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Evaluation of the sensitivity and specificity of bovine tuberculosis diagnostic tests in naturally infected cattle herds using a Bayesian approach

Julio Álvarez [a], Andrés Perez [b,c], Javier Bezos [d], Sergio Marqués [e], Anna Grau [e], Jose Luis Saez [f], Olga Mínguez [e], Lucía de Juan [d,g,*], Lucas Domínguez [d,g]

[a] Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13071 Ciudad Real, Spain
[b] Center for Animal Diseases Modeling and Surveillance, One Shield Avenue, University of California, Davis, CA 95616, USA
[c] CONICET/Facultad de Ciencias Veterinarias UNR, Boulevard Ovidio Lagos y Ruta 33, Casilda, Santa Fe, Argentina
[d] Centro VISAVET, Universidad Complutense de Madrid, Avda. Puerta de Hierro S/N, 28040 Madrid, Spain
[e] Dirección General de Producción Agropecuaria, Servicio de Sanidad Animal, Junta de Castilla y León, C/Rigoberto Cortejoso 14, 47014 Valladolid, Spain
[f] Subdirección General de Sanidad de la Producción Primaria, Dirección General de Recursos Agrícolas y Ganaderos, Ministerio de Medio Ambiente y Medio Rural y Marino, C/Alfonso XII, 62, 28071 Madrid, Spain
[g] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Avda. Puerta de Hierro S/N, 28040 Madrid, Spain

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ABSTRACT

Test-and-slaughter strategies have been the basis of bovine tuberculosis (BT) eradication programs worldwide; however, eradication efforts have not succeeded in certain regions, and imperfect sensitivity and specificity of applied diagnostic techniques have been deemed as one of the possible causes for such failure. Evaluation of tuberculosis diagnostic tools has been impaired by the lack of an adequate gold standard to define positive and negative individuals. Here, a Bayesian approach was formulated to estimate for the first time sensitivity (Se) and specificity (Sp) of the tests [single intradermal tuberculin (SIT) test, and interferon-gamma (IFN-γ) assay] currently used in Spain. Field data from the first implementation of IFN-γ assay (used in parallel with SIT test 2–6 months after a first disclosure SIT test) in infected beef, dairy and bullfighting cattle herds from the region of Castilla and Leon were used for the analysis. Model results suggested that in the described situation: (i) Se of SIT test was highly variable (40.1–92.2% for severe interpretation, median = 66–69%), and its Sp was high (>99%) regardless interpretation criteria; (ii) IFN-γ assay showed a high Se (median = 89–90% and 83.5% for 0.05 and 0.1 cut-off points respectively) and an acceptable Sp (85.7% and 90.3% for 0.05 and 0.1 thresholds) and (iii) parallel application of both tests maximized the combined Se (95.6% using severe SIT and 0.05 cut-off point in the IFN-γ assay). These results support the potential use of the IFN-γ assay as an ancillary technique for routine BT diagnosis.

1. Introduction

Bovine tuberculosis (BT) is a globally distributed zoonotic disease of cattle (Michel et al., 2010). Its control and in certain cases eradication has been achieved through the application of test-and-cull strategies for extended periods of time – typically, decades (Collins, 2006; Cousins and Roberts, 2001). Success of eradication strategies is based on early detection and removal of infected animals from a herd; thus the use of accurate diagnostic tests is of crucial importance.

Diagnostic tests used for detection of infected cattle are mainly based in the detection of the cellular mediated immune (CMI) response, which is triggered in the early

* Corresponding author. Tel.: +34 913943992; fax: +34 913943795.
E-mail address: dejuan@visavet.ucm.es (L. de Juan).

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stages of infection (Pollock et al., 2001); one of the most widely applied diagnostic technique for this purpose, the single intradermal tuberculin (SIT) test, is based on the inoculation of the bovine PPD in the skin of the neck or in the caudal fold (Monaghan et al., 1994). European Union (EU) legislation (Council Directive 64/432/EEC as last amended) establishes conditions for identification of positive animals but, depending on the epidemiological situation, different interpretations can be applied to increase diagnostic sensitivity (Anon, 2006), although this sensitivity usually entails a decrease in the specificity of the test. Accuracy of the skin test is greatly affected by a variety of factors, both related with the host and with the test itself; for these reasons accurate estimation of its sensitivity and specificity in the field has been difficult.

In the last 20 years an additional diagnostic tool also aiming at the detection of the CMI immune response, the gamma-interferon (IFN-γ) detection test, has been increasingly applied (Wood et al., 1990). In areas of the EU with a high prevalence of BT, its use is recommended to detect the maximum number of infected animals in a herd or region; however, its application in areas in which herd prevalence is low is not recommended due to specificity limitations (Anon, 2006). In an attempt to optimize the accuracy of IFN-γ assay different interpretations of the test have been considered (Wood and Jones, 2001), mainly changing the threshold for positivity to maximize diagnostic sensitivity or specificity depending on the aim of each use.

Latent class analysis is being increasingly applied for estimation of operating characteristics of diagnostic tests when true disease status cannot be determined, i.e., for which it is not possible to assume the use of perfect reference tests (Fosgate et al., 2006; Muma et al., 2007; Enoe et al., 2000). This is the case of BT, as common reference tests (detection of lesions and/or isolation of the agent) have a limited sensitivity compared to immunological tests (Vordermeier et al., 2004). Latent class models have been used for evaluation of the accuracy of tuberculosis diagnosis in meerkats (Drewe et al., 2009), badgers (Drewe et al., 2010) and cattle (Muller et al., 2009; Cleggs et al., 2011). However, to the authors' knowledge, accuracy of diagnostic techniques currently applied in cattle in Spain (SIT test and IFN-γ assay applied in parallel) has never been quantified in the field using latent class models.

The study here presents field estimates of the sensitivity and specificity of the different interpretations of the diagnostic tests currently used in Spain for BT diagnosis on infected herds after the first BT disclosure SIT test.

2. Materials and methods

2.1. Study population

BT diagnostic tests results from 6202 cattle in 42 herds located in the region of Castilla y Leon, in the west-central area of Spain, were available to us. Cattle herds were randomly selected among those in which BT was detected in the period 2007–2009, including farms belonging to all types of cattle industry present in the region: beef ($n = 11$, 1141 cattle), dairy ($n = 18$, 2563 cattle) and bullfighting ($n = 13$, 2498 cattle). BT-infection was confirmed in the 42 herds by either 1, isolation of *Mycobacterium bovis*/*Mycobacterium caprae* or 2, detection of macroscopical lesions compatible with BT infection. Tests results were obtained coincidently with the first implementation of the IFN-γ assay on each farm, which always followed the application of an intradermal test (performed at least 8 weeks before application of the IFN-γ assay) in which positive reactors were detected (and removed from the herd).

2.2. Diagnostic tests

2.2.1. Single intradermal tuberculin test

SIT test was performed by field veterinarians using bovine PPD (CZ Veterinaria, Porriño, España) according to official rules (RD2611/1996, transposition of annex A of Council Directive 64/432/EEC). Cattle were inoculated with 0.1 mL of a solution containing 0.1 mg of bovine PPD (2500 CTU) on the left side of the neck, and test results were determined by measuring the increase of the skin-fold thickness 72 h later. According with the criteria specified in the Council Directive, results were considered positive when an increase of 4 mm or more in the injection site and/or presence of oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in the region or in the lymph nodes was observed; inconclusive if clinical lesions were absent and the increase was 2–4 mm; and negative if the increase was not larger than 2 mm and clinical signs were absent. Two criteria were used for definition of the infection status:

- *Severe interpretation*: all positive and inconclusive reactors were considered infected.
- *Standard interpretations*: only cattle showing a positive reaction to the SIT test were considered infected.

2.2.2. IFN-γ detection assay

Heparinized blood samples were collected from every animal before PPDs for skin test were inoculated, and delivered to the laboratory within 8 h of collection at room temperature. Stimulation with avian and bovine PPDs was carried out as described elsewhere (Wood et al., 1990) and plasma samples were analysed using a sandwich EIA for detection of bovine IFN-γ (Bovigam™ Bovine Gamma Interferon Test, Prionics, Schlieren, Switzerland). Results were interpreted following procedures described elsewhere (Aranaz et al., 2006). Two cut-off points were selected to define two interpretation criteria for the test; the "severe interpretation" identified an animal as infected if the mean optical density (OD) of a sample stimulated with bovine PPD minus the mean OD of nil antigen was greater than 0.05 and greater than the same value of the sample stimulated with avian PPD (interpretation prescribed in the Spanish eradication program); whereas the "standard interpretation" considered an animal as infected if the same value was above 0.1 and greater than the value obtained after stimulation with avian PPD.