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Short communication

Flock-level risk factors associated with *Neospora caninum* seroprevalence in dairy goats in a semiarid region of Northeastern Brazil

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ABSTRACT

A cross-sectional study based on a planned sampling was carried out to determine flock-level risk factors associated with *Neospora caninum* antibody prevalence in dairy goat flocks in a semiarid region of Northeastern Brazil. Serum samples from 975 adult dairy goats from 110 flocks were examined by the indirect immunofluorescent antibody test, using cut-off point at dilution of 1:50. From the 110 flocks 18 presented at least one seropositive animal, corresponding to a prevalence of 16.4% (95% CI: 10.0–24.6%), and 26 (2.7%; 95% CI: 1.7–3.9%) animals tested positive for *N. caninum* antibodies. Graze at communal pasture (OR = 10.34; $P = 0.034$), not using disposable syringes (OR = 3.78; $P = 0.023$) and flock size >25 goats (OR = 6.51; $P = 0.007$) were identified as risk factors. Further studies are needed to elucidate the importance of the identified risk factors in the epidemiology of this infection.

1. Introduction

Neospora caninum is the major cause of abortions in cattle in some countries (Dubey, 2003), and can also cause abortion or neonatal mortality in goats (Corbellini et al., 2001; Dubey, 2003). However, the rates of *N. caninum* infection and the significance of the disease in this species have been poorly investigated.

The use of convenience sampling in epidemiological works to determine the occurrence of infectious diseases is very common and allows the determination of important information; however, epidemiological inference should not be made based on this procedure because of the occurrence of biases. In Brazil, although many serological studies on *N. caninum* infection in goats are available, works based on a planned sampling are few. The aim of this study was to identify risk factors associated with flock-level prevalence of *N. caninum* infection in dairy goats from a semiarid region of Northeastern Brazil based on a planned sampling.

2. Materials and methods

2.1. Study design and sampling

The present study was carried out from March 2009 to March 2010 in the county of Monteiro (7°53′S, 37°5′W), Cariri Occidental micro region, semiarid region of the State of Paraíba, Northeastern of Brazil. The study was designed as a cross-sectional study of randomly selected dairy goat flocks. Blood samples were collected from female goats that were ≥12 months old. A two-stage sample design was followed. Firstly, dairy goat flocks were randomly selected. The number of flocks to be sampled was determined considering the number of dairy goat flocks in the region ($n = 180$, according to the data of the Center for Integrated Development of Goat Production, Paraíba State), an expected flock prevalence of 50% (considering no a priori knowledge of the herd prevalence), and a 10%

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desired accuracy for a 99% level of confidence (Thrusfield, 2007), resulting in 86 herds to be sampled. Secondly, the sample size of goats to be selected was individually determined for each flock so as to detect the presence of the infection. Calculations were made in accordance with the formula commonly applied in veterinary epidemiological investigations (Thrusfield, 2007):

$$n = [1 - (1 - p)^{1/d}] \times \left(N - \frac{d}{2}\right) + 1$$

where n is the sample size; p is the probability of detection of at least one seropositive goat; N is the herd size; and d is the number of seropositive goats in the herd.

The probability of detection of at least one seropositive goat in a flock was determined at 95% ($P = 0.95$), and the number of seropositive goats in each herd (d) was calculated assuming within flock prevalence of 6.4% (Figliuolo et al., 2004).

Selection of goats to be sampled from each flock was based on a systematic random sampling, where goats were put in a crush pen and systematically selected. In situations where handling infrastructure was absent, true random sampling was difficult to attain. In such situations, animals were put in a kraal and randomly captured.

A total of 975 female goats in 110 flocks were randomly selected and examined for *N. caninum* antibodies.

2.2. Epidemiological data collection

A structured questionnaire focusing on risk factors for neosporosis was given to each farmer at the time of blood collection. Information was collected on a total of 23 flock-level factors that included: management system, main activity in the farm, flock size, predominant goat breed, presence of cattle, swine, cats, dogs and wildlife, presence of toxic plants, availability of veterinary services, animal purchasing, mineral supplementation, use of disposable syringes, lend buck for breeding, graze at communal pasture, use of disinfectants and use of maternity pens. History of abortions, infertility, premature birth, stillbirths and birth of weak animals were also included.

2.3. Serological diagnosis

For the detection of anti-*N. caninum* antibodies the indirect fluorescent antibody test (IFAT) was performed according to Paré et al. (1995), adopting 1:50 dilution as the cut-off point (Lindsay et al., 1995). NC-1 strain of *N. caninum* cultured in bovine monocytes was used as the antigen. Positive and negative controls were also used. The conjugate used was an anti-goat IgG (whole molecule; Sigma, St. Louis, MO, USA) in a 1:400 dilution in sterile PBS (0.105 M Na_2HPO_4, 0.018 M KH_2PO_4, 1.37 M NaCl, and 0.027 M KCl), pH 7.6.

2.4. Statistical analysis

Flocks that presented at least one seropositive animal were considered positive. Prevalence of positive flocks was estimated from the ratio of positive flocks to the total number of flocks investigated, with the exact binomial confidence interval of 95% (Thrusfield, 2007), using the program EpiInfo version 6.04.

Risk factor analysis was performed in two steps: univariate and multivariate analysis. Univariate analysis was performed using the Chi-square test or Fisher's exact test, and those variables that presented $P \leq 0.20$ were used for multiple logistic regression. The multivariate analysis was then performed, using the stepwise forward method (Hosmer and Lemeshow, 2000). The significance level in multivariate analysis was 5%. The adjustment of the final model was checked using the Hosmer and Lemeshow test, and $P \geq 0.05$ was taken to indicate a satisfactory fit. The tests were performed using the SPSS for Windows software package, version 13.0.

3. Results

From the 110 flocks 18 presented at least one seropositive animal for *N. caninum*, corresponding to a prevalence of 16.4% (95% CI: 10.0–24.6%). Within-flock prevalence ranged from 8.3% to 40%. Twenty-six (2.7%; 95% CI = 1.7%-3.9%) animals tested positive for *N. caninum* antibodies. Overall, 4 (15.4%), 6 (23.1%), 3 (11.5%), 2 (7.7%), 2 (7.7%), 2 (7.7%) and 7 (26.9%) animals presented titers 100, 200, 400, 800, 3200, 6400 and 12,800, respectively.

In the univariate analysis, variables management system, flock size, presence of swine, use of disposable syringes, lend buck for breeding and graze at communal pasture were associated ($P < 0.20$) with flock-level prevalence of *N. caninum* and then were selected for the multivariate analysis (Table 1). When these independent variables were subjected to the multivariate analysis, graze at communal pasture (OR = 10.34; $P = 0.034$), not using disposable syringes (OR = 3.78; $P = 0.023$) and the presence of more than 25 goats in the flock (OR = 6.51; $P = 0.007$) were identified as risk factors (Table 2). Final model had a good fit (Hosmer and Lemeshow test: $P > 0.05$).

4. Discussion

Neosporosis is a relative new disease and little information is available about this in goats (Iovu et al., 2012). In the present work, 18 flocks (16.4%; 95% CI: 10.0–24.6%) had at least one seropositive animal for *N. caninum*. In animals, prevalence was 2.7% (95% CI: 1.7–3.9%). Although these results indicate a low prevalence, it was found within-flock prevalence of up to 40%, and in 66.4% (73/110) of the flocks the presence of dogs was reported, demonstrating that infection by this agent is spread in dairy goats in the region. These flock-level and within-flock prevalence found may be associated with the extensive management system and unlimited contact with free-roaming dogs, both of which are important risk factors (Czopowicz et al., 2011).

Related to anti-*N. caninum* antibody titers, 42.3% (11/26) of seropositive animals showed titers >800, and most frequent titer was 12,800 with 26.9% (7/26) of seropositive animals. Different results were found by Faria et al. (2007), in goats slaughtered in the county of Patos, Paraíba State, Northeastern Brazil, in which seropositive animals showed only titers <800. According to Çoškun et al. (2000), titers lower than 800 are suggestive of subclinical infection. These results indicate that 42.3% of the seropositive animals in this work might be acutely infected, which is important given the possibility of occurrence of transplacental infection as all investigated animals were adult females.

Risk factors associated with flock-level prevalence for *N. caninum* were graze at communal pasture (OR = 10.34; 95% CI: 2.00–89.35), not using disposable syringes (OR = 3.78; 95% CI: 1.20–11.88) and flock size >25 goats (OR = 6.51; 95% CI: 1.66–25.51). In all these variables, 95% confidence intervals for OR were large probably due to small number of observations (number of positive flocks) in at least one category in each variable because the confidence interval can be influenced by sample size (Thrusfield, 2007). However, it had no interference on the results since the confidence intervals did not include value 1.

Graze at communal pasture was also identified as risk factor by Al-Majali et al. (2008) in goats in Jordan. This finding may further suggest the idea of horizontal transmission and indicates the possibility of contamination of pasture and water with canine feces. *N. caninum* oocysts are excreted in the feces of dogs, sporulated outside the