

## Brucellosis in Dogs

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### Abstract

The brucellosis is a cronical contagious disease that affects animals and humans, been characterized as a zoonosis. The present study aimed to the detection of brucellosis in 66 dogs examined at the Veterinary Hospital of the Federal University of Uberlândia, naturally infected with *Ehrlichia sp.* For this study, two species of *Brucella* were selected, *B.abortus* and *B.canis*. For *B.abortus*, the disease was diagnosed with the Rose Bengal Test, confirmed by the ME-RSAT test, and the *B.canis* was diagnosed by a Veterinary Diagnostic commercial kit specifically for it. The prevalence of *B.abortus* was found in 16 of the 66 dogs evaluated (24.24%), with five confirmed by the ME-SAT test, and no reagent for *B.canis*. Positive animals had mainly reproductive problems. With the presented results, it can be concluded that brucellosis is a present disease in dogs and probably have being neglected by the occurrence of erlichiosis at the same time.

**Keywords:** Breeding; *B.abortus*; *B.canis*; Brucellosis; Erlichiosis; Canine

### Introduction

Brucellosis is an infectious disease that affects most domestic animals and even humans, being characterized as a zoonotic disease [1]. It is a chronic disease caused by facultative intracellular bacteria of the genus *Brucella spp.* with the main species are *B.abortus*, *B.canis*, *B.ovis* and *B.melitensis* [2].

The International Organization of Epizoonoses (OIE) classified this disease in their list B because of the significant consequences for international trade of animals and animal products, besides the socio-economic importance and / or public health impact.

With the growth of small animal market and its proximity to their responsible and their families, a correct diagnosis of brucellosis is important, especially if this is contagious and poses risks to humans. The dogs are susceptible to infection by strains of the species *B.abortus*, *B.ovis* and *B.canis*, and may be found in acute or chronic phases [2].

Brucellosis in dogs has no specific clinical signs and when there are symptoms, it is usually of the reproductive tract [3]. For this reason, it is often overlooked clinically, being confused with other reproductive disorders. Therefore, veterinarians do not request tests for diagnosis for brucellosis, keeping it in the environment in which the animal is inserted, whether rural or urban, lending itself as a source of infection to other animals and humans. Dogs can contract this disease when in contact with contaminated material such as meat, milk, blood, placenta or other biological materials [4].

As brucellosis, ehrlichiosis is an infectious disease of dogs and is characterized as zoonosis [5]. Very common in dogs in Brazil, it is characterized by thrombocytopenia and increased spleen and liver volume. The vector of *Ehrlichia canis* is the tick *Rhipicephalus sanguineus*, a very common ectoparasites in dogs in Brazil. Therefore, it is a disease of high prevalence and difficult to eradicate [6].

Given that the ehrlichiosis is one of the most common diseases in dogs in Brazil, leaving debilitated dogs and susceptible to other infections, this study aimed to research antibodies anti *B.abortus* and *B.canis* in dogs with presence of morula of *Ehrlichia sp.*

### Materials and Methods

This study was approved by the Ethics Committee on the Use of Animals (CEUA-UFU 036/14).

A total of 66 samples of blood serum from dogs diagnosed with ehrlichiosis by the morula detection in peripheral blood smear, from September 2013 to April 2014, were taken at the Veterinary Hospital of the Federal University of Uberlândia.

To detect the presence of antibodies anti *B.abortus*, was applied to all 66 samples the Rose Bengal Test (Paraná Institute of Technology-TECPAR, Curitiba, Paraná, Brazil). For this was used a glass plate with four centimeters enclosed square, which was applied 30 µL serum to be tested and, next to this but still in the same square, 30 µL of the *B.abortus* antigen. After that, the serum and the antigen were mixed so as to form a circle of approximately two centimeters.

This mixture was homogenized using a circular motion to the plate for four minutes and the results were interpreted on the plate over an indirect light box, where it was considered as positive result the presence of lumps, and negative result the absence of lumps. The agglutination reactions that occurred after four minutes were not considered [7].

Slow Agglutination Test (SAT) and 2-mercaptoethanol (ME) were used as a confirmatory test. For those tests were used two rows with four tubes each for individual's samples, following dilutions 1:25, 1:50, 1:100, 1:200. Were homogenized two mL of antigen diluted in phenol solution and 100 µL of saline solution in the first's four tubes (T). In the remains four tubes (M) was added one mL of 2-ME inhomogeneous solution, and after 30 minutes, added one mL of 50% antigen. Tubes of both series (T and M) were kept in a 37°C oven for

48 hours and the results interpreted as positive (presence of lumps in SAT and ME), negative (no lumps of SAT and ME) or inconsistent (presence of lumps in one of tests) after reading above a light source in a dark room [8].

The diagnosis of *B.canis* was performed with Alere Brucellosis Canine Test Kit, a kit for rapid immunochromatographic test (Alere, Waltham, Massachusetts, USA). The test was performed according to manufacturer's specification, which after 20 minutes was possible to verify a pink line on the tag control (C) independent of the result. If positive, a pink line on the tag T appears.

The clinical records of the animals were evaluated for age, sex, race, residence (urban or rural perimeter), food (homemade food or dog food), as well as clinical signs of ocular, neurological, reproductive changes, liver, spleen and palpable lymph nodes. Fisher's exact test was used for statistical interpretation of data [9].

## Results

For the Rose Bengal Test, 24.24% (16/66) of the evaluated animals were positive for *B.abortus*. Of these, 7.57% (5/66) had confirmation by 2-ME test and SAT for this bacterial specie. All animals tested for *B.canis* by the immune chromatographic analysis were negative.

In medical records was found that 43.75% (7/16) of the positive dogs for Rose Bengal Test had ocular manifestations such as purulent secretions, teary eyed, episcleral congested vessels and the presence of calcium deposits. In 12.5% (2/16) of the dogs was noted neurological clinical signs, which are ataxia, head tilt, head pressing and absence of movement of the hindquarters.

In 43.75% (7/16) of positive animals for *B.abortus* by the Rose Bengal Test, was observed presence of reproductive changes, such as the presence of nodules in the mammary glands, pyometra, vulvar swelling, uterine hyperplasia, extended heat, penile edema. Changes in the spleen and liver were also found, including swelling detected by palpation, and presence of granulation by ultrasound in 43.75% (7/16) dogs.

Lymph nodes were increased when touched in 25% (4/16) of positive dogs for *B. abortus* by Rose Bengal Test.

## Discussion

Of the 16 positive animals for *B.abortus* by Rose Bengal Test, 56.25% (9/16) lived in urban area and were fed with dog food, while 37.5% (6/16) lived in urban area and were fed with homemade food (rice and meat, for example), according to analysis of medical records. Only 6.25 (1/16) lived in the countryside, feeding with homemade food. However, the relationship between food, housing and positivity for *B.abortus* was not significant ( $p = 0.4375$ ).

Although brucellosis is a chronic disease in dogs [8], clinical signs consistent with *Brucella sp* infection were found by other authors [2]. There were noted changes in the eyes, reproductive tract, lymph nodes, spleen, liver and, to a lesser extent, neurological findings. However, it is important to remember that the ehrlichiosis in dogs is also able to cause eye, spleen and liver abnormalities, which could cause confuse diagnosis.

Although no significant results co-relating housing, food and infection with *B.abortus*, were found 93.75% (15/16) positive urban animals for this bacterium. Therefore, raises the hypothesis of

contaminated meat or milk consumed, which suggests that animals positive for brucellosis have been slaughtered [10,11].

Urban dogs infected with *B.abortus* may have contracted from the consumption of contaminated animal products. Thus, not only increases the concern for the health of dogs, but also to public health. [12]. Given that these positive dogs have contact with other animals of the same species and also humans, there is a concern with regard to contamination through contact with secretions with bacterial load, such as urine, faeces, vaginal secretion, and ejaculate [4].

Knowledge of brucellosis in dogs is information that not only alerts veterinary medical community and infectious disease physicians, but also the kennels owners, who may suffer serious financial losses if there is an infected animal on their property [13], or even already has been losing litters with this disease, not investigated.

As for *B.canis*, all dogs of this research were negative. The results obtained in this work does not imply the absence of *B.canis*, since previous studies confirm infection by this bacterium in 14.2% (90/635 animals) dogs through Agar Gel Immunodiffusion Test (AGID) with rough surface strains [8]. The application of test with specific antigen in Colombia obtained positivity for 27.7% (61/220) dogs and 17.3% (9/52) humans [14]. For this reason, *B.canis* should not be neglected.

## Conclusion

The presence of antibodies anti *B.abortus* in dogs with *Ehrlichia sp.* morula demonstrated the possibility of brucellosis and ehrlichiosis at the same time. This information highlights the need to increase the investigation of brucellosis because, not only cause clinical signs similar to those of ehrlichiosis, but also the brucellosis can be transmitted to humans.

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