

Original Research Paper

Association of Reproductive Problems and Dairy Cow Productivity in a Farm with an Outbreak of Neosporosis Abortion in Brazil

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Abstract: Neosporosis is a disease caused by the protozoan *Neospora caninum* and its only clinical symptom in adult cows is abortion. In order to determine whether *N. caninum* was associated with an outbreak of abortion on a high milk production dairy farm with excellent livestock and health control standards, blood was collected from all the animals in the herd to obtain their serum and perform indirect ELISA. To this end, we followed the procedures of the HerdChek anti-*Neospora caninum* test kit to obtain epidemiological information about isolated estrus repetition, production, recurrent estrus, isolated abortion and recurrent abortion on the dairy farm. Relative risk and Odds ratio were calculated and all the calculations were performed using SAEG 9.1 software. Of the 111 blood samples collected, 42% tested seropositive and among the 58 lactating cows, 16 (28%) were seropositive. High milk producing cows were more vulnerable to infection by *N. caninum* getting to have 4.5 lower risk of infection than lower milk producing cows. It was also observed that low milk productivity is associated with protection factor. No association was observed between isolate estrus repetition and seropositive and seronegative animals as well as between the presence of recurrent estrus and positive and negative cows. Significant association was observed between isolated abortion and seropositive and seronegative animals as well as between recurrent abortion and positive and negative animals evaluated. Therefore, we conclude that neosporosis does not interfere in fertility, but this is related strictly to abortions in the herd and this problem is most evident in high milk producing dairy cows.

Keywords: Neosporosis, Abortion, Fertility, ELISA

Introduction

Neospora caninum is an emergent protozoan of the phylum Apicomplexa, which can cause reproductive problems in cows worldwide (Dubey *et al.*, 2007). This parasite is responsible for neosporosis-one of the most important diseases that cause abortions in dairy cows (Gennari, 2004; Guimaraes *et al.*, 2004; Goodswen *et al.*, 2013). In Brazil, *N. caninum* has been diagnosed in aborted fetuses since 1999 and in serological surveys of cattle and dogs in different states, as well as in other domesticated and wild animals (Cerqueira-Cézar *et al.*, 2017).

Serological testing, an important tool that can be applied to diagnose infection in herds by detecting specific anti-*N. caninum* antibodies, involves techniques such as Indirect Fluorescence Antibody Test (IFAT), Neospora Agglutination Test (NAT), Enzyme-Linked Immunosorbent Assay (ELISA), rapid Immunochromatographic Test (RIT) and the immunoblot assay (Dubey and Schares, 2006). In Brazil the seroprevalence of *N. caninum* varies with the type of cattle (beef, dairy), different regions, within region and with the type of serological tests used (Cerqueira-Cézar *et al.*, 2017).

In Latin America, neosporosis is considered an emergent disease in dairy cattle being responsible for economic losses related to abortion (Fávero *et al.*, 2017). Basso *et al.* (2010) have shown in their study that *N. caninum* is the main cause of reproductive disorders in cows worldwide, including abortion, estrus repetitions and temporary anestrus (Bruhn *et al.*, 2013). Animal age was observed by Guimarães *et al.* (2004) as a risk factor for *N. caninum* infection, where older animals showed greater seroprevalence, indicating that the horizontal transmission has great influence on the herds. In addition, a study in South America demonstrated that neosporosis in dairy cattle has many more risk factors related to the infection (Moore, 2005). These factors are increasing age of cows (between 2 and 6 years old), as well as the intensive management of dairy cattle in the farm, indicating high rates of horizontal transmission in the herds (Moore, 2005).

The purpose of this study was to determine the frequency of anti-*N. caninum* antibodies and investigate the association between reproductive problems through indicators such as fertility, repetition of isolated and recurrent estrus, presence of isolated and recurrent abortion in the herd, as well as whether milk production interfered in the infection on a Brazilian dairy farm which presented an outbreak of epizootic bovine abortion.

Materials and Methods

The present research was done in March 2010 in a property located in the municipality of Boa Esperança in the South of Minas Gerais, Brazil. Data collected indicated the occurrence of expressive increase of cows that were aborting in the initial third of gestation. This dairy farm had 200 Girolando cattle raised in a semi-intensive system, where cows had access to forage and concentrate in the trough and produced an average 30 kg of milk/cow/day. The farm has a good management system based on a vaccination program to control diseases such as foot-and-mouth disease, rabies, clostridiosis, mastitis, salmonellosis, brucellosis, leptospirosis, infectious bovine rhinotracheitis (IBR) and Bovine Viral Diarrhea (BVD), which causes abortion and reproductive problems. According to the report of the owner and responsible veterinarian sporadic undiagnosed bovine abortions have occurred, despite the excellent sanitary control of the herd. However, in a short period of time, mainly in the last 6 months, several cases of abortion occurred on the farm, leading the owner to seek help from the Veterinary Hospital of the State University of North Fluminense (UENF) to investigate possible causes.

A history of the dairy farm, containing information on reproduction type, estrus recurrence and abortions was obtained from an epidemiological questionnaire applied to the veterinarian and individual milk production of the cows was obtained from the production control record provided by the owner.

After local antiseptics with 2% iodinated alcohol solution, blood samples were drawn from the jugular vein of 111 cows, using 40×1.2 mm size disposable needles and 8.5 mL tubes with separator gel (BD Vacutainer®, New Jersey, EUA). The samples were labeled, placed in isothermal boxes (8-10°C) and sent to the laboratory of parasitology at UENF, where they were centrifuged at 350g for 10 min to separate the serum. They were then placed in 2.0 mL screw capped plastic tubes, in duplicate, labeled and stored at -20°C until testing.

The indirect ELISA was used for the detection and titration of anti-*N. caninum* antibodies, following the protocol of the HerdChek anti-*Neospora caninum* test kit for the detection of anti-*N. caninum* antibodies in ruminants (Herdchek®, Westbrook, USA). Absorbance values were measured using an ELISA Multiskan EX reader (Thermo Fisher Scientific®, Vantaa, Finland) equipped with a 620 nm wavelength filter.

The relationship between overall reproductive problems (abortion or estrus repetition) and the frequency of anti-*N. caninum* antibodies was assessed by calculating the Relative Risk (Rr) using the χ^2 statistical test. The Rr was calculated using Fisher's test with katz approximation to determine whether the frequency of antibodies was associated with cows presenting isolated estrus repetition, recurrent estrus, isolated abortions and recurrent abortions and as well as their influence on milk production. Odds ratio (Or) with 95% Confidence Intervals (CI) were also calculated to determine the chance of the event happening. All the calculations were performed using System of Statistical Analysis (SAEG 9.1) software.

Results

Since in the farm was only used artificial insemination, the method of reproduction could not be evaluated as risk in the cases of abortion observed in the present study.

Of the 111 cows evaluated, 46 had anti-*N. caninum* antibodies in their sera, indicating a frequency of 42% of seropositive animals (Fig. 1). Among the 58 lactating cows, 16 (28%) were seropositive (Fig. 2).

High milk producing cows (above 24 kg/day) were more vulnerable to infection by *N. caninum* (Rr=4.5158; IC95%: 1.8290-11.1494), that is, it has 4.5 lower risk of infection than lower milk producing cows, a fact that is extremely significant in terms of infection (P=0.0011). It was also observed an association when the analysis was performed by Or (Fig. 2), however, determining that a lower milk production is a protective factor for neosporosis.

No association was observed between isolated estrus repetition and positive and negative animals evaluated by Rr=0.8728 (95% CI: 0.5575-1.3660) with P=0.5518, as well as by OR analysis (Fig. 1). Also, no association between positive and negative cows was observed for the presence of recurrent estrus when Rr=1.5568 (95% CI: 0.9592-2.5267) was evaluated with P=0.0733, as well as by OR analysis (Fig. 3).

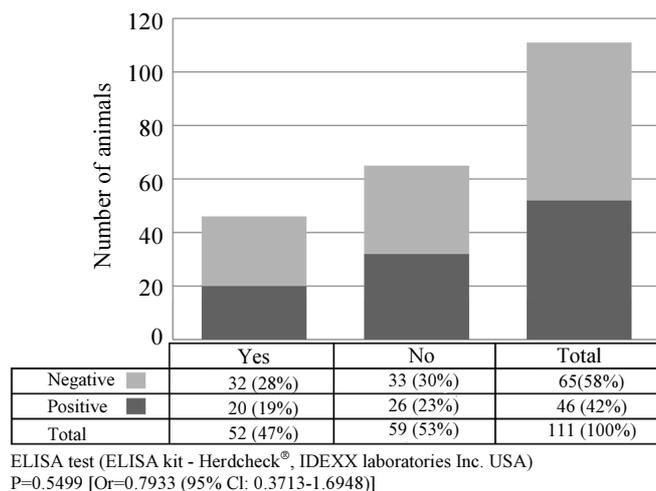


Fig. 1: Relationship of isolated estrus repetition and frequency of anti-*Neospora caninum* antibodies in Giroland cows of high zootechnical standard, from a farm with an epizootic abortion, in the city of Boa Esperança, Minas Gerais State, Brazil

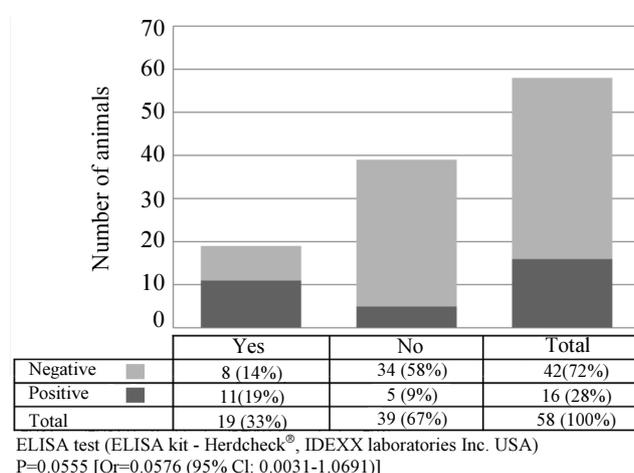


Fig. 2: Relationship of daily production >24 kg and frequency of anti-*Neospora caninum* antibodies in Giroland cows of high zootechnical standard, from a farm with an epizootic abortion, in the city of Boa Esperança, Minas Gerais State, Brazil

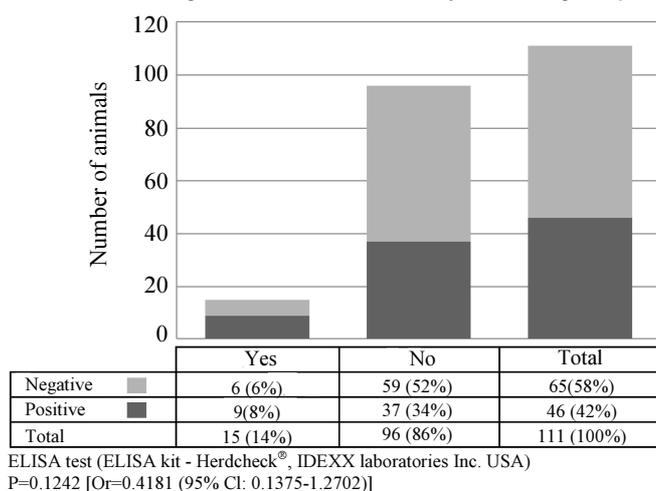


Fig. 3: Relationship of recurrent estrus and frequency of anti-*Neospora caninum* antibodies in Giroland cows of high zootechnical standard, from a farm with an epizootic abortion, in the city of Boa Esperança, Minas Gerais State, Brazil

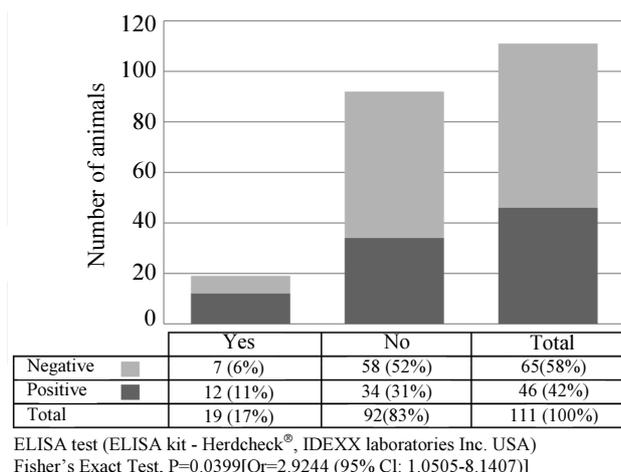


Fig. 4: Relationship of isolated abortion and frequency of anti-*Neospora caninum* antibodies in Giroland cows of high zootechnical standard, from a farm with an epizootic abortion, in the city of Boa Esperança, Minas Gerais State, Brazil

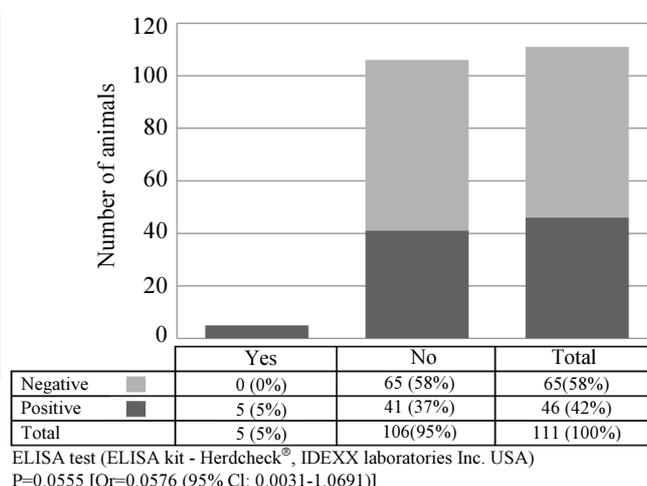


Fig. 5: Relationship of recurrent abortion and frequency of anti-*Neospora caninum* antibodies in Giroland cows of high zootechnical standard, from a farm with an epizootic abortion, in the city of Boa Esperança, Minas Gerais State, Brazil

Significant association was observed between isolated abortion and seropositive and seronegative animals evaluated by $R_r=1.7090$ (95% CI: 1.1060-2.6402) with $P=0.0157$. This association is indicating that cows seropositive for *N. caninum* were 1.7 times more likely to abort than seronegative cows. When this association was assessed by the log-normal distribution through Or, it was indicative that the seropositive cows are almost three times more likely to abort than negative cows (Fig. 4).

We also observed an extremely significant association between recurrent abortion and positive and negative animals evaluated by $R_r=2.5854$ (95% CI: 2.0343-3.2857) with $P<0.0001$. This indicated that positive cows were 2.5 times more risk to have recurrent abortion. When we checked this association using Or, it was observed that the chance of recurrent abortion in seropositive cows is not a valid association for cases of neosporosis outbreaks (Fig. 5).

Discussion

The frequency rates of anti-*N. caninum* antibodies in the serum of dairy cows around the world vary considerably and these rates increase when associated with the occurrence of abortions in herds (Dubey *et al.*, 2007). In this study, the frequency rate of 42% of seropositive cows was higher than the rates of 23.3%, 19% and 10.9% reported, respectively, by Munhoz *et al.* (2009), Bôa-Morte and Oliveira (2009) and Aguiar *et al.* (2011) on other Brazilian farms. Including the frequencies observed in the same state where our research was carried out and using the same test (Melo *et al.*, 2004; 2006; Mineo *et al.*, 2006; Bruhn *et al.*, 2013).

These authors carried out their research on cows without actual problems of abortion in the herds. The

significant difference in the frequency rates observed by these authors, unlike the high frequency rate in the cows of this research, explains the outbreaks of epizootic bovine abortion at the farm under study. The effective disease control program of reproductive problems on the farm, except for neosporosis, reinforces the relationship between abortions and the frequency of anti-*N. caninum* antibodies in the sera of the cows investigated here, which must be a determining factor for the presence of the parasite in the herd.

The non-interference of seropositivity in the repetition of estrus observed in the present research, including in the animals in which this problem is recurrent, is in agreement with authors who have stated that neosporosis does not imply fertility problems (López-Gatius *et al.*, 2005; Brickell *et al.*, 2010; Landmann *et al.*, 2011; Gonzalez-Warleta *et al.*, 2011). However, although Xia *et al.* (2011) did not find a significant difference with respect to abortions, the high frequency rate and significant association between abortions in the cows analyzed in this study and the group of seropositive animals are in agreement with the findings reported by Landmann *et al.* (2011) and Gonzalez-Warleta *et al.* (2011). This confirms the strong association between this parasite and abortions that occur on several farms where neosporosis is highly frequent, with the Or varying according to the research site and the animal species under analysis, including buffaloes (Nasir *et al.*, 2011), sheep (Machado *et al.*, 2011) and goats (Topazio *et al.*, 2014), including when in cases of epizootic outbreaks.

According to Aguiar *et al.* (2011), more productive dairy farms have a higher prevalence of anti-*N. caninum* antibodies, corroborating the findings of this study, which found a significant association between higher milk producing cows and the presence of anti-*N. caninum* antibodies in their sera. Xia *et al.* (2011) also reported that cattle are more susceptible to disease between 5 and 6 years of age, as cows have their highest production peak in this age group, perhaps this factor is collaborating with the greater seroreactivity of the cows of higher production of our research and of Aguiar *et al.* (2011). In contrast to this hypothesis, our results when analyzed by the log transformation method determined by Or showed that the lower dairy production of cows is a protection factor (Table 2). This may explain why neosporosis-related problems are not reported in properties with low milk production as occurs in most Brazilian farms (Bôa-Morte and Oliveira, 2009; Munhoz *et al.*, 2009; Aguiar *et al.*, 2011) where production in the vast majority is below 24kg of milk per animal per day. In contrast to more developed countries where production losses due to disease are significant (Dubey *et al.*, 2007).

Conclusion

In this study, we described an epizootic outbreak of neosporosis-related abortion at the farm under investigation. It was also confirmed that neosporosis does not interfere with the fertility of the herd, but is strictly related to abortion and that this problem is more evident in high milk producing cows.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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Author's Contributions

Francisco Carlos Rodrigues de Oliveira: Experimental, design, statistical analysis and writing of the manuscript.

Samira Salim Mello Gallo: Aided in interpreting the results and worked on the manuscript.

Murilo de Oliveira Bôa-Morte: Conducted the experiment, summarized the data.

Nicole Brand Ederli: Worked out almost all of the technical details and worked on the manuscript.

Ethics

The authors declare there are no ethical issues related to this observational study.

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