Short Communication

Tuberculosis due to *Mycobacterium bovis* and *Mycobacterium caprae* in sheep

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ABSTRACT

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 tuberculosis.

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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 sheep in Spain: four in Aragón (*M. bovis* and *M. caprae*), four in Salamanca (*M. bovis*, spoligotype SB0124), one in Cataluña (*M. bovis*, SB0415) and one in Huelva (*M. caprae*, SB0157) (García Marín et al., 1989; Gutiérrez et al., 1997; MARM/VISAVET-Spanish Database of Animal Mycobacteriosis mycoDB¹).

In this study, TB is described in three flocks of sheep in Galicia, north western 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 Veterinaria; 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 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

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¹ See: www.marm.es.

Table 1

History, lesions and results of the standard comparative intradermal tuberculin test and culture in three flocks of sheep with tuberculosis.

Flock	Year	Clinical signs ^a	SCITT	Number sacrificed/total number	Macroscopic lesions	Microscopic lesions	Culture from sheep (strain isolated)	Other ruminant species on farm (strain isolated)
A	2009	5/177	NT	177/177	5/177	NS	5/7 (5 <i>M. caprae</i> SB0157)	Goats and a calf (<i>M. caprae</i> SB0157 in goats)
B	2010	2/30	2/28	30/30	3/30	8/8	1/8 (1 <i>M. bovis</i> SB0886)	Cattle (<i>M. bovis</i> SB0886)
C	2010	ND	NT	3/71	3/3	NS	3/3 (3 <i>M. bovis</i> SB0886)	Goats and cattle (<i>M. bovis</i> SB0886 in goats and cattle)

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

^a Clinical signs consisted mainly of cough and dyspnoea.

showing clinical signs but no macroscopic lesions and two with no clinical signs or macroscopic lesions). After fixation in 10% neutral buffered formalin and routine processing, several 4 µm 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.

Pools of tissues (2 g) from the lungs, retropharyngeal, submandibular, tracheobronchial and mediastinal lymph nodes from each of 18 ewes (with clinical signs, reacting in the SCITT or with macroscopic lesions) were inoculated into the Mycobacteria Growth Indicator Tube (MGIT) liquid medium system and Löwenstein–Jensen solid media with sodium pyruvate for isolation of MTC (Idigoras et al., 2000) (Table 1). Real time (RT)-PCR to identify species was performed on culture isolates using the MTC forward primer 5'-TAGTGCATGCACCGAATTAGAACGT-3', the MTC reverse primer 5'-CGAGTAGGTCATGGCTCCTCC-3' and the TaqMan probe YY/BHQ 5'-AATCGCGTCGCCGGGAGC-3', which amplifies a 184 base pair fragment (Coetsier et al., 2000). MTC isolates were characterised by direct variable repeat (DVR)-spoligotyping (Kamerbeek et al., 1997). The amplified product was detected by hybridisation of the biotin-labelled PCR product onto spoligotyping membrane (Isogen Bioscience BV). The results were recorded in SB code followed by four digits (*M. bovis* Spoligotype Database website²).

MTC species were isolated and identified by RT-PCR from 9/18 (50%) ewes, all of which had macroscopic lesions (Table 1). The two ewes from flock B that reacted in the SCITT were negative by culture. Isolates were characterised by spoligotyping as *M. caprae* SB0157 (flock A) and *M. bovis* SB0886 (flocks B and C).

Our study provides evidence of TB in sheep on three farms that also shared facilities with cattle or goats. Macroscopic and microscopic lesions in sheep were confined to the thoracic cavity and retropharyngeal lymph nodes, suggesting an aerogenous route of infection. Sheep appear to be susceptible to MTC infection when there is high environmental contamination (Cordes et al., 1981). Two ewes that were positive in the SCITT did not exhibit gross

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.

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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.

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² See: www.mbovis.org.

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