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Original Research

## Risk Factors for Occurrence of Anti-*Neospora* spp. Antibodies in Horses From Alagoas, Brazil



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#### ABSTRACT

The aim of the present study was to investigate the frequency of *Neospora* spp. infection, as well as its associated risk factors, in horses in the state of Alagoas, Brazil. In total, 427 samples from 36 properties in 23 districts, covering three mesoregions, were studied. An immunofluorescence assay was used to quantify anti-Neospora caninum immunoglobulin G antibodies, and risk factors were assessed through investigative questionnaires that focused on the productive, reproductive, and sanitary management of herds. Data from the questionnaires were evaluated by two statistical analyses. The total frequency of anti-Neospora spp. antibodies found was 18% (95% confidence interval [95% CI]: 14.4-22.1). At least one seropositive horse was found in 72.2% of the farms evaluated (26 of 36). The following risk factors were associated with infection: the access of other species to the horses' water source (P = .017; odds ratio [OR] = 1.84; 95% CI: 1.11–3.04); no to consume hay (P = .000; OR = 3.33; 95% CI: 1.87–5.94); the informal purchase of animals (P = .000; OR = 8.43; 95% CI: 3.06–23.16); and an absence of quarantine management (P = .000; OR = .000); OR = .0008.70; 95% CI: 3.10–24.39). The results of the present study show that horses in the state of Alagoas are at risk for Neospora spp. infection, and care should be implemented based on identified risk factors to prevent infection in horses.

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#### 1. Introduction

Neospora spp. genus is composed of two protozoa species: Neospora caninum and Neospora hughesi, which are intracellular obligate parasites. Neospora caninum has been reported in dogs (definitive hosts) and several other animal species, whereas N. hughesi has been isolated from horses [1]; however, the definitive host remains unknown, and there are doubts about the intermediary host and the form of exposure to the parasite [2].

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According to Dubey et al [3], *Neospora* spp. transmission can occur either horizontally or vertically. In horses, *N. caninum* infection has been associated with reproductive problems and neonatal disease [4], whereas *N. hughesi* infection has been shown to cause neurologic disorders [5,6].

In Brazil, some studies were performed to detect anti-*Neospora* spp. antibodies [7–11]. Hoane et al [2] reported a prevalence of 2.5% in serum samples from 10 federal states, whereas Villalobos et al [7] found a prevalence of 15.1% in animals living in São Paulo state. Locatelli-Dittrich et al [8] reported prevalence rates ranging from 30% to 47% in the state of Paraná. In Rio Grande do Sul, Toscan et al [12] reported a prevalence of 13.8%, and Sangioni et al [13] found a

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prevalence of 15.4%. Little is known about the frequency of this parasite among horses in the Northeast of the country.

Few studies have been conducted in Brazil assessed the risk factors associated with *N. caninum* [12,14,15].

The aim of the present study was to investigate the frequency of *N. caninum* infection, as well as its associated risk factors, among horses in the state of Alagoas in the Northeast of Brazil.

#### 2. Materials and Methods

#### 2.1. Study Area

The present study was conducted on horse breeding farms in Alagoas, Brazil. Alagoas is located in the centereast of the Brazilian Northeast on an intertropical strip that has long periods of solar irradiation throughout the year: ranging from 2,200 to 2,600 hours of sun. Temperatures are high during most of the year, ranging from 22°C to 28°C. The state is divided into three mesoregions: the Leste Alagoano, Agreste Alagoano, and Sertão Alagoano [16].

The Leste mesoregion is characterized by a wet tropical coastal climate, with a relative air humidity of 79.2% and an average rainfall of 1,410 mm/year. The sun dominates from September to May, and during this period, temperatures range from 19°C to 32°C, whereas during the rainy season between June and August, temperatures range from 15°C to 26°C. The *Sertão* mesoregion, by contrast, has a semiarid climate, with irregular rainfall and low relative air humidity. Finally, the Agreste mesoregion lies between the *Sertão* and the *Leste* mesoregions and consequently exhibits characteristics of both.

#### 2.2. Sampling

Samples were collected by convenience, based on the sample size for a finite population with an expected frequency of 50%, a confidence level of 95%, a statistical error of 5% [17], and population of 56,310 the horses in the state of Alagoas. These parameters indicated that a minimum of 385 animals should be used in this study. Sampling was conducted between August and November of 2012.

A cross-sectional study was conducted using 427 samples of horse serum. Male and female horses older than 36 months were used. Animals were selected from 36 farms located in 23 districts of the Leste Alagoano, Agreste Alagoano, and Sertão Alagoano mesoregions. The samples were collected by jugular venipuncture using a blood collection vacuum system (BD Vacutainer).

Horses of different breeds were used from farms that used either natural mating and/or artificial insemination. Farms from Leste Alagoano and Agreste Alagoano mesoregions used a semi-intensive raising system, whereas those in the *Sertão Alagoano* used an intensive system. Each animal's diet was primarily based on green forage and/or hay as well as balanced ration.

#### 2.3. Serology

To measure anti-*N. caninum* immunoglobulin G, an indirect immunofluorescence assay was performed used *N.* 

caninum (NC-1 strain) tachyzoites fixation in slides. Anti-immunoglobulin G horse antibodies conjugated to fluorescein isothiocyanate (Sigma Chemical) were used as secondary antibodies. All the reactions involved previously established positive and negative controls. Samples were considered positive when they exhibited maximum fluorescence at a dilution of 1:50 [18]. The positive samples were submitted to twofold serial dilutions until the greatest positive dilution was obtained. The titer of the serum was defined as the reciprocal of the greatest dilution that provided a positive result.

#### 2.4. Statistical Analysis

Descriptive statistical analysis was used to calculate the relative and absolute frequencies of the results obtained from the serology. Risk factors were assessed based on investigative questionnaires consisting of objective questions related to the productive, reproductive, and sanitary management of the animals. To identify the risk factors associated with Neospora spp. infection, univariate analysis of the variables of interest was first conducted using Pearson's chi-squared test or Fisher's exact test when necessary, with the level of significance set at 5%. Subsequently, multivariate analysis was conducted using a logistic regression, where the serologic status of the animal (positive or negative) was the dependent variable. The independent (or explanatory) variables considered in the model were those that exhibited a statistical significance of < 0.20. The farm when defined as a focus farm when at least one positive animal was found. Epi Info software, version 3.5.2, was used to execute statistical calculations (Center for Disease Control and Prevention).

#### 3. Results

Of the 427 samples analyzed, 77 (18%; 95% confidence interval: 14.4–22.1) were positive in the indirect immunofluorescence assay. Titer 50 was identified in 24 samples (31.17%), whereas titer 100 was found in 28 samples (36.36%), titer 200 was observed in 19 samples (24.68%), titer 400 was identified in five samples (6.49%), and titer 800 was found in one sample (1.3%).

Regarding the number of foci, 26 (72.2%) of the farms contained at least one positive animal. The occurrence of positive animals on the farms analyzed ranged from 9.1% to 100.0%.

A significant difference was found among the different mesoregions studied in terms of frequency. The greatest frequency rates were found in the Agreste mesoregion (Fig. 1).

The results found in the univariate analysis are in Table 1. The following risk factors were identified: the access of other species (dog and wild animals) to the horses' water source (odds ratio [OR] = 1.84); no to consume hay (OR = 3.33); the informal purchase of animals (OR = 8.43); and an absence of quarantine management (OR = 8.70; Table 2).

#### 4. Discussion

The frequency of seropositive horses (18%) found in the present study is relatively high compared to the results of

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