



Case study of canine brucellosis by *brucella abortus*: epidemiological inquiry

Inquérito epidemiológico e estudo de casos da brucelose canina por *Brucella abortus*

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ABSTRACT: The objective of this research was to identify brucellosis by *Brucella abortus* and the possible risk factors associated with the transmission of the infection in dogs in the urban area of the municipality of Viçosa-AL, as well as to alert health authorities about the risk of this potential zoonosis, create a booklet to alert the population about the risks of the disease and the possibility of creating a test protocol in clinical care at the Veterinary Hospital of the Federal University of Alagoas. A study was carried out in the city of Viçosa-AL to investigate the presence of *B. abortus* in 30 adult male dogs. The animals that showed symptoms suggestive of reproductive disease were referred for evaluation of the hematological pattern and clinical and surgical treatment. Parallel to the blood collection, a questionnaire was applied to the owners of these dogs. Buffered acid antigen (AAT) techniques were used as a screening test and 2-Mercaptoethanol (2-ME) as a confirmatory test for AAT positives with symptoms of brucellosis. The study aimed to analyze the number of positive dogs, the clinical and anatomopathological changes and the risk factors for the occurrence of brucellosis in dogs in the municipality of Viçosa-AL. As dogs are a means of transmission for humans, health authorities must pay special attention to this disease, including the control of dogs in the Program for Control and Eradication of Brucellosis of the Ministry of Agriculture. In addition to intensifying control of vaccination and diagnosis in cattle.

KEYWORDS: Orchitis; epidemiology; diagnosis; zoonosis.

RESUMO: O objetivo desta pesquisa foi identificar a brucelose por *B. abortus* e os possíveis fatores de risco associados à transmissão de infecção em cães na área urbana de Viçosa-AL, bem como alertar as autoridades de saúde sobre o risco dessa potencial zoonose, criar cartilha para alertar a população sobre os riscos da doença e a possibilidade de criação de um protocolo de teste no atendimento clínico do Hospital Veterinário da Universidade Federal de Alagoas. Um estudo foi realizado no município de Viçosa-AL para investigar a presença de *B. abortus* em 30 cães machos adultos. As técnicas do Antígeno Acidificado Tampado (AAT) foram usadas como teste de triagem e o 2-Mercaptoetanol (2-ME) como confirmatório dos positivos para AAT com sintomas de brucelose. Dos 30 animais testados com AAT, 15 eram domiciliados e 15 semi-domesticados, nos quais 60% (9/15) e 47% (7/15) desses cães eram reativos ao soro, respectivamente. Dos 16 cães reativos séricos para AAT, apenas 25% (4/16) apresentaram sintomas e foram testados com 2-ME, sendo confirmado 100% (4/4). Nos exames anatomopatológicos, foram observadas características típicas de animais brucélicos. Somente o contato com animais da fazenda foi associado à brucelose ($p < 0,05$), sendo considerado fator de proteção.

PALAVRAS-CHAVE: Orquite; epidemiologia; diagnóstico; zoonose.

INTRODUCTION

Brucellosis is a chronic infectious disease caused by bacteria of the genus *Brucella* sp, a facultative intracellular pathogen, gram-negative coccobacillus, non-capsulated,

immobile and unable to form spores, which has six species called: *Brucellae melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae* (VIDAL, J L.; ORTIZ, L F.; OLIVERA, M, 2018).

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The dog can be infected by *B. canis*, *B. abortus*, *B. suis* and *B. melitensis*, the last three occur with dogs in contact with cattle, pigs and goats (MEGID et al., 2007). Generally, transmission occurs directly or indirectly by penetration of the microorganism into the mucosa, leading to bacteremia (SUZUKI et al., 2008). In rural dogs, the *B. abortus* bacteria occurs sporadically due to contact or ingestion of placental remains and fetuses, but there are reports of dogs being contaminated in urban areas due to approaching fairs that sell meat from contaminated production animals (CARMICHAEL; GREENE, 1998).

Clinical signs in male dogs are rare, but may present with epididymitis, prostatitis, testicular atrophy and infertility. In some dogs, *Brucella abortus* associated with transitory bacteremia may present with lymphadenopathy, abortions, orchitis, epididymitis and joint lesions (AZEVEDO et al., 2004).

Diagnosis is made by detecting the bacteria by isolation or serological testing. (AZEVEDO et al., 2004). But the success of the diagnosis depends on the type of material to be cultivated. Clinical diagnosis in dogs is very difficult, usually in the early stages of the disease, infected animals may not show clinical signs or may not be clearly identified (CARMICHAEL; GREENE, 1998). In pathology, it can cause inflammatory changes in the genitals, but not all organs and tissues invaded show visible changes and areas of necrosis (XAVIER, 2009).

The objective of this research was to identify brucellosis by *Brucella abortus* and the possible risk factors associated with the transmission of the infection in dogs in the urban area of the municipality of Viçosa-AL, as well as to alert health authorities about the risk of this potential zoonosis, to create a booklet to alert the population about the risks of the disease and the possibility of creating a test protocol in clinical care at the Veterinary Hospital of the Federal University of Alagoas (UFAL).

MATERIAL AND METHODS

The municipality of Viçosa - AL is located in the mountainous micro-region of Alagoas, 85 kilometers from the capital Maceió, with the main economic activities being trade and agriculture with dairy and beef cattle.

According to research released by the IBGE, in 2014 the population of Viçosa-AL was 26.249 inhabitants, having, in 2016, approximately 30.650 head of cattle. The municipality has many stray and semi-domesticated dogs in the city, which, due to their close connection with the countryside, end up having close contact with rural animals, such as cattle or their by-products, such as meat and raw milk.

Thirty randomly chosen male dogs were used. The type of sampling used was non-probabilistic, which according to Babbie (1999) is used in situations where probabilistic sampling is unnecessary or costly, which, in the case of this study, opted for the use of non-probabilistic sampling for convenience.

The analyzed dogs were classified as domiciled and semi-domiciled. The domiciled ones were those who depended totally on the tutor and left accompanied by their tutors. Semi-domesticated animals were animals that did not completely depend on their guardians, but needed them to receive food and water.

According to data from the health surveillance of the municipality of Viçosa-AL, the estimated canine population in the 2018 vaccination campaign was 3.665 thousand dogs in the rural area and 2.378 thousand dogs in the urban area, totaling 6.043 thousand dogs, not counting the lost ones. The data shows that there are more dogs in rural areas than in urban areas, showing a much greater connection between dogs and livestock.

From July to November 2019, 30 adult male dogs, domiciled and semi-domiciled, with no defined breed and of different ages from the municipality of Viçosa-AL, were selected. The choice was made at random and according to the demand of the veterinary service of the Veterinary Hospital of UFAL by those responsible for these animals. The dogs underwent clinical evaluation and samples were collected for the immunological diagnosis of canine brucellosis. The animals that showed symptoms suggestive of reproductive disease were referred for evaluation of the hematological pattern and clinical and surgical treatment.

A clinical evaluation was carried out in the dogs, observing the clinical parameters of these animals, in addition to the evaluation of the reproductive system regarding symptomatology or testicular alterations. All animals were able to reproduce.

From each dog, 1 to 2 mL of blood was collected in 5 mL syringes and a 25mm x 0.7mm needle, through the puncture of the cephalic vein and placed in sterile test tubes without anticoagulants (dry tubes) and taken to the infectious diseases laboratory from the Federal University of Alagoas (UFAL). To obtain serum, the clot was retracted and the tubes subsequently placed in a centrifuge at 3000rpm for 10 to 15 minutes for complete separation of blood and serum. After this procedure, the serum was placed in a polyethylene tube with a conical bottom to be frozen at -20°C until the time of processing the samples for performing the serological test.

For hematological evaluation, 2 mL of blood were collected, using 5 mL syringes and a 25mm x 0.7mm hypodermic needle and vacuum stopwatch, through the puncture of the cephalic vein and placed in a sterile test tube and taken to the analysis laboratory UFAL's hematological departments analyze the amount of neutrophils and leukocytes to certify that the animal was fit for the surgical procedure and subsequently release it for surgery.

This test was chosen due to the close contact between urban and rural areas, which could be a means of transmission of *B. abortus* between species, in which dogs were exposed to the risk of infection by this *Brucella* sp. *lisa*. The choice of indirect diagnosis for brucellosis complied with the technical standards

of the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (PNCEBT).

The antigen consists of an inactivated suspension of the *Brucella abortus* 1119-3 sample, stained with Rose Bengal, diluted to 8.0% in buffer solution pH 3.65 ± 0.05 , standardized by comparison with the reference antigen. Due to its pH, some non-specific agglutinins are inhibited, providing an increase in the specificity of the test.

Slow serology in tubes with 2-mercaptoethanol (2ME) was used as a confirmatory test for the screening test, only in animals that showed reagents in the Acidified Buffered Antigen (AAT) test with symptoms of brucellosis. The test was chosen in compliance with the norms of the technical regulation of the PNCEBT.

The proof is based on the action of compounds containing the thiol radical (2-ME), which degrade the pentamer configuration of IgM, determining the loss of its agglutinating activity. This process makes the test sensitive to the agglutinations established by the IgG class, which does not change its activity in the presence of this reagent, making the test more specific (NICOLETTI, 1980).

The preoperative clinical evaluation was carried out following the pattern of the HVU-UFAL anamnesis form. In which it was verified: body temperature, mucous membranes, lymph nodes, skin and hair, presence of ectoparasites, presence of brucellosis symptoms, testicular enlargement, heart and respiratory rate, capillary refill time and pulse.

The animals that were fit in the clinical and hematological examination were referred for pre-scrotal orchiectomy performed in the surgical center of the HVU-UFAL by the hospital's surgeon and anesthesiologist.

The anesthetic protocol contained pre-anesthetic medication: morphine at a dose of 0.5 mg/kg, associated with midazolam at a dose of 0.2 mg/kg. Ten minutes were waited for the trichotomy to be performed. With the animal calmed down, trichotomy of the pre-scrotal region was performed. Anesthetic induction was performed with propofol at a dose of 5 mg/kg and maintenance with isoflurane, in a semi-open system. Antisepsis of the pre-scrotal region was performed with the animal in dorsal decubitus and after positioning the field cloths, fixed with backhaus tweezers.

The surgery was performed as follows: one of the testicles was pushed into the pre-scrotal region. An incision was made in the skin and subcutaneous tissue along the medial raphe above the displaced testis and through the spermatic fascia to exteriorize the testis. The parietal vaginal layer over the testis was incised. A hemostat was placed in the tunica vaginalis where it attaches to the epididymis. Digitally, the tail of the epididymal ligament has been separated from the tunica. The testicle was exposed and the structures of the spermatic cord identified, clamped and connected with 2-0 nylon thread. The same procedure was performed on the contralateral

testicle. The skin was closed with simple isolated stitches and 2-0 nylon thread.

After the surgical procedure, the animal was medicated with meloxicam at a dose of 0.1mg/kg and ceftriaxone at a dose of 30mg/kg, intramuscularly. The animal was only discharged after recovering from anesthesia and receiving medical discharge.

The testicles underwent anatomopathological evaluation to verify consistency, size and texture before being submitted to histopathological procedures.

Testes and annexes were evaluated for macroscopic changes related to size, consistency and presence of inflammatory sequelae. Subsequently, for histopathological examination, a fragment of each testicle was removed and placed in a sterilized container with a volume of buffered formalin fixative 10 times greater than the tissue to be fixed. Once fixed, the piece was transferred to 70% alcohol, where it could remain indefinitely (JUNQUEIRA; JUNQUEIRA, 1983).

The pieces were included, dehydrated, clarified and impregnated. The paraffin blocks were passed through a microtome to obtain thin and uniform cuts. To visualize the tissue structures, the slides were stained with Eosin (HE). The final assembly of the slide occurred by depositing a drop of liquid resin on the cut adhered to the glass slide and covered with a coverslip and thus cataloged to be analyzed under the microscope (JUNQUEIRA; JUNQUEIRA, 1983).

Canine brucellosis, unlike other diseases, does not have vaccines for prevention. Other means of prevention are necessary, such as laboratory tests, which can be indirect by serology and direct by isolation (MEGID et al., 2007).

In order to cure the *Brucella abortus* infection, in addition to orchiectomy, the tutor was prescribed to administer at home as a continuation of the dipyrrone treatment at a dose of 25mg/kg every 8 hours for 3 days, meloxicam at a dose of 0.1 mg/kg every 24 hours for 3 days and enrofloxacin at a dose of 10 mg/kg every 24 hours for 28 days. Due to the intracellular persistence of the agent, the results of antibiotic therapy are not very satisfactory, but it is considered a treatment.

Parallel to the blood collection, a questionnaire was applied to the owners of these dogs, with the objective of obtaining information related to handling, symptoms, feeding and coexistence with other animals in the rural area, in order to identify risk factors.

The research comprised a cross-sectional epidemiological study with a quantitative approach in which the situation of canine brucellosis was determined in relation to the risk factors for its occurrence.

Data analysis was performed using the statistical program BioEstat 5.0. Several groups were compared, obtaining odds ratios (OR), 95% confidence intervals and significance verified by chi-square (X^2) and Fisher's exact tests. The established significance level was $p < 0.05$.

RESULTS

Fifteen semi-domesticated male dogs and 15 domesticated male dogs were analyzed. Most animals did not show clinical symptoms. Only 25% (4/16) had testicular alterations, 13.33% (2/15) lived at home and 13.33% (2/15) lived at home.

Of the 25% (4/16) symptomatic, 50% (2/4) had hypertrophy of one of the testicles and 50% (2/4) had bilateral enlargement, one of them with ocular discharge. One of those who had hypertrophy of one of the testicles also had scrotal dermatitis, fever and apathy. The remaining dogs, 86.66% (26/30), had normal clinical features, no testicular symptoms and capable of reproduction.

Dogs with symptoms underwent hematological examination before surgery, which is a pre-surgical requirement. One animal had anemia with low neutrophil and leukocyte counts. This animal only underwent surgery after recovery. The veterinary doctor at the UFAL Veterinary Hospital prescribed medication and after two months the blood count was redone, which was normal and the animal underwent surgery.

Of the 30 samples of adult male canines evaluated in the city of Viçosa - AL, 16 gave reagents to the Acidified Buffered Antigen (AAT) test, presenting seropositivity, in general, of 53.3%. When analyzed by category, 60% (9/15) of the samples from domesticated dogs and 47% (7/15) of the samples from semi-domesticated dogs were seroreactive. Two reactive households belonged to the same guardian, accounting for 15 outbreaks of *B. abortus*. Only 12.5% (2/16) of those positive for AAT ate pet food and did not walk alone, the other 87.5% (14/16) ate raw meat and milk, and of these, 7.1% (1/14) had the habit of eating leftover placenta. Of the AAT reagents, only 25% (4/16) dogs that showed symptoms with testicular changes were tested with 2-mercaptoethanol (2-ME) and 100% (4/4) were reagent. Of these four 2-ME-reactive dogs, two were semi-domiciled from the same owner and two were domiciled from different owners, resulting in three different foci of confirmed *B. abortus*.

The surgeries were performed in only 25% (4/16) of the dogs, which presented clinical symptoms and were positive in the 2-mercaptoethanol test. After the surgical procedure of orchietomy, which was uneventful, the testes were analyzed anatomopathologically and histopathologically. Anatomically, 50% (2/4) had hypertrophy in both testes, 50% (2/4) had hypertrophy in only one testicle, 75% (3/4) had normal features, 25% (1/4) had both the testicles with a very firm consistency and the presence of several nodules, dog two from chart 1. One of the testicles had dermatitis, dog one from chart 1.

Pathologically, 75% (3/4) of the analyzed testes showed small cytoplasmic vacuoles in the epididymal lining epithelium. Another 50% (2/4) had thickening of the tunica albuginea of the testis, with increased connective tissue. In addition, 50% (2/4) had atrophy of the seminiferous tubules, with reduced sperm, 25% (1/4) with reduced sperm and extensive fibrosis,

50% (2/4) the seminiferous tubules had reduced spermatogenesis, 100% (4/4) the tubules of the epididymis had the presence of spermatozoa, 50% (2/4) thickening of the tunica albuginea of the testis, 50% (2/4) the spermatids were degenerated and with the presence of multinucleated giant cells, resulting from the its merger.

As a form of treatment, the positive dogs in the 2-ME test were submitted to castration followed by antimicrobial therapy and to confirm the therapy, the animals must undergo the AAT and 2-ME tests again. After 6 months, the animals must undergo examinations again to confirm the absence of the disease.

Regarding the situation of canine brucellosis and the risk factors for its occurrence, according to the results presented, only contact with production animals was associated with brucellosis ($p < 0.05$), being considered a protective factor (RR = 0.33). The other evaluated variables showed no association with brucellosis ($p > 0.05$).

DISCUSSIONS

In this study, preference was given to semi-domiciled and domiciled dogs to observe the rates of *B. abortus* in dogs from the city of Viçosa-AL, as it is close to the countryside. Generally, infections in dogs caused by *B. abortus* occur sporadically in dogs that have contact with animals in rural areas, through contaminated animal products or by ingestion of abortion remains (AZEVEDO et al., 2004). Brazil has one of the largest cattle herds, being a risk factor for the increase in the prevalence of the disease caused by *B. abortus* in humans (PAPPAS, 2006).

Preferences were given in the study to adult men of any age, without race determination, as it was not pertinent to this research. In the study carried out in Uberlândia - MG, there was no statistically significant difference ($p > 0.05$) between gender and age of dogs reactive for *B. abortus* (SALABERRY, 2010). Thus concluding that both males and females and dogs of different age groups are equally exposed to the risk of infection, corroborating what was said by Azevedo et al. (2004). According to Almeida et al. (2000), the highest frequency of reactive dogs occurs with age greater than one year or in reproductive age. In this study, all dogs analyzed were able to reproduce.

In males, brucellosis presents itself in the form of epididymitis, prostatitis, uni or bilateral testicular atrophy, scrotal dermatitis, sperm abnormalities and infertility (CARMICHAEL; GREENE, 1998). According to data from this study, of all animals that showed symptoms, 25% (4/16) had testicular changes characteristic of reproductive disease. In a study carried out by Keid et al. (2007) demonstrated that the proportion of positive dogs detected by each of the diagnostic methods used (RSAT, ME-RSAT, blood culture and PCR) was higher in animals that showed clinical evidence of brucellosis than in animals that did not show clinical signs. ($p < 0.05$).

Contrary to what was found in this study, in which 75% (12/16) of those positive for AAT did not present symptoms.

Some dogs with epididymitis are in pain and may lick the area frequently, causing dermatitis. In the cases of the dogs studied, only 25% (1/4) had scrotal dermatitis and were seropositive in the 2-ME. These dermatitis can also be accompanied by *Staphylococcus aureus* (CARMICHAEL; GREENE, 1998).

Regarding laboratory tests, the blood count may show anemia, normal or low leukocyte count, with relative lymphocytosis and thrombocytopenia. (CUNHA, M.; MIGUEL, N.; MANSO, J. A., 1992). Similar to what was found in this work in operated dogs, that in the blood count, a surgical prerequisite, three of the four dogs did not show alterations in leukocytes and neutrophils. Only one had anemia and the treatment was performed before surgery. Thus contributing to reinforce the thesis of Cunha, Miguel e Manso. (1992).

Of the 30 samples of adult male canines evaluated from the city of Viçosa - AL, 53.3% (16/30) were reactive to the AAT test, 60% (9/15) being domiciled and 46.67% (7/15) were semi-domiciled. Much lower results were detected in a study carried out in Patos de Minas - Paraíba, in which seropositivity for *Brucella abortus* was 0.85% (1/118). On the other hand, other studies have shown absence of antibodies to *B. abortus* (AGUIAR et al., 2005; MALEK DOS REIS et al., 2008). Serological tests for the detection of antibodies to *Brucella* are the best means of diagnosing the infection (CARMICHAEL; GREENE, 1998).

B. abortus and *B. canis* are the most pathogenic serotypes for dogs, causing chronic or subclinical disorders that are difficult to diagnose due to their similarity with other diseases (GUIMARÃES et al., 2000). In the first stage of infection, dogs may not show clinical signs of the disease or may not be clearly identified (CARMICHAEL; GREENE, 1998). As many dogs present the asymptomatic form, they become an important source of infection and spread of the disease (BAEK et al., 2003). Corroborating this study, that of the 53.3% (16/30) of the AAT-reactive animals, only 25% (4/16) had symptoms and the remaining 75% (12/16) were asymptomatic, data similar to those found by other authors. Clinical manifestations by *B. abortus* in dogs are rare (AZEVEDO et al., 2003).

The prevalence of *B. abortus* found can be explained by the type of sample studied, the presence of animals at the beginning of the infection, where antibodies may not be detected, cross-reactions with other microorganisms and the diagnostic method (CARMICHAEL; GREENE, 2006; NICOLETTI, 1980). Most seroepidemiological surveys show that the prevalence of anti-*B. abortus* ranges from 0 to 18.4% and are found mainly in the southeastern and southern regions of the country (PAZ et al., 2015). Results well below those found in the present study, which was 53.3% by AAT and is located in the northeast region of the Zona da Mata Alagoana.

Non-specific reactions generated by the serum activity of Immunoglobulin M (IgM) by AAT may result from recent

infections, in which the presence of Immunoglobulin G (IgG) may not be detected. Under these conditions, the 2-ME test is important as confirmation of the AAT test (POESTER, 1975). In this research there were no nonspecific reactions, which demonstrates that these are old infections, in the AAT test, 53.3% (16/30) were positive and of the symptomatic ones tested in the 2-ME, 100% (4/4) were positives.

Some national studies detected a low occurrence of anti-*B. abortus*, such as the work by Almeida et al. (2001) in Minas Gerais, who did not find animals reactive to *B. abortus*. In Pará Lopes, Molnár e Molnár et al. (1999), studying several seropositive samples of animal and human species, detected a dog (4.3%) reactive for *B. abortus* through the ELISA technique. It is concluded that seropositivity may be linked to the region, due to the local culture, eating habits and proximity to the countryside.

In this study of *Brucella abortus* in dogs, the testes were analyzed anatomically and histologically. Histopathological examination showed 25% (1/4) of granulomatous reaction and 50% (2/4) presence of multinucleated giant cells resulting from the fusion of several spermatids, characterizing an inflammatory reaction by *Brucella* sp, in agreement with reports of the described brucella orchitis by Gomes et al. (1999). 25% (1/4) had both testicles with a very hard appearance and the presence of several nodules, 50% (2/4) had atrophy of the seminiferous tubules, which could indicate a chronic phase.

All the analyzed testicles presented inflammatory alterations corroborating with what was said by Xavier (2009). The bacteria multiplies in the genitals, which can lead to inflammatory and anatomopathological changes and, consequently, hyperplasia and lymphadenitis. Not all organs and tissues invaded show visible changes and areas of necrosis (XAVIER, 2009). In this study, 75% (3/4) of the testicles analyzed had a normal appearance, with only a change in size.

As a treatment, dogs positive in the 2-ME test were subjected to castration followed by antimicrobial therapy and to confirm the therapy, the animals must undergo AAT and 2-ME tests again. The use of only one antimicrobial is not recommended (FLORES-CASTRO et al., 1997). And they must be retested from three to six months after the treatment protocol, mainly if the therapy is interrupted to certify that they are cured (CARVALHO et al., 1995). Until the completion of this article, the animals continued the treatment, which can take up to 1 year. Thus requiring a more in-depth study.

A study by Megid et al. (2007) showed that antimicrobial therapy combined with castration is effective. Of the 12 animals naturally infected and followed serologically for two months, 91.6% were cured after two months of antibiotic therapy (MEGID et al., 2007). It is necessary to carry out a broader study in order to identify the best therapeutic protocols for dogs in the city of Viçosa-AL. The treatment is suggested for those dogs that have a high affective and economic value and the therapy must be rigorously evaluated by the veterinarian and indicated exclusively in these specific

conditions (MEGID et al., 2007). Due to the sentimental value that the studied dogs presented to their owners, this study opted for treatment through sterilization and antibiotic therapy to obtain a cure.

In this study, 60% (9/15) of the domesticated dogs and 46.6% (7/15) of the semi-domesticated dogs were AAT-reactive. The number of infected households is greater than semi-domiciled ones. This demonstrates that the contagion occurred within the residence and not through direct contact with animals in the rural area. Corroborating with a study carried out in Uberlândia - MG, which showed the occurrence of 5.93% of *B. abortus* antibodies in dogs in the urban perimeter of Uberlândia - MG, semi-domiciled and domiciled (CARVALHO et al., 2000; SALABERRY, 2010).

Of the sixteen AAT positives, only 12.5% (2/16) of the households ate animal feed, had no contact with animals in the rural area or had contact with stray animals, and one had already reproduced. These probably could have been infected indirectly through contact with secretions from brucella dogs when walking with their tutors or copulating with infected females, further increasing the spread of the disease. The other 87.5% (14/16) of households ate raw meat and milk, including one that used to eat leftover placenta. Demonstrating that the animals became infected through contaminated food, agreeing with what was said by Charmichael and Greene (1998), that the infection of dogs by *B. abortus* has been related to the consumption of food of animal origin and remains of bovine abortion.

Generally, infections in dogs caused by *B. abortus* occur sporadically in dogs that have contact with animals in rural areas, through contaminated animal products or by ingestion of abortion remains (AZEVEDO et al., 2004).

Regarding the semi-domiciled, all 46.6% who responded to the AAT ate at home, consuming meat and raw milk. Still 28.57% (2/7) attended the municipal fair in the city of Viçosa, where beef without origin is sold. One of the semi-domiciled individuals, in addition to eating raw meat, lived with animals from the rural area. In addition, 57.14% (4/7) did not reproduce, 14.28% (1/7) had already reproduced and 28.57% (2/7) did not know and it was even the two who presented symptoms and gave reagents to the 2-MINE. Corroborating once again what some authors have said about contamination by food and contact with excretions or animals (AGUIAR et al., 2005; MALEK DOS REIS et al., 2008).

The two semi-domiciled animals positive for AAT and 2-ME, which showed symptoms, had no contact with animals in the rural area, did not know if they had copulated with stray animals, did not live close to the rural area, had no contact with the municipal fair, but they fed on meat and raw milk, even the tutors bought meat at the city's municipal fair. Demonstrating once again that contamination occurred through food. Different results from those found by Malek et al. (2008) in São João da Bela Vista - SP with 500 dogs from the Centro de Zoonoses, of both sexes and ages, which were negative for

Brucella abortus, justifying that they are animals from the urban area, which had no direct contact with livestock Of region. The reactive animals in this study had no direct connection with rural animals, but had contact with their by-products.

Although Viçosa -AL is a region with significant dairy and beef activity and close to rural properties, the study showed that this approximation was not a risk factor for contamination of dogs, since no AAT and 2-ME reagent dogs lived or were close to rural properties. The presence of positive animals in the screening and confirmatory test for the diagnosis of *B. abortus* infection may be linked to the high risk of infection to which the animals were submitted due to their eating habits.

In the study carried out by Almeida et al. (2004) in Afenas (MG) detected a prevalence of 2.8% of dogs reactive to *B. abortus*, due to the contact of dogs with cattle, since the region has an expressive dairy activity, which in the case of this study was not one of the main contamination factors. Thus, noting that we must pay attention to the cultural part of each city and region, as proximity to the countryside is not always a contamination factor, as demonstrated by several authors. The city of Viçosa-AL has a municipal fair where the population buys meat without refrigeration, supervision and with terrible hygiene conditions.

In this work, the variables studied were not considered risk factors, contact with animals in rural areas was considered a protective factor. Although Viçosa is closer to the rural area, the dogs did not contract the disease through direct contact with cattle, a crucial factor for the transmission of *B. abortus*, but indirectly through its by-products, serving as a warning to the authorities of the risks of contamination. due to the culture of each region. Each region must carry out its control according to the risks, as in Viçosa-AL, which at the veterinary clinics and at the HV-UFAL must have a protocol for brucellosis tests in patients arriving with reproductive symptoms.

CONCLUSION

The frequency of *B. abortus* in domesticated and semi-domesticated dogs in the city of Viçosa-AL is high. Infected and symptomatic animals have a high frequency of uni or bilateral orchitis and scrotal dermatitis, in addition to other pathological changes. The treatment of infected animals is important to prevent the spread of the disease and must be carried out with the use of combinations between castration and antibiotic therapy, preceded by the evaluation of the animal's surgical aptitude. It is not considering contact with live cattle as a risk factor in this situation, reinforcing the need to control the population of stray dogs and guide the population regarding the food and health management of animals in the municipality of Viçosa-AL. With the results of this study, the diagnosis of canine brucellosis will become part of the routine diagnostic protocol of the Veterinary Hospital of UFAL, to meet the needs of this region. To inform the population, an educational guide was prepared, which will be distributed in the city of Viçosa-AL.

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