Veterinary Parasitology: Regional Studies and Reports 10 (2017) 71–74

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Original article

Prevalence and risk factors associated with anti-*Neospora caninum* antibodies in dairy herds in the central region of Minas Gerais State, Brazil

Rafael Romero Nicolino[a,*], Camila Stefanie Fonseca de Oliveira[b], Luciano Bastos Lopes[c], Rogério Oliveira Rodrigues[d], João Paulo Amaral Haddad[e]

[a] Federal University of Jequitinhonha and Mucuri Valleys - UFVJM, Agronomic & Animal Sciences Institute, Av. Ver. João Narciso, 1380 – Cachoeira, Unaí, Minas Gerais, Brazil
[b] Centro Universitário de Belo Horizonte, UniBH. Av. Prof. Mário Werneck, 1685 - Estoril -, Belo Horizonte, Minas Gerais, Brazil
[c] Brazilian Agricultural Research Corporation, Embrapa. Rodovia MT-222, Km 2,5, Zona Rural, Sinop, Mato Grosso, Brazil
[d] Secretary of Agriculture/SC, Estrada do Conde, n° 6000, Eldorado do Sul, Rio Grande do Sul, Brazil
[e] Federal University of Minas Gerais, UFMG. Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte, Minas Gerais, Brazil

ARTICLE INFO

Keywords:
Dairy herd
Neospora caninum
Risk factors
Prevalence
Minas Gerais (Brazil)

ABSTRACT

Neospora caninum is one of the parasites that frequently causes reproductive loses in cattle herds all over the world. Surveys have shown a high prevalence of the parasite in herds; in certain locations, 90% to 100% of dairy holdings are positives according serological tests. The aim of this study was to estimate the prevalence and risk factors associated with the increased number of seropositive dairy cattle in the central region of Minas Gerais, the largest milk producer state in Brazil. Samples were collected from 151 dairy herds and from 2915 lactating cows, and were evaluated by an indirect ELISA assay. According to results, animal prevalence was 21.9% (CI 95%: 18.9 to 24.9%), while the herd prevalence it was 98.5% (CI 95%: 97.0 to 99.9%). Poisson regression estimate the herd risk factors associated with the increased number of cases. An increased number of positive animals was related to the previous history of abortion and the presence of dogs. The use of individual natural colostrum feeding and reproductive techniques such as embryo transfer and *in vitro* fertilization are associated with a reduced number of seropositive animals in the herd. In conclusion, *Neospora caninum* is overspread across the dairy herds of the region, and it may be causing major economic losses for dairy farmers. The use of reproduction techniques, the access of dogs to the herds and the management of calf colostrum are important issues to be considered in the control of the disease.

1. Introduction

Neospora caninum is an obligate intracellular protozoan, and experimental studies show that the domestic dog (*Canis lupus familiaris*), the Australian dingo (*Canis lupus dingo*), and the coyote (*Canis latrans*) are the definitive hosts of the parasite, and that a wide range of animals of importance to the world's livestock, such as cattle, sheep and buffaloes, act as intermediate hosts (Dubey et al., 1988; McAllister et al., 1998; Gondim et al., 2004; King et al., 2010). The agent was first reported in 1984 in dogs with nervous clinical signs in Norway (Bjerkås et al., 1984), but a new genus and species, called *N. caninum* it was only isolated and described by Dubey in 1988. Since then, neosporosis has been associated to major economic impacts on cattle herds all over the world, mainly due to abortions and reproductive failures (Reichel et al., 2013).

In dairy cattle herds, the main transmission route of the agent is transplacental (vertical), responsible for up to 95% of transmissions. However, the major abortion outbreaks it's been reported when horizontal transmission occurs, especially in primarily infected heifers. Horizontal transmission takes place when the cattle ingests the spore form of the oocyst released by the definitive host via feces; contaminating cattle's, feed or water sources (Schares et al., 1998; Dubey et al., 2007).

Abortion is the main clinical sign manifested by cows infected with *N. caninum*. Fetal death usually occurs between the third and eighth months of pregnancy due to direct damage to the fetal or placental tissues. Fetuses dying before the fifth month of pregnancy may be mummified and retained in the uterus for several months; in contrast, fetuses affected in early stage of development tend to be reabsorbed. The antibody peak occurs between the fourth and fifth months before

* Corresponding author at: Laboratory of Epidemiology and Geoprocessing, Agronomic & Animal Sciences Institute - UFVJM, Av. Ver. João Narciso, 1380 - Cachoeira, Unaí, Minas Gerais, Brazil.
E-mail address: rafael.nicolino@ufvjm.edu.br (R.R. Nicolino).

http://dx.doi.org/10.1016/j.vprsr.2017.08.008
Received 3 January 2017; Received in revised form 12 July 2017; Accepted 15 August 2017
Available online 17 August 2017
2405-9390/

parturition or abortion cases, decreasing after that period (Stenlund et al., 1999). Nicolino et al. (2015) reported an increase of 10.2% (9.8 to 11.8%) on abortion risk, demonstrating the negative impact of the agent on dairy herds in Brazil. The country is the fourth cow's milk producer in the world, behind USA, India and China, producing 34.3 billion kilograms (Sheth, 2017).

Despite the large representation of the sector, few researchers have estimated the economic impact of *N. caninum* in Brazilian herds. Reichel et al. (2013) estimated the losses related to abortion in US$ 51.3 million. Nicolino (2015) found losses related to abortion, milk yield, and involuntary culling around US$ 100 million (US$ 63 to US$ 150 million) for dairy herds, using stochastic-simulation modeling.

Cross-sectional studies show a high prevalence of antibodies in Brazilian's dairy cattle, with rates ranging from 12.4% in Rio Grande do Sul (Vogel et al., 2006), 21.7% in Minas Gerais (Bruhn et al., 2013), 30.0% in Goiás (Melo et al., 2006), to 35.0% of dairy cows in São Paulo State (Sartor et al., 2005). The main risk factors on dairy farms are the advanced age of the animals, the presence of dogs and their proximity to the herd, climatic conditions favorable for oocysts on environment, the use of heifers from the farm for herd replacement, high density of animals, and improper biosecurity practices (Corbellini et al., 2006; Dubey et al., 2007; Vanleeuwen et al., 2010; Piagentini et al., 2012; Sousa et al., 2012). The aim of this study was to estimate the prevalence and indicate the risk factors associated with the increased number of cases of seropositive animals in dairy herds in the micro-region of Sete Lagoas, Minas Gerais State, Brazil.

2. Material and methods

2.1. Sample

The study is a cross-sectional observational investigation, from January to July of 2010, with simple random sampling. All farms were registered in the Institute of Agriculture of Minas Gerais State (IMA), and had the same probability of being sampled. For the definition of the sample size, 1446 dairy cattle herds were included in the calculation, all from eleven municipalities in the micro-region of Sete Lagoas – Minas Gerais, Brazil (Fig. 1). The sampled municipalities were Araçaí (48 herds), Caetanópolis (43 herds), Cachoeira da Prata (26 herds), Cordisburgo (287 herds), Fortuna de Minas (96 herds), Funilândia (92 herds), Inhaúma (79 herds), Jequitibá (259 herds), Prudente de Morais (38 herds), Paraopeba (198 herds), and Sete Lagoas (280 herds). The expected herd prevalence was 70%, with a maximum error of 7%, and a 95% confidence level. Using StatCalc module from EpiInfo 6.04, the minimum number of herds necessary for the estimation of prevalence was 149. To determine the animal prevalence it was used Herdacc 3.0® software (Herdacc, 1995) with a diagnostic sensitivity of 0.96; specificity of 1.0 (data provided by the distributor of the test); population size fixed as 20 dairy cows per farm (average of cows per herd according to IMA's database); and expected animal prevalence of 25%, resulting in a sample of 19 dairy cows per farm.

2.2. Diagnostic test

Blood samples were collected from each cow, centrifuged at 1000 RPM for 10 min, the serums were stored at − 20 °C until to the laboratorial analysis. An indirect commercial ELISA kit (Herdcheck®) with 96.6% sensitivity and 100% specificity (IDEXX Laboratories, Inc., USA) was used to check the presence of anti-*N. caninum* antibodys. The tests were performed according to the recommendations in the manufacturer's manual.

2.3. Statistical analysis

The animal and herd prevalences were estimated by defining the specific weight of each sample unit in the universe of herds and animals included in the study, according to Dargatz and Hill (1996) and Dohoo et al. (2003). Risk factors associated with the presence of anti-*N. caninum* antibodies were estimated by applying a structured questionnaire on each farm. The questions addressed economic aspects, animal management, and biosecurity practices. A dataset was built and then analyzed using the statistical software STATA® (version 11). It was adopted a Poisson regression model to run the analysis due the high animal and herd prevalence. The regression uses the count of seropositive animals as the outcome to estimate the Incidence Rate Ratio (IRR). Initially, the univariate model selected variables with a significance level below 0.15, and later, in the multivariate model, the significance level was 0.05. Thus, the statistically significant variables should be understood as herd risk factors that elevate the number of seropositive animals inside the herd.

3. Results and discussion

3.1. Prevalence

A total of 151 dairy herds were visited and samples were collected from 2915 dairy cows, averaging 19.3 cows per farm. The herd prevalence was estimated at 98.5% (CI 95%: 97.0 to 99.9%), while animal prevalence was estimated at 21.9% (CI 95%: 18.9 to 24.9%). These values show that the agent is widespread, and with a high prevalence in dairy herds in the central region of Minas Gerais. The state is responsible for 25.7% of milk production in Brazil, with > 9 billion liters of milk produced and 5 million cows milked in 2015 (IBGE, 2015). Together, the eleven municipalities sampled produced > 97 million liters of milk, with 39,424 cows milked, according to data from IBGE (2015). The prevalence is similar to others studies in Minas Gerais. Santos et al. (2009) reported 100% of prevalence on the tested herds and 41% of animals and Bruhn et al. (2013) reported the presence of antibodys in 21.7% of cows and 95% of dairy herds in Minas Gerais as well. According to Orlando et al. (2013), *N. caninum* is an important agent associated with abortion cases in Minas Gerais.

High seropositivity may be related to lack of knowledge leading to the adoption of control and prevention measures with low effectiveness by the producers and veterinarians. The questionnaire revealed that 90% (136/151) of owners declared to have dogs on their farms and do not control the locations to which they have access. An important issue involving biosecurity is that on 20% (30/151) of the farms, some of the equipments used to distribute feed for cows it was also used to collect the animal feces, including shovels and hoes. This practice can be one of the causes for food contamination with oocysts eliminated in the feces of dogs, increasing prevalence rates due to horizontal transmission within herds. In addition, no farmer reported to have requested *N. caninum* tests before purchasing new animals.

3.2. Risk factors

As shown in Table 1, the results reveal that the presence and proximity of dogs to dairy cows constitute a significant factor that increase the number of cases in the studied dairy herds. The variable “presence of dogs” on the farm was not statistically significant ($p = 0.67$), possibly due to the high percentage of farms with dogs (over 90%) and the high prevalence of the protozoan among the herds. Invariably, even the farms that do not have dogs cannot accomplishing the control of stray dogs, which are very common in the social context of the livestock areas of Brazil.

However, the variables related to location of whelping on the farm, such as unknown location to the producer (IRR 1.24), in the doghouse (IRR 1.32), and in the cow pen (IRR 1.36), were all significant, increasing the number of seropositive dairy cows. It is expected that these dogs have free access to the facilities of the farm, contaminating food and water with oocysts. The presence and proximity of dogs to dairy cows are very important risk factors recurrent in the literature