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SEROLOGICAL DIAGNOSIS OF INFECTIOUS DISEASES IN DOGS

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ABSTRACT
Infectious diseases are of great importance in veterinary medicine. The use and improvement of serological diagnostic techniques have proven effective in reducing infectious diseases by rapidly diagnosing infectious diseases and consequently favoring the adoption of prevention and control measures. Serological tests are essential in the diagnosis of infectious diseases, as they are effective in concluding diagnoses and prognoses, in addition to contributing to the epidemiological surveillance of diseases. This study aims to contribute relevant information for the serological diagnosis of brucellosis, parvovirus, and leptospirosis. Laboratory diagnosis of infectious diseases is important in dogs to isolate infected animals and prevent secondary infections of susceptible animals that have contact with sick dogs. Clinical diagnosis is indefinite, as several other viral pathogens can cause common symptoms in dogs, such as coronavirus, adenovirus, morbillivirus, rotavirus, reovirus, and norovirus. Therefore, serological diagnosis is a fundamental tool in veterinary medicine to diagnose and specifically treat the occurrence of common pathologies in dogs.

Keywords: Epidemiological Surveillance. Infectious Diseases. Serological Tests.

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INTRODUCTION

In clinical medicine, laboratory diagnosis of infectious diseases is generally a complementary diagnostic resource that confirms or denies an initial suspicion, sometimes requiring additional tests to clarify each condition and outline therapeutic, preventive, and control guidelines for a given disease.

Among the laboratory tests, there are serological tests that consist of the detection and quantification of antigens and antibodies. These tests have several advantages, including speed, simplicity, possibility of automation, storage of biological material, low operating cost, and provision of standardized commercial kits.

The use of serological tests has several applications: presumptive and differential diagnosis; differentiation of disease phases; diagnosis of allergies; diagnosis of autoimmune diseases; diagnosis of congenital immunodeficiencies; disease prognosis; evaluation of the effectiveness of the established therapy; evaluation of specific immunity (vaccination); antigen research in cells or tissues; epidemiological research; basic and applied research.

In this sense, the diseases brucellosis, parvovirus, and leptospirosis were selected to carry out a descriptive study of the serological diagnosis. The serodiagnosis of canine brucellosis can be performed using the following tests: rapid serum agglutination (RAS), slow serum agglutination (SAL), agar gel immunodiffusion (AGID), and ELISA (KEID, 2006). Among these serological tests, the most used in the diagnosis of canine brucellosis, by Brucella canis, is the agar gel immunodiffusion test (AGID).

To diagnose canine parvovirus, the virus or viral antigens are detected in feces, thus several methods can be used, such as electron microscopy (EM) or electron immunomicroscopy (IME), viral isolation in cell cultures. The serological techniques used are: hemagglutination reaction followed or not by hemagglutination inhibition, enzyme-linked immunosorbent assays (ELISA), immunofluorescence reactions (IF), and immunoperoxidase.

The test of choice for the serological diagnosis of canine leptospirosis in reference laboratories is the microscopic agglutination test (MAS) since serovars that are predominant in certain regions can be selected for the diagnosis of the disease.

Given the importance of serological diagnoses for these infectious diseases of relevant occurrence in the medical clinic of small animals, the objective of this work is to elucidate the serological diagnosis of these infectious diseases. Since these diseases are important from an epidemiological point of view, some of them have zoonotic potential, as

is the case of brucellosis and leptospirosis. Furthermore, if not diagnosed and treated correctly, they can lead to the death of the animal.

LITERATURE REVIEW

BRUCELLOSIS

Etiology

The genus Brucella is composed of facultative intracellular bacteria, with six recognized species, each of which affects a preferred host. Brucella species are identified according to the preferred host, morphological characteristics, metabolic properties, serotyping, and phenotyping (ALTON et al, 1988).

Brucellosis in dogs caused by Brucella canis is a contagious disease, transmitted sexually or orally, characterized mainly by abortions in the final third of gestation, usually after 45 days (MINHARRO et al, 2005).

B. canis is a Gram-negative bacterium in the form of a coccus rod, aerobic, slow-growing, non-motile, and non-spore-forming. It is biochemically similar to B. suis, being a urease producer, H2S negative, nitrate reducer, non-fermentative, and oxidase positive (ALTON et al, 1988).

B. canis infection is responsible for reproductive problems, such as abortion, conception failure, orchitis, epididymitis, and infertility. Non-reproductive clinical signs can be observed associated with tissues rich in endothelial cells and asymptomatic infections can also be observed (KEID, 2006). B. canis parasitizes a limited number of species, domestic dogs and wild canids being the most affected.

Epidemiology

Canine brucellosis has already been reported in America, Europe, Asia, and Africa (CARMICHAEL, 1990). In Brazil, Cortes et al (1988) evaluated blood serum from 3,386 stray dogs captured by the zoonosis control program of the Zoonosis Control Center of the Health and Hygiene Department of the city of São Paulo, during the period from 1981 to 1985, in 14 locations, distributed throughout the four regional divisions of the city. Of the samples analyzed, 254 (7.50%) were positive in the AGID test.

VARGAS et al (1996) reported a case of isolation of B. canis from samples of placenta, aborted fetuses, and neonates, from a kennel located in the city of Uruguaiana, Rio Grande do Sul. In addition, these authors observed through the AGID test, that 72.7% (8/11) of animals were reactive to the test.

MEGID et al (1999) observed canine brucellosis in four different kennels. The kennels presented animals with a history of abortion, neonatal mortality, and premature births. The percentage of animals seropositive for canine brucellosis, by the agar gel immunodiffusion test, ranged from 4.6 to 57.1%. In addition, the authors observed a positive association between the percentage of positive animals and reproductive aspects and crowding conditions.

MORAES et al (2002) evaluated the prevalence of anti-Brucella canis antibodies in dogs from the Serra de Botucatu microregion, São Paulo State, through the rapid serum agglutination test (SAR) and rapid serum agglutination test with 2-mercaptoethanol (SAR-2ME). Of the 1,072 dog sera examined in the study, positive reactions were found in 19 (1.77%) in the SAR test and nine (0.84) in the SAR-2ME test.

MORAES et al (2002b), intending to study the prevalence of B. canis in the West Zone of the city of Rio de Janeiro, used 119 dogs originating from the neighborhoods that make up the region of the city. Using the plate agglutination technique to identify seropositivity for B. abortus and the agarose gel immunodiffusion technique to identify agglutinins for B. canis, they observed that 9.2% (n=11) of the animals were reactive for B. canis and that there was no positive reaction for B. abortus in any of the animals tested. AZEVEDO et al (2003) investigated the prevalence of brucellosis caused by Brucella canis in dogs from the municipality of Santana de Parnaíba, SP, Brazil. For this purpose, 410 blood serum samples from dogs collected during the animal anti-rabies vaccination campaign were examined. Agar gel immunodiffusion (AGID) using lipopolysaccharide antigen and proteins from Brucella ovis, sample Reo 198, was used in normal sera as a screening test, and for confirmation, the same technique was applied to sera treated with 2-mercaptoethanol (AGID-ME). The complement fixation reaction (CFR) using B. ovis antigen, sample 63/290, was also used as a confirmatory test. The determination of prevalence was considered positive for the animals that reacted positively in both confirmatory tests (AGID-ME and CFR). The prevalence of B. canis was 2.2%.

ALMEIDA et al (2004) to evaluate the prevalence of canine brucellosis caused by B. canis and B. abortus in the city of Alfenas, Minas Gerais, analyzed blood serum samples from 635 dogs. The prevalence of B. canis was 14.2% (90/635) and that of B. abortus was 18.1% (115/635); only 2.8% (18/635) were confirmed in the screening test.

KEID et al (2004) analyzed samples from 171 dogs from 12 commercial kennels in the state of São Paulo. The laboratory tests used were agar gel immunodiffusion (AGID) and blood culture. Of the 171 dogs examined, 39 (22.80%) presented at least one clinical

sign compatible with brucellosis, 58 (33.91%) were positive by AGID, and 24 (14.03%) by blood culture.

AGUIAR et al. (2005) evaluated 304 dogs from rural and urban environments in the municipality of Monte Negro, Rondônia, using Buffered Acidified Antigen (AAT), Slow Serum Agglutination in Tubes (SAL) and 2-Mercaptoethanol (2-ME) to detect anti-Brucella abortus antibodies and Agar Gel Immunodiffusion (AGID) and Agar Gel Immunodiffusion with 2-Mercaptoethanol-treated serum (AGID-ME) for Brucella canis. Samples that reacted in the confirmatory 2-ME and AGID-ME tests were considered positive. There were 56 (18.4%) animals that reacted to AAT and 12 (4.0%) that reacted to SAL. Only one dog (0.3%) was considered positive, confirmed by the 2-ME test. Eleven (3.6%) reactions to AGID were observed, but there was no confirmation in the AGID-ME test. CAVALCANTI et al (2006) aimed to investigate anti-Brucella canis antibodies in dogs living in the metropolitan region of Salvador. Eighty-five blood serum samples from domesticated dogs were analyzed. For the serological diagnosis of Brucella canis infection, the agar gel immunodiffusion test with Brucella ovis membrane antigen was used. The results indicated a seropositivity of 5.88% (5/85), demonstrating the presence of anti-Brucella canis antibodies in dogs living in the metropolitan region of Salvador.

REIS et al (2008) conducted a serological study to investigate the frequency of canine brucellosis by Brucella canis and Brucella abortus in 500 stray dogs in the city of São João da Boa Vista/SP - Brazil, using the techniques of agar gel immunodiffusion (B. ovis cell wall antigen) and immune agglutination on a plate with buffered acidified antigen. They observed a low frequency of dogs infected with B. canis 4/500 (0.8%) and an absence of positive sera for B. abortus.

FERNANDES et al (2013) to determine the occurrence of anti-Brucella rugosa and anti-Bruc ella Lisa in dogs from the city of Natal, Rio Grande do Norte State, Brazil, as well as to identify risk factors associated with positivity and perform molecular detection in seropositive animals, used blood sera from 416 dogs treated at veterinary clinics. For the serological diagnosis of Brucella rugosa infection, the agar gel immunodiffusion test (AGID) was used, using lipopolysaccharide antigen and proteins from Brucella ovis, sample Reo 198, and for the diagnosis of Brucella Lisa infection, the buffered acidified antigen test (AAT) was used. Blood samples with sodium citrate were collected from seropositive animals for diagnosis by polymerase chain reaction (PCR). The frequency of anti-Brucella rugosa antibodies was 28.9% (120/416). All animals were negative for anti-Brucella Lisa antibodies. Among 80 seropositive animals, the DNA of Brucella spp. was amplified in three animals (3.8%).

In this sense, data on the occurrence of canine brucellosis caused by B. canis in Brazil are specific and mostly based on serological tests. An occurrence ranging from 1.32% to 72.7% is observed, according to the region, the population of dogs examined, and the diagnostic test used (KEID, 2006).

Serological Diagnosis

Due to the limitations of laboratory procedures for isolating microorganisms of the genus Brucella, serological methods have become the main diagnostic methodology (ALTON et al, 1988). The first antibodies to appear after infection are of the IgM class, indicating recent infection. Followed by IgG class antibodies, which remain for long periods, mainly in chronic infections. Since canine brucellosis is a chronic disease, the main immunoglobulin to be detected by diagnostic tests is IgG. Despite being the most widely used method for diagnosing canine brucellosis, serology presents many problems in our environment, mainly related to the availability of antigens and kits for serological diagnosis (MINHARRO et al, 2005).

Serodiagnosis of canine brucellosis can be performed using the following tests: rapid serum agglutination (RAS), slow serum agglutination (SAL), agar gel immunodiffusion (AGID) and ELISA (KEID, 2006). Among these serological tests, the most widely used in the diagnosis of brucellosis by Brucella canis in dogs is the agar gel immunodiffusion test (AGID). The sharing of antigens between Brucella canis and Brucella ovis allows the indiscriminate use of reagents produced from these two microorganisms for the diagnosis of brucellosis in sheep and dogs. AGID has been particularly widely used. With this test, antibodies can be detected from eight to 12 weeks after infection and persist for several years (MINHARRO, 2005).

SAR uses an antigen prepared with B. ovis stained with rose bengal. However, positive results should be interpreted with caution, since a significant proportion of false-positive results can occur with this test (GEORGE and CARMICHAEL, 1978). This test has good sensitivity, but its specificity is very low, i.e., a negative result is strong evidence that the animal is not infected, but only 50% of the animals whose sera show agglutination are positive. Therefore, animals that test positive in SAR cannot be considered infected before undergoing a confirmatory test (MINHARRO, 2005).

SAL, in turn, is the classic serological test for diagnosing canine brucellosis. It provides results in title (semiquantitative) and is often used to confirm SAR-2ME (CARMICHAEL, 1998). SAL is less sensitive and slightly more specific than SAR (KEID, 2006).

In this sense, it is observed that laboratory diagnosis is a fundamental tool for understanding the prevalence of brucellosis and for preventing and controlling infection in dog breeding. Rapid identification of infected animals is necessary to contain the spread of the infection (KEID, 2006).

PARVOVIRUS

Etiology

Canine parvovirus is an important viral disease in dogs. The etiological agent is the virus of the Parvovirus genus, of the Parvoviridae family. Canine parvovirus is a single-stranded, non-enveloped, hemagglutinating DNA virus (DEZENGRINI et al, 2007). There are currently two canine parvoviruses: CPV type 1, also called canine parvovirus diminutive (CnMV), with little clinical importance defined in gastroenteritis, causing mainly mild diarrhea, and CPV-2, which has three subtypes: CPV2a, CPV2b, and CPV2c.

CPV2b is the most prevalent in the canine population and, consequently, is used in vaccines (TRUYEN, 1995). CPV-2 is responsible for myocarditis and hemorrhagic gastroenteritis in puppies between six weeks and six months of age (DEZENGRINI et al, 2007). CPV-2 It was gradually replaced in the canine population by new antigenic variants, or biotypes designated CPV-2a and CPV-2b (PRATELLI et al, 2001), and a third biotype, CPV-2c, has already been identified (NAKAMURA et al, 2004).

Epidemiology

Canine parvovirus is an infectious disease that has been emerging worldwide since the 1970s. In Brazil, it appeared in the 1980s. Initially, it affected animals of all ages, causing myocarditis in newborns and enteritis in young dogs; currently, the disease occurs mainly in puppies (SANTOS et al, 1997). Since the first reports of the disease in Brazil (ANGELO et al, 1980; HAGIWARA et al, 1980), CPV has been present in the country's canine population and several studies have demonstrated its presence in various regions of the country (BARCELOS et al, 1988).

Since it emerged in 1978 as a new pathogen of dogs, CPV has continued to evolve. Through the use of specific monoclonal antibodies and restriction enzymes, CPV-2a became prevalent in the canine population. From 1984 onwards, a new variant emerged, CPV-2b; only 10-30% of CPV samples isolated in Europe and the United States are currently type 2a.

CPV variants appear to be more adapted to replication in dogs, facilitating the spread of the virus in the canine population. CPV-2 is transmitted mainly by the orofecal

route, with contaminated feces or fluids being the primary source of canine parvovirus infection (SANTOS et al, 1997). Studies have shown that there are antigenic and genetic similarities between the parvovirus virus and the feline panleukopenia virus (GREENWOOD, 1995).

In 2000, a new antigenic variant, CPV-2c, was detected in Italy and quickly spread to several countries. Compared to the original CPV-2 type, the antigenic variants exhibit increased pathogenicity in dogs and are capable of infecting and causing disease in cats. Epidemiological surveys indicate that the newer CPV-2c type is becoming prevalent in different geographic regions, and is considered a serious disease in puppies and adult dogs, as well as in dogs that have completed the vaccination protocol. However, the main cause of vaccination failure is maternally derived immunity deficiency (DECARO AND BUONAVOGLIA, 2012).

Mortality rates may be high in puppies but are generally less than 1% in adult dogs. Dogs may present hemorrhagic enteritis of the small intestine and enlarged mesenteric lymph nodes and Peyer's patches (DECARO AND BUONAVOGLIA, 2012).

Parvovirus is diagnosed by examining feces, where it is investigated using hemagglutination tests (HA), immunoenzymatic assay, polymerase chain reaction (PCR), and viral isolation in enzymatic culture (STROTTMANNI et al, 2007).

Isolation in cell culture is considered the standard test, but PCR and HA have been widely used mainly due to the high specificity and practicality of these tests (STROTTMANNI et al, 2007).

Parvovirus infection has a high morbidity and mortality rate due to the lack of immunity in dogs against parvovirus, especially in puppies between 6 weeks and 6 months of age (MORAES AND COSTA, 2007). Puppies are more likely to have the disease, but dogs of any age can have hemorrhagic gastroenteritis. Dogs of breeds such as Doberman, Labrador, Pitt Bull, Rottweiler, and German Shepherd are more susceptible to developing the disease (MORAES AND COSTA, 2007).

The prophylaxis of CPV infection depends mainly on vaccination. Since inactivated vaccines are capable of inducing short-term immunity, modified live virus vaccines are widely used. These vaccines, based on the CPV-2 virus or its variant CPV-2b, are highly effective and are capable of protecting dogs against parvovirus, and post-vaccination reactions are very rarely observed. A recent study showed that most dogs that contracted the disease after vaccination were infected by the virus alone or with the attenuated vaccine virus. (DECARO et al, 2007).

The main causes of failure of vaccination against parvovirus are related to the immunity transmitted by bitches to their offspring through colostrum and, to a lesser extent, through maternal milk (DECARO et al, 2007).

Adult dogs are resistant to parvovirus infection due to specific immunity induced by vaccination or previous infections (often subclinical). Although CPV infection is generally restricted to young animals, it is noted that adult dogs also contract this disease (DECARO et al, 2007b).

Serological Diagnosis

Laboratory diagnosis of CPV infection is important in dogs to isolate infected animals and prevent secondary infections of susceptible animals that have contact with sick dogs. The clinical diagnosis is unclear, as several other viral pathogens can cause diarrhea in dogs, such as coronavirus, adenovirus, morbillivirus, rotavirus, reovirus, and norovirus. Therefore, a suspected clinical case should always be confirmed by laboratory tests. Several methods have been developed for the laboratory diagnosis of CPV infection, using feces (or intestinal contents if the animal is dead) from affected dogs (DECARO et al, 2007b).

For the result of CPV-2 detection in fecal samples to not be false negative, the collection must be done early during the infection. The immune response to this virus generalizes rapidly, beginning four to five days after infection. Consequently, the virus can only be detected in feces for a short period (three to four days) after the onset of clinical signs (SANTOS et al, 1997). CPV-2 is very resistant to the environment, remaining stable for up to six months outside the cell if kept at 4°C (SANTOS et al, 1997). Viral particles can be detected in the feces of suspected patients using viral isolation, cell culture or ELISA, electron microscopy, and direct hemagglutination (DE MARI et al, 2003).

Indirect serological tests, such as hemagglutination, serum neutralization, indirect ELISA, and immunofluorescence, can detect past infections. In the first week, elevated serum IgM concentrations are observed, either due to the onset of infection or vaccination with the attenuated virus (DE MARI et al, 2003).

From the second week onwards, an increase in serum IgG concentrations is observed (DE MARI et al., 2003). Isolation in cell culture is considered the standard test, but the polymerase chain reaction (PCR) is widely used due to the high specificity and sensitivity of the test when compared to ELISA. The detection of genetic material by PCR is currently the method of choice, as it has helped to exclude many false positives and false negatives (DE MARI et al, 2003).

Immunoperoxidase (IPX) is a technique that can be applied to cell monolayers, called immunocytochemistry, and to smears or directly to tissues, called immunohistochemistry. These methods detect the multiplication of the virus in cell cultures or tissues, confirming the presence of the agent (DE MARI et al, 2003).

The hemagglutination (HA) test is used to identify and quantify CPV. For this test, plates of serial dilutions of the fetal suspension in an equal volume of saline solution are used. The hemagglutination inhibition (HI) test detects anti-CPV antibodies. To this end, serial dilutions (base 10) of serum (inactivated at 56ºC and treated with 25% kaolin and 50% swine red blood cells) were performed in an equal volume of BBS containing 2% FBS. Subsequently, eight HA units (UHA) of the viral sample were added and the plate was incubated in a humid chamber at 37ºC for two hours. Afterward, a suspension of swine red blood cells was added, followed by incubation at 4ºC for two hours (SENDA et al, 1986). The antibody titer was considered the reciprocal of the highest dilution that inhibited HA. The HI technique was also used to confirm the identity of CPV in fecal samples (STROTTMANN et al, 2008). To prevent false negative results for CPV-2 detection in fecal samples, collection must be performed early in the course of the infection. The immune response to this virus is rapidly generalized, beginning four to five days after infection. Consequently, the virus can only be detected in feces for a short period (three to four days) after the onset of clinical signs (SANTOS et al., 1997). CPV-2 is very resistant to the environment, remaining stable for up to six months outside the cell if kept at 4°C (SANTOS et al., 1997).

LEPTOSPIROSIS

Etiology

Leptospirosis is an infectious disease that affects domestic animals and humans, caused by bacteria, and spirochetes, which belong to the Leptospiraceae family, Leptospira genus. They are long, thin, and spiral-shaped, may have hooked ends, and are classified by more than 200 serovars, including Canicola, Icterohaemorrhagiae, Pomona, Grippotyphosa, Autumnalis, Bratislava, Hardjo, Pyrogenes, Copenhagen, Ballum, Tarassovi, among others (MELLO AND MANHOSO, 2007).

The disease has a strong socio-economic-cultural significance and is spread by factors such as the disorderly growth of large urban centers, migration, deficiencies in basic sanitation conditions, and the disorderly accumulation of garbage, which promotes the expansion of the rodent population. The persistence of the agent in nature and the high potential for infection is ensured by the diversity of serological identities, the

multiplicity of host species, and the relative degree of survival in the environment without parasitism (in conditions of high humidity, protection from sunlight, adequate temperatures and neutral or slightly alkaline pH), even though pathogenic leptospires do not move to multiply outside the host organism (CÔRTES, 1993).

Leptospirosis is an agent commonly involved in reproductive problems, abortions, and infertility (GREENE AND CARMICHAEL, 2006). Fever and jaundice may accompany or precede abortions, death of neonates, and death of newborns within a few weeks of life. Reproductive diseases have been described in kennels and are generally associated with the Bratislava serovar (GRAHAM AND TAYLOR, 2012).

The most commonly associated and known serovars of classic canine leptospirosis are Icterohaemorrahagiae and Canicola (SCANZIANI et al, 1994). Some of the serovars that have been found, including in Brazil, infecting dogs and causing morbid conditions or benign infections are: Pomona, Castellonis, Pyrogenes, and Copenhageni (DICKESON and LOVE, 1993; BRIHUEGA and HUTTER, 1994). The prevalence found in Brazilian canine populations has varied between 10 and 22% (ALVES et al, 2000).

Epidemiology

The occurrence of leptospirosis varies in different regions of the world and can be either sporadic or endemic, and the occurrence of Leptospira spp serovars varies according to the geographic region. In Rio de Janeiro, in 1940, 11 dogs with clinical manifestations compatible with leptospirosis were subjected to necropsy to confirm the presence of the causative agent of leptospirosis in dogs in Brazil (DACORSO FILHO, 1940). In Pelotas, Rio Grande do Sul, to know the prevalence and risk factors. 489 serological samples from dogs from 213 properties were examined. The samples were subjected to the microscopic agglutination test (MAS), with 13 (2.66%) positive samples being detected with antibody titers ranging from 50 to 800, for the serovars Icterohaemorragiae, Australis, Copenhageni, Pyrogenes, Sentot and Canicola (JOUGLARD AND BROD, 2000). LILENBAUM et al (2000), when evaluating the occurrence of serological evidence of leptospirosis among the canine population of an urban center located in the Amazon region, with identification of the prevalent serovars, examined blood samples from 185 canines from the municipality of Oriximiná, Pará, located within the Amazon region, using the microscopic agglutination test (MAS). Of the total samples analyzed, 34 (18.4%) were reactive, with a minimum titer of 100. The most frequently found serovars were Canicola and Icterohaemorrhagiae, in addition to Copenhagen, also belonging to the lcterohaemorrhagiae serogroup.

MASCOLLI et al (2002) evaluated the zoonotic potential of the canine population of the municipality of Santana de Parnaíba, São Paulo, about leptospirosis. For this purpose, 410 canine serum samples were collected and leptospirosis was determined using the microscopic agglutination technique, using a collection of 22 serological variants. A positivity rate of 15% was found, with the most frequent variants being Copenhagen (24%), Canicola (16%) and Hardjo (16%). QUERINO et al (2003) evaluated the frequency of seropositive dogs for leptospira, treated at the Veterinary Hospital of the State University of Londrina. A total of 160 dogs of both sexes and not vaccinated against leptospirosis were studied between March 1997 and April 1998. All animals were subjected to the microscopic agglutination test and direct urine examination. Antibody titers of 100 were detected in 40 dogs, with the highest frequency against the Pyrogenes serovar (45.00%), and 24 animals were positive in the direct urine examination. The authors emphasized that the results serve as a warning regarding the possibility of human exposure to some risk factors for leptospirosis to which these dogs are exposed.

In Botucatu – SP, leptospirosis was seroepidemiologically investigated in 775 dogs, in blood samples obtained during the annual anti-rabies vaccination campaign. For diagnosis, microscopic agglutination was performed, using 12 serovars of Leptospira spp. 119 (15.3%) positive samples were obtained, with the reaction for 11 serovars, with greater importance for canicola, in 48 (40.3%) samples, and pyrogens, in 41 (34.5%) (MODOLO et al, 2006). MAGALHÃES et al (2007) when processing 3417 serum samples, observed positive reactions to SAM in 448 (13.1%) samples, for one or more serovars of Leptospira spp., with titers ranging from 200 to 25,600. The highest frequencies of positive reactions were for the serovars Canicola (7.0%), Ballum (6.1%), Pyrogenes (3.2%), and Icterohaemorrhagiae (2.9%), the others presented frequencies below 1.0%.

Given this epidemiological distribution, it is noted that dogs are considered an important source of leptospirosis infection since several studies report the presence of reactive animals. And in this sense, close contact with humans, eliminating live leptospires in the urine for several months, without presenting characteristic clinical signs, represents a risk to human health, and therefore Prevention and control of the disease in dogs is important.

Serological Diagnosis

Laboratory tests are required to diagnose canine leptospirosis, with bacteriological tests considered definitive (SANTA ROSA, 1970; FAINE et al, 1999). Direct visualization of leptospires under a dark-field microscope has been used mainly in urine samples during

the leptospira phase. Among the serological tests, microscopic agglutination test (MAS) with live antigens is the most widely used worldwide (FAINE et al, 1999). Laboratory tests such as complete blood count, measurement of serum urea and creatinine levels, and urinalysis can be used as complementary tests, as they indicate functional changes in the different organs affected.

For the diagnosis of leptospirosis using serology, the following should be considered: choosing the confirmatory test; the collection of antigens used, since for a more accurate diagnosis the prevalence of certain serovars in certain regions must be taken into account; the antigen and antibody reaction, since there are cross-reactions between serovars and other diseases such as babesia and brucellosis. Among the variables related to location, areas considered endemic due to factors such as poor basic sanitation, the time of year in which the collections were made, depending on the greater or lesser rainfall and the animal species, since some are natural reservoirs for some serovars (FAVERO et al, 2002).

In clinical routine, confirmation of the clinical and epidemiological diagnosis is done by testing for specific antibodies in the serum, using the microscopic serum agglutination test (MAS), or macroscopic slide agglutination test (LEVETT, 2001). Among the serological methods, microscopic agglutination (COLE JR et al, 1973) is the most commonly used and is designated as a reference test by the OIE (OIE, 2008). In this test, blood serum reacts with live Leptospira antigens and, for its performance, a battery of antigens with the serovars representing each serogroup is used. Sera from individuals with positive titers generally present cross-reactions to a variety of serovars, making it difficult to identify the infecting serovar (WHO, 1967). Therefore, microscopic agglutination can be considered a serogroup-specific test (FAINE, 1999). The battery of antigens should include serovars representative of the region studied. For serological confirmation of leptospirosis in an individual, a four-fold increase in the titer of agglutinating antibodies between the acute phase and convalescence is recommended (GALTON et al, 1965). In endemic areas, a single sample with a titer equal to or greater than 800 may be considered diagnostic, but it is recommended to use titers equal to or greater than 1,600 for this decision (WHO, 2003). Other serological methods, such as ELISA, have been used mainly to distinguish between IgM and IgG antibodies (HARTMAN et al, 1984) and several modifications have been applied (LEVETT, 2001). Furthermore, methods such as macroscopic slide agglutination (BRANDÃO et al, 1998), radioimmunoassay (KAWAOKA et al, 1979), and indirect hemagglutination (SULZER and JONES, 1973) have been used, but are more appropriate for the diagnosis of human leptospirosis.

To perform the Microscopic Serum Agglutination (MAS) technique, considered the “gold standard” (OIE, 2008), the Leptospira spp. Antigens used in serological tests are replicated weekly in EMJH liquid culture medium (Ellighausen, McCullough, Johnson, and Harris), using 10% of the volume of the medium to be seeded as inoculum, and kept in a B.O.D bacteriological incubator at 29°C ± 1°C (OIE, 2008).

Blood serum samples are diluted in saline solution, with the initial dilution being 1/25. Aliquots of 25 µL are placed on flat-bottomed polystyrene plates, and an equal amount of antigen from the serovars prevalent in the region is added, resulting in a dilution of 1/50. The serum-antigen mixture is homogenized and incubated in a BOD incubator at 28°C for 40 to 120 minutes, and then read using dark-field microscopy, with a 10x objective and eyepiece, directly from the plate wells.

Samples in which 50% agglutination occurred will be considered reactive, with half of the leptospires agglutinated in the microscopic field at 100x magnification. Reactive samples in the initial dilution will be tested with serial dilutions of a ratio of two, with the first dilution being 1/100, as recommended by the OIE (2008).

CONCLUSION

Laboratory diagnosis of brucellosis, leptospirosis, and parvovirus in dogs is important to isolate and treat infected animals and prevent secondary infections in susceptible animals that have contact with sick dogs. Clinical diagnosis is often unclear, as several other viral pathogens can cause clinical signs. The symptoms of these diseases. Therefore, a suspected clinical case should always be confirmed by laboratory tests. Several methods have been developed for the laboratory diagnosis of these diseases.

Brucellosis infection, for example, has socioeconomic importance, as it is responsible for reproductive problems, such as abortion, conception failure, orchitis, epididymitis, and infertility. Diagnosis of this disease is of fundamental importance to control the economic losses generated in cattle herds by this pathology.

In the case of leptospirosis, the disease has a strong socioeconomic-cultural significance and is transmitted by factors related to poor sanitation conditions and the disorderly accumulation of garbage, which promotes the expansion of the rodent population. It is an important zoonosis and should therefore be properly diagnosed to achieve greater control of the disease in the population. Parvovirus is a disease with high morbidity and mortality in dogs, with clinical signs common to other diseases that affect dogs. Therefore, clinical diagnosis alone is insufficient to confirm this disease. Therefore,

laboratory diagnosis is important to confirm the disease and consequently treat affected dogs specifically, thus minimizing the spread of the pathogen within the canine population.

This study aims to demonstrate the need for serological diagnosis as a fundamental tool in veterinary medicine to diagnose and specifically treat the occurrence of common pathologies among dogs.

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