



CANINE BRUCELLOSIS: An Emerging Disease

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ABSTRACT

Canine brucellosis is an infectious disease caused by *Brucella canis*, discovered by Carmichael in 1966. *B. canis* bacteria are gram-negative, immobile, coccobacillary, measuring between 1.0 and 1.5 μ m, facultative intracellular, aerobic, with a rough surface and non-spore forming. The bacteria is present in different countries around the world, including Brazil, and is easily spread. Resistance to *Brucella* infection is mainly involved in cellular immunity, as it depends on the activation of macrophages, but also on humoral immunity. Antibodies provide only partial protection and are mainly directed against lipopolysaccharide (LPS). There are several diagnostic methods for the disease, with their respective particularities. However, confirmatory diagnosis is only possible with isolation and identification of the bacteria, even though a negative result cannot confirm the absence of the pathogen, as several factors may interfere. It is necessary to obtain more information about the disease, as although it does not yet have great pathogenic potential, it has a clear capacity for infection, including in humans.

Keywords: Zoonosis; *Brucella canis*; Infection; LPS (lipopolysaccharide); Bacterium.

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INTRODUCTION

Canine brucellosis is an infectious disease, which is not mandatory to be notified to the World Organization for Animal Health (WOAH), which negatively results in the notification of positive cases and, therefore, in obtaining truthful data. (Djokic, Vitomir, et al.; 2023a). Due to the lack of constant vigilance regarding this disease, the change in geographical barriers can be considered constant (Cosford, Kevin L; 2018).

The main agent involved in the maintenance of the disease is *Brucella canis*, however, there are reports of infection by *Brucella abortus*, *Brucella melitensis* and *Brucella suis* (Greene; Carmichael, 2015). *B. canis* is a gram-negative bacterium that has a rough, aerobic, capsuleless, immobile, coccobacillus-shaped, non-spore-forming, and obligate intracellular morphological characteristic (Whatmore, 2009). When compared to other *Brucella* spp., *B. canis* induces less inflammation and more insidious lesions (Chacón-Díaz, et al.; 2015), a factor that can be explained by being devoid of the somatic O antigen, indicating reduced cell wall, and with little endotoxin (Morisset et. al.; 1969).

Updates on the study of canine brucellosis highlight the importance of this disease in its recognition as an anthrozoosis. Although its pathogenicity is not as high or concerning compared to other strains of brucellosis, it represents a clear and established zoonotic potential. This risk is especially relevant due to the close relationship between dogs and humans, which is aggravated in people with weakened immune systems, as in the case of a 46-year-old woman with HIV (Lawaczek, E. et al., 2021), or in individuals with an immune system in formation, such as a 3-year-old child (Dentinger, C. M., 2015).

The survey of canine brucellosis is important to improve the understanding of the disease, considering the need for greater collection of information, especially for the production of diagnostic tools. Studies such as this one contribute to this collection, in addition to making it possible to obtain data on the reality of some neighborhoods in the municipality of Cruz das Almas, Bahia.

MATERIALS AND METHODS

The present study consists of a bibliographic research for analysis, comparison and compilation of information on *Brucella canis*, focusing on the diagnostic area, in several academic repositories, such as PubMed, SciELO, and websites of organizations, in addition to the pages of universities responsible

for Course Completion Papers, Dissertations and Theses. The following keywords were used, among others: *Brucella*, *Canis*, *Canine*, brucellosis and tests.

LITERATURE REVIEW

Historical Context of *Brucella canis*

According to Kevin L. Cosford (2018), *Brucella canis* was first described in 1966, in the USA, by Carmichael, when it was associated with cases of abortion in beagles. This pathogen was isolated from aborted tissues and vaginal discharge. For this reason, Faig E.L. (1969) suggested calling canine brucellosis "beagle fever". There are also reports of the presence of the bacterium in Quebec in the 1970s and in southwestern Ontario in the 1980s.

Initially, due to genotypic and phenotypic similarities, since they share a common ancestor, *B. canis* was considered a biotype of *Brucella suis* (Moreno, Edgardo; Cloeckert, Axel; Moriyón, Ignacio, 2002). To differentiate the two strains, it was necessary to optimize a conventional multiplex polymerase (PCR) chain reaction (Goñi-López, Ignacio, et al., 2011).

In Latin America, specifically in Brazil, the first description occurred in the state of Minas Gerais, in 1977, by Godoy et al., who isolated the bacterium from a bitch with a history of miscarriage and a slow agglutination-proof reagent (SAL). In 1980, Larsson et al. isolated three samples of *B. canis* in São Paulo, one of which was isolated from a female with a history of infertility, another from an asymptomatic female and the last from an asymptomatic male. Subsequently, in 1996, in the city of Santa Maria, Vargas et al. isolated *B. canis* from the placenta of aborted neonates and fetuses in a kennel with canine breeders of different origins. In Porto Alegre, in 1999, Gomes et al. isolated *B. canis* from the epididymis and testicle of a dog with clinical orchitis and epididymitis.

Etiological Agent, Morphological and Antigenic Characteristics

Bacteria of the genus *Brucella* are divided into two groups, rough and smooth, due to their distinct antigenic characteristics. This classification is based on the constitution of the lipopolysaccharide (LPS) of the cell wall of the bacterium. In rough species, LPS contains only lipid A and the nucleus of the oligosaccharide, while in smooth species, LPS includes lipid A, nucleus oligosaccharide, and the O chain (Corbel, 1997).



One of the determining factors in the virulence of the bacterium is the LPS (Cardoso et al., 2006), specifically the O chain present in it, since this structure can protect the bacterium from the immune response, as discussed by J.A. Smith (2018). The O chain interacts with lipid rafts on the surface of macrophages, allowing bacteria to enter the cell. On the other hand, *Brucella* strains with R-LPS, such as *B. canis*, do not interact with lipid rafts and quickly connect to lysosomes (Lapaque et al., 2005).

The most characteristic structure of gram-negative bacteria is their cellular envelope, formed by a cytoplasmic membrane, a periplasmic space rich in intermediate soluble proteins, and an outer membrane (Moriyon et al., 2002).

Bacteria of the genus *Brucella* have a coccobacillary shape, with dimensions between 1.0 and 1.5 μm . They are facultative, aerobic, gram-negative, immobile, non-spore-forming intracellular cells, and *B. canis* has a rough surface, in addition to having as a peculiar characteristic the ability to form colonies with a mucoid aspect (Greene; Carmichael, 2015; Keid, 2015; Carmichael; Kenny, 1970).

Examples of smooth colonies, according to the chemical characteristics of the cell wall, include *Brucella abortus*, *B. melitensis*, and *B. suis*. On the other hand, rough colonies include *B. ovis* and *B. canis* (BRASIL, 2006). Meanwhile, *B. neotomae* is described as another example of a bacterium with smooth LPS (lipopolysaccharide), according to Waldrop S.G. and Sriranganathan N. (2019).

The characterization of the molecular genetics of the genus *Brucella* occurred almost exclusively during the 1990s. The genus, by its nature, is extremely homogeneous, with all members showing a homology greater than 95%. Based on DNA-DNA hybridization studies carried out by Verger et al. (1985) with 51 *Brucella* strains of all species, this genus was considered monospecific (Dees et al., 1981; Correa; Correa, 1992).

Only domestic and wild canids are susceptible to infection by *B. canis*. On the other hand, felines are relatively resistant, with limited reports of experimental infections, in which they present transient bacteremia (Greene; Carmichael, 2015; Keid, 2015). Although there is a preference for *B. canis* strains to infect canids, *B. abortus* to cattle, *B. suis* to swine, *B. ovis* to sheep and *B. melitensis* to goats, cross-contamination can occur. It is important to emphasize that *B. melitensis* is exotic in Brazil (Poester et al., 2002).

Four of the six most classic species of *Brucella* can infect both dogs and humans, and are therefore a zoonosis. These are: *B.*

canis, *B. melitensis*, *B. suis* and *B. abortus* (Carmichael, L.E.; Greene, C.E.; Hollett, R.B., 2006). According to Kevin L. Cosford (2018), the remaining two species of *Brucella*'s classics, *B. neotomae*, associated with rodents and desert rats, and *B. ovis*, found in sheep, are not associated with diseases in dogs.

In addition, other species, such as terrestrial forms (*B. microti*, *B. inopinata*) and marine forms (*B. maris*, *B. pinnipediae*, *B. ceti*), present uncertain pathogenicity for dogs.

Suitable humidity and temperature conditions make *Brucella* spp. viable in soil, milk, water, and urine for more than 10 weeks. However, these bacteria are sensitive and can be inactivated by common disinfectants, light, and heat. They survive freezing and thawing, and the main means of transmission is contact with abortion products and vaginal secretions (Hollett, R.B., 2006). The bacteria can also remain viable for up to two months in buried contaminated cadavers or tissues (Greene; Carmichael, 2015).

Bacteria of the genus *Brucella*, including *B. canis*, have a predilection for female and male reproductive tracts in sexually mature animals. This tropism is attributed to the microorganism's affinity for steroid-producing tissues. However, it can also occur in other organs, such as the eyes, spinal cord, liver, spleen and lymphnodes (Makloski, 2011).

Epidemiology

The presence of *B. canis* in several countries of the world has been demonstrated from the isolation of the etiological agent or even by suspicion based on the serological response. In Asia and the southern U.S., canine brucellosis has been reported as endemic, as well as in Central and South America (ME, Hensel; M, Negron; AM, Arenas-Gamboa, 2018), including Brazil, which has a high population of dogs (Keid, L. B. et al., 2017). In Europe, *Brucella canis* is becoming the leading cause of canine brucellosis; however, the reports largely reflect the occurrence of signs and symptoms in dogs and humans.

The zoonotic character of the infection was identified in 1969, when the first records occurred due to accidents in laboratories (Morisset and Spink, 1969). Currently, the absence of surveillance programs and the scarcity of data prevent the exact understanding of which countries can be considered endemic for the disease (Djokic, Vitomir, et al., 2023).

There is no predilection for race, age or sex, and females and males are equally affected by the disease (Germano et al., 1987;



Moraes et al., 2002; Azevedo et al., 2003). The season of the year and the climate do not influence the presentation of the disease (Corrêa; Corrêa, 1992). The high incidence of well-documented outbreaks in beagle dogs may be related to the widespread use of the breed for research purposes (Spink, W.W.; Morisset, R., 1970).

Infection caused by *B. canis* is more common among stray dogs, shelter dogs, or commercial breeding kennels (Carmichael, L.E.; Joubert, J.C., 1988), presenting higher frequencies among stray dogs than among those with responsible tutoring (Lovejoy, G.S., et al., 1976), probably due to the absence of control in mating (Santos, Renato L., et al., 2021). The disease, when introduced into a kennel, spreads quickly (Carmichael, L.E.; JOUBERT, J.C., 1988; Ferreira, Vicente A., et al., 2020).

Azevedo et al. (2003) concluded that, after analyzing whether age could be a risk factor associated with seropositivity for *B. canis*, unpubescent and sexually mature animals are equally exposed to the risk of infection. However, unpubescent animals, if they acquire the infection, become abacteremic, usually developing unilateral or bilateral lymphadenopathy. After puberty, these animals may manifest clinical reproductive signs.

Route Of Elimination

In females, excretion through milk may occur, although in small concentrations and with little importance in the infection of the offspring, since there is usually intrauterine contamination (Carmichael; Greene, 1998).

In males, elimination by semen is the result of the presence of bacteria in the prostate and epididymis. In the first six to eight weeks after infection, the amount of these microorganisms is considered high. However, elimination was found in low concentration for up to 60 weeks after infection, which can extend up to two years (Carmichael; Greene, 1993; Johnson; Walker, 1992). In acute infections, urine may contain about 10^3 to 10^6 *Brucella canis*/ml (Suzuki, Erika Yuri et al., 2008).

The comparison of the urine of females and males suggests that the amount isolated in females is lower than in males. An explanation for this fact would be the anatomy of the male, due to the close relationship between the prostate, the epididymis and the urinary vesicle. However, although the risk of transmission through urine is higher in males, both sexes are potentially infectious when there is prolonged and close contact (Moore, 1969; Serikawa et al., 1978; Carmichael; Joubert, 1988; Johnson; Walker, 1992; Carmichael; Greene, 1998). Although, in comparison with bacterial loads, the urine load is up to 10^6

bacteria/ml (Marloes A.M. Van Dijk, Marc Y. et al., 2021), while that of genital discharges is up to 10^{10} bacteria/ml (Carmichael L.E.; Joubert J.C., 1988), the time of exposure to urine can play an important role in transmission (Djokic, Vitomir, et al., 2023).

Source of Infection

The sources of infection range from natural to artificial means, with greater or lesser importance. However, Wanke (2004) suggests that dogs are capable of transmitting the disease to other dogs and to humans.

Natural transmissions usually occur due to the inhalation or ingestion of microorganisms present in aborted fetal tissues, vaginal discharges from labor or abortion, as well as in urine. Venereal transmission can occur to females and males, either by eliminating the agent in the vaginal discharges of females infected during estrus, because it contains a high concentration of the pathogen, or by semen (Moore; Gupta, 1970; Carmichael; Greene, 1993; Johnson; Walker, 1992; Miranda et al., 2005). Puppies can be infected by intrauterine vertical transmission or by breastfeeding infected females (Santos, Renato L., et al., 2021).

Artificial transmissions involve vaginoscopy, blood transfusion, artificial insemination, and contaminated syringes. However, because they contain a higher concentration of microorganisms, vaginal secretions and semen are the most likely vehicles of infection due to mucosal contamination (Hollett, R.B., 2006; Greene; Carmichael, 2015).

Reservoir

According to Silveira et al. (2015), sexually intact animals should be neutered before starting canine brucellosis chemotherapy to decrease the risk of transmission and remove potential reservoirs of infection.

In a study carried out by Nicolás Galarce et al. (2021), in Chile, the presence of *Brucella canis* in wild canids was found, which can have their fertility and reproduction affected, consequently threatening their conservation. Forty-six blood samples of *Lycalopex culpaeus* (fox) and *L. griseus* (gray fox) were obtained, detecting 10.9% of seropositivity for *B. canis*. These could then act as a reservoir, highlighting the need to establish surveillance programs for these emerging pathogens. However, the domestic dog is the main reservoir, also affecting wild canids and, rarely, cats (Carmichael; Joubert, 1988; Wanke, 2004).



Entrance Door

In dogs, the main entry points for the bacterium are especially the conjunctival, oronasal and genital mucous membranes (Currier et al., 1982; Carmichael; Joubert, 1988; Carmichael; Greene, 1998), in addition to the digestive tract or skin continuity solutions, with the oropharyngeal mucosa being the most important (Nelson & Couto, 2010). Experimental infections have already been induced by the subcutaneous, intravenous, intraperitoneal and intravaginal routes (Serikawa; Muraguchi, 1979; Meyer, 1983).

The minimum infective dose for oral infection is about 10^6 microorganisms, this being the most frequent form. On the other hand, the minimum infective dose is 10^4 to 10^5 microorganisms, while the venereal route contains the infective dose (Johnson; Walker, 1992; Carmichael; Joubert, 1988; Carmichael; Greene, 1998).

Humoral and Cellular Immune Response

The acute inflammatory response is developed, with IgM antibodies being the first to be produced, followed by IgG antibodies, which persist. Slowly, acute inflammation is replaced by pyogranulomatous inflammation. The bacterium escapes degradation mechanisms when phagocytosed by neutrophils and macrophages, being able to grow and replicate within macrophages and dendritic cells, with no phagolysosomal fusion, since it acidifies the phagosome. Bacterial growth and multiplication, accompanied by the death of infected macrophages, consequently generate pyogranulomatous inflammation in genital tissues and other organs (Zachary, 2013).

Acquired resistance to *Brucella* infection involves both humoral and cellular immunity. The antibodies offer only partial protection and are directed primarily against lipopolysaccharide (LPS) (Carmichael; Shin, 1996).

However, *B. canis* infection induces mainly cell-mediated immune responses, since they depend on the activation of macrophages. These responses vary according to factors such as the pathogenicity of the infecting strain, age, host immunity, nutritional status, and previous antibiotic treatments. Circulating antibodies also play a role in immunity; however, there is little correlation between antibody titers and the degree of resistance. The concentration of IgM increases after infection, being detected in the first weeks after infection and decreasing after 3 months. IgG begins to increase in the second week of infection and persists for at least a year in untreated patients. If there is treatment, it decreases to 6 months. If the increase is persistent, it

is attributed to the presence of viable intracellular microorganisms in the reticuloendothelial tissue or to foci of infection (Cottrino et al., 2003).

The increased activity performed by macrophages to eliminate the bacterium is due to lymphokine, a type of interleukin, released by specific T lymphocytes, which are activated by the recognition of the bacterial antigen and by the components of the major histocompatibility complex on the surface of the macrophage (González et al., 2004).

Chacon-Diaz et al. (2015) conducted a murine study confirming the pathogenic strategy of *B. canis* as an intracellular bacterium, with an intracellular trafficking route indistinguishable from that of *B. abortus*. In that study, a less robust response was documented in mice infected with *B. canis* compared to *B. abortus* in terms of pro-inflammatory cytokines (TNF-alpha, IL-6, IL-12), IFN-gamma levels, splenic inflammation, and liver granulomas. This demonstrates that *B. canis* may be less pathogenic than other *Brucella* species in this murine model, corroborating the clinical observations.

Pathogeny

The main routes of entry of the pathogen are the oronasal, conjunctival, or genital mucous membranes. After penetration into the host, the bacterium is phagocytosed by macrophages and other phagocytic cells. These microorganisms have the ability to survive inside macrophages and escape fusion with phagolysosomes, being carried to the lymphatic (lymph nodes and spleen) and reproductive (steroid-dependent) organs, where they multiply. In males, the prostate, testicle and epididymis; and in females, the fetuses, the pregnant uterus and the placenta. The bacteria is also found in the stomach contents of the fetus, which suggests contamination in utero. The aborted placenta presents foci of coagulative necrosis of the chorionic villi, necrotizing arteritis and numerous bacteria in the trophoblastic epithelial cells (Wanke, 2004; Hollett, R.B., 2006).

Inflammation of the testicles and epididymis causes sperm extravasation, inducing cellular and humoral immune response. Such responses contribute to epididymitis, infertility and even the interruption of spermatogenesis (Hollett, R.B., 2006). Especially in the final third of pregnancy, infection of placental trophoblasts promotes the loss of placental integrity, leading to abortifacient conditions (Roop II, Martin R., et al., 2009). In males, replication of the organism and delayed hypersensitivity reactions contribute to epididymitis and infertility.



Inside leukocytes, bacteremia occurs, which spreads by hematogenous route to other systems of the body (Johnson; Walker, 1992; Carmichael; Greene, 1998), between one and four weeks after infection, and can last for a period of six months to five years (Keid, 2015). Examples of other tissues affected by bacteremia are the intervertebral disc, eyes, kidneys, and meninges, triggering, respectively, discospondylitis, anterior uveitis, glomerulopathy, and meningoenzephalitis, resulting from the deposition of immune complexes (Greene; Carmichael, 2015).

After bacteremia, persistent and nonprotective antibody titers against *B. canis* begin to be detected. However, they seem to have little influence on bacteremia and the number of microorganisms found in tissues (Carmichael; Greene, 1998; Wanke, 2004).

According to Chacón-Díaz et al. (2015), among the zoonotic species of *Brucella*, *B. canis* is the one that induces the lowest level of pro-inflammatory response, with low concentrations of tumor necrosis factor alpha and interleukins (IL) 6 and 12 being detected in infected animals. Despite this, hyperglobulinemia may occur; however, the antibodies produced are not protective, since the bacterium has the characteristic of being located inside the cell, and therefore cellular immunity is a more effective protective mechanism (Roop II et al., 2009). If there is balance in the relationships of metabolic and enzymatic interactions between the phagocyte and the phagocytosed microorganism, there may be a state of intracellular parasitism, which would explain the chronicity of the disease (Corrêa; Corrêa, 1992).

Spontaneous recovery can occur from one to five years after the initial infection. Consequently, dogs become abacteremic and have low titers of agglutinating antibodies (1:25 or 1:50), which suggests the elimination of the bacteria. In these cases, reinfections do not occur, as a result of the efficiency of cellular immunity. However, in cases of dogs with chronic infections that were successful using antibacterial drugs, there is susceptibility to oronasal reinfection 12 weeks after treatment interruption.

In some cases where *B. canis* persists in body tissues, there may be a negative culture associated with decreased serum agglutination titer. In males, the prostate can be a site of persistence of microorganisms (Hollett, R.B., 2006; Greene; Carmichael, 2015). In untreated dogs, the bacteremic phase can persist for up to five years (Forbes; Pantekoek, 1988; Ledbetter et al., 2009).

Clinical Signs

Canine brucellosis is manifested by prolonged bacteremia, without the occurrence of fever, starting between one and four weeks after infection and persisting for at least six months, and may intermittently last 64 months or more (Currier et al., 1982; Wanke, 2004). At this stage, the agent can be found in various organs, such as the spleen, lymph nodes, liver, bone marrow and reproductive system. Rarely, adult dogs manifest severe systemic clinical signs, even though it is a systemic disease, with the main problems being related to reproductive performance (Johnson; Walker, 1992).

Described by Nelson and Couto (2010), the clinical signs cause great impacts on reproduction and, therefore, canine brucellosis is considered a disease of the reproductive sphere. However, it can also cause claudication or nonspecific discospondylitis (Djokic, Vitomir, et al., 2023); arthritis, uveitis, meningitis, and encephalitis may occur less commonly (Megid, 2002). Other signs cited include peripheral lymphadenopathy, accompanied or not by infertility in both sexes, and rarely accompanied by fever (Forbes; Pantekoek, 1988; Corrêa; Corrêa, 1992; Keid et al., 2004). Congestion, subcutaneous edema and subcutaneous hemorrhage in the abdominal region can also occur (Rodrigues et al., 2016).

There are descriptions in the literature of ocular manifestations, such as panuveitis, endophthalmitis, chorioretinitis, panophthalmitis, retinal detachment, vitreitis, and keratoconjunctivitis. However, reports on ophthalmopathies related to natural infection by *Brucella canis* are still considered scarce (Santos, L. G. dos et al., 2021).

The signs are usually specific according to sex, although some may be asymptomatic and others may be common to both. Most dogs infected with *B. canis* do not develop clinical signs other than increased lymph nodes, and the clinical manifestation may vary with less frequent signs, especially in neutered dogs (Santos, Renato L. et al., 2021).

The main clinical sign of female dogs is abortion in the final third of gestation (Megid, 2002). However, abortions can also occur in another phase of pregnancy, and are usually characterized by fetal autolysis and dark and/or greenish serosanguinous vaginal secretion, lasting from one to six weeks. The estrous cycles of females show few noticeable changes or remain normal. Surviving pups may present generalized lymphadenopathy and maintain the infection until sexual maturity (Forbes; Pantekoek, 1988; Corrêa; Corrêa, 1992).

Sexually mature dogs may present orchitis and epididymitis and, consequently, testicular enlargement, with accumulation of



serosanguineous fluid, in addition to unilateral or bilateral testicular atrophy, reduction in the number of spermatozoa and presence of inflammatory cells in the ejaculate (Forbes; Pantekoek, 1988; Corrêa; Corrêa, 1992; Quinn et al., 2005). From the 4th week post-infection, a reduction in sperm motility, sperm concentration and volume, as well as sperm defects and a decrease in seminal quality, become evident. Dermatitis in the scrotum due to licking may occur, favoring the emergence of secondary infections, especially by *Staphylococcus aureus* (Forbes; Pantekoek, 1988; Carmichael; Greene, 1998).

Around the eighth week post-infection, abnormalities become more evident, such as a tightly curled tail, deformed acrosomes, folded middle piece, decapitations and reduced motility (there may be only 10% of motile sperm). From the twelfth week onwards, sperm agglutinations can be noticed, due to the autoimmune response with the production of antibodies against spermatozoa (GEORGE et al., 1979).

In humans, most cases involve mild symptoms of the disease, such as headache, weakness, and fever. Occasionally, the infection can be severe, including miscarriages, osteomyelitis, and infectious aneurysms associated with brucelic endocarditis (Krueger et al., 2014; Dentinger et al., 2015). In addition, depression, jaundice, joint pain may occur (Nelson; Couto, 2010), chills, sweats, weight loss, hepatomegaly, splenomegaly, lymphadenopathy, fatigue, malaise and oral lesions. Fever is often periodic and nocturnal (Carmichael, L.E.; Greene C.E., 2006; Hollett R.B., 2006; Nasphv, 2017). Neurobrucellosis (due to meningoencephalitis) is uncommon in dogs, unlike humans (Greene; Carmichael, 2015).

Nonspecific signs are not often observed; however, depression, lethargy, loss of vigor, weight loss, anorexia and reduced exercise tolerance are also described as dry and dull fur (Keid, 2015).

Anatomopathological Findings

In females, the infection is usually associated with placentitis, metritis, and abortion, with focal necrosis of the chorionic villi and numerous bacteria in the trophoblastic cells (Carmichael L.E.; Kenney R.M., 1968a). According to Ramírez (2006), glandular hypertrophy of the uterus also occurs with infiltration of the lamina propria by lymphocytes and formation of granulomas, with neutrophil infiltration. Remnants of the placenta may remain in the uterus, presenting focal coagulative necrosis of the chorionic villi. The dog will excrete a grayish-green, brownish, or reddish uterine discharge (metrorrhea) for a period of one to six weeks post-abortion (Holst et al., 2012).

The lesions induced in the canine gravid uterus and fetuses are similar to the lesions induced by *Brucella* spp. in other species (Carvalho Neta, A.V., et al., 2010; Poester, F.P.; Samartino, L.E.; Santos, R.L., 2013).

Aborted fetuses may present myocarditis, renal hemorrhage, hepatitis, lymphadenitis, and bronchopneumonia, in addition to the birth of weak offspring, which have a high neonatal mortality rate (Carmichael L.E.; Kenney R.M., 1968b; Souza T.D. et al., 2018). They commonly have an autolyse appearance, with stillbirths exhibiting findings of generalized bacterial infection, such as subcutaneous edema, hemoperitoneum, and hepatosplenic, renal, and intestinal degenerative lesions (Holst et al., 2012). The bacterium has been detected in a wide range of tissues of naturally infected neonates, such as lymph nodes, stomach, kidneys, navel, liver, lungs, spleen, and central nervous system (Souza T.D. et al., 2018).

In males, epididymitis seems to be the most common primary lesion than orchitis (Carmichael L.E.; Kenney R.M., 1968c). The condition is also often associated with inflammatory changes in the renal pelvis and prostate (Moore, J.A.; Kakuk T.J., 1969). Epididymitis, orchitis, hydrocele, scrotal dermatitis due to constant licking are also observed, in addition to focal secondary bacterial infection and uni or bilateral testicular atrophy in chronic cases. There are also reports of prostatitis in the literature (Greene; Carmichael, 2015; Keid, 2015).

Diagnosis

The diagnosis of *Brucella canis* remains a challenge due to the frequent false-negative results in direct and indirect diagnostic methods used to detect infection in adult dogs and humans (Carmichael L.E., S.J., 1996; Lucero N.E. et al., 2005; Taques I.I.G.G. et al., 2016). However, the efficiency of the association of direct and indirect methods, such as blood culture associated with PCR, especially genital PCR, has been proven as important tools for the diagnosis of canine brucellosis (Keid et al., 2009).

The determinant diagnosis of brucellosis is possible only with bacteriological isolation of the causal agent (Pessegueiro et al., 2003; Keid et al., 2007; Mantur et al., 2007). The differential diagnosis includes *Streptococcus* β -hemolytics, *Ureaplasma*, *Escherichia coli*, *Streptomyces*, *Salmonella*, *Mycoplasma*, *Campylobacter*, *Canine Herpesvirus*, *Neospora caninum* and *Toxoplasma gondii* (Corrêa; Corrêa, 1992; Keid et al., 2007).

It is important to emphasize that the negative result of the culture cannot confirm the absence of infection by *B. canis*, due to several factors, such as the intermittent elimination of the



bacteria, poorly collected or poorly preserved material, in addition to the use of antibiotics, which can reduce the sensitivity of the test (Flores-Castro; Carmichael, 1981; Minharro et al., 2005).

Clinical-Epidemiological

The history of recent miscarriage in females and the appearance of males with orchitis, epididymitis and/or other clinical signs of brucellosis increase clinical suspicion (Megid et al., 2002), as well as reproductive deficiencies, testicular atrophy and low seminal quality, which should lead to the investigation of the disease (Flores-Castro; Carmichael, 1981).

However, since the symptoms of brucellosis are often nonspecific, it is important for clinical suspicion to obtain a detailed history based on epidemiological information (Pessegueiro et al., 2003a; Mantur et al., 2007).

Ultrasonography can be applied to obtain more detailed information about affected tissues (Egloff, S. et al., 2018), and on radiographic examination of the spine, changes characteristic of discospondylitis can be observed (Tipold; Stein, 2010).

However, in order to reach a definitive diagnosis, anamnesis and clinical data should always be used in conjunction with serology and bacteriology (Wanke, 2004). The blood count is not very specific (Megid et al., 2000), and may indicate anemia, normal or low leukocyte count, with relative lymphocytosis and thrombocytopenia. C-reactive protein (CRP) is commonly elevated, and sedimentation rate (SV) is variable, having little diagnostic importance. There may also be an elevation of liver enzymes, which is also nonspecific (Pessegueiro et al., 2003b).

Laboratorial

Routine laboratory tests: Although it is a systemic disease, animals infected with brucellosis generally do not present hematological, biochemical or urinary alterations (Johnson; Walker, 1992). Studies carried out in kennels with cases of *B. canis* infection have shown that infected dogs that exhibited clinical signs did not show any changes in urinalysis (Megid et al., 2000), nor significant hematological changes (Chacón-Díaz et al., 2015). In dogs with chronic infection, hyperglobulinemia associated with hypoalbuminemia and a positive Coombs test may be found. In the cytological analysis of aspirates from hypertrophied lymph nodes, lymphoid hyperplasia with a large number of plasma cells can be observed (Greene; Carmichael, 2015). The cytological findings of orchitis and epididymitis by *B. canis* are similar to those present in inflammation of other

tissues. Macrophages and multinucleated giant cells may be present; however, the observation of the microorganism may be rare (Zinkl, 2009).

Bacteriological: Cultivation, isolation and identification are carried out by obtaining the samples collected. The material must be stored in an appropriate place and sent to the laboratory as soon as possible. Depending on the time for the biological material to arrive at the laboratory, it is important that the sample is frozen at -20°C, in order to avoid the loss of *B. canis* viability (Carmichael, 1998). As *B. canis* is considered a level 3 biosafety pathogen, isolation must be carried out in laboratories with adequate facilities for this purpose (Teixeira; Valle, 1996a). The isolation and identification of *Brucella canis* are considered methods of high diagnostic specificity (Teixeira; Valle, 1996b). However, they have low sensitivity due to intermittent elimination of the agent, inadequate collection and packaging of biological material, or the use of antibiotics (Keid, 2006). Isolation of the agent is the only form of definitive diagnosis (Wanke, 2004), although there are several diagnostic methods. Culture can be performed from samples of blood, vaginal discharges, semen, urine, milk, lymph nodes, spleen, liver, bone marrow, uterus, prostate, epididymis and testicles (Keid, 2015), in addition to the placenta, aborted fetuses and neonates (Vargas et al., 1996). However, blood culture may reveal false-negative results in chronically infected dogs, when bacteremia is typically absent or intermittent (Greene; Carmichael, 2015).

Serology: Serological tests present difficulties related to the availability of antigens (MINHARRO et al., 2005), in addition to being subject to misinterpretations caused by the possibility of cross-reactions with infections by other organisms, such as *Bordetella bronchiseptica*, *Pseudomonas* and *Actinobacillus equuli* (ETTINGER; FELDMAN, 1997). Routinely used serological tests detect *B. abortus* antigens, which cross-react with *B. melitensis* and *B. suis*, but not with *B. canis*. The identification of *B. canis* requires specific tests, which are rarely available (PESSEGUEIRO et al., 2003; MINHARRO et al., 2005). Since *B. canis* is a *Brucella* that does not have the lipopolysaccharide (LPS) O chain of the complete cell wall and has a rough morphology, antigens prepared with smooth samples of *Brucella*, such as *B. abortus* used in the diagnosis of bovine brucellosis, are not capable of detecting anti-*B. canis* antibodies, requiring the use of antigens prepared with samples of *B. canis* or *B. ovis* (Minharro et al., 2005). The four main serological tests used for the diagnosis of canine brucellosis are: TAT, TARP, TARP-ME and AGID (Carmichael et al., 1984). TAT and TARP, with or without the addition of 2-mercaptoethanol, are recommended for screening procedures, while AGID is considered the confirmatory test for the diagnosis of the disease



(Minharro et al., 2005). It is important to establish that serological tests should be used in the diagnostic routine with the aim of screening suspected animals. None of the serological tests used in the diagnosis of canine brucellosis is definitive, mainly due to the presence of nonspecific antibodies, thus indicating the performance of at least two serological tests (Carmichael; SHIN, 1996).

Immunochromatography: The diagnostic technique of immunochromatography showed high sensitivity and specificity in comparison with blood culture and TARP-ME, and is therefore a useful, fast and effective tool to assist in the diagnosis of the disease (Kim et al., 2007).

Slow Tube Serum-Agglutination (SALT): the Slow Tube Serum Agglutination (SALT) is considered a less sensitive and slightly more specific test than the SAR (Carmichael; Greene, 1998a). Also known as the tube agglutination test, it is used to reduce the incidence of false positive SARs, and is therefore commonly used to confirm infection in dogs that have shown a reactive result in the simple SAR or one added to 2-ME. It will show positive results around two to four weeks after infection. Although it is not a very specific test, it has the advantage of being semi-quantitative. A titer equal to or greater than 2:200 demonstrates active infection, while individuals with titers below 1:200 should be retested in approximately two weeks. The addition of 2-ME to this test increases specificity by reducing cross-reactions with other microorganisms (Wanke, 2004; HOLLET, 2006; Makloski, 2011). Titres of up to 1:50 are considered negative (Carmichael; Greene, 1998b).

Agar Gel Immunodiffusion (AGID): The agar gel immunodiffusion test (AGID) is used to confirm positive results in SAR and SAL alone or associated with 2-ME. Two types of AGID are standardized: a more specific one that uses cytoplasmic protein antigens and one that uses bacterial cell wall antigens (Makloski, 2011). It reveals positivity in infected animals from 12 weeks after infection until 36 months after the end of bacteremia, a factor that makes AGID the most appropriate test for the diagnosis of animals with chronic infection (Greene; Carmichael, 2015). AGID using cytoplasmic antigens is the most specific method for identifying *Brucella rugosa* using cytoplasmic antigens, in addition to having the advantage of being able to detect circulating antibodies up to 3 years after the bacteremia has ceased (Oliveira, 2011).

Rapid Serum Agglutination on a Slide: rapid serum agglutination on a slide has been the most widely used serological test for screening animals possibly infected with *B. canis*, and has the advantage of low cost, ease of performance and rapid results

(Costa, Mizael M. et al.; 2017). The antigen used in SAR comes from *B. ovis* stained with rose bengal, and allows the use of hemolyzed blood, a limiting factor in the use of tube serum agglutination. This technique is able to reveal antibodies from 3 to 4 weeks after infection. However, the interpretation of the RAS must be cautious, because although it has good sensitivity, the specificity is low and there may therefore be false positives and, when negative, it is strong evidence of not being infected (Cavalcante, 2006). Both the SAR with and without 2-mercaptoethanol (2-ME) use stained *B. ovis* (Makloski, 2011). The false positives presented, between 50 and 60%, are due to cross-reactions with antibodies directed at other microorganisms, such as *Bordetella*, *Pseudomonas* and *Moraxella*. Therefore, animals positive for this test should have their samples analyzed by other specific laboratory tests for a confirmatory diagnosis. The association with 2-ME reduces heterologous agglutination and, as a consequence, reduces false-positive reactions by increasing the specificity of the test. In animals with more advanced stages of the disease or in cases of chronic infections, titres decrease and remain at low levels (Hollet, 2006; Makloski, 2011; Keid, 2015).

Complement Fixation Reaction (CFT): the complement fixation reaction (CFT) has high specificity and sensitivity, and is used to confirm the diagnosis of *B. ovis* and *B. abortus* infections, however it is rarely used in the routine diagnosis of *B. canis* infection in dogs (Azevedo et al., 2004).

Enzyme-Linked Immunosorbent Assay (ELISA): several ELISA approaches have been proposed for the serodiagnostic diagnosis of infection. However, they present results that vary according to the properties of the antigen used in the assay. Antigens from the wall of *B. canis* and the wall of *B. abortus*, which is common to several species of *Brucella* (Serikawa; Muraguchi; 1979; Johnson And Walker, 1992; Mateus-De-Antonio et al., 1993a; Baldi et al., 1994; Baldi et al., 1997; Letesson et al., 1997). The advantage of these tests is that they do not present cross-reactions with other bacteria that are not of this genus. Indirect ELISA is quite specific, however it is less sensitive than SAL, when it comes to screening infected dogs (Carmichael; Greene, 1998), more sensitive than agglutination serological tests (Wanke, et al., 2002), and more specific than AFI (indirect fluorescent antibody test), being able to detect positive dogs within 30 days after infection (Hollet, 2006; Makloski, 2011). The use of purified antigens has been indicated for the ELISA test, in confirmatory diagnoses to screening tests, replacing tests with 2ME or diffusion gel (Lucero, et al., 2002; Ebani, et al., 2003). Different techniques used for extraction of *Brucella canis* antigens can cause interference in the protein composition or alter the primary structure of the epitopes, a factor that would affect their function.



Some authors have demonstrated that cytosolic antigens can provide more sensitive and specific serotests compared to *B. canis* outer membrane antigenic preparations (Carmichael; Joubert, 1987). Indirect ELISA tests with antigens extracted by heated saline solution (HSS) from a non-mucoid sample of *B. canis* demonstrated better sensitivity (M-variant) (mateus-de antonio et al., 1993b). Others, however, argue that there are no important differences in antigen-independent ELISAs (Wanke et al., 2002). The heat-extracted antigen, according to De Oliveira MZ et al., (2011), presented better ELISA results than the ultrasound-extracted antigen. Another factor that favors heat testing is that it does not need more sophisticated equipment and/or highly trained technicians, which favors the application of the test for field studies or population surveys, being reliable and safe. She completes the study by highlighting the important advantages of ELISA over other serological tests commonly used for the diagnosis of canine brucellosis, such as providing readily measurable results and being easy to perform and standardize.

Indirect Fluorescent Antibody Test (IFA): the sensitivity of the indirect fluorescent antibody (IFA) test has not yet been well established, which means that some infected dogs may show negative results in this method (Hollet, 2006; Makloski, 2011).

Polymerase Chain Reaction (PCR): PCR is increasingly being used to diagnose brucellosis in various animal species and in humans. It is a highly sensitive and specific method - 100% sensitivity and specificity in blood samples. The test can be carried out on whole blood, semen or vaginal samples, with sensitivity of 86.6 and 67.3% (respectively) and 100% specificity for the latter two (Keid, 2015).

The technique can be carried out when animals showing clinical signs compatible with brucellosis obtain negative results in other laboratory tests (Greene; Carmichael, 2015).

Compared to blood culture and TARP-ME, using blood and milk samples from infected animals, PCR proved to be effective in helping to diagnose *B. canis* (Oliveira et al., 2011).

Molecular techniques are the most effective methods for detecting brucellosis, such as classical PCR and real-time PCR. The PCR method applies several pairs of primers to amplify different fragments of the genome. Examples of the genes used to identify *Brucella* spp. are: BCSP 31 (primers: B4/B5), 16S rRNA sequence (primers: F4/R2), omp2 gene (primers: JPF/JPR) (Badour And Alkhalifa, 2008).

Immunohistochemistry (IHC): the use of formalin-fixed, paraffin-embedded (FFPE) testicular tissues offers the opportunity to detect *B. canis* by PCR or immunohistochemistry (IHQ) in the absence of samples available for culture (Camargo-Castañeda AM et al. 2021a).

The results of the study carried out by Camargo-Castañeda et al. (2021b) on dogs clinically diagnosed with orchitis suggest that rtPCR and IHQ are promising techniques that can be used on FFPE tissues to detect *B. canis* when other detection techniques are not available. It was considered reasonable to use IHQ as an adjunct test for detecting *B. canis* infection in canine male reproductive tissues, with greater certainty of detection based on the results of rtPCR.

However, using this technique to detect *B. canis* is difficult due to the lack of commercially available antibodies and the production of anti-*Brucella* antibodies in house is restricted to biosafety level 3 facilities (Brennan et al., 2008).

Spermogram: according to Greene and Carmichael (2015), semen examination of infected dogs usually reveals abnormal sperm and a severe reduction in motility. Animals that have been infected for more than five weeks have a significant decrease in ejaculate volume. After eight weeks of infection, there is a marked number of sperm with morphological alterations, which include immature sperm, deformed acrosomes, curved tails, an increase in the midpiece and retained protoplasmic droplets, detached heads, agglutination between heads and also phagocytosis of the sperm head by inflammatory cells.

Treatment

Recovery of the infected animal can occur spontaneously, however treatment can speed up recovery and there may also be specific treatment for other specific organs (Carmichael; Greene, 2006). Treatment is generally based on antibiotic therapy, however, the results are uncertain and relapses are common (Wanke, 2004).

The period of antimicrobial therapy for the treatment of canine brucellosis is prolonged (longer than six weeks), and can still result in the infection not being definitively eradicated. The best antimicrobial therapy for canine brucellosis is currently unknown. Antibiotic therapy in canine brucellosis reduces symptoms and the recurrence of complications. Various antibiotics have been used, alone or in combination, and none has been 100% effective in eradicating the disease. It has also been shown that combined therapy should be used, since monotherapy (treatment with just one drug) is not only unsuccessful, but also has excessively high relapse rates (Pessegueiro et al., 2003; Wanke, 2004).

Wanke et al. (2000) observed a case in which an infected dog showed negative titers immediately after treatment, but at the same time bacteria were isolated in its semen (Wanke, 2004). This demonstrates negative antibody titers, but the presence of



the pathogen, making it necessary to consider the possibility of transmission to other animals and humans, as well as the financial cost.

The combination of two antimicrobials, one being the aminoglycoside streptomycin and the other a tetracycline, such as tetracycline hydrochloride, doxycycline, minocycline, when administered during the first 3 months of infection, provides more experienced and more effective results in the treatment of canine brucellosis *in vivo*. Some authors unanimously conclude that polytherapy reduces the number of relapses, especially if streptomycin is one of the antibiotics used (Schin; Carmichael, 1999; Pessegueiro et al., 2003; Quinn et al., 2005).

Prevention Control Measures

There is no vaccine available to prevent infection, identifying and segregating infected animals is considered the main measure to control brucellosis in a confined population (Makloski, 2011; Pickerill; Carmichael, 1972). If prophylactic and control measures are not implemented, breeding kennels can maintain the infection (Greene; Carmichael, 2015).

According to Borie, C. et al., (2022), genetic studies of *B. canis* for vaccine preparation are relatively new and smaller compared to more traditional *Brucella* species. The antibodies induced by the BLSOmp31 formulation, by Clausse et al., (2013), tested in mice, were intended to promote the death of *B. canis* by complement activation, opsonization and phagocytosis or by promoting NK cell-dependent or other killer cell toxicity during the extracellular life of this pathogen in serum or mucosal tissues, which would prove effective in preventing the risk of infection by *Brucella canis*. This would be the first report of a recombinant vaccine conferring protection against *B. canis* in mice.

In 2017, Clausse et al., analyzed the recombinant BLSOmp31 vaccine in 5 beagles, which induced Th2 and humoral mucosal response (IgA and IgG) in saliva, preputial secretion and tears, in addition to highlighting the bactericidal activity of serum and the opsonizing activity of antibodies, which may play a protective role in the initial phase of bacteremia, preventing *B. canis* from entering the white tissues. It is intended, in the future, to increase the number of dogs for further studies and vaccine strategies under field conditions.

Eckstein et al., (2020) used a mutant strain of *Brucella ovis* in an ABC transporter for a protective study against *B. canis* in a murine model and in canine macrophages. At the end of the

study, it was concluded that the safety of the vaccine, as it did not present clinical signs, in addition to the local reaction at the inoculation point that returned 4 weeks after the injection. Seroconversion against *Brucella rugosa* was detected in 80% of the dogs (4/5 used) in the fourth week post-immunization.

Even though there are currently varied vaccine proposals in terms of vaccine type, protection, dose, strain, and experimental model, there is still no commercial preparation to stop the expansion of canine brucellosis considering genetic variability in relation to virulence factors (BORIE, C. et al., 2022).

Measures to prevent brucellosis by *B. canis* are based on sanitary aspects, regular serological control of kennel animals, castration of infected dogs, isolation of females in calving, elimination of positive ones, systematic disinfection of the kennel and quarantine before the introduction of new animals (Flores-Castro; Carmichael, 1981; Berthelot; Bastuji, 1996)

The Georgia Department of Agriculture and the U.S. Department of Agriculture have proposed detailed prevention strategies in breeding facilities, although they are not standardized measures, such as: dogs must have a negative result in serial screening tests performed 8 weeks apart before admission to a kennel or breeding program. If positive, they should be isolated and decisions made about euthanasia, or treatment and monitoring, including castration. Regarding biosecurity, infection control principles will include the use of single-use PPE (gloves, goggles, masks, boots, gowns); proper handling of samples; thorough hand washing; routine disinfection (2.5 sodium hypochlorite, quaternary ammonium compounds or 70% ethanol with a minimum of 10 minutes of contact); prevention of biofilm (minimize organic material), drying and exposure to sunlight; employee and customer education; and notification of the laboratory personnel receiving samples regarding the suspected diagnosis (Carmichael, LE; Greene, CE, 2006; Hollett, RB, 2006; Nasphv, 2017; USDA, 2017; CFSPH, 2017; AGR, 2017).

CONCLUSÃO

Canine brucellosis is an emerging disease and needs attention from the authorities, although it does not have as high a pathogenicity as other species of *Brucella* due to its rapid power of dissemination, because there are no commercial vaccines available so far and, mainly, because of the role played by pets today, taking into account that it is an anthroozoonosis.



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