SEROLOGICAL DIAGNOSIS OF INFECTIOUS DISEASES IN DOGS

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Luanda Ferreira Cipriano[1], Nicole Amoêdo Luvison[2], Ketlin Milena Zardin[3], Camille Moreira Bergamo Barros[4], Gabriela Victoria Araújo Saraiva[5], Dreyd Rodrigues Medeiros[6], Barbara Fernandes Werneck Teixeira[7], Édios Meurer Lana da Silva[8], Carolina Aires Martins[9] and Renata Ferreira dos Santos[10].

[1] Doctorate student in Veterinary Medicine
Unesp -SP
luanda.cipriano@unesp.br/
https://orcid.org/0000-0002-8246-3257/
http://lattes.cnpq.br/6176296921118786
[2] Undergraduate student in Veterinary Medicine
University of Caxias do Sul - UCS
naluvison@ucs.br
https://orcid.org/0009-0005-5783-8810
http://lattes.cnpq.br/7395426062661654
[3] University of Caxias do Sul - UCS
ketlinkolling@gmail.com
https://orcid.org/0009-0004-1683-2271
http://lattes.cnpq.br/0685734131130293
[4] Undergraduate student in Veterinary Medicine
Pioneer Union of Social Integration - UPIS
bergamovetz@gmail.com
0009-0000-6688-3000
https://lattes.cnpq.br/9445983420853659
[5] Undergraduate student in Veterinary Medicine
gvictoriasaraiva@gmail.com
61 993068888
0009-0002-0616-0956
Catholic University of Brasilia - UCB
Lattes: https://lattes.cnpq.br/9933238062460353
[6] Undergraduate student in Veterinary Medicine
drmedeiro@me.com
0009-0002-5714-9642
https://lattes.cnpq.br/9504913789155844
[7] Undergraduate student in Veterinary Medicine
barbarafwt@gmail.com
Catholic University of Brasilia - UCB
https://orcid.org/0009-0008-7426-4769
https://lattes.cnpq.br/1011296974890263
[8] Undergraduate student in Veterinary Medicine
Pioneer Union of Social Integration - UPIS
edmeurerls@gmail.com
0009-0002-0311-5834
https://lattes.cnpq.br/6899580204036046
[9] Undergraduate student in Veterinary Medicine
carol.aires@gmail.com
0009-0008-4152-0594
Catholic University of Brasilia -UCB
Lattes: https://lattes.cnpq.br/8985736732153179
[10] Dr. in Preventive Veterinary Medicine
Unesp-SP
renatafdsantos@hotmail.com
https://orcid.org/0000-0002-1033-275X.
http://lattes.cnpq.br/5559547541688954

ABSTRACT

Infectious diseases are of great importance in veterinary medical clinics. Brazil has shown a significant reduction in many infectious diseases, through effective instruments in the early diagnosis of infectious diseases and consequently the adoption of prevention and control measures. Serological tests are fundamental in the diagnosis of infectious diseases, as they are effective in concluding diagnoses and prognosis, in addition to contributing to the epidemiological surveillance of diseases. This study aims to contribute with relevant information for the serological diagnoses of brucellosis, parvovirus and leptospirosis diseases. Laboratory diagnostics of infectious diseases are important in dogs to isolate infected animals and prevent secondary infections of susceptible animals that have contact with sick dogs. The clinical diagnosis is undefined, as several other viral pathogens can cause common symptoms in dogs, such as coronavirus, adenovirus, morbillivirus, rotavirus, reovirus, norovirus. Therefore, serological diagnosis is a fundamental tool within veterinary medicine, to diagnose and treat in a specific way the occurrence of common pathologies among dogs.

Keywords: Serological diagnosis. Infectious diseases.

INTRODUCTION

In clinical medicine, the laboratory diagnosis of infectious diseases is generally a complementary diagnostic resource, which confirms or not an initial suspicion, and sometimes it is necessary to carry out additional tests so that each condition can be clarified and the therapeutic, preventive and control guidelines for a given disease can be outlined.

Among the laboratory tests, there are serological tests, which consist of the detection and quantification of antigens and antibodies. These tests have several advantages, among them: speed, simplicity, possibility of automation, storage of biological material, low operating cost, offer 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; prognosis of the disease; evaluation of the efficacy of the therapy instituted; assessment 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 agglutination (SAR), slow 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).

For the diagnosis of canine parvovirus, the virus or viral antigens are detected in feces, so 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), immunoperoxidase.

The test of choice for the serological diagnosis of canine leptospirosis, in reference laboratories, is microscopic agglutination (SAM), as serovarieties predominant in certain regions can be chosen for the diagnosis of the disease.

Given the importance of serological diagnoses for these infectious diseases of relevant occurrence in the small animal medical clinic, 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. In addition, if not diagnosed and treated correctly, they can lead to the death of the animal.

LITERATURE REVIEW

BRUCELOSE

Etiology

The genus Brucella is made up of facultative intracellular bacteria, with six recognized species, each of them affecting a preferential host. Brucella species are identified according to the preferred host, morphological characteristics, metabolic properties, serotyping and phage typing (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 urease-producing, H2S-negative, nitrate-reducing, non-fermenting, and oxidase-positive (ALTON et al, 1988).

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

Epidemiology

Canine brucellosis has already been found in America, Europe, Asia and Africa (CARMICHAEL, 1990). In Brazil, Cortes et al (1988) evaluated blood serum from 3386 stray dogs captured by the zoonoses control program of the Zoonoses Control Center of the Department of Hygiene and Health of the city of São Paulo, during the period from 1981 to 1985, in 14 localities, distributed among 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 municipality of Uruguaiana, Rio Grande do Sul. In addition, these authors observed through the AGID test that 72.7% (8/11) of the animals were reactive to the test.

MEGID et al (1999) observed canine brucellosis in four different kennels. The kennels had animals with a history of abortion, mortality in neonates 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 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 microregion of the Serra de Botucatu, State of São Paulo, using the technique of rapid agglutination on cardboard (SAR) and rapid agglutination on cardboard with 2-mercaptoethanol (SAR-2ME). Of the 1,072 dog sera examined in the study, positive reactions were observed in 19 (1.77%) in the SAR test and nine (0.84) in the SAR-2ME test.

MORAES et al (2002b), with the intention of studying the prevalence of B. canis in the West Zone of the city of Rio de Janeiro, used 119 dogs from the neighborhoods that make up the city region. By the plate agglutination technique to identify seropositivity for B. abortus and for the agarose gel immunodiffusion technique for the identification of 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 in the municipality of Santana de Parnaíba, SP, Brazil. To do this, 410 samples of blood serum from dogs collected during the animal rabies vaccination campaign were examined. Agar gel immunodiffusion (AGID), using lipopolysaccharide antigen and Brucella ovis proteins, sample Reo 198, was used in normal sera as a screening test, and, for confirmation, the same technique was applied in sera treated with 2-mercaptoethanol (IDGA-ME). The complement fixation reaction (CFR), using B. ovis antigen, sample 63/290, was also used as confirmatory evidence. The determination of prevalence considered as positive the animals that reacted positively in the two confirmatory tests (IDGA-ME and RFC). The prevalence of B. canis was 2.2%.

ALMEIDA et al (2004) in order 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); In the screening test, only 2.8% (18/635) were confirmed.

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%) had 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 Tube Seroagglutination (SAL) and 2-Mercaptoethanol (2-ME) for the search for anti-Brucella abortus antibodies and Agar Gel Immunodiffusion (IDGA) and Agar Gel Immunodiffusion with 2-Mercaptoethanol-treated Serum (IDGA-ME) for Brucella canis. The reactive samples were considered positive in the confirmatory tests of 2-ME and IDGA-ME. There were 56 (18.4%) animals reactive to AAT and 12 (4.0%) reactive to SAL. Only one dog (0.3%) was considered positive, confirmed by the 2-ME test. 11 (3.6%) reactions to AGID were observed, but there was no confirmation in the IDGA-ME test.

CAVALCANTI et al (2006) aimed to investigate anti-Brucella canis antibodies in dogs living in the metropolitan region of Salvador, 85 blood serum samples from domiciled dogs were analyzed. For the serological diagnosis of Brucella canis infection, the agar gel immunodiffusion test was used, with Brucella ovis membrane antigen. The results indicated a seropositivity of 5.88% (5/85), demonstrating the presence of anti-Brucella canis antibodies in dogs residing 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 plate immunoagglutination with buffered acidified antigen. They observed a low frequency of dogs infected by B. canis 4/500 (0.8%) and absence of positive sera for B. abortus.

FERNANDES et al (2013) in order to determine the occurrence of anti-Brucella rugosa and anti-Brucella lisa antibodies in dogs from the municipality of Natal, State of Rio Grande do Norte, 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 in veterinary clinics. For the serological diagnosis of Brucella rugosa infection, the agar gel immunodiffusion test (AGID) was used, using lipopolysaccharide antigen and Brucella ovis proteins, Reo 198 sample, and for the diagnosis of Brucella lisa infection, the buffered acidified antigen (AAT) test 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 punctual and mostly based on serological tests. An occurrence ranging from 1.32% to 72.7% is observed, depending on the region, the population of dogs examined and the diagnostic test used (KEID, 2006).

Sorologic diagnosis

Due to the limitations of laboratory procedures for the isolation of 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. This was followed by IgG antibodies, which remain for long periods, especially in chronic infections. As canine brucellosis is a chronic disease, the main immunoglobulin to be detected by diagnostic tests is IgG. Despite being the most widespread methodology for diagnosing canine brucellosis, serology presents many problems in Brazil, mainly related to the availability of antigens and kits for serological diagnosis (MINHARRO et al, 2005).

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

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

SAL, in turn, is the classic serological test for the diagnosis of canine brucellosis. It provides the results in a titer (semi-quantitative) and is often used for the confirmation of SAR-2ME (CARMICHAEL, 1998). SAL is less sensitive and somewhat more specific than SAR (KEID, 2006).

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

REVIEWS

Etiology

Canine parvovirus is an important viral disease in dogs. The etiological agent is the virus of the genus Parvovirus, of the family Parvoviridae. Canine parvovirus is a single-stranded, non-enveloped, hemagglutinating DNA virus (DEZENGRINI et al, 2007). Currently, there are two dog parvoviruses: CPV type 1, also called miniature dog parvovirus (CnMV), with little defined clinical importance 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, 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 has been 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 emerging worldwide since the 70s. In Brazil, it emerged in the 80s. At first, 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 occurrence of the disease in Brazil (ANGELO et al, 1980; HAGIWARA et al, 1980), CPV has been maintained in the country's canine population and several studies have demonstrated its presence in various regions of the country (BARCELOS et al, 1988).

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

CPV variations seem to be more adapted to replication in dogs, facilitating the spread of the virus in the canine population. CPV-2 is transmitted mainly through the oro-fecal route, contaminated feces or fluids are the primary source of canine parvovirus infection (SANTOS et al, 1997). Through studies, it has been found 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 type of CPV-2, the antigenic variants exhibit increased pathogenicity in dogs and are able to infect and cause disease in cats. The epidemiological survey indicates that the newest type CPV-2c is becoming prevalent in different geographic regions, being considered a serious disease in puppies and adults and also in dogs that have completed the vaccination protocol. However, the main cause of vaccination failure is the deficiency of maternal immunity (DECARO AND BUONAVOGLIA, 2012).

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

The diagnosis of parvovirus is made through stool testing, where hemagglutination tests (HA), enzyme-linked immunosorbent assay, polymerase chain reaction (PCR), and viral isolation in enzymatic culture are investigated (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 has a high morbidity and mortality rate, due to the lack of immunity of dogs against parvovirus, especially in puppies between 6 weeks and 6 months (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, PittBull, Rottweiler and German Shepherd are more susceptible to developing the disease (MORAES AND COSTA, 2007).

Prophylaxis of CPV infection depends primarily on vaccination. Since inactivated vaccines are capable of inducing immunity in the short term, the live virus modified in vaccines are widely used. These vaccines based on the CPV-2 virus or its variant CPV-2b, are highly effective, being able to protect 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 with 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 to their offspring through colostrum and, to a lesser extent, to breast milk (DECARO et al, 2007).

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

Sorologic 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 undefined, as several other viral pathogens can cause diarrhea in dogs, such as coronavirus, adenovirus, morbillivirus, rotavirus, reovirus, norovirus. Thus, a suspected clinical case should always be confirmed by laboratory tests. Several methods have been developed for the laboratory diagnosis of CPV infection, using the feces (or intestinal contents if the animal is dead) of affected dogs (DECARO et al, 2007b).

In order for the CPV-2 detection result in fecal samples not to be false negative, it is necessary that the collection be done early during the course of infection. The immune response to this virus generalizes rapidly, starting four to five days after infection. Consequently, the virus can only be detected in feces for a short period of time (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). The detection of viral particles in the feces of suspected patients can be performed through the methods of viral isolation, cell culture or ELISA, electron microscopy, right hemagglutination (DE MARI et al, 2003).

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

From the second week onwards, an increase in serum IgG concentration is observed (DE MARI et al., 2003). Isolation in cell culture is considered the standard test, but 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 contributed to exclude many false positives and false negatives (DE MARI et al, 2003).

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

The hemagglutination test (HA) is used to identify and quantify CPV. For this test, serial dilution plates of the fetal suspension are used, in an equal volume of saline solution. The hemagglutination inhibition (HI) test detects anti-CPV antibodies. For this purpose, serial dilutions (base 10) of serum (inactivated at 56ºC and treated with 25% caolin and 50% porcine red blood cells) were performed in an equal volume of BBS, containing 2% of FBS. Subsequently, eight HA units (UHA) were added to the viral sample and the plate was incubated in a humid chamber at 37ºC for two hours. Afterwards, a suspension of porcine red blood cells is 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 the CPV in the fecal samples. (STROTTMANN et al, 2008). In order for the CPV-2 detection result in fecal samples not to be false negative, it is necessary that the collection be done early during the course of infection. The immune response to this virus generalizes rapidly, starting four to five days after infection. Consequently, the virus can only be detected in feces for a short period of time (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).

LEPTOSPIROSE

Etiology

Leptospirosis is an infectious-contagious disease that affects domestic animals and humans, caused by bacteria, spirochetes, which belong to the Leptospiraceae family, genus Leptospira. They are long, slender and spiral in shape, can have hooked ends and are classified by more than 200 serovars, especially Canicola, Icterohaemorrhagiae, Pomona, Grippotyphosa, Autumnalis, Bratislava, Hardjo, Pyrogenes, Copenhageni, 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, migrations, 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 are 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 against solar rays, adequate temperatures and neutral or slightly alkaline pH), although pathogenic leptospires do not multiply outside the organism of the hosts (CÔRTES, 1993).

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

The most commonly associated and well-known serovarieties of classic canine leptospirosis are Icterohaemorrahagiae and Canicola (SCANZIANI et al, 1994). Some of the serovarieties 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 ranged from 10 to 22% (ALVES et al, 2000).

Epidemiology

The occurrence of leptospirosis varies in different regions of the world, and can present itself in both sporadic and endemic forms; and the occurrence of Leptospira serovarieties

In Rio de Janeiro, in 1940, 11 dogs with clinical manifestations compatible with leptospirosis were submitted 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, Brazil, the study was conducted in order to determine the prevalence and risk factors. A total of 489 serological samples of dogs from 213 farms were examined. The samples were submitted to the microscopic agglutination technique (SAM), and 13 (2.66%) positive samples were 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) evaluated 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 serovaries, and examined by the microscopic agglutination method (SAM) blood samples from 185 canines from the municipality of

Oriximiná, Pará, located within the Amazon region. Of the total samples analyzed, 34 (18.4%) were reactive, with a minimum titer of 100. The most frequently found assorvarieties were Canicola and Icterohaemorrhagiae, as well as Copenhageni, also belonging to the serogroup Icterohaemorrhagiae.

MASCOLLI et al (2002) evaluated the zoonotic potential of the canine population in the municipality of Santana de Parnaíba, São Paulo, in relation to leptospirosis. To do this, they collected 410 samples of canine serum and leptospirosis was determined through the microscopic agglutination technique, using a collection of 22 serological variants. A positivity rate of 15% was found, with a higher frequency of the Copenhageni (24%), Canicola (16%) and Hardjo (16%) variants.

QUERINO et al (2003) evaluated the frequency of leptospira-seropositive dogs 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 submitted to the microscopic agglutination test and to direct urine examination. 100 antibody titers were detected in 40 dogs, being more frequent against the Pyrogenes serovar (45.00%) and 24 animals were positive in the direct urine test. The authors reinforced 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 rabies vaccination campaign. For the diagnosis, microscopic agglutination was performed, using 12 serovars of Leptospira spp. A total of 119 (15.3%) positive samples were obtained, with a reaction to 11 serovars, with greater importance for the kennel, in 48 (40.3%) samples, and pyrogenes, 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 serovarieties of Leptospira spp., with titers ranging from 200 to 25,600. The highest frequencies of positive reactions were for the serovarieties Canicola (7.0%), Ballum (6.1%), Pyrogenes (3.2%) and Icterohaemorrhagiae (2.9%), the others presented a frequency lower than 1.0%.

Given this epidemiological distribution, it is noted that dogs are considered an important source of leptospirosis infection, since several studies have reported the presence of reactive animals. And in this sense, the close contact with humans, eliminating live leptospires through urine for several months, without showing characteristic clinical signs, represent a risk to human health, thus being important the prevention and control of the disease in dogs.

Sorologic diagnosis

For the diagnosis of canine leptospirosis, laboratory tests are necessary, and the bacteriological test is 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 leptospiruria phase. Among the serological tests, microscopic agglutination (SAM) with live antigens is the most widely used worldwide (FAINE et al, 1999). Laboratory tests such as blood count, serum urea and creatinine levels, and urinalysis can be used as complementary tests, as they indicate functional changes in the different affected organs.

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

In the clinical routine, the confirmation of the clinical and epidemiological diagnosis is given by the search for specific antibodies in the serum, by means of the microscopic agglutination test (SAM), or macroscopic agglutination test in slide (LEVETT, 2001). Among the serological methods, microscopic agglutination (COLE JR et al, 1973) is the most commonly used, being designated as a reference test by the OIE (OIE, 2008), in which the blood serum reacts with the live leptospira antigens and, for its performance, a battery of antigens with the serovars representing each serogroup is used. Serums from individuals with positive titers often have cross-reactions to a variety of serovars, making it difficult to identify the infecting serovar (WHO, 1967) and, therefore, microscopic agglutination can be considered a serogroup-specific test (FAINE, 1999).

The antigen battery should include serovars representative of the region studied. For serological confirmation of leptospirosis in an individual, a fourfold increase in the value of the agglutinative antibody titer between the acute phase and convalescence is recommended (GALTON et al, 1965). In endemic areas, a single sample with a titre equal to or greater than 800 can be considered diagnostic, but it is recommended to use 1,600 or more 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). In addition, 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 they are more appropriate for the diagnosis of human leptospirosis.

To perform the Microscopic Seroagglutination (SAM) technique, considered the "gold standard" (OIE, 2008), the Leptospira spp. antigens used in the serological tests are rechopped weekly in liquid culture medium of EMJH (Ellighausen, McCullough, Johnson and Harris), with 10% of the volume of the medium to be seeded as inoculum, and kept in a bacteriological B.O.D greenhouse at 29°C ± 1°C (OIE, 2008).

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

Samples in which 50% agglutination occurred will be considered reactive, with half of the leptospires agglutinated in the microscopic field at a magnification of 100 times. The reagent samples at the initial dilution will be tested with serial dilutions of ratio two, with the first dilution being 1/100, according to the recommendation of the OIE (2008).

CONCLUSION

Laboratory diagnosis of brucellosis, leptospirosis and parvovirus diseases are important in dogs to isolate and treat infected animals and prevent secondary infections of susceptible animals that have contact with sick dogs. Clinical diagnosis is often undefined, as several other viral pathogens can cause clinical signs of these diseases. Thus, 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 a socio-economic importance, as it is responsible for reproductive problems, such as miscarriage, conception failures, orchitis, epididymitis and infertility. The diagnosis of this disease is of fundamental importance, in order to control the economic losses generated in cattle herds by this pathology.

In the case of leptospirosis, the disease has a strong socio-economic-cultural significance, and is transmitted by factors related to poor basic sanitation conditions and the disorderly accumulation of garbage, which promotes the expansion of the rodent population,

being an important zoonosis, and should therefore be well diagnosed, in order to obtain greater control of the disease in the population.

Parvovirus is a disease of high morbidity and mortality in dogs, having common clinical signs of other diseases that affect dogs, so only the clinical diagnosis becomes insufficient to confirm this disease, and therefore the importance of laboratory diagnosis is observed, which can confirm the disease and consequently treat the affected dogs in a specific way, In this way, it is possible to minimize the occurrence of the dissemination of pathological agent within the canine population.

This study aims to show the need for serological diagnosis, as a fundamental tool within veterinary medicine, to diagnose and treat in a specific way the occurrence of common pathologies among dogs.

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