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Review Article

Aspects and Applications of Epidemiological Chain of Bovine Neosporosis as Prophylaxis Tool on Rural Properties

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Abstract

Bovine Neosporosis (NB) directly interferes with the reproductive indices of bovine farming, being the main cause of abortions in bovine herds not only in Brazil, but also throughout the world. Given the importance of this disease for animal health, the present work points to the need for a reflection about the knowledge of the epidemiological chain of NB as a prophylactic tool in rural properties. The objectives of this work were to describe and point out the importance of knowledge about the biology and epidemiology of *Neospora caninum*, in order to subsidize effective measures of prophylaxis in rural properties. In the face of the analysis, it was possible to observe that in addition to the reproductive impacts, NB can cause abnormalities and neurological disorders in newborn calves, resulting in a reduced weight gain and growth delay, which generates large financial and sanitary losses to breeders. Measures such as the use of diagnostic tests for the identification of cases, sealing the presence of dogs in the production areas in order to avoid infection through feces in contact with water or food, as well as preventing the ingestion of placental remains or dead animals in the areas of the site are effective actions of prophylaxis of NB. In addition, integrated actions of sanitary, reproductive, nutritional and environmental handling represent tools to avoid the disorders caused by NB.

Keywords: Epidemiology; Abortions; Reproduction; *Neospora caninum*

Introduction

Systemic parasitic infections are responsible for serious damage to livestock. In the reproductive sphere of bovine farming, Bovine Neosporosis is described as one of the main diseases causing abortions in bovine herds, generating considerable reproductive and, consequently, economic impacts in the Brazilian livestock sector. This disease is caused by the protozoan *Neospora caninum*, a coccyde found in the form of tissue cysts and intra-cellular tachyzoites. This condition gives the parasite an efficient capacity of spread and transmission within cattle flocks, and can infect up to 90% of animals in confinement with milking or for slaughter [15].

The protozoa *Neospora caninum* was first described in 1988 in Norway, involving puppies of dogs, 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, based on serological studies, the disease has been endemically in several regions of the country, such as Bahia, São Paulo, Rio Grande do Sul, Paraná, Mato Grosso do Sul and Minas Gerais [4].

The biological cycle of the *Neospora caninum* is heterogeneous, with the domestic dog of the subspecies Canis lupus familiaris described as the usual and definitive host of the parasite, and can also encounter several intermediate hosts such as equines, 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 [22]. In addition to the domestic dog, the coyote (Canis latrans), the wolf (*Cannis lúpus*) and the dingo (Canis lúpum dingo) can be considered definitive hosts. Moreover, more 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 positive for anti-Neospora antibodies and negative for Toxoplasma. gondii antibody, suggesting that the disease can be considered zoonotic [25].

Basically, there are two forms of transmission of bovine neosporosis that determine the spread between herds, and can occur horizontally through the consumption of water or food contaminated with oocysts of dogs or other infected canids; or vertically, where transplacentary transmission occurs. It is important to point out the importance of endogenous transplacentary transmission in the maintenance of the disease in the flocks, since about 95% of the seropositive cows will abort or generate seropositive calves [9].

In addition, it is important to highlight that the economic damage caused by *Neospora caninum* is related to diseases of the reproductive system, which mainly include disorders, such as return to the stomach, with regular or irregular intervals, abortions, birth of weak and inviable animals, with neurological signs or still in the condition of persistently infected [2]. Barros et al. [6] that the damage caused by neosporosis repercussions in different proportions, according to the technological level of the rural enterprise and with negative consequences for the state economy.

The main clinical sign associated with adult female infection is miscarriage, which is most common between the fifth and sixth months of gestation. Already in neonates, neurological changes, deaths, mummification, bad formations, myocarditis and also polymyositis can be observed. If it occurs in the late period of gestation, the delivery will occur normally, but the calf can be congenitally infected, and the infection can be repeated in future generations [17].

The control of the disease is made through the correct and accurate diagnosis, which can be made through laboratory tests, such as immunohistochemical tests, histopathology and polymerase chain reaction. (PCR). Also, the method of insulation of the parasite in cell cultures can be used. It is important to stress that serological methods end up standing out from the tests described earlier, because they are a valuable tool used in longitudinal and transversal epidemiological studies. Several serological tests, including ELISA, RIFI, TAD can be used to identify anti-Neospora antibodies in serum and cavitary fluids [11]. Studies of seroprevalence of Neosporosis point out that the parasitosis is endemic in national territory and that, therefore, may not be easily perceived by the rural producer; it is necessary to awaken him to the economic losses caused by the disease, as well as the need to adopt economically viable control strategies for the rural enterprise.

In view of the above, the general objective of the work was to analyze, from the literature, the economic and reproductive impacts caused by *Neospora caninum* on infected cattle flocks. The specific objectives were to describe and highlight the importance of prevention and knowledge on the biology and epidemiology of the etiological agent of bovine neosporosis, in order to subsidize effective measures of prophylaxis in rural properties.

Methodology

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

The study included articles presenting the topic of bovine neosporosis, totalling 17 selected articles. The inclusion and exclusion criteria were defined based on the proposal that guides the research, the identification of relevant studies through

different sources, the composition of the elucidative content based on the search and inclusion/exclusion criteria, and the extraction of data related to the research question, including general information about the study. Based on the collected material, a qualitative analysis of the selected articles was carried out, with the aim of seeking to highlight the risk factor, economic impacts, epidemiology of the disease and its prophylaxis, as well as the recognition of infected animals and the importance of the diagnosis for the control of the infection in the flocks.

Results and Discussion

Since its description, *Neospora caninum* has been identified in much of the world as an important abortion-causing agent [13]. However, the inclusion of this protozoa in bovine health control programmes, as well as the assignment of clinical or subclinical disease to the parasite remained for a few years without proof and clarification. 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 agent of neosporosis as belonging to the phylum Apicomplexa, class Sporozoa, order Eucoccidiorida, suborder Eiomeriose, family Sarcocystidae and subfamily Toxoplasmatinae [22].

Serological studies from several countries have shown the parasite to be the major cause of abortions in dairy herds. The disease is widely spread on different continents. The economic importance of Bovine Neosporosis is mainly attributed to the costs associated with abortion, the value of fetuses, artificial insemination or covering, decreased milk production, increased culling, and replacement of animals [12]. Possible risk factors associated with the prevalence of this disease have been associated with variables such as location with endemic geographic coordinates, measures adopted to prevent and control infection, individual characteristics of each animal, as well as health data were addressed. Thus, it is possible to perceive that Bovine Neosporosis is widely disseminated in rural properties, presenting an association with a history of abortion on the properties, presence of dogs and sale of cattle for reproduction, and the relative risk of the disease is constant [14].

In addition, it is important to point out that the risk of infection with *Neospora caninum* in cattle is related to numerous variables or determining factors of its natural history of occurrence, such as the type of farm (beef, milk or mixed), the type of breeding (confined or extensive), the use of artificial insemination, the predominant breed (European breed of milk, and the type of breed of *Neospora caninum*). Zebu or crossbred), presence of dogs, sheep, goats and horses on the property, presence of wild species, deer and capybaras living freely on the property, history of abortions, destination of aborted fetuses and placentas, acquisition of females or males for the purpose of reproduction and origin of the animals acquired (auction/exhibition, cattle trader or other properties) [26].

In addition, seropositivity, in general, increases with age, suggesting that horizontal transmission of *Neospora caninum* plays an important role in some herds. The age of females is associated with animal seropositivity. From 24 months, each year of life, there is a 1.012-fold increase in the chance of the animal becoming seropositive to the protozoan. Farms with higher prevalence for *Neospora caninum* had animals with nutritional status classified as poor, while in those where the animals had regular nutritional status, the prevalence was lower [5].

The life cycle of *Neospora caninum* consists of three evolutionary stages: bradizoites, tachizoites and sporozoites. The bradizoite forms (from the Greek bradys, which means slow multiplication) are ovid, represent the latency period and have a slowed multiplication. At this stage, the parasite is able to form tissue cysts. The cysts usually have an oval, lunar or globular shape, measuring about 107 µm in diameter and are found in the cells of the nervous system. The tachizoites (from the Greek tachys, which means rapid multiplication) have a fast multiplication, the moon-shaped, measuring about 6.0 µm in length. They can be found in nerve cells, in macrophages, endothelial cells of the kidney tubes, fibroblasts, hepatocytes and in various tissues of sick animals. Tachizoites and tissue cysts are found intracelularly in intermediate and definitive hosts [12].

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cells of the nervous system. Tachizoites and tissue cysts are found intracelularly in intermediate and definitive hosts [12]. Oocysts are 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 sexuated multiplication, which occurs in the process of enteroepithelial infection in dogs. *Neospora caninum* has an optional heteroxene cycle. This means that the parasite can complete its cycle only in the definitive host or, still, can have several intermediate hosts. Thus, the infection covers different species within the properties [14].

Bovine Neosporosis can be transmitted from mother to child via transplacental, which is called vertical transmission or congenital infection. This is considered the largest source of infection, as it can occur more than once in the same animal and through offspring through many generations. Another way of infection is the horizontal transmission also called postnatal infection, where intermediate hosts ingest tissues infected by cysts or food and water contaminated by spore oocysts of *Neospora caninum* [3].

When the spore oocysts reach the stomach of the intermediate host, breakage occurs by mechanical action, resulting in the release of sporozoites in the intestine. These follow to the tissues where asexual multiplication occurs generating mobile tachizoites, which initiate an intense increase of protozoa in a short time. Through the bloodstream, they spread through different tissues such as uterus, hepatocytes, vascular endothelium, heart muscle, kidney cells, alveoli and placental appendages. In these tissues, tachizoites cause cell destruction resulting in acute infection [19].

To understand the epidemiology of *Neospora caninum*, it is important to know its prevalence and its geographical distribution [14]. Several countries have already confirmed Neosporosis in their flocks such as Africa, the United States, Germany, Mexico and Brazil. In Brazil, serological studies have been conducted in several regions, such as Bahia, São Paulo, Rio Grande do Sul, Paraná, Mato Grosso do Sul and Minas Gerais [5].

In Brazil, it was highlighted the prevalence of 14,09% among dairy cattle in Bahia [26]. In the South region studies show that about 23% of the flocks with a history of abortion tested positive for *Neospora caninum* infection, the indices reach almost 25% of seropositivity when adhered positive animals with no history of miscarriage. In Minas Gerais seroepidemiological studies show a significant variation between regions, the seroprevalence of the parasite ranges from 6.8% to 91.2%. In addition, the protozoa was detected in 81.8% of the fetuses examined for immunohistochemistry. It is worth noting that the prevalence is variable and depends on the type of sampling used and the laboratory techniques employed [9].

The Amazon was considered a Neosporosis-free region, but studies carried out in the state found a prevalence of 8.8% in cows and 72% among farms [1]. These studies are of the utmost 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 making it difficult the epidemiological reality of *Neospora caninum* in Brazil and the identification of infected females, which are responsible for the proliferation of the parasite through the endogenous and vertical pathways of transmission. Studies show that approximately 95% of HIV-positive cows will abort or generate infected calves [10,21].

Frequently, abortions induced by *Neospora caninum* occur between 5 and 6 months of gestation [3]. During this period, the fetus is unable to recognize pathogens and becomes more susceptible to infection. In the early trimester of pregnancy, the bovine fetus does not recognize the pathogens, being vulnerable to *Neospora caninum* [7]. Abortion can occur sporadically, endemically or epidemically, at any time of the year [12]. Some signs that can also be observed are the death of the fetus in the uterus, mummification, autolised fetuses, death after birth, or birth with the chronic form of the disease, which does not present clinical signs, but is extremely worrying as the animal has the ability to transmit infection and infertility. The fetuses can die in the womb and be reabsorbed, aborted mummified, born dead or born alive, but chronically infected [14].

Infected females most often have a high number of antibodies that act against the parasite, but it is of utmost importance to highlight that this fact does not represent maternal protection. The bovine fetus has no competence to assemble an immune response against pathogens before the 100 days of gestation [20].

This increase in antibodies means a higher rate of passage of *Neospora caninum* through the cow's placenta, injuring the fetus

and thus causing abortion. Normally, infected cows show no other clinical signs besides those of a reproductive character [2].

The diagnosis of Bovine Neosporosis is given by a set of factors. It is necessary to associate the history of the flock in partnership with the presented clinical signs and laboratory data. Abortion is the most relevant clinical sign presented in old animals, in young animals is observed the appearance of neurological signs and polymyositis. Direct methods detect forms of the parasite or parts thereof, such as antigenic substances, while indirect methods depend on clinical, immunological and biochemical evidence associated with infection [19].

Laboratory confirmation is given by means of histopathological and immunohistochemical tests in aborted tissues or fetuses. Serological methods such as ELISA, RIFI and NAT are used, the RIF being the most indicated for showing 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, as it may show false negative or false positive results. Another method used is molecular, where PCR is widely used. PCR is a very sensitive and extremely assertive method in the diagnosis of *Neospora caninum*. This technique is mainly applied in post-mortem diagnosis of neosporosis in fetal tissues [25]. Annual economic losses resulting from neosporosis reach hundreds of thousands of dollars per year in the world [15].

Control of the disease becomes difficult because there is no vaccine or specific treatment. Some ongoing studies show that the inactive vaccine can help in preventing vertical transmission, but it is worth noting that there has not yet been any confirmation of the effectiveness of the vaccine and the subject still causes a lot of debate. There is a growing demand for an effective vaccine to be developed in order to prevent abortions in cattle and prevent the excretion of oocysts in the final hosts [8].

Given that the largest form of transmission of the disease occurs vertically, one way of prevention would be to use serological screening in calves and cows, in order to identify seropositive animals in the herd, thus making it possible to dispose of contaminated animals and reposition the flock using seronegative animals, minimizing the case rate present on the property and the losses by abortions. Seropositive cows have a high risk of miscarriage and there is a high probability of congenital infection in calves born of these animals [23]. Another form of contamination occurs by contact of spore oocysts with intermediate hosts. Therefore, it is extremely important to prevent or control the presence of dogs in the same environment as animals. From an epidemiological point of view, it is also important to carry out serology in farm dogs [18].

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 tissue, this type of material should be collected from the environment. The fate of carcasses and placental remains, which must be buried or incinerated, significantly impact on the control of neosporosis [22]. The best way to prevent and control the disease is to perform seroepidemiological analyses and know the reproductive history. The purchase of animals proven negative for *Neospora caninum* is an important form of prevention [24].

Conclusion

Bovine Neosporosis is one of the main diseases affecting herds in several countries, and is considered one of most widespread and frequent causes of abortion in cattle throughout the world. In Brazil, it is considered the disease with the greatest impact by abortions that usually occur in the fifth month of gestation, and by mortality, leading to large economic losses.

In this context, greater attention is needed to this disease, which is often neglected, both by rural producers and by veterinarians, because its impacts, which go beyond pathologies, also directly affect the global economy. Furthermore, biosafety measures must be used in sanitary, reproductive, nutritional and facility handling together with prophylaxis in order to have real control and then end the disorders caused by Bovine Neosporosis.

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