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Infectious Causes of Abortion, Stillbirth and Neonatal Death in Bitches

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Additional information is available at the end of the chapter

Abstract

Problems in gestational development in dogs can be determined by infectious and non-infectious causes. Among the non-infectious causes, trauma during pregnancy, genetic characteristics of the animal, deficit nutrition, thyroid dysfunction, maternal problems and hormonal disorders are found. The majority of the cases are in relation to infectious diseases, one should consider viral, bacterial, fungal and protozoal, which can interfere directly or indirectly in the foetal development. The progression of foetal development may be affected by the direct action of the microorganisms to overcome the placenta, but they are also able to affect pregnancy and release placental toxins by inflammatory processes and, may still cause maternal pathologies, which entail problems such as hyperthermia, hypoxia and endotoxemia, which can result in abortion. Several diseases can trigger pregnancy loss in dogs. This action can be direct by microorganisms, as well as indirectly triggering other problems that lead to abortion. This chapter discusses the infectious aetiologies of reproductive failures (abortion, stillbirth and neonatal death) in bitches.

Keywords: Puppies, Bitches, reproductive failure, infectious causes, diagnostic

1. Introduction

Problems in gestational development in dogs can be caused mainly by infectious diseases. The progression of foetal development may be affected by the direct action of microorganisms to

overcome the placenta, but they are also able to affect pregnancy and release placental toxins by inflammatory processes and may still cause maternal pathologies, which entail problems such as hyperthermia, hypoxia and endotoxemia, which can result in reproductive failures (abortion, stillbirth and neonatal death) [1].

2. Bacterial diseases

2.1. Brucellosis

The bacterium *Brucella canis* (small, aerobic Gram-negative coccobacilli, which stain red using the modified Ziehl-Neelsen technique) stands out as one of the main bacterial causes of pregnancy loss in bitches [2]. The species *B. abortus*, *B. melitensis* and *B. suis* have also been found in dogs, where it is believed that the natural infection occurs after the ingestion of placenta and aborted foetuses. The main source of infection is through vaginal and seminal secretions from infected animals, although bacteria are shed in faeces, milk, saliva, and nasal and ocular secretions [2]. The *B. canis* may be present for a long time in dogs without exhibiting clinical signs. After the initial exposure, the bacteria reach the bloodstream in about three weeks. Subsequently, the pathogen can infect the genital tissues enabling a continuous release of the agent, which may be recurring for months or even years. In turn, canine brucellosis can result in infertility, difficulties in pregnancy, early embryonic death, foetal resorption and late abortion. The clinical signs associated with Brucellosis in dogs are not pathognomonic and due the lack of the lipopolysaccharide antigen associated with endotoxemia in bitches, it is rarely systemic ill and fever [3]. Clinical signs reflect the localization of the bacteria in extra repro-ductive tract sites such as the eye, intervertebral disc spaces, and reticuloendothelial system. Brucellosis causes spontaneous late abortion in a healthy bitch, most commonly occurs from days 30 to 57, accompanied by a vaginal discharge lasting up to 6 weeks. Earlier abortions can occur but may be incorrectly reported as conception failure since the bitch typically ingests aborted foetuses. Early embryonic death and foetal resorption can occur within 10 – 20 days post-mating. Many bitches that abort will subsequently have normal litters, although puppies born to infected bitches contain both live and dead pups, although most live pups die shortly thereafter. Aborted puppies usually appear partially autolysed, with lesions of generalized bacterial infection, including subcutaneous oedema and degenerative lesions in the liver, spleen, kidneys and intestines. Seroprevalence studies indicate that canine brucellosis is wide-spread in the Americas. Isolation (placenta, lymph nodes, prostate, and spleen are suitable samples for culture) and identification of *B. canis* is the gold standard, however, serology (the most accurate serological test currently available is the agar gel immunodiffusion test-AGID) and polymerase chain reaction (PCR) are widely used to diagnose canine brucellosis [3, 4]. *B. canis* is not considered a significant zoonosis and, serious illness can occur in immunocompro-mised patients by direct contact with infected animals or through occupational aerosol expo-sure, but the owners should be aware of the zoonotic potential of this disease [4].

2.2. Others bacterial agents and Rickettsias

The *Ehrlichia canis* and *Anaplasma platys* have been found in dogs attended in veterinary hospitals and clinics in various countries and states in Brazil [1]. Anaemia and thrombocytopenia are the

main clinical signs found in dogs infected with these agents in Brazil. However, it is noted that these dogs more often develop anaemia and thrombocytopenia. So, clinical signs may vary according to geographical variations and pathogenicity. These agents are not yet known to cause abortion directly but have been reported in abortion in anaemic bitches infected. Results also indicate that animals with infectious anaemia may be more susceptible to suffer reproductive failures than animals with normal haematological values [1] (**Figure 1**).
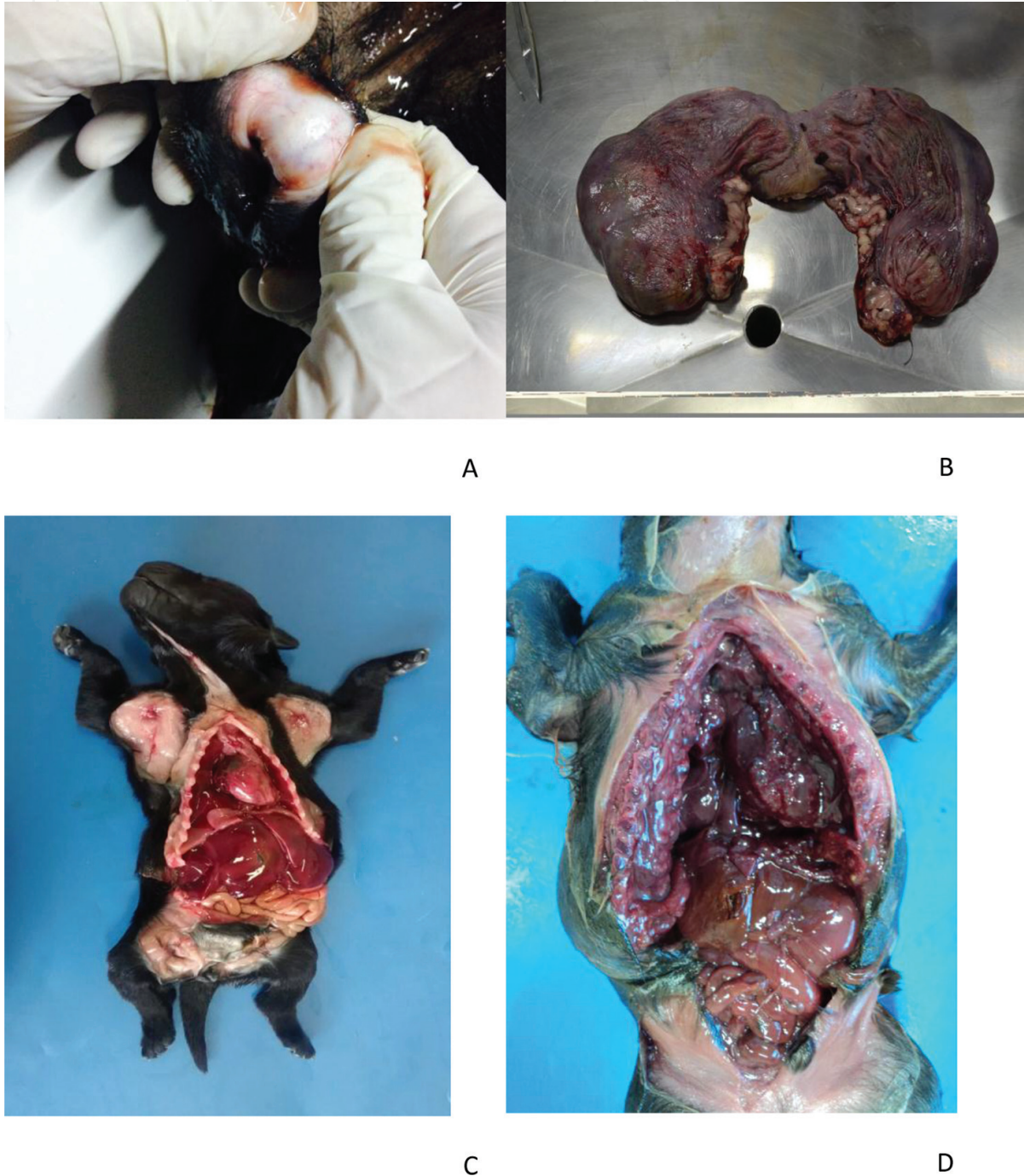

A

B

C

D

Figure 1. Macroscopic lesions caused after abortion due anaemia occasioned by vector-borne disease. (A). Anaemic vulva after intense parasitaemia by ticks; (B). uterus with haemorrhagic serous after episode of abortion; (C). hepatosplenomegaly in aborted foetus with 56 days of gestation; (D). foetus with blackened organs and in the autolysis process after stillbirth in late pregnancy.

Escherichia coli is the most common bacterium isolated from the canine vagina and is also commonly cultured from the uteri of bitches with metritis and pyometra. *E. coli* produces an endotoxin that may result in pregnancy loss in the bitch. *Streptococcus* spp are bacteria that are physically present in the skin and mucosa of dogs and cats. Some microorganisms belonging to this group have been related to the occurrence of neonatal sepsis, abortion and metritis [2, 3]. βH *Streptococcus* is known cause metritis, pyometra, placentitis and abortion, commonly linked to ascending infection. *Streptococcus* βH is a major cause of neonatal death. Typically, newborns are infected in the mother's birth canal, through the umbilical cord or, less commonly, from the udders with mastitis. Bitches have high bacterial load in the vaginal canal, which persist throughout pregnancy leading to the infection of the uterus and foetus [2, 3].

3. Protozoal diseases

Some protozoan species are capable of infecting dogs and can also infect humans, triggering zoonoses. The existence of protozoal coinfection in dogs are already established, one of the combination of *Toxoplasma gondii* and *Neospora caninum* sometimes increased by the presence of *Leishmania* spp. These types of infections exacerbate the clinical state of the animal by co-existence of various diseases [5].

The *T. gondii* causes toxoplasmosis and its definitive hosts are the Felidae. However, its prevalence in dogs is high. Transmission can occur vertically through congenital infection, however, horizontal transmission is the main mean of infection. Dogs are the definitive hosts of *N. caninum* that causes Neosporidiose disease, which can be transmitted horizontally or vertically, and it has maintained the parasite for generations amongst the canines [84].

3.1. Toxoplasmosis

Toxoplasma gondii, the protozoan that causes a coccidiosis in felines can infect mammals (including humans), birds and reptiles, mainly affecting the central nervous system and occasionally, the reproductive system, muscles and visceral organs [6]. This protozoan is an obligate intracellular parasite and its definitive hosts are the Felidae, which excrete oocysts that house sporozoites, an infectious stage of the parasite. As for the intermediate hosts and therefore not Felidae, which shelter tissue cysts, it was possible to observe two infectious stages of the organism: tachyzoites and bradyzoites [7]. The oocytes containing the sporozoites have a double-walled, spherical shape and resistance to environmental conditions, Being the parasitic manner that promotes their strength and dissemination. They are developed by a entero-epitelial cycle inside the felids through sporogony, and are then excreted in their immature form with the faeces of these animals. The tachyzoites Form occurs in the active proliferation stage of *T. gondii*, or the acute phase of infection Parasitizing preferably macrophages and monocytes but with the potential to infect any nucleated cell. This parasitic stage has an arc shape, is mobile and provides rapid multiplication by endodyogeny. In the chronic phase of infection are found the bradyzoites located in parasitophorous vacuole of a cell whose membrane forms the capsule of the tissue cyst. They have slow multiplication, also endodyogeny,

intracisto, where it is protected from the action of the host immune system and drugs, allowing its existence for months and even years. They are poorly resistant to high temperatures [8].

T. gondii has worldwide distribution and is considered as the most cosmopolitan of all the causative parasite zoonoses. It is an opportunistic organism, independent of the host and has relevance related to animal production and public health by having transmitted through food from infected animals. In transmission between animals, the Felidae assume the lead role as a source of infection [8]. Thus, the transmission can occur by congenital way, via ingestion of infected tissue or contaminated food and water, in addition to transmission by breastfeeding, through transfusion of body fluids or by transplantation of tissues or organs [7].The entero-epitelial biological cycle is unique to the definitive hosts in which the oocysts are released in the faeces. Intermediate hosts are infected by ingestion of oocysts and cysts. Oocysts must sporulate in the environment to become infective and once sporulated and ingested by potential hosts, will promote asexual reproduction and infect the animal that ingested it, leading to the formation of tissue cysts. Cysts, in turn, may be ingested by host carnivores, infecting them. Ingestion of raw or undercooked meats are the main sources of this type of transmission. Congenital transmission and placenta are more common in women, sheep, goats and nuts. However, studies have shown that infected female dogs with 56, 40 and 32 days of gestation showed congenital infection that may have led to abortion and thus confirm this relationship.

The evidence of facts relating to such a manifestation of the parasite in dogs is scarce. What is known is that in its life cycle, the *T. gondii* reaches different host tissues, including male and female reproductive organs of intermediate hosts, which may cause certain adverse effects on the reproductive function. In animals, toxoplasmosis is associated with reproductive failure in males related with some evidence of venereal transmission of *T. gondii* [9]. Researchers have shown that four bitches inseminated with canine semen samples containing 1×10^6 tachyzoites of *T. gondii* RH strain, had embryonic resorption in two of them and the protozoan was found in the brain of the four offsprings [10]. The results of this study show, among other things, that dogs with infected semen can cause reproductive problems and, consequently, the vertical transmission to offspring. Additionally, transmission of tachyzoites to the milk intake neonates (via lactogenic) shortly after birth [11] has been reported. The severity and type of clinical signs presented by the Animals infected with *T. gondii* are variable depending on the degree of tissue injury and location. There is no established reason regarding the different presentations of clinical signs and their absence in dogs. It is assumed that all types of cells are susceptible and some of these differences can be assigned to factors such as age, sex, host species, *T. gondii* strain, body number and stage of ingested parasites [8]. Specifically in dogs, the occurrence of clinical signals from toxoplasmosis is associated with concomitant infections such as distemper, ehrlichiosis, neosporidiose and leishmaniasis.

Animals infected with *T. gondii* usually show no clinical symptoms and undergo the primary infection for a latent infection or a chronic stage. In dogs, infection with this parasite is very common, being demonstrated by several studies of serological prevalence. Already the clinical form of infection is rather sparse, usually presenting itself in young animals, usually associated with immunosuppressive factors, infections or to canine distemper virus. Canine symptoms can concentrate in the gastrointestinal tract and respiratory systems and in neuromuscular or

be from generalized infection. Therefore, clinical signs usually identified in dogs with toxoplasmosis are ataxia, diarrhoea and respiratory disease and focal necrosis in areas of the lung, liver and brain may also occur, triggering numerous clinical signs [12]. Therefore, toxoplasmosis in dogs and other animals can show symptoms quite similar to many infectious diseases, the Neosporidiose one with the greatest similarity.

The possible interference of Toxoplasmosis in the reproduction of dogs as a whole has been studied in 1970 and found out that toxoplasma infection in dogs at various gestational stages can cause mortality of the puppies from the 4th to 75th postnatal days [13]. Still, abortion and foetal death has been observed, in the middle third and final pregnancy in dogs experimentally infected with *T. gondii* orally (oocysts) and subcutaneously (tachyzoites) [14]. Later, reproductive problems related to canine toxoplasmosis were again observed, when naturally infected dogs with 30 days of gestation were artificially reinfected. The puppies from infected mothers were positive serologically at birth, but without symptoms, except a neonate who was weakened. With additional tests, the parasite was detected in saliva samples, milk and urine of these animals still being detected positive immunostaining of cysts and / or tachyzoites by immunohistochemistry in 23 organs of experimental animals [15, 16]. Soon, placental transmission and subsequent foetal infection with *T. gondii* in cats and dogs, have been experimentally observed and their association with foetal death have been found.

Diagnosis of toxoplasmosis is mainly based on clinical suspicion since the symptoms can be similar to other infectious diseases. Therefore, attention should be paid to the epidemiological information and data collected during the interview, making a request for additional tests, a prerequisite. In dogs, the presence of apathy, rhinorrhea, conjunctivitis, pneumonia, fever, convulsions, paralysis, diarrhoea and lymphadenopathy may be clinical signs of *T. gondii* infection, requiring additional tests to confirm the diseases [6]. The identification of *T. gondii* presence can be accomplished by in vitro and in vivo isolation, serology, histopathology, immunohistochemistry and PCR. In general, the serological diagnosis is the most used, mainly through modified agglutination test (MAD) and indirect immunofluorescence (IFA), which is the preferred test [17]. Whichever method is used, it is important to collect samples at intervals of about two weeks to determine seroconversion, indicating recent infection [18]. When there is abortion, should be referred to the placenta and the foetus (paying attention to the foetal brain), cooling temperature, as soon as possible to enable the laboratory diagnosis for toxoplasmosis. Submissions will be used for histopathology associated or not with immunohistochemical tissues with compatible lesions [8].

In cases of acute systemic infection in dogs changes can be observed in haematological parameters, such as nonregenerative anaemia, neutrophilic leucocytosis, lymphocytosis, monocytosis and eosinophilia. The biochemical changes are consistent with increased serum activities of alanine aminotransferase (ALT) and alkaline phosphatase may occur when there is liver necrosis. If necrosis is acute, bilirubin levels are likely to be increased. In the event of muscle necrosis, serum creatine kinase activity is also increased [7]. The establishment of the diagnosis can be made through serological tests, but there is no absolute serologic test that can definitely confirm the diagnosis of toxoplasmosis. Furthermore, only the detection of antibody against the parasite

is not sufficient for the establishment of serological diagnosis since previously infected dogs may also exhibit antibodies response. Some tests that may be ordered in addition to the IFT and MAD, such as the reaction of Sabin-Feldman (SF), the enzyme linked immunosorbent assay (ELISA), complement fixation test (CF) and the reaction of indirect haemagglutination (HI) [8].

The IFA identifies antibodies through specific fluorescent conjugates of IgG and IgM. The presence of IgG is related to a previous exposure, suggesting active and recent infection. The MAD evaluates IgM antibodies indirectly by subtracting antibody titres present in treated and untreated sera [19]. The SF reaction has a high sensitivity and specificity and allows the antibody titration within a few days post-infection. The enzyme linked immunosorbent assay allows detection of IgM response. In the CF test, different parasite antigens are used and that can be identified as testing positive in case of an early infection. In short, the interpretation of the results: a high titre of IgM with low or zero IgG indicates a disease evolving, otherwise, the high quantitation of IgG and low or zero IgM indicate a chronic state of the disease. Whatever the outcome, a new serology test should be done after 15–21 days due to the possibility of severe immunosuppression until the second post-infection week, which may lead to false-negative results [8]. Identifying the *T. gondii* in tissue or bodily fluids is possible by polymerase chain reaction that detects the protozoa in biological samples. However, this detection which is based on PCR is still quite limited, as it requires special equipment, which are not available in any clinical laboratory. In addition, the realization of body isolation requires relatively vast and considerable experience time. However, care should be taken that the molecular detection of the parasite does not replace the serological methods in the diagnosis of the disease, making it imperative to associate the two diagnostic methods to identify the presence of infection and the determination of the disease stage [8].

Various infectious and non-infectious diseases should be evaluated in the differential diagnosis of toxoplasmosis. In dogs, the most relevant are distemper, neosporosis, isosporiasis and strongyloidiasis [8]. In aborted foetuses, macroscopically it is possible to observe necrotic foci white-greyish punctate located in the lungs and liver, as well as pulmonary congestion and heart pallor. The central nervous system (CNS) is marked with congestion of the brain and cerebellum [7].

Treatment of toxoplasmosis is based on suppression of the replication agent when the disease presents itself in the acute form, and emphasizes the importance of early diagnosis and consistent implementation of measures leading to the reduction in disease transmission [20]. Clindamycin is the drug of choice for the treatment of dogs and cats. The drug administration once started, the clinical signs tend to initiate regression after 24 – 48 hours. In addition to clindamycin, the combined use of pyrimethamine and fast-acting sulphonamides (e.g., sulfa-diazine, sulfamethazine and sulfamerazin) is valid in the treatment of systemic toxoplasmosis infection [7].

Prevention should be taken to avoid ingestion and contact with oocysts and cysts. So for pets that eat meat, the meat should be well cooked. As a complement to prevention, measures should be taken to avoid these same animals hunt and/or eating mechanical hosts such as cockroaches, flies, worms and rodents, potential or intermediate hosts. The prevention becomes even more important because there is no vaccine available for humans or animals [8]. General care in food

hygiene, whether in urban or rural areas, considering proper cooking of meat and milk boiling, are the main preventive measures of toxoplasma infection to humans. Personal hygiene and comprehensive hand washing performed after handling raw meat, for example, assists in preventing the disease. Generally, prevention of human toxoplasmosis is based on the maximum avoidance of exposure to susceptible hosts. This disease is not notifiable, except for outbreaks [7].

3.2. Neosporosis

Neospora is a protozoan belonging to the phylum Apicomplexa, Sporozoa class, Eucoccidiida order and Sarcocystidae family [21]. *Neospora caninum* is a parasite coccidia, intracellular exclusively, forming cysts that causes neosporidiose, a disease that has been disseminated in the continents generating a high rate of infected cattle and dogs [22]. In the genus *Neospora* were identified only two species *N. caninum* [23] isolated from dog brain and *N. hughesi* [24, 25] isolated from the brain and spinal cord of horses [24]. It has not been described any cases of the disease in humans [26]. Dogs (*Canis lupus familiaris*) [27], coyotes (*Canis latrans*) [28], dingo (*Canis lupus dingo*) [29] and gray wolves (*Canis lupus*) [30] have been identified as definitive hosts of the parasite and are significant in the transmission of the parasite to other animals. The prevalence of antibodies against *N. caninum* in dogs is the result of research in various parts of world, which has reached from 4 to 54.2% [31, 32]; this percentage can be varying because of habitat, age, living of dogs with cattle, diet and serological technique employed etc., among others [33]. The life cycle of *N. caninum* has three forms: tachyzoites, bradyzoites in the cysts and sporozoites in the oocysts. However, the tachyzoites and bradyzoites are intracellular stages identified in intermediate hosts [34]. The tachyzoites are responsible for the acute phase and has the ovoid or circular shape. Bradyzoites are in latent stages, through tissue cysts that are resistant to acid solution pepsin [35].

Horizontal transmission (postnatal) is due to the ingestion of water or food contaminated with oocysts eliminated by dogs, especially in cases of abortion outbreaks. It is identified as the association between seroprevalence and abortions in bovines, when the presence of dogs could increase the incidence of the disease in both species. Dogs present in farms were identified with greater prevalence of infection than in urban area [36]. This is due to the dogs ingest infected bovine foods such as foetuses, foetal membranes and fluids [37]. In dogs, neosporidiose can develop neuromuscular, cardiac, pulmonary and skin lesions changes, there still descriptions of dermatitis, cardiomyosite and pneumonia in this species [38, 39]. Vertical transmission via lactogenic have been reported in calves experimentally and the presence of DNA of *N. caninum* was also observed in the colostrum of cows infected, reporting the possibility of transmission of *N. caninum* by colostrum [40].

Contamination can occur vertically when a bitch with subclinical infection transmits *N. caninum* to its foetuses and litters can be born infected [41]. The congenitally infected animals are the most severe cases; however, the infection can occur in animals of all ages. Young animals manifest hind limb paralysis that occurs at an accelerated rate, and incoordination can cause paresis of hind limbs in adult dogs [42, 43]. For a reliable diagnosis: detailed history and description, good physical examination and laboratory tests are necessary [42]. Haematological and biochemical tests are not enough to confirm the diagnosis of this

disease, since the laboratory diagnosis is affected by means of serological and parasitological examinations. However, the muscle biopsy is considered important for the diagnosis of neosporidiose [23, 44]. The use of immunohistochemistry is also of paramount importance to identify tachyzoites and cysts in the tissues [37]. Analysis of the cerebrospinal fluid is another form of diagnosis that can facilitate the identification of this disease. The inflammatory cell count demonstrates an inflammatory or infectious situation and tachyzoites display may indicate protozoal encephalomyelitis, and can also identify bradyzoites in cerebrospinal fluid. In the dog faeces, the oocysts of *N. caninum* can be observed using the flotation technique [37, 45].

The PCR is essential to detect the DNA of the parasite in the faeces of the definitive host and thus used to confirm the diagnosis. This technique is also used to perform DNA detection in the intermediate host [46, 47]. Serological tests such as indirect immunofluorescence assay, agglutination test Neospora (NAT) and multiple tests of ELISA were made for diagnosis in dogs [48]. IFA was the initial test to identify antibodies against *N. caninum*. Immunofluorescence with greater than or equal to title 1:50 demonstrates dog contact with the agent. The NAT test demonstrates the agglutination of tachyzoites in the presence of specific antibodies contained in the serum, abolishing the use of secondary antibodies used in the previous tests. This test has identified specificity and similar sensitivity of the IFA [21, 49].

Macroscopic lesions caused by *N. caninum* is rare, however in experimental infections, it was identified necrosis of the foetal placental villi, necrosis and inflammation [50]. For the treatment of dogs clindamycin (11-22 mg/kg, BID, TID), sulphonamides (15 mg/kg bid) and pyrimethamine (1mg/kg SID) [51] are used. However, the treatment efficiency is not effective, but there are data that demonstrate an effective response against neosporosis in the symptoms of adult dog administration associated with pyrimethamine and sulphadoxine for 1 month [52]. For the prophylaxis and control of *N. caninum* infection, the reproduction of positive dogs that have already demonstrated compatible symptoms or have calved infected puppies and sick should be prevented. It should also be able to prevent these carnivores eat foods such as meat or raw entrails, and especially farm animals [42]. To prevent the ingestion of aborted foetuses and placental membranes should discard these materials in an appropriate place, since we do not have efficient commercial vaccines on the market [53].

4. Viral diseases

4.1. Canine herpesvirus

The canine herpesvirus 1 (CHV-1) has a worldwide distribution and is associated with respiratory and reproductive diseases in dogs [54]. The CHV-1 was isolated in several countries, with a disease considered enzootic for dogs [55]. The first study to report this agent associated with fatal haemorrhagic disease in puppies was Carmichael et al. in 1965 [56]. The *Canid alphaherpesvirus* 1 species refers to family *Herpesviridae*, subfamily *Alphaherpesvirinae* and genus *Varicellovirus* [57]. This virus consists of double-stranded DNA, has

icosahedral infecting only dogs or cells canine origin. This specificity is due to the presence of specific receptors on the cell surface, such as the glycoprotein D (haemagglutinin) [54, 58]. As for CHV-1 characteristics of the environment, there is a higher incidence and spread of the virus in kennels than in the home environment. The presence of this agent in serum from canis can get up to 100%. This fact is mainly due to agglomeration and poor hygiene conditions in places [58]. Moreover, the disease manifests itself in seasonal way, accentuating in cold weather because the virus is unstable and sensitive to higher temperatures [58, 59].

Among the infectious diseases of viral origin, the CHV-1 stands out as one of the main viral cause of abortion and neonatal mortality in dogs [60]. The infection caused by this virus during pregnancy can lead to abortion, stillbirth, embryonic resorption, premature birth and neonatal death [61, 62]. It can result in infertility, birth of mummified foetuses, weak puppies, or premature sick [55]. Its horizontal transmission can occur due to direct or indirect contact between dogs. This contact can happen through nasal secretions, semen and contaminated aerosols, regardless of sex or age distinction, being observed an increased susceptibility in puppies less than 2 months [63]. Animals without updated immunization record, with vaccination failures and maternal immunization also become more prone to infection [64]. Vertical transmission occurs from mother to foetus through the placenta [55]. In some cases the infection can reach the uterus resulting in foetal death and still birth of the offspring [65]. After infection of the cell by the virus in the cell nucleus will be synthesis of viral DNA and nucleocapsids. As the viral envelope from the nuclear membrane. The virus then travels through the endoplasmic reticulum and Golgi, and subsequently released to infect new cells [58].

Infected adult dogs often do not show apparent symptoms. In them, the infection is often subclinical. However, in newborns and puppies with 1–2 weeks of life may develop systemic disease that may result in a generalized necrotizing haemorrhagic disease [55, 56]. Still, this pathogen has an important characteristic of latent infection remaining in a state of latency in lymph nodes and lymphoid tissues of the oronasal mucosa and genital [58, 66]. So that makes the diagnosis difficult due to the absence of clinical signs. In this condition, the presence of factors such as pregnancy, stress, immunity reduction, diseases and use of corticosteroids can reactivate the virus [58], with the possible occurrence of necrosis in the placenta in pregnant infected bitches [65]. In females, papulovesicular in genitalia and oral lesions can be visualized, and in males, may be similar lesions on the penis and release the virus by semen [58]. The histopathologic examination of the liver, lung and kidney in adult dogs reveals haemorrhages with necrotic foci and intra-nuclear inclusion bodies. Puppies contaminated by CHV-1 usually die resulting from systemic disease. The consequences of pathogen infection during pregnancy in bitches will depend on the stage of gestation when infection occurred. Histopathological and post-mortem exams may be observed mummified and calcified foetuses, foetuses with progressive multifocal haemorrhagic necrosis in various organs, and haemorrhagic foci in uteri and placentas. An increased virus concentration has also been observed in the adrenal glands, kidneys, lungs, spleen and liver. In the post-mortem examination the presence of serous fluid and haemorrhage in the pleural and

peritoneal cavities is observed. And especially in canine puppies infected by the oronasal route may have meningoencefatite after birth [58].

Diagnosis can be accomplished through fluids and vaginal swabs collected and nasal secretions, or using tissues from foetal organs or adult dogs after necropsy. The antigen or genetic material in aborted foetuses or newborns can be extracted from humours, liver, adrenal glands, lungs, spleen, kidneys and lymph nodes. With the collection of the appropriate material, histopathological tests, biochemical, immunofluorescence microscopy and molecular tests can be performed. The CHV-1 presence can be confirmed in samples by polymerase chain reaction [63] and by the sequencing, comparing similarity between the surrounding sequences. PCR allows for viral detection even in animals that have the latent infection [58]. Obtaining the history and medical records of these animals is also essential for a complete and accurate diagnosis [67]. The treatment of this disease in puppies is difficult due to the rapid development of infection and mortality that occur before the diagnosis is established [58]. However, despite this agent being seen as the main cause of abortion and foetal mortality in dogs, other pathogens may be associated directly or indirectly with these consequences, such as loss of pregnancy by systemic infectious anaemia [1]. Vaccination and appropriate sanitary measures are essential to prevent viral spread among animals in kennels [63].

4.2. Canine minute virus

Another viral agent that can cause severe disease in newborns, transplacental infections and embryonic resorption in dogs is *Canine minute virus* (CnMV) or CPV-1 (*Canine Parvovirus*-1) [68]. This agent sets off a disease currently considered endemic [69]. The consequences of infection with this virus in pregnant females may vary according to the time of infection during pregnancy and may cause embryonic resorption, stillbirth, neonatal mortality and abortion [65, 70], or initiate respiratory, cardiac and enteritis problems in puppies [65]. The CnMV is a parvovirus that belongs to *Parvoviridae* family, subfamily *Parvovirinae* and genus *Bocavirus* [57]. It is a very small virus approximately 22 nm in diameter, single-stranded DNA and has no viral envelope. This virus has tropism for lymphoid and embryonic tissues, bone marrow, myocardium and intestinal epithelium [69].

The clinical signs found in CnMV infection may vary, be unapparent, or apparent as respiratory problems, enteric disease and reproductive disorders [71]. Infected puppies less than 1 month of age may have mild symptoms or accelerated death, depression, lack of appetite, acute myocarditis, respiratory failure and enteritis. The viral action mechanism contributes to reducing phagocytosis by monocytes promoting immunosuppression [68, 72]. The transplacental infections can cause disease in subclinical phase, deformations of foetuses and the loss of pregnancy. The consequences in pregnant bitches may vary according to the time of infection. In the first half of the pregnancy, stillbirth and embryo resorption process can occur after infection. In the second half, observed a larger number of stillborn and weak puppies [71].

As for post-mortem examinations on puppies, pneumonia, enteritis, myocarditis, oedema and atrophy of the thymus have been observed. In relation to the histopathologic findings, viral presence in the epithelial cells of the intestinal crypts and cardiomyocytes are observed. Other changes found are hyperplasia of the interstitial crypts, myocardial necrosis, pneumonia, and depletion of lymphocytes in the thymus and other lymphoid tissues [71]. The samples for diagnosis may vary mostly from foetal or neonatal tissues of the myocardium, intestine, lungs, kidneys and faeces. The diagnosis of the CnMV can be accomplished by direct methods such as viral isolation, immunohistochemistry, electron microscopy, direct ELISA, conventional PCR and RT-PCR, of the tissue, and/or faeces and/or enteric contents. For detection of intranuclear inclusion bodies, haematoxylin-eosin staining or immunofluorescence using specific antibodies [73] can be used. As for indirect methods of serological diagnosis may used the indirect ELISA and haemagglutination inhibition, allowing the study of the prevalence of disease [74].

The treatment of parvovirus is based on the recovery of electrolyte balance and in the prevention of secondary infections using antibiotics. Attenuated vaccines of CnMV in dogs provide superior immunity than inactivated, and are safer. In newborn puppies, it is suggested the warming of them and to maintain nutrition and adequate hydration [74].

4.3. Other viral diseases

Other viral infections known to cause abortions, stillbirths and neonatal death in bitches are Bluetongue (BTV), canine distemper and canine adenovirus-1 [75]. The Bluetongue is a disease transmitted by arthropods especially in ruminants. Infection of dogs is currently thought to be by oral ingestion of infected meat or meat products rather than through vector feeding [76]. There is evidence of direct transmission of the agent to the dog. Abortion and stillbirth are consequences of infection of this agent in pregnant bitches [77]. Direct transmission and differences in canine susceptibility to certain serotypes of the virus are not well elucidated in dogs [78]. The canine distemper virus (CDV) may also affect gestation by the weakness of maternal health inducing abortion, and in rare cases can cross the placenta and lead to abortion or foetal infection [79, 80]. The abortion can originate from a systemic infection in dogs or transplacental infection [81]. The infection by this virus can still result in stillbirth and congenital infections in dogs. Transplacentally infected puppies can develop neurologic signs within 6 weeks after birth [79, 82]. The canine adenovirus-1 (CAV-1) may be associated with fatal pneumonia in pups less than 1 month of age [83]. However, this virus can result in miscarriage, with or without foetal infection. Abortion can be a result of stress caused by the disease [79, 80].

5. Final considerations

Infectious causes are still the most responsible for reproductive failure in dogs through the direct action of the pathogen in the foetus and in placenta; however, we must always try to reduce the chances of reproductive failures that occur due to systemic action of the infectious agent in the mother through the early diagnostic and treatment.

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