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ROBERTO ALVES BEZERRA

Epidemiologia e dinâmica sorológica na infecção natural por *Neospora caninum* em ovinos no Semiárido Brasileiro

Patos/PB
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Dissertação submetida ao Programa de Pós-Graduação em Ciência e Saúde Animal, da Universidade Federal de Campina Grande, como requisito parcial para obtenção do grau de Mestre em Ciência e Saúde Animal.

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ROBERTO ALVES BEZERRA

EPIDEMIOLOGIA E DINÂMICA SOROLÓGICA DE IGG DA INFECÇÃO NATURAL POR *NEOSPORA CANINUM* EM OVINOS NO SEMIÁRIDO BRASILEIRO

Dissertação apresentada ao Programa de Pós-Graduação em Ciência e Saúde Animal como pré-requisito para obtenção do título de Mestre em Ciência e Saúde Animal.

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SUMÁRIO

RESUMO

A neosporose é uma doença causada por um parasito intracelular obrigatório, *Neospora caninum*, capaz de infectar diversos hospedeiros e de provocar falhas reprodutivas graves em fêmeas. Esta dissertação é constituída por dois capítulos: o primeiro capítulo intitulado **Dynamics of *Neospora caninum* transmission in naturally infected sheep under semiarid conditions** teve como objetivo realizar um estudo longitudinal da ocorrência de *N. caninum* para determinar o perfil sorológico de cordeiras fêmeas no primeiro ano de vida, indicando qual é a principal faixa etária para a ocorrência da primo-infecção em condições de semiárido. Neste estudo, foram acompanhadas 59 crias fêmeas desde o primeiro até o décimo segundo mês de vida para a detecção de anticorpos IgG anti-*N. caninum* por meio da Reação de Imunofluorescência Indireta (RIFI). Das cordeiras acompanhadas, 61% (36/59) foram positivas para anticorpos anti-*N. caninum* em pelo menos um dos 12 meses de avaliação. Nas filhas de mães positivas, foi observada uma menor oscilação da produção de anticorpos ao longo do período. Houve correlação entre os títulos de anticorpos das mães e das filhas no primeiro mês de vida ($r = 0.59$; 95% CI = 0.39 – 0.73; $P < 0.0001$). Observou-se, ainda, que 76,7% (23/30) das infecções ambientais ocorreram até a idade reprodutiva (seis meses) ($p < 0,0001$). Concluiu-se que a primo-infecção, na maioria dos animais, acontece até a idade reprodutiva. O segundo capítulo intitulado **Detection of anti-*Neospora caninum* IgG in blood serum and colostrum samples in naturally infected sheep** objetivou detectar, por meio da RIFI, a correlação de anticorpos IgG para *N. caninum* em amostras de soro e colostro de matrizes ovinas e avaliar a presença desta imunoglobulina no soro dos filhotes recém-nascidos, após a ingestão do colostro. Para esse estudo foram coletadas 162 amostras de sangue e colostro de matrizes que se apresentavam hígidas não apresentavam nenhuma enfermidade no exame físico geral, recém paridas (máximo 5 dias pós-parto) e 182 amostras de sangue de neonatos. Das matrizes analisadas, 27,8% (45/162) foram positivas para a presença de IgG anti-*N. caninum*, das quais 53,8% (24/45) não tiveram anticorpos para este protozoário detectados no colostro. Todas as matrizes que apresentaram o colostro positivo tiveram suas crias reagentes. Concluiu-se que os resultados obtidos demonstram boa concordância entre a detecção de anticorpos anti-*N. caninum* no colostro se comparado à matriz e ao borrego, sendo uma alternativa no diagnóstico da doença em rebanhos.

PALAVRAS-CHAVE: Colostro; imunidade; neosporose; Reação de Imunofluorescência Indireta.

ABSTRACT

Neosporosis is a disease caused by an obligate intracellular parasite, *Neospora caninum*, capable of infecting several hosts and causing severe reproductive failure in females. This dissertation consists of two chapters: the First Chapter entitled **Dynamics of *Neospora caninum* transmission in naturally infected sheep under semiarid conditions**, aimed to carry out a longitudinal study of the occurrence of *N. caninum* to determine the serological profile of female lambs in the first year of life, indicating the main age group for the occurrence of primary infection in semiarid conditions. In this study, 59 female pups were followed from the first to the twelfth month of life, for the detection of IgG anti-*N. caninum*, through the Indirect Immunfluorescence Reaction. 61.0% (36/59) of the ewes followed were positive for anti-*N. caninum* in at least one of the 12 months of evaluation. In the daughters of positive mothers, a smaller fluctuation in the production of antibodies was observed over the period. There was a correlation between the antibody titers of mothers and daughters in the first month of life ($r = 0.59$; 95% CI = 0.39 – 0.73; $P < 0.0001$). It was also observed that 76.7% (23/30) of environmental infections occurred until reproductive age (six months) ($p < 0.0001$). It was concluded that primary infection, in most animals, occurs until the reproductive age. The second Chapter entitled **Detection of anti-*Neospora caninum* IgG in blood serum and colostrum samples in naturally infected sheep**, aimed to detect through IFRI the correlation of IgG antibodies to *N. caninum* in serum and colostrum samples from sheep matrices, and also to evaluate the presence of this immunoglobulin in the serum. of newborn pups after colostrum ingestion. For this study, 162 blood and colostrum samples were collected from sows that did not present any disease in the general physical examination, newly born with a maximum of 5 days postpartum and 182 samples from neonates. 27.8% (45/162) of the matrices were positive for the presence of IgG anti-*N. caninum*, in which 53.8% (24/45) had no antibodies to this protozoan detected in colostrum. All sows that showed positive colostrum had their offspring reacting. It was concluded that the results obtained demonstrate good agreement between the detection of anti-*N. caninum* in colostrum compared to matrix and lamb, being an alternative in the diagnosis of the disease in herds.

KEY-WORDS: Colostrum; immunity; neosporosis; Indirect Immunofluorescence Reaction.

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CAPÍTULO 1

LISTA DE ABREVIAÇÕES

DNA - Deoxyribonucleic acid

Ha - Hectare

IFAT - Indirect Fluorescence Aantibody

IgG - Immunoglobulin G

IFPB - Instituto Federal da Paraíba

LIID - Laboratory of Immunology and Infectious Diseases

LTh1 - T helper 1 lymphocytes

LTh2 - T helper 2 lymphocytes

Log - Logarithm

ml - milliliter

mm - millimeter

PCR - *Polymerase Chain Reaction*

RIFI - Reação de Imunofluorescência Indireta

UFCG - Universidade Federal de Campina Grande

°C - Degrees Celsius

INTRODUÇÃO GERAL

A ovinocultura destaca-se como uma importante atividade pecuária no semiárido brasileiro, essa exploração apresenta inúmeras vantagens, como necessidade de menor área de criação, menor consumo de alimentos, facilidade de manejo e grande diversidade de produção de carne e couro de boa qualidade, servindo como alternativa de renda. Esses fatores associados à boa adaptabilidade dos ovinos aos ecossistemas locais, torna essa atividade pecuária muito frequente nessa região (REIS et al., 2015; AZAMBUJA-RIBEIRO & GONZÁLEZ-GARCÍA, 2016).

O estado da Paraíba tem um rebanho de 712.632 mil cabeças ovinas, com mais da metade inseridas na zona semiárida, apresentou crescimento considerável entre 2016 e 2020, com aumento entre 2019/2020 de 6,5% (MAGALHÃES et al., 2020). A grande maioria da criação é caracterizada pelo manejo semi-intensivo, com propriedades com área entre 15 e 40 ha. Os animais normalmente são criados sem suplementação alimentar e mineral, alimentando-se apenas da vegetação nativa. Os rebanhos têm em média 50 animais, representados por animais rústicos como ovinos da raça Santa Inês e mestiços. No manejo reprodutivo, as fêmeas, em sua grande maioria, entram na fase reprodutiva entre quatro e oito meses de idade, com parição por volta de um ano a um ano e meio. A maior parte dos produtores tem a ovinocultura para subsistência e não comercialização, sendo importante fonte de alimento para as populações do meio rural, fornecendo carne e leite (SILVA et al., 2018; BEZERRA et al., 2022).

No entanto, problemas sanitários na ovinocultura brasileira são comuns, causando sérios prejuízos econômicos para os criadores, principalmente na reprodução, ocorrendo comumente casos de abortos e ineficiência reprodutiva nessa espécie no semiárido brasileiro e no estado da Paraíba (NÓBREGA JR et al., 2005). Neste contexto, às doenças que tradicionalmente fazem parte do manejo sanitário vem se juntar as enfermidades emergentes, dentre as quais se destaca a neosporose pelas alterações reprodutivas que esta pode causar (RIZZO et al., 2018).

A neosporose é uma doença causada por um parasito intracelular obrigatório, *Neospora caninum*, capaz de infectar diversos hospedeiros como bovinos, ovinos, caprinos equinos, caninos e animais silvestres. A eliminação dos oocistos ocorre nas fezes de canídeos infectados, hospedeiros definitivos, e quando são ingeridos pelos hospedeiros intermediários, como os ovinos, podem desenvolver a doença e desencadear surtos de abortos (DUBEY et al., 2007).

Os pequenos ruminantes, assim como os bovinos, estão susceptíveis a parasitos como *N. caninum*, sendo constantemente associados a distúrbios reprodutivos. A grande maioria das pesquisas sobre a importância de *N. caninum* como causador de enfermidades que levam às

perdas reprodutivas são realizadas de forma experimental. De acordo com Azevedo-Filho et al. (2017), estudos observacionais que refletem o que ocorre quando parâmetros como a dose de infecção, o estágio de gestação e a virulência do parasito não são controlados são extremamente importantes para estudar a dinâmica natural das infecções.

Portanto, devido à importância econômica que tem *N. caninum* e carência de estudos no estado da Paraíba sobre a infecção por este protozoário em ovinos, para o primeiro capítulo, objetivou-se estudar sua epidemiologia e transmissão em ovinos naturalmente infectados no semiárido paraibano, avaliando o perfil do *status* sorológico desses animais do nascimento à idade reprodutiva, definindo se as fêmeas chegam protegidas com anticorpos anti-*N. caninum* na sua primeira gestação. Foi realizado no segundo capítulo dessa dissertação um estudo de detecção de anticorpos anti-*N. caninum* no colostro de ovinos, avaliando a eficiência da imunização passiva do neonato para esse agente, e a determinação da concordância dos anticorpos séricos e colostrais da matriz pela Reação de Imunofluorescência Indireta (RIFI), considerando a passagem dos mesmos para a glândula mamária.

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CAPÍTULO I

DYNAMICS OF *NEOSPORA CANINUM* TRANSMISSION IN NATURALLY INFECTED SHEEP UNDER SEMIARID CONDITIONS

Autores

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Dynamics of *Neospora caninum* transmission in naturally infected sheep under semiarid conditions

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Abstract

This study longitudinally assessed the serological profile of *N. caninum* occurrence among female lambs in the first year of life to determine the main age at which primary infection occurred under semiarid conditions. Blood samples were collected from 53 pregnant ewes from farms in Paraíba state, Brazil in the month before giving birth, and their 59 female offspring were sampled monthly up to one year of age. The indirect fluorescence antibody test was used,

with a cutoff point of 1:50. Among the pregnant ewes, 26.4% (14/53; CI = 23.8%-29.3%) were positive. Among the offspring, 61% (36/59; CI = 57.2%-63.4%) of the lambs monitored were positive for anti-*N. caninum* antibodies in at least one of the 12 months of evaluation. Transient antibody production was observed for a short period after seroconversion. Among the offspring of positive mothers, a smaller oscillation in the production of antibodies was observed over the period. There was a correlation between the antibody titers of mothers and offspring in the first month of life ($r = 0.59$; $P < 0.0001$). It was also observed that 76.7% (23/30) of post-natal infections occurred before reproductive age (six months) was reached ($p < 0.0001$). It was concluded that in most lambs, primary infection occurs up to reproductive age. Furthermore, infection by *N. caninum* occurs throughout the year in this semiarid environment, giving rise to transitory production of antibodies for this protozoon. This emphasizes the importance of longitudinal studies.

Keywords: immunity, serology, neosporosis, reproduction.

1. Introduction

Neospora caninum is a protozoon of the phylum Apicomplexa. It is an obligate intracellular parasite belonging to the family Sarcocystidae, with wide geographic distribution, and is constantly listed as one of the main causes of abortion and reproductive failure in cattle (Dubey, 2013; Reichel et al., 2013). Over recent years, neosporosis has been reported as a cause of reproductive diseases in small ruminants (Nunes et al., 2017; García-Sánchez et al., 2020), with abortion outbreaks reported in different regions of the world (Moreno et al., 2012; Pinto et al., 2012; González-Warleta et al., 2014; Al-Shaeli et al., 2020). Sánchez-Sánchez et al. (2021) verified that *N. caninum* was the cause of low reproductive performance in a sheep flock in Spain. Similarly, Della Rosa et al. (2021) reported *N. caninum* was the main pathogen detected in abortions and perinatal deaths in different flocks in Argentina, suggesting that *N. caninum* is efficiently transmitted to small ruminants and a frequent cause of ovine reproductive failure, in contrast to Australia, which Clune et al. (2020) did not observe that *N. caninum* is a cause of abortion and stillbirth. In Brazil, Pinto et al. (2012) reported an abortion outbreak in ewes during the final third of pregnancy associated with *N. caninum* infection, and neurological signs associated with neosporosis have been reported in newborn and adult sheep in Brazil (Bishop et al., 2010; Pereira et al., 2021).

The definitive hosts for this protozoon are canids, which can eliminate oocysts through their feces. This leads to infection of intermediate hosts such as ruminants (exogenous transmission), mainly through ingestion of contaminated water and food (Cerqueira-Cézar et al., 2017). *N. caninum* has already been described in different species of intermediate hosts, such as cattle, buffaloes, sheep, goats, horses and pigs, in different regions of the world (Mahajan et al., 2019; Al-Shaeli et al., 2020; Gui et al., 2020; Leszkowicz Mazuz et al., 2020; Oliveira Junior et al., 2020).

Infection of ewes during the gestational period, through exogenous transmission, is capable of triggering acute neosporosis, given that during pregnancy there is a shift in the response from T helper 1 lymphocytes (LTh1) to T helper 2 lymphocytes (LTh2), which is less effective against intracellular pathogens. On the other hand, in ewes whose primary infection occurred prior to pregnancy, cysts are more likely to form, thus characterizing a chronic stage of the infection. However, when these ewes become pregnant, it is possible that with the modulation of the immune response caused by pregnancy, recrudescence of infection may occur (endogenous transmission). In these cases, bradyzoites become reactive and transform to the infective form of tachyzoites, which leads to infection of placenta and/or the fetus (Dubey et al., 2017; Tizard, 2018). Recrudescence of *N. caninum* infection in infected ewes can compromise pregnancy and the fetus and is directly dependent on the immune response produced by the mother (Gutiérrez-Expósito et al., 2020). Among ruminants, the main means of maintenance of *N. caninum* in herds is through the endogenous route (Dubey et al., 2017).

Epidemiological studies in Brazil have shown frequencies of seropositivity for anti-*N. caninum* antibodies ranging 20.3% - 60.6% among sheep in the states of Rio Grande do Sul, Rondônia and Sergipe, respectively, using indirect fluorescence antibody test (IFAT) (Rizzo et al., 2017; Consalter et al., 2020; Maia et al., 2021). The majority of *N. caninum* seroprevalence studies on have depicted a single point in time, thus reflecting the animals' immune status without distinguishing between new and old cases. In contrast, longitudinal seroprevalence studies identify frequency of cases over a period of time (Thrusfield, 2018) and provide a better indication of the occurrence of the disease in a given population as this may address variation in the duration of antibody production according to the infecting dose and the individuals' immune status.

Sheep-rearing is of growing economic importance in Brazil, but infection due to *N. caninum* has been gaining prominence in some sheep-rearing regions (Liu et al., 2015; Maia et

al., 2021). Moreover, studies on natural infection by this protozoon in the semiarid region of Brazil remain at an initial stage. Therefore, the aim of the present study was to determine the serological profile among female lambs in this region, in their first year of life, so as to indicate the main age range over which primary infection by *N. caninum* occurs.

2. Materials & Methods

2.1. Experimental design

During the years from 2018 to 2020, blood samples were collected from 182 ewes, of no defined breed, that were in their final period of gestation. Among these samples, those from ewes that gave birth to male lambs and those from ewes, which for some reason it was not possible to monitor their female lambs until they reached reproductive age, i.e. until at least the age of six months, were discarded from the experiment. Therefore, 59 female offspring from 53 ewes were monitored monthly, through blood samples, from the first to the twelfth month of life, to detect IgG anti-*N. caninum* antibodies.

2.2. Characterization of farms and herds

The farms studied are located in the semiarid region of Paraíba. This climate is characterized by high annual temperatures, ranging from 20º to 38ºC throughout the year. The dry season lasts about eight months, while the wet season lasts just four months, with rainfall from 528 to 750 mm (INMET, 2010; Azevedo et al., 2017).

Pregnant ewes were selected from seven farms in the Sertão region of the state of Paraíba, northeastern Brazil, which is an area of semiarid climate (Fig. 1). Farms and were located in the municipalities of Aparecida (farm 1, n=4 ewes and n=4 lambs), Sousa (farm 2 with n=8 ewes and n=9 lambs; farm 3 with n=6 ewes and n=6 lambs; farm 4 with n=13 ewes

and n=15 lambs; farm 5 with n=7 ewes and n=8 lambs), Paulista (farm 6, n=12 ewes and n=14 lambs) and Pombal (farm 7, n=3 ewes and n=3 lambs).

To avoid potential sources of bias, the matrices, at the time of selection, were identified with numbers, as well as their respective offspring, this identification was essential so that the matrices and their offspring could be compared. Another measure adopted, was the inclusion in the study of only the offspring who were able to carry out the 12 collections.

Farms were selected for inclusion according to convenience sampling. Occurrences of abortions and birth of premature lambs were reported on all of the study farms. Ewes were identified with numbered tags at the time of selection, as well as their respective offspring. A requirement for inclusion in the study was ability to sample the offspring for 12 collections at one-month intervals. The main management systems used on these farms were extensive and semi-intensive systems, in which sheep reproduction, rearing and fattening were undertaken with the purpose of slaughter. High turnover of animals in the flocks was reported with frequent purchase and selling of stock, but only ewes and lambs that were born and raised up to twelve months on the same farm were included in the study. Rams used on the study farms were borrowed from other properties in some instances, but the study farms did not have any history of acquisition of animals from each other.

2.3. Collection of blood samples

Blood samples were taken from ewes that were in the final third of gestation and from their female offspring. Among the offspring, blood samples were collected monthly, commencing in the first month of age until lambs were 12 months old. Blood was collected by means of venipuncture of the external jugular vein. Approximately 5 ml were collected in vacuum tubes without anticoagulant. These samples were kept cool in an isothermal box and were sent to the laboratory, where they were centrifuged at 1000 × g for 10 minutes. From this,

blood serum was obtained, which was then divided into identified aliquots and stored at a temperature of -20 °C, until the time of serological tests.

2.4. Serological tests

Anti-*N. caninum* IgG was detected by means of IFAT at the Laboratory of Laboratory of immunology and infectious diseases, from the Instituto Federal da Paraíba.

The serum dilution used as a cutoff point was 1:50, as described by Helmick et al. (2002), using the NC-1 *N. caninum* tachyzoites as the antigen (Dubey & Beattie, 1988). Fluorescein isothiocyanate-labeled anti-sheep IgG conjugate (whole molecule, SIGMA, St. Louis, MO, USA) was used. The slides were evaluated under a fluorescence microscope. Positive and negative control samples that had previously been tested for validation of the reaction, were added to all slides. Samples were considered positive when complete peripheral fluorescence was identified in more than 50% of the tachyzoites in different fields of each well (Kim et al., 2019). Serum samples with titers ≥ 50 were titrated from sequential dilutions at base two until negative.

2.5. Statistical analysis

To verify the correlation between antibody titers from positive ewes and their offspring, Pearson's correlation analysis was performed. Association analysis was performed between ewe serological status in pregnancy and lamb serological status at birth/before 6 months of age by Chi-square or Fisher's exact test. The analysis on the antibody titers of the offspring from positive and negative mothers, over the 12-month period, was performed using a general linear model (two-way ANOVA) for repeated measures, including the individual lamb as a random factor nested within the serological status of the ewe during pregnancy. For this analysis, antibody titers were transformed into $\log_{10}{}^{+1}$ to approximate normal distribution. The

significance level was set at 5% and all analyses were done in the R environment R Team (2021), using the packages *dplyr, car, rstatix, DescTools, emmeans* and *ggplot2*.

3. Results

Among the 53 ewes selected for the experiment, 14 (26.4%; CI = 23.8%-29.3%) were seropositive, with titers ranging from 1:50 to 1:12,800. Twin offspring were born to 11.3% (6/53; CI = 9.2%-13.7%) ewes included in the study, however none of these ewes were seropositive for *N. caninum*. Ewes that were positive for *N. caninum* were identified on all the farms. The litter size between positive and negative ewes did not differ statistically (P = 0.850), and was 1.0 and 1.3 offspring per ewe, respectively.

It was found that 61% (36/59; CI = 57.2%-63.4%) of the offspring were positive for anti-*N. caninum* antibodies in at least one of the 12 months of evaluation. The six positive offspring that were positive in the first month, all of them had positive mothers and there was a correlation between the mothers' and offspring's titers in the first month (Table 1, r = 0.59; P < 0.0001). Among the offspring of negative mothers (78%; 46/59), post-natal seroconversion was observed in 50% (23/46), occurred after the third month of life, with a peak of seropositivity in the IFAT at six months (Fig. 2).

For the 30 offspring for which seropositivity detected up to 12 months of age but were not positive in the first month, seroconversion was more likely to be detected by 6 months of age (23/30 offspring) compared to the period 7-12 months (7/30; P < 0.0001).

Among the offspring of positive ewes, a low rate of passive immunization for *N. caninum* was found: only 42.8% (6/14) were reactive in the first month of evaluation and the titers varied between 1:100 and 1:6400 (Table 2). It was also found that among the six offspring from positive ewes, three were seroreactive from the first to the last month of evaluation and in

these animals, there was less oscillation in the monthly titration of antibody levels for *N. caninum*, which suggested that vertical transmission occurred in these offspring.

It was observed that among the offspring that seroconverted from the 4th month onwards, only 13.8% (4/29) remained seropositive at the times of all subsequent collections. In 86.2% (25/29), transient antibodies against *N. caninum* were observed, with great variation between the months evaluated: lambs that had high titers in one month or in sequential months were negative in the following month (Table 2).

During the 12 months of evaluation, among the offspring of negative ewes, there were low titers during the experiment, over a range from 50 to 200. On the other hand, among the offspring of positive ewes, a range of titers from 50 to 12800 was observed (Fig. 3). There were effects of the number of months of life of the offspring (F statistics = 3.714; degrees of freedom [df] = 11; P = 0.002), the serological status of the ewes (F statistics = 239.09; df = 1; P = 0.001) and the interaction these two factors (F statistics = 2.330; df = 11; P = 0.029) on the offspring's antibody titers. The most common titer found over the twelve months of evaluation was 1:100, and this titer was present in at least one animal over the twelve months of evaluation (Table 2).

4. Discussão

The seroprevalence of anti-*N. caninum* antibodies in ewes was 26.4%, with detection of positive animals on all the farms monitored in this study. This corroborates the high prevalence and wide distribution of this protozoon that was previously described in Brazilian herds of sheep (Paiz et al., 2015; Consalter et al., 2020; Maia et al., 2021). In other countries, research carried out shows similar seroprevalences, such as 32% and 19.3% in Spain and Italy, respectively (Gazzonis et al., 2016; Roberto-Sánchez et al., 2021). There is a variation in seroprevalence reported in other regions of the world and these variations can be attributed to different climates, livestock management systems, exposure to canids or different methodologies (IFAT vs.

ELISA). In South Australia, *N. caninum* is not considered an important cause of reproductive diseases, Clune et al. (2021) performed a cross-sectional study using the indirect ELISA test and found only 0.16% (2/1279) of positive sheep.

Among the offspring, 61% were seroreactive against the parasite in at least one month, during the first year of life. This percentage reflects the high incidence of this protozoon in sheep and indicates that the point prevalence may not demonstrate the true rate of exposure of these animals to *N. caninum* in a herd or a region, due to the high fluctuation rate among the monthly serological tests on the animals evaluated, with high prevalence on 6th month of the study. These results corroborate the data obtained by Azevedo-Filho et al. (2017), who carried out a study on the incidence of *N. caninum* infection among naturally infected sheep, in which the initial prevalence was 26% (13/50) and the final prevalence among the same animals was 72% (36/50), after serological follow-up of six months.

The monthly results from the exogenously infected offspring were variable, thus demonstrating transient antibody production. The lowest monthly prevalence was 10.1% at 12 months of age, while the highest number of reactive animals was seen at the age of six months, with a percentage of 45.7%. In a two-year longitudinal study carried out by Cardoso et al. (2012), on three cattle farms, with quarterly evaluations on calves, heifers and cows, high variation in the prevalence of anti-*N. caninum* antibodies was also observed. The percentage of positive samples in each period evaluated ranged from 3.3% to 37.1% on the three farms. Among horses, this same pattern of variation was also observed: Kormann et al. (2008) studied the monthly kinetics of antibodies against *N. caninum* referring to the last four months of pregnancy and observed a pattern of transient immunity similar to that of our study, such that mares might be positive in a given month of evaluation and then becoming negative in subsequent months. Cross-sectional studies provide a “snapshot” at the time of blood collection

and, with this transient antibody production for *N. caninum*, the prevalence of this protozoon may be underestimated.

Among the offspring born from *N. caninum*-seropositive ewes, three of them showed antibody titers against the parasite during all the months of evaluation, with little fluctuation in the titers measured. This finding is suggestive of occurrence of transplacental transmission, since there was no decline in antibody levels after the third or fourth month of life in these animals, which would normally occur with colostral antibodies. In a study carried out among cattle by Cardoso et al. (2008), it was observed that calves that were born seronegative and ingested colostrum from positive cows became seropositive, but their colostrum antibody levels began to reduce from the fourth month of age onwards, disappearing completely after the fifth month of life. Mesquita et al. (2013) observed that among naturally infected goats, all offspring had vertical transmission, i.e. they were seropositive before ingesting colostrum and remained positive throughout the study period, even after the metabolization of colostral antibodies.

It is noteworthy that *N. caninum* is capable of forming cysts, thereby escaping immunity and causing a decrease in serum antibody levels. However, according to Dubey et al. (2017), at any time that some immunosuppression occurs in the host, the parasite can be reactivated, thus enabling emergence of clinical signs and reproductive problems. However, animals with reduced or undetectable serum antibody titers can still be protected and produce an efficient immune response, due to persistence of memory B and T lymphocytes, which are capable of responding quickly to reinfection (Tizard, 2018).

It was observed that 30.5% of the offspring were infected for the first time after the sixth month of life. Therefore, horizontal infection, by oocyst ingestion, potentially occurred during the reproductive phase in these animals. Ewes that were already infected before mating are capable of developing a good immune response and consequently producing anti-*N. caninum* antibodies. This provides protection against new exogenous infections and/or reactivation of

bradyzoites capable of causing vertical transmission during the gestation period. The moderate serological response in the mother and the increased production of interleukin-17 reduced the expression of interferon-γ, which is related to pathological changes in the placenta and fetal death (Gutiérrez-Expósito et al., 2020).

Passive immunization failures regarding *N. caninum* were also described by Hoffmann (2007) in a study monitoring passive transfer of anti-*N. caninum* antibodies in horses. The researchers found that foals born to positive mares did not seroconvert after ingestion of colostrum in the first months of life and even suggested that this could have been due to low concentrations of antibodies in colostrum, caused by low titers in the mothers, which made it impossible to detect the antibody levels through conventional serological methods. In our study, we observed that there were two ewes with titers of 6400 and 12800 (3-P5 and 6-P2), and even so, the offspring were not positive in the first few months. In contrast, in a study on the immune response of sheep naturally infected by *Toxoplasma gondii*, which is an similar apicomplexan protozoon, Costa et al. (2021) observed that, excluding animals with vertical transmission, 48% (20/41) of the lambs that were offspring of mothers with titers from 1:64 to 1:8192 were positive for colostral antibodies in the first month.

Colostrum consists of the accumulated secretions of the udder at the end of pregnancy, it is mainly composed of fat and proteins. Among the proteins, the most important are immunoglobulins, in decreasing order of concentration, such as IgG, IgA and IgM. Low rates of passive immunization of progeny can be caused by several factors, such as failure of the mother to produce antibodies, failure of the offspring to ingest colostrum, or failure of intestinal absorption (Tizard, 2018).

Thus, neosporosis may constitute an important reproductive disease among sheep in the semiarid region of northeastern Brazil, while it has already been recognized as the most important cause of abortions and neonatal losses among cattle worldwide (Reichel et al., 2013).

Among sheep, although it has recently been described as an emerging disease capable of causing neosporosis in these animals, it is sometimes neglected or underreported (González-Warleta et al., 2014; Liu et al., 2015). It is important to note that, assuming that reproductive disease (abortion) occurs when naïve ewe become infected during gestation, it is worth considering whether ewes mated after 12 months are likely to be immune and probably less susceptible to reproductive disease (abortion) in event of point source exposure (ie contaminated feed, water). However, fetal infection is possible even in animals that have been exposed prior to pregnancy with recrudescence of latent infection causing vertical transmission reported in 31.6% offspring (Feitosa et al., 2021).

5. Conclusion

It can be concluded that, under semiarid conditions, the prevalence of seropositivity for *N. caninum* in sheep is high, with high fluctuation of antibody titers in the offspring of both positive and negative ewes. In most cases, there is only transient immunity, which can lead to underestimation of prevalence in cross-sectional epidemiological studies. It is also suggested that most animals become infected up to six months of age, such that the primary infection mainly occurs no later than when they reach reproductive age.

Declaration of competing interest

We declare that there were no competing interests.

Ethical appoval

This study was approved by the Ethics Committee for Use of Animals (CEUA) of the Instituto Federal da Paraíba (IFPB), under protocol 23000.000526.2018-22.

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Table 1. Association analysis between ewe serological status in pregnancy and lamb serological status at birth/before 6 months of age.

Offspring status	At birth			Before 6 months of age		
	Positive ewes	Negative ewes	P-value	Positive ewes	Negative ewes	P-value
Positive (%)	6 (42.9)	0 (0)		11 (78.6)	19 (42.2)	
Negative (%)	8 (57.1)	45 (100)	0.0001	3 (21.4)	26 (57.3)	0.039

Table 2. Anti-*Neospora caninum* antibodies in prepartum ewes and their offspring that were positive for at least one month, identified through the indirect fluorescence antibody test (IFAT) for IgG.

Mothers - Farms		**Female offspring born from positive ewes - monthly IFAT titre**											
	IFAT titre	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1 - P2	12800	3200	<1:50	200	400	1600	3200	6400	12800	6400	6400	3200	<1:50
2 - P2	12800	1600	<1:50	<1:50	200	<1:50	<1:50	<1:50	<1:50	800	400	<1:50	<1:50
3 - P5	12800	<1:50	<1:50	6400	1600	1600	<1:50	3200	6400	6400	6400	3200	N/C
4 - P7	6400	6400	6400	1600	800	800	1600	3200	1600	1600	1600	800	800
5 - P6	6400	3200	<1:50	1600	800	400	400	800	800	800	200	1600	1600
6 - P2	6400	<1:50	<1:50	<1:50	<1:50	3200	3200	6400	6400	6400	N/C	N/C	N/C
7 - P5	3200	100	100	100	100	100	100	100	100	100	100	100	N/C
8 - P6	100	<1:50	<1:50	<1:50	<1:50	50	50	100	50	<1:50	<1:50	<1:50	<1:50
9 - P1	100	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	100	50	<1:50	<1:50	<1:50	<1:50
10 - P5	50	100	50	50	50	50	50	50	100	100	50	N/C	N/C
11 - P4	50	<1:50	<1:50	<1:50	200	200	200	200	200	200	200	<1:50	<1:50
12 - P5	50	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	50	50	<1:50	<1:50	<1:50
13 - P3	50	<1:50	<1:50	<1:50	<1:50	<1:50	50	N/C	N/C	N/C	N/C	N/C	N/C
16* - P2	<1:50	<1:50	<1:50	<1:50	<1:50	50	50	50	<1:50	<1:50	<1:50	<1:50	<1:50
	<1:50	<1:50	<1:50	<1:50	50	50	50	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50
17 - P2	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	50	<1:50	<1:50	<1:50	<1:50	<1:50
21 - P3	<1:50	<1:50	<1:50	N/C	<1:50	<1:50	<1:50	<1:50	100	50	<1:50	<1:50	<1:50
22 - P3	<1:50	<1:50	<1:50	<1:50	<1:50	100	100	100	100	100	<1:50	<1:50	<1:50

23 - P3	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	50	50	50	50
24 - P3	<1:50	<1:50	<1:50	<1:50	50	50	50	50	50	<1:50	N/C	N/C	N/C
26 - P4	<1:50	<1:50	<1:50	<1:50	50	200	100	50	50	50	<1:50	<1:50	<1:50
27 - P4	<1:50	<1:50	<1:50	<1:50	<1:50	50	50	50	<1:50	50	50	<1:50	<1:50
28 - P4	<1:50	<1:50	<1:50	<1:50	50	50	50	50	100	100	<1:50	<1:50	<1:50
31 - P4	<1:50	<1:50	<1:50	<1:50	200	100	50	50	50	100	50	100	100
32 - P4	<1:50	<1:50	<1:50	<1:50	50	50	50	50	<1:50	<1:50	<1:50	<1:50	<1:50
33 - P4	<1:50	<1:50	<1:50	<1:50	<1:50	200	200	100	<1:50	<1:50	<1:50	<1:50	<1:50
34* - P4	<1:50	<1:50	<1:50	<1:50	<1:50	100	100	<1:50	<1:50	<1:50	<1:50	100	100
35* - P4	<1:50	<1:50	<1:50	<1:50	50	100	200	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50
44 - P6	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	50	50	<1:50	50	<1:50	<1:50	<1:50
45 - P6	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	50	50	50	<1:50	<1:50	<1:50	<1:50
46* - P6	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	50	<1:50	<1:50	<1:50	<1:50	50	<1:50
50 - P7	<1:50	<1:50	<1:50	<1:50	50	50	<1:50	<1:50	N/C	N/C	N/C	N/C	N/C
51 - P5	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	50	50	50	<1:50	<1:50	<1:50
52* - P5	<1:50	<1:50	<1:50	<1:50	<1:50	50	50	50	50	<1:50	<1:50	<1:50	<1:50
	<1:50	<1:50	<1:50	<1:50	50	100	50	50	<1:50	<1:50	<1:50	<1:50	<1:50
53 - P5	<1:50	<1:50	<1:50	<1:50	<1:50	<1:50	50	50	50	50	50	50	50

* Mother with twin birth.
34* and 35* = Mothers with twin births and only one seroreactive progeny during the months of evaluation.
N/C = not collected.
P = Farm

Captions of Figures

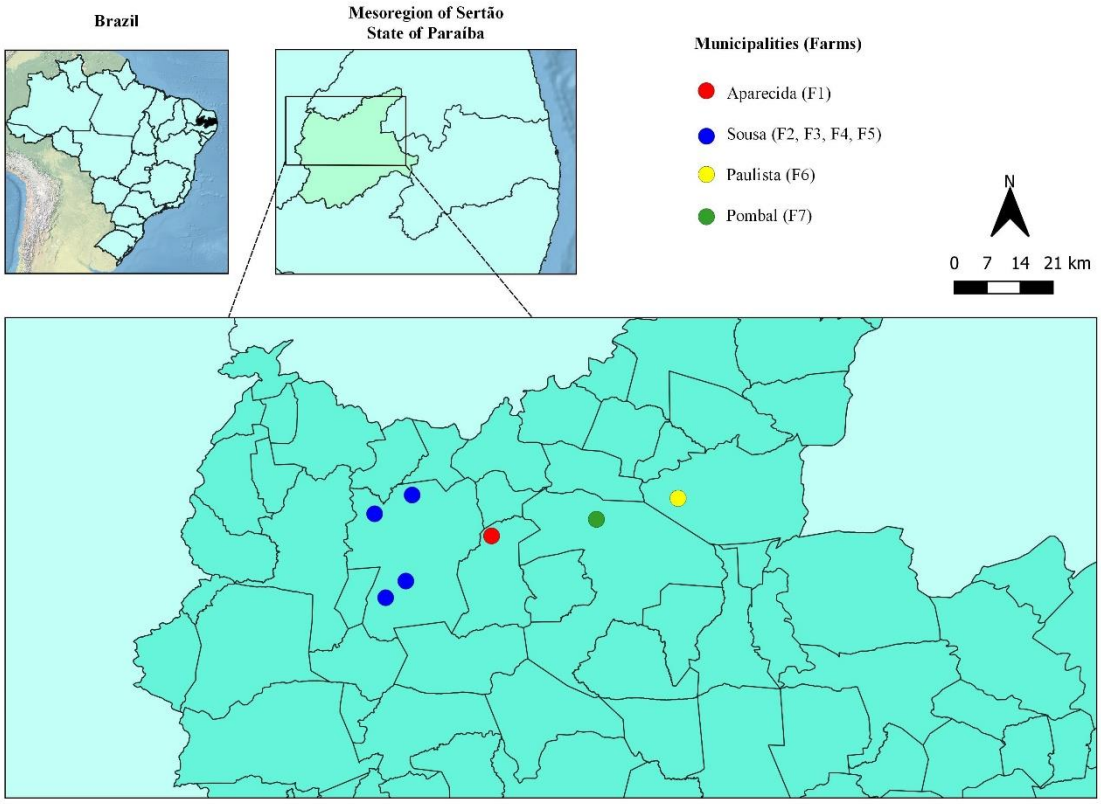

Figure 1. Geographical locations of the sheep herds analyzed in the semiarid region of the state of Paraíba, northeastern Brazil.

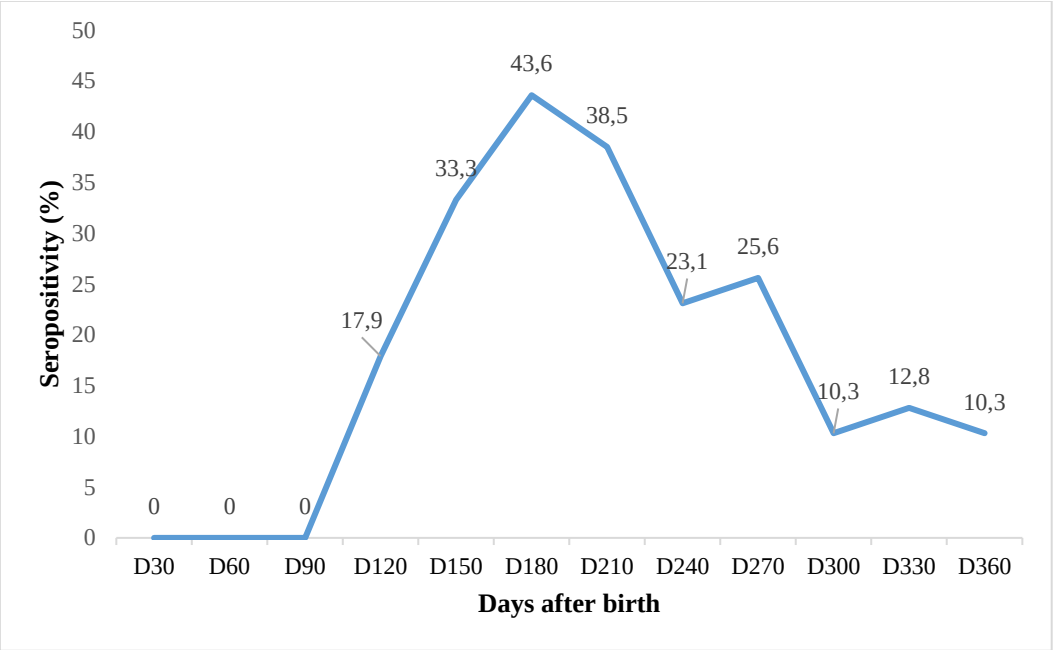

Figure 2. Proportion of positive offspring, born from ewes that were negative for anti-*Neospora caninum* antibodies, determined through the indirect fluorescence antibody test (IFAT), according to age, during the first year of life.

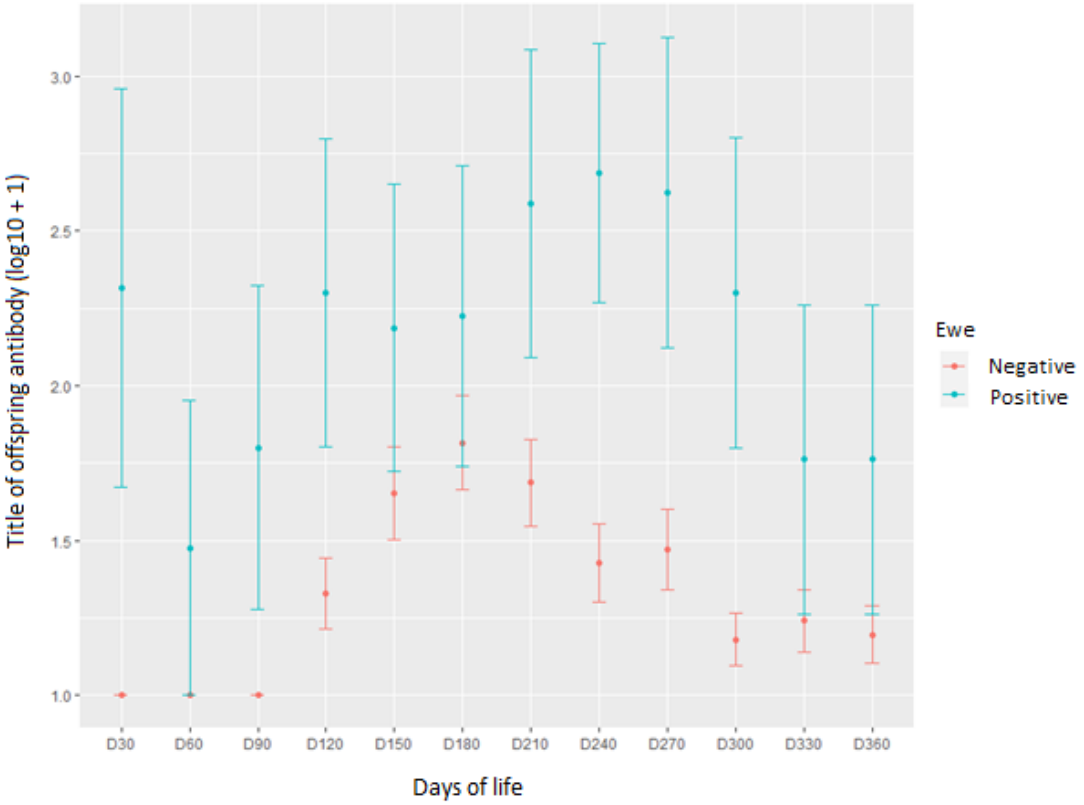

Figure 3. Mean and standard error of the antibody titers of female offspring according to months of life of offspring and serological status of the ewes.

CAPÍTULO II

DETECTION OF ANTI-*NEOSPORA CANINUM* IgG IN BLOOD SERUM AND COLOSTRUM SAMPLES IN NATURALLY INFECTED SHEEP

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Detection of anti-*Neospora caninum* IgG in blood serum and colostrum samples in naturally infected sheep

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Abstract:

The aim was to detect correlations of IgG antibodies against *N. caninum* in serum and colostrum samples from ewes, through the IFAT, and to evaluate the presence of this immunoglobulin in the serum of newborn lambs after colostrum ingestion. Blood samples from 162 ewes that did not show any disease in the general physical examination and from their newborn lambs, not more than 5 days postpartum, along with 162 colostrum samples and 182 blood samples from the neonates. 27.8% (45/162) of the mothers were positive for anti-*N. caninum* IgG, among which antibodies were detected in colostrum in 46.7% (21/45). All the ewes with positive

colostrum had reactive offspring. The kappa agreement for the correlation between the serological tests on the ewes and the colostrum results was 0.558. This correlation increased as the antibody titers of the mothers increased, and reached 1.000 from the titer of 1:400 from the mothers. Comparison of the antibody detection results between the offspring's serum and colostrum showed kappa agreement of 1.000. In conclusion, there was a good agreement regarding detection of anti-*N. caninum* IgG between colostrum samples and lambs' serum; the use of colostrum forms a noninvasive alternative for diagnosing *N. caninum* in sheep herds.

Keywords: Neosporosis; Offspring; Serology; Ovine.

1. Introduction

Neosporosis is a disease caused by the protozoon *Neospora caninum* (Apicomplexa: Sarcocystidae), which mainly causes reproductive problems such as miscarriage in intermediate hosts. However, it can also give rise to neurological symptoms in cases of congenital infection of neonates [1] and can form viable cysts in tissues. Under suitable conditions of low immunity, the latter may undergo recrudescence and can cause clinical signs in animals [1-3].

N. caninum needs to be controlled in order to prevent infection. Once an individual has become exposed to this protozoon, the infection may persist for the entire lifetime. Sheep can acquire the infection before birth, via the placenta [2,4]. This can cause significant damage to their fetuses, since the syndesmochorial nature of their placenta does not allow passage of antibodies. In this way, the transfer of maternal immunity to the offspring only occurs after ingestion of colostrum, which contains antibodies [5].

Studies have shown that *N. caninum* can have major impacts on pregnancy in sheep. Infection in the first and second trimesters of pregnancy causes high abortion rates, while in the final trimester it can cause premature, infected, weak or unhealthy births [6].

Sheep farming stands out as an important livestock activity and has numerous advantages, such as needing a smaller breeding area, less forage consumption, ease of handling and production of multiple products from the same animal, such as meat, milk and good-quality leather [7].

Because sheep herds are mainly dedicated to production of meat and leather, reproductive diseases can cause great damage, thus leading to losses for producers [8]. Neosporosis is of great importance in small ruminants, as it is a disease that leads to formation of cysts that can undergo reactivation under conditions of low immunity. Such conditions commonly occur during pregnancy and lead to abortion [9-12].

Therefore, taking into account the impacts that *N. caninum* can cause in sheep herds, the aim of this study was detect the correlation of IgG antibodies against *N. caninum* between serum and colostrum samples from ewes, and to evaluate the presence of this immunoglobulin in the serum of newborn lambs after ingestion of colostrum.

2. Materials and Methods

2.1 Experimental design

Twenty sheep breeding farms in the semiarid region of the state of Paraíba, Brazil, were selected according to convenience. During the study, 162 blood and colostrum samples were collected from ewes and 182 samples from their neonates (in cases of twin births, samples were collected from both lambs).

On each farm, ewes that were found to be healthy in a general physical examination were selected for serological and colostral samples to be taken. All of them had recently lambed and were not more than five days postpartum. Blood samples were also collected from the respective lambs, for detection of anti-*N. caninum* and evaluation of passive immunization against this agent.

2.2 Blood and colostrum sampling

From each selected ewe, blood was collected by means of venipuncture of the jugular vein. Soon after this, colostrum was collected by manual milking. Simultaneously, blood samples were also taken from the newborn lambs of these selected ewes, between the second and fifth day after birth, to investigate the passive transfer of anti-*N. caninum* IgG.

Colostrum was collected aseptically, in sterile test tubes. The first three milk jets were always discarded in order to avoid contamination and then approximately 5 mL of colostrum

was collected from each ewe. Animals that presented clinical mastitis were not included in this study.

The blood samples were centrifuged at 1000 x g for 10 minutes, to obtain serum for analysis. The colostrum was also centrifuged and, after this procedure, the superficial fat layer (supernatant) was discarded and only the colostrum precipitate was used for the analyses. The blood serum and colostrum precipitate from each animal were properly identified and then stored at -20 °C until the time of examination.

2.3 Serological and colostral tests

The serum samples (ewes and lambs) and samples of colostrum precipitate were subjected to the indirect immunofluorescence reaction (IFAT), always using the same protocol.

The IFATs for anti-*N. caninum* IgG were carried out at the Laboratory of Immunology and Infectious Diseases (LIID) of the Instituto Federal da Paraíba, Sousa campus.

The dilution used as a cutoff point for blood serum [13] and colostrum, adapted from Camillo et al. [14], was 1:50. The Nc-1 strain of tachyzoites, fixed on a slide, was used as the antigen [15].

A whole-molecule sheep anti-IgG conjugate was used (SIGMA, St. Louis, MO, USA).

Serum and colostrum precipitate samples that reacted at dilutions greater than or equal to 1:50 were considered positive. The reactive serum and colostrum precipitates were titrated in sequential dilutions at base two until negative.

2.3 Statistical analysis

A concordance analysis was performed on the results from the IFATs between serum samples from the ewes and their offspring and the colostrum samples, using the kappa coefficient [16]. The sensitivity and specificity of detection of antibodies in colostrum through

IFAT were calculated in comparison with detection of antibodies in the blood serum samples. To assess the sensitivity and specificity of detection of antibodies in colostrum through IFAT, detection of antibodies in the blood serum samples from the ewes and lambs was used as a standard [14].

3. Results

Among the 162 serological samples from ewes, 27.8% (45/162) were positive for anti-*N. caninum* IgG, with titers of between 1:50 and 1:6400 (Table 1).

Table 1. Detection and titration of anti-*Neospora caninum* IgG antibodies (Ab) by means of the indirect immunofluorescence reaction (IFAT) in blood serum and colostrum samples from ewes and in their lambs' serum.

No. of ewes and lambs	Postpartum days	Ewes (serum)	Colostrum	Lambs
2	4	200	200	100
3	3	3200	1600	100
5	3	200	100	50
6 - L1	4	200	100	100
6 - L2			-	50
9	2	200	-	-
10	5	50	-	-
11	2	200	-	-
12	3	50	-	-
18	4	50	-	-
21	2	50	-	-
27 - L1	4	200	100	50
27 - L2				50
28	2	50	-	-
29	2	50	-	-
31	3	100	100	100
34	3	6400	6400	100
35	4	200	200	200
37	2	50	-	-
38	2	100	50	50
39	4	50	-	-
42	4	50	50	50
61	2	100	50	50
63	2	200	-	-
64	2	400	400	400
71	3	50	50	50
81	4	50	-	-
82	3	50	-	-
83	2	50	-	-
101	5	50	-	-
103	5	100	100	100
107	4	50	-	-
108	3	50	-	-
112	3	50	50	50
113	3	50	50	50
115	4	100	50	50
123	4	50	-	-
125	4	50	-	-

No. of ewes and lambs	Postpartum days	Ewes (serum)	Colostrum	Lambs
126	3	50	-	-
127	3	50	-	-
128	2	50	-	-
134	5	50	-	-
135 - L1	5	100	100	100
135 - L2				100
140	5	50	-	-
146	5	50	50	50
147	4	100	50	50
148	2	50	50	50

L1: lamb 1; L2: lamb 2

It was observed that for 53.3% (24/45) of the ewes that were positive for anti-*N. caninum* in serum, no antibodies to this protozoon were detected in their colostrum. The titers for these ewes were between 1:50 and 1:200 (Table 1). Among all the ewes that had positive colostrum, their offspring were also reactive to anti-*N. caninum* antibodies. The mothers with titers greater than 1:200 showed higher possibilities of immunizing their offspring.

There was a pattern among the samples that were positive in the IFAT. The serum samples from the ewes had levels greater than or equal to the levels of the colostrum titers, and the titers of their offspring were lower than or equal to the colostrum titers.

Moderate agreement was observed in the results from the IFATs on the ewes, in correlations with the results from colostrum, with a kappa coefficient of 0.558 (Table 2). It was also found that the titer results from the animals had an influence on the kappa coefficient results: the ewes with low titers showed results that were discordant with those from the respective colostrum, whereas the ewes with higher titers showed almost perfect kappa agreement (Table 2).

Table 2. Ewes that were positive and negative for anti-*Neospora caninum* IgG antibodies by means of the indirect fluorescence antibody test (IFAT) in colostrum and serum samples. Result stratified according to antibody titers obtained from the ewes' serum, with comparison of the analyses on the ewes' serum and colostrum according to the kappa coefficient.

Titer of the ewes	Positive ewes/analyzed ewes (%)	Positive colostrum/positive ewes (%)	Kappa
1:50	27/162 (16.7%)	6/27 (22.2%)	0.323
1:100	7/162 (4.3%)	7/7 (100%)	1.000
1:200	8/162 (4.9%)	5/8 (62.5%)	0.760
1:400	1/162 (0.6%)	1/1 (100%)	1.000
1:3200	1/162 (0.6%)	1/1 (100%)	1.000
1:6400	1/162 (0.6%)	1/1 (100%)	1.000
Total	45/162 (27.7%)	21	0.558*

*Kappa values: 0.20 to 0.40 indicates reasonable agreement; 0.40 to 0.60 indicates moderate agreement; 0.60 to 0.80 indicates substantive agreement; and 0.80 to 1.00 indicates perfect diagnostic agreement between the tests evaluated.

Comparison of the detection results regarding the anti-*N. caninum* levels between lamb serum and colostrum showed a kappa agreement of 1.000 (Table 3).

Table 3. Positive and negative animals according to IFAT examination of colostrum and offspring serum, comparing the techniques according to the Kappa coefficient.

	Seropositive lambs	Seronegative lambs	Total	Kappa
Positive colostrum	21	0	21	1,000*
Negative colostrum	0	141	141	
	21	141	162	

*Kappa values: 0.20 to 0.40 indicates reasonable agreement; 0.40 to 0.60 indicates moderate agreement; 0.60 to 0.80 indicates substantive agreement; and 0.80 to 1.00 indicates perfect diagnostic agreement between the tests evaluated.

4 Discussion

In this study, anti-*N. caninum* antibodies were found through IFAT in colostrum samples from the ewes evaluated. This is the first study worldwide to have evaluated detection of antibodies against *N. caninum* in colostrum from sheep and the agreement of IFAT regarding the serum antibody levels in ewes and lambs. A similar study was conducted by Ooi et al. [17] with regard to detection of anti-*N. caninum* in the main fluids (serum, milk, vaginal secretion and saliva) from cattle through IFAT. High sensitivity was observed in that study, but antibodies against the parasitic agent were more frequently detected in blood serum, followed by the milk of these animals. Meirelles et al. [18] investigated detection of *Toxoplasma gondii* in cows' milk and observed that IFAT was not indicated for detection of anti-*Toxoplasma gondii* antibodies in this milk, since there was no agreement between blood serum and milk. Possibly the sensitivity observed in the present study was due to the fact that colostrum was used instead of milk.

Failure to pass on anti-*N. caninum* antibodies through colostrum was observed in the cases of 53.3% (24/45) of the ewes of the present study. Thus, for their offspring, passive immunization failed. One observed characteristic was that among these 24 ewes that did not have antibodies against *N. caninum* in their colostrum, 21 had a titer of 1:50. The kappa index observed in the analysis between serum from the ewes and their colostrum showed moderate agreement (0.558; Table 2). This may have occurred because the vast majority of the mothers evaluated that were positive in blood serum and negative in colostrum (21/24) had a serum titer of 1:50 (Tables 1 and 2). This low titer may have meant that these animals did not have enough antibodies for their transfer to be detected in colostrum and in their offspring [19], such that they would have a positive result in the IFATs, which would have considerably reduced the level of agreement. Feitosa et al. [4] carried out a study evaluating vertical transmission of IgG antibodies against *N. caninum* in naturally infected Santa Inês ewes. They observed that the

ones with serum titers of 1:50 and 1:100 did not demonstrate good immunization of their offspring in the first month of life. In this regard, low titers of serum IgG in the mothers can influence the presence and concentration of anti-*N. caninum* in colostrum, which interferes with passive immunization of their offspring.

In an experimental study on cattle, Cardoso et al. [20] used colostrum from cows that were positives for anti-*N. caninum* and observed that three of the eight suckled calves were not passively immunized against the parasite. In this case, as in our study, there were several factors that might have led to the failure of passive immunization of these calves through colostrum. It is known that the antibodies in colostrum are influenced by the levels of serum immunoglobulins, which are also produced in the udder. A reduction in antibody levels occurs rapidly over the first few weeks of lactation [21].

Considering that the low-titer ewes did not pass enough antibodies to their offspring for them to become immunized, these animals may have suffered parasite recrudescence due to lowering of their immune response during pregnancy. This would generate the possibility that lactogenic transmission to the neonates could occur. There are no studies demonstrating lactogenic transmission of *N. caninum*, but there are studies demonstrating the presence of this agent's DNA in cow`s milk, through the polymerase chain reaction (PCR) [22, 23].

It was observed in the present study that the kappa agreement was higher among ewes with titers greater than or equal to 1:200. Similar results were observed among cattle by Camillo et al. [14] and Meirelles et al. [18], who found 100% agreement between blood serum and milk among cows with serum antibody titers above 1:100. This indicated that *N. caninum* in colostrum could be diagnosed through IFAT, but with restrictions, especially among animals with titers of 1:50. In studies on outbreaks of abortions due to *N. caninum* infection, high titers were observed among those suffered abortions. Thus, detection of IgG in colostrum can be used as a screening test in these cases [24, 25].

In the present study, the kappa index obtained in comparative analysis between the lambs' serum and colostrum showed perfect agreement, regardless of the titer obtained from colostrum (1.000; Table 3). This was because immunization of lambs only becomes possible if the colostrum presents enough anti-*Neospora caninum* IgG antibodies.

The analysis for diagnosing *N. caninum* demands specialized labor and a lot of time. Therefore, it can be suggested that, in sheep herds, IFAT should be performed on colostrum for diagnosing *N. caninum* in ewes. This makes it possible to identify the presence of this parasite in the herd and also makes it possible to ascertain whether passive immunization of the lambs has occurred, according to the titration. Therefore, when the colostrum presents titers greater than or equal to 1:200 and there was no failure of ingestion of colostrum by the lamb, this indicates that passive immunization of the lamb occurred. It is well known that low titers are commonly associated with old infections, while higher titers indicate recent exposure to the agent [5, 19].

Among cattle, excellent results have been demonstrated in studies evaluating the detection of anti-*N. caninum* in cow's milk through IFAT. The main advantage of using this biological sample is that the material is collected noninvasively, which reduces the risks of disease transmission and high stress among the animals tested [14,18].

Among the ewes whose serum titers through IFAT were greater than or equal to 1:200, the concordance with the results from colostrum and from the lambs' serum was greater. This allows combined analysis of colostrum and the lambs' serum, using only the serum or colostrum from adult females, given that seropositive females with titers of 1:200 will have similar titers in the colostrum. Consequently, these ewes' offspring will be passively immunized. In this manner, analysis exclusively on serum or colostrum from ewes with titers greater than or equal to 1:200 can make the analysis more practical for herds.

5 Conclusions

It was concluded from the present study that there was a high level of agreement in detection of anti-*N. caninum* antibodies between the serum of the ewes and their colostrum, especially with higher titers among the ewes. Moreover, there was perfect agreement between the colostrum and the lambs' serum. This makes it possible to use IFAT on colostrum as a noninvasive alternative for diagnosing *N. caninum* in sheep herds or mothers that present reproductive problems.

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