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Short Communication

Serological reactivity to MPB83 and CFP10/ESAT-6 antigens in three suid hosts of *Mycobacterium bovis* infection

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ABSTRACT

Domestic pigs and wild suids are susceptible to *Mycobacterium bovis* (*M. bovis*) infection and may even serve as reservoir hosts in some situations. Therefore, detection of infected animals is important for understanding their role in the epidemiology of the disease as well as for management and control of bovine tuberculosis. Infected suids develop strong humoral responses, making serological screening a feasible approach to disease surveillance. However, to optimize sensitivity of the antibody assays, it is necessary to identify and incorporate immunodominant antigens recognized by the target species. The objective of this study was to characterize the antigen recognition by three suid species in a commercially available serological test, DPP VetTB Assay. Serum samples from naturally *M. bovis*-infected domestic pigs, wild boar and common warthogs were tested. MPB83 protein appeared to be the immunodominant antigen recognized by antibodies in all three species. Overall test sensitivity was increased in wild suids when seroreactivity to CFP10/ESAT-6 antigen was included. Infected animals with visible lesions showed more robust antibody responses than those without gross lesions. The high sensitivity and specificity of the DPP VetTB Assay demonstrated in the present study supports the utility of antibody tests employing these antigens in serological screening of the suid species for *M. bovis* infection.

1. Introduction

Mycobacterium bovis, the main causative agent of bovine tuberculosis (bTB), infects a wide range of hosts, including domestic and wild animals, as well as humans. Some of these species may act as reservoir hosts, as has been demonstrated for wild boar (*Sus scrofa scrofa*) and domestic pigs (*Sus scrofa domesticus*) in Spain and Italy (Parra et al., 2003; Naranjo et al., 2008; Di Marco et al., 2012; Cano-Terriza et al., 2018). Suids, especially feral or wild species, often share grazing and water resources with other livestock and wildlife, therefore it is important to be able to detect *M. bovis* infection to understand their role in mycobacterial transmission and management in order to prevent it. However, there are limited tests available for bTB diagnosis in these species (Pesciaroli et al., 2012), and detection often relies on post-mortem examination, and culture or PCR of tissues (Cardoso-Toset

et al., 2015).

Diagnostic tests for bTB in animals typically rely on detecting cell-mediated immune responses, including tuberculin skin tests and interferon-gamma release assays (Schiller et al., 2010). However, these have limitations including multiple handling events required or time-sensitive processing of fresh blood samples. Alternatively, serological assays measuring humoral responses can provide simple rapid identification of infected animals as well as retrospective surveillance. However, this requires a better understanding of immune recognition of *M. bovis* antigens in each host species.

Unlike many other animals, suids appear to develop detectable humoral responses soon after *M. bovis* exposure, which are maintained during the development of disease (Garrido et al., 2011; Roos et al., 2016). This allows rapid detection of infected animals using serological assays (Boadella et al., 2011). However, to optimize sensitivity of these

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Table 1
Summary of sera tested in the DPP VetTB Assay from *M. bovis*-infected and uninfected suids.

Infection status	Domestic pigs	Wild boar	Warthogs
Uninfected	50	30	35
Infected with NVL ^a	1	21	4
Infected with lesions	41	35	17
Total	92	86	58 ^b

^a NVL – no visible lesions.

^b Two *M. bovis*-infected warthogs did not have report regarding presence or absence of lesions.

assays for use in multiple hosts, it is necessary to identify and incorporate immunodominant antigens recognized by each of the target species. Therefore, the aim of this study was to determine seroreactivity patterns of antibody detection to MPB83 and CFP10/ESAT-6 antigens in three suid species, domestic pigs, wild boar, and warthogs (*Phacochoerus africanus*) naturally infected with *M. bovis*.

2. Materials and methods

Serum specimens were obtained from: 1) 42 *M. bovis*-infected domestic pigs of extensive managed systems (Iberian pig) and 50 uninfected domestic pigs of intensive managed systems (white pig) in Spain; 2) 56 *M. bovis*-infected and 30 uninfected free-ranging wild boar in Spain; and 3) 23 *M. bovis*-infected and 35 uninfected free-ranging warthogs in South Africa (Table 1). Diagnosis of *M. bovis* infection was based on presence of *M. bovis*-compatible gross lesions, culture confirmation and speciation as previously described (Boadella et al., 2011; Roos et al., 2018; Thomas et al., 2019). In the infected groups, 41/42 domestic pigs, 35/56 wild boar, and 17/21 warthogs had gross lesions and *M. bovis* was isolated from all animals (including 2 additional warthogs that did not have records regarding presence of lesions) (Table 1). Uninfected animals were culture-negative endemic controls with no tuberculosis-compatible gross lesions. DPP VetTB Assay (Chembio Diagnostics Inc., Medford, NY, USA) was used to detect serum IgG antibodies to the mycobacterial antigens MPB83 (test line 1) and CFP10/ESAT-6 (test line 2), as previously described (Greenwald et al., 2009). This commercial test is approved by the United States Department of Agriculture for use in cervids and elephants, and it has also shown diagnostic potential in other wildlife species (Lyashchenko et al., 2008). An optical reader (Chembio Diagnostics Inc.) was used to measure antibody binding at each test line in relative light units (RLU). Receiver operator characteristic (ROC) curve analyses were performed using data from known uninfected and *M. bovis*-infected suids to determine species-specific cut-off values. The cut-off values were calculated according to the Youden's index (Youden, 1950). Overall DPP VetTB Assay sensitivity and specificity were calculated using the number of samples that were considered positive on either test line 1 or 2. Sensitivity was calculated as the proportion of test positive samples over the total number of samples from *M. bovis*-infected animals. Specificity was calculated as the proportion of test negative samples over the number of uninfected animals. Seroreactivity rates for each test line (line 1- antibodies to MPB83; line 2-antibodies to CFP-10/ESAT-6) were

Table 2
DPP VetTB assay seroreactivity rates (% and confidence intervals) for MPB83 and CFP10/ESAT-6 antigens in domestic pigs, Eurasian wild boar, and warthogs naturally infected with *M. bovis*.

Host species	Animal number	MPB83 total	CFP10/ESAT-6 total	MPB83 alone	CFP10/ESAT-6 alone	MPB83 and CFP10/ESAT-6	MPB83 or CFP10/ESAT-6
Pig	42	95.2% (83.4-99.0)	80.9% (66.4-90.3)	14.3% (6.3-28.2)	0.0% (0-10.0)	80.9% (66.4-90.3)	95.2% (83.4-99.0)
Wild boar	56	78.6% (66.0-87.4)	46.4% (34.0-59.3)	33.9% (22.9-47.0)	1.7% (0-10.3)	44.6% (32.4-57.6)	80.4% (68.0-88.8)
NVL	35	94.3% (80.4-99.4)	60.0% (43.5-74.5)	37.1% (23.1-53.7)	2.9% (0-15.8)	57.1% (40.8-72.0)	97.4% (84.2-100)
Warthog	23	52.3% (32.4-71.7)	23.8% (10.2-45.5)	28.6% (13.6-50.2)	0% (0-18.2)	23.8% (10.2-45.5)	52.4% (32.4-71.7)
Warthog	23	78.3% (57.7-90.8)	60.9% (40.7-77.9)	21.7% (9.2-42.3)	4.4% (0-22.7)	56.5% (36.8-74.4)	82.6% (62.3-93.6)

calculated individually as well as seroreactivity of animals that were positive for both test lines or the combination if either test line was positive (MPB83 or CFP-10/ESAT-6). Confidence intervals for proportions were calculated using the modified Wald method in Graph Pad (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

The species-specific cut-off values for the DPP VetTB Assay test line 1 (MPB83) and test line 2 (CFP10/ESAT-6), using ROC curve analyses, were 60 and 10 RLU for domestic pigs, 16 and 10 RLU for wild boar, and for warthogs, 3.4 and 4.5 RLU, respectively. Comparing the reactivity to the individual test lines, MPB83 protein was found to elicit more robust IgG antibody responses than did CFP10/ESAT-6 antigen during *M. bovis* infection in all three suid species (Table 2, Fig. 1). The seroreactivity to MPB83 ranged from 78.3% in warthogs to 95.2% in domestic pigs. A lower and more variable response rate was observed for CFP10/ESAT-6, with the highest seroreactivity in *M. bovis*-infected domestic pigs (80.9%) compared to 46.4% in wild boar, with very low numbers of individuals (0–4.4%) showing a response to this antigen solely. In all suid species, most individuals recognized CFP10/ESAT-6 in the presence of detectable responses to MPB83. Interestingly, when the *M. bovis*-infected wild boar cohort was divided into sub-groups that have visible lesions (VL) consistent with bTB or no visible lesions (NVL), both groups had similar patterns of antigen recognition with MPB83 being the immunodominant antigen, although the VL positive animals had much higher rates of seroreactivity to both MPB83 and CFP10/ESAT-6 (Table 2, Fig. 1). While the frequency of MPB83 seroreactivity in VL wild boar was 1.8 times greater than that in NVL animals, that difference was 2.5-fold for CFP10/ESAT-6 reactivity.

Overall sensitivities and specificities of the DPP VetTB Assay in the three suid species relied on the detection of combined responses to MPB83 or CFP10/ESAT-6 (Table 3). High sensitivity was found in all three species, ranging from 80.4% in wild boar to 95.2% in domestic pigs. However, sensitivity was significantly higher in *M. bovis*-infected wild boars with VL (97.4%) than in those with NVL (52.4%) (Table 2, Fig. 1). The DPP VetTB Assay had high specificity in domestic pigs, wild boars and warthogs, ranging between 94.3% and 100%.

4. Discussion

The mycobacterial antigen MPB83 was serodominant in the three naturally *M. bovis*-infected suid species in this study, based on responses in the DPP VetTB Assay. However, seroreactivity to CFP10/ESAT-6 was also observed in infected domestic pigs, wild boars, and warthogs, albeit at lower levels compared to MPB83. Overall sensitivity and specificity of the DPP VetTB Assay (combination of responses to MPB83 or CFP10/ESAT-6 antigens) in these species was high, suggesting that this test has promising diagnostic potential.

MPB83 is one of the immunodominant antigens recognized most commonly by antibodies from *M. bovis* infected cattle, white-tailed deer, wild boar, warthogs, and badgers (Lyashchenko et al., 2008, 2018; Roos et al., 2016). This may be related to the high level of protein expression by *M. bovis* (Wiker, 2009). The majority of *M. bovis*-infected pigs, wild boar, and warthogs in this study had antibodies to this single

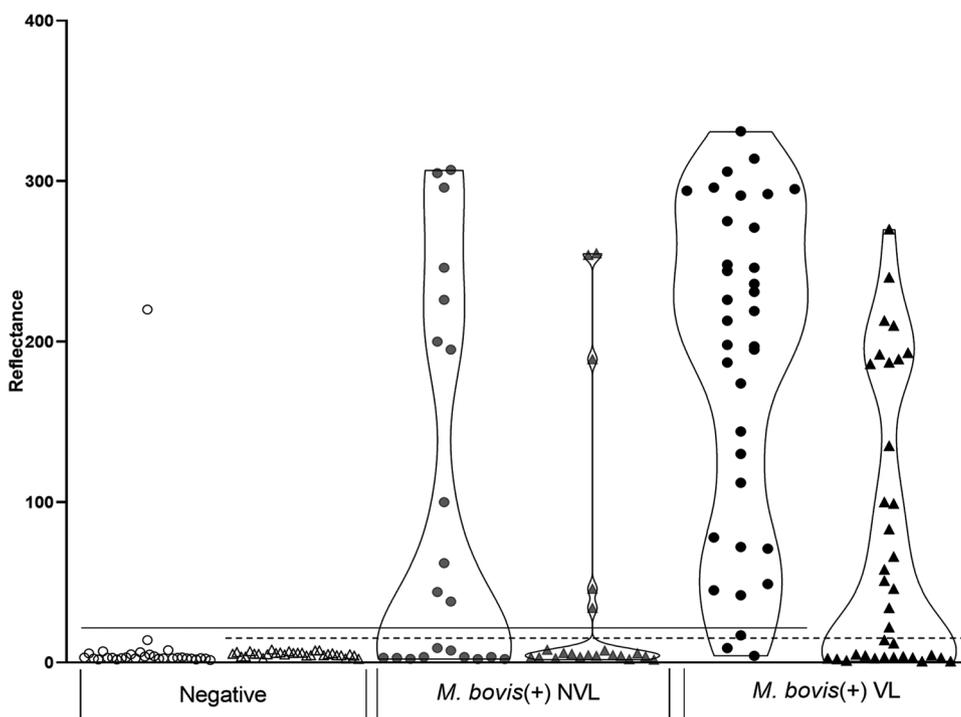


Fig. 1. Antibody responses to MPB83 and CFP10/ESAT-6 in wild boar infected with *M. bovis*. Results are shown as DPP reader values (reflectance in RLU) obtained in DPP VetTB Assay with serum samples from 30 uninfected (open symbols) and 56 *M. bovis*-infected wild boar including 21 NVL (gray symbols) and 35 VL animals (black symbols). Individual antibody levels to MPB83 and CFP10/ESAT-6 in each group are shown as circles and triangles, respectively. Horizontal solid line and dotted line represent cutoff values, 16 and 10 RLU for MPB83 and CFP10/ESAT-6, respectively. Violin plot (Prism, version 8.0) was applied to characterize data distribution and highlight differences between the animal groups.

Table 3

Overall diagnostic sensitivity and specificity (confidence intervals) of DPP VetTB Assay in different suid species.

	Domestic pigs	Wild boar	Warthogs
Sensitivity	95.2% (83.4–99.5)	80.4% (68.0–88.8)	82.6% (62.3–93.6)
Specificity	100% (91.5–100)	96.7% (81.9–100)	91.4% (76.9–97.8)

antigen, which suggests it should be included in any serological assay developed for suids. However, additional studies should investigate cross-reactivity to MPB83 in non-tuberculous mycobacteria-infected suids. Since it is also an immunodominant antigen in other hosts of *M. bovis* infection, it supports the use of the DPP VetTB Assay for bTB screening of a wide range of species.

The proteins CFP10 and ESAT-6 are absent in most non-pathogenic mycobacteria, therefore are ideal candidate targets for accurate diagnostic tests (Lyashchenko et al., 2008). Although this study showed lower seroreactivity to CFP10/ESAT-6, especially by wild boar, combined responses to this antigen and MPB83 provided optimal sensitivity of the DPP VetTB Assay. These antigens have previously shown improved sensitivity of serological assays in *M. tuberculosis*-infected elephants, *M. bovis*-infected deer, badgers and wild boar (Lyashchenko et al., 2008; Greenwald et al., 2009). In addition, these antigens are present in *Mycobacterium caprae*, which has been isolated from wild boar and pigs in Spain (Rodriguez et al., 2011). Therefore, the DPP VetTB Assay may be a valuable tool for screening suids for both *M. bovis* and *M. caprae* infections.

Although MPB70 is not included as an antigen in the current DPP VetTB Assay, studies have shown that humoral responses to this antigen can be used for diagnosis of bovine tuberculosis (Bezoz et al., 2014). Therefore, addition of this antigen to future serological tests for TB in suids should be investigated.

The *M. bovis*-infected wild boar in this study could be divided into two subsets based on the presence or absence of VL consistent with bTB. As expected, the sensitivity of the DPP VetTB Assay was increased in the VL animals. However, the pattern of predominant antigen recognition did not differ between the sub-groups.

Among the three suid species studied, domestic pigs had

particularly impressive diagnostic accuracy of the DPP VetTB Assay demonstrating 95.2% sensitivity and 100% specificity. In light of the wild boar data on stratification between VL and NVL animals, it should be noted that, since the vast majority of the *M. bovis*-positive pigs had gross lesions (41/42), the true sensitivity of the antibody tests for bTB might be overestimated. Despite this, the DPP VetTB Assay appears to be more sensitive than other previously reported serological assays for suids, including the bovine purified protein derivative ELISA in wild boar (Se = 72.6%; Aurtentxe et al., 2008) and warthogs (Se = 88%; Roos et al., 2016). Therefore, using a combination of specific mycobacterial peptides for antibody detection appears to increase sensitivity while maintaining high specificity.

The heterogeneity of humoral responses between and within the three suid species described in the present report reflects the natural diversity in host-pathogen relationships during *M. bovis* infection. Our recent analyses of differential immune recognition of the two antigens used in the DPP VetTB Assay in tuberculous cattle, elephants, and three cervid species revealed even more variation in antibody specificity profiles among the hosts (Lyashchenko et al., 2018). Therefore, development of new and improved serologic tests for bTB that could be used for multiple domestic and wildlife animals will require identification of novel disease biomarkers, detailed characterization of the antibody profiles in each target species, and balanced combinations of rationally selected mycobacterial antigens.

5. Conclusion

The patterns of seroreactivity to MPB83 and CFP10/ESAT-6 in *M. bovis*-infected domestic pigs, wild boar and warthogs were similar among the host species, with predominant recognition of MPB83 by the majority of animals. Elevated antibody responses to both antigens were associated with the presence of VL in the infected wild boar. The high sensitivity and specificity of the DPP VetTB Assay in suids demonstrates the utility of this assay for bTB surveillance in these species.

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