Tuberculosis due to Mycobacterium bovis and Mycobacterium caprae in sheep

Marta Muñoz Mendoza a, Lucía de Juan b, Santiago Menéndez c, Antón Ocampo d, Jorge Mourelo a, José L. Sáez e, Lucas Domínguez b, Christian Gortázar f, Juan F. García Marín g, Ana Balseiro h, i, n,*

a Servicio de Sanidad Animal, Subdirección de Ganadería, Consellería do Medio Rural, Xunta de Galicia, Edificio Administrativo San Caetano, 15781 Santiago de Compostela, A Coruña, Spain
b Centro de Vigilancia Sanitaria Veterinaria (VISAVET) y Departamento de Sanidad Animal (Facultad de Veterinaria), Universidad Complutense, Avenida de Puerta de Hierro s/n, 28040 Madrid, Spain
c Departamento de Biología Molecular del Laboratorio de Sanidad y Producción Animal de Galicia, Consellería do Medio Rural, Xunta de Galicia, Avenida de Madrid, 77, 27002 Lugo, Spain
d Departamento de Patología Animal, Medicina Animal (Anatomía Patológica), Facultad de Veterinaria, Universidad de León. Campus de Vegazana, s/n, 24071 León, Spain
e Subdirección General de Sanidad de la Producción Primaria, Dirección General de Recursos Agrícolas y Ganaderos, Ministerio de Medio Ambiente y Medio Rural y Marino, 28071 Madrid, Spain
f IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n. 13071 Ciudad Real, Spain
g SERIDA, Servicio Regional de Investigación y Desarrollo Agroalimentario, Camino de Rioseco, 1225, 33394 La Olla, Deva, Gijón, Asturias, Spain
h Área Veterinaria de Lalín, Servicio Provincial de Ganadería de Pontevedra, Consellería do Medio Rural, Xunta de Galicia, Calle Arenal 27, 36500 Lalín, Pontevedra, Spain
i Departamento de Patología Animal, Medicina Animal (Anatomía Patológica), Facultad de Veterinaria, Universidad de León. Campus de Vegazana, s/n, 24071 León, Spain
j SERIDA, Servicio Regional de Investigación y Desarrollo Agroalimentario, Camino de Rioseco, 1225, 33394 La Olla, Deva, Gijón, Asturias, Spain

A R T I C L E  I N F O
Article history:
Accepted 7 May 2011

Keywords:
Sheep
Tuberculosis
Mycobacterium bovis
Mycobacterium caprae
Spain

A B S T R A C T
Tuberculosis was diagnosed in three flocks of sheep in Galicia, Spain, in 2009 and 2010. Two flocks were infected with Mycobacterium bovis and one flock was infected with Mycobacterium caprae. Infection was confirmed by the comparative intradermal tuberculin test, bacteriology, molecular analysis and histopathology. Sheep have the potential to act as a reservoir for tuberculous.

© 2011 Elsevier Ltd. All rights reserved.

Tuberculosis (TB) in sheep, caused by members of the Mycobacterium tuberculosis complex (MTC), has been reported in New Zealand, Sudan, Italy, Ireland and the United Kingdom (Cordes et al., 1981; Davidson et al., 1981; Tag El Din and Nour Gamaan, 1992; Malone et al., 2003; Marianelli et al., 2010; Van Der Burgt, 2010). Ten clinical cases of TB have been reported previously in Spain (1992; Malone et al., 2003; Marianelli et al., 2010; Van Der Burgt, 2010). In this study, TB is described in three flocks of sheep in Galicia, northwestern Spain (Table 1). Sheep in all three flocks shared facilities with cattle or goats that had been diagnosed with TB. In flocks A and B, clinical signs in ewes were mainly coughing and dyspnoea. TB was discovered during routine abattoir surveillance in one ewe from flock B and in three ewes from flock C.

Twenty-eight ewes from flock B were tested using the standard comparative intradermal tuberculin test (SCITT) to determine reactivity to bovine and avian purified protein derivative (PPD, CZ Veterinary; European Ethics Community Directive 86/609/EC). SCITT was performed by the intradermal inoculation of 0.1 mL (1 mg/mL bovine PPD and 0.5 mg/mL avian PPD) in the shoulder (Spanish Real Decreto 2611/1996). Two ewes exhibited an increased thickness of 2–4 mm to bovine PPD > avian PPD. Ewes from flocks A (n = 177), B (n = 30) and C (n = 3) were sacrificed and examined postmortem (Table 1). Macroscopic evidence of TB was found in 11/210 (5.2%) ewes. Lesions in 10 ewes ranged from 2 to 3 cm white foci in the lungs and single lymph nodes to extensive, soft, yellow, caseous lesions involving the mediastinal lymph nodes and 7–8 cm confluent tubercles in the lungs (Supplementary Fig. 1). In one sheep from flock B, an isolated 1 cm nodule was observed in a retropharyngeal lymph node. The two ewes that reacted to bovine PPD did not have any macroscopic lesions.

Ewes from flocks A (n = 177), B (n = 30) and C (n = 3) were sacrificed and examined postmortem (Table 1). Macroscopic evidence of TB was found in 11/210 (5.2%) ewes. Lesions in 10 ewes ranged from 2 to 3 cm white foci in the lungs and single lymph nodes to extensive, soft, yellow, caseous lesions involving entire lymph nodes (mainly the mediastinal lymph nodes) and 7–8 cm confluent tubercles in the lungs (Supplementary Fig. 1). In one sheep from flock B, an isolated 1 cm nodule was observed in a retropharyngeal lymph node. The two ewes that reacted to bovine PPD did not have any macroscopic lesions.

Tissues (retropharyngeal, submandibular, tracheobronchial, mediastinal and mesenteric lymph nodes, lungs and intestine) were collected for histopathology from eight ewes in flock B (two reactors in the SCITT, three showing macroscopic changes, one...
Two ewes that were positive in the SCITT did not exhibit gross macroscopic lesions in sheep. Sheep appear to be susceptible to MTC infection when there is high environmental contamination (Cordes et al., 1981). Infection. Sheep appear to be susceptible to MTC infection when showing clinical signs but no macroscopic lesions and two with no clinical signs or microscopic lesions). After fixation in 10% neutral buffered formalin and routine processing, several pm sections were stained with haematoxylin and eosin (HE) and by the Ziehl–Neelsen (ZN) method for acid-fast bacteria (AFB).

All eight ewes examined histologically had microscopic TB lesions. Lesions in retropharyngeal lymph nodes ranged from small granulomas consisting mainly of macrophages and Langhan’s-type giant cells (n = 6 ewes; Supplementary Fig. 2A) to a tuberculous nodule consisting of caseous necrosis with central mineralisation (n = 1 ewe; Supplementary Fig. 2B). Six ewes (including the two that reacted in the SCITT and one showing clinical signs) had early stage granulomatous lesions in the lungs (Supplementary Fig. 2C) and mediastinal and tracheobronchial lymph nodes (Supplementary Fig. 2E). The two ewes with extensive macroscopic lesions had granulomas typical of TB in the lungs and lymph nodes (Supplementary Fig. 2D and 2F). Langhan’s-type giant cells were observed occasionally. AFB were detected on ZN staining in the lungs and mediastinal lymph node only in one animal with extensive macroscopic lesions. 

Lesions and were culture negative, although both had microscopic TB lesions. In a study by Cordes et al. (1981), the SCITT had a sensitivity of 81.6% on the basis of gross lesions. The source of the infection for sheep in the present study appeared to be through close contact with cattle or goats infected by the same spoligotypes (Table 1). Although it was not possible to determine whether sheep were a source of infection for cattle, goats or other species, this study suggests that sheep have the potential to act as an additional domestic reservoir for TB. To minimise the spread of TB among sheep and from sheep to other species on the same farm, consideration should be given to (1) obligatory sacrifice of sheep cohabiting with cattle or goats with TB; (2) segregation of cattle from other ungulates where possible; and (3) active surveillance in abattoirs to detect TB in sheep.

**Conflict of interest statement**

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

**Acknowledgements**

The authors thank Jorge González from University of León and the Official Veterinary Services from Lalín and Trives for assisting with abattoir sampling and processing of samples. We would like to thank the National and Regional Animal Health authorities (MARM) for their support. We also thank Jose Montoya of the Animal Health Veterinary Service for helping with the database, Carmen Calvo from the Laboratorio de Sanidad y Producción Animal de Galicia for helping with the culture of samples and Nuria Moya from VISAVET Health Surveillance Centre for molecular characterisation of the MTC strains. We are grateful to The Spanish Network on Surveillance and Monitoring of Animal Tuberculosis Working Group on Bovine Tuberculosis Surveillance for their continuous contribution to mycobDB. Dr. Mark A. Chambers is thanked for critically reviewing the manuscript. Ana Balseiro is the recipient of a Contrato de Investigación para Doctores from the Instituto Nacional de Investigación Agraria y Agroalimentaria (INIA). This Project is supported by INIA RTA2008-00041-00-00 and TB-STEP Grant 212414, EU.

**Appendix A. Supplementary material**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tvjl.2011.05.006.

**References**


### Table 1

<table>
<thead>
<tr>
<th>Flock</th>
<th>Year</th>
<th>Clinical signs</th>
<th>SCITT number sacrificed/total number</th>
<th>Macroscopic lesions</th>
<th>Microscopic lesions</th>
<th>Culture from sheep (strain isolated)</th>
<th>Other ruminate species on farm (strain isolated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2009</td>
<td>5/177</td>
<td>NT</td>
<td>177/177</td>
<td>5/177</td>
<td>NS</td>
<td>5/7 (5 M. caprae SB0157) (M. caprae SB0157 in goats)</td>
</tr>
<tr>
<td>B</td>
<td>2010</td>
<td>2/30</td>
<td>2/26</td>
<td>30/30</td>
<td>3/30</td>
<td>NS</td>
<td>1/8 (1 M. bovis SB0886) (M. bovis SB0886)</td>
</tr>
<tr>
<td>C</td>
<td>2010</td>
<td>ND</td>
<td>NT</td>
<td>3/71</td>
<td>3/3</td>
<td>NS</td>
<td>3/3 (3 M. bovis SB0886) (M. bovis SB0886 in goats and cattle)</td>
</tr>
</tbody>
</table>

SCITT, standard comparative intradermal tuberculin test; NS, no sample; NT, not tested; ND, no data.

* Clinical signs consisted mainly of cough and dyspnoea.

**Appendix A. Supplementary material**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tvjl.2011.05.006.

**References**


