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Serological Study of Equine Brucellosis in the Legal Amazon Region

Marco Augusto Giannopccaro Silva[1]*, Thais Evelin Freitas de Oliveira[2], Daniela Costa Pereira[1], Samara Rocha Galvão[1], Katyane Sousa Almeida[1], Helcileia Dias Santos[1]

[1]Postgraduate Program in Animal Health and Public Health in The Tropics, Brazil

[2]Student, Postgraduate Program in Animal Health and Public Health in The Tropics, Brazil

***Corresponding author:** Marco Augusto Giannopccaro Silva, Postgraduate Program in Animal Health and Public Health in The Tropics, Brazil, Tel: +5563992695335, E-mail: marcogiannopccaro@uft.edu.br

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Abstract

Equine brucellosis is a zoonosis caused mainly by the bacterium *Brucella abortus*, which can cause debilitating lesions and manifests itself mainly through fistulas in the region of the withers. Euthanasia is recommended for affected animals. Few studies have been developed in equines, making it difficult to observe the distribution of the disease nationally and worldwide. Thus, the aim of the current study was to determine the seroprevalence of *Brucella abortus* in horses from the city of Araguaína, Tocantins. In total, 388 horses were used in the study, 236 males and 152 females, of different breeds and from different regions in the municipality of Araguaína, Tocantins. For diagnosis, Buffered Acidified Antigen (BAA) tests were used and for the reactive animals in this test, the 2-mercaptoethanol test (2-ME) was performed. A total of 17 (4.38%) animals were reactive in the BAA and, of these, 14 were non-reactive and 3 were inconclusive in the 2-ME. From the results obtained, it is possible to conclude that the animals tested were negative for infection by *B. abortus*; care and prevention measures are essential to maintain this state; the association of screening and confirmatory serological tests are of fundamental importance for the diagnosis of equine brucellosis; and, finally, the control and eradication plan for bovine Brucellosis in the city, through vaccination and inspection, has reduced the incidence of the disease, since most animals cohabited with cattle and other animal species, which could reflect on the positivity of the animals studied.

Keywords: AAT; horse; *Brucella abortus*; public health; 2-Mercaptoethanol.

Introduction

Among the various infectious diseases that affect horses is brucellosis, a zoonosis that deserves attention, as due to their use in various activities, horses have a close relationship with humans [4].

Brucellosis is a contagious zoonosis caused by bacteria of the genus *Brucella* spp., characterized by infection of cells of the mononuclear phagocytic system [1]. In horses, it is mainly caused by *B. abortus* and is characterized by lesions in the cervical region, withers, bursae, tendons, and joints [2].

Despite the clinical signs and possible risks of perpetuating the disease due to the close contact of these animals with other species and humans, in Brazil, there are few studies related to brucellosis in horses, compared to other species such as cattle, which report a low prevalence of the disease [3].

The National Plan for the Control and Eradication of Brucellosis and Tuberculosis (PNCEBT) does not have detailed norms for equine brucellosis, which makes it extremely difficult to control and prevent the disease in this species. In view of this, horses are usually considered "accidental" hosts of *B. abortus*, of relative importance in the epidemiological chain of transmission within or between equine herds and to other species. This lack of standardization and interpretation of serodiagnostic methods in equine brucellosis creates difficulties in terms of health management of the diseases [4].

According to an epidemiological bulletin issued in 2008 in Araguaína-TO by the Secretary of Health Surveillance, several cases of brucellosis in humans were reported between 2006 and 2008; from 11,463 patients seen in clinics, 28 patients met the definition of a suspected case, and of these, 23 were confirmed by a laboratory diagnosis, demonstrating an impact of brucellosis on public health in the city [5].

In Araguaína, due to the lack of studies on equine brucellosis and the possible involvement of animals of this species, contributing to the epidemiological chain of the disease and, consequently, the importance of brucellosis to public health, the current study was developed to identify the presence or absence of *B. abortus* in horses in the city. This information is essential for the adoption of prevention and control strategies. To this end, serological tests were used to estimate the seroprevalence of *Brucella abortus* in horses in Araguaína-TO, Legal Amazon region.

Material and Methods

This work was approved by the Ethics Committee on the Use of Animals of the Federal University of Tocantins (UFT) under number 23.101.008520/2019-46. The experiment was conducted between October 2019 and December 2020, taking into account the blood collections and the performance of serological tests.

Calculation of the sample and origin of the animals

The sample size was calculated using OpenEpi software, Version 3, open source calculator-SSPropor, considering a population size (N) of 4631 animals (ADAPEC 2019), a hypothetical frequency (p) of 50% (corresponding to disease of unknown occurrence in a given population), confidence limit (d) of 5%, and design effect ($EDFF$) equal to 1, which resulted in a sample N of 388 horses. The equation used for the calculation was:

$$\text{Sample size (n)} = [EDFF^{*}Np(1\text{-}p)] \,/\, [(d^2/Z^2_{1-\alpha/2})^{*}(N\text{-}1) + p^{*}(1\text{-}p)]$$

In all, 388 horses were used, 236 males and 152 females, with a mean age of 6.10±3.87 years, belonging to different regions of the city of Araguaína - TO.

Sample collection

Blood samples were obtained by jugular venipuncture performed aseptically, using a vacuum system (Vacutainer®) consisting of 25x0.8mm disposable needles and previously identified tubes without anticoagulants. After collection, the samples were placed in an isothermal box containing recyclable ice, until arrival at the Laboratory of Veterinary Parasitology at the Federal University of Tocantins (UFT), where they were then centrifuged for five minutes at 5,000 rpm to obtain serum. Serum from each animal was placed in labeled sterile *Eppendorf* tubes and frozen at –20 °C.

Serological tests

The serological analysis took place in two stages, following the recommendations of the National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis of the Ministry of Agriculture, Livestock and Supply (MAPA) [6]. It is noteworthy that as there are no standardized diagnostic methods described for horses, those established for bovines and buffalos were followed. First, the samples were analyzed by the Buffered Acidified Antigen (BAA) test, also known as Rose Bengal, at the UFT Parasitology Laboratory; subsequently, the samples considered as reactive in the previous step (characterized by the presence of clots) were submitted to the confirmatory test of 2-Mercaptoethanol (2-ME), which includes the Slow Agglutination Serum in Tubes (SAL) and the 2- Mercaptoethanol (2-ME), carried out by the National Agricultural Laboratory -LANAGRO/MG.

Statistical analysis

Data analysis was performed using descriptive statistics, in which the test results are presented as percentage analysis for reactive and non-reactive animals in the serological tests.

Results

This work brings the first information about the prevalence of antibodies to *Brucella* spp. in horses from the State of Tocantins. Of the 388 animals tested, 17 (4.38%) were reactive in the BAA test (06 females and 11 males), however, of these, 14 (82.35%) were non-reactive and 03 (17.65%) were inconclusive in the test confirmation of 2-Mercaptoethanol.

Discussion

The results of this research corroborate the findings of [7] who, despite identifying animals that were reactive in the BAA, among these, did not find animals reactive to the confirmatory tests. The authors also detected an inconclusive animal to 2-Mercaptoethanol, confirmed by the complement fixation test with a negative result. In the present study, three animals were inconclusive in the 2-Mercaptoethanol test, two of the Quarter Horse breed (8 years and 6 years) and one Paint Horse (14 years), all kept under intensive care, but with frequent contact with cattle due to the sport in which they were used (Team Roping). However, it was not possible to perform the complement fixation test, as there was no longer enough sample to perform it, nor was there the possibility of a new collection, since the animals were no longer on the properties, as they had been sold and the new destination was unknown. [8] obtained a null prevalence in 187 blood samples analyzed from draft horses in the municipality of Uberlândia-MG in the BAA test and concluded that brucellosis is of low epidemiological importance for horses in the studied area, as reported by [9]. In the same direction, [10] observed BAA reactive horses that were not confirmed in the 2-Mercaptoethanol test, demonstrating that the agent was not present in horses from the Zona da Mata of the State of Minas Gerais. [11] tested 123 draft horses, of which eight were reactive to BAA, but only one was confirmed by 2-Mercaptoethanol. [12] drew attention to the large number of animals reactive to BAA (40%) and the small percentage (5%) that reacted positively to 2-Mercaptoethanol.[13] reported a low number of animals in the BAA (3.7%) and low positivity in the 2-Mercaptoethanol confirmation (0.6%).

The explanation for the false positives found in the BAA in this and other works was related by [14] to the fact that other Gram-negative bacteria have a bacterial wall constitution similar to the genus *Brucella* spp., and therefore, cross-reaction oc-

curs. These facts show the importance of confirmatory testing and the need to associate the screening test with the confirmatory test(s), as recommended by the National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis [6].

The correlation established between the infection of horses by *Brucella* spp. and cohabitation with other species of domestic animals [4], especially cattle, was not detected in this study, even though all the animals included in the research were in frequent or intermittent contact with cattle. This is possibly related to the fact that the main source of infection is through the secretion eliminated by a pregnant female during abortion or birth [15], a fact that was non-existent in the current study and, to the contact of horses with Brucellosis-negative cattle, demonstrating the advance of the vaccination program in the region. [16] detected an apparent prevalence of outbreaks of 6.42% and that of animals of 2.21%, demonstrating both a significant reduction in comparison with a previous study that found, respectively, 21.2% and 4.4% [17], and that the vaccination program adopted since 2010 with vaccination coverage above 70% plays a preponderant role in this result.

Unlike the result found in the current study, [18] detected a prevalence of 73.1%, however, it is considered that the animals evaluated presented lesions and had a clinical suspicion of Brucellosis, unlike the animals included in this study. Therefore, in animals with clinical signs of equine brucellosis, the probability of confirming the diagnosis either by serological or secretion tests is great.

In a study carried out in Araguaína [19], the researchers recommended that the BAA test be performed from the seventh day after vaccination with B19, as a diagnostic test, considering a minimum seroconversion coefficient of 75%, aiming at monitoring the vaccination process and better control of the disease in cattle. If adopted by competent agencies, this could further reduce cases of bovine brucellosis in the state and maintain the number of cases in horses, as found in the current research.

Despite the absence of positive cases of equine brucellosis in the city of Araguaína, it is important to make owners and caretakers aware of the care and need to maintain prevention measures, so that the absence of cases is maintained, including periodic tests of horses on the property, vaccination of cattle as recommended by competent agencies, inclusion of new animals (bovine and equine) on the property only after a negative test for the condition, and proper destination of abortion material with disinfection of the site. To prevent brucellosis in humans on rural properties, in addition to the measures mentioned above, attention must be paid to hygiene in the management of equines and to avoiding the consumption of animal products from animals whose status in relation to the disease is unknown.

Conclusions

From the results obtained, it is possible to conclude that the animals tested were negative for infection by *B. abortus*; that the care and maintenance of preventive measures should remain in place to maintain of the obtained data; and also, that the association of screening and confirmatory serological tests for the diagnosis of equine brucellosis is of fundamental importance.

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