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Evaluation of the performance of cellular and serological diagnostic tests for the diagnosis of tuberculosis in an alpaca (*Vicugna pacos*) herd naturally infected with *Mycobacterium bovis*

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ABSTRACT

Tuberculosis (TB) in llamas and alpacas has gained importance in recent years since they are imported into the European Union mainly for serving as pets and for production of natural fibre. The intradermal tuberculin test has been widely used for diagnosis of TB in these species showing lack of sensitivity (Se) although little information has been previously reported evaluating the effect on its performance of different PPD inoculation sites and time of readings. Moreover, different cost-effective serological assays have been developed in the recent years for TB diagnosis in camelids obtaining a variety of results and, for this reason, new assays still being developed. The main objectives of this study were: (1) to evaluate the performance of the intradermal tuberculin test using different inoculation sites (axillary, prescapular and cervical) and times of reading (72 and 120 h) and (2) to test a novel serological assay based on MPB83 antigen in a *Mycobacterium bovis* naturally infected alpaca herd in Spain. In regards to skin test, single intradermal tuberculin (SIT) test at the prescapular site and reading at 72 h showed the highest proportion of test-positive-culture positive animals among all culture positive animals (T+/C+), ranging from 53.8% (95% CI, 37.2–69.9) to 80% (95% CI, 44.4–97.5) using a more stringent interpretation than typically prescribed although, in general, low T+/C+ was achieved using both SIT and single comparative intradermal tuberculin (SCIT) tests alone. T+/C+ of the serological assay increased using samples collected 15–30 days after PPD injection [76.9% (95% CI, 60.7–88.9) – 100% (95% CI, 69.2–100)]. The best results of T+/C+ were obtained applying in parallel the most sensitive SIT test and serology using samples collected 15–30 days after PPD inoculation [90% (95% CI, 55.5–99.7)-100% (95% CI, 69.2–100)]. Therefore implementation of serology in parallel with the most sensitive skin test could maximize the detection of infected animals.

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1. Introduction

Tuberculosis (TB) in New World Camelids (NWC) is increasingly gaining importance in recent years, especially in regions where TB is endemic in cattle or wildlife (Barlow et al., 1999; Dean et al., 2009; García-Bocanegra et al., 2010; Twomey et al., 2007; Twomey et al., 2012). Llamas (*Lama glama*) and alpacas (*Vicugna pacos*) are the main NWC imported into the European Union with the aim of serving as pets or pack animals or for production of natural fibre. Tuberculosis in these species is mainly caused by *Mycobacterium bovis* and *Mycobacterium microti* and it has been reported in several European countries including Spain (Álvarez et al., 2011a). Infection by *M. bovis* in llamas and alpacas mainly occur by the respiratory route and lesions are generally located in lung, pleura and associated lymph nodes in form of small multifocal white–yellowish caseous nodules or larger gross coalescent lesions (Barlow et al., 1999; García-Bocanegra et al., 2010; Twomey et al., 2007; Twomey et al., 2012). Clinical signs are unspecific and generally related to the route of infection, including respiratory distress and cough. Lethargy and anorexia has been also described in infected animals and, in some outbreaks, sudden death of animals without external signs of infection may occur (Barlow et al., 1999; Twomey et al., 2007; Zanolari et al., 2009).

Due to its unspecific clinical presentation, diagnosis based on the detection of the immune response is the most frequent approach used for detection of TB infection in NWC. The intradermal tuberculin test has been widely used for diagnosis of TB in llamas and alpacas showing, in general, a poor performance in these species (Lyashchenko et al., 2007; Ryan et al., 2008; García-Bocanegra et al., 2010). Accurate evaluation of Se in NWC is limited by the lack of studies in a high number of animals with a known infection status (Álvarez et al., 2011a). Both single and comparative intradermal tuberculin (SIT and SCIT respectively) tests have been applied in NWC using different inoculation sites and times of readings after PPD (purified protein derivative) injection (Stevens et al., 1998; García-Bocanegra et al., 2010; Álvarez et al., 2011a;). Concerning SIT test, several studies were performed in experimentally infected (*M. bovis*) llamas and alpacas. Proportion of test-positive-culture positive animals among all culture positive animals (T+/C+) ranged between 80% (95% CI, 28.4–99.5) and 100% (95% CI, 29.2–100 and 79.4–100) although the number of animals used in each study was low (Stevens et al., 1998; Álvarez et al., 2011a). SCIT test has been used more frequently in NWC in order to minimize false positive reactions caused by sensitization against environmental mycobacteria. As reported in other species, SCIT test showed a higher Sp than the SIT test to the detriment of Se.

Previous studies reported a T+/C+ of the SCIT test between 0% (95% CI, 0–84.2) and 14.3% (95% CI, 1.8–42.8), and between 76.2% (95% CI, 52.8–91.8), and 87.5% (95% CI, 67.6–97.3) in naturally and experimentally infected llamas and alpacas respectively (Lyashchenko et al., 2007; García-Bocanegra et al., 2010). However, few animals were also tested in these studies. Axillary site has been the preferred site for PPD inoculation and readings were generally performed at 96 h post-inoculation although in several studies this information was not stated. Other sites such as the neck have been tested for PPD inoculation although skin in that region has been originally considered thick and resilient making the interpretation difficult.

Since interferon-gamma (IFN-γ) assay for NWC is difficult to standardize and has shown low Se and Sp (Rhodes et al., 2012), several in-house and commercial diagnostic tests based on humoral immune response have been developed (Lyashchenko et al., 2007; Dean et al., 2009; Rhodes et al., 2012; Twomey et al., 2012). In general, these techniques have been assayed in a low number of animals showing high Sp (Lyashchenko et al., 2007) but a lack of T+/C+ (Lyashchenko et al., 2007; Dean et al., 2009; Rhodes et al., 2012) probably due to the absence of high levels of antibodies in the first stages of TB infection (Pollock and Neill, 2002) even using sera samples collected after intradermal PPD injection with the aim of increasing the detection of infected animals (Dean et al., 2005; Rhodes et al., 2012).

The aim of this study was to (1) evaluate the performance of the intradermal tuberculin test using different inoculation sites (axillary, prescapular and cervical) and times of reading (72 and 120 h) and (2) to assess a novel serological technique based on MPB83 antigen in a *M. bovis* naturally infected alpaca herd in Spain.

2. Materials and methods

2.1. Herd

A TB outbreak was detected in an alpaca farm located in central Spain in December 2011. The herd was made up of 115 male (25%) and female (75%) alpacas of Suri and Huacaya breed. The age of the animals ranged between one and 10 years. First alpacas were imported from Chile and New Zealand in 2001 and no previous history of tuberculosis was reported until this outbreak. In December 2011, field veterinarians detected clinical signs (anorexia, cachexia, respiratory distress) and/or sudden deaths in three alpacas. Compatible TB lesions were observed in the necropsy of one of these alpacas and *M. bovis* infection was subsequently confirmed. Thirty-eight animals died by natural causes (due to TB infection) along the study (24 between January and March and 14 between March and June).

The animals used in this study were not experimental animals. All handling and sampling procedures were carried out in accordance with local and Spanish legislation (Royal Decree 53/2013).

2.2. Study design

Three herd testing events were carried out during the study (January, March and June 2012). Two times of reading (72 h and 120 h) and three inoculation sites for SIT and SCIT tests were evaluated in each animal: axillary (January, March and June), prescapular (March and June) and cervical (June) sites (Table 1). Sera samples from all the alpacas were collected just before PPD inoculation for detection of specific humoral response and a proportion of animals were