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A review of tests available for use in the diagnosis of tuberculosis in non-bovine species

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Summary
Bovine tuberculosis is an important disease that has impacts on regional and international trade. The disease can affect both social and economic stability and have a deleterious affect on species diversity. The intradermal tuberculin test has been in use for almost a century and, despite the technological advances of the last two decades, is still the only prescribed test for the diagnosis of tuberculosis in cattle. Many other species of animal, including humans, can be infected with *Mycobacterium bovis*. This paper reviews the various tests that have been used by researchers for detecting infection with *M. bovis* in a variety of animal species, and attempts to prioritise or comment on the importance of having appropriately validated diagnostics for the different species. The difficulties of test validation using small numbers of animals, especially when tuberculosis occurs in only a few instances or the species of animal affected is rare and/or valuable, are discussed.

Keywords
Diagnosis – Enzyme-linked immunosorbent assay – Interferon assay – Intradermal skin test – Mycobacterium bovis – Tuberculin test – Wildlife.

Introduction

It is well known that *Mycobacterium bovis* has an extraordinary host range, especially when compared to other *M. tuberculosis* complex species (54). The list of animals susceptible to *M. bovis* is extensive: domesticated animals that can be infected include cattle, farmed buffalo, goats, various species of deer, sheep and pigs, and a variety of wildlife species, both in the wild and in captivity, are also susceptible (21) (Table I).

An infected wild animal population may be classed as either a maintenance or a spillover host depending on the dynamics of the infection (50). In a maintenance host, infection can persist by intraspecies transmission alone, and may also be transmitted to other species. In a spillover host, infection will not persist indefinitely unless either there is re-infection from another species or a temporary and reversible change occurs in the population and enhances intraspecies transmission. Identifying whether a species has the status of a maintenance or spillover host is important when determining whether disease control within a host species is necessary, or in predicting whether infection will persist once the source of infection is removed or the behaviour changes reversed. The status of a species may change over time or between regions where conditions are different, for example where population densities differ or where different management systems exist (7). Maintenance and spillover hosts can both act as vectors of disease to other species.

The status of some wild animal species as either maintenance or spillover hosts has been clearly resolved. Wildlife species such as the badger (*Meles meles*) in the United Kingdom (UK) and Ireland (27, 32), the brushtail possum (*Trichosurus vulpecular*) in New Zealand (51), and the Cape buffalo (62) and Kudu in southern Africa (3) are considered to be maintenance hosts for *M. bovis*, and may

Table I
Examples of free living wildlife or captive wildlife reported with *Mycobacterium bovis

Free wildlife hosts	Captive wildlife hosts
Antelope, marsh *(Kobus leche)*	Baboons *(Papio hamadryas)*
Baboon, olive (*Papio cynocephalus anubis*)	Baboons (*Papio papio*)
Baboon, chacma (*Papio ursinus*)	Camel, Bactrian (*Camelus bactrianus*)
Badger (*Meles meles*)	Chimpanzee (*Pan troglodytes*)
Bear, black (Ursus americanus)	Deer, axis (*Axis axis*)
Bison (*Bison bison*)	Deer, fallow (*Dama dama*)
Bobcat (*Lynx rufus)*	Deer, red (*Cervus elaphus*)
Buffalo, African (*Syncerus caffer*)	Deer, roe (*Capreolus capreolus*)
Buffalo, water (*Bubalus bubalis*)	Deer, sika (*Cervus nippon*)
Cat, feral (*Felis catus*)	Dusky langur (*Presbytis obscurus*)
Cheetah (*Acinonyx jubatus*)	Fox, fennec (*Vulpes zerda*)
Coyote (*Canis latrans*)	Gibbon, siamang (*Symphalangus syndactylus*)
Deer, axis (*Axis axis*)	Kudu, greater (*Tragelaphus strepsiceros*)
Deer, fallow (*Dama dama*)	Lemur, Mayotte (*Lemur mayottensis mayottensis*)
Deer, mule (*Odocoileus hemionus*)	Leopard (*Panthera pardus*)
Deer, red (*Cervus elaphus*)	Leopard, snow (*Uncia uncia*)
Deer, roe *(Capreolus capreolus*)	Macaque, lion-tailed (*Macaca silenus*)
Deer, sika (*Cervus nippon*)	Macaque, stump-tailed (*Macaca arctoides*)
Deer, white-tailed (*Odocoileus virginianus*)	Monkey, colobus (*Colobus guereza caudatus*)
Duiker, common (*Sylvicapra grimmia*)	Monkey, rhesus (*Macaca mulatta*)
Ferret (*Mustela putorius furo*)	Oryx, Arabian (*Oryx leucoryx*)
Fox, red (*Vulpes vulpes*)	Rhinoceros, black (*Diceros bicornis*)
Goat, feral (*Capra hircus*)	Rhinoceros, white (*Ceratotherium simum*)
Hare, European	Sea lion, Australian
(*Lepus europaeus occidentalis*)	(*Neophoca cinerea*)
Hedgehog (*Erinaceus europaeus*)	Sea lion, South American (*Otaria byronia*)
Kudu, greater (*Tragelaphus strepsiceros*)	Sea lion (*Otaria flavescens*)
Leopard (*Panthera pardus*)	Seal, New Zealand fur *(Arctocephalus forsteri*)
Lion (*Panthera leo*)	Tiger *(Panthera tigris*)
Lynx, Siberian (*Lynx pardinus*)	
Mink, American (*Mustela vison*)	
Mole, European (*Talpa europaea*)	
Pig, feral (*Sus scrofa*)	
Possum, brushtail (*Trichosurus vulpecula*)	
Rabbit, European (*Talpa europaea*)	
Raccoon (*Procyon lotor*)	
Rat (*Rattus norvegicus*)	
Seal, Australian fur (*Arctocephalus pusillus doriferus*)	
Seal, New Zealand fur (*Arctocephalus forsteri*)	
Seal, subantarctic fur (*Arctocephalus tropicalis*)	
Sea lion, South American (*Otaria flavescens*)	
Stoat (*Mustela erminea*)	
Warthog (*Phacochoerus aethiopicus*)	

* Examples where *M. bovis* or a closely related variant has been isolated are included in this list (adapted from [21] and [14]).

act as reservoirs for infection of both domestic animals and other wildlife species. In particular, the badger has caused almost insurmountable difficulties to conventional control and eradication programmes in both Great Britain and Ireland, as has the possum in New Zealand, and the Cape buffalo is causing significant problems for the management of the Kruger and other national parks in South Africa.

An example of a wild animal species that was initially classified as a maintenance host but whose current status may better be understood as a spillover host is the white-tailed deer in Michigan. Here changes in the management of the wild population, which resulted in decreased population density and less social interactive behaviour (46), have apparently led to a decrease in disease prevalence in this species.

Diagnostic tests for tuberculosis may be based on the detection of:

a) *M. bovis* organisms (culture or deoxyribonucleic acid [DNA] detection) or the host's pathological response to *M. bovis* (histopathology)

b) a cellular immune response to infection

c) antibody response to infection.

Culture is still recognised as the gold standard for diagnosis of infection with *M. bovis*. Some studies use histopathology as the gold standard, but histopathology or the presence of a granuloma itself is not specific for *M. bovis*. Obviously, culture cannot be used as a herd-based test since tuberculosis is primarily a respiratory disease, and it is neither practical nor feasible to sample the tissue samples that are most likely to harbour infection while the animal is alive. Numerous studies reporting detection of *M. bovis* in samples using DNA methodologies such as polymerase chain reaction (PCR) have been reported. However, most of these report a lower sensitivity for PCR than culture, and hence PCR will not be addressed in detail in this review.

Tests for cell-mediated immunity

The tuberculin test, which involves intradermal injection of *M. bovis* purified protein derivative (PPD) tuberculin and the subsequent detection of a swelling (delayed hypersensitivity) at the site of injection three days later, has been in use since the early 1900s and is still the tool of choice for most bovine tuberculosis eradication and control programmes. The tuberculin test is the only test for tuberculosis in cattle prescribed by the World Organisation for Animal Health (OIE), although it may be used by different countries in different ways. For example, in Australia, the single caudal fold test is applied using an increased amount of tuberculin, and the comparative tuberculin test is used only rarely (for more details on the

use of the tuberculin test in the Australian eradication programme see www.animalhealthaustralia.com.au/programs/adsp/tfap2/tfap2_home.cfm). In many European countries, the single tuberculin test using bovine PPD is only rarely used. For example, in the Republic of Ireland and Great Britain, the comparative tuberculin test is used routinely and is applied to the neck of the animal. Each country establishes its own protocols for use, and interpretation of the tuberculin test is based on local circumstances and programme requirements. Both the single and comparative tuberculin tests are accepted by OIE, as are caudal fold and cervical sites of injection.

Other methods of measuring cell-mediated immunity have been developed and have been applied to both cattle and other animal species, including the interferon-γ assay (IFN-γ) (35, 82), which is gaining increasing popularity in tuberculosis eradication and control programmes, and the lymphocyte proliferation assay (LPA), which is primarily a research tool since it suffers from logistical problems when large numbers of tests are necessary. The terms lymphocyte transformation assay or test may be used interchangeable with LPA.

Tests for humoral response

It is generally considered that the detection of humoral antibody is a poor indicator of tuberculosis infection. The humoral immune response rises towards the end stage of the disease process when the host may be at its most infectious, and although many tests for humoral antibody were trialled in the 1980s and 1990s, these tests have not had a role in eradication or control programmes to date. Enzyme-linked immunosorbent assays (ELISA) have been developed and evaluated in a number of animal species.

Some authors promote the use of antibody tests (with culling of positives) as a method of potentially reducing the likelihood of transmission within herds (41). However, because of the biology of the disease, it is not envisioned that antibody-based tests alone will have real application in tuberculosis eradication and control programmes. There has been renewed interest in antibody-based technology in recent times, due mainly to the availability of purified antigens and some new technologies for antibody detection. A number of newer technologies have been proposed for their potential to rapidly detect a humoral response to tuberculosis and provide indicators of disease. These include the fluorescent polarisation assay (FPA), Chembio's rapid test and the multi-antigen print immunoassay (MAPIA).

The FPA is based on the principle that an antigen bound in an immunocomplex will have a higher polarisation value than free antigen (a small antigen will more rapidly depolarise 'plane-polarised' light than will the same antigen coupled to its antibody). The principal advantage of FPA is that no separation of bound from free antigen is required. The entire assay is performed in solution in a single tube, with no precipitation or washing steps (19).

The MAPIA consists of a cocktail of antigens applied by micro-aerosolisation to nitrocellulose membranes in narrow bands. Strips cut perpendicular to the antigen bands are subject to a blocking step and incubated with serum samples, and this is followed by immunodetection using standard chromogenic methods (41).

The Chembio rapid test is based upon the detection, in infected animals, of antibodies to a set of recombinant tuberculosis (TB) antigens. The format is a proprietary lateral flow test that uses a drop of blood and gives a visual result within 15 minutes (28).

Although serological-based assays have many advantages in terms of logistics, lower cost and ease of application, few of them have been found useful when evaluated under field conditions.

This paper reviews the various tests reported for a number of animal species, and attempts to prioritise or comment on the importance of having appropriately validated diagnostics for the different species. The paper discusses the difficulties of test validation using small numbers of animals, especially when tuberculosis occurs only occasionally or the species of animal affected is rare and/or valuable.

Materials and methods

The review was conducted by performing a literature review and contacting a wide range of international contacts known to be working in the area of tuberculosis diagnostics. The authors e-mailed 35 contacts during April 2005 and sent a second round of 12 e-mails in late August of that year, following discussions with colleagues at the Fourth International Conference on *Mycobacterium bovis*, held in Dublin from 22 August to 26 August. A summary table was prepared listing the various species of animals, the types of tests reported for use in each of the species, estimates of the effectiveness of the tests, and a comment section for describing key details of a study.

In the light of background knowledge of tuberculosis disease and epidemiology, the authors analysed the data, considered the importance of having a diagnostic test for various types of animals, and identified gaps in knowledge. An attempt was made to prioritise where research funds, if available, should be focused, although the authors recognise that individual countries and individual scientists or policy makers may have differing opinions.

Results and discussion

Of the 47 contacts e-mailed, 27 (57.4%) responded and 24 (51.1%) provided information that could be included in the review. This is considered to be above average for such surveys. A summary of the review data can be found in Table II. Information was gathered on 15 different families and 25 different species.

Cattle were included in the summary table for comparison purposes. Buffaloes and bison were also included despite the fact that they are in fact 'bovine' (and hence do not fit the original definition of 'non-bovine species') because they are known to be significant hosts to bovine tuberculosis in some countries (e.g. Australia, South Africa, Canada). Humans are also included for comparison purposes, although in most cases, the data relates to infection with *M. tuberculosis* since the authors are not aware of any published studies that consider the diagnostic validity of tests only for patients infected with *M. bovis*. In the case of elephants, the data mostly relates to *M. tuberculosis* infection as this is the most common cause of tuberculosis in this species. Similarly tuberculosis in non-human primates may also be due to *M. tuberculosis* or *M. bovis*.

Animal species in this review included some that are well-recognised maintenance hosts of *M. bovis* such as the buffalo, badger and possum, as well as animals that are considered spillover or incidental hosts.

Test validation issues

The OIE has recently published guidelines for the validation of new tests (http://www.oie.int/vcda/eng/en_background_VCDA.htm) to OIE standards. In normal circumstances, estimates of diagnostic sensitivity should be made in populations of animals that are as close as possible to the populations that the test will be used on. With bovine tuberculosis, this will mean populations that range from non-infected animals to those in the early stages of infection to ones that are diseased. Estimates of specificity should be made in populations of animals that are known to be disease free. In the case of tuberculosis, particularly when disease occurs in wildlife or rare animal species, it is often difficult to test a sufficient number of animals to provide a robust estimate of diagnostic sensitivity and diagnostic specificity.

In reality, very little validated information is available for many of the tests listed in this review, and the presence of sensitivity or specificity values in Table II does not necessarily mean those figures will apply under all circumstances. In many – if not most – cases, the tests have been evaluated under sub-optimal conditions. In fact, because of the factors described above, the evaluation or experimental study is more often a small study performed with a 'convenient' number and type of available animals. In non-agricultural species, the available numbers are generally insufficient to truly validate a test. For example, llamas and alpacas experimentally infected with *M. bovis* (or 'vaccinated' with *M. bovis* bacillus Calmette-Guérin [BCG]) may react to the tuberculin test, but the results do not provide sufficient evidence to validate a test.

While culture of *M. bovis* continues to be the gold standard for evaluation of new tests, it is often not used, as researchers prefer to use skin test results, the finding of visible lesions or histopathology. Thus the use of different tests as the reference standard influences the outcome (in terms of sensitivity) and does not allow for comparative assessment of various studies. In addition, in natural infection, the pattern of reaction may vary greatly and anergy is not uncommon. Moreover, while a test may work in a heavily infected herd, this does not mean that the same test will work as well in a low-prevalence population.

Most tests for tuberculosis are used for herd control or eradication rather than as tests of individual animals. When a number of animals are grouped together for a few months, as in the case of animals intended for export, they may be considered as a herd. However, data gained from these 'herds' is questionable, and a good herd history is required for genuine evaluation. In many cases, what may be considered as an accepted test in a species is only accepted because there is nothing better available. The record of a test working once is not enough for it to be accepted as a validated test. Clearly the diagnosis of tuberculosis in animals other than cattle and buffalo remains a significant problem for veterinarians, farmers of unusual species, import and export authorities, and managers of zoological collections.

Validation data for different animal species

In elephants, culture of trunk washes is accepted as the gold standard diagnostic test. The tuberculin test is considered to perform poorly and is not validated and there is limited data available from alternate tests. It should be remembered that most of the cases of tuberculosis in elephants are due to *M. tuberculosis* rather than *M. bovis*; *M. tuberculosis* presents an important zoonotic risk to zookeepers, animal handlers and the public, despite the fact that the animals may have contracted the disease from their own keepers or handlers. Elephants are valuable animals and treatment is sometimes attempted, so improved diagnostics would be useful for the few zoos that are affected.

A number of ELISAs and other antibody-based tests have been tried in the badger with limited success in terms of both sensitivity and specificity. The comparative LPA provided reasonable sensitivity but poorer specificity. This

Table II
Summary of data collected on the use of tests for diagnosis of tuberculosis in animals (with a focus on *Mycobacterium bovis* infection) *

Species (name)		Test	Sensitivity	Specificity	References	Comment
Common	Latin					
Badger	*Meles meles*	Comparative tuberculin skin test	70% (7/10)	73% (27/37)	43	Sensitivity and specificity based on the evaluation of 47 badgers (10 culture-positive, 37 culture-negative) trapped in East Sussex, UK
			NE	NE	Fiona Stuart (personal communication, 2005)	Test is not sensitive in experimentally infected badgers and would not be of use in a field situation
			NE	NE	Mark Chambers (personal communication, 2005)	Test may be useful in captive animals
		IFN-γ assay (ELISA and quantitative RT-PCR)	NE	NE	Mark Chambers (personal communication, 2005)	Test has been developed and is currently under evaluation
		Comparative LTA	87.5%	84.6%	18	Test is unsuitable for routine use antigens: bovine and avian tuberculin Using indirect ELISA on samples from the same animals, sensitivity was 62.5% and specificity was 100%
		Chembio Rapid test (lateral flow technology)	29.5% (gold-based test)	88% (gold-based test)	28	Sensitivity and specificity based on the evaluation of 78 culture-positive and 100 culture-negative samples
			44.9% (latex-based test)	96% (latex-based test)	Mark Chambers (personal communication, 2005)	Sensitivity is greater in 'super-shedders' Improved time efficiency and potential to be performed animal-side
		Fluorescence polarisation assay (antigen: MPB70)	92%	93%	Data from correspondence between Ed Corrigan and VLA Weybridge (2005), 12	Sensitivity and specificity based on the evaluation of 40 samples, 20 known *M. bovis*-positives and 20 known-negatives
		ELISA (antigen: *M. bovis* MPB83)	37%	98%	26	Sensitivity and specificity based on the evaluation of a badger population of known culture status
			40.7% (at the individual animal level) 62.3% (in badgers with a history of *M. bovis*)	94.3%	9	Sensitivity and specificity based on the evaluation of 1982 badgers captured during statutory badger removal operations in south-west England
			68.2% (in badgers with a history of *M. bovis*)	NE	8	Sensitivity based on the evaluation of 128 badgers trapped in Woodchester Park, UK (1985-1998); 4.7% of badgers in this area were culture-positive for *M. bovis* during this time
			47.4% (37/78)	89% (89/100)	28	Sensitivity and specificity based on the evaluation of 78 culture-positive and 90 culture-negative MPB83 is serodominant in badgers with TB
		MAPIA	59% (46/78)	84% (84/100)	28	Sensitivity and specificity based on the evaluation of 78 culture-positive and 100 culture-negative Sensitivity is greater in 'super shedders' Additional antigenic targets identified including MPB70, CFP-10, Mtb48, Mtb8 yet MPB83 is serodominant
Bison	*Bison bison*	Single tuberculin skin test (intra-dermal caudal fold)	66.7% (116/174)	89.6% (164/183)	52	Sensitivity and specificity based on the evaluation of 174 necropsy-positive samples and 183 necropsy-negative samples
		Fluorescence polarisation assay (antigen: MPB70)	100% (3/3)	100% (6/6)	40	Sensitivity and specificity based on the evaluation of three *M. bovis*-positive samples and six negative samples The small number of samples used for research evaluation were selected on the basis of MPB70 ELISA results
Buffalo (Asian)	*Bubalus bubalis*	Comparative tuberculin skin test (adaptation of bovine test; caudal fold)	NE	NE	Kevin de Witte (personal communication, 2005)	Use of modified comparative tuberculin test reduced non-palpable lesion (NPL) reactor rates from 10% to 1% in disease-free herds Test is not approved for routine use

Table II (contd)

Species (name) Common	Species (name) Latin	Test	Sensitivity	Specificity	References	Comment
Buffalo (African)	*Syncerus caffer*	Tuberculin skin test (intradermal)	95.3% in infected populations (143/150)	97.7% in infected populations (1452/1486) 99.5% in uninfected populations (1345/1352)	Anita Michel (personal communication, 2005), 45	Specificity estimates based on 1,486 buffalo in an infected population and 1,352 uninfected African buffalo
		IFN-γ assay (Bovigam™ Assay; CSL Limited)	NE	NE	66	Monoclonal antibody to bovine IFN-γ cross-reacts with buffalo antibodies
			84.6% (143/169)	99.3% (1381/1390)	Anita Michel (personal communication, 2005)	
		Modified IFN-γ assay (Bovigam™ Assay; CSL Limited)	84.6%	99.4%	33, 45	Modification of Bovigam™ test increases specificity
Camelids (Alpaca)	*Lama pacos*	Single tuberculin skin test	100% (16/16)	100%(12/12)	Ricardo de la Rua (personal communication, 2005)	Test approved by the Animal Health Board (New Zealand) as a 'validated' primary test for TB screening of alpacas Test performed 100 days after inoculation with *M. bovis*
		Comparative tuberculin skin test	76.2% (16/21)	100%(12/12)	Ricardo de la Rua (personal communication, 2005)	Test approved by the Animal Health Board (New Zealand) as a 'validated' ancilliary test for TB screening of alpacas Test performed 104 days after inoculation with *M. bovis*
Camelids (Llama)	*Lama glama*	Comparative tuberculin skin test (intradermal)	87.5% (21/24)	100% (12/12)	Fiona Stuart (personal communication, 2005)	Sensitivity and specificity based on the evaluation of 24 llamas experimentally infected with *M. bovis* and 12 uninfected llamas
			Detected single infected animal	NE	Sharon Redrobe (personal communication, 2005)	Infection confirmed at post-mortem by culture and PCR (individual animal basis)
		ELISA (antigen: bovine and avian PPD)	100% (24/24)	NE	Fiona Stuart (personal communication, 2005)	Sensitivity and specificity based on the evaluation of 24 llamas experimentally infected with *M. bovis* and 12 uninfected llamas
		Fluorescence polarisation assay (antigen: MPB70)	100% (3/3)	100% (6/6)	40	Sensitivity and specificity based on the evaluation of three *M. bovis*-positive samples and six negative samples The small number of samples used for research evaluation were selected on the basis of MPB70 ELISA results
Cats (domestic)	*Felis domesticus*	Tuberculin skin test	NE	NE	66; Merck Veterinary Manual (8th Ed. online) – accessed on 5 October 2005; Ricardo de la Rua (personal communication, 2005)	The tuberculin skin test is considered unreliable in cats
		ELISA (antigen not specified)	20% (4/20)	NE	36	Sensitivity based on the evaluation of 20 domestic cats exposed to a cat with laboratory-confirmed *M. bovis* infection All cats were negative by tuberculin skin test, histology and culture All cats with positive ELISA responses were offspring of the cats with TB
Cattle	*Bovidae*	Single tuberculin skin test	68%-95%	96%-99% (high percentage of false-positive tests in animals with non-TB mycobacteria)	47	Large-scale field evaluation Sensitivity and specificity of test increased when injected into CCT rather than caudal fold In Australia, single caudal fold skin test used for eradication of bovine TB with concurrent increase in tuberculin concentration
			53.6% (15/28)	99.8% (5653/5666)	69	Sensitivity and specificity based on the evaluation of 28 culture-positive samples and 5,666 presumed-negative samples
		Comparative tuberculin skin test	NE	> 99%	47	Validated in UK and used since 1942 Used in Ireland since 1954

Table II (contd)

Species (name) Common	Latin	Test	Sensitivity	Specificity	References	Comment
Cattle	*Bovidae*	IFN-γ assay (Bovigam™ Assay; CSL Limited)	76%-93.6%	96.2%-98.1%	80	Sensitivity and specificity based on the evaluation of > 6,000 cattle from TB-positive herds and > 6,000 cattle from TB-free herd Large scale field evaluation Sensitivity increases to 95.2% when both an IFN-γ assay and a single intradermal tuberculin test are used
			96.6% (201/208)	98% (392/400)	24	Field evaluation Sensitivity and specificity based on the evaluation of 28 culture-positive samples and 5,666 presumed-negative samples
			81.8%-100%	94%-100%	81	Cattle from Australia, Brazil, Ireland, USA, Northern Ireland, Italy, New Zealand, Spain, Romania IFN-γ assay detects *M. bovis* infection earlier than the skin test In New Zealand, test is applied to detect skin test negative cattle with TB and is approved for serial skin testing skin test positive cattle when non-specificity is suspected IFN-γ assay accredited as official diagnostic test for bovine TB in Australia
			86.7% (26/30)	99.7% (362/363)	Anita Michel (personal communication, 2005)	Field evaluation
					5, 60, 61, 72, 73	Use of MTC-specific antigens (e.g. ESAT-6 and CFP-10) enhances specificity
		Post-mortem examination and bacteriological culture	NE	NE	48	Six cats were histo-positive Three out of six were cultured: all three *M. bovis*-positive
			NE	NE	22	6/36 cats tested (1971-1996) in the UK were positive
		ELISA (antigens: ESAT-6, MTSA-10, MPTS1, MPT63, MPB59,MPB64, MPB70, MPB83)	57.1% (16/28 reactors)		2	Sensitivity and specificity based on the evaluation of 28 cattle (16 skin test-positive and 12 skin test-negative; all 28 classified as reactors by IFN-γ assay) and 21 officially-certified TB-free cattle (all skin test-negative; 9 were avian-positive and 12 were non-reactive by IFN-γ assay) Detected *M. bovis*-infected but skin test-negative cattle (7/16 ELISA-positive cattle were skin-test negative) May be employed in skin test-negative cattle to confirm results from IFN-γ assay
		Commercial enzyme immunoassay (EIA) (antigens: ESAT-6, MPB70)	98.6% (ESAT-6) 96.8% (MPB70)	98.5% (ESAT-6) 90.1% (MPB70)	38	Sensitivity and specificity based on the evaluation of 300 naturally-infected cattle (all cattle were skin test-positive and culture-positive), 20 experimentally-infected cattle (19/20 were skin test-positive, culture-positive and histo-positive) and 155 healthy animals
		Immunochromato-graphic assay (antigen: recombinant MPB70)	83% (MPB70)	99.4% (MPB70)	38	
		Latex bead agglutination assay (antigen: ESAT-6, MPB70)	94.8% (ESAT-6) 86.7% (MPB70)	92.6% (ESAT-6) 97.8% (MPB70)	38	
		Fluorescence polarisation assay (antigen peptide derived from MPB70)	79%	99.8%	12	Sensitivity and specificity based on the evaluation of 85 culture-positive or TB lesion-positive status and 5,092 presumed-negative samples Currently under evaluation
Deer (various)	Cervidae	Comparative tuberculin skin test	91.7% (55/60)	98.7% (1142/1157)	13	Sensitivity and specificity based on results for 60 deer experimentally infected with *M. bovis* and 1,157 deer from uninfected herds with a history of non-specific tuberculin test reactions
		IFN-γ assay	NE	NE	63	Monoclonal antibody to bovine IFN-γ does not cross-react with cervine IFN-γ

Table II (contd)

Species (name) Common	Latin	Test	Sensitivity	Specificity	References	Comment
Deer (Elk)	*Cervus elaphus nelsoni*	Comparative tuberculin skin test (CCT)	100% (7/7)	100% (3/3)	76	Sensitivity and specificity based on results for seven elk vaccinated with *M. bovis* BCG and three elk confirmed as negative for *M. bovis* by Bovigam™ Small number of animals used for research evaluation Maximal response at 24 h and 48 h post-PPD administration Diminished reactivity at 72 h post-PPD administration
		PBMC proliferation (antigen: *M. bovis* PPD)	NE	NE	76	Immune responses based on results for seven elk vaccinated with *M. bovis* BCG and three elk confirmed as negative for *M. bovis* by Bovigam™ Small number of animals used for research evaluation Measurable but low difference between test and control animals
		Fluorescence polarisation assay (antigen: MPB70)	100% (3/3)	100% (9/9)	40	Sensitivity and specificity based on the evaluation of three *M. bovis*-positive samples and six negative samples The small number of samples used for research evaluation were selected on the basis of MPB70 ELISA results
Deer (North American red)	*Cervus elaphus*	Comparative tuberculin skin	80%	61.3%	68	Sensitivity and specificity based on the evaluation of 51 samples and expressed percentages are relative to subsequent cultural tests on tissues
			NE	46.9% (100/218)	29	Specificity based on 218 disease-free deer from uninfected herds
		IFN-γ assay	75% (3/4 infected deer)	100% (5/5)	65	Sensitivity and specificity based on results for four deer (two experimentally infected with *M. bovis* and two histo-positive tuberculous deer) and five uninfected controls
		Blood tuberculosis test (comparative: use antigens from *M. bovis* and *M. avium*)	95.7%-95.9% (in herds with < 2.0% or > 30% incidence of TB)	98.0%	29	Sensitivity and specificity based on the evaluation of 150 deer culture-positive for *M. bovis* and 218 disease-free deer from uninfected herds
		Antibody test	85.3% (87/102)	100% (218/218)	29	Used to diagnose *M. bovis* in skin-test negative anergic deer from infected herds
			95% (when used in combination with skin test)	NE (when used in combination with skin test)		Blood taken from deer ten days after reading skin test Sensitivity and specificity based on the evaluation of 102 deer culture-positive for *M. bovis* and 218 disease-free deer from uninfected herds
		LTA (antigens: *M. bovis* PPD, MPB70)	Increased with bovine PPD	Increased with MPB70	31	Most effective in deer with disseminated disease
		ELISA (antigens: *M. bovis* PPD, MPB70)	NE	Increased when antigens are used in conjunction	31	Most effective in deer with disseminated disease
		MAPIA	64%	100%	K. Lyashchenko (unpublished observations) Ray Waters (personal communication, 2005)	Studies with serum obtained from experimentally- and naturally-infected animals indicate potential for this test However, test has a low sensitivity
Deer (Reindeer)	*Rangifer tarandus*	Single tuberculin test	NE	Low specificity	55	
		Comparative tuberculin skin test	92.3% (12/13)	25%(3/4)	55	TB in reindeer is extremely rare and false-positives are common Sensitivity and specificity based on results for 13 reindeer experimentally infected with *M. bovis* BCG and four deer uninfected controls

Table II (contd)

Species (name) Common	Latin	Test	Sensitivity	Specificity	References	Comment
Deer (Reindeer)	*Rangifer tarandus*		NE	NE	78	Skin testing is the only approved test for tuberculosis surveillance of reindeer in the USA IFN-γ responses were decreased immediately after skin testing
		IFN-γ assay (Cervigam™ Assay; CSL Limited)	100% (13/13)	100% (4/4)	78	Sensitivity and specificity based on results for 13 reindeer experimentally infected with *M. bovis* BCG and four deer uninfected controls
			NE	NE	55	Improved specificity with use of antigens specific for virulent tubercle bacilli e.g. ESAT-6 and CFP-10
		MAPIA	100% (11/11 positive for MPB83) 81.8% (9/11 positive for MPB70)	50% (2/4; after skin testing)	74	Sensitivity and specificity based on results for 11 reindeer experimentally infected with *M. bovis* BCG and four deer uninfected controls Serum response boosted by previous tuberculin skin test MPB83 is serodominant
		Comparative tuberculin skin test (CCT)	100% (8/8)	50% (1/2)	58	Sensitivity and specificity based on results for eight deer experimentally infected with *M. bovis* and two uninfected controls
			97%	81%	59	Sensitivity and specificity based on results for 116 deer
		IFN-γ assay (Cervigam™ Assay; CSL Limited)	40% (2/5 based on repeat testing)	100% (5/5 based on repeat testing)	79	Sensitivity and specificity based on results for five deer experimentally infected with *M. bovis* and five uninfected controls Assay from nine days post-infection and include results from repeat testing
Deer (White-tailed)	*Odocoileus virginianus*	LPA (using blood mononuclear cells)	NE	NE	58	Both sensitivity and specificity are improved with the use of MPB70 Results are based on eight deer experimentally infected with *M. bovis* and two uninfected controls Requires processing of blood sample within 24 h Subject to complications associated with overnight delivery
		LPA (using blood mononuclear cells)	80% (4/5 based on repeat testing)	40% (3/5 based on repeat testing)	79	Sensitivity and specificity based on results for five deer experimentally infected with *M. bovis* and five uninfected controls Assay performed 126 days post-infection and based on repeat testing The three controls with positive results were also responsive to avium PPD
		ELISA (antigens: *M. bovis* PPD, MPB70)	NE	Low level of cross-reaction to *M. avium* PPD	58	Test performed in eight deer experimentally infected with *M. bovis* and two uninfected controls Antibody response most prominent in deer with disseminated disease but response did not correlate with inoculum dose
			NE	NE	57	Test performed in 26 deer experimentally infected with *M. bovis* and seven uninfected controls IFN-γ production greater in infected than in uninfected deer in response to *M. bovis* PPD
		Production of NO from infected PBMC stimulated with *M. bovis*	NE	NE	77	Test performed in five deer experimentally infected with *M. bovis* and five uninfected controls Infected deer release higher concentrations of NO upon stimulation with *M. bovis* PPD, *M. bovis* culture filtrate and whole cell sonicate as compared to NO release from uninfected deer This test cannot be used in red deer since macrophages from red deer are deficient in their ability to produce NO
		Lipoarabinom annan-based ELISA	NE	81.8% (9/11)	77	Specificity based on results for 25 deer experimentally infected with *M. bovis* and 11 uninfected controls Sensitivity increased by detecting antibodies at non-proteinaceous and proteinaceous epitopes Serum response boosted by previous tuberculin skin test

Table II (contd)

Species (name) Common	Latin	Test	Sensitivity	Specificity	References	Comment
Deer (White-tailed)	*Odocoileus virginianus*	MAPIA	80% (20/25)	100% (11/11)	75	Sensitivity and specificity based on results for 25 deer experimentally infected with *M. bovis* and 11 uninfected controls MPB83 is serodominant but must identify additional seroreactive antigens for use as a highly sensitive test
			72%	97%	Ray Waters (personal communication, 2005)	Sensitivity and specificity based on results based on 422 samples from experimentally-infected animals and field cases
Dogs	*Canis familiaris*	Single tuberculin skin test	NE	NE	67	USDA tuberculin 10/29 dogs exposed to humans with TB were positive 2/70 dogs without known exposure to TB were positive
			NE	NE	Merck Veterinary Manual (8th Ed. online) – accessed on 5 October 2005	False negative tuberculin tests are common in dogs
Dolphins	*Delphinidae*	Comparative tuberculin skin test (CCT)	NE	High level of cross-reactions with avian PPD	15	Seven animals (from TB-free population) were tested
		ELISA (antigens: bovine and avian PPD)	NE	No cross-reaction with avian PPD	15	Seven animals (from TB-free population) were tested Simple to perform and time-effective (24 h)
Elephants	*Elephus maximus*	Tuberculin skin test	Poor	Poor	Ray Waters (presentation to Committee on Captive Wildlife and Alternative Livestock, 2004)	Tuberculin test is currently not validated in elephants
		Culture of trunk wash samples	NE	Excellent	Ray Waters (presentation to Committee on Captive Wildlife and Alternative Livestock, 2004)	Gold standard; mostly *M. tuberculosis*: 30 cases in Asian elephants in zoos in the USA (1994-2004); single *M. bovis* case in African elephant
		Immunoblot assay (antigen: whole *M. bovis* sonicate)	Detected *M. bovis* before positive trunk wash culture in a single animal	NE	Ray Waters (presentation to Committee on Captive Wildlife and Alternative Livestock, 2004)	Antigen preparation lacks secreted antigens Used to monitor reactivation of infection
		MAPIA	Detected antibody response before positive trunk wash culture in a single animal	No antibody response in non-infected elephants	Ray Waters (presentation to Committee on Captive Wildlife and Alternative Livestock, 2004)	Used prior to rapid test to indicate which antigens will show strongest reaction (primary or confirmatory test)
			Detected antibody response years prior to positive trunk wash culture	NE	K. Lyashchenko (personal communication, 2005), Ray Waters (personal communication, 2005)	Comments based on the evaluation of 63 known-negatives and 17 known-positives
		Chembio Rapid test (laternal flow technology)	Detected antibody response years prior to positive trunk wash culture in a single animal	NE	Ray Waters (presentation to Committee on Captive Wildlife and Alternative Livestock, 2004)	Results similar to MAPIA Used to monitor therapy and reactivation of infection (promising screening test)
			Detected antibody response years prior to positive trunk wash culture	No antibody response in non-infected elephants	K. Lyashchenko (personal communication, 2005)	Sensitivity and specificity based on the evaluation of 63 known-negatives and 17 known-positives

Table II (contd)

Species (name) Common	Species (name) Latin	Test	Sensitivity	Specificity	References	Comment
Elephants	*Elephus maximus*	IFN-γ assay	NE	NE	Ray Waters (presentation to Committee on Captive Wildlife and Alternative Livestock, 2004)	Currently under development
		Multi-antigen ELISA	Detected antibody responses years prior to positive trunk wash culture	NE	Scott Larsen (personal communication, 2005)	Improved with the addition of MTC-specific antigens, particularly ESAT-6 Antigens included in the test: *M. bovis* CF, MPB70, ESAT-6, Ag85, MTP-64, MPT32
Goat	*Caprinae*	Single tuberculin skin test	100% (1/1)	100% (18/18)	17	Sensitivity and specificity based on the evaluation of 19 goats exposed to a TB-infected cow (18 culture-negatives and 1 culture-positive); prevalence of *M. bovis* infection was 5.3% (small number of animals tested)
			38.3% (18/47)	Inconclusive result for single *M. bovis* negatives sample	39	Sensitivity and specificity based on the evaluation of 47 *M. bovis*-positive samples and 1 *M. bovis*-negative sample
			> 95%	NE	Juan Francisco Garcia Marin (personal communication, 2005)	Sensitivity based on the evaluation of herds with suspected or confirmed TB
		Comparative tuberculin skin test	83.7% (41/49)	100% (25/25)	34	Sensitivity and specificity based on the evaluation of 51 culture-positive samples and 25 culture-negatives samples
		IFN-γ assay (Bovigam™ Assay; CSL Limited)	NE	NE	64	Monoclonal antibody to bovine IFN-γ cross-reacts with caprine IFN-γ
			100% (1/1)	38.9% (7/18)	17	Sensitivity and specificity based on the evaluation of 19 goats exposed to a TB-infected cow (18 culture-negative and 1 culture-positive); prevalence of *M. bovis* infection was 5.3% (small number of animals tested)
			83.7% (41/49)	96% (24/25)	34	Sensitivity and specificity based on the evaluation of 51 culture-positive samples and 25 culture-negative samples
			87.2% (41/47)	NE	39	Sensitivity based on the evaluation of 47 *M. bovis*-positive samples and one *M. bovis*-negative sample
		ELISA (antigen: bovine PPD)	54.9% (28/51; test performed in conjunction with the tuberculin test)	88% (22/25; test performed in conjunction with the tuberculin test)	34	Sensitivity and specificity based on the evaluation of 51 culture-positive samples and 25 culture-negative samples
			88.6% (39/44; test performed 15 days after the tuberculin test)	95.8% (23/24; test performed 15 days after the tuberculin test)		Sensitivity and specificity based on the evaluation of 44 culture-positive samples and 24 culture-negative samples Anamnestic ELISA (performed after the tuberculin test) offers higher sensitivity than the standard ELISA test
		IFN-γ assay	NE	NE	64	Monoclonal antibody to bovine IFN-γ does not cross-react with human IFN-γ
Human	*Homo sapiens*	Tuberculin skin test	75%-90%	70%-100%	23	Sensitivity and specificity based on the evaluation of patients with active disease Numbers vary widely depending on the population, BCG vaccination rates, and PPD source
			NE	NE	49, 37, Stephen Jones (personal communication, 2005)	Results are grossly confounded by BCG vaccination in the population

Table II (contd)

Species (name) Common	Latin	Test	Sensitivity	Specificity	References	Comment
Human	*Homo Sapiens*	IFN-γ assay (Quantiferon™-TB Gold; Cellestis Ltd; uses peptide antigens that simulate ESAT-6 and CFP-10)	82%-89% (untreated active disease)	NE	37	IFN-γ assay performs better than tuberculin skin test for detecting both active and latent tuberculosis infection in a BCG-vaccinated population
			82%-89% (untreated active disease)	98.1% (in populations with no risk of TB)	49	IFN-γ assay is more sensitive and specific than tuberculin skin test in a heavily BCG-vaccinated population
			NE	99.8% (in populations with no risk of TB)	Stephen Jones (personal communication, 2005)	
		MAPIA	56%-72%	98.5%-100%	41	Sensitivity and specificity based on the evaluation of 75 patients culture-positive for TB and 67 healthy controls Sensitivity unaffected by the number of antigens used Test not yet validated
Non-human primates (various)		Tuberculin skin test	Considered poor and therefore, requires serial testing	Prone to false positives and false negatives	Candace McCombs (presentation to Committee on Captive Wildlife and Alternative Livestock, 2004)	TB is considered to be the most important disease of captive primates TB test requires anaesthesia and injection is administered into the eyelid
		Tuberculin skin test	Detected 4/6 experimentally infected cynomolgus monkeys and 7/8 experimentally infected rhesus monkeys		71	Sensitivity and specificity based on the evaluation of 343 samples (225 rhesus monkeys, 82 cynomolgus monkeys, 19 chimpanzees, 17 new world monkeys) 15/82 cynomolgus monkeys and 8/225 rhesus monkeys were experimentally infected with *M. tuberculosis*
		IFN-γ assay (Primagam™ Assay; CSL Limited)	NE	NE	Candace McCombs (presentation to Committee on Captive Wildlife and Alternative Livestock, 2004)	Received provisional USDA approval
			100% (23/23)	100% (320/343)	71	Sensitivity and specificity based on the evaluation of 343 samples (225 rhesus monkeys, 82 cynomolgus monkeys, 19 chimpanzees, 17 new world monkeys) 15/82 cynomolgus monkeys and 8/225 rhesus monkeys were experimentally infected with *M. tuberculosis*
		Chembio Rapid test	90.2% (46/51)	98.1% (154/157)	Candace McCombs (presentation to Committee on Captive Wildlife and Alternative Livestock, 2004)	Sensitivity and specificity based on the evaluation of 51 infected monkeys and 157 negative monkeys (4 different primate species) Specificity studies are being conducted with different mycobacterium species Needs more work for validation
Non-human primates (gorilla)	*Gorilla gorilla*	Comparative tuberculin skin test (intradermal)	Detected two individual animals	NE	Sharon Redrobe (personal communication, 2005)	Infection confirmed post-mortem by culture and PCR (individual animal basis)
Non-human primates (baboon)	*Papio* sp.	FN-γ assay (Primagam™ Assay; CSL Limited)	100% (5/5)	100% (10/10)	A.L. Michel (personal communication, 2005)	Sensitivity and specificity based on the evaluation of five culture-positive samples (four baboons and one chimpanzee) and ten uninfected baboons Small number of animals used
Pig		Comparative tuberculin skin test (intradermal)	100% (19/19)	100% (25/25)	1	Pigs are rarely infected by human or bovine TB but are commonly infected by the *M. avium/intracellulare* complex Data is from pigs infected with *M. africanum* Small numbers of animals
		IFN-γ assay	NE	NE	63	Monoclonal antibody to bovine IFN-γ does not cross-react with porcine IFN-γ

Table II (contd)

Species (name) Common	Latin	Test	Sensitivity	Specificity	References	Comment
Pig (feral)	*Sus scrofa*	Single tuberculin skin test (intradermal, ear)	NE	100% (17/17)	53	Specificity based on the evaluation of 17 pigs from a TB-free area
Possum	*Trichosurus vulpecula*	Tuberculin skin test	NE	NE	6	Weak responses observed Skin testing impractical – possums are handled twice over a 2-3 day interval, possums captured for the first time exhibit lower cellular immune responses than those captured frequently
		ELISA using:			4	Sensitivity and specificity based on the evaluation of 100 possums from a TB-free area and 29 possums infected with *M. bovis* Combination of all three tests gives sensitivity of 51% and specificity of 93%
		M. bovis AN5 culture filtrate	45%	96%		
		M. bovis MPB70	21%	98%		
		Monoclonal antibody to MPB70	28%	99%		
			NE	NE	6	Serological responses more commonly found in possums in the terminal stage of the disease
		LTA (antigen: bovine PPD)	83% (5/6) 100% (5/5)	NE	10	Tests performed with 11 brushtail possums experimentally infected with *M. bovis*; six possums were euthanased for week-three test and five possums for week-four test (macroscopic TB lesions were observed at time of euthanasia)
			NE	NE	6	Positive LTA responses coincide with onset of clinical signs of TB
Raccoon	*Procyon lotor*	ELISA (antigens: *M. bovis* PPD, *M. bovis* whole cell sonicate and *M. avium* PPD)	17.2% (5/29)	100% (8/8)	56	Sensitivity and specificity based on the evaluation of 29 raccoons experimentally infected with varying doses of *M. bovis* and eight uninfected raccoons Test is most effective in animals with disseminated disease
Seals	Phocidae	Comparative tuberculin skin test (CCT)	7/10 positive	3/10 negative	15	Ten animals tested Positive reactors confirmed as positive by culture High levels of cross-reaction with avian PPD in negative reactors
		Detection of antibodies by ELISA (antigens: bovine and avian PPD)	100% (7/7)	No cross-reaction with avian PPD	15	Difficult to collect blood (requires animal to be restrained) Serum response boosted by previous tuberculin skin test Simple to perform and time-effective (24 h)
Sheep	*Ovis* Linnaeus	Single tuberculin skin test (intradermal)	81.6%	99.6%	11	Sensitivity and specificity based on the evaluation of 281 sheep from an area with a history TB in cattle and possums
						30/31 reactors were histo-positive for tuberculosis
						243/250 non-reactors were histo-positive for tuberculosis
		Single tuberculin skin test (intradermal)	NE	NE	20	597 sheep potentially exposed to TB-infected cattle were tested and 108/597 sheep were reactors 70 were selected for necropsy and 43/70 were histo-positive
			66.7% (4/6 reactors were histo-positive)	NE	44	Sensitivity based on the evaluation of sheep potentially exposed to a high level of infection from in-contact cattle
		IFN-γ assay (Bovigam™ Assay; CSL Limited)	NE	NE	64	Monoclonal antibody to bovine IFN-γ cross-reacts with ovine IFN-γ
			6/6 reactors were positive for IFN-γ	NE	44	Sensitivity based on the evaluation of sheep potentially exposed to a high level of infection from in-contact cattle
Tapir	*Tapirus terrestris*	Comparative tuberculin skin	Detected two individual animals test (intradermal)	NE	Sharon Redrobe (personal communication, 2005)	Infection confirmed at post-mortem by culture and PCR (individual animal basis)

* Much of the information incorporated in this table is anecdotal and most does not conform to World Organisation for Animal Health standards for a validated test

BCG: bacillus Calmette-Guérin
CCT: cervical region
CFP: culture filtrate protein
ELISA: enzyme-linked immunosorbent assay
ESAT: early secretory antigenic target
IFN-γ: interferon-γ
LPA: lymphocyte proliferation assay
LTA: lymphocyte transformation assay
MAPIA: multi-antigen print immunoassay
MTC: *Mycobacterium tuberculosis* complex
NE: no estimate
NO: nitric oxide
PBMC: peripheral blood mononuclear cells
PPD: purified protein derivative
RT-PCR: reverse transcriptase-polymerase chain reaction
TB: tuberculosis
UK: United Kingdom
USA: United States of America
USDA: United States Department of Agriculture

assay is generally considered a research tool since it is impractical for testing large numbers of animals. The tuberculin test has also been found to be of limited value in badgers. It is hard to see diagnostic tools being used in any routine manner in badgers, other than in the study of pathogenesis.

Few tests have been reported for use in bison. One study using the single caudal fold tuberculin test suggested a sensitivity of 66.7% and specificity of 89.6% based on necropsy findings (52). A small study found that the FPA was as effective as the MPB70 ELISA for detecting antibodies (40) in bison. The development of diagnostic tests for bison would be of value for screening animals on entrance to zoological collections or for monitoring the health of such collections (e.g. the Hook Lake Wood Bison Recovery Project in Canada), or in farmed bison if a country was considering eradication or control of disease by test and slaughter.

The tuberculin test and a modified Bovigam™ assay have been sufficiently validated for use in the Cape buffalo, and both tests have good specificity (45). Sensitivity is also acceptable, at least as good as in cattle, and the Bovigam™ offers advantages in that it is a test-and-release method that does not require secondary capture to read the test. Selected animals can be culled by rifle from a helicopter. However, it is unlikely that either test will be used in a routine test or cull eradication programme. The main purpose of the tests is to monitor the spread of infection in zoological parks, and to screen animals prior to entry into disease-free herds. Testing may also be used to monitor the prevalence of disease in various populations.

A modified, comparative caudal fold tuberculin test was used in Australia's test and slaughter campaign in a small number of Asian-buffalo herds run under northern Australian farm management conditions. Although the test was never scientifically validated, it was used to monitor disease-free herds in a programme that decreased non-visible lesion reactor rates from 10% to 1%. It was also used in diseased herds. However, because of the progress of the Australian programme, it was never used to test a herd to freedom. The remaining infected herds were depopulated to achieve the aims of the national eradication programme before the test could be fully evaluated. Asian buffalo were included in the early field evaluation of the IFN-γ in Australia.

Camelids, including llama, alpaca and camels, are occasionally reported to be infected with *M. bovis*. They are not considered to be maintenance hosts and infection is usually transmitted via contact with other infected animals. A well-validated test would be useful for trade purposes. The comparative tuberculin test has been reported to provide reasonable sensitivity and good specificity in an experimentally infected llama in a study performed in the UK (F. Stewart, personal communication, 2005). In the same study, the ELISA also provided good sensitivity and specificity. In a study described by Lin *et al.* (40), the FPA was as effective at detecting antibody levels in llamas as a normal ELISA using MPB70 antigen, but the test has not been validated and there is no evidence to suggest it would be useful in a field situation. No information on diagnostic tests was available for camels.

Test for tuberculosis have been applied to a number of species of Cervidae, including red deer, white-tailed deer, reindeer and elk. The single tuberculin test for deer is most often applied to the mid-cervical region (MCST), with the comparative test applied to the cervical region (CCT). Because deer do not have a caudal fold, the test cannot be applied to that site. The tuberculin test is particularly difficult to apply in deer because many species of deer have very thin skins and it may be difficult to inject the tuberculin intradermally. In addition, stags develop extremely thick skins during the rut, which can interfere with the accurate measurement of changes in skin thickness (30). A high level of non-specific sensitisation which results from exposure to saprophytic mycobacteria or *M. paratuberculosis* can also complicate diagnosis in deer. In New Zealand, it is generally accepted that the CCT is less sensitive than the MCST (30), which has a sensitivity of 80%. The IFN-γ test designed for cattle works poorly in deer and a cervine test has been specially developed (Cervigam™). Overall, the tests applied in deer lack the robust validation that has been applied to diagnosis in cattle.

In red deer, results from the comparative tuberculin test suggest the test has a reasonable sensitivity (80%) but low specificity (46.9% to 61.3%). The low specificity may be due to the interference of *M. avium* species infection, to its presence in the environment or to infection with *M. paratuberculosis*. Many of the studies have been done with small numbers of animals. Griffin *et al.* (29) report improved specificity and sensitivity using the comparative LPA and ELISA tests in parallel. The ELISA is considered most useful when used in conjunction with the skin test (as an indicator of anamnestic response). Recent reports suggest eradication of tuberculosis in deer can be achieved by using the skin test and ELISA in combination if the disease is detected early (30).

Several experimental infections have accumulated data for diagnostic tests in white-tailed deer. Most of these experiments have used small numbers of animals. In one study of 116 animals, a sensitivity of 97% and a specificity of 81% were achieved (59).

Tuberculosis in reindeer is considered to be extremely rare, and skin testing is the only approved test in the United States of America (USA). All results for reindeer are based on small numbers of experimentally infected reindeer and

deer negative controls. Estimates of the sensitivity of the single and comparative tuberculin test have been made using experimentally infected animals. The Cervigam™ assay has been used and found to be slightly better in terms of specificity than the tuberculin test. When applied to reindeer, the MAPIA provided reasonable to good sensitivity in experimentally infected animals that have been boosted by previous tuberculin tests; however, specificity is again poor (50%).

Little information was available for the single tuberculin test in goats. Sensitivity results varied from 38.3% to 95% and very few estimates of specificity were available. However, in a study of 521 culture-positive animals, the comparative tuberculin test provided good sensitivity (83.7%) and specificity (100%). Estimates of sensitivity and specificity for the IFN-γ were better than for the skin test in one study (39), but another study found IFN-γ to be less sensitive (34). The results of an ELISA test performed 15 days after a skin test provided an increased sensitivity compared with a normal ELISA (which lacked both sensitivity and specificity); this was attributed to an anamnestic response. A combination of the comparative tuberculin test and the IFN-γ assay offered the highest sensitivity (95.8%) and also high specificity: 96% in one study (34). More information is needed on the specificity of various tests in goats.

The mantoux (skin) test has been used as a screen test for human tuberculosis for many years. In humans, tuberculosis diagnosis may also be achieved by using smear examination and/or culture (of three consecutive sputum samples) and/or chest X-ray. In recent years, several versions of the IFN-γ assay (Quantiferon™, Quantiferon™ TB Gold, Cellestis Ltd) have been evaluated, and some publications suggest the assay has equivalent sensitivity and specificity to the skin test. In patients vaccinated with BCG, the IFN-γ assay is considered to be more sensitive and specific than the skin test, and this technology is now approved by the US Food and Drug Administration.

The tuberculin test has long been the recognised test for diagnosis of tuberculosis in non-human primates. However, the sensitivity of the tuberculin test, generally applied to the upper eyelid, is considered poor and serial testing is necessary. More recently, the IFN-γ assay has been developed for use in these species and is marketed as the Primagam™ test. This test appears to be gaining acceptance as an alternative test for these species and has gained provisional approval from the Department of Agriculture of the USA. The Chembio rapid test claims an acceptable sensitivity and a good specificity on limited numbers of animals, and the test is being evaluated further for sensitivity and for specificity using animals infected with other *Mycobacterium* spp. (42). Tuberculosis is considered to be the most serious disease of captive non-human primates, and a good diagnostic test is important to those working in the management of non-human primate colonies and primate rehabilitation programmes.

A report (25) of the use of diagnostic tests in marine mammals (seals and dolphins) suggests that the comparative tuberculin test may be of use in detecting infection in captive seals. Many infected seals will show no clinical signs. Culture of bronchial washes has also been used as a screening test for animals in zoological collections (unpublished data) but the sensitivity of the test is unknown. Tuberculin testing in wild-caught (trapped) seals can only be done if the animals can be held for the 72 hours required for reading of the injection site. Animals have to be sedated to clip the injection site and to inject the tuberculin, and again for the reaction to be read (unless the reaction is extensive, in which case it may be observed). Tuberculosis is present in at least seven different seal species in the southern hemisphere (16) and it is a known zoonosis (70). It is therefore important when managing standings or introducing new animals into a collection to screen them for tuberculosis if the species originate from the southern hemisphere. Zoological collections that include seals would benefit from the development of alternative tests that require minimal animal handling (e.g. IFN-γ assay).

The comparative tuberculin test was reported to perform with perfect sensitivity and specificity in a study that involved small numbers of pigs infected with *M. africanum* (1). The tuberculin test was compared to macroscopic lesions at slaughter and *M. africanum* was isolated in some animals. A small study using the single tuberculin test provided a specificity of 100% in pigs from an *M. bovis*-free area (53). No reports could be found on the sensitivity of the tuberculin test in *M. bovis*-infected pigs. The existing Bovigam™ assay cannot be applied to pigs. Infection of pigs with *M. africanum* or *M. tuberculosis* is considered very rare. These animals are usually an end host for *M. bovis* and are most commonly infected by the ingestion of contaminated milk or offal or by scavenging infected carcasses. There is some suggestion that wild boar may act as maintenance hosts in Spain (A. Aranaz, personal communication, 2005). There is likely to be a limited need for validated diagnostic tests in pigs unless such tests are important for trade purposes. If tuberculosis is found in domestic pigs, it is normal practice to depopulate the animals.

A number of ELISAs have been evaluated for the diagnosis of tuberculosis in possums. These tests appear to have low sensitivity but reasonable specificity. The LPA provided better sensitivity than the ELISA but, as discussed previously, LPA remains a research tool. As in the case of badgers, no diagnostic tool is likely to be of value except for use in pathogenesis studies.

An ELISA with low sensitivity but good specificity has been reported for raccoons. As is the case with possums and badgers, described above, it is difficult to see the need for a validated test for tuberculosis in raccoons.

Tuberculosis in sheep is considered to be rare, and generally only occurs when animals are in close contact with heavily infected cattle. The tuberculin test is seldom used in sheep but the single tuberculin test has been reported to have a sensitivity of 67.7% (44) and 81.6% (11) in two separate studies on six and 31 histopathology-positive animals respectively. The Bovigam™ test is reported to be acceptable for use in sheep, and when used in the small study reported by Malone *et al.* (44), it resulted in a sensitivity of 100%.

The tuberculin test was successful in diagnosing tuberculosis in two tapirs in a British zoo. These animals were thought to have contracted the disease from infected seals in an adjacent enclosure. As with many wildlife species, there are no validated tests for tapirs.

Conclusions and recommendations

M. bovis is well known to have the widest species range of any of the *M. tuberculosis* complex members, infecting an extensive range of animals, from cattle to humans, domestic animals to feral or wild ones. Some wildlife species have a considerable impact on eradication and control programmes for tuberculosis around the world. In addition, captive animals infected with tuberculosis create problems in the management of zoological collections, increasing the risk of infection to other valuable animals as well as to their keepers. Although tuberculosis in such animals is an important problem, there is a dearth of well-validated data for the diagnosis of the disease in animals other than cattle. As is evident from Table I, many different species can become infected with tuberculosis. Table II and the text above have attempted to summarise the diagnostic tests that have been applied to the detection of tuberculosis in a variety of species. As noted at the bottom of Table II, however, many of the tests recorded here have not been properly validated and would fail to meet the validation criteria currently required by the OIE.

The types and circumstances of animals are relevant in assessing the importance or need to have validated diagnostic tests for tuberculosis. Animals other than cattle may be classified into the following four groups:

a) domestic or farmed maintenance hosts that cannot be eradicated and may have an impact on the prevalence of tuberculosis in cattle (e.g. Asian buffaloes in Australia, goats in Spain)

b) wild or domestic species in zoological collections where the infection provides a transmission risk to other animals in the collection or where there is a zoonotic risk (e.g. non-human primates, seals, oryx)

c) wild animals where the spread of disease can directly affect the value of the collection, and hence the value of tourism or related economic benefits to the country (e.g. African buffaloes, kudu, oryx)

d) spillover hosts that have a negligible ability to re-infect cattle (and where control of tuberculosis in cattle will lead to a corresponding decrease in prevalence rates in the spillover host; e.g. feral pigs in Australia).

Groups *a* to *c* are considered to be of higher importance than group *d* in terms of the importance of developing validated tests for tuberculosis, but the tests are presumably only of value if they are to be used for eradication or control purposes or to facilitate trade.

The information accumulated during this review suggests there may be adequate data available for the validation of the tuberculin test and the IFN-γ assay for South African buffaloes. There appears to be a lack of valid specificity data for diagnostic tests in goats. In the case of the Asian buffalo in Australia, there is no requirement to further validate diagnostic tests for this species. Whether there is a requirement for such a test in Asian countries is yet to be determined.

By comparison, there has been a reasonable amount of work done in non-human primates, and it is important to have a test validated for these species. Collection of further data is encouraged so as to build up sufficient validation data over time. The Primagam™ test in particular holds considerable promise for these species.

The OIE requires certain numbers of tests to be conducted in particular species for the purpose of validation. In many cases, because tuberculosis may occur in rare animals or because the occurrence of disease in a particular species is very low, the numbers of tests that are required cannot be achieved. Because of this, it will be necessary to accumulate validation data over time as cases occur. In many instances, it appears that data is not published or collated; an international effort may be required to collect information so that it can gradually be accumulated for validation purposes. In some cases, it is expected that there will still be insufficient data to meet the rigorous guidelines established by the OIE.

Recommendations

In order to collate validation data on the diagnosis of tuberculosis in species other than bovine, the OIE should:

a) develop a suitable template for submitters that will help in the collection of key information to allow integrated data analysis of tests used for diagnosis of tuberculosis

b) encourage veterinarians and researchers to submit data from test evaluation studies using the developed template, so that over time the data can be accumulated

c) make the information available to interested parties as appropriate for the purpose of further study and test validation.

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Examen des épreuves utilisables pour le diagnostic de la tuberculose chez des espèces autres que les bovins

D.V. Cousins & N. Florisson

Résumé

La tuberculose bovine est une maladie importante qui a des répercussions sur le commerce régional et international. La maladie peut nuire à la stabilité sociale et économique et avoir un effet néfaste sur la diversité des espèces. Le test tuberculinique intradermique a été utilisé pendant près d'un siècle et, malgré les progrès technologiques accomplis ces vingt dernières années, il reste la seule épreuve prescrite pour le diagnostic de la tuberculose chez les bovins. De nombreuses autres espèces animales, y compris l'homme, peuvent être infectées par *Mycobacterium bovis*. Le présent article passe en revue différentes épreuves utilisables pour la détection de l'infection par *M. bovis* chez diverses espèces animales et tente d'établir un ordre de priorité ou d'expliquer à quel point il est important de disposer d'épreuves diagnostiques correctement validées pour les différentes espèces. Les difficultés posées par la validation des épreuves à l'aide d'un petit nombre d'animaux, notamment quand la tuberculose ne concerne que quelques cas ou quand l'espèce animale touchée est rare et/ou très utile, sont exposées.

Mots-clés

Diagnostic – Épreuve à l'interféron – Faune sauvage – Méthode de dosage immuno-enzymatique – Mycobacterium bovis – Test intradermique – Test à la tuberculine.

Repaso de las pruebas existentes para el diagnóstico de la tuberculosis en especies no bovinas

D.V. Cousins & N. Florisson

Resumen
La tuberculosis bovina es una importante enfermedad, que influye en el comercio regional e internacional y puede minar la estabilidad social y económica y tener efectos deletéreos sobre la diversidad de especies. La prueba intradérmica de la tuberculina, que se aplica desde hace casi un siglo pese a los avances técnicos de los últimos veinte años, sigue siendo la única prueba prescrita para diagnosticar la tuberculosis en el ganado vacuno. Pero *Mycobacterium bovis* puede infectar a otras muchas especies, incluido el hombre. Los autores pasan revista a diversas pruebas evaluadas para detectar la infección por *M. bovis* en una serie de especies animales, tratan de definir un orden de prioridades entre ellas y formulan observaciones sobre la importancia de disponer de métodos de diagnóstico convenientemente validados para las distintas especies. También comentan las dificultades de validar una prueba empleando un pequeño número de animales, sobre todo cuando la especie en cuestión es infrecuente o valiosa o cuando la tuberculosis se presenta sólo esporádicamente en ella.

Palabras clave
Diagnóstico – Ensayo inmunoenzimático – Fauna salvaje – Mycobacterium bovis – Prueba del interferón – Prueba intradérmica – Prueba de la tuberculina.

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