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Bovine neosporosis in Rio Grande do Sul, Brazil: Elevated antibody detection rate in comparison to previous decades[1]

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ABSTRACT.- Roman I.J., Rosa G., Rodrigues F.S., Cargnelutti J.F., Sangioni L.A. & Vogel F.S.F. 2024. **Bovine neosporosis in Rio Grande do Sul, Brazil: Elevated antibody detection rate in comparison to previous decades.** *Pesquisa Veterinária Brasileira 44:e07476, 2024.* Graduate Program in Veterinary Medicine, Laboratório de Doenças Infecciosas e Parasitárias, Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil. E-mail: fernanda.vogel@ufsm.br

This study aimed to determine the frequency of anti-*Neospora caninum* antibody detection in three samples and sampling methods: A prevalence study, routine diagnostic laboratory samples, and fetal bovine serum samples. These samples were collected from cattle in Rio Grande do Sul (RS), southern Brazil, and analyzed using the indirect immunofluorescence reaction technique. For each sampling method, a historical study was used as a reference for comparison. In the prevalence study, 1,248 serum samples were collected from 2020 to 2022. The prevalence of *N. caninum* in the RS state was 22.8% (285/1248). This figure was statistically different compared to previous studies conducted in 2002, which reported a prevalence of 11.2% (p<0.001). In the routine diagnostic samples, an average rate of 29.95% (985/3289) of anti-*N. caninum* antibodies were detected. This rate was statistically higher than that of a previous study conducted in 2003, which reported a rate of 20% (p=0.01). Similar data were found in the fetal bovine serum samples, which showed an increase compared to previous studies conducted in 2010 that reported a rate of 15% (p=0.003). The increase in the detection rate of *N. caninum* antibodies underscores the need for measures to control and prevent bovine neosporosis.

INDEX TERMS: Protozoan, reproduction, diagnosis, serology, fetal bovine serum, bovine neosporosis, antibody.

RESUMO.- [Neosporose bovina no Rio Grande do Sul, Brasil: elevada taxa de detecção de anticorpos em comparação com décadas anteriores.] Este estudo teve como objetivo determinar a frequência de detecção de anticorpos anti-*Neospora caninum* em três diferentes amostras e métodos de coleta: um estudo de prevalência, amostras de laboratório de diagnóstico de rotina e amostras de soro fetal bovino. Essas amostras foram coletadas de bovinos no Rio Grande do Sul (RS), região Sul do Brasil, e analisadas usando a técnica de reação de imunofluorescência indireta. Para cada método de coleta, um estudo histórico foi usado como referência para comparação. No estudo de prevalência, foram coletadas 1.248 amostras de soro entre 2020 e 2022. A prevalência de *N. caninum* no estado do RS foi de 22,8% (285/1248). Esse valor foi estatisticamente diferente quando comparado a estudos anteriores realizados em 2002, que relataram uma prevalência de 11,2% (p<0,001). Nas amostras de diagnóstico de rotina, foi detectada uma taxa média de 29,95% (985/3289) de anticorpos anti-*N. caninum*. Essa taxa foi estatisticamente maior do que a de um estudo anterior realizado em 2003, que relatou uma taxa de 20% (p=0,01).

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Dados semelhantes foram encontrados nas amostras de soro fetal bovino, que mostraram um aumento em comparação com estudos anteriores realizados em 2010 que relataram uma taxa de 15% (p=0,003). O aumento na taxa de detecção de anticorpos anti-*N. caninum* destaca a necessidade de medidas para controlar e prevenir a neosporose bovina.

TERMOS DE INDEXAÇÃO: Protozoário, reprodução, diagnóstico, sorologia, soro fetal bovino, neosporose bovina, anticorpos, bovinos.

INTRODUCTION

Neospora caninum, a protozoan from the phylum Apicomplexa, was initially proposed as a new species in 1983. This suggestion came after its identification in lesions that were consistent with *Toxoplasma gondii* infection but with observed protozoa and cystic structures that differed from those typically found in *T. gondii* (Bjerkas et al. 1984). The proposed new protozoan was completely described and characterized in 1988 (Dubey et al. 1988).

N. caninum has a heteroxenous life cycle, with definitive hosts including *Canis lupus familiaris*, *Canis latrans*, *Canis lupus*, and *Canis lupus dingo*. A broad spectrum of species serve as intermediate hosts (McAllister et al. 1998, Dubey et al. 2002, Gondim et al. 2004, King et al. 2010).

Contrary to observations in dogs (initially associated with neuromuscular disorders), the primary clinical signs in cattle are linked to reproductive disorders, such as abortion and stillbirth. Furthermore, *N. caninum* infection has been found to negatively impact milk and meat production (Dubey et al. 2006, Reichel et al. 2013).

Cattle can contract infections through horizontal (oral) and vertical (transplacental) transmission (Gondim et al. 2002, Benavides et al. 2012). Horizontal transmission takes place when cattle ingest oocysts excreted in the feces of definitive hosts (Gondim et al. 2002). Vertical (transplacental) transmission occurs during the gestational period due to an acute infection, which can result from oral transmission or the reactivation of persistent infections (Benavides et al. 2012, Cabrera et al. 2019). Vertical transmission is considered the most significant mode of transmission in cattle, accounting for up to 95% of the generative capacity of persistently infected animals (Bartley et al. 2012, 2013, Benavides et al. 2012).

Antibody detection techniques are commonly employed to determine the distribution of *N. caninum*, a protozoan that causes persistent infection (Paré et al. 1995, Dubey et al. 2007). These serological tests can be conducted using various techniques, with enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence being the most frequently utilized for cattle (Ribeiro et al. 2019).

The initial detection of anti-*N. caninum* in Brazilian cattle was reported in dairy cattle from the state of São Paulo and beef cattle from Mato Grosso do Sul (Brautingam et al. 1996). Subsequent reports of seroprevalence in Rio Grande do Sul (RS) confirmed the circulation of the agent within the bovine population, with rates fluctuating between 11.2% and 20% (Corbellini et al. 2002, Ragozo et al. 2003). However, these detection rates do not encompass all the intermediate regions of the state (Corbellini et al. 2002, Ragozo et al. 2003).

Prior research has been conducted to determine the prevalence of neosporosis in cattle within the RS state. However, the majority of the data documented in the literature pertains to antibody detection rates in serum samples. These samples were either obtained without applying a sample calculation or were derived from animals with a history of reproductive issues. Consequently, this study aimed to determine the frequency of anti-*N. caninum* antibody detection across three distinct samples and sampling methods. These include a prevalence study, samples from a routine diagnostic laboratory, and fetal bovine serum (FBS) samples from cattle in the RS state.

MATERIALS AND METHODS

Ethical approval. No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with diagnostics clinical samples and samples for slaughterhouses, as per the approval of the "Conselho Nacional de Controle de Experimentação Animal" (CONCEA).

Three distinct methods were employed to ascertain the detection frequency of anti-*Neospora caninum* antibodies in cattle from the RS state: (i) a prevalence study involving a substantial sample of the state's cattle population; (ii) the frequency of antibody detection in routine diagnostic specimens; and (iii) the rate of antibody detection in FBS samples.

Prevalence study. The state of RS is divided into 497 municipalities, eight intermediate regions (Porto Alegre, Pelotas, Santa Maria, Uruguaiana, Ijuí, Passo Fundo, Caxias do Sul, and Santa Cruz), and 43 immediate regions (IBGE 2017). The StatCalc program from Epi Info® version 7.2.5.0 (CDC 2021) was utilized to determine the sample size ("n"). The cattle count for each municipality was sourced from the "Secretaria da Agricultura, Pecuária e Desenvolvimento Rural" (Secretariat of Agriculture, Livestock, and Rural Development – SEAPDR 2022) and subsequently stratified according to the corresponding intermediate regions. The effectiveness of each intermediate region was employed as a population size factor. The expected detection frequency used was the average of 11.4%, as derived from the study by Vogel et al. (2006). The standard error in the test was 5%, and the sample size was determined based on a reliability coefficient of 95%. The goal was to collect an average of 156 samples per intermediate region, amounting to a minimum of 1,248 samples across the eight intermediate regions.

A total of 1,248 samples were collected and sent for diagnostic serology from 2020 to 2022. Each sample was accompanied by a completed request form, including location, farm staff, gender, age, test purpose, reproductive issues, details of purchase and sale, and serological monitoring data.

A screening was conducted based on the information provided in the requisition form. The inclusion criteria mandated the use of samples from the RS state. Additionally, serological screening was performed when it was reported that samples from all animals on a property were collected, with a maximum limit of 25 samples per property.

Routine samples. To determine the detection rate of anti-*N. caninum* antibodies, we collected 3,289 bovine serum samples from the RS state. These samples were sent for diagnostic serology from 2020 to 2022. The screening was conducted based on the information provided in the requisition form, and the samples were selected based on the following criteria: Clinical suspicion of neosporosis, a descriptive history of reproductive issues indicative of neosporosis or otherwise, and sampling from a herd constituting ≤10% of all animals on the property.

Fetal bovine serum samples. In 2022, 100 samples of FBS were collected from pregnant, non-clinical beef cows at an officially inspected abattoir in Santa Maria/RS, southern Brazil. The procedures

for animal slaughter and evisceration were conducted in compliance with Brazilian legal protocols for ethics and animal welfare under the supervision of technicians from the Official Veterinary Inspection Service.

Serology. Slides featuring 15 multispot wells were utilized. These slides were sensitized using *N. caninum* strain NC-1 tachyzoites, aiming for an average dispersion of 10-20 tachyzoites per focus under a 40× objective. Following sensitization, the slides were fixed with acetone and subsequently stored at -20°C until required.

Serum samples underwent anti-*N. caninum* antibody research using the indirect immunofluorescence technique, as outlined by Dubey et al. (1988) and Paré et al. (1995). The serum samples utilized in the prevalence study and routine samples were diluted 1:200, following the method described by Corbellini et al. (2002). FBS samples were diluted 1:25 as per Cadore et al. (2010) and tested for the presence of immunoglobulins G. The secondary antibody used was rabbit anti-bovine IgG, conjugated with fluorescein and diluted according to the manufacturer's recommendations (Rabbit Anti-Bovine IgG FITC®, F7887, Sigma-Aldrich, San Louis/MO, USA). Reactions were considered positive if all the tachyzoite surfaces were fluorescent (Conrad et al. 1993, Paré et al. 1995). Each slide tested included negative and positive control serum samples.

Statistical analysis. The GraphPad Prism 6 program was utilized to analyze serological data, employing the chi-square test and a 95% confidence interval. Studies employing identical sample selection criteria, cut-off points, and analysis techniques were selected for comparison. The results obtained by Corbellini et al. (2002) served as a reference for calculating the difference in prevalence. Data from Ragozo et al. (2003) was employed to compute the differences between routine samples. Similarly, the data from Cadore et al. (2010) was used to calculate the difference between FBS samples. Differences were considered significant when the *p* values were less than 5% ($p<0.05$).

RESULTS

The RS state reported a 22.8% (285/1248) prevalence of *Neospora caninum* in cattle. A significant statistical difference ($p<0.001$) was observed when comparing our findings with those of Corbellini et al. (2002). Detection rates varied across intermediate regions, ranging from 12.2-38.3%. The region of Pelotas recorded the highest prevalence, while Santa Cruz do Sul reported the lowest (Fig.1).

The average detection rate for routine samples was 29.95% (985/3289). A significant statistical difference ($p=0.01$) was observed compared to the results obtained by Ragozo et al. (2003), who reported a detection rate of 20.4%. The detection rate varied from 10-100% for records with n≥5 samples.

The average detection rate for FBS sampling was 35% (35/100). A significant statistical difference ($p=0.003$) was observed compared to the results obtained by Cadore et al. (2010). The detection range was between 28.6% and 80% for records with n≥5 samples.

DISCUSSION

A neosporosis seroprevalence of 22.8% was identified using samples from all intermediate regions of the RS state. Previous studies have confirmed the presence of *Neospora caninum* in the RS state, with seroprevalence rates ranging from 11.2-11.4% (Corbellini et al. 2002, Vogel et al. 2006). A comparison with the study by Corbellini et al. (2002) revealed a discrepancy in detection rates, indicating increased agent circulation within the state territory.

In routinely collected samples from herds with a history of reproductive issues, we observed an elevated rate of antibody detection. This finding aligns with the study conducted by Ragozo et al. (2003), which also utilized samples from a herd experiencing reproductive difficulties to estimate the detection rate in the RS state. While the study by Ragozo et al. (2003) relied on a single sample from one farm, our study analyzed samples from all intermediate regions. Consequently, our results indicate a heightened detection rate in samples associated with a history of reproductive problems.

The results from 35% of the FBS samples confirm bovine transplacental transmission and an increased detection of anti-*N. caninum* antibodies compared to other studies (Cadore et al. 2010, Alves et al. 2020). The gestational age of the fetuses could not be determined, suggesting that they may have been at different stages of gestation. Given that these samples were collected from fetuses at varying gestational ages, it cannot be definitively stated that all would have been born, as pregnancy termination could still occur owing to *N. caninum* infection (Bartley et al. 2013, 2012).

The detection rates of *N. caninum* varied from 12.2-38.3% in the prevalence study and between 10 and 100% in routine samples. Several factors may contribute to these variations in detection rates, including the presence of dogs on the property, a history of reproductive issues, the age of the animals, and the production system in stables, whether confined or grazing (Moore et al. 2013, Topazio et al. 2014, Klun et al. 2019). Another factor that may influence detection rates and the incidence of reproductive problems is the variability in genetics and virulence depending on the isolate. This is an intriguing point, and we believe it may explain the absence of a history of reproductive problems among seropositive

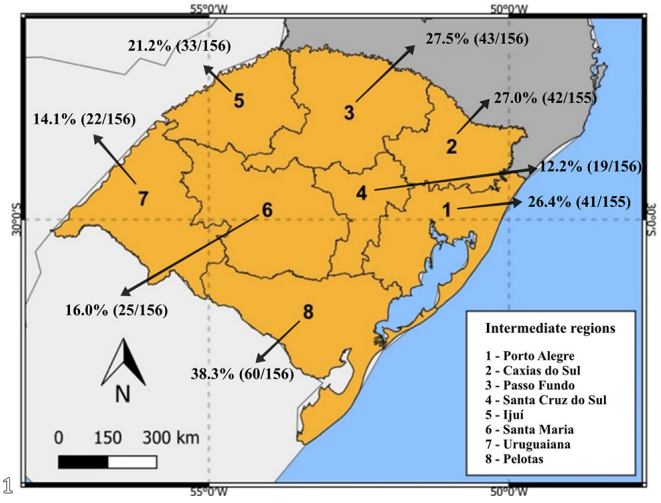

Fig.1. 1 = Intermediate region of Porto Alegre, 26.4% (41/155). 2 = Intermediate region of Caxias do Sul, 27.0% (42/155). 3 = Intermediate region of Passo Fundo, 27.5% (43/156). 4 = Intermediate region of Santa Cruz do Sul, 12.2% (19/156). 5 = Intermediate region of Ijuí, 21.2% (33/156). 6 = Intermediate region of Santa Maria, 16.0% (25/156). 7 = Intermediate region of Uruguaiana, 14.1% (22/156). 8 = Intermediate region of Pelotas, 38.3% (60/156).

individuals in the populations studied (Regidor-Cerrillo et al. 2008, Jiménez-Pelayo et al. 2019).

In the samples utilized to determine the prevalence of anti-*N. caninum* antibodies, detection occurred in populations with no prior history of reproductive issues. Serological data serve as a valuable tool for implementing control and monitoring measures within a herd (Horcajo et al. 2016). The correlation between positive serology and reproductive problems has been used as a criterion for removing an animal from the herd. However, studies indicate a variance in pathogenesis among different *N. caninum* strains (Regidor-Cerrillo et al. 2008, Jiménez-Pelayo et al. 2019).

Regional characteristics precluded the maintenance of homogeneity between dairy and beef cow samples. This discrepancy should not be construed as an indication of a higher disease prevalence in dairy cows. As Dorsch et al. (2021) have noted, the infection's behavior and subsequent clinical manifestations do not vary based on the individual's productive aptitude.

CONCLUSION

The antibody detection rate for neosporosis in bovines from the Rio Grande do Sul state showed an increase compared to historical studies employing similar methodologies and experimental designs. This data underscores the necessity for control measures and prophylaxis against bovine neosporosis. Furthermore, it can provide a foundation for future studies focused on the control and prophylaxis of bovine neosporosis.

Authors' contributions.- CRediT taxonomy. Conceptualization: Isac Junior Roman, Juliana Felipetto Cargnelutti, Fernando de Souza Rodrigues, Fernanda Silveira Flôres Vogel. Data curation: Isac J. Roman, Gilneia da Rosa, Fernanda S.F. Vogel. Formal analysis and investigation: Isac J. Roman, Fernanda S.F. Vogel, Juliana F. Cargnelutti, Luis A. Sangioni. Methodology: Isac J. Roman, Fernanda S.F. Vogel. Writing – original draft preparation: Isac J. Roman. Writing – review and editing: Isac J. Roman, Fernando S. Rodrigues, Fernanda S.F. Vogel. Resources: Luis A. Sangioni, Fernanda S.F. Vogel. Supervision: Fernanda S.F. Vogel.

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