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Evaluation of single and comparative intradermal tuberculin tests for tuberculosis eradication in caprine flocks in Castilla y León (Spain)

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ABSTRACT

Goats can act as reservoirs for tuberculosis (TB) infection. The main etiological agents of TB in goats are *Mycobacterium caprae* and *Mycobacterium bovis* and they infect also a wide range of domestic and wild animals and humans. Control programmes based mainly on the application of single and comparative intradermal tuberculin (SIT and SCIT respectively) tests are being implemented in certain regions of Spain with a high density of caprine flocks as Castilla y León, including goats with epidemiological relationship with cattle. The aim of this study was to evaluate the performance of the intradermal tests in naturally TB-infected caprine flocks from this region. The study was performed using data from 17,450 goats in 54 different flocks that were classified as TB-infected in the control programmes executed in 2010 and 2011. Data from 1237 goats from 7 dairy flocks depopulated after the first intradermal testing were used to estimate the sensitivity (Se) using bacteriology as the gold-standard. Overall Se of the SIT test using the severe interpretation was 43.9% (CI 95%, 40.4–47.4) and decreased to 38.8% (CI 95%, 35.5–42.3) using the standard interpretation. Overall Se of the SCIT test ranged between 21.3% (CI 95%, 17.6–25.4) and 7% (CI 95%, 4.9–9.8) depending of the interpretation criteria. A significant weak positive correlation was found between age and skin fold thickness (Spearman's test $p < 0.05$). Results from this study yielded, in general, low Se values probably due the systematic detection and slaughter of reactors as a consequence of the eradication programme in previous years or the presence of factors that may interfere in the diagnosis. Therefore, these results suggest the necessity of including ancillary diagnostic tools and/or strict interpretation criteria to maximize detection of positive animals in infected settings.

1. Introduction

Mycobacterium caprae and *Mycobacterium bovis* are the main etiological agents of tuberculosis (TB) in goats, producing a chronic infectious zoonotic disease (Aranaz et al., 2003; Crawshaw et al., 2008) with both significant economic and sanitary implications mostly derived from a decrease in milk and meat production of infected animals and the possibility of transmission to humans or other domestic and wildlife species (Cvetnic et al., 2007; LoBue et al., 2010). In Spain, TB in goats is not subjected to official eradication campaigns (except goats co-existing with cattle in the same farm or sharing pastures or with epidemiological links with cattle). In spite of the lack of official data, a high prevalence of infection (>5%) has been detected in certain region (Bezos et al., 2012b). For this reason, control programmes based mainly on the application of single and comparative intradermal tuberculin (SIT and SCIT respectively) tests and, in particular cases, the ancillary use of the interferon-gamma (IFN-γ) assay, are being implemented in certain regions in Spain with a high density of caprine flocks such as Castilla y León, Murcia, Andalucía or Islas Canarias. These programmes aim to reduce the prevalence of caprine TB and, simultaneously, decrease the risk of transmission from goats to cattle or other domestic or wildlife species that could become reservoirs of infection (Gortázar et al., 2005; Matos et al., 2010).

The *ante-mortem* diagnosis of TB in goats is mostly performed using the same tests based on the cell-mediated immune response and interpretation criteria applied in cattle: SIT and SCIT tests, using only bovine PPD or both bovine and avian PPDs respectively. The SCIT test is used to differentiate between animals infected with *M. bovis*/*M. caprae* from those sensitized to bovine PPD as a result

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of exposure to other non-tuberculous mycobacteria showing, therefore, a higher specificity (Bezos et al., 2012a).

In terms of sensitivity (Se), a wide range of values (53.2–87.2%) has been reported depending on the test used (SIT or SCIT test), the interpretation criteria applied, the presence of non-tuberculous mycobacterial co-infections and the age of the animals (Gutiérrez et al., 1998; Liébana et al., 1998; Álvarez et al., 2008; Bezos et al., 2011a).

However, most of these studies were carried out using a low number of animals from the same flock and/or confirmation of the true infection status by bacteriology in a low proportion of them (Gutiérrez et al., 1998; Liébana et al., 1998; Álvarez et al., 2008; Bezos et al., 2011a).

The caprine TB control programs performed in certain regions of Spain and, particularly, in Castilla y León, where TB voluntary control programmes started in 2005, have yielded a large amount of data to evaluate the usefulness of SIT and SCIT test for the diagnosis of TB in goats. In 2010 and 2011, 80,644 and 91,489 goats from 666 and 927 flocks respectively were tested in the frame of the TB control programmes in this region yielding a flock prevalence of 10.6% and 4.9% (Marqués et al., 2013). Information from officially TB-free negative flocks in Castilla y León was recently used to evaluate the specificity of the diagnostic assays (Bezos et al., 2012a) but similar studies to evaluate their Se in infected flocks are still lacking.

The aim of this study was (1) to evaluate prevalence of reactors and (2) the Se of the SIT and SCIT tests in a large number of TB-infected caprine flocks in Castilla y León in order to determine the usefulness of these techniques for detection of TB infected goats and (3) the molecular characterization of the isolates to find any evidence of TB transmission between goats and cattle in the region.

2. Materials and methods

2.1. Flocks and animals

The study was performed using data from 17,450 goats mainly of mixed (57.8%) and Murciano-granadina breeds (25.7%) from 54 infected flocks detected in the TB programmes executed in 2010 and 2011 by the regional authorities. Only 7 out of the 54 flocks (885 goats) were for meat production while the rest (16,565 goats from 47 flocks) were dairy animals (including males in dairy flocks). Information about the response to the diagnostic test, the age and the flock of origin was available for each animal. The mean number of goats in meat and dairy flocks was 126 (range 17–275) and 352 (range 5–2254) respectively. The mean age of the animals was 4.4 (range 0.4–14.2) and 2.8 (range 0.2–15.5) years in meat and dairy flocks years respectively. The true status of infection was assessed by bacteriology in 1926 goats (46 and 1880 from meat and dairy flocks respectively).

Data from 1237 goats (median age: 3.4 years, range 0.3–12.4) from 7 dairy flocks (1–7) depopulated due to the high proportion of reactors detected after the first intradermal test (excluding flock 4) were used to calculate the Se at the individual level. Vaccination against PTB was performed in flocks 1, 2, 3 and 6 (Table 1).

The animals used in this study were not experimental animals. All handling and sampling procedures were carried out in accordance with Spanish legislation (R.D. 727/2011).

2.2. Tuberculin tests

SIT and SCIT tests were carried out according to the R.D. 2611/96, Council Directive 64/432/EEC and Orden del 29 de Abril de 2002 de la Consejería de Agricultura y Ganadería de Castilla y León using the commercial bovine and avian PPDs (CZ Veterinaria S.A., Porriño, Spain). The goats were inoculated with 0.1 mL bovine PPD on the left side of the neck, and 0.1 mL avian PPD on the right side using a McLintock syringe (Bar Knight McLintock Limited, Clydebank, UK). The results of the tests were determined by measuring the increase of the skin-fold thickness 72 h later.

Two interpretations were considered for SIT and three for SCIT test. In the SIT test, animals were classified as positive if a bovine reaction (increase of the skin fold thickness after the application of the bovine PPD) of 2 mm or more or the presence of clinical signs such as oedema, exudation, necrosis, pain or inflammation at the injection site were observed (severe interpretation) (Álvarez et al., 2008). Alternatively, positive animals were those showing a skin fold thickness increase of 4 or more mm or the presence of clinical signs (standard interpretation). Animals were considered positive to the SCIT test if a positive bovine reaction of 4 or more mm was observed which was greater than the avian reaction, or if the presence of clinical signs at the injection site of the bovine PPD were observed (standard interpretation based on Orden del 29 de Abril de 2002). In addition a severe interpretation, in which animals showing a positive bovine reaction of 1 or more mm which was greater than the avian reaction and/or with clinical signs at the bovine PPD injection site were considered positive, "SCIT severe 2002" in the manuscript) was also considered. Afterwards, in 2010, another interpretation for reactor to the SCIT test was suggested: animals showing a bovine reaction of 8 or more mm and equal to lower than the avian reaction ("SCIT severe 2010" in the paper).

2.3. M. bovis/M. caprae culture

One thousand nine hundred and twenty-six goats (46 meat and 1880 dairy goats respectively) were sampled for bacteriology. In particular, 991 out of those 1926 goats belonged to 7 dairy depopulated flocks and infection was confirmed in 775 of them. Tissue samples from lung and lymph nodes from head and thorax collected in the slaughterhouse were cultured using BACTEC MGIT 960 Mycobacterial Detection System (Becton Dickinson, USA). Isolates were identified and characterized by Direct Variable Repeat (DVR)-spoligotyping (Kamerbeek et al., 1997).

2.4. Statistical analysis

Comparison of proportions of test reactors between age groups (<24, 24–60 and >60 months) was performed using Pearson's Chi Square. Quantitative outcomes of the tests were assessed for normality and differences between groups' values were evaluated using non-parametric tests (Kruskal–Wallis tests). Correlation between quantitative test results and age of animals was analyzed using Spearman's and Mann–Whitney's tests. Statistical analysis was performed using IBM SPSS Statistics 19.

3. Results

3.1. Intradermal test: overall results

3.1.1. Meat flocks (n = 7)

Forty-eight (5.4%) and 61 (6.9%) goats out of 885 goats were classified as positive reactors using the standard and severe interpretation of the SIT test respectively. Five hundred and ninety-two goats were also tested using SICT test, yielding 31 (5.2%) and 36 (6.1%) positive reactors using standard and severe interpretation (2002 and 2010) respectively (Table 2).

Positive reactions measured at the bovine PPD injection site (SIT test severe interpretation) ranged from 3 to 18 mm (median = 6). The highest number of reactors and the largest increase of the skin