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Animal-side serologic assay for rapid detection of *Mycobacterium bovis* infection in multiple species of free-ranging wildlife

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Abstract

Numerous species of mammals are susceptible to *Mycobacterium bovis*, the causative agent of bovine tuberculosis (TB). Several wildlife hosts have emerged as reservoirs of *M. bovis* infection for domestic livestock in different countries. In the present study, blood samples were collected from Eurasian badgers ($n = 1532$), white-tailed deer ($n = 463$), brushtail possums ($n = 129$), and wild boar ($n = 177$) for evaluation of antibody responses to *M. bovis* infection by a lateral-flow rapid test (RT) and multiantigen print immunoassay (MAPIA). Magnitude of the antibody responses and antigen recognition patterns varied among the animals as determined by MAPIA; however, MPB83 was the most commonly recognized antigen for each host studied. Other seroreactive antigens included ESAT-6, CFP10, and MPB70. The agreement of the RT with culture results varied from 74% for possums to 81% for badgers to 90% for wild boar to 97% for white-tailed deer. Small numbers of wild boar and deer exposed to *M. avium* infection or paratuberculosis, respectively, did not cross-react in the RT, supporting the high specificity of the assay. In deer, whole blood samples reacted similarly to corresponding serum specimens (97% concordance), demonstrating the potential for field application. As previously demonstrated for badgers and deer, antibody responses to *M. bovis* infection in wild boar were positively associated with advanced disease. Together, these findings suggest that a rapid TB assay such as the RT

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may provide a useful screening tool for certain wildlife species that may be implicated in the maintenance and transmission of *M. bovis* infection to domestic livestock.

Keywords: Tuberculosis; *Mycobacterium bovis*; Wildlife; Serology

1. Introduction

Bovine tuberculosis (TB) remains an important zoonotic disease with significant impacts on the economy in many countries (Corner, 2006; Cosivi et al., 1998; Michel et al., 2006). Several wild mammal species are implicated in the maintenance and transmission of *Mycobacterium bovis* infection and thereby impede national bovine TB control programs and international trade (Cousins, 2001; Palmer, 2007). Well-known examples of wildlife maintenance hosts include Eurasian badgers (*Meles meles*) in Great Britain and Ireland (Clifton-Hadley et al., 1993; Griffin et al., 2005), white-tailed deer (*Odocoileus virginianus*) in the United States (Schmitt et al., 1997; O'Brien et al., 2002, 2006), brushtail possums (*Trichosurus vulpecula*) in New Zealand (Coleman et al., 2006; Porphyre et al., 2007), wild boar (*Sus scrofa*) in Spain (Gortázar et al., 2003; Naranjo et al., 2008), and African buffalo (*Syncerus caffer*) in South Africa (Michel et al., 2006).

The successful eradication of bovine TB from livestock in countries with a wildlife reservoir is likely to require focusing on the wildlife reservoir(s) of *M. bovis* infection (Palmer, 2007). Current methods of diagnosis in live animals, such as the intradermal tuberculin test or interferon-gamma (IFN-γ) release assays, are not fully validated for species other than cattle (Monaghan et al., 1994). Rapid and accurate assays that could be used in a variety of *M. bovis*-susceptible mammals under field conditions would significantly improve wildlife TB surveillance efforts worldwide.

Prior studies have demonstrated the utility of membrane-based assays to detect specific antibodies during TB in cattle (Waters et al., 2006), cervids (Waters et al., 2004, 2005), elephants (Lyashchenko et al., 2006), camelids (Wernery et al., 2007; Lyashchenko et al., 2007a,b), and tapirs (Moser et al., 2008). The objectives of the present study were to (1) characterize the antibody responses of wild mammals infected with *M. bovis*, including badgers, deer, possums, and wild boar, (2) evaluate the usefulness of a lateral-flow test under field conditions, and (3) determine if a single serologic assay can be used for TB surveillance in a variety of different wildlife host species.

2. Methods

2.1. Animals and samples

Sera were collected from Eurasian badgers in Great Britain, white-tailed deer in the United States, brushtail possums in New Zealand, and wild boar in Spain and Portugal (Table 1).

Badger sera were obtained from two sources: (1) 1464 animals killed as part of the Randomised Badger Culling Trial (RBCT) (Donnelly et al., 2007), and (2) 68 animals captured, bled, and released as part of an on-going ecological study by the Central Science Laboratory (CSL) in Woodchester Park, south-west

Table 1
Study populations

Species	Institute, country	Number of animals	
		TB	Control
Badger	VLA, UK	386	1078
	CSL, UK	68	0
Deer	MDNR, USA	9	425
	NADC, USA	19[a]	10[b]
Possum	AgR HRI, New Zealand	38	91
Wild boar	IREC, Spain	50	50
	ICVS, Portugal	14	63[c]

[a] Inoculated with *M. bovis* intratonsilarly.
[b] Includes three animals experimentally infected with *M. avium* subsp. *paratuberculosis.*
[c] Includes three animals with *M. avium* and five animals with other non-TB mycobacteria identified by culture and PCR at necropsy.

England (Chambers et al., 2008). All work with animals was conducted under licenses issued by the Home Office, UK, following ethical clearance by the VLA and CSL. All sera were stored frozen at −20 °C until used for testing. Each badger from the RBCT was subjected to routine post-mortem examination and culture for the presence of *M. bovis* as described in Sawyer et al. (2007). The infection status of Woodchester Park badgers was determined by bacterial culture of clinical specimens (feces, urine, sputum, purulent exudate from abscesses, and bite wound swabs) collected from anaesthetized animals (Clifton-Hadley et al., 1993; Delahay et al., 2000). Since Woodchester Park badgers were live-sampled, only those animals excreting *M. bovis* were included in the present study.

Deer samples were obtained from experimentally infected animals at different stages of disease. White-tailed deer (1–3 years of age) were either raised within a TB-free herd at the National Animal Disease Center (NADC), Ames, Iowa, USA, or obtained from farmed herds with no history of TB. Groups consisted of seven non-infected animals, three deer inoculated with *M. avium* subsp. *paratuberculosis*, and 19 deer inoculated via the intratonsilar route with various doses of *M. bovis* (3×10^2–2×10^8 colony-forming units), as described previously (Waters et al., 2004; Palmer et al., 2007). All animals were euthanized at 4–11 months post-infection. Blood, diaphragm fluid and aqueous humor were collected during necropsy of experimentally infected deer. Specimens were extracted from diaphragms by freeze/thaw and mechanical disruption (using a garlic press). Aqueous humor was obtained by fine needle aspiration. Various tissues were collected for bacteriologic culture and microscopic examination. Disease was confirmed at necropsy in each infected deer by the presence of gross lesions, histopathological examination, and mycobacterial culture. The Institutional Animal Care and Use Committee approved protocols detailing procedures and animal care prior to initiation of the experiments.

In addition, fresh whole blood samples were collected from 434 free-ranging white-tailed deer in Michigan State where *M. bovis* infection is persistent in wild deer (Schmitt et al., 1997). The deer enrolled in the study inhabited the ''core'' area of the Michigan TB outbreak area (O'Brien et al., 2002). Blood samples were obtained from four sources: (1) culls of free-ranging deer conducted by hunters during 2005/2006 and 2006/2007 winter seasons as part of herd-health checks; (2) depopulation of a fenced captive deer shooting preserve in late 2006 following culture confirmation of grossly lesioned *M. bovis*-positive deer; (3) nuisance deer shot on cattle farms under disease control permits; and (4) deer tested as part of an ongoing live trap/test/cull project. Tissue samples used for culture included medial retropharyngeal lymph nodes, parietal pleura and lungs.

A group of brushtail possums naturally infected with *M. bovis* consisted of 29 animals captured at Castlepoint, Wairarapa and nine possums from the Orongorongo Valley near Wellington. All these had macroscopic TB lesions, from which *M. bovis* was cultured. Blood was collected from all of the animals immediately prior to euthanasia. A negative control group included 91 possums captured in Manawatu, an area of New Zealand that is free of bovine TB in domestic livestock and wild animals.

Hunter-harvested wild boar were sampled in Spain between November 1999 and February 2005 ($n = 100$) and in Portugal between December 2005 and January 2007 ($n = 77$). Various lymph nodes were examined for gross lesions and cultured for *M. bovis* as described previously (Gortázar et al., 2003; Martin-Hernando et al., 2007). Portuguese mycobacterial isolates were identified by PCR for a panel of selected genes: 16S RNA, IS1081, Rv3120, Rv1510, and IS1245, and confirmed by spoligotyping. According to Huard et al. (2003) and Bartos et al. (2006), this set of genes allows for differentiation between *M. bovis* or other members of the *M. tuberculosis* complex and *M. avium* or other non-TB mycobacteria.

2.2. Multiantigen print immunoassay (MAPIA)

We used a panel of 12 mycobacterial antigens including 8 purified recombinant proteins (ESAT-6, CFP10, MPB64, MPB59, MPB70, MPB83, Acr1, and the 38 kDa protein), two protein fusions (CFP10/ESAT-6 and Acr1/MPB83), and two native antigens, bovine protein purified derivative (B-PPD) and *M. bovis* culture filtrate (MBCF). MAPIA was performed as described previously (Lyashchenko et al., 2000). Briefly, antigens were immobilized on a nitrocellulose membrane (Schleicher & Schuell, Keene, N.H.) at a protein concentration of 0.05 mg/ml using a semi-automatic micro-aerosolization device (Linomat IV,

Camag Scientific Inc., Wilmington, Delaware) to generate invisible parallel bands. After antigen printing, the membrane was cut into 3 mm wide strips, perpendicular to the antigen bands, so that each strip carried all antigens. Strips were blocked for 1 h with 1% non-fat skimmed bovine milk in phosphate-buffered saline containing 0.05% Tween 20 and then incubated with individual serum samples diluted 1:50 in blocking solution for 1 h at room temperature. After washing, the strips were incubated for 1 h with peroxidase-conjugated protein G (deer, wild boars) or protein A (badgers, possums) diluted 1:1000 (Kirkegaard & Perry Laboratories), and subsequently washed again. IgG antibodies bound to immobilized antigens were visualized with 3,3′,5,5′-tetramethyl benzidine (Kirkegaard & Perry Laboratories). MAPIA results were read visually, with a band of any intensity being considered as a positive reaction.

2.3. Rapid test (RT)

A simple and rapid antibody detection assay was developed by Chembio Diagnostic Systems, Inc. using colored latex-based lateral-flow technology and a cocktail of selected *M. bovis* antigens including ESAT-6, CFP10, and MPB83 (Greenwald et al., 2003; Waters et al., 2006). Serum specimens were tested for the presence of specific antibody as previously described (Lyashchenko et al., 2006). Results were read at 20 min after adding sample buffer. Any visible band in the test area of the RT, in addition to the control line, was considered an antibody positive result, whereas no band in the test area in addition to the visible control line was considered a negative result.

2.4. Data analysis

Diagnostic performance of the RT was evaluated against the gold standard of *M. bovis* culture by calculating test sensitivity, specificity, and accuracy (% of RT results in agreement with TB status determined by culture) using available software (Lowry, 2007) and reported with the 95% confidence interval (CI). Test of significance between proportions (Fisher's exact test) and the calculation of odds ratio were performed using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA, www.graphpad.com. The statistical significance of the proportion of antibody detection rate in lesion positive wild boar in relation to those without visible lesions was performed by Chi-square tests (SPSS 11.0 Statistical Program).

3. Results

3.1. Antibody responses and antigen recognition

To compare antibody responses to *M. bovis* infection in different hosts, serum samples of all animals (possums) or of randomly selected culture-positive and culture-negative animals from naturally infected populations (badgers, deer, wild boar) were tested by MAPIA. Fig. 1 provides representative examples of the individual antigen reactivity patterns in each of the four mammal species. Table 2 shows the frequencies of IgG reactivity to *M. bovis* antigens found in infected and control animals. The MAPIA results confirmed our previous observations that MPB83 protein is serodominant in badgers with *M. bovis* infection and in experimentally infected white-tailed deer (Greenwald et al., 2003; Waters et al., 2004). The present study further revealed that this molecule is the most reactive antigen in brushtail possums and wild boar. In the naturally infected animals, the MPB83 seroreactivity rates ranged from 34% in possums to 89% in deer, whereas in culture-negative controls it varied from 1% in deer to 10% in badgers. ESAT-6 and CFP10 antigens were the second and third most frequently recognized antigen in the MAPIA in all the species, except for wild boar. MPB70 protein elicited serological responses in greater numbers of infected wild boar (68%) than did ESAT-6 (58%) or CFP10 (52%), although levels of MPB70 antibodies (evaluated visually by band intensity) were commonly lower than those of ESAT-6, CFP10, or MPB83 antibodies (Fig. 1). The other single proteins of *M. bovis* used in MAPIA reacted with variable numbers of sera from infected animals, ranging from 0% to 44%. Significant seroreactivity was found for MBCF, with a few *M. bovis*-infected animals displaying antibody only to this antigen (Fig. 1, B4). However, the crude native preparation of *M. bovis* showed high rates of false-positive results (from 10% in badgers to 26% in deer).

Heterogeneous antigen recognition patterns were observed in each host species, so that there was no

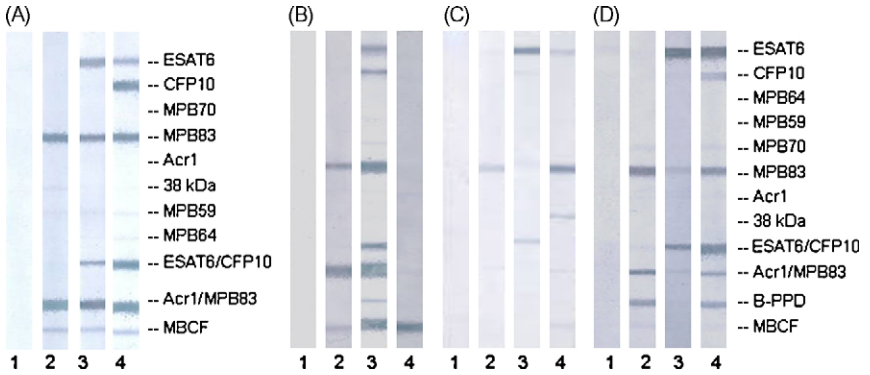

Fig. 1. Antibody responses detected by MAPIA in Eurasian badgers (A), white-tailed deer (B), brushtail possums (C), and European wild boar (D) naturally infected with *M. bovis*. Antigens printed onto nitrocellulose membrane, as described in Section 2, are indicated on the right. One control serum from culture-negative animal (strip 1) and three sera from different culture-positive animals (strips 2–4) were selected to show variable antigen recognition patterns in each host species. Visible bands of any intensity observed with certain antigen are IgG antibody positive reactions with respective antigens.

single antigen target common to all seropositive animals. It was demonstrated that the serological performance of the two antigen fusion proteins, CFP10/ESAT-6 and Acr1/MPB83, reflected the reactivity of the corresponding single antigens (Fig. 1). Subjective evaluations of the MAPIA band intensities and the numbers of antigens recognized by each animal in this study suggested that the strongest antibody responses were found in wild boars, whereas possums produced relatively weak antibody responses to *M. bovis* infection.

3.2. Antibody detection by rapid test

Serological evaluation of the RT revealed variable diagnostic performance when used in different host species (Table 3). Higher test sensitivity and specificity were found for deer and wild boar, compared to those observed in badgers and possums. The overall accuracy of the test ranged from 74% in possums to 97% in deer. When the diagnostic sensitivity was analyzed separately for deer with experimental and natural *M. bovis* infections, a lower rate of serological detection was found in the latter group (67%) than in animals inoculated intratonsilarly with high dose of *M bovis* (79%). To further gauge the RT specificity, serum samples from three deer developing experimental paratuberculosis (all three had strong antibody responses, as previously shown by Palmer et al., 2007) and from eight wild boar naturally infected with *M. avium* or other non-TB mycobacteria were tested. None of these 11 animals produced a positive RT result.

Table 2
Seroreactivity rates (%) of *M. bovis* antigens obtained in MAPIA with sera from naturally infected and uninfected animals

Antigen	Badger		Deer		Possum		Wild boar	
	TB ($n = 15$)	Control ($n = 29$)	TB ($n = 9$)	Control ($n = 98$)	TB ($n = 38$)	Control ($n = 91$)	TB ($n = 50$)	Control ($n = 50$)
MPB83	67	10	89	1	34	3	78	2
ESAT-6	27	0	67	2	21	1	58	8
CFP10	13	0	56	1	3	4	52	2
MPB70	7	0	44	0	2	0	68	0
38 kDa	7	0	11	1	2	3	30	0
Acr1	7	0	22	0	0	1	22	0
MPB64	13	0	11	1	0	0	2	0
MPB59	27	7	44	3	0	0	0	0
MBCF	53	10	89	26	34	20	44	12

Table 3
Diagnostic performance of rapid test in different host species

Species	Prevalence of infection (%) (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Accuracy (%) (95% CI)
Badger	29.6 (27.6–32.2)	50.7 (46.0–55.3)	93.1 (91.4–94.5)	80.5 (78.5–82.5)
Deer	6.0[a] (4.1–8.7)	75.0 (54.8–88.6)	98.9 (97.2–99.6)	97.4 (95.4–98.6)
Possum	29.5 (21.9–38.2)	44.7 (29.0–61.5)	85.7 (76.4–91.9)	73.6 (65.0–80.8)
Wild boar	36.2 (29.2–43.7)	76.6 (64.0–85.9)	97.3 (91.9–99.3)	89.8 (84.2–93.7)

[a] Calculated prevalence of *M. bovis* infection is not representative for wild deer populations, as the TB group in this study included experimentally infected deer.

3.3. Antibody in various types of biological samples

Serum, plasma, fresh whole blood, diaphragm fluid, and aqueous humor were collected from three white-tailed deer experimentally infected with *M. bovis* and one non-infected deer. All the samples from the infected deer, except for the aqueous humor, yielded positive responses (Table 4). The intensity of RT reactions obtained with plasma, whole blood, and diaphragm fluid were comparable to that found for serum samples. No antibody reactivity was detected in any of the biological samples collected from the control deer. The MAPIA demonstrated that the antigen recognition patterns of antibodies found in diaphragm fluid and serum were similar (Fig. 2).

The use of whole blood in RT was more extensively evaluated in the Michigan study where samples were collected from 434 deer and tested under field conditions, followed by testing sera from the same animals in a blind-coded fashion. Nine deer were found to have TB lesions, from which *M. bovis* was cultured. Six of the infected animals were RT-reactive when serum samples were used, of which five deer produced antibody positive results with whole blood samples as well. Overall, the concordance between use of serum and whole blood was 96.8% (94.5–98.2 at 95% CI).

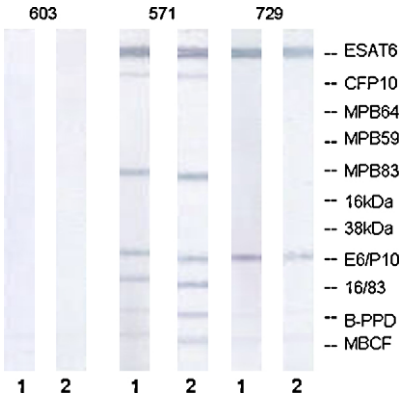

Fig. 2. MAPIA results obtained with sera (strip 1) and diaphragm fluids (strip 2) of white-tailed deer experimentally infected with *M. bovis* (571 and 729) and negative control (603). Antigens printed onto nitrocellulose membrane, as described in Section 2, are indicated on the right. Visible bands of any intensity observed with certain antigen are IgG antibody positive reactions with respective antigens.

3.4. Serology and severity of disease

A close association between strong antibody responses and the presence of gross lesions in wild boar infected with *M. bovis* was noticed (Table 5). While the overall sensitivity of the RT was 77%, the antibody detection rate in the boar with macroscopic lesions was five times higher (83%) than that in

Table 4
Antibody detection by rapid test in various types of biological samples from white-tailed deer experimentally inoculated with *M. bovis*

Group	Animal ID no.	Serum	Plasma	Whole blood	Diaphragm juice	Aqueous humor
Uninfected	603	–	–	–	–	–
M. bovis-infected	528	++	++	++	++	–
	571	+++	+++	+++	++	–
	729	++	++	+	++	–

Test results were scored visually as strong (+++), moderate (++), or weak (+) reactions, or non-reactive (–).

Table 5
Association between the presence of gross lesions and positive rapid test results in infected wild boar

Test	Visible lesions	No visible lesions	Total
RT-positive	48	1	49
RT-negative	10	5	15
Total	58	6	64

animals without visible lesions (17%). The difference was highly significant (χ^2 13.24, d.f. 1, $p = 0.0003$; $p = 0.002$, Fisher's exact test), with an odds ratio of 24 (2.5–228.4, 95% CI). In other words, the boar with gross lesions were on average 24 times more likely to be positive in the RT than those without visible lesions. With samples from Portugal, where most of the *M. bovis*-infected wild boar without lesions originated, all 10 animals with gross lesions were RT-reactive, whereas none of the four culture-positive boar without lesions had detectable antibodies.

4. Discussion

The present study characterized serological responses in infected animals of four species of wild mammals that are implicated in the persistence of *M. bovis* infection in cattle in different countries. Our results demonstrated that tuberculous badgers, white-tailed deer, brushtail possums, and wild boar produced variable levels of IgG antibodies against several *M. bovis* antigens. Importantly, the responses could be detected in each host by a recently developed 'point-of-care'-type lateral-flow test for bovine TB (Lyashchenko et al., 2006; Waters et al., 2006). MAPIA revealed antibodies to multiple proteins of *M. bovis* with heterogeneous antigen recognition patterns observed in the infected animals. These findings are in agreement with our previous reports on human TB serology (Lyashchenko et al., 2000) as well as other mammal species susceptible to organisms of the *M. tuberculosis* complex (Lyashchenko et al., 2004, 2006, 2007a,b; Waters et al., 2005, 2006). The serodominance of the MPB83 protein known to elicit the strongest antibody responses to experimental TB infection in cattle, badger, and deer (Lesellier et al., 2008; Lyashchenko et al., 2004; Waters et al., 2004) was extended in the present study to naturally infected animals as well as other, previously untested species (possums and wild boar).

Antibody detection methods are generally simple, rapid, and inexpensive. The lateral-flow assay format offers important features that make the RT evaluated in the present study an attractive screening tool for field applications. This is an easy-to-perform animal-side disposable kit which can use serum, plasma, whole blood, or other samples to provide ''yes-or-no'' visual read-outs within 15–20 min. The test speed is particularly useful for wildlife surveillance where a ''euthanize or release'' decision may be needed quickly on a physically restrained animal. The RT kits are stable at room temperature for up to 18–24 months, and they do not require refrigeration for storage, a power source, equipment, laboratory environment, or skilled personnel to perform the assay and interpret results. The test is suitable for use under field conditions, as it can be minimally affected by extremes of ambient temperature. Test results are unambiguous; high levels of reproducibility (lot-to-lot, operator-to-operator, laboratory-to-laboratory, and day-to-day) have been demonstrated in our unpublished studies, with the overall precision of 98.5% (Lci = 96.3%). Although the immunoassay itself is straightforward, obtaining the actual blood sample for testing may be less practical for some species (e.g. badgers) than others (e.g. deer). The requirement to anaesthetise an animal in order to obtain a blood sample may complicate surveillance or control programmes but not necessarily render them unfeasible.

The RT described here has been designed to detect specific antibodies of three major classes, IgM, IgG, and IgA. Moreover, the immunoassay format makes it independent of antibody origin, as long as the molecule has at least two functional antigen-binding sites. This multi-host diagnostic potential was demonstrated in the present study as an additional important feature of the testing technology. In fact, the majority of animals in naturally infected populations of the four species studied were correctly identified by the same device. The data described here suggest that the use of such a quick and easy field test may accommodate wildlife surveillance and bovine TB control strategies in many countries where persisting *M. bovis* infection in free-ranging wild mammals poses a constant threat to livestock.

Suboptimal RT sensitivities found in this study for badgers and possums (51% and 45%, respectively) are in line with previous attempts to detect antibodies to *M. bovis* in these animals (Buddle et al., 1995; Chambers et al., 2002, 2008; Greenwald et al., 2003). The strong IgG responses in wild boar appear in agreement with the reported observation that Ig heavy and light chains were up-regulated during *M. bovis* infection (Naranjo et al., 2006). This finding offers the possibility of using serology for large-scale testing of wildlife in epidemiological surveys. Additionally, the availability of the RT makes test-and-cull schemes an attractive approach in situations where mere reduction of wild populations is not satisfactory.

In wild boar, for which pathology data were available for analysis, strong antibody responses were commonly associated with the presence of gross lesions. Similar observations have been previously made for other host species (Chambers et al., 2002; Lesellier et al., 2008; Lyashchenko et al., 2004; Waters et al., 2004). This supports the view that serological assays may predominantly target animals in the advanced stages of disease progression typically characterized by higher rates of shedding, which may reflect enhanced potential for transmission (Chambers et al., 2008).

White-tailed deer experimentally infected with *M. bovis* produced antibodies that could be found by MAPIA and RT in serum, whole blood and other biological fluids equally well. The demonstrated feasibility of antibody detection from non-serological samples, such as tissue exudates from animal carcasses, provides a useful option for RT application under field conditions when a fresh blood specimen cannot be collected, although a recent badger study suggested that sample quality could affect the diagnostic performance of the RT by reducing its sensitivity if haemolized or lipaemic blood specimens were used (Chambers et al., 2008).

Although deer MAPIA analyses showed no significant difference between experimental and natural *M. bovis* infection in terms of the magnitude of antibody responses and variable antigen recognition profiles, the RT sensitivity appeared higher in animals inoculated intratonsilarly, when compared to that found in Michigan free-ranging deer. This difference may be due to (1) the use of high dose of *M. bovis* for most of the experimentally infected deer, and (2) collection of blood samples for serological testing at certain time-points of well-synchronized infection when most of the infected animals could have detectable antibody responses already developed. Recent studies on nonhuman primates found no difference in the diagnostic performance of similar serologic assays between natural and low-dose experimental infections with *M. tuberculosis* or *M. bovis* (Lyashchenko et al., 2007a).

As was shown for wild boar, our unpublished field studies on Michigan white-tailed deer also suggest that the RT is most effective at detecting animals with severe disseminated TB (based on post-mortem examination and culture) and that it typically yields a strong positive result within 3–5 min. Because deer with more advanced disease are most likely to be excreting *M. bovis* in significant quantities (Schmitt et al., 1997; Palmer, 2007), they pose the greatest risk of exposure to uninfected wildlife and livestock. Consequently, they are the most important animals to cull from the population. A similar strategy may also be appropriate for other countries with established wildlife reservoirs of *M. bovis* infection. Thus, serologic assays such as the RT may provide useful screening tools for controlling bovine TB in populations of different wildlife species, especially where the sensitivity is at its highest and the practical limitations can be overcome.

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