Bovine tuberculosis in Europe from the perspective of an officially tuberculosis free country: Trade, surveillance and diagnostics

Irene Schiller a,*, W. Ray Waters b, H. Martin Vordermeier c, Thomas Jemmi a, Michael Welsh d, Nicolas Keck e, Adam Whelan c, Eamonn Gormley f, Maria Laura Boschirolig, Jean Louis Moyen h, Carmen Vela i, Monica Cagiola j, Bryce M. Buddle k, Mitchell Palmer b, Tyler Thacker b, Bruno Oesch l

a Federal Veterinary Office, Animal Health Division, Schwarzenburgstrasse 155, CH-3003 Bern, Switzerland
b National Animal Disease Center, Agricultural Research Service, US Department of Agriculture, Ames, IA, USA
c Veterinary Laboratory Agency, Addlestone, Great Britain, United Kingdom
d AFBI—Veterinary Sciences Division, Stormont, Northern Ireland, Ireland
e Laboratoire Départemental Vétérinaire de l’Hérault, Montpellier, France
f University College Dublin, Dublin, Ireland
ghi Laboratoire Conseil Général de la Dordogne, France
j Ingenasa, Madrid, Spain
k Istituto Zooprofilattico dell’Umbria e delle Marche, Perugia, Italy
l AgResearch, Palmerston North, New Zealand
m Prionics AG, Schlieren, Switzerland

ABSTRACT

Switzerland has been officially free of bovine tuberculosis (OTF) since 1960. Since 1980 the control of bovine tuberculosis (bTB) has been reduced to passive abattoir surveillance. Isolated cases of bTB, partly due to reactivation of human Mycobacterium bovis infections with subsequent transmission to cattle, have been noticed in the last years. In Europe, the overall prevalence of bTB is slightly increasing. Both OTF and non-OTF countries report increases in the proportion of bTB positive cattle herds.

Current bTB eradication and control programs in Europe are facing a range of challenges. Whole herd depopulation is becoming a less attractive option for economic reasons and due to animal welfare concerns. Live animal trade is increasing both at national and international levels. Regarding these tendencies and taking into account the chronicity of bTB infection, pre-movement testing is becoming increasingly important as a central tool for eradication and for protection against re-introduction of bTB. Pre-movement testing, however specifically focuses on the infection status in individuals, requiring a high level of diagnostic accuracy to correctly diagnose infected animals. Current screening tests for bTB, however, have been designed to meet demands as herd tests. This illustrates that the modification of existing and/or the development of new diagnostics for bTB might be needed.

The tuberculin skin test (TST), the primary screening test for bTB may in certain situations have low sensitivity. The interferon gamma (IFN-γ) assay is accepted to be more sensitive compared to TST. Reduced specificity, however, especially in areas of low bTB prevalence raises concerns. New antigen combinations including Rv3615c, OmpATb and others have been shown to complement ESAT-6 and CFP-10 in the whole blood IFN-γ
1. Introduction

Bovine tuberculosis (bTB), caused primarily by Mycobacterium bovis and to a lesser extent by Mycobacterium caprae, is endemic in many European countries and constitutes a significant economic burden to the agricultural industries. The control and eradication of bTB is mainly based on a test and slaughter policy and/or abattoir surveillance. Despite intensive eradication efforts over decades, bTB continues to be a problem with global perspective. In some countries wildlife reservoirs constitute a continuous source of re-infection of cattle (Corner, 2006). Current bTB eradication and control programs are facing a range of challenges. Whole herd depopulation is becoming less of an option for economic reasons and due to animal welfare concerns. Live animal trade is increasing both on national and international levels. As proposed by international organizations (Anon., 2008) trade agreements increasingly utilize concepts like regionalisation, zoning, and compartmentalisation as principles of disease control. Regarding these tendencies and taking into account the chronicity of bTB, pre-movement testing is becoming increasingly important as a central tool for eradication and for protection against re-introduction of bTB. Pre-movement testing, however, specifically focuses on the infection status of individual animals. This shows that requirements for diagnostic tests have changed. A high level of reliability to correctly diagnose infected animals is needed. Current screening tests for bTB were originally designed as herd tests (De la Rua-Domenech et al., 2006). This illustrates that a modification of existing and/or development of new diagnostics for bTB might be required. In this article we address these issues by summarizing published data on diagnostic tests for bTB in addition to new data and suggestions for possible improvements. We limited our reflections to validated and/or development of new diagnostics for bTB might be required. In this article we address these issues by summarizing published data on diagnostic tests for bTB in addition to new data and suggestions for possible improvements. We limited our reflections to validated and approved tests, mainly ante mortem screening tests, and we do not consider new test platforms in this paper.

2. Eradication and control of bTB in Europe

In the EU, the overall prevalence of bTB is currently increasing. In 2007, 0.53% of cattle herds were bTB positive compared to 0.48% in 2006 (Anon., 2009a). Both countries officially free of bTB (OTF) and non-OTF report increases in the proportion of bTB positive cattle herds. Ireland and the United Kingdom are currently facing the highest herd prevalences in Europe (4.37 and 3.27% respectively; Anon., 2009a).

Even in countries with low bTB prevalence it is difficult to achieve eradication. In Italy, the overall herd prevalence of bTB is 0.95%. The country has had a split bTB status for several years; various provinces in 10 regions have a prevalence of <0.1% and have been declared OTF, while the non-OTF provinces and regions still report prevalences from 0.2 to 5.3% (data from 2007, http://ec.europa.eu/food/committees/regulatory/scca/animal_health/presentations/tb_34033403_it.pdf; Anon., 2009b). Recent bTB outbreaks in the region of Trentino in the North of Italy are probably due to the importation of M. caprae-infected cattle from Austria and Germany. Trade and direct animal contact on alpine pastures have caused continued outbreaks in this region, illustrating the high infectious potential of circulating mycobacterial strains (Maria Pacciariini and Franco Chin, personal communication). In Spain, herd prevalence increased from 1.52% in 2005 to 1.76% in 2006, and declined to 1.63% in 2007 and 1.59% in 2008 (www.rasve.es; http://ec.europa.eu/food/committees/regulatory/scca/animal_health/presentations/tb_34032009_es.pdf; Anon., 2009a). The recent decrease is most likely due to more effective testing strategies based on sensitive methods (intensified testing in high prevalence areas, increased use of the interferon-γ assay and modified use of the assay at a lower cutoff point). A high density of wild deer in some areas (such as the South of Spain) that act as a reservoir of bTB and which live in close proximity to farmed cattle is a major concern regarding the eradication of bTB (Lucas Dominguez and Alicia Aranaz, personal communication).

Some of the OTF countries are currently observing rising bTB prevalences although the increases are only slight. In France, herd prevalence increased from 0.04% in 2006 to 0.05% in 2007 (Anon., 2009a). Higher detection rates of bTB may in part be linked to improved surveillance, as in the region of Dordogne (Jean-Louis Moyen, personal communication). Another contributing factor in France is spillover infection from wild boar and wild deer in the Brotonne Forest of Upper Normandy (Zanella et al., 2008) and poor test performance in infected bull fighting herds in the region of Camargue that have a herd prevalence of about 10% (Nicolas Keck, personal communication). Fighting bulls are a unique cattle breed and are a protected...
species, but as herds with less than 10% positives may be derogated from the French regulation (according to which one or more confirmed case of bTB would normally lead to herd depopulation) and undergo progressive disease eradication by repeated testing.

In Germany, 119 outbreaks were recorded between 1995 and 2008, but the number recently increased with 12 outbreaks in 2007 and 23 outbreaks in 2008. Trade was identified as the major epidemiological factor for the spread of bTB in Germany. Strain typing indicated a clear epidemiological link between 12 of the 23 outbreaks in 2008, all located in the Northern part of the country (Lower Saxony, Northrhine-Westfalia and Schleswig-Holstein).

Bovine TB has not been detected in German wildlife in the last few years, except for sporadic cases of *M. caprae* in wild deer in the South of the country, close to the German-Austrian border (Bavaria; I. Moser, personal communication). In contrast, regions in Austria along the border of Germany and Switzerland (Tirol and Vorarlberg) hold wild red deer with endemic tuberculosis caused by *M. caprae*. Recently, increased numbers of red deer infected with tuberculosis have been found in these areas, a tendency which may be closely associated with the practice of supplementary feeding leading to a marked increase of the total deer population. There have been increased numbers of bTB outbreaks in cattle and strain typing has indicated that the livestock infections probably originated from wildlife (Josef Köfer, personal communication).

Based on requirements of the OIE International Animal Health Code Switzerland has been OTF since 1960 (Flückiger, 1960; Thomas Jemmi, personal communication). The OTF status is recognized by the European Union (EU; Bilateral Agreement between the EU and Switzerland on Trade with Agricultural Products, Annex 11, 1999). In the 1930s bTB was widely spread in Switzerland similarly to other European countries. A voluntary program to fight bTB was started in 1934. Due to its limited success it was transformed into a mandatory eradication program in 1950 (Flückiger, 1960). Herd prevalence at that time exceeded 25% and about 10% of human cases of TB were caused by *M. bovis*. Based on the experience and the success from the eradication of bTB in the USA, the Swiss eradication program was fundamentally built on tuberculin skin testing and removal of all reactors, including inconclusive reactors (Fritschi, 1960). The focus was on the whole cattle population and not on individual animals. During the ten years of eradication, about 400,000 cattle were killed and the cost was estimated to have been 400 million Swiss Francs (Hügli, 1960). Between 1960 and 1980, active surveillance was performed by testing the whole cattle population every second year. Since 1980 the control of bTB has been reduced to passive abattoir surveillance. Single cases of bTB have been detected in the last 30 years, partly due to reactivation of human *M. bovis* infections with subsequent transmission to cattle (T. Jemmi, personal communication). In 1997, a randomized survey using tuberculin skin testing was conducted in about 10% of Swiss cattle holdings (111,394 cattle from 4874 farms) and did not reveal any positive skin test reactors (Anon., 2009c). Farmed deer (*n* = 343) was investigated in 1998 (Wyss et al., 2000). The proportion of deer with positive mycobacterial culture results was high (75.7%), but none of the isolates were *Mycobacterium tuberculosis* complex bacteria. Like in other countries without wildlife reservoirs, the test and slaughter strategy has proven successful in eradicating bTB. Yet, factors like trade, direct contact of bTB-free animals with animals from herds with a risk of being infected, as in the mixing of herds from different countries on alpine summer pastures and with infected wild animals, especially in border areas, represent continuous possibilities to re-introduce the disease into Switzerland and emphasize the importance of effective surveillance.

3. Diagnosis of bTB

3.1. Ante mortem screening tests

3.1.1. Tuberculin skin test

The tuberculin skin test (TST) constitutes the primary screening test for bTB, either as caudal fold test (CFT), as single cervical intradermal test (CIT) or as comparative cervical test (CCT). Skin testing has been used as cornerstone for the successful eradication of bTB in many countries. Summary of data from international studies indicate 68–96.8% sensitivity and 96–98.8% specificity for CFT, 80–91% sensitivity and 75.5–96.8% specificity for CIT, 55.1–93.5% sensitivity and 88.8–100% specificity for CCT (Vordermeier et al., 2008). Differences in performances are largely due to a broad variation of TST techniques, including differences in tuberculin doses, tuberculin preparations and the interpretation of skin reactions. Other important factors include differences in the design of the various studies, the selection of animals and most importantly, how the infection status of the animals was determined. Recent data from Northern Ireland and a particular region in France (Camargue) indicate a markedly reduced sensitivity of TST in certain situations: only 59% (CCT, Northern Ireland) and 10.6% (CIT, Camargue) of animals with confirmed *M. bovis* infection were detected (Michael Welsh, unpublished data; Nicolas Keck, unpublished data). The observation in Camargue refers to fighting bulls. These data may represent the lower end of the performance spectrum of TST and not average values. Still, a lack of sensitivity constitutes a serious threat for eradication and control programs and it illustrates that live cattle trade may be linked to an unavoidable level of risk of re-introduction of bTB in OTF herds, regions or countries where freedom is defined using a TST.

Improvement of purified protein derivatives (PPD) would have the potential to enhance sensitivity and specificity of TST. In fact, more active and more specific tuberculins would be desirable. To date, limited progress has been achieved in this field, mainly due to the ill-defined nature of the antigens in PPD as well as the complexity of PPD production. Improved and extended control of PPD activity after production by using standardized methods resulting in higher reproducibility than in vivo guinea pig and cattle potency testing could markedly improve TST (Bakker et al., 2005; Good et al., 2008). Defined antigens may eventually prove useful to improve TST. Use of ESAT-6 at a high dose in TST has resulted in
increased specificity but decreased sensitivity relative to PPD (Pollock et al., 2003). Co-administration of a synthetic lipopeptide resulted in slightly lower concentrations of ESAT-6 inducing cell-mediated immune response (CMI); however, the addition of an adjuvant could harbour the risk of inducing tuberculin sensitization (Whelan et al., 2003). Alternative tools for the interpretation of TST may improve its ease of use. Infrared thermography (IRT) is currently being evaluated in the USA to assess CCT reactions from a distance (Johnson and Dunbar, 2008). Preliminary data from 15 cattle sensitized to either bovine tuberculin, avian tuberculin or nothing (control) indicate that 86% of cattle were correctly classified compared to 80% with skin thickness measurements. In addition, reading by IRT may be done after only 24 h (compared to 72 h of traditional reading) and, importantly, IRT may provide a more objective method.

3.1.2. Interferon gamma assay

The interferon gamma assay (IFN-γ; Wood et al., 1990; Bovigam®, Prionics, Switzerland) is used complementary to TST as a screening test for bTB, generally in herds with TST reactors. The tuberculin skin and IFN-γ tests detect not only overlapping, but also distinct, populations of M. bovis-infected cattle (Neill et al., 1994; Vordermeier et al., 2006; Coad et al., 2008).

Sensitivity and specificity of the IFN-γ assay estimated in international studies ranged from 73% to 100% and from 85.0 to 99.6% (De la Rua-Domenech et al., 2006). Again, variations in assay protocols may have resulted in disparate results between studies, therefore more standardized procedures would be desirable. In recent studies, parameters of the IFN-γ assay have been analyzed with a view to defining a range of possible conditions. Delayed initiation of culture in a PPD-based assay (24 h compared to 8 h after blood collection) resulted in a significant improvement of specificity (97 compared to 85%), whereas there was only a modest reduction of sensitivity (from 96 to 90%), which was statistically not significant. Stimulation requires a temperature of 33 °C or higher, carbon dioxide is not needed for stimulation, and various plate formats ranging from 24- to 96-well plates can be utilized. The produced IFN-γ is stable at 4 °C for at least 28 days as well as after repeated freeze-thaw cycles (Schiller et al., 2009a). Moreover, a tool for the standardization of PPD activity in the IFN-γ assay named Relative Potency 30 (RP30) has been proposed (Schiller et al., 2010).

The IFN-γ assay is accepted to be more sensitive compared to skin test (Vordermeier et al., 2008). Follow-up studies of TST-negative cattle in Great Britain showed that IFN-γ positive cattle had an odds ratio (OR) of 11.1 for confirmed bTB compared with TST and IFN-γ negative animals (Coad et al., 2008). This observation is consistent with findings of a study performed in the Republic of Ireland, where TST-negative/IFN-γ positive cattle were 8.9 times more likely to become a TST-reactor at the next testing occasion than TST and IFN-γ negative cattle from the same herds (Gormley et al., 2006). In Northern Ireland, a TST-negative/IFN-γ positive animal had an 18% increased risk of being TST-positive within one year as compared to a TST-negative/IFN-γ negative animal (Michael Welsh and Jim McNair, unpublished data).

Similar results were obtained with the aforementioned fighting bull herds in Camargue, France. The IFN-γ assay achieved a herd and animal sensitivity of 96 and 66.2% (respectively), compared to only 58 and 10.6% (respective) with CIT (Nicolas Keck, unpublished data). Yet, IFN-γ sensitivity was clearly lower in these fighting bulls compared to data from international studies (De la Rua-Domenech et al., 2006). IFN-γ specificity in fighting bulls was 99.7% (1 unconfirmed IFN-γ reactor out of 382 cattle, 98.6–99.9, CI 95%, N. Keck, unpublished data).

In areas of low bTB prevalence, however, there are concerns regarding the Bovigam specificity, which may limit the wider application of this test as a routine herd surveillance or pre-movement test (Monaghan et al., 1997; Lauzi et al., 2000; Pollock et al., 2000; Palmer et al., 2006). The replacement of tuberculins by defined antigens is regarded as have the greatest potential for improving the specificity of Bovigam. ESAT-6 and CFP-10 have been shown to give a significant improvement in specificity in the whole blood IFN-γ assay (Pollock et al., 2000; Vordermeier et al., 2001, 2005; Buddle et al., 2003; Waters et al., 2004; Aagaard et al., 2006; Cockle et al., 2006; Ewer et al., 2006). Results from a recent field trial in Great Britain indicate that a peptide cocktail composed of peptides from ESAT-6 and CFP-10 (E/C) achieved a sensitivity of 86% (76.6%, 92.5%, 95% CI, n = 85) and specificity of 99% (92.5%, 99.9%, 95% CI, n = 72), as opposed to 97% (90.0%, 99.3%, 95% CI, n = 85) sensitivity and 94% (86.4%, 98.5%, 95% CI, n = 72) specificity with PPD-based IFN-γ assay (Vordermeier et al., 2009). Interestingly, sensitivity of the IFN-γ assay based on E/C alone is still superior to that of CCT (at a mean sensitivity value of 80% for CCT [De la Rua-Domenech et al., 2006]).

New antigen combinations for the IFN-γ assay have recently been described that resulted in improved sensitivity compared to E/C alone and without reducing specificity (Cockle et al., 2006; Sidders et al., 2008; Schiller et al., 2009b). Importantly, Rv3615c and OmpATb were found to identify cattle with confirmed bTB that did not react to E/C. In recent data a sensitivity of 94% (86.8%, 98.1%, 95% CI, n = 85) and specificity of 99% (92.5%, 99.9%, 95% CI, n = 72) was achieved for E/C combined with Rv3615c (Vordermeier et al., 2009).

An IFN-γ assay based on defined antigens along with other modifications may allow further improvements. A modified interpretation of the IFN-γ ELISA, such as measuring the percentage positivity compared to a standardized positive control, could be done, and having a shortened pre-culture time before whole blood stimulation: these have the potential to further improve sensitivity and overall performance and require warrant further examination. In addition changes in the format of the Bovigam assay are proposed. An “in-tube” or “in-plate” stimulation device that would allow lymphocytes to be rapidly exposed to antigen stimulation after blood collection could be beneficial and overcome logistical difficulties. In addition, the inclusion of a stimulation control [e.g., pokeweed mitogen (PWM) or staphylococcal enterotoxin B (SEB)] in the routine assay procedure offers an important
tool to control the validity of whole blood samples. Currently the costs of the IFN-γ assay constitute a disadvantage in its current application, and curtail its wider use. Increased use of the assay may allow lower price from a high scale production. Importantly, additional laboratory cost reductions could be achieved by automation of the assay, as would modification to use a rapid ELISA. Thus, the assay may be adapted to provide a highly specific and sensitive screening test for use as a stand-alone test or in conjunction with other screening tests. In addition, a multispecies IFN-γ assay usable also for non-bovine species, such as camelids, dogs and cats, would be of use for bTB controls.

3.2. Post mortem examination ("passive" bTB surveillance at slaughter)

Lesion detection during the commercial slaughter of cattle represents a cost-effective procedure for passive surveillance of bTB, especially in areas of low prevalence or in OTF regions. Generally, the method has low sensitivity but is used for surveillance in both OTF and non-OTF countries to supplement live cattle testing. In many OTF countries it is the only means of surveillance. Lesions may vary in size and when small and localized in large organs or lymph nodes, are easily overlooked during abattoir inspection. A study in Australia analyzing reactors to the tuberculin test showed that abattoir inspection failed to detect an estimated 47% of cattle with lesions (Corner et al., 1990). In the USA, slaughter surveillance sensitivity has been estimated to be as low as 28.5% (Anon., 2009d). In that study, slaughter sensitivity was calculated as the probability of an infected animal having visible lesions times the probability of the lesion being detected by slaughter inspectors times the probability of the lesion being submitted to the laboratory times the probability of the laboratory tests being conducted on the sample and the tests being positive. This illustrates the low probability of infection in a carcase being detected and consequential delay in detecting a re-introduction of bTB in OTF regions or countries.

There are limited ways of improving the sensitivity of post mortem detection of bTB. Continuous education and training of slaughter inspectors are certainly of major importance. In addition, the combined use of liquid and solid media has been reported to improve culture sensitivity (Hines et al., 2006).

3.3. Comparison of diagnostic tests in different epidemiologic settings

The choice of the diagnostic strategy within programs in general and for specific situations, such as outbreaks within herds and movement of individual animals, should include consideration of epidemiological risk factors. This concept can be quantified using the predictive values: the probability that a positive or a negative test result respectively reflects a truly infected or non-infected animal, and may provide additional information on the individual test results. The negative predictive value (NPV) is indirectly proportional to the prevalence of infection in the population screened (Hüsler and Zimmermann, 2005). This means, the greater the risk of infection, the less confidence can be attached to a negative test result.

From a policy maker’s perspective, which is primarily focused on limiting disease transmission, the NPV is critical. Table 1 shows the comparative performance of selected diagnostic techniques for bTB in different epidemiological settings. At low animal prevalence (0.1%) all NPVs are very high (99.9% for lesion and culture, 100% for the other assays), meaning that the probability that an animal with a negative test result might actually be infected is extremely low, even for tests with a low sensitivity, such as lesion and culture. At high prevalences, as they might occur within an infected herd, however, differences between diagnostics are more evident. At 40% prevalence, the NPV of CCT is 88%, meaning that about one in ten animals with a negative test result might be infected, as opposed to 98% of the PPD-IFN-γ assay or 96% of the E/C/Rv3615c-IFN-γ assay. These differences may be of relevance if only reactors but not herds are depopulated, as for this strategy it would be crucial to identify and eliminate each infected animal from the herd.

From an economic point of view, the PPV is important and differences between the screening tests might be especially relevant at low and medium infection pre-

### Table 1

The performance of selected diagnostic assays for bovine tuberculosis in different epidemiological settings.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Se (%)a</th>
<th>Sp (%)a</th>
<th>Reference</th>
<th>PPVb 0.1%</th>
<th>1%</th>
<th>10%</th>
<th>20%</th>
<th>40%</th>
<th>NPVb 0.1%</th>
<th>1%</th>
<th>10%</th>
<th>20%</th>
<th>40%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCT</td>
<td>80.0</td>
<td>96.8</td>
<td>De la Rua-Domenech et al. (2006)</td>
<td>2.4</td>
<td>20.2</td>
<td>73.5</td>
<td>86.2</td>
<td>94.3</td>
<td>100</td>
<td>99.8</td>
<td>97.8</td>
<td>95.1</td>
<td>87.9</td>
</tr>
<tr>
<td>PPD IFN-γ</td>
<td>97.0</td>
<td>94.0</td>
<td>Vordermeier et al. (2009)</td>
<td>1.6</td>
<td>14.0</td>
<td>64.2</td>
<td>80.2</td>
<td>91.5</td>
<td>100</td>
<td>100</td>
<td>99.6</td>
<td>99.2</td>
<td>97.9</td>
</tr>
<tr>
<td>E/C IFN-γ</td>
<td>86.0</td>
<td>99.0</td>
<td>Vordermeier et al. (2009)</td>
<td>7.9</td>
<td>46.5</td>
<td>90.5</td>
<td>95.6</td>
<td>98.3</td>
<td>100</td>
<td>99.9</td>
<td>98.5</td>
<td>96.6</td>
<td>91.4</td>
</tr>
<tr>
<td>E/C/Rv3615c IFN-γ</td>
<td>94.0</td>
<td>99.0</td>
<td>Vordermeier et al. (2009)</td>
<td>8.6</td>
<td>48.7</td>
<td>91.3</td>
<td>95.9</td>
<td>98.4</td>
<td>100</td>
<td>99.9</td>
<td>99.3</td>
<td>98.5</td>
<td>96.1</td>
</tr>
<tr>
<td>Lesion detection and culture</td>
<td>28.5</td>
<td>100%</td>
<td>Anon. (2009d)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>99.9</td>
<td>99.1</td>
<td>91.4</td>
<td>82.5</td>
<td>63.8</td>
</tr>
</tbody>
</table>

- a: Sensitivity and Sp: specificity. The values established in these studies represent the best balance between sensitivity and specificity.
- b: PPV: positive predictive value and NPV: negative predictive value, calculated for 5 different prevalences of infection.
- c: Median sensitivity and specificity evaluated in international field studies.
- d: Field evaluation in Great Britain.
valences. At 1% prevalence, the IFN-γ assays with defined antigens have PPVs of 49% (E/C/Rv3615c) and 47% (E/C), compared to CCT with 20% and PPD-IFN-γ with 14%, clearly demonstrating the benefit of defined antigens for the diagnosis of bTB.

4. Conclusions

Tools and settings for the control and eradication of bTB in Europe are changing, with a tendency towards increased trade, the facilitation of trade and free trade generally. There is shift away from whole herd depopulation as an eradication tool, with an emphasis towards other principles of disease control, such as effective surveillance and pre-movement testing. These latter tools are becoming important but require a high level of accurate identification of individual infected animals. Current screening tests, originally designed to meet demands as herd tests, could be significantly improved by the replacement of tuberculin by defined antigens. Thus limitations on test accuracy due to the current interpretation of tests based on the relative response to bovine and avian PPDs could be overcome. Defined antigens stimulate an in vitro CMI response in the IFN-γ assay and therefore a modified version of the IFN-γ assay using these antigens could represent an efficient primary screening tool for bTB with improved accuracy and as individual animal test.

In conclusion, the development of improved diagnostic tests will be important for future eradication of bTB, in line with international commitment to high standard animal health programs.

Conflict of interest statement

The authors have no conflict of interest.

Acknowledgements

We gratefully acknowledge Jim McNair (AFBI-Veterinary Sciences Division, Stormont, Northern Ireland), Maria Pacciarini (Istituto Zooprofilattico Sperimentale, Brescia, Italy), Franco Chin (U.O. Igiene e Sanità Pubblica Veterinaria, Trent, Italy), Lucas Dominguez and Alicia Aranzaz (Universidad Complutense, Madrid, Spain), Irmgard Moser (Friedrich-Loeffler-Institute, Jena, Germany), Josef Köfer and Petra Winter (AGES, Vienna, Austria), Karl Schöpf (AGES, Innsbruck, Austria) and Pauline Nol (USDA, Fort Collins, USA) for providing valuable information and unpublished data.

References


