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Evaluation of the use of a needle-free injection syringe as a cause of non-specific reactions in the intradermal tuberculin test used for the diagnosis of bovine tuberculosis

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ABSTRACT

The objective of the study was to elucidate whether the use of the needle-free Dermojet syringe, which is based on a high pressure inoculation and is used to inject tuberculin in cattle in several countries, may, in itself, cause skin reactions that can be interpreted as positive reactions to the intradermal tests that are not, in fact, related to the real infection status of the animals.

Forty-four cattle from an officially tuberculosis-free (OTF) herd were selected, and four single intradermal tuberculin (SIT) tests were performed on each animal, two on each side of the neck. Three different Dermojet (D1, D2 and D3) and one McLintock (M4) syringes were used to carry out sterile phosphate buffer saline (PBS) with 10% of glycerol and bovine PPD injections.

No positive reactions to the SIT test were observed when using the D1-D3 syringes in the case of either bovine PPD or PBS. With regard to M4 (PBS), all the tests were negative when using a standard interpretation but three were positive in the case of the severe interpretation. Significant differences ($p < 0.05$) in the skin fold thickness measured were found only between certain Dermojet and McLintock syringes at certain inoculation sites. The results showed that the needle-free Dermojet syringe used for PPD intradermal testing in cattle did not cause significant reactions that could be misunderstood as positives.

1. Introduction

The intradermal tuberculin test is the official test for the diagnosis of bovine tuberculosis (TB) and is also widely used for the diagnosis of TB in other domestic and wildlife species (Alvarez et al., 2012; Bezos et al., 2012; Chambers, 2013; Bezos et al., 2014; Che-Amat et al., 2016). According to the European Legislation that describes how to perform the intradermal tuberculin test in cattle, the dose of tuberculin (not exceeding 0.2 mL) should be injected using a method that ensures that the tuberculin is delivered intradermically, such as a short sterile needle, with the bevel edge outwards and a graduated syringe charged with tuberculin, inserted obliquely into the deeper layers of the skin (European Directive 64/432/CEE).

In order to make the intradermal testing carried out by the veterinarians involved in eradication campaigns easier and faster, multi-dose syringes with needles have been traditionally used in the field, examples of which are the McLintock (Bar Knight McLintock Limited, UK), Hauptner (Jorgensen Labs, USA), Henke (Henke Sass Wolf, Deutschland) or Hauptner Muto (Medinova, Italy) syringes. The McLintock multi-dose syringe is probably that most widely used for intradermal tuberculin testing throughout Europe, and allows the accurate intradermal injection of a volume of 0.1 mL of PPDs in each shot. This syringe has a short needle (a length of 3.9 mm is recommended) in order to ensure that the tuberculin injection takes place intradermally and that subcutaneous injection does not occur. Moreover, the needle should be examined at regular intervals during a herd testing event and

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replaced if it is in any way bent or damaged. Disinfection is also very important, since the same needle is used for all the animals and is, therefore, a potential cause of cross-infections. A cap containing disinfectant has consequently been developed for its specific use with McLintock syringes (Sterimatic, UK). In order to avoid the use of non-interchangeable needles, so as to increase safety, other systems for intradermal tuberculin injection have also been developed. The Dermojet syringe (AkraDermojet, France) is a needle-free multi-dose device that can be used for the high-pressure intradermal injection of 0.1 mL doses of different liquids, including tuberculin. Dermojet is or has been used for intradermal tuberculin injections in different regions of European countries, such as Spain, Belgium or Bulgaria, and the benefits and drawbacks of this system in comparison to others using needles has been previously reported (SciCom, 2016).

The accuracy of Dermojet and McLintock syringes as regards inoculating the 0.1 ml dose has been previously demonstrated (MAPAMA, 2011), although it has been highlighted that both systems should be used by experienced veterinarians and that the syringes must be regularly maintained if the quality of the results is to be ensured. Dermojet syringes have been expressly prohibited by the legislation in France, which argues that the use of a needle-free system based on high pressure does not ensure an adequate intradermal injection (Direction Générale de l'alimentation. Service des actions sanitaires en production primaire. Ministère de l'agriculture, de l'agroalimentaire et de la forêt., 2015). Moreover, field veterinarians have suggested that inoculation on the basis of high pressure may be a cause of injuries to the skin that could be misinterpreted as positive reactions to the intradermal tuberculin test.

The aim of the present study was to elucidate whether the Dermojet syringe may, in itself, cause skin reactions that can be interpreted as positive reactions to the intradermal tests but that are not, in fact, related to the real infection status of the animals.

2. Material and methods

2.1. Animals and design of the study

In the present study, Forty-four 6–14 month-old male Avileño cross-breed cattle were selected from an officially tuberculosis-free (OTF) herd with no previous history of infection. Four single intradermal tuberculin (SIT) tests were performed on each animal, two on each side of the neck, corresponding to four inoculation sites: cervical and caudal on the left and right sides of the neck, respectively (CR1, CD1, CR2, CD2) (Fig. 1). This signifies that 44 tests were performed with each syringe in a total of 176 tests. Three different Dermojet (D1, D2 and D3) and one McLintock (M4) syringes were used. All the syringes had been previously subjected to the regular adjustment and maintenance according manufacturer's instructions. Since the study was focused on the effect of the syringe, regardless the infection status of the animals, sterile phosphate buffer saline (PBS) with 10% of glycerol was injected using D2, D3 and M4 to avoid any effects on the results of exposure to other non-tuberculous mycobacterial infections. Moreover, PPD tuberculin (CZ Veterinaria, Spain) was injected using D1 as a control. The syringe used for each inoculation site was randomly changed for each animal, thus ensuring a significant number of tuberculin tests with all the syringes on all the sites.

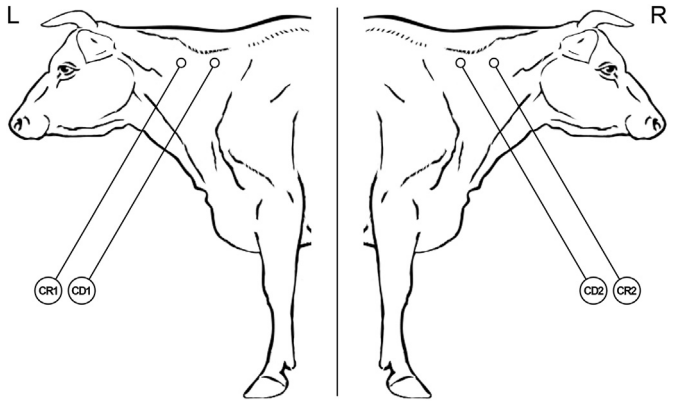

Fig. 1. Scheme of the injection sites for intradermal testing using Dermojet and McLintock syringes.

All husbandry practices and animal procedures were authorized by the scientific and animal experiments committee of the Animal Research Committee from the Madrid Region (ref. 99/127657.9/16).

2.2. Intradermal tuberculin tests

The SIT test was conducted by an accredited veterinarian in accordance with European and Spanish legislation (EU Council Directive 64/432/CEE and R.D. 2611/1996). The skin fold thickness was measured and recorded by the same veterinarian following a protocol accredited by the Entidad Nacional de Acreditación (ENAC, Spain, Reference 817/LE1410). The animals were inoculated with 0.1 ml of each product (PBS or bovine PPD) using Dermojet and McLintock syringes. The results were expressed as the difference in skin thickness in millimeters (mm) between the pre- and post-skin test measurements (72 ± 4 h later). Moreover, the presence of local clinical signs was recorded (edema, erythema, necrosis) and the overall score was obtained using a semi-quantitative system (ranging from 0 to 3). European Legislation was employed as a basis on which to classify the animals as positive reactors to the SIT test if the increase in the skin fold thickness after the application of bovine PPD was equal or greater than 4 mm and/or the presence of severe local clinical signs (scores 2 and 3) was observed. A more severe interpretation of the test was additionally applied (recommended in infected herds) that considered a reactor to be positive when the skin fold thickness difference was over 2 mm and/or the presence of severe local clinical signs was observed. The veterinarian in charge of the tuberculin tests did not know the content of each syringe; the day of interpretation, information about the syringe used in each position was not available for the veterinarian.

2.3. Statistical analysis

Differences observed in the skin fold thickness between inoculation sites and syringes used were analyzed using Kruskall-Wallis test and pairwise comparisons were used as post hoc tests for the former. Calculations were carried out using SPSS Statistics 20 (IBM, New York, NY, USA), and interpreted considering a p-value of 0.05 to determine statistical significance.

3. Results

No positive reactions to the SIT test (standard and severe interpretation) were observed when employing Dermojet syringes in the case of either bovine PPD or PBS (Table 1). When using D1 (bovine PPD), the skin fold thickness was no > 2 mm in any of the cases, and no severe local reactions or local lymphadenopathy were observed. Only one reading of 2 mm was observed at the CR1 inoculation site. Similar skin fold thickness was observed when using D2 and D3 (both with PBS). In this case, three tests showed readings of 2 mm, two at the CR1 site and one at the CD1 site. No severe local reactions or local lymphadenitis were observed when using either D2 or D3. With regard to M4 (PBS), all the tests were negative when employing a standard interpretation. Nevertheless, three reactions of 3 mm (at the CR2, CD1 and CD2 sites, respectively, in three different six-month old animals) were measured and interpreted as positive when using the severe interpretation of the SIT test. Moreover, there were nine reactions of 2 mm (three, one, four and one at CR1, CD1, CR2, and CD2 sites, respectively). No severe local clinical signs or lymphadenitis were