



Original Article

Comparison of the cutaneous response of the comparative cervical tuberculin (CCT) test with different reading times in buffaloes

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ABSTRACT

Tuberculosis is an infectious disease that affects ruminants, causing losses in milk and meat production. Thus, the aim of this research was to compare the cutaneous response of the comparative cervical tuberculin test in buffaloes. Thus, the Comparative Cervical Test (CCT) examined 473 buffaloes, with 440 females and 33 males of different ages and races. Measurement of the allergic skin response was performed at 24, 48, 72 and 96 hours after tuberculinization. For avian tuberculin, the mean skin increases ranged from 12.24mm (0 hour) to 14.82mm (96 hours). As for bovine tuberculin ranged from 11.99mm (0 hour) to 13.68mm (96 hours). The maximum point of measurement of the allergic skin reaction was observed at 72 hours after the application of avian tuberculin, the same was observed for bovine tuberculin. However, there was no statistically significant difference in relation to the amplitude of the reactions observed at 48, 72 and 96 hours. Therefore, it was concluded that the skin allergic reaction occurred in a gradual manner and that the maximum amplitude value of the cutaneous allergic response in buffalo in the diagnosis of tuberculosis was at 72 hours.

INTRODUCTION

Research on the health of buffaloes in Brazil is still insufficient. Almost all of them, the herds are exploited with minimal disease control, using bovine breeding parameters. For many breeders, the buffalo is an animal of high rusticity, which in a way neglects the health care with these animals. However, buffaloes are susceptible to the great majority of infectious etiological agents that affect cattle, such as *Mycobacterium bovis* (MOTA et al., 2002).

Tuberculosis is an infectious disease that affects ruminants. It presents a chronic character and is characterized by the development of specific granulomas, called tubers (OLIVEIRA et al., 2007). Tuberculosis affects buffaloes as much as cattle, as far as

the etiological agent, pathogenicity, clinical signs, pathological, epidemiological and zoonotic aspects are concerned (ALBERNAZ et al., 2015; MANSOUR, 1994).

Roxo et al. (1998) conducted studies evaluating the tuberculin test response in buffaloes and cattle, and observed that at 72 hours buffalo reactions were higher than in cattle. This occurrence can be attributed to the high susceptibility of buffaloes to atypical mycobacteria, which can cause a large number of false positive reactions due to cross-reactions to bovine PPD (EID; MOUSA; SELIM, 2001). Ribeiro (2003), carried out a study to define criteria for the use of intradermal tuberculinization in buffaloes, since it affirmed the variety of obstacles to an effective approach in the diagnosis of tuberculosis in this species.

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The lack of data on tuberculosis, as well as the absence of specific criteria for the interpretation of the tuberculin test in goats and sheep, led Silva et al. (2006) and Cyrillo et al. (2007) to evaluate the allergic skin response at different measurement times in goats and sheep, respectively, and observed a higher reaction intensity at 48 hours in both species.

Some studies have been carried out in the diagnosis of tuberculosis in buffaloes in several regions of the country (BARBOSA et al., 2014; MINHARRO, 2016; PEREIRA et al., 2009). However, all following the recommendations of the diagnosis for cattle. Tuberculosis plays an important role, especially in developing countries, not only in terms of public health, but also because it causes a drop of up to 18% in production, in addition to the condemnation of carcasses at slaughter and the implications for national and international trade in animals and products (NEVES et al., 2011). In view of this, and the need to establish specific reading parameters for the different species of ruminants, this research aimed to evaluate the cutaneous response of the Comparative Cervical Test (CCT) at different times, of buffaloes reared on lower marenhense, Maranhão.

MATERIALS AND METHODS

This research was developed with buffalo herds created at lower marenhense. This region, located in the northwestern part of the state of Maranhao (1 ° 00' - 4 ° 00'S and 44 ° 21 ' - 45 ° 21'W), encompasses extensive areas liable to flooding and has ecological tension among the Cocais formations in South; Close to the East; Amazon Forest in the West and Marine Systems in the North (IMESC, 2017).

Seventeen herds were surveyed from the municipalities of Arari, Matinha, Olinda Nova do Maranhao, Pinheiro, Sao Joao Batista, Viana and Vitoria do Mearim. The population examined consisted of 440 females and 33 males, mostly murrah hybrid aged 1.5 to 216 months.

The animals were contained in asqueeze chuteor using zoo technical containment methods. The inoculation sites were demarcated by clipping them, avoiding areas with lesions or nodules of parasites and vaccine abscesses. Subsequently, the thickness of the skin fold was measured with the aid of a cutimeter (Hauptner®). 0.1 mL of avian PPD (2,500 / IU) cranially and 0.1 mL of bovine PPD (5,000 / IU) (Tecpar® Laboratory) were injected caudally into the cervical region, posterior to the spinal process of the scapula at 20 cm from the withers, via with a minimum distance between applications of 15 to 20 cm. The inoculation was performed using defined-dose syringe (Hauptner®). The interpretation of the results was performed according to the recommendations of the National Program for the Control and Eradication of Brucellosis and Tuberculosis -

PNCEBT (BRASIL, 2016). However, for evaluation, the skin fold thickness was measured at 24, 48, 72 and 96 hours after tuberculinization.

The paired t test was used for the results of the comparative cervical method at different reading times. The level of significance used in the statistical test analysis was 5% and the confidence interval was 95%. The software used for data analysis was Minitab® 16/2013.

This research was authorized by the Ethics and Animal Experimentation Commission - EAEC of the Veterinary Medicine Course of State University of Maranhao, according to protocol No. 008/2014.

RESULTS AND DISCUSSION

In this study among the 473 tuberculin tested buffaloes, only a buffalo cow aged over 7 years showed clinical signs suggestive of tuberculosis. At the clinical examination, this animal showed dyspnea, cough, enlargement of the superficial cervical lymph node and progressive weight loss. The other buffaloes had no clinical alteration of their vital functions (respiratory rate, heart rate, ruminal movements and body temperature). These results corroborate with those described by Silva et al. (2006) in goats, differing only by the fact that the present research was carried out under real field conditions. The tuberculin test for manifesting a delayed type IV hypersensitivity reaction does not present clinically significant physiological changes, since according to Tizard (2000), tuberculin stimulates a local inflammatory response.

The verification of the response evidenced an increase in cutaneous thickness gradually. For avian tuberculin, the mean skin increases ranged from 12.24mm (0 hour) to 14.82mm (96 hours). As for bovine tuberculin ranged from 11.99mm (0 hour) to 13.68mm (96 hours). The maximum point of measurement of the allergic skin reaction was observed at 72 hours after the application of avian tuberculin, the same was observed for bovine tuberculin (Table 1). However, there was no statistically significant difference in relation to the amplitude of the reactions observed at 48, 72 and 96 hours.

Silva et al. (2006) in goats described that the maximum thickness of the skin occurred at 48 hours; however, there was no statistical difference in comparison to the magnitude of the reactions verified at 72 hours. Doherty et al. (1996) reported that the highest intensity of reactions occurred between 48 and 72 hours. Taking into consideration the immunological profile of the allergic response during the test, the results of this research show that there is no variation in the field, a fact described by Tizar (2000), when he stated that the inflammatory response of tuberculinization reached its highest intensity from 24 to 72 hours. There was a

decrease for both reactions with the 96-hour reading, which may be explained by a delayed reaction of T lymphocytes (Th1), which migrate to the site of antigen injection and release cytokines such as interleukin-2 (IL-

2), and interferon-gamma (IFN- γ). This makes it possible to sensitize the vascular endothelium and the displacement of cells of the immune system (GONZÁLEZ LLAMAZARES et al., 1999; JANEWAY; TRAVERS 1997).

Table 1 – Arithmetic mean and standard deviation (mm) of the skin thickness values of buffaloes induced by avian and bovine tuberculin, applied in the cervical region, at different reading times, in buffaloes of lower marenhense, Maranhão, 2014.

Tuberculins	Nº of animals	0 hours (at)	24 hours (pt)	48 hours (pt)	72 hours (pt)	96 hours (pt)
Avian	473	12,24 ^A (3,36)	13,87 ^B (4,21)	14,64 ^C (4,40)	14,97 ^{CD} (4,65)	14,82 ^{CDE} (4,64)
Bovine	473	11,99 ^A (5,24)	12,90 ^B (3,95)	13,61 ^C (4,45)	13,80 ^{CD} (4,79)	13,68 ^{CDE} (4,81)

Means with different capital letters, on the same line, indicate statistical difference between the intensity of the reactions caused by *M. avium* and *M. bovis* at different reading times ($P < 0.05$). at = measurement of skin thickness in mm, prior to tuberculinization. pt = measurement of skin thickness in mm after inoculation of tuberculinization. Av = avian tuberculin. Bov = bovine tuberculin.

The results of skin thickness measurement obtained in this study are similar to those found by Ribeiro (2003), who performed studies to determine parameters for intradermal tuberculinization in buffaloes at different reading times (24, 48, 72, 96 and 120 hours after inoculation), observed that at 77.3 hours was the time in which the maximum increase in skin thickness occurred.

Roxo et al. (1998), evaluating the test reaction in buffaloes and cattle, observed that the peak skin thickness occurred at 72 hours and that buffalo reactions were 1.69 times higher than in cattle. This can be explained as a consequence of the breeding type, making buffaloes more susceptible to atypical mycobacteria, and may cause a large number of false positive reactions due to cross-reactions to bovine PPD (EID; MOUSA; SELIM, 2001). This fact, which may compromise the interpretation of the test, once Freitas; Guerra; Panetta (2001) reported that the influence of atypical mycobacteria in the diagnosis of tuberculosis in buffaloes has not been well described.

In this study, comparative cervical method was used to reduce the interference of atypical mycobacteria, because according to Haagsma (1995), the comparative test is used to distinguish *Mycobacterium bovis* infected animals from other mycobacteria of genera and the simultaneous use of avian and bovine PPD in the same animal is of paramount importance, since avian PPD serves as a sensitivity index and bovine specificity, and therefore the latter is positive for bovine tuberculosis.

The criteria for conducting and interpreting the tuberculinization test in buffaloes may follow the suggested pattern for cattle as recommended by the PNCEBT (BRASIL, 2016), since the peak skin reaction was observed 72 hours after tuberculinization, similar to the PNCEBT recommendation. However, it is suggested to consider a margin of interval of 24 hours for more or less to perform the reading, unlike the reading interval of ± 6 hours advocated by PNCEBT (BRASIL, 2016).

However, some researchers state that the interpretation parameters used for the diagnosis of tuberculosis in

buffaloes require review of the test, as well as the sensitivity and specificity of the test (RIBEIRO, 2003; ROXO et al., 1998). Lopes et al. (2006) suggest the elaboration of a new table of interpretation of cutaneous reactions, aiming at the reduction of cases of false-positive animals, as well as the review of reading time and the regions of tuberculin injection. Thus, it is necessary to form a research network in the various regions of the country, in order to carry out further studies aiming at a standardization for buffaloes, regarding the diagnosis of tuberculosis.

CONCLUSIONS

According to the results of this research, it was concluded that the peak of cutaneous reaction occurred at 72 hours with a variation of ± 24 hours in the comparative cervical tuberculin test (CCT) for the diagnosis of tuberculosis in buffaloes.

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