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Short communication

Spectrum of antibody profiles in tuberculous elephants, cervids, and cattle

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ABSTRACT

Using multi-antigen print immunoassay and DPP® VetTB Assay approved in the United States for testing captive cervids and elephants, we analyzed antibody recognition of MPB83 and CFP10/ESAT-6 antigens in Asian elephants (*Elephas maximus*) infected with *Mycobacterium tuberculosis* and in white-tailed deer (*Odocoileus virginianus*), fallow deer (*Dama dama*), elk (*Cervus elaphus*), and cattle (*Bos taurus*) infected with *Mycobacterium bovis*. Serum IgG reactivity to MPB83 was found in the vast majority of tuberculous cattle and cervid species among which white-tailed deer and elk also showed significant CFP10/ESAT-6 recognition rates with added serodiagnostic value. In contrast, the infected elephants developed antibody responses mainly to CFP10/ESAT-6 with MPB83 reactivity being relatively low. The findings demonstrate distinct patterns of predominant antigen recognition by different animal hosts in tuberculosis.

1. Introduction

Tuberculosis (TB) has a broad range of mammalian host species (Schiller et al., 2010; Waters et al., 2014). *Mycobacterium bovis* is most commonly isolated from infected cattle and cervids, whereas certain captive wildlife may be also susceptible to *M. tuberculosis* (Greenwald et al., 2009; Lyashchenko et al., 2008, 2012a,b). Both free-ranging and farmed deer are involved in *M. bovis* transmission to cattle (Buddle et al., 2010; Waters et al., 2011; Busch et al., 2017). With both *M. bovis* and *M. tuberculosis*, infection may be transmitted from animals to man and vice versa (Murphree et al., 2011; Lyashchenko et al., 2012a,b; Müller et al., 2013; Ramdas et al., 2015).

Ante-mortem testing for TB in various host species is traditionally based on measuring cell-mediated immune responses, such as tuberculin skin tests or in vitro interferon-gamma release assays (Schiller et al., 2010). In addition, serological approaches have recently offered attractive options for simple and rapid identification of infected animals (Greenwald et al., 2009; Boadella et al., 2012; Waters et al., 2017). The DPP® VetTB Assay (Chembio Diagnostic Systems, Inc., Medford, NY, USA), which is approved by the United States Department of Agriculture for use in elephants and cervids, was designed to detect antibodies against two key antigens, MPB83 and CFP10/ESAT-6, independently (Greenwald et al., 2009; Lyashchenko et al., 2013). This feature of the immunoassay provides useful information for better understanding of immune recognition. In the present study, we compared antibody reactivity rates obtained for the two antigens in tuberculous cattle (*Bos taurus*), white-tailed deer (WTD) (*Odocoileus virginianus*), elk (*Cervus elaphus*), fallow deer (*Dama dama*), and Asian elephants (*Elephas maximus*).

2. Materials and methods

Serum specimens were obtained from: 1) 27 cattle with naturally acquired *M. bovis* infection in a dairy herd, New Mexico, USA (Connell, 2008); 2) 32 free-ranging WTD infected with *M. bovis* in Michigan, USA (Lyashchenko et al., 2013); 3) 34 farmed elk and 32 fallow deer identified in one *M. bovis*-infected herd in Nebraska, USA (Waters et al., 2011); 4) 33 *M. bovis*-infected fallow deer from Spain including both free-ranging (n = 21) and farmed (n = 12) animals (Boadella et al., 2012); and 5) 12 Asian elephants diagnosed with TB due to *M. tuberculosis* in the USA (Lyashchenko et al., 2012a,b).

Samples from cattle and elk in the USA, and fallow deer in Spain were collected several weeks to several months after tuberculin skin tests performed in accordance with government regulations, as described (USDA and APHIS, 2007; Waters et al., 2011; Boadella et al., 2012). No tuberculin skin tests were administered to the elephants,

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Table 1
Antibody reactivity rates (%) for MPB83 and CFP10/ESAT-6 antigens in MAPIA testing of tuberculous cattle, fallow deer, elk, and elephants.

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
Cattle	27	77.8%	14.8%	63.0%	0.0%	14.8%	77.8%
Fallow deer	32	96.9%	25.0%	71.9%	0.0%	25.0%	96.9%
Elk	34	67.7%	76.5%	5.9%	14.7%	61.8%	82.4%
Elephants	12	33.3%	91.7%	8.3%	66.7%	25.0%	100.0%

For fallow deer, MAPIA results were only available for 32 specimens from the USA.
Data shown in the last column (MPB83 or CFP10/ESAT-6) reflects diagnostic sensitivity estimates for each host species.

WTD, and fallow deer in the USA.

Post-mortem diagnosis was based on the presence of mycobacteriosis-compatible gross and microscopic lesions as well as isolation of *M. bovis* or *M. tuberculosis* from lesioned tissues (Waters et al., 2011, 2017; Boadella et al., 2012; Greenwald et al., 2009). Serum IgG antibodies to MPB83 and CFP10/ESAT-6 were detected by DPP® VetTB and multi-antigen print immunoassay (MAPIA) as described previously (Lyashchenko et al., 2012a,b, 2013).

3. Results

We found that the relative rates of serum antibody reactivity to MPB83 and CFP10/ESAT-6 proteins used in DPP VetTB Assay and MAPIA varied significantly in different animal species (Table 1, Fig. 1A). Cattle and fallow deer infected with *M. bovis* predominantly recognized MPB83, with no reactivity to CFP10/ESAT-6 alone. In contrast, the majority of tuberculous elephants had antibodies to CFP10/ESAT-6. In WTD and elk, serum antibodies to the two antigens were detected at similar rates; furthermore, the proportion of elk with seroreactivity to CFP10/ESAT-6 alone was slightly higher than that found for MPB83 alone (Fig. 1B). As a result, the frequency of MPB83 recognition in the absence of antibody to CFP10/ESAT-6 in fallow deer was over 10-fold of that observed in elk from the same *M. bovis* outbreak in Nebraska (Table 1).

The USA findings with fallow deer were consistent with the results of DPP VetTB testing of fallow deer in Spain showing nearly 2-fold higher seroreactivity rates for MPB83 (72.7%) as compared to CFP10/ESAT-6 protein (36.4%). For this host species, no significant differences in antibody profiles were found between the two countries or between free-ranging and farmed cervids (data not shown).

Fig. 2 shows MAPIA data comparing the antibody responses in the infected elephants and fallow deer, illustrating differential antibody reactivity profiles. The last column of Table 1 reflects the serodiagnostic sensitivity estimates for each host species irrespective of the antigen recognition pattern.

4. Discussion

In the present study, we used MAPIA and DPP VetTB Assay to compare antibody reactivity profiles with two antigens, MPB83 and CFP10/ESAT-6, in different animal species. Elephants and cervids were selected as the animal groups approved for DPP VetTB Assay application, whereas cattle were included in MAPIA studies as an important bovine TB reference. Depending on the host, the diagnostic sensitivity of DPP VetTB Assay ranged from 79% in elk to 100% in elephants. The specificity evaluated previously in elephants and cervids ranged from 98 to 100% (Greenwald et al., 2009; Waters et al., 2011; Boadella et al., 2012).

The predominant recognition of MPB83 antigen by serum antibodies during *M. bovis* infection in various animal species is well-documented (Lyashchenko et al., 2008; Schiller et al., 2010; Waters et al., 2014, 2017; Roos et al., 2016). This makes the findings of relatively high CFP10/ESAT-6 seroreactivity rates reported here for elk and WTD somewhat surprising. It is well-known that during *M. bovis* infection antibody responses to certain antigens can be boosted by a preceding skin test administration (Waters et al., 2014). Therefore, one may speculate that the unexpected antigen recognition pattern in elk could stem from the antibody boosting effect following injection of tuberculin 3–4 months before blood collection; given that the fallow deer predominantly recognized MPB83 and were not skin tested (Waters et al., 2011). However, this assumption would hardly explain why the free-ranging WTD (not skin tested) recognized the two antigens equally well and why MPB83 response rates in the USA fallow deer (not skin tested) were still considerably higher (~91%) than that found in the skin tested elk (~79%); which is in sharp contrast to the CFP10/ESAT-6 reactivity rates (~28% in fallow deer vs. ~74% in elk). Another contributing factor could stem from differing *M. bovis* strains, but this possibility is unlikely, as the elk and fallow deer infected in the Nebraska outbreak were infected with identical *M. bovis* spoligotypes (Waters et al., 2011) and whole genome sequencing of 27 isolates from this outbreak revealed no significant difference between fallow deer

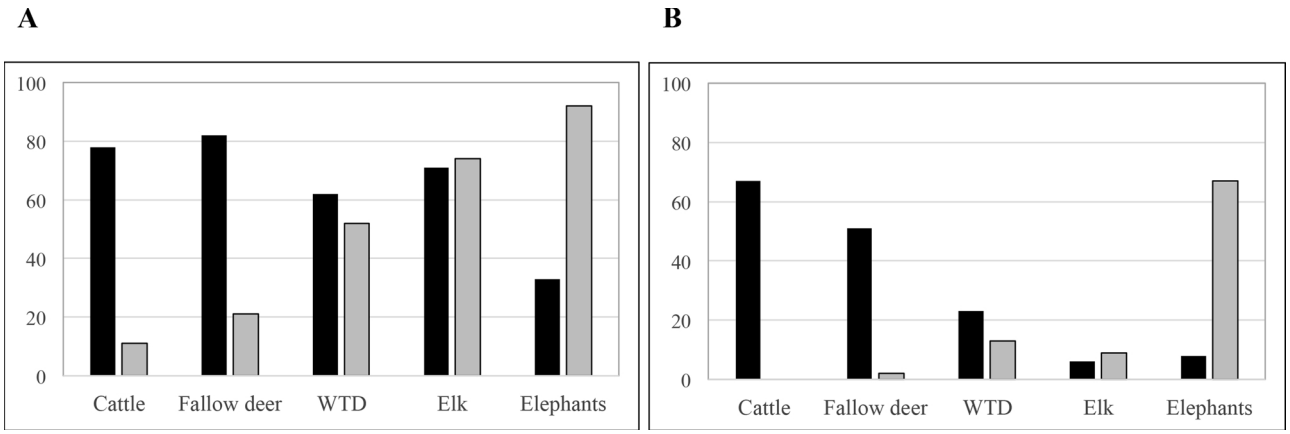

Fig. 1. Antigen recognition rates (%) obtained for MPB83 (dark bars) and CFP10/ESAT-6 (gray bars) in DPP VetTB Assay with serum samples from tuberculous cattle (n = 27), fallow deer (n = 65), white-tailed deer (WTD) (n = 32), elk (n = 34), and elephants (n = 12). A, total rates of IgG seroreactivity per antigen; B, rates of IgG seroreactivity to each antigen alone in the absence of antibody to the other antigen.