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Unexpected high responses to tuberculin skin-test in farmed red deer: Implications for tuberculosis control

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ABSTRACT

Tuberculosis (TB) in deer is a serious zoonotic disease of worldwide distribution. Detection of infected animals is usually performed using single or comparative skin-testing (SST/CST), although false responses due to sensitization to other mycobacteria may occur, hampering diagnostic specificity. We describe the evolution of the responses to the SST, CST and to an in-house serological assay in a red deer farm subjected to regular TB testing in southern Spain in an attempt to understand the dynamics of possible non-specific reactions occurring under field conditions. We performed 2288 skin-tests and ELISAs in nine sampling periods between May 2009 and January 2011. In May 2010, a strong increase in skin fold thickness in response to avian purified protein derivative (PPD) (mean = 4.0 mm, 95% CI = 3.5–4.5) and bovine PPD (mean = 1.8 mm, 95% CI = 1.6–2.0) was observed in yearling deer hinds ($n = 150$), compared to values recorded for the same individuals in November 2009 (avian PPD: mean = 0.7 mm, 95% CI = 0.6–0.8 and bovine PPD: mean = 0.7 mm, 95% CI = 0.6–0.7) and in January 2011 (avian PPD: mean = 2.2 mm, 95% CI = 1.9–2.4 and bovine PPD: mean = 1.1 mm, 95% CI = 1.0–1.2). Using SST, 54 animals (36%) of the yearlings tested in May 2010 would have been classified as positive reactors, while none of them was positive in the CST. The five animals with highest skin fold increases to mycobacterial antigens were culled and subjected to post-mortem analysis, which confirmed the absence of *Mycobacterium tuberculosis* complex (MTBC) infection but demonstrated the presence of environmental mycobacteria and closely related bacteria in four out of the five analyzed animals. Our results demonstrated how non-specific responses to mycobacterial antigens can adversely affect the specificity of TB diagnosis based on the SST. Thus, once TB infection has been ruled out using confirmatory techniques, application of comparative diagnostic tests is highly advisable to maximize test specificity and avoid the slaughter of false positive reactors.

1. Introduction

Deer have been farmed for centuries under a variety of management systems, including farms, extensive ranching conditions, hunting parks, zoological parks and private estates (Mackintosh et al., 2004). In Spain, deer farming is

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a growing activity, with most of the farms devoted to the production of red deer (*Cervus elaphus*) for restocking of fenced estates for hunting purposes (Fernández-de-Mera et al., 2009). Tuberculosis (TB) due to *Mycobacterium bovis* and closely related members of the *Mycobacterium tuberculosis* complex (MTBC), and paratuberculosis (PTB; Johne's disease) caused by *Mycobacterium avium paratuberculosis*, are two of the most important health issues in deer farming (Clifton-Hadley and Wilesmith, 1991; Griffin and Buchan, 1994; Mackintosh et al., 2004). The fact that these diseases represent a threat to livestock makes their surveillance a priority in wildlife management and sanitary control (Fredriksen et al., 2004; Gortázar et al., 2007).

For TB control, the tuberculin skin-test, based on the inoculation of purified protein derivatives (PPDs), has been employed worldwide as the standard diagnostic method designated by the World Organization for Animal Health (OIE, 2002). This test, based on the measurement of the increase in the skin fold thickness induced by the inoculation of PPD of *M. bovis* (bovine PPD) or *M. avium* (avian PPD), is the basis of tuberculosis diagnosis in deer. As in cattle, it can be based on a single inoculation of bovine PPD (single skin-test, SST) (Corrin et al., 1987). However, PPDs consist of a complex mixture of proteins, lipids, sugars and nucleic acids, and include a great variety of antigens, many of which are shared with other mycobacterial species and close related bacteria (Karlson, 1962; Monaghan et al., 1994); this can lead to a lack of specificity of the test, which means false positive reactors (de Lisle and Havill, 1985). To increase the specificity of the test, a comparison of the responses measured after bovine PPD and avian PPD inoculation can be performed (comparative skin test, CST) (Corrin et al., 1993); in this case, the reactivity to bovine PPD is expected to be greater in deer infected with MTBC members. If animals are infected with mycobacteria other than the MTBC (MOTT), they usually develop greater responses to avian PPD than those observed to bovine tuberculin (Cooney et al., 1997; Köhler et al., 2001; Amadori et al., 2002; Hope et al., 2005). Although specificity of CST is greater than that observed in the SST, its sensitivity is usually reduced (Corrin et al., 1993). Studies carried out using CST with farmed red deer in New Zealand described 82–86% sensitivity and 46–76% specificity (Griffin et al., 1991; Corrin et al., 1993; Norden et al., 1996). A number of factors such as age, sex, season, body condition and management type among others can affect the responses detected in the skin tests in deer compromising both sensitivity and specificity (Fernández-de-Mera et al., 2011).

The usefulness of serological tests has been evaluated to overcome diagnostic limitations of skin-test. However, to date no assay detecting circulating antibodies to *M. bovis* has shown adequate sensitivity or specificity for standalone routine TB diagnosis in deer (Harrington et al., 2008). Nevertheless, these tests can be used as complement to other tests, and have the advantage of requiring only one handling of the subjects (Lyashchenko et al., 2008). The situation is different in the case of PTB, as the developed serological tests allow the detection of positive animals with acceptable levels of sensitivity and specificity. Nonetheless, there are few references on the application of these diagnostic tests in wildlife, making it difficult to establish the cut-off points and make a correct interpretation of results (Reyes-García et al., 2008). Moreover, recent results on wild deer from TB endemic regions suggest that care should be taken in interpreting serological results, because of possible cross-reactions (Carta et al., in press).

The aim of this study was to describe the evolution of the responses to the skin-test and to an in-house serological assay in a red deer farm subjected to regular TB testing in southern Spain in an attempt to understand the dynamics of possible non-specific reactions occurring under field conditions.

2. Material and methods

2.1. Animals

The present study was carried out in a red deer farm in southern Spain. It is a farm with a semi-intensive management scheme, with pasture-rotation and year-round food supplementation. Deer are separated by age in calves (<6 months old), yearling hinds (7–24 months old), adult hinds (>2 years old), yearling stags (7–24 months old) and adult stags (>2 years old). Animals are handled at least once a year (calves in November; yearling hinds in May; yearling stags in August; adult hinds and adult stags in January and August) for skin-testing, biometry measurement, blood sampling and administration of antiparasitic drugs. Deer sampled in this study included all hinds and reproductive stags and their calves in the farm during the sampling years (2009–2011), with focus on the female deer born in April/May 2009 ($n = 150$). During the study period we performed 2288 skin-tests and ELISAs in nine testing rounds. Handling procedures and sampling frequency were designed to reduce stress and health risks for subjects, according to European (86/609) and Spanish laws (RD 223/1988; RD 1021/2005), and current guidelines for ethical use of animals in research (ASAB, 2005).

2.2. Diagnostic tests

2.2.1. Skin-test

Standardized skin-tests and sampling procedures were performed during the study period on different dates depending on the age group (Table 1). Animals were handled twice during the procedure, at times 0 and 72 h. Deer were moved from the paddocks to the farm enclosures and then immobilized by physical restraint, in a hydraulic crush. At time 0, each animal was identified by the eartag number, weighed and blood samples were collected from the jugular vein. Three areas of 3 cm × 3 cm were shaved at the right side of the neck with an electric shaver (Moser Avalon 1290; Moser, Valencia, Spain), and skin fold thickness was measured using a manual calliper (Mitutoyo, Cardiff, UK) three different times in each area, to the nearest 0.1 mm, by the same operator. Then 0.1 ml of each antigen [avian and bovine PPD (Cooper-Zeltia, Spain) and the plant derived mitogen phytohaemagglutinin (250 μg of PHA; Sigma–Aldrich, Missouri, USA) diluted in phosphate buffered saline as positive control] were inoculated using 1-ml syringes fitted with a 25-G ½-in. needle. At time 72 h,