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Short communication

Frequency of *Neospora caninum* infections in beef cow–calf operations under extensive management

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ABSTRACT

The aim of this study was to evaluate the frequencies of *Neospora caninum* horizontal and vertical transmissions in beef cow–calf operations under three different extensive management systems: group A: 0.75 head per hectare pasturing on natural grass; group B: 1.1 head per hectare on natural grass and improved cultured pastures; and group C: 2 head per hectare on natural grass, improved cultured pasture and whole corn silage. Serum samples from 72 multiparous cows assigned to each beef cow–calf operations were obtained every 3 months during 2 years. A group of 30 replacement heifers from each group were tested similarly since they were 10–21 months old. Twenty four, 20 and 34 calves from groups A, B and C respectively, were bled before colostrum intake and again 6 months later. The samples were analyzed by indirect fluorescence antibody test (IFAT) for detection of total IgG against *N. caninum* at a serological titre ≥200 for multiparous cows and replacement heifers, and a serological titre ≥25 for calves. Serum samples from seropositive cows were assessed by ELISA to evaluate the avidity of their specific antibodies. There were no differences in the proportion of seropositive cows from groups A, B and C at the beginning of the trial ($p > 0.05$). Interestingly, the lowest serological titres in seropositive cows from all groups were observed during the first trimester ($p < 0.05$). Although seropositive cows had medium to high avidity antibodies, suggesting chronic infection; seroconversion associated with low antibody avidity was found in 2, 3 and 3 seropositive cows from groups A, B and C. All replacement heifers remained seronegative. No abortions were recorded but 2, 1, and 2 calves from groups A, B and C were seropositive before colostrum intake, respectively. Seropositive calves born from cows having intermediate or high avidity remained with the same serostatus at 6 months of age. Even under varying extensive management conditions, both *N. caninum* horizontal and vertical transmission methods do occur in beef cow–calf operations.

1. Introduction

Neosporosis is a parasitic disease of major concern for the cattle industry (Dubey et al., 2007). In beef cattle, economic losses have been estimated in 455.4 million dollars due to bovine abortion (Reichel et al., 2013). Although vertical transmission is considered the most frequent route of infection (Anderson et al., 1997; Davison et al., 1999), horizontal transmission has also been experimentally demonstrated (Trees et al., 2002). Despite the efficiency of endogenous transplacental transmission, it is evident from theoretical modelling that *Neospora caninum* infection cannot be sustained in herds without horizontal transmission (French et al., 1999). In agreement, epidemiological studies in dairy cattle provided some evidence for the occurrence of horizontal infection (Davison et al., 1999; Hietala and Thurmond, 1999; Dijkstra et al., 2002). Also in beef cattle, exogenous transplacental transmission and epidemic abortion outbreak have been associated with horizontal transmission (McAllister et al., 2000). Nevertheless, horizontal

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transmission has been scarcely reported in cattle worldwide (Dubey et al., 2007).

Specific antibodies develop after the ingestion of oocysts (Trees et al., 2002). Also changes in antibody titres through the gestation have been well documented in chronically naturally infected cattle (Stenlund et al., 1999). Indeed, an increase in the antibody titres is suggested as a good indicator of parasitemia after the reactivation of latent infections during pregnancy (Stenlund et al., 1999). On the other hand, the level of antibodies in an infected host may be low and not enough to reach the cut-off point in several serological tests (Dubey et al., 2007).

Interestingly, the avidity of IgG has been successfully used to distinguish between acute and chronic *N. caninum* infections in cattle (Björkman et al., 1999). Moreover, chronically infected beef cattle with high antibody avidity were less likely to abort than recently infected pregnant beef cattle with low antibody avidity (McAllister et al., 2000). Avidity indices have been also used as indicator of mature antibody response in *Neospora* infections (Björkman et al., 1999; McAllister et al., 2000).

Dubey et al. (2007) reviewed risk factors associated with bovine neosporosis. Some of them are: presence of definitive hosts, immunosuppression by presence of mycotoxins in food or concomitant viral infections, feed supplementation, use of own replacement heifers and higher stocking rate. There is also some controversy regarding the seasonal effect or use of working dogs (Sanderson et al., 2000; Barling et al., 2001). Although it has been suggested that management practices associated with further intensification may increase the prevalence of *N. caninum* in cattle (Dubey et al., 2007), little is known about the risk factors that contribute to the exposure of beef cattle to *N. caninum* (Sanderson et al., 2000; Waldner et al., 2001).

This study was designed to evaluate frequencies of *N. caninum* horizontal and vertical transmission in beef cow–calf operations under three different extensive management systems in the humid pampas, one of the most important livestock production regions of the world.

2. Materials and methods

2.1. Animals, experimental design and location

The study was conducted at an Experimental Station of the National Institute of Agrarian Technology (INTA), located in the Buenos Aires province (37°05′07″ South; 57°52′50″ West), Argentina. Angus multiparous cows belonging to a herd with a seroprevalence of 5.3% of 645 head, were randomly assigned to three different groups of 72 cows each one. The experimental design and differences in management systems are shown in Table 1. In all cases, the forage availability was appropriate to maintain body condition at level 5 (scale of 1–9) throughout the experimental period.

Cows from group A were exposed to 2 bulls between November 1 to January 31. On the other hand, fixed time artificial insemination (FTAI) was used for females in groups B and C, but 2 weeks after FTAI each group was exposed to 1 bull.

The health control program was similar in the three experimental groups. All cows were vaccinated before breeding with a vaccine containing inactivated viruses of bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD), and parainfluenza-3 (PI-3) and killed *Leptospira* sp., *Campylobacter* sp., and *Haemophilus somnus* (Biogenesis™, Argentina). A few weeks prior to parturition the dams were vaccinated with inactivated vaccines against rotavirus (serotype 6 and 10) and *Escherichia coli* J5 (Biogenesis™, Argentina).

All cows were brucellosis and tuberculosis free and a vaccination program against foot and mouth disease and anthrax were routinely performed in accordance with current schedule of Argentina National Animal Health Service. All the cows received an application of parenteral copper supplement in the last month of pregnancy and had access to magnesium salts after the first trimester of gestation. The animals had no history of reproductive failure. Forty five days after finishing the mating season pregnancy diagnosis was made by trans-rectal examination. During that time, non-pregnant cows were culled from the system and replaced with 21 month old pregnant heifers. Therefore 30 replacement heifers from each experimental group were also involved in the study.

Heifers were in level 6 (scale of 1–9) of body condition and fed oat pasture and supplemented with whole maize grain during the winter at a rate of 3 kg/animal/day. During the mating season they were kept on natural grass. The health control program was similar to that applied to cows, except that double doses with an interval of 30 days were used for vaccines against reproductive diseases and neonatal diarrhea. Preventive anthelmintic treatment was also applied.

During pregnancy, daily observations of the animals were performed to detect possible abortions. After birth, all calves were vaccinated against clostridiosis (Biogénesis™, Argentina) at four and five months of age. Females at the age of 6–9 months were immunized against brucellosis with *Brucella abortus* strain 19 vaccine.

2.2. Sampling

From April 2012 to April 2014, blood samples were obtained 8 times with an average interval of 3 months. Due to tooth wear, 7, 6 and 5 cows from groups A, B and C were culled, respectively. Therefore, continuous serological monitoring was achieved in 65, 68 and 67 multiparous cows from groups A, B and C, respectively. Blood samples (5 mL) were obtained by coccygeal vein puncture. Similarly, 30 blood samples from replacement heifers from groups A, B and C were obtained 5 times with an average interval of 72 days from April 2012 to April 2013.

Previous to colostrum intake, blood samples from 24, 20 and 34 calves from groups A, B and C were obtained during the 2013 calving season. Only sera having ≤50 IU/L of gamma glutamyl transferase (GGT) (De Magalhães et al., 2014) were maintained at −20 °C and tested for presence of specific antibodies against *N. caninum*. Blood samples were also obtained from these calves at 6 months of age.

2.3. Serological tests

2.3.1. Indirect fluorescent antibody test (IFAT)

IFAT was performed as previously described by Moré et al. (2009). *Neospora*-specific antibodies were measured using dilutions of serum from 1:200 to endpoint titre for cows and heifers, and 1:25 for their calves. A polyclonal rabbit anti-bovine IgG labeled with fluorescein isothiocyanate (Sigma, St. Louis, MO) was used. Positive and negative control sera were used (VMRD, Inc., USA). Slides were examined with an epifluorescence microscope (Nikon Fluophot, 40 × 1.3). Antibody titres were expressed as the reciprocal of the highest serum dilution that showed distinct whole parasite fluorescence.

2.3.2. ELISA for avidity of antibodies against N. caninum

In order to characterize the infections based on the avidity of the specific antibodies, a commercial IgG avidity enzyme-linked immunosorbent assay (ELISA) (CIVTEST, Hipra BOVIS *Neospora*™, Spain) was applied. The avidity of IgG antibodies directed to *N. caninum* was assessed in IFAT seropositive cows. As recommended by the ELISA manufacturers, low-affinity antibodies were eluted by adding an incubation step with urea after the serum incubation. The antibody titers obtained with and without incubation with urea

Table 1
Experimental design and different treatments applied in beef cow–calf operations under extensive management systems.

	Experimental groups		
	A	B	C
Stocking rate (heads per hectare)	0.75	1.1	2
Nutritional management	Natural grass	Natural grass and improved cultured pastures	Natural grass, improved cultured pasture and whole plant corn silage
Reproductive management	Natural service	FTAI[a]	FTAI

[a] FTAI: fixed time artificial insemination.

were then used to calculate the IgG avidity values. Briefly, samples were analyzed in duplicate, and the mean value of the optical density (OD) was converted into a relative index per cent (RIPC) by employing the following formula: RIPC = (OD sample–OD negative control)/(OD positive control–OD negative control) × 100. For samples having an RIPC higher than 6, a second index was calculated for the antibody avidity (AAI) as follow: (OD sample diluted 1/25–OD sample diluted 1/100)/(OD sample diluted 1/25 and incubated with urea–OD sample diluted 1/100 incubated with urea). The interpretation of serological results was: high avidity ≤1; intermediate avidity = between 1 and 2; ≥2 low avidity.

2.4. Data analysis

2.4.1. Frequency of horizontal transmission

The frequency of horizontal transmission was estimated in two ways: (1) the number of seronegative heifers and cows that became seropositive during the study period; (2) The percentage of pre colostrum seronegative calves that became seropositive 6 months later (Moré et al., 2009).

2.4.2. Frequency of vertical transmission

The frequency of vertical transmission was estimated as follow: (number of seropositive calves before colostrum intake/seropositive dams) × 100 (Moré et al., 2009).

2.5. Statistical analysis

The proportions of *N. caninum* seropositive cows among groups through the time were analyzed by Fisher ís test using the PROC-FREQ, SAS Inst., Inc. IFAT titers were compared by using PROC-MIXED SAS for repeated measures analysis of variance (Littell et al., 1998). The natural logarithm of reciprocal antibody titer was considered as the dependent variable; and the stocking rate, the sampling moment (mating or first, second or third trimester of gestation) and their interactions were included as fixed effects. Symmetry and homoscedasticity of the residuals were assessed using graphical methods and the Bartlett's test, respectively. For all the analysis the significance level was $\alpha = 0.05$.

3. Results and discussion

After assessing 1830 serum samples (1134, 450 and 156 blood samples from multiparous cows, replacement heifers and calves, respectively), the frequency of *N. caninum* infections in beef cow–calf operations under extensive management were recorded. Although no significant differences in the proportions of *N. caninum* seropositive cows among groups neither at the beginning nor during the period of the study were observed ($p > 0.05$), the percentage of seropositive animals in groups A, B and C ranged from 1.5–9.7, 2.8–4.4 and 0–6.1, respectively, over 2 years.

The horizontal transmission of *N. caninum* observed in this study was low; nevertheless, it is interesting to mention that a few cows from each experimental group had seroconversion associated with low IgG avidity. Seroconversion to *N. caninum* was detected in 4, 2 and 4 cows from groups A, B and C, respectively. As expected, that seroconversion was associated with low avidity except in one cow in group C which had intermediate avidity results. This finding is in agreement with the fact that new infections are needed for the maintenance of the disease in the herd (French et al., 1999) even in extensively managed beef cattle.

The odds of being seropositive have been already associated with increased stocking rates (Barling et al., 2000, 2001). A higher exposure to excretions from definitive hosts is a clear risk factor, increasing the frequency of horizontal transmission (Dubey et al., 2007). Probably due to the extensive management in all experimental groups, the frequency of horizontal transmission was not affected by increasing from 0.75 to 2 head per hectare nor by grazing on different types of pasture. Although more than 0.5 head/hectare increased the odds of seropositivity to *N. caninum* in beef cattle in Texas, US (Barling et al., 2001), our results should be taken with caution because an stocking rate of 1 head per hectare, as used in this study, means one head per hectare over one year. Indeed, the concept of "instantaneous stocking rate", which means hundreds of head**s** grazing one hectare, should be considered when analyzing the risk factors for *Neospora* infections. The low frequency of postnatal transmission could be influenced also for the period of time for this study (2 years) being not sufficient to show significant differences among the experimental groups.

Similarly, no effect in the fluctuation of antibody titers on seropositive cows due to treatment was observed ($p > 0.05$); however, the lowest antibody titres were observed during the first trimester of gestation independently of the experimental group ($p < 0.05$) (Fig. 1). As clearly described previously (Stenlund et al., 1999) there is a huge fluctuation in the antibody titres through the gestation in chronically infected cattle. This fact should be considered when detection of *N. caninum* infected cattle are recommended for health management programs. In order to identify chronically infected cattle for culling from the herd the best moment to bleed cattle should be after the 4th month of pregnancy. In this regard, the period of gestation should be recorded when cross-sectional studies for bovine neosporosis are designed.

Although one calf from a seropositive cow having high IgG avidity was born prematurely and died with a low weight (14 kg) in group C; no abortions were recorded at any time. However, the efficiency of vertical transmission was 100 (2 seropositive calves from 2 seropositive cows; IC 95: 15.8–100), 50 (1 seropositive calf from 2 seropositive cows; IC 95: 1.3–98.7) and 100% (2 seropositive calves from 2 seropositive cows IC 95: 15.8–100) for each group, respectively. All seropositive cows delivering seropositive calves had high or intermediated IgG avidity.

The other calves from all experimental groups remained seronegative until weaning, at 6 months of age. Similarly, all replacement heifers were seronegative all the time. In contrast, multiparous cows had specific antibodies suggesting that age is a risk factor for *Neospora*-infection (Dubey et al., 2007).

Although specific antibody titers were detected in asymptomatic calves is noteworthy that none of replacement heifers

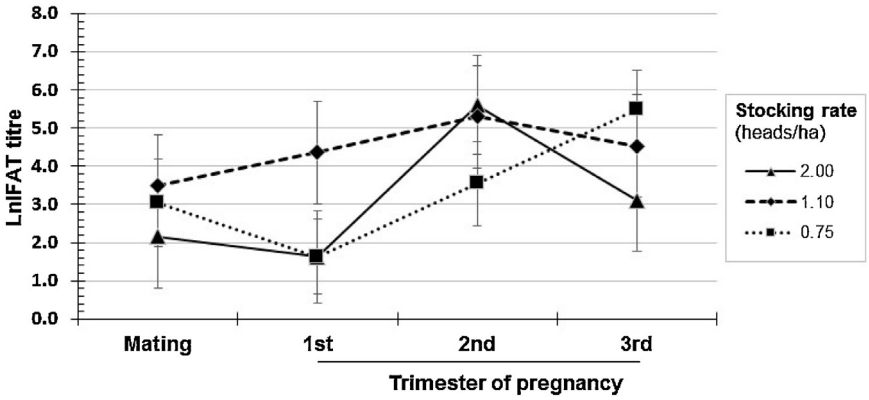

Fig. 1. Reciprocal antibody titers (least squares means ± standard error) by IFAT for *N. caninum* in two consecutive pregnancies in seropositive cows throughout mating and pregnancy under extensive management systems.

was seropositive at serological titres of ≥1:200. Interestingly, as suggested by Dubey et al. (2007) the antibodies titres may decrease below the serological cut-off level after birth. Moreover, 2, 1 and 2 heifers from the groups A, B and C, respectively had serological titres of ≤1:200 (data not shown). Eventually, conversion from bradyzoite to tachyzoite stages may be influenced by immune factors and hormones during pregnancy, and the associated parasitemia may cause the antibody titre to rise above the cut-off, in which case the avidity is likely to be high (Bjorkman et al., 1999). Even when all replacement heifers were seronegative, some heifers could suffer a recrudescence of a chronic infection during their first pregnancy, unfortunately this fact was not evaluated in this study.

Finally, even in beef cow–calf operations under extensive management systems, both *N. caninum* horizontal and vertical transmission methods are likely to occur in the humid pampas. Other risk factors like the presence of wildlife, definitive hosts, immunosuppression by presence of mycotoxins in food or concomitant viral infections, should be evaluated in further studies.

Conflict of interest

There is no conflict of interest including any financial, personal or other relationships with other organizations that could have inappropriately influenced this work.

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