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ORIGINAL PAPER

Serologic profiles for *Sarcocystis* sp. and *Neospora caninum* and productive performance in naturally infected beef calves

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Abstract *Sarcocystis* sp. and *Neospora caninum* infections affect cattle worldwide causing important economic losses. The objective of the present study was to trace serologic profiles for *Sarcocystis* sp. and *N. caninum* in naturally infected beef calves and analyze their relationship with transmission routes and productive performance. Samples were collected in two cow-calf operations located in Buenos Aires province, Argentina. In farm 1, 43 calves were bled and weighed three times. In farm 2, 69 calves were bled and weighed six times. *Sarcocystis* sp. and *N. caninum* immunofluorescence antibody test (IFAT) titers were averaged for each sampling point in order to trace serologic profiles for each infection. Categories were created to evaluate differences in daily weight gain. For *S. cruzi* antigen, animals were separated in a low-titer (≤200) and high-titer group (>200); for *N. caninum*, animals were grouped as infected and uninfected. *Sarcocystis* sp. antibody titer as well as the number of infected animals increased gradually over time in both farms. In farm 2 the low-titer group had significantly higher daily weight gain than the high-titer group. For *N. caninum* 44% (farm 1) and 65% (farm 2) of calves were considered infected, and the serological profile was horizontal or decreasing over time. However, seroprevalence increased in both farms and vertical and horizontal transmission frequency were estimated between 18.5%–29% and 22–25.5%, respectively. No differences were detected in daily weight gain between *N. caninum* groups from both farms. This is the first report of serological profiles for *Sarcocystis* sp. and *N. caninum* by IFAT in naturally infected beef calves and their relationship to different transmission routes and productive performance.

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Introduction

Sarcocystis sp. and *Neospora caninum* infections affect cattle worldwide causing important economic losses, mostly due to occasional systemic disease and abortions, respectively (Dubey 2003; Dubey et al. 1989; Giles et al. 1980). In addition, studies have reported low weight gain associated to high *N. caninum* antibody titers in beef calves and steers (Barling et al. 2001; Barling et al. 2000). Experimental infection with *Sarcocystis cruzi* caused low weight gain (Daugschies et al. 2000); however, whether this occurs in naturally infected animals remains unknown.

Sarcocystosis and neosporosis can be diagnosed by several serologic methods, with the immunofluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) being the most commonly used (Dubey and Schares 2006; Tadros and Laarman 1982; Uggla and Buxton 1990). For *Sarcocystis*, both serological tests are genus specific with cross reactions among several species

(Tadros and Laarman 1982). Few studies have evaluated antibody titers in longitudinal studies or associated the serological results to weight gain in calves naturally infected with *N. caninum* or *Sarcocystis* sp. (Barling et al. 2001; Barling et al. 2000; Piergili Fioretti et al. 2000). Serologic profile evaluation in relationship to animal age as well as cow-calf serology may contribute to determine the most prevalent infection route in a herd (Bergeron et al. 2000; Davison et al. 1999; Dubey and Schares 2006; Hietala and Thurmond 1999; Moré et al. 2009; Waldner et al. 1998), which would aid in determining control strategies for these infections. Therefore, the objective of the present study was to trace the serologic profiles for *Sarcocystis* sp. and *N. caninum* in naturally infected beef calves and analyze their relationship with transmission routes and productive performance.

Materials and methods

Animals and sampling

The study was conducted in two cow-calf operations located in Buenos Aires province, Argentina. In farm 1, 43 calves (21 male and 22 female) were bled and weighed three times between July and October 2005 (45 days between sampling points). Calves were 15–120 days old at the beginning of the sampling period. In the first sampling, blood was also collected from dams. In farm 2, 69 calves (40 male and 29 female) were bled and weighed monthly from November 2005 to April 2006. Calves were 15–60 days old at the beginning of the sampling period. All cattle in both farms were given recommended vaccinations for infectious diseases and dewormed every 2 months with ivermectin and benzimidazoles. All calves were weaned at the end of the study.

Animals were kept without food 12–18 h prior to sampling. Blood samples were collected by puncture of the coccigeal vein, refrigerated, and transported to the laboratory within 24 h. Samples were centrifuged and sera were stored at −20°C until analysis.

IFAT for *N. caninum* and *Sarcocystis* sp.

Detection of *N. caninum* and *Sarcocystis* sp. antibody in sera was performed by IFAT as described previously (Moré et al. 2008) using *N. caninum* NC-1 tachyzoites and *S. cruzi* bradyzoites as antigens, respectively. Samples collected from each animal were processed simultaneously. Titers for each sampling point were averaged in order to trace serologic profiles for each infection. Sera from cows from farm 1 were processed at dilutions 25 and 200.

Animal categories were created to evaluate differences in weight gain according to antibody titer. For *Sarcocystis* sp., animals were separated in a low (≤200 throughout all samplings) and high-titer groups (>200 in at least one sampling). For *N. caninum*, animals were grouped as infected and uninfected, and the infected animals were further divided as follows: seropositive animals throughout all time points and seropositive animals only at 140 days of age or more, Seropositive animals that did not meet these criteria were not classified in any category. Daily weight gain (DWG) for each animal category was calculated by averaging total weight gain/days between sampling points. Differences in DWG were analyzed using ANOVA. Statistical significance was declared at $p<0.05$.

Results

Farm 1

All cows (43) were positive for *Sarcocystis* sp. (18 had a titer of 25 and 25 cows had a titer of 200) and 71% of cows (31/43) were positive for *N. caninum* (26 had a titer of 25 and five cows had a titer of 200). Table 1 shows antibody titer distribution for *Sarcocystis* sp. and *N. caninum* at each sampling point in farm 1. Serologic profiles for both infections are shown in Fig. 1. Thirty nine calves increased their *Sarcocystis* IFAT titer and seroconverted by the 3rd sampling; three calves had the same titer through all samplings and one calf had a decreasing titer. For *N. caninum* 51% ($n=22$) of calves were classified as uninfected and 44%

Table 1 Distribution of IFAT antibody titers to *Sarcocystis* sp. and *Neospora caninum* in calves from farm 1

	Time point	IFAT titer						
		<25	25	50	100	200	400	800
Sarcocystis sp.	1	6	19	7	9	2	0	0
	2	1	5	13	12	8	2	2
	3	0	3	15	10	15	8	2
Neospora caninum	1	33	6	1	2	0	0	1
	2	24	13	2	3	0	0	1
	3	22	14	3	3	0	1	0

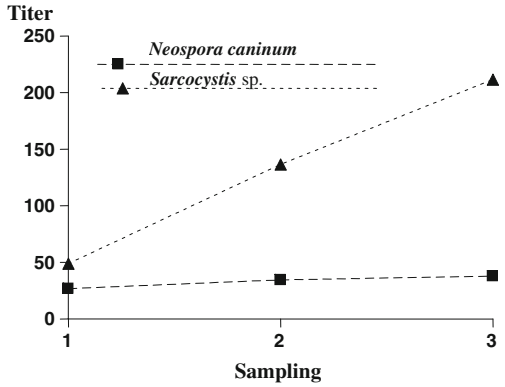

Fig. 1 Serologic profile for *Sarcocystis* sp. and *Neospora caninum* infection in calves from farm 1. The *lines* represent the mean antibody titer at each sampling point

(n=19) as infected; 25.5% (n=11) were seropositive only at ≥ 140 days of age and 18.5% (n=8) were seropositive throughout all time points. Two seropositive calves less than 140 days of age at the 3rd sampling were not classified in any category.

Average calf weight was 102, 144, and 188 kg at each sampling point, with DWG of 840 g and 1,030 g between the 1st and 2nd and 2nd and 3rd sampling point, respectively. No differences were observed in DWG between the *Sarcocystis* sp. low-titer group (n=31) and high-titer group (n=12) or between *N. caninum*-infected and uninfected calves.

Farm 2

Table 2 shows antibody titer distribution for *Sarcocystis* sp. and *N. caninum* at each sampling point in farm 2. Serologic profiles for both infections are shown in Fig. 2. Sixty two calves increased their *Sarcocystis* sp. IFAT titer and seroconverted by the 6th sampling point. Two calves had the same *Sarcocystis* sp. titer through all samplings and one had a decreasing IFAT titer.

For *N. caninum*, 35% (n=24) of calves were considered uninfected (20 were seronegative throughout all sampling points and 4 were seropostive only before 140 days of age). Sixty five percent (n=45) of calves were considered infected; 29% (n=20) were seropositive throughout all time points, 22% (n=15) were seropositive only at ≥ 140 days age, and 14% (n=10) of seropositive calves were not classified in any category.

Table 3 shows average weight at each sampling point and DWG for *Sarcocystis* sp. low-and high-titer groups. For *Sarcocystis* sp., the low-titer group (n=36; 21 male and 15 female) had significantly higher DWG (116 g; $p<0.05$) than the high-titer group (n=33; 19 male and 14 female) between the 4th and 5th sampling point. No differences in DWG were observed in other time intervals. No differences in DWG were observed between *N. caninum*-infected and uninfected calves.

Discussion

Seroprevalence for *Sarcocystis* sp. observed in both farms is in agreement with other studies from several countries that indicate up to 100% prevalence, especially for *S. cruzi* (Böttner et al. 1987; Dubey et al. 1989; Latif et al. 1999; Van Knapen et al. 1987; Vercruysse et al. 1989). *Neospora caninum* seroprevalence may vary widely depending on diagnostic technique and cutoff titer used (Dubey et al. 2007); however, in the present study seroprevalence in cows and calves was similar to that reported previously in Argentina (Moré et al. 2009). Serologic profiles for *Sarcocystis* sp. and *N. caninum* infection evidenced

Table 2 Distribution of IFAT antibody titers to *Sarcocystis* sp. and *Neospora caninum* in calves from farm 2

	Time point	IFAT titer								
		<25	25	50	100	200	400	800	1600	3200
Sarcocystis sp.	1	1	34	25	6	3	0	0	0	0
	2	0	21	35	10	3	0	0	0	0
	3	0	8	27	13	20	1	0	0	0
	4	0	3	20	17	15	12	2	0	0
	5	0	2	6	25	18	13	2	2	1
	6	0	0	4	19	16	17	6	6	1
Neospora caninum	1	41	5	2	2	2	6	2	7	2
	2	43	5	1	2	4	5	3	5	0
	3	36	15	1	1	6	8	2	1	0
	4	36	14	2	3	9	4	1	1	0
	5	34	13	7	3	9	3	1	0	0
	6	30	19	7	6	2	5	0	0	0

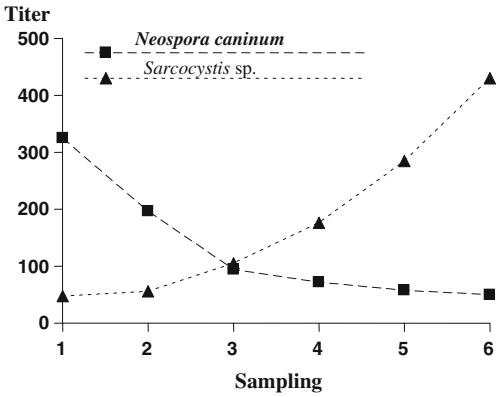

Fig. 2 Serologic profile for *Sarcocystis* sp. and *Neospora caninum* infection in calves from farm 2. The *lines* represent the mean antibody titer at each sampling point

characteristics that may be associated to different transmission routes. For *S. cruzi* antigen, high titers (≥400) were detected in later sampling points. In Farm 1, 6 calves were seronegative at the 1st sampling despite of all dams being seropositive. This could be due to an initial low maternal antibody concentration and consequent antibody decrease to undetectable levels by the first months of life. Antibody titer as well as number of infected animals increased gradually over time in both farms resulting in ascending serological profiles. Furthermore, ~90% of calves in both farms seroconverted by the last sampling point. These findings suggest primarily a horizontal transmission route. Taking into account the antibody response after experimental infection (Gasbarre et al. 1984; Lunde and Fayer 1977), the IgG increase from approximately 120–150 days of age observed in most calves would suggest that infection likely occurred 30–45 days earlier. At this time point (~ 3-4 months of age), calves increase grass intake which would result in contact with sporocysts.

Higher *N. caninum* antibody titers were found in earlier sampling points and serological profiles were horizontal or decreasing over time in farms 1 and 2, respectively. However, seroprevalence increased in both farms but this was not evident in the serological profiles due to the low titers of newly infected calves. Taking into account that *N. caninum* colostral antibodies were undetectable after 128 days of age (Hietala and Thurmond 1999), we considered seropositive calves throughout all time points as vertically infected and seropositive calves at ≥ 140 days of age as horizontally infected. Therefore, horizontal transmission was estimated at 25.5% and 22% in farms 1 and 2, respectively, which is in agreement with values reported by (Björkman et al. 2003) in beef cattle using an avidity ELISA and to those reported for dairy heifers (Moré et al. 2009). However, our estimations are higher than those reported by others (Bergeron et al. 2000; Davison et al. 1999; Hietala and Thurmond 1999; Waldner et al. 1998) which may be due to different diagnostic methods and cut off titers used. Alternatively, these discrepancies may be due to farm management practices, since in Argentina many dogs are present in most farms which could favor oocyst ingestion by cattle and therefore increase horizontal transmission. Furthermore, the high seroprevalence to *S. cruzi* antigen and efficient horizontal transmission evidenced in this study suggest frequent contamination of pastures with dog feces, which is a risk factor for *N. caninum* infection (Dijkstra et al. 2002; Moore et al. 2002). Estimation of *N. caninum* vertical transmission in farm 2 was 29%, similar to that reported by Moré et al. (2009) in dairy calves using the same diagnostic technique and cutoff titer. Although vertical transmission is considered the most important infection route for *N. caninum*, our results indicate equal relevance for both transmission routes in beef cattle, and similar vertical transmission frequency to that reported previously in dairy cattle.

The observed difference in DWG between *Sarcocystis* sp. low- and high-titer groups suggests that productive losses reported in experimental infections could also occur in natural infections in our country; moreover, the magnitude of productive losses could be reduced in nursing calves. Further studies are necessary to elucidate the importance and

Table 3 Average weight (in kilograms) at each sampling point and daily weight gain (DWG) in calves from farm 2

Sarcocystis sp. titer group	Sampling point					
	1	2	3	4	5	6
High						
Weight	82.1 (13.6)	109.1 (14.7)	134.3 (17.5)	154.1 (18.1)	178.5 (16.3)	191.5 (20.4)
DWG		0.793 (0.12)	0.700 (0.15)	0.708 (0.19)	0.843* (0.18)	0.419 (0.20)
Low						
Weight	71.8 (17.4)	95.8 (20.0)	119.5 (22.3)	139.1 (24.1)	166.9 (18.7)	180.7 (23.6)
DWG		0.707 (0.17)	0.657 (0.16)	0.701 (0.21)	0.959* (0.19)	0.446 (0.25)

*$p<0.05$; standard deviation in parenthesis

distribution of productive losses as well as their occurrence in other animal categories.

In the present study, no differences in DWG were observed between *N. caninum*-infected and uninfected calves. It is possible that *N. caninum* vertical infection does not impact DWG, or that production losses are minimized in nursing calves. Furthermore, decreased DWG in *N. caninum*-infected calves were reported in weaned animals (Barling et al. 2001; Barling et al. 2000; Venturini et al. 2002).

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