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Current *ante-mortem* techniques for diagnosis of bovine tuberculosis

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ABSTRACT

Bovine tuberculosis (TB), mainly caused by *Mycobacterium bovis*, is a zoonotic disease with implications for Public Health and having an economic impact due to decreased production and limitations to the trade. Bovine TB is subjected to official eradication campaigns mainly based on a test and slaughter policy using diagnostic assays based on the cell-mediated immune response as the intradermal tuberculin test and the gamma-interferon (IFN-γ) assay. Moreover, several diagnostic assays based on the detection of specific antibodies (Abs) have been developed in the last few years with the aim of complementing the current diagnostic techniques in the near future. This review provides an overview of the current ante-mortem diagnostic tools for diagnosis of bovine TB regarding historical background, methodologies and sensitivity (Se) and specificity (Sp) obtained in previous studies under different epidemiological situations.

1. Introduction

Diagnosis of bovine tuberculosis (TB), mainly caused by *Mycobacterium bovis*, is still a challenge since available diagnostic tools have limitations regarding sensitivity (Se) and specificity (Sp) that affect the detection of infected animals and the advance, in part, of the TB eradication programmes towards their final objective. Current bovine TB eradication programmes are based on a screening and slaughter policy using mainly the intradermal tuberculin test. The intradermal tuberculin test is recognised by the World Organisation of Animal Health (OIE) and the European Commission as the primary screening test for detection of tuberculosis in cattle (Karolemeas et al., 2012; Schiller et al., 2010). Other diagnostic tools have been also developed over the years as the *in vitro* interferon-gamma (IFN-γ), lymphocyte proliferation or serological assays showing some benefits or lacks regarding Se and Sp in comparison with the intradermal test. IFN-γ assay is approved for use in the European Union since 2002 [Council Directive 64/432/EEC, amended by (EC) 1226/2002], received approval by the United States Department of Agriculture (USDA) in 2003 (USDA:APHIS, 2005), and was accredited by the Standing Committee on Agriculture as an official diagnostic test for bovine TB in Australia in 1991. In Europe, the single and comparative intradermal tuberculin (SIT and SCIT respectively) tests at cervical site are used. The intradermal test in the caudal fold is used in the United Stated and New Zealand and was also used in Australia during their bovine TB eradication campaign (Good and Duignan, 2011).

Advantages of the intradermal tuberculin test and reasons for its wide use are low cost, low logistical demands, well-documented use and, for a long time, lack of alternative methods to detect bovine TB. Still, this test has many known limitations including difficulties in administration and interpretation of results, need for a second-step visit, low degree of standardisation and imperfect test accuracy (Rua-Domenech et al., 2006). The fact that a significant proportion of outbreaks are detected in the slaughterhouse suggest that the intradermal test has some limitations or it is not performed permanently in the most adequate way. In this sense, an appropriate training of the veterinary practitioners is essential to ensure that the results are reliable (Working Document on Eradication of Bovine Tuberculosis in the EU accepted by the Bovine tuberculosis subgroup of the Task Force on monitoring animal disease eradication, SANCO/10067/2013).

Although the IFN-γ assay overcomes many of the disadvantages associated with the skin test, its use as a primary test has been limited only to certain countries (Flores-Villalva et al., 2012) or to difficult TB situations (Keck et al., 2010). However, recently the European Commission requested the European Food Safety Authority (EFSA) to issue a scientific opinion on the suitability of the IFN-γ test for inclusion in Directive 64/432/EEC as an official primary or stand-alone test and as equivalent to the intradermal test to define

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the infectious status. In response to this request, EFSA published a scientific opinion in which EFSA acknowledged that the performance of the PPD-based IFN-γ test is comparable to that of the intradermal tests. In this sense, they reported that the IFN-γ test should be considered after harmonisation for inclusion in the official tests for the purpose of granting and retaining official TB-free herd status and for certification for intra-European Union trade of cattle. Nevertheless, the EFSA expert panel recognised that in certain conditions, the Sp of the IFN-γ test might not be as high as the SIT test. Therefore, and in consideration of ongoing research on improved antigens, it was recommended to further evaluate the performance of specific antigens to be used for blood stimulation in the IFN-γ assay with the aim to improve its Sp (EFSA, 2012).

As the TB infection progresses, a shift from the predominant cell-mediated immune response towards humoral response occurs (Pollock and Neill, 2002). These antibodies (Abs) are generally targeted at immunodominant antigens that elicit a humoral response, notably MPB70 and MPB83 released in large amounts by *M. bovis* in the later stages of the disease. In general, the profile of the humoral response varied individually and among animal species affecting significantly to the Se achieved using these assays. For these reasons, the detection of Abs continues being a bottleneck and it has not played an important role in eradication programmes yet since it is not included as an official diagnostic tool. A variety of ELISA tests have been developed that rely on the detection of circulating Abs against the immunodominant antigens of *M. bovis* (Green et al., 2009; Waters et al., 2011; Whelan et al., 2008). In general, these assays are simple, rapid and inexpensive although they have shown lower Se than the assays based on cell mediated immune response.

2. Intradermal tuberculin test

2.1. Historical background

The tuberculin test has been used for *ante-mortem* diagnosis of latent and active TB in man and animals for more than 100 years (Good and Duignan, 2011; Monaghan et al., 1994). Finland was the first country in the late 1890s to start a TB eradication campaign using the tuberculin test (Francis, 1958). Once bovine TB programme based on a test and slaughter policy began, the incidence of clinical TB decreased since a high proportion of infected cattle was removed. Other countries gradually applied eradication programmes using different methodologies of the tuberculin test (ophthalmic and palpebral test, Stormont test, vulval test, etc.) that are discarded nowadays. Neck was finally selected as the site of tuberculin injection due to the higher Se and Sp values in cattle in comparison with the caudal fold (Good and Duignan, 2011). Moreover, different studies were carried out to determine which part of the neck was more suitable for tuberculin injection. Good et al. (2011a) reported that, in a practical sense, location of the PPD sites is of great importance and the middle and anterior third of the neck is recommended. However, the avian and bovine PPD measurements were not significantly different when sites anterior or posterior to this were chosen (Good et al., 2011a).

The Koch's old tuberculin described in 1890 is replaced nowadays by the PPD tuberculin prepared after a heat-treatment and lysis of *M. bovis* AN5 (bovine PPD) and *M. avium* D4ER or TB56 (avian PPD). The current PPD tuberculins consist of a mixture of small water-soluble proteins and lack of some non-specific components of the Koch's tuberculin (Tameni et al., 1998).

2.2. Potency of PPD tuberculins

Tuberculin potency is critical for the outcome of the intradermal test since a significant difference in the number of reactors detected using high and low potency tuberculins has been reported (Good et al., 2011a). Production of PPD tuberculins are standardised and regulated by the EU. Manufacture must fulfil the Good Manufacturing Practice conditions and comply with the European Pharmacopeia and OIE requirements (OIE, 2009; Good and Duignan, 2011). The protein content of the tuberculins is not correlated with the biological activity and therefore, potency assays of the tuberculin batches must be performed in guinea pigs and cattle (Haagsma, 1986). The requirement to check the potency in the bovine bioassay was included in the original Directive 64/432/EEC and was recommended in the OIE technical reports. However, this requirement was modified mainly due to the high cost and logistical demands of the assays and removed from the Directive in 2002 (Good and Duignan, 2011). Nowadays it is rarely conducted and only some laboratories, including the European Reference Laboratory for Bovine TB are carrying out potency tests of bovine PPDs from different manufacturers in cattle.

In these experiments, potency of the tuberculins is compared to an international standard (National Institute for Biological Standards and Control-NIBSC, UK) with an established potency of 32,500 IU/mg. The standardisation is based on an eight-point and four-point assay which use four and two different dilutions in guinea pigs and cattle respectively (OIE, 2009). Assessment of tuberculin potency in cattle requires naturally infected animals that are reactors in the intradermal test whereas guineas pigs are experimentally infected 5–7 weeks prior to the assay using a low dose of *M. bovis*. The results are statistically evaluated using the parallel-line assays according to Finney (OIE, 2009). A bovine tuberculin is considered acceptable for diagnosis in the eradication programmes if it has a minimum potency of 2,000 IU per dose and if the estimated potency is between the 66% and the 150% of the potency stated by the manufacturer on the label. Potency estimations in guinea pigs can be imprecise due to the inherent variability of the tuberculin PPD and the biological variations of the *in vivo* models. An imprecision of the potency estimated in the guinea pigs bio-assay has been reported although the potency test in cattle may have the same lacks (Good et al., 2011a). Moreover, according to current legislation, a reduction in the use of experimental animals and the use of *in vitro* alternative assays when possible is mandatory (Directive 2010/63/EU) and, for this reason, some *in vitro* methodologies are being developed with the aim of replacing the potency test in guinea pigs in the future (Ho et al., 2006).

2.3. Performance of the intradermal test

The intradermal tuberculin test measures dermal swelling primarily because of a cell-mediated immune response (CMI) 72 hours after intradermal injection of purified protein derivative (PPD) in the skin of the neck or the caudal fold. The skin of the neck is considered more sensitive to a tuberculin-related hypersensitivity reaction than the skin of the caudal fold and, therefore, higher doses of PPD may be used in the caudal fold to compensate this difference (Schiller et al., 2010).

Two approaches for the intradermal tuberculin test are currently in use. The SIT test measures the cell-mediated delayed type hypersensitivity against bovine PPD injected in the mid-cervical region (Member States) or in the caudal skin fold (United States, Canada and New Zealand). The SCIT test compares the response against bovine PPD and avian PPD in the cervical region with the aim of increasing the Sp. The test procedure are described by the OIE (2009) and the Animal Plant Health Inspection Service of the USDA (USDA: APHIS, 2005). According to the protocols, the injection site should be clipped and cleansed. Afterwards skin fold thickness should be measured using a caliper. The tuberculin can be injected using different syringes: the most frequently used are McLintock (Bar Knight McLintock Limited, UK) and Dermojet (Akra Dermojet, France) syringes. The main difference between them is