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Short communication

A longitudinal study of *Neospora caninum* infection on three dairy farms in Brazil

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ABSTRACT

Neospora caninum is an apicomplexan protozoan parasite that is one of the most important infectious causes of abortion in both dairy and beef cattle in many countries. The objectives of this longitudinal study were to determine the prevalence, rates of vertical and horizontal transmission of *N. caninum* and hazard for culling of *N. caninum*-seropositive animals in three Brazilian dairy herds. Blood samples from all animals were collected nine times at each of the three farms over a two-year period. Serum was tested for antibodies against *N. caninum* using the indirect fluorescent antibody test with a cutoff value of 1:100. The percentage of *N. caninum*-positive samples at each sampling time ranged at Farm I from 3.32% to 11.71%, at Farm II from 3.90% to 22.06% and at Farm III from 3.90% to 22.06%. The number of positive serological reactions varied in relation to the number of repeated samples taken from individual animals at each farm. In all herds, there was a high degree ($P < 0.05$) of association between the *N. caninum* serological status of dams and daughters. The seropositive conversion rate was estimated as 0.37%, 3.00% and 6.94% per 100 cow-years at Farms I, II and III, respectively. The seronegative conversion rate was estimated as 31.58% and 11.11% per 100 cow-years at Farms I and III, respectively. In all herds, there was no difference ($P > 0.05$) in the culling rate between the cattle that were seropositive cattle and seronegative for *N. caninum* infection. The results from this study confirm the importance of vertical transmission in the epidemiology of *N. caninum*. Although a few positive seroconversions indicated horizontal transmission, it does not appear to be the major route of infection for *N. caninum*.

1. Introduction

Neospora caninum is an apicomplexan protozoan parasite that causes neuromuscular disease in dogs, and it is one of the most important infectious causes of abortion in both dairy and beef cattle in many countries (Dubey and Lindsay, 1996; Dubey et al., 2007).

Transplacental transmission is frequently assumed to be the major route of *N. caninum* infection in cattle. Serological studies using precolostral blood samples have shown that 81–95% of *N. caninum*-infected cows transmit the infection to their offspring and in consecutive pregnancies (Paré et al., 1997; Wouda et al., 1998; Hietala and Thurmond, 1999; Dijkstra et al., 2001).

Dogs, coyotes and dingoes are considered to be both the definitive and intermediate hosts for *N. caninum* (McAllister et al., 1998; Gondim et al., 2004; King et al., 2010). Presence of dogs on farms has been shown to be a risk factor for occurrences of *N. caninum* horizontal

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transmission in cattle. However, despite the presence of dogs, a low level of postnatal infection, less than 8.5% has been reported (Paré et al., 1998; Wouda et al., 1999; Dijkstra et al., 2002a).

High hazard for culling has been found both to be associated (Thurmond and Hietala, 1996; Waldner et al., 1998; Hobson et al., 2005; Bartels et al., 2006) and not to be associated (Cramer et al., 2002; Pfeiffer et al., 2002; Tiwari et al., 2005) with *N. caninum*-seropositive cattle.

The objectives of this study were to determine the prevalence, rates of vertical and horizontal transmission of *N. caninum* and hazard for culling of *N. caninum*-seropositive animals in three Brazilian dairy herds.

2. Materials and methods

2.1. Cattle and herd management

A prospective longitudinal study was carried out in three dairy herds, designated Farms I, II and III, located in the municipalities of Caçapava, Pindamonhangaba and Lagoinha, state of São Paulo, Brazil. The herds were selected and included in the study because they had at least one *N. caninum* seropositive animal at the first sampling and had a records system for individual zootechnical data.

At all three farms, the cattle were of Holstein–Friesian crossbreed and were reared in a semi-intensive system, kept on pasture. The newborn calves were usually given the first colostrums, either milked from or by suckling from their dams, within a few hours after birth and they were separated from their dams approximately after 12 h after birth. The calves were kept in individual pens until weaning at about 2 months of age, when they were transferred to the young stock area, composed of outdoor pens. Calves older than 4 month, heifers, milking and dry cows were kept in pasture.

Concentrate and mineral supplements were offered in accordance with to animal stock type and milk production status. During the rainy season, forage grass that was produced on the farm was harvested and offered to the cattle in troughs. During the dry season, the animals were fed with corn silage that was produced and stored at the farms. All animals were bred by means of artificial insemination and pregnancy diagnoses were performed on day 40 post-insemination by palpation per rectum. The cows and heifers calved all year round and were milked twice per day. All animals were tuberculosis and brucellosis-free, and vaccination programs were followed for prevention of the main bovine diseases, such as brucellosis, leptospirosis, IBR/BVD, clostridiosis and rabies.

Birds, chickens, rodents and feral and domestic cats and dogs lived among these herds, with free access to stables and pastures.

2.2. Blood sample collection and serology

Blood samples were collected from the coccygeal or jugular vein of all cattle in each herd on the respective visit. Each farm was visited over a two-year period, at three-month intervals. It was not possible to collect precolostral samples from calves. Blood samplings of calves younger than four months were not used to avoid false-positive as result of the presence of colostral antibodies against *N. caninum* (Cardoso et al., 2008). The samples were collected at Farm I from March 2004 to March 2006, at Farm II from September 2004 to September 2006 and at Farm III from October 2005 to October 2007.

After centrifugation at $1000 \times g$ for 20 min, the sera were removed and stored at −20 °C until analysis. Serum was tested for antibodies against *N. caninum* by means of the indirect fluorescent antibody test (IFAT), using whole culture-derived tachyzoites (NC-1 strain) as the antigen, with a cutoff value of 1:100 (Dubey et al., 1988). Fluorescein isothiocyanate conjugated rabbit anti-bovine IgG (Sigma, St. Louis, MO, USA) was used at a dilution of 1:3000. Positive and negative control samples were added to each slide.

2.3. Statistical analysis

The prevalence of *N. caninum* infection in herds was defined as the number of seropositive animals divided by the total number of animals tested within that herd in a given sample collection. Only animals sampled more than once were used in analyzing *N. caninum* vertical transmission and seroconversion. To minimize the impact of a false-positive or false-negative result, the animals were classified as seropositive if the first two consecutive blood samples were positive, but otherwise, they were considered seronegative if the first two consecutive blood samples were negative (Hietala and Thurmond, 1999).

All dam-daughter serological results were recorded. The efficiency of vertical transmission was estimated as the proportion of seropositive daughters born to seropositive dams (seropositive calves/seropositive dams × 100). The association between the serological status in dams and their offspring was measured and compared using 2 × 2 tables and the χ^2 test.

Seroconversion was designated when an animal classified as *N. caninum* negative or positive had, respectively, two consecutive positive or negative samples thereafter. The seroconversion rate and the proportion culled were expressed per 100 cow-years at risk (Thrusfield, 2007). Differences in the rates of positive and negative seroconversion and culling and the association between age of seropositive cows and congenital infection rate were assessed by comparison between two independent proportions. Values are expressed as the Mean ± Standard Deviation (S.D.). Differences and association were considered statistically significant when $P < 0.05$. The statistical analysis was performed using EPIDAT (Pan-American Health Organization, USA).

3. Results

3.1. Seroprevalence

Blood samples from all animals were collected nine times at each of the three farms over the two years of observation. A total of 2750 blood samples from 466 females were included in the analysis of Farm I and the percentage of *N. caninum*-positive samples at each sampling period ranged from 3.32% to 11.71%. A total of 1291 blood samples

Table 1
Seroprevalence of anti-*Neospora caninum* antibodies at Farm I, Farm II and Farm III in relation to stock class: calf (≤12 months), heifer (>12 ≤24 months) and cow (>24 months) during the sampling period.

Sampling	No. seropositive/No. sampled animals (%)											
	Farm I				Farm II				Farm III			
	Calf	Heifer	Cow	Total	Calf	Heifer	Cow	Total	Calf	Heifer	Cow	Total
1	6/61 (9.8)	11/58 (19.0)	18/180 (10.0)	35/299 (11.7)	1/24 (4.2)	0/34 (0.0)	5/96 (5.2)	6/154 (3.9)	16/42 (38.1)	12/29 (41.4)	54/150 (36.0)	82/221 (37.1)
2	9/72 (12.5)	10/51 (19.6)	13/188 (6.9)	32/311 (10.3)	8/31 (25.8)	1/33 (3.0)	19/96 (19.8)	28/160 (17.5)	10/41 (24.4)	12/38 (31.6)	48/144 (33.3)	70/223 (32.9)
3	3/80 (3.7)	4/59 (6.8)	11/179 (6.1)	18/318 (5.6)	5/31 (16.1)	0/19 (0.0)	10/95 (10.5)	15/145 (10.3)	15/53 (28.3)	8/35 (22.8)	50/150 (33.3)	73/238 (32.2)
4	4/82 (4.9)	4/61 (6.5)	13/165 (7.9)	21/308 (6.8)	7/30 (23.3)	2/18 (11.1)	20/94 (21.3)	29/142 (20.4)	16/66 (24.2)	9/37 (24.3)	41/146 (28.1)	66/249 (28.6)
5	2/63 (3.2)	2/65 (3.1)	6/161 (3.7)	10/289 (3.5)	2/28 (7.1)	4/20 (20.0)	18/95 (18.9)	24/143 (16.8)	12/63 (19.0)	13/38 (34.2)	49/155 (31.6)	74/256 (32.3)
6	3/70 (4.3)	2/68 (2.9)	9/165 (5.4)	14/303 (4.6)	5/29 (17.2)	7/23 (30.4)	16/89 (18.0)	28/141 (19.8)	9/54 (16.7)	9/43 (20.9)	56/163 (34.3)	74/260 (31.9)
7	2/64 (3.1)	4/67 (6.0)	9/175 (5.1)	15/306 (4.9)	6/26 (23.1)	6/26 (23.1)	18/84 (21.4)	30/136 (22.1)	13/40 (32.5)	12/37 (32.4)	54/159 (33.9)	79/236 (35.3)
8	3/81 (3.7)	2/63 (3.2)	7/171 (4.1)	12/315 (3.8)	5/23 (21.7)	6/25 (24.0)	13/88 (14.8)	24/136 (17.6)	18/50 (36.0)	13/42 (30.9)	54/156 (34.6)	85/248 (35.8)
9	1/63 (1.6)	2/60 (3.3)	7/178 (3.9)	10/301 (3.3)	5/23 (21.7)	2/23 (8.7)	16/88 (18.2)	23/134 (17.1)	13/47 (27.6)	9/36 (25.0)	52/140 (37.1)	74/223 (34.7)

from 213 females were included in the analysis of Farm II with prevalence that ranged from 3.90% to 22.06%. A total of 2154 blood samples from 348 female were included in the analysis of Farm III and the prevalence over the period ranged from 28.57% to 37.10% (Table 1).

The number of positive serological reactions varied in relation to the number of repeated samples taken from individual animals at each farm. Out of the 466 cows sampled at Farm I, 408 (87.44%) and 15 (3.22%) were, respectively, seronegative and seropositive at all sampling. Out of the 213 cows sampled at Farm II, 160 (75.12%) were seronegative and 9 (4.23%) were seropositive at all sampling. Out of the 348 cows sampled at Farm III, 208 (59.77%) and 83 (23.85%) were, respectively, seronegative or seropositive at all sampling times.

3.2. *Vertical transmission and age of the seropositives cows*

In all herds, there was a high degree ($P < 0.05$) of association between the *N. caninum* serological status of dams and daughter. The proportions of vertical transmission at Farms I, II and III were 50% (3/6), 83.33% (5/6) and 83.33% (20/24), respectively. The percentages of seronegative dams and seronegative daughters was 100% (111/111), 96.77% (30/31) and 95.89% (70/73), respectively at Farms I, II and III.

The mean ages of the seropositive dams that had seropositive and negative calves were, respectively, 4.13 ± 0.45 years (range, 3.64–4.53 years) and 3.33 ± 1.19 years (range, 2.07–4.43 years) at Farm I; 4.85 ± 1.33 years (range, 3.55–6.66 years) and only one animal with 4.87 years at Farm II; and 5.07 ± 1.56 years (range, 2.22–9.00 years) and 5.16 ± 3.57 years (range, 2.07–10.24 years) at Farm III. No association between age of seropositive cows and congenital infection rate was found ($P > 0.05$).

3.3. *Seroconversion*

The seropositive conversion rate was estimated as 0.37% (95% CI: 0.01–2.05%), 3.00% (95% CI: 0.83–7.52%) and 6.94% (95% CI: 2.86–11.01%) per 100 cow-years at Farms I, II and III, respectively. The mean age at the time of conversion was 2.67 ± 1.19 years (range, 1.75–4.38 years) and 2.27 ± 1.56 years (range, 1.09–5.66 years) at Farms II and III, respectively. Only one animal seroconverted at Farm I, and it was 4.98 years old. All seroconverted cattle remain positive over the follow-up period.

The seronegative conversion rate was estimated as 31.58% (95% CI: 12.58–56.56%) and 11.11% (95% CI: 2.56–19.67%) per 100 cow-years at Farms I and III, respectively. No seronegative conversion occurred at Farm II. The mean age at the time of conversion was 2.61 ± 1.18 years (range, 1.37–4.54 years) and 4.25 ± 3.79 years (range, 0.59–10.92 years) at Farms I and III, respectively. Three of the seven animals that converted to seronegative at Farm III became seropositive again, and two of these three animals were kept for six months and the other for nine months. Two of these three animals were pregnant during this transitory seronegative period.

3.4. *Culling*

The decision to cull a cow was made by the owner without knowledge of the *N. caninum* serological status of the animals. The proportions of culled *N. caninum*-seropositive and seronegative cattle per 100 cow-years were, respectively, 22.22% and 23.60% at Farm I; 11.77% and 15.24% at Farm II; and 32.97% and 23.21% at Farm III. The mean ages at the time of culling the seropositive and seronegative cattle were, respectively, 4.69 ± 3.00 years (range, 3.29–9.14 years) and 4.83 ± 2.63 years (range, 0.57–9.87 years) at Farm I; 4.68 ± 3.76 years (range, 0.67–9.75 years) and 4.29 ± 3.34 years (range, 0.71–11.66 years) at Farm

II; and 5.17 ± 2.82 years (range, 0.69–10.68 years) and 5.58 ± 3.68 years (range, 0.66–15.39 years) at Farm III. In all herds, there was no significance difference ($P < 0.05$) in culling rate between the cattle that were seropositive and seronegative for *N. caninum* infection.

4. Discussion

There was a wide variation in *N. caninum* prevalence in the herds investigated and, within herds, variations were observed over the sampling times and stock classes. These values are within the range of previous studies in Brazil, in which a wide range in prevalence values among cattle was also observed, from 0.0 to 91.2% (Gondim et al., 1999; De Melo et al., 2001; Guedes et al., 2008).

The high vertical transmission rate of *N. caninum* at Farms II and III (83.33% at both farms) is very similar to the congenital infection values (81–95%) reported in other studies (Paré et al., 1997; Wouda et al., 1998; Hietala and Thurmond, 1999; Dijkstra et al., 2001). The lower value found at Farm I (50%) was also in agreement with other studies (Bergeron et al., 2000; Chanlun et al., 2007; Moré et al., 2010).

Bergeron et al. (2000) and Chanlun et al. (2007) suggested that disparities between rates of vertical transmission may be explained by the variability in prevalence of seropositive animals. In fact, the high degree of correlation between the vertical transmission rate and the prevalence of seropositive animals at Farms II and III suggests that only in herds with high prevalence were high levels of transmission observed. Two explanations for this correlation are worth examining: first, high herd prevalence of seropositive animals may reflect a high proportion of active versus latent infections; second, the positive predictive value of IFAT must be considered in interpreting herd results. In low-prevalence herds, like Farm I, the predictive value of a positive test is low, because of the high proportion of negative animals. Therefore, in low-prevalence herds, a higher proportion of false-positive results may be expected, in relation to high-prevalence herds. It appears that estimated vertical transmission rates are more reliable in high-prevalence herds than in low-prevalence herds.

No association between age and congenital infection rate was found in the present study, as also reported by Paré et al. (1996). However, Dijkstra et al. (2003) suggested that the proportion of congenital infection decreased with increasing parity of the mother, possibly due to increased immunity to transplacental infection with increasing age.

Transient false-positive results, i.e. animals classified as *N. caninum*-negative with one or more isolated serological responses to *N. caninum*, were reported from the present study, in agreement with other studies (Hietala and Thurmond, 1999; Chanlun et al., 2007; Dijkstra et al., 2008).

The low seropositive conversion rates found in this study are consistent with other longitudinal studies, in which rates less than 8% were shown (Paré et al., 1998; Wouda et al., 1999; Dijkstra et al., 2002a). The high seronegative conversion rates at Farms I and III are similar to results found by other studies (Waldner et al., 2001; Dijkstra et al., 2002b; Pfeiffer et al., 2002; Moré et al., 2010).

Studies on both experimentally and naturally infected cattle have shown that the antibody levels can fluctuate, especially during gestation, and sometimes fall below the cutoff levels of the commonly used serological assays (Stenlund et al., 1999; Guy et al., 2001; Trees et al., 2002). This hypothesis may explain the return to seropositive condition in two of the three animals at Farm III that had seronegative conversion during the pregnancy period. However, Hietala and Thurmond (1999) reported that a few seropositive animals had a period of negative samples, and this may have occurred in these three negative seroconverted animals.

Although many studies have shown that *N. caninum*-seropositive cattle were more likely to be culled than were seronegative cattle (Thurmond and Hietala, 1996; Waldner et al., 1998; Hobson et al., 2005; Bartels et al., 2006), there was no significant difference in culling rate in the present study, between cattle that were *N. caninum*-seropositive and seronegative, as previously reported (Cramer et al., 2002; Pfeiffer et al., 2002; Tiwari et al., 2005).

This is the first longitudinal study on the seroprevalence of *N. caninum* in dairy herds in Brazil. The results confirm the importance of vertical transmission in the epidemiology of the parasite. Although there were indications for horizontal transmission, it does not appear to be the major route of *N. caninum* infection. High seronegative conversion was demonstrated at all the farms studied, and the culling rate of the animals was not associated with *N. caninum* infection.

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