Aspects of the epidemiological chain of bovine neosporosis and applications as a prophylaxis tool

Aspectos da cadeia epidemiológica da neosporose bovina e aplicações como ferramenta de profilaxia

Isabela Gonçalves da Silva

Lara Ellen Correa Nogueira Silva

Renata Vieira Chaves Gabriel

Alexandra Cristina Silva

Sérgio Eustáquio Lemos da Silva

ABSTRACT
Systemic parasitic infections are responsible for serious losses in agriculture. In the reproductive sphere of cattle farming, bovine neosporosis is described as one of the main diseases that causes abortions in cattle herds, generating considerable reproductive and, consequently, economic impacts on the Brazilian livestock sector. This disease is caused by the protozoan Neospora caninum, a coccid found in the form of tissue cysts and intracellular tachyzoites. This condition gives the parasite an efficient capacity for dissemination and transmission within cattle herds and can infect up to 90% of animals in confinement with dairy or beef aptitude (DUBEY et al. 2006).

Keywords: Neosporosis, Epidemiological, Parasite.

RESUMO
As infecções parasitárias sistêmicas são responsáveis por sérios prejuízos na agropecuária. Na esfera reprodutiva da bovinocultura, a neosporose bovina é descrita como uma das principais doenças causadora de abortos em rebanhos bovinos, gerando consideráveis impactos reprodutivos e, consequentemente, econômicos no setor pecuário brasileiro. Essa enfermidade é causada pelo protozoário Neospora caninum, um coccídeo encontrado na forma de cistos teciduais e de taquizoítos intracelulares. Essa condição, confere ao parasita uma eficiente capacidade de disseminação e de transmissão dentro dos rebanhos bovinos, podendo infectar até 90% dos animais em confinamento com aptidão leiteira ou para corte (DUBEY et al. 2006).

Palavras-chave: Neosporose, Epidemiológica, Parasita.

1 INTRODUCTION

Systemic parasitic infections are responsible for serious losses in agriculture. In the reproductive sphere of cattle farming, bovine neosporosis is described as one of the main diseases that causes abortions in cattle herds, generating considerable reproductive and, consequently, economic impacts on the Brazilian livestock sector. This disease is caused by the protozoan *Neospora caninum*, a coccid found in the form of

tissue cysts and intracellular tachyzoites. This condition gives the parasite an efficient capacity for dissemination and transmission within cattle herds and can infect up to 90% of animals in confinement with dairy or beef aptitude (DUBEY et al. 2006).

The protozoan *Neospora caninum* was first described in 1988 in Norway, affecting puppies, whose species is described as the definitive host of the protozoan. In Brazil, the first report of neosporosis was made in 1999, in the state of Bahia. Since then, from serological studies, the disease has been reported endemic in several regions of the country, such as Bahia, São Paulo, Rio Grande do Sul, Paraná, Mato Grosso do Sul and Minas Gerais (ANDREOTTI et al., 1999; CORBELINI et al., 2000; LOCATELLI-DITTRICH et al., 2001).

The biological cycle of *Neospora caninum* is heteroxenous, and the domestic dog of the subspecies *Canis lupus familiaris* is described as the usual and definitive host of the parasite and may also affect several intermediate hosts such as horses, ruminants, humans, foxes (*Vulpes vulpes)* and wild rats (*Rattus novergicus).* In some cases, although not well elucidated, the dog can assume the role of intermediate host (OSHIRO, 2006; LLANO, 2013). In addition to the domestic dog, the coyote *(Canis latrans),* the wolf (Canis lupus) and also the *dingo (*Canis lupus dingo) can be considered definitive hosts. In addition, greater attention should be paid to neosporosis, since not only animals, but also humans can be equally affected, and therefore this disease is a public health issue. Indirect immunofluorescence (IFI) techniques were used in humans, which were positive for anti-N. *caninum* antibodies and negative for *T. gondii* antibodies, suggesting that the disease can be considered zoonotic (TRANAS et al., 1999).

Fundamentally, there are two forms of transmission of neosporosis that determine the spread between herds, which can occur horizontally through the consumption of water or food contaminated with oocysts of dogs or other infected canids; or vertically, where transplacental transmission occurs. It is important to point out the importance of endogenous transplacental transmission in the maintenance of the disease in herds, since about 95% of seropositive cows will abort or generate seropositive calves (DINIZ et al., 2019; MCALLISTER, 2016).

In addition, it is important to highlight that the economic losses caused by *Neospora caninum* are related to diseases of the reproductive system, which mainly include disorders, such as return to estrus, with regular or irregular intervals, abortions, birth of weak and unviable animals, with neurological signs or even in the condition of persistently infected (ALMERIA et al., 2009a; Almeria et al., 2010; DUBEY, SCHARES AND ORTEGA-MORA, 2007). Barros et al. (2014) reported that the losses caused by neosporosis have different repercussions, according to the technological level of the rural company and with negative consequences for the state's economy.

The main clinical sign related to infection of adult females is abortion, which is more frequent between the fifth and sixth month of gestation. Already, in neonates, neurologic changes, stillbirths,

mummification, malformations, myocarditis, and polymyositis can be observed. If it occurs in the final period of gestation, the delivery will happen normally, but the calf can be congenitally infected, and this infection may be repeated in future generations (GUIMARÃES, 2007).

The control of the disease is done through the correct and accurate diagnosis, which can be done through laboratory tests, such as immunohistochemistry tests, histopathology, and polymerase chain reaction. Also, the method of isolation of the parasite in cell cultures can be used. It is important to emphasize that serological methods end up standing out from the tests previously described, as they are a valuable tool used in longitudinal and cross-sectional epidemiological studies. Several serological tests, including ELISA, RIFI, TAD can be used to identify anti-Neospora antibodies in serum and cavitary fluids (DUBEY, 1999). Studies of the seroprevalence of neosporosis indicate that parasitosis is endemic in the national territory and that, therefore, it may not be easy to perceive by the rural producer; it is necessary to awaken them to the economic losses caused by the disease, as well as the need to adopt economically viable control strategies for the rural company.

2 GOAL

Thus, the general objective of this study was to analyze, from the literature, the economic and reproductive impacts caused by *Neospora caninum* in infected herds. The specific objectives were to describe and point out the importance of prevention and knowledge about the biology and epidemiology of the etiological agent of bovine neosporosis, to subsidize effective prophylaxis measures in rural properties.

3 METHODOLOGY

The present study was carried out from exploratory bibliographic research with the scientific databases SciElo, PubMed, CAPES, LILACS, MEDLINE, and Google Scholar. For the research, we used the time frame of publications made between the years 2003 to 2020, using the following keywords: dogs, neosporosis, abortion, cattle, reproduction, *Neospora caninum*, epidemiology, diagnosis, veterinary medicine and protozoa.

Articles on bovine neosporosis were included in the study, totaling 17 selected articles. Based on the material collected, a qualitative analysis was made about the selected articles, in order to try to evidence the risk factors, economic impacts, epidemiology of the disease and its prophylaxis, as well as the recognition of infected animals and the importance of diagnosis for the control of infection in herds.

4 DEVELOPMENT

Since its description, *Neospora caninum* has been identified in much of the world as an important causative agent of abortion (DUBEY; LINDAY, 1996). However, the insertion of this protozoan in bovine

health control programs, as well as the attribution of the clinical or subclinical disease to the parasite was for some years without proof and elucidation. This reality can be associated with the late description of the parasite, and is therefore very commonly confused with *Toxoplasma gondii* due to the close phylogenetic relationship between these two parasites. After the description, the international taxonomy committee classified the neosporosis agent as belonging to the phylum *Apicomplexa*, class *Sporozoa*, order *Eucoccidiorida*, suborder *Eiomeriose*, family *Sarcocystidae* and subfamily *Toxoplasmatinae* (OSHIRO, 2006).

The life cycle of *Neospora caninum* comprises three evolutionary stages: bradizoites, tachyzoites and sporozoites. The bradizoite forms (from the Greek bradys, meaning slow multiplication) are ovoid, represent the latency period, and have slowed multiplication. At this stage, the parasite is able to form tissue cysts. The cysts are usually oval, lunar or globular in shape, measuring about 107 µm in diameter and are found in the cells of the nervous system. Tachyzoites (from the Greek *tachys*, meaning rapid multiplication) exhibit rapid multiplication, the shape of a moon, measuring about 6.0 µm in length. The same can be found in nerve cells, macrophages, endothelial cells of the renal tubes, fibroblasts, hepatocytes and in various tissues of sick animals. Tachyzoites and tissue cysts are found intracellularly in intermediate and definitive hosts (DUBEY et al., 2002a). The bradizoites are ovoid, represent the latency period and have a slowed multiplication, in this period the parasite is able to form tissue cysts. The cysts are usually oval, lunar or globular in shape, measuring about 107 µm in diameter and are found in the cells of the nervous system. Tachyzoites and tissue cysts are found intracellularly in intermediate and definitive hosts (DUBEY et al., 2002a). Oocysts constitute the environmentally resistant form of the parasite. Each oocyst has in its interior two sporocysts, each with four sporozoites, which are the result of gametogonic sexual multiplication, which occurs in the process of enteroepithelial infection in dogs (WILLIAMS et al., 2009).

Neospora caninum has a facultative heteroxene cycle. This means that the parasite can complete its cycle only in the definitive host, or it can have several intermediate hosts. Thus, the contamination covers different species within the properties (ORTEGA-MORA et al., 2007). Bovine neosporosis can be transmitted from mother to child by transplacental route, which is called vertical transmission or congenital infection. This is considered the largest source of infection, because it can occur more than once in the same animal and through progeny through many generations (ANDERSON et al., 2000). Another form of infection is horizontal transmission also called postnatal infection, where intermediate hosts ingest cyst-infected tissues or food and water contaminated by sporulated oocysts of *Neospora caninum*. When these reach the stomach of the intermediate host, a disruption will occur that happens by mechanical action, resulting in the release of sporozoites in the intestine. These will proceed to the tissues where asexual multiplication will be carried out generating mobile tachyzoites, which will initiate an intense increase of protozoa in a small space of time. Through the bloodstream they spread to different tissues such as: pregnant

uteruses, hepatocytes, vascular endothelium, heart muscle, renal cells, alveoli, and placental appendages, in these tissues tachyzoites cause a cellular destruction resulting in an acute infection (MCALLISTER et al., 2016)

To understand the epidemiology of *Neospora caninum* it is important to know its prevalence and its geographical distribution (DUBEY et al; SCHARES et al; ORTEGA-MORA et al., 2007). Several countries have already confirmed neosporosis in their herds such as Africa, the United States, Germany, Mexico, Brazil, among others. Serological studies have been performed in several regions of the country, such as Bahia, São Paulo, Rio Grande do Sul, Paraná, Mato Grosso do Sul and Minas Gerais (ANDREOTTI et al., 1999; CORBELINI et al., 2000**;** LOCATELLI-DITTRICH et al., 2001).

In Brazil, the prevalence of 14.09% among dairy cattle in Bahia was highlighted (GONDIM et al., 1999). In the South region studies show that about 23% of herds with a history of abortion tested positive for the *Neospora caninum infection*, the rates reach almost 25% of seropositivity when positive animals without a history of abortion are adhered to. In Minas Gerais, seroepidemiological studies show a relevant variation between regions, the seroprevalence of the parasite ranges from 6.8% to 91.2%. In addition, the protozoan was detected in 81.8% of the fetuses examined by immunohistochemistry (CORBELLINI et al., 2002). It is noteworthy that the prevalence is variable and depends on the type of sampling used and the laboratory techniques employed.

The Amazon was considered a region free of neosporosis, studies done in the state found a prevalence of 8.8% in cows and 72% among farms (AGUIAR et al., 2006). These studies are of paramount importance in epidemiology, because in this way it is possible to have more information and rates of infection of the disease. Some states have not registered the presence of the infection, one of the reasons is usually the lack of tests and seroepidemiological studies, which ends up hindering the epidemiological reality *of Neospora caninum* in Brazil and the identification of infected females, which are responsible for the proliferation of the parasite through endogenous and vertical transmission routes. Studies prove that approximately 95% of seropositive cows will abort or generate infected calves (DINIZ et al., 2019; MCALLISTER, 2016).

Frequently induced abortions by *Neospora caninum* occur between 5 and 6 months of gestation (ANDERSON et al., 1991; WOUDA, 1998). In this period the fetus is not able to recognize pathogens and becomes more susceptible to infection. In the initial third of pregnancy, the bovine fetus does not recognize the pathogens, being vulnerable to *Neospora caninum* (BUXTON et al., 2002). Abortion can occur sporadically, endemic or epidemic, at any time of the year (DUBEY, 2003; Garcia, 2003; LLANO, 2013). Some signs that can also be observed are the death of the fetus in the womb, mummification, autolyzed fetuses, post-birth death, or birth with the chronic form of the disease, which does not present clinical signs, but is extremely worrisome because the animal has the ability to transmit infection and infertility. Fetuses

can die in the womb and be reabsorbed, mummified aborted stillborn or born alive but chronically infected (DUBEY; LINDSAY, 1996).

Infected females most often have a high number of antibodies that act against the parasite, but it is of paramount importance to emphasize that this fact does not represent maternal protection. The bovine fetus does not have the competence to mount an immune response against pathogens before 100 days of gestation (MALEY et al., 2003). This increase in antibodies means a higher rate of passage of *Neospora caninum* through the cow's placenta, injuring the fetus and thus causing miscarriage. Usually infected cows do not present any other clinical signs, besides these of reproductive character (ALMERIA et al., 2010).

The diagnosis of bovine neosporosis is made by a set of factors. It is necessary to associate the history of the herd in partnership with the clinical signs presented and the laboratory data. Abortion is the most relevant clinical sign presented in old animals, in young animals the appearance of neurological signs and polymyositis is observed. Direct methods detect forms of the parasite or parts of it, such as antigenic substances, while indirect methods depend on clinical, immunological, and biochemical evidence associated with infection (LINDSAY; DUBEY, 2020; CALLEFE et al., 2021). Laboratory confirmation is given by histopathological and immunohistochemical examinations in aborted tissues or fetuses. Serological methods such as ELISA, IFAT and NAT are used, and the RIF is the most indicated for presenting a higher performance among the three. Some authors such as Hasler et al. (2006) warn that it is necessary to be cautious when using ELISA, because it may present false-negative or false-positive results. Another method used is the molecular one, where the Polymerase Chain Reaction (PCR) is used a lot. PCR is a very sensitive and extremely assertive method in the diagnosis of *Neospora caninum.* This technique is mainly applied in the post-mortem diagnosis of neosporosis in fetal tissues (SUTEU et al., 2010). Annual economic losses due to neosporosis reach hundreds of thousands of dollars per year in the world (DUBEY, SCHARES AND ORTEGA-MORA, 2007).

The control of the disease becomes difficult because it does not have a vaccine or specific treatment. Some ongoing studies show that the inactive vaccine can help prevent vertical transmission, but it is worth mentioning that there has not yet been any confirmation of its effectiveness and the subject still causes many debates. There is a growing demand for the development of an efficient vaccine in order to prevent abortions in cattle and prevent the excretion of oocysts in the definitive hosts (CERQUEIRA-CÉZAR et al., 2017).

Considering that the greatest form of transmission of the disease occurs vertically, one way of prevention would be the use of serological screening in heifers and cows, in order to identify seropositive animals in the herd, thus being possible to dispose of contaminated animals and replace the herd using seronegative animals, minimizing the case rate present on the property and the losses due to abortions. Seropositive cows have a high risk of miscarriage and there is a high probability of congenital infection in calves born from these animals (MOEN et al., 1998). Another form of contamination occurs by the contact

of sporulated oocysts with the intermediate hosts. Therefore, it is extremely important to prevent or control the presence of dogs in the same environment as the animals. From the epidemiological point of view, it is also important to perform serology in dogs of the property (KATO, 2009).

Domestic dogs should not be fed raw meat and it should be avoided that they feed on carcasses of dead animals and remains of fetal tissues, this type of material should be collected from the environment. The responsible destination of carcasses and placental remains, which should be buried or incinerated, significantly impact the control of neosporosis (MEGID et al., 2016). The best way to prevent and control the disease is to perform seroepidemiological analysis and know the reproductive history. The purchase of animals proven to be negative for *Neospora caninum* is an important form of prevention (DUBEY, SCHARES E ORTEGA-MORA, 2007)

5 FINAL CONSIDERATIONS

Neosporosis is one of the main diseases that affects the herds of several countries. Being considered the biggest cause of abortions in Brazil, it is majestic that greater attention is given to it, which is often neglected by both farmers and veterinarians. Its impact goes beyond pathologies and directly affects the economy. Biosecurity measures should be used in sanitary, reproductive, nutritional and facility management along with prophylaxis to have a real control and then end the disorders caused by bovine neosporosis.

REFERENCES

CARVALHO, Rodrigo Pereira de et al. Neosporose bovina-revisão de literatura. 2014.

DIAS, Luzia Renata Oliveira et al. Doenças parasitárias reprodutivas em bovinos-neosporose. 2014.

MOTA, R. A. Situação da neosporose bovina no Brasil e métodos de diagnóstico. Medicina Veterinária (UFRPE), v. 2, n. 1, p. 38-48, 2008.

PARRA, Bruno César; PARRA, Brenda Silvia; NEVES, F. N. Neosporose uma doença que acomete abortos em bovinos. Rev Cient El Med Vet, v. 6, n. 1, p. 1-5, 2008.

SCHMIDT, Ana Carolina. Epidemiologia da neosporose bovina no Pantanal mato-grossense. 2017.

BRUHN, Fábio Rapahel Pascoti et al. Neosporose em ruminantes. PUBVET, v. 6, p. Art. 1270-1276, 2012.

ORLANDO, Débora R. et al. Abortos por Neosporacaninum em bovinos do sul de Minas Gerais. Pesquisa Veterinária Brasileira, v. 33, p. 1332-1338, 2013.

ANDREOTTI, Renato. Neosporose: um possível problema reprodutivo para o rebanho bovino. Campo Grande: Embrapa Gado de Corte, 2001.

ANDREOTTI, Renato et al. Diagnóstico e controle da neosporose em bovinos. Campo Grande: Embrapa Gado de Corte, 2003.

MELO, Debora Pereira Garcia. Neosporose bovina: prevalência, transmissão e diagnóstico em fetos abortados, nas microrregiões de Anápolis e Goiânia-Goiás. 2005.

JUFFO, Gregory Duarte. Aborto em bovinos principais causas infecciosas. 2010.

CARDOSO, José Márcio Sbruzzi. Aspectos epidemiológicos da infecção por Neospora caninum em bovinos leiteiros da região do Vale do Paraíba Paulista. 2010. Tese de Doutorado. Universidade de São Paulo.

DE OLIVEIRA, Charles Mendes et al. Situação da neosporose na bovinocultura brasileira. Pubvet, v. 14, p. 163, 2020.

VARASCHIN, Mary S. et al. Fatores associados a soroprevalência de Neospora caninum e Toxoplasma gondii em rebanhos caprinos na região sul de Minas Gerais. Pesquisa Veterinária Brasileira, v. 31, p. 53-58, 2011.

RIVERA, Hermelinda. Causas frecuentes de aborto bovino. Revista de Investigaciones Veterinarias del Perú, v. 12, n. 2, p. 117-122, 2001.

GASPARETTO, Raíssa et al. UTILIZAÇÃO DE PCR NA IDENTIFICAÇÃO DE NEOSPORA CANINUM EM BOVINOS DA FRONTEIRA OESTE DO RIO GRANDE DO SUL. Anais do Salão Internacional de Ensino, Pesquisa e Extensão, v. 13, n. 3, 2021.

LANGONI, Helio et al. Avaliação sorológica para Neospora caninum em propriedades de bovinos leiteiros com alterações reprodutivas. Veterinária e Zootecnia, v. 20, n. 1, p. 124-130, 2013.