International Journal of Advanced Engineering Research and Science (IJAERS)
Peer-Reviewed Journal
ISSN: 2349-6495(P) | 2456-1908(O)
Vol-9, Issue-9; Sep, 2022
Journal Home Page Available: https://ijaers.com/
Article DOI: https://dx.doi.org/10.22161/ijaers.99.53

Epidemiology and Risk Factors of Brucellosis in Veterinary Medicine Professionals and Academics in the Middle-North Region of Mato Grosso State, Brazil

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Received: 20 Aug 2022,

Received in revised form: 13 Sep 2022,

Accepted: 18 Sep 2022,

Available online: 30 Sep 2022

***Keywords*— Brucella sp., Brucella abortus, Population study, Prevalence, Poisson regression, Veterinary Physicians.**

Abstract*—The present study aimed to evaluate the seroprevalence of anti* - Brucella abortus *antibodies in Veterinary Medicine students at the Federal University of Mato Grosso (UFMT) - Sinop campus and Veterinary Physicians in the mid-north region of Mato Grosso, as well as the risk factors associated with its transmission. Blood samples were collected from the participants to assess the frequency of anti*-Brucella abortus *antibodies and sent to the Infectious Diseases Laboratory at UFMT Campus Sinop, where the samples were processed. The blood samples collected from indirect diagnostic methods, based on the detection of antibodies. This is an observational, cross-sectional, retrospective study, in which the results were evaluated through the analysis of the chi-square association by Fisher's exact test and for the identification of factors associated with the reagents for anti-Brucella abortus, it was carried out crude analysis using Poisson regression. Of the 213 volunteers, seven were tested for the Buffered Acidified Antigen (42.9%) and with a predominant range of age from 18 to 25 years (42.9%) %). The variable "Frequent Laboratory that diagnoses Brucellosis" (p = 0.028) was the only variable that remained significant after regression. It was concluded the lack of preparation and responsibility of some professionals when exercising the profession, not fulfilling the basics: protecting themselves and helping others to do the same, putting their health at risk and that of the population in general.*

I. INTRODUCTION

Brucellosis is an important zoonosis caused by *Brucella* species (Brucellaceae). The disease affects a wide range of domestic and wild animals as well as humans (PROCH et al., 2017). In livestock, it causes chronic disease with reproductive failures that contribute to production losses and, in humans, it causes an often chronic febrile illness that is underdiagnosed in many low- and middle-income countries (LINDAHL et al., 2020). According to Ghanbari (2020), in the world, the number of unreported human cases that present unspecified clinical symptoms is ten times greater, therefore, it is one of the most significant public health concerns. It can affect all age and sex groups, and its control in humans depends on limiting infection in animals through vaccination and care programs.

Brucellosis is considered an occupational disease, in which professionals commonly infected are veterinarians, microbiologists, butchers, slaughterhouse workers and animal handlers (LAWINSKY et al.; 2010), as they are often exposed to infected animals, contaminated biological materials and live attenuated anti-*Brucella* spp vaccines capable of causing the disease in humans (MEGID, 2016). In addition, these professionals, as well as the general population, can also contract the disease through the ingestion of unpasteurized milk and dairy products (PEREIRA et al., 2020). Human-to-human transmission is rare, but has been reported following blood transfusion, bone marrow transplantation, and sexual intercourse (MEGID, 2016).

The National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis - PNCEBT was established in Brazil by Ministerial Normative Instruction nº 02/2001 and regulated by Normative Instruction SDA nº 10/2017 with the objective of reducing the negative impacts of these zoonoses on human health and animal (BRASIL, 2006). The PNCEBT is mainly based on the mandatory vaccination of bovine females aged between 3 and 8 months with the B19 vaccine and the vaccination of bovine females that have not been vaccinated at this age with RB51, the program also includes transit control for breeding and slaughter of positive animals (PEREIRA et al., 2020). Vaccination against brucellosis in Brazil is only performed by veterinarians accredited by the PNCEBT or by vaccinators registered under their responsibility (BRASIL, 2006). Veterinarians and their assistants are among the most susceptible to human brucellosis, because in addition to dealing directly with infected animals, they are also exposed to a vaccine that has the live attenuated *Brucella* spp agent (PEREIRA et al, 2020). Unfortunately, there is no vaccine available for use in humans, largely due to safety concerns associated with potential residual virulence of live attenuated vaccines (KHALAF et al., 2020).

Brucellosis is distributed worldwide, being considered one of the main zoonoses by the World Health Organization (WHO). The countries with the highest occurrence of human brucellosis are Syria (1,603.4 cases per 1,000,000 individuals), Mongolia (391.0) and Tajikistan (211.9). According to the World Bank for Animal Health Information (WAHID), Mexico had the highest number of reported outbreaks, 5,514 in 2014. Followed by China (2,138), Greece (1,268) and Brazil (1,142). Most of these outbreaks are from Brucella abortus, the etiologic agent of bovine brucellosis (HULL, 2018).

Given the high prevalence of bovine brucellosis in underdeveloped and developing countries and its zoonotic risk, several authors have evaluated the frequency of seropositivity in cattle and humans from different parts of Brazil and the world, generally in individuals whose profession is intrinsically related to contact with animals. . In Brazil, Carvalho et al. (2016) evaluated this relationship in the central region of the state of Maranhão and found seropositivity in 4.95% of dairy cows analyzed and 1.66% in seropositive computers.

Schneider et al. (2013) evaluated higher frequencies in their study carried out in a slaughterhouse in Cuiabá, Mato Grosso. In this study, a frequency of 7.5% and 4.5% of seropositivity was observed in slaughtered and slaughtered cows, respectively. Pereira et al. (2020), identified the epidemiological picture due to accidental exposure to the B19 and RB51 vaccine among veterinarians representing the PNCEBT in Minas Gerais, Brazil, where approximately one third of the professionals interviewed had already been accidentally exposed to the B19 and RB51 vaccines and observed that the adoption of measures of individual protection and knowledge about the disease were especially important factors in preventing occupational exposure to *B. abortus*.

However, published data from other countries show alarming results, as described by Madut et al. (2018) in several regions of Sudan, with average rates of 31% and 33% in seropositive cattle and rural producers, respectively, 31% and 33.3% in seropositive cattle and rural producers. Proch et al. (2017) specifically evaluated Veterinarians in India, according to the results of these authors, 21.9% of these professionals had seroagglutination in the Buffered Acidified Antigen Test and 24% in the Slow agglutination tube test. The authors stated that the intimate contact of these professionals with the animals is an important risk factor for infection.

In Brazil, there is a lack of literature relating to zoonotic brucellosis specifically in veterinarians, nor the possible risk factors for brucella infection. In this way, this study carried out an important approach in the relationship of brucellosis in academics of Veterinary Medicine and Veterinary Doctors in the north region of Mato Grosso State.

II. MATERIAL AND METHODS

This is an observational, cross-sectional, retrospective study. The study is being carried out at the Universidade Federal de Mato Grosso – Sinop campus. It is located in a region of the State of Mato Grosso that stands out for its location close to the Amazon Basin. In addition to Sinop, collections were carried out in three more municipalities in the North region of Mato Grosso: Sorriso, Guarantã do Norte and Alta Floresta.

This is a cross-sectional observational study, in which students of the Veterinary Medicine course, Resident Veterinarians and Veterinary Physicians in the mid-north region of Mato Grosso were evaluated. It was estimated that there are around 2000 veterinarians in the aforementioned region and 500 students enrolled in the Veterinary Medicine course at the Universidade Federal de Mato Grosso Campus Sinop, the only university in the mid-north region of Mato Grosso that provides this course.

Questionnaires with information on age, sex and habits were applied prior to collection, in which all 213 participants agreed to answer a brief questionnaire applied before blood collection. The variables used in the elaboration of the questionnaire were based on the study by Tenório et al. (2008). In addition, all participants signed the Free and Informed Consent Form (ICF).

Blood samples were collected by intravenous puncture, using 25 x 8 mm needles and syringes with a capacity of 10 mL. Then, the blood was deposited in previously identified sterile test tubes, 5mL in each, one with the addition and the other without the addition of the anticoagulant ethylenediaminetetraacetic acid (EDTA). Then, serum was obtained from the blood aliquot without anticoagulant, by means of centrifugation at 3000 rpm for five minutes. The blood sample with the addition of EDTA and the serum obtained from the blood without anticoagulant, were aliquoted 0.5 mL in each previously identified polypropylene microtube, with two microtubes destined for serum and two for blood without anticoagulant. placed in specific boxes also already identified and kept frozen at -20 °C until analysis.

For the serodiagnosis of the samples, a buffered acidified antigen (AAT) test was performed to detect anti-*Brucella* antibodies as a screening test.

The characterization of the significance between the differences observed in the frequencies of seropositive individuals according to aspects related to risk factors was determined using the SPSS version 2.0 program. The significance level adopted was 5% (AZEVEDO et al., 2004). All 213 participants agreed to answer a brief questionnaire applied before blood collection. In order to extract better information from the descriptive approach, the chi-square association analysis was performed using Fisher's exact test. To identify factors associated with anti-Brucella abortus reagents, a crude analysis was performed using Poisson regression.

III. RESULTS AND DISCUSSION

Of the 213 volunteers who underwent serodiagnosis for Brucellosis, seven individuals were reactive to the Buffered Acidified Antigen (AAT) test. Of these individuals, four were inhabitants of the municipality of Sinop, two from Alta Floresta and one from Sorriso. The reagent population was mostly self-employed veterinarians (40.3%), male (85.7%) and predominantly aged between 18 and 25 years (42.9%). In the other occupations in the area of veterinary medicine, there were only two reactive individuals (28.6%) to the AAT, who were academics in veterinary medicine, reporting that the relationship of inherent veterinary practices during graduation is also a potential risk factor. in *Brucella abortus* infection.

Of the total population studied, 40.8% of the individuals were male and 59.2% were female, both with a predominant age group of 26 to 35 years (44.6%). Of the reactive individuals, only 1 (14.3%) was female and the other 6 (85.7%) were all male and the variable “Sex” (p=0.019) was statistically significant. According to Soares et al. (2015) and other reports in the literature, portrayed that the disease is more common in adults aged between 55 and 64 years, however, in the present study the occurrence of contact with *Brucella abortus* was in a younger age group, which can be explained by the fact that human brucellosis is an occupational disease and consequently affects the population at an age considered productive for work.

The high proportion of women observed among the participants did not make them the majority among the reactive individuals, which may be due to the occupation profile described in the country, in which the average professional experience of the participants showed a predominance of male veterinarians among them, those who work with large animals. Therefore, veterinary services related to reproduction and vaccination against brucellosis are frequent in this area, which implies a greater possibility of contact with *B. abortus*, compared to professionals who work in other areas (PEREIRA et al., 2020). Some studies report the prevalence in females, but it cannot be said that brucellosis has a predilection for the female organism, precisely because women seek the health service more often than men (SOARES et al., 2015).

Among all the variables, the following were significant in the chi-square test: “Sex” (p=0.019); “Contact with the B19 vaccine” (p=0.001); “Contact with the RB51 vaccine” (p=0.008) and “Attends a laboratory that diagnoses brucellosis” (p=0.008). To identify the factors associated with the reagents for anti-*Brucella abortus*, a crude analysis was performed using Poisson regression of those variables that would reach a value of p<0.05 in the chi-square test.

We must take into account that the vaccine used in animals has the characteristic of being live attenuated,

when accidentally inoculated in humans or improperly manipulated, forming aerosols, it can end up generating human brucellosis (LOURENCETTI et al., 2018; SOUSA et al., 2020). Poor knowledge about the symptoms of human brucellosis and lack of proper use of PPE are likely causes of unintentional contact with vaccine strains.

Pereira et al. (2020), carried out a study to determine the prevalence of accidental exposure to vaccine strains B19 and RB51 and occupational brucellosis among veterinarians registered with the PNCEBT in Minas Gerais, Brazil. Data were collected through an online questionnaire. Approximately one-third of veterinarians registered to administer bovine brucellosis vaccination in Minas Gerais, 32.83% (108/329), reported being accidentally exposed to vaccine strains B19 or RB51. Exposure factors associated with this outcome included a personal protective equipment (PPE) score at work (OR = 0.94; 95% CI: 0.89-0.98) and a knowledge score about symptoms of brucellosis, classified as poor (base category), intermediate (OR = 0.26; 95% CI: 0.07-0.87) or good (OR = 0.22; 95% CI: 0.07- 0.62).

The role of livestock vaccination in the prevention and control of brucellosis is very important and it is almost impossible to control and eradicate the disease without it (LINDAHL et al., 2020). Therefore, a high level of knowledge about the disease on the part of those who will handle the vaccine is extremely necessary, as it has a potential risk of human infection. Considering the large number of viable bacteria in the brucellosis vaccine, in addition to being highly contagious, it is believed that the use of prophylactic antibiotic therapy in cases of accidental exposure to the vaccine, as well as adequate clinical and laboratory monitoring of the injured person, are fundamental in reducing of the complications of this occupational risk (HYEDA et al., 2011).

Although the Poisson regression analysis the contact variables with the B19 and RB51 vaccines (p=0.256; p=0.388) did not give statistically significant, we must also take into account that not only individuals who have a routine of direct contact with animals are subject to being infected with the disease, but also those who are still in academic training are not taking adequate preventive measures during contact with animals or during the handling of vaccines. As the disease is chronic and has a low mortality rate, generally little attention is given to disease prevention measures in humans, unlike the attention given to bovine brucellosis, which causes great economic losses (GHANBARI et al., 2020).

The variable “Attends a laboratory that diagnoses Brucellosis” (p=0.028) was the only one that remained significant (Table 3). Brucella spp. has a high potential to be aerosolized and has an infective dose of 10 to 100 organisms, these agent characteristics contribute to the associated risk in a laboratory environment (TRAXLER et al., 2013; LOURENCETTI et al., 2018). It is considered one of the most common pathogens responsible for laboratory-acquired infections, along with *Shigella*, *Salmonella*, *Mycobacterium tuberculosis* and *Neisseria meningitides* (SINGH, 2009).

In 2013, Rodrigues et al. reported the first outbreak of Brucella abortus infection in a Brazilian laboratory, where exposure was a result of damage to a biological safety cabinet and failure of the unidirectional airflow ventilation system. The epidemiological investigation identified 3 seroconverted individuals, of whom 1 had clinical manifestations and laboratory results compatible with infection at the time of exposure (n = 11; attack rate = 9.1%).

More recently, there was an outbreak caused by a leak at a Chinese biopharmaceutical company in 2019, where 3,245 people contracted brucellosis despite no deaths being recorded, according to the country's health authority (YEUNG and CHEUNG, 2020).

Of all the pathogens already mentioned in the literature, laboratory-acquired Brucella represents the greatest relative risk: one study found that the incidence of Brucella spp. was 641 cases per 100,000 laboratory technicians, compared to 0.08 cases per 100,000 people in the general population (SINGH, 2009). Although these professionals are generally well educated about the risk of contracting a zoonotic infection during work activities, many adopt attitudes that put their own health and that of their colleagues at risk, such as working outside the security booth and sniffing the plates containing samples of *Brucella* spp. (PEREIRA et al., 2020).

Brucella spp. should only be handled in laboratories with biosafety level 3 or higher. Contamination in laboratories can be associated with aspiration of bacteriological cultures, direct contact with the skin, formation of aerosols, mouth pipetting and spraying on the conjunctiva, nose and mouth. It should be noted that anyone who is in the laboratory during the work of identifying a pathogenic isolate of Brucella spp. is considered an exposed worker (LAWINSKY et al., 2010; PEREIRA et al., 2020).

In the work carried out by Langoni et al (2009), when evaluating knowledge regarding risk factors, it was verified, for some situations, equivocal knowledge of veterinary medicine students, mainly in relation to elimination routes and routes of agent transmission. Even though the majority indicated in the questionnaire that the use of gloves in hospital and laboratory procedures was

important, only 48% of them reported using them frequently in these procedures.

The "use of disposable gloves" (p=1.000) was statistically proven to be a protective factor. What stands out is that only 2 (28.5%) of the reagent cases stated that they did not use gloves regularly, contradicting the variables, in which they stated that at some point they had "Contact with raw beef"; "Contact with bovine fetuses and abortion fluids"; "Contact with the B19 vaccine" and "Contact with the RB51 vaccine". We can observe that despite the level of education and knowledge about the importance of prevention through the use of gloves, few were really aware of its importance as an individual protection measure at the time of practicing activities related to the profession. In addition, there was a bias when filling out the questionnaire, in which the participants probably omitted the lack of wearing gloves for fear of being judged, because it was applied in person by a professional colleague and there were few individuals at a time, the which led to being able to identify who had answered the questionnaire even though there was no identification name, only a number.

A greater relevance in relation to the risk factors related to the veterinary occupation and the symptoms of brucellosis in humans during graduation, perhaps, would bring their awareness to the use of adequate personal protective equipment during the practices inherent to the profession. As well as the importance of your role as a professional in spreading adequate information and being an example for others. Thus, it is important to carry out more studies addressing these factors in this academic and professional occupation, especially in regions that have a high prevalence of bovine brucellosis. With the proper use of PPE's during the practices inherent to veterinary medicine, it is believed that these individuals who were reagents probably would not be, taking into account the existing literature and the variables that were significant in this study.

IV. CONCLUSION

The veterinarian has as one of his professional responsibilities, as a health profession, to bring information to the general population, warning about the risks of direct contact of owners or caregivers with sick animals. Explaining how infection by zoonotic agents works and warning about the ingestion of food of animal origin when these have not been properly prepared and inspected. With this study, we can observe the lack of preparation and responsibility of some professionals when exercising the profession, not fulfilling the basics: protecting themselves and helping others to do the same, putting their health at risk and that of the population in general.

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