. caprae* | SB0157 |
| **9** | Goat | - | - | - | N/D |
| **10** | Goat | - | - | - | N/D |
| **11** | Sheep | Lung, Mediastinal, tracheobronchial, mesenteric LNs, Spleen | + | *M. caprae* | SB0157 |
| **12** | Sheep | - | - | - | N/D |
| **13** | Sheep | Lung | + | *M. caprae* | SB0157 |

[a]ZN: Ziehl Neelsen stain; [b]LN: lymph node.

*Manuscript (latest version with changes highlighted)

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Furthermore, recent studies in sheep cohabiting with infected cattle indicate that they may be also involved in bovine TB outbreaks in certain epidemiological situations (Broughan et al., 2013; Malone et al., 2003; Muñoz-Mendoza et al., 2016) . Our results support the fact that sheep is a susceptible species to TB infection in an outbreak scenario and may also represent a potential TB domestic reservoir to goats. Therefore, sheep testing for TB status is strongly recommended when cohabiting with other TB-positive susceptible species such as cattle or goats. Also, *M. caprae* has already been reported to cause tuberculosis in human patients in Spain (Rodríguez et al., 2009). Thus, public health risks need to be considered, particularly since unpasteurized milk from goats and ewes may be consumed or used to manufacture dairy products.

Most caprine and ovine Spanish herds are not subjected to routine TB diagnostic tests. The present outbreak was detected because the farmer joined a voluntary program aimed to qualify the Catalan goat herds as TB-free. Thus there is no recorded TB historical data of the herd. No epidemiological relationship of this herd with infected cattle (such as shared pastures) was reported, and the contact with potential wildlife reservoirs was deemed minimal. Moreover, taking into account that Catalonia is a low-prevalence area of TB in cattle (0.32 % in 2015, according to the Spanish Ministry of Agriculture and Fisheries, Food and Environment data), the entry of an undiagnosed goat or sheep from a TB infected herd was established as the most likely source of infection.

Accordingly, ~~TB outbreak due to~~ a single *M. caprae* strain (SB0157) was confirmed in the investigated herd. To date, this spoligotype profile is the most frequent *M. caprae* isolate in Spain and the fourth most frequent spoligotype among all the MTBC isolated from animals in Catalonia, including cattle (mycoDB.es, MAPAMA~~GRAMA~~-UCM, Spain).

Cross-reactions caused by other mycobacteria cannot be excluded in the four skin test positive reactors (3 goats and one ewe) that did not show TB gross lesions and were negative to bacteriological culture. However, a high specificity of the SICCT test has been previously reported for goats under different epidemiological situations (Bezos et al., 2012) and, in addition, there were no confirmed cases of paratuberculosis in the herd. Therefore, the most likely explanation is that these animals were still in an early stage of TB infection and, thus visible lesions had not yet developed.

Animal TB is a multi-host disease which requires a holistic approach. Infected sheep and goats may hinder bovine TB eradication programs. This paper highlights the critical role of a thorough laboratory diagnosis in both the management of multispecies TB outbreaks and the elucidation of their epidemiological relationships.

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## FIGURES

**FIGURE 1**. **Tuberculosis lesions in a sheep. A:** Caseous-necrotizing lesions in the lungs (arrowheads point to the granulomas). **B:** Granulomatous lymphadenitis in mesenteric lymph nodes. **C:** Hematoxylin and eosin staining micrograph showing granulomatous pneumonia lesions in A: central mineralized necrotic core surrounded by epithelioid macrophages and a few multinucleated giant cells (arrowhead) and fibrosis in the outer layers. **D:** Spleen with TB granulomatous lesions. Hematoxylin and eosin stain. Necrotic area surrounded by macrophages and the occasional Langhans cell (arrowhead).

## TABLES

**TABLE 1. Pathological and bacteriological findings.**

| Animal | Species | Location of TB compatible lesions | ZN[a] | Culture | Spoligotype |
|---|---|---|---|---|---|
| **1** | Goat | Mediastinal and tracheobronchial LNs[b] | + | *M. caprae* | SB0157 |
| **2** | Goat | Lung | + | *M. caprae* | SB0157 |
| **3** | Goat | Mediastinal and tracheobronchial LNs | + | *M. caprae* | SB0157 |
| **4** | Goat | Tracheobronchial LN | + | *M. caprae* | SB0157 |
| **5** | Goat | - | - | - | N/D |
| **6** | Goat | Lung, Tracheobronchial LN | + | *M. caprae* | SB0157 |
| **7** | Goat | Lung, Tracheobronchial LN | + | *M. caprae* | SB0157 |
| **8** | Goat | Lung | + | *M. caprae* | SB0157 |
| **9** | Goat | - | - | - | N/D |
| **10** | Goat | - | - | - | N/D |
| **11** | Sheep | Lung, Mediastinal, tracheobronchial, mesenteric LNs, Spleen | + | *M. caprae* | SB0157 |
| **12** | Sheep | - | - | - | N/D |
| **13** | Sheep | Lung | + | *M. caprae* | SB0157 |

[a]ZN: Ziehl Neelsen stain; [b]LN: lymph node.

Figure
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