Risk Factors Associated With Infection By Neospora Caninum In Dual- Purpose Cattle In The Central Region Of Veracruz, Mexico

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Citation

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Abstract

The aim of the present study was to determine the frequency of Neospora caninum-seropositive dual-purpose cattle from two municipalities in Veracruz, Mexico, and to determine possible risk factors associated with seropositivity. The study examined 28 farms in the municipalities of Jamapa and Veracruz with a total population of 1889 cattle. From these cattle, 555 serum samples were obtained. Anti-Neospora caninum antibodies were identified using ELISAs. The risk factors considered included the presence of dogs and/or wild canids, occurrence of abortions and neonatal deaths, origin of replacement animals, feed, and vaccination programs. The strength of the association of each factor (variable) with the serological results was evaluated by odds ratios (ORs) and chi-square or Fisher's exact tests. Of the 555 sera, 105 (18.91%) were positive for anti-Neospora caninum antibodies. Factors significantly associated with seropositivity for Neospora caninum were the presence of abortions [OR = 7.34; 95% confidence interval ($CI_{95\%}$) = 2.35–22.92; $p< 0.05$] and replacement animals from other Mexican states [OR = 2.23; $CI_{95\%}$= 1.13–4.39; $p< 0.05$]. In conclusion, many of the dual-purpose cattle examined here had been exposed to Neospora caninum as evidenced by positive serological tests for anti-Neospora caninum antibodies in cattle from 23 of the 28 farms tested (82%). The association between the abortions and Neospora caninum seropositivity suggests that cattle could be previously infected with the parasite. Due to there was not a correlation between the presence of canids and Neospora caninum seropositivity, the main route of transmission must be between cattle (endogenous and exogenous).

INTRODUCTION

Neospora caninum is a protozoan parasite that can cause abortion in cattle, the intermediate host, which results in great economic losses for the livestock industry worldwide. The main transmission route is vertical, and this can occur over several generations (endogenous transplacental transmission). Horizontal transmission occurs to a lesser degree (exogenous transplacental transmission) by means of sporulated oocytes that are present in water, on prairies, and in forages or other foods. The definitive hosts are dogs (Canis familiaris) and coyotes (Canis latrans) that shed unsporulated oocytes in their feces (McAllister et al. 1998; Lindsay et al. 1999; Gondim et al. 2004).

In dairy cattle, the frequency of animals positive for neosporosis by serological tests varies considerably throughout the world. For example, in Sweden and Germany frequencies of 1.3% and 1.6%, respectively, have been recorded (Bartels et al. 2006), while in Uruguay and Argentina the seroprevalence is 61.3% and 64.5%, respectively (Venturini et al. 1999; Kashiwazaki et al. 2004). These variations in frequency depend on several factors, such as the place of study, number of animals and herds sampled, the sampling design, type of serological test used, and the cut-off for each test (Dubey, 2003; Dubey et al. 2007). In Mexico, neosporosis in dairy cattle has been recorded since 1997 (Morales et al. 1997). Nevertheless, there is little epidemiological information on this disease in beef and dual-purpose cattle (Salinas et al. 2005; Garcia-Vazquez et al. 2009; Romero-Salas et al. 2009).

With regard to the Mexican state of Veracruz, a study performed by Romero-Salas et al. (2009) identified antibodies against Neospora caninum in 24.6% of the 3555 animals sampled. When the results were categorized according to zones, the central zone had the highest prevalence (27.1%) of neosporosis, while the northern zone had the lowest prevalence (20.9%). With regard to zootechnic function, dual-purpose cattle had the highest prevalence (25.8%) of neosporosis.

Previously-identified risk factors associated with bovine neosporosis include (1) the presence and abundance of dogs or wild canids on farms, which can result in environmental contamination with oocytes (Paré et al. 1998; Bartels et al. 1999; Mainar-Jaime et al. 1999; Barling et al. 2000; Sánchez et al. 2003; Schares et al. 2004; Corbellini et al. 2006; Gutiérrez et al. 2007), (2) the presence of different intermediate hosts in addition to bovids, such as poultry (Bartels et al. 1999; Otranto et al. 2003; Costa et al. 2008), ducks, rats, mice, and rabbits (Ould-Amrouche et al. 1999; Huang et al. 2004; Hughes et al. 2006; Dubey et al. 2007), (3) high environmental temperatures that can favor the sporulation of oocytes present in feed (pastures and forage) or water sources (Schares et al. 2004; Rinaldi et al. 2005; Dubey et al. 2007; Medina-Esparza et al. 2009), and (4) an association with other diseases, mainly of viral origin (Björkman et al. 2000; Dubey et al. 2007; Rinaldi et al. 2007).

Determination of the risk factors for Neospora caninum infection is important for the development and application of preventive measures and disease control programs. The analysis of cross-sectional studies allows, in general, for the identification of factors associated with risk or protection. Nevertheless, conclusive data can only be obtained by prospective cohort studies, and experimental or case-controlled studies.

The aims of the present study were to determine the frequency of Neospora caninum-seropositive dual-purpose cattle in two tropical municipalities of Veracruz, Mexico, to identify risk factors associated with seropositivity, and to report the geographical locations of the production units evaluated.

MATERIALS AND METHODS

SPATIAL LOCATION OF FARMS

The study examined dual-purpose cattle from the Mexican municipalities of Jamapa and Veracruz that are located in the central region of the state of Veracruz (Figure 1). Using the Köppen climate classification modified by García (1973), this zone is characterized by a warm, humid climate with a dry season (Aw). The geographic coordinates of the examined farms were established by global positioning system (GPS).

MINIMUM SAMPLE SIZE AND SAMPLING DESIGN

Sampling was performed on 28 farms from the two municipalities, which at the time of the study had a population of 1889 cattle. A pilot study was performed with 100 cattle with the aims of estimating the frequency of Neospora caninum infection and determining a minimum sample size. Serum antibodies against Neospora caninum were identified by enzyme-linked immunosorbent assays (ELISAs), which indicated a frequency of 10% seropositivity. With this indicator, the minimum sample size was determined using the following equation that was proposed by Navarro (1988):

Figure 1

$$n = \frac{1-p}{(p)(d)}$$

Where p = expected proportion and d = estimated error, which in this case was 0.02. Thus, a minimum sample size of 450 animals was required. However, our sample consisted of 555 serum samples from cows between 1 and 5 years of age.

BLOOD SAMPLING

The median coccygeal artery or adjacent vein was punctured for blood collection using a vacutainer. Each sample was identified individually and transported to the parasitological laboratory in Torreon del Molino, Faculty of Veterinary Medicine and Zootechnics of the Universidad of Veracruz (FMVZ-UV). Samples were centrifuged at 6,04 X g for 5 min, and the serum was removed and stored at –20°C.

SEROLOGIC TESTS

Serum samples were tested using an indirect ELISA test (Pourquier Institute version: P00511/01) for the detection of antibodies against Neospora caninum in accordance with the manufacturer’s instructions (Hall et al. 2006). These tests were performed in the immunohistochemical and molecular biology laboratory of the Pathology Department of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico (UNAM).

RISK FACTORS

Herd surveys gathered the following information: the presence of dogs and/or wild canids, occurrence of abortions and neonatal deaths in the cattle, origin of herd replacements, and feeding and vaccination programs. A database was developed using Epi Info version 3.4.3 to analyze each factor (variable). Each variable was evaluated to measure the strength of its association with the serological test result through univariate analysis of the odds ratio (OR), and its statistical significance was measured using chi-square or Fisher's exact tests. Variables with $P \leq 0.05$ were included in multivariate logistic regression analyses.

RESULTS

Serum samples from one or more animals from 23 of the 28 farms evaluated (82%) were positive for antibodies against Neospora caninum as determined by ELISAs. Of the 555 sera, 105 (18.91%) gave a positive result (Table 1).

Figure 2

Table 1. Frequencies of -seropositive serum samples from 28 dual-purpose cattle farms from the municipalities of Jamapa and Veracruz, Veracruz, Mexico as determined by ELISAs.

Farm	Total Animals tested	Positive animals	Percent positive (%)
1	20	1	5.0
2	10	0	0.0
3	9	0	0.0
4	15	2	13.3
5	25	1	4.0
6	62	20	32.2
7	39	6	15.3
8	22	8	36.3
9	23	6	26.0
10	21	3	14.2
11	42	9	21.4
12	17	4	23.5
13	10	2	20.0
14	17	4	23.5
15	34	12	35.2
16	11	0	0.0
17	9	2	22.2
18	15	0	0.0
19	14	1	7.1
20	9	3	33.3
21	16	3	18.7
22	30	5	16.6
23	6	1	16.6
24	6	2	33.3
25	21	3	14.2
26	7	1	14.2
27	42	6	14.2
28	3	0	0.0
Total	555	105	18.9

Division of the serum samples based on municipality indicated that 23.92% (89 of 372) of the samples from Jamapa were seropositive for Neospora caninum. However, only 8.74% (16 of 183) of the samples from the municipality of Veracruz were seropositive.

Figure 1 indicates the GPS location of the 28 farms examined and the frequencies of animals seropositive for Neospora caninum on each farm.

Figure 3

Figure 1. Frequencies of dual-purpose cattle seropositive for on 28 farms in two municipalities of Veracruz, Mexico.

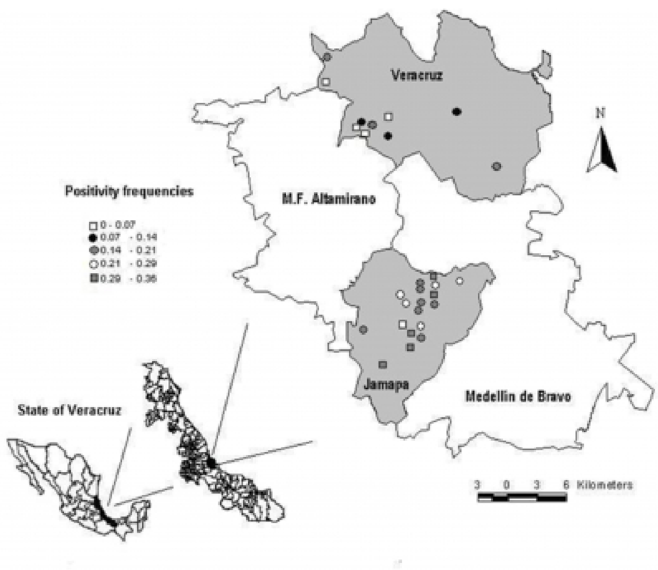

Table 2 displays the results of the univariate analysis for the determination of risk factors associated with the presence of Neospora caninum in dual-purpose cattle. Of the variables analyzed, only the presence of abortion in cows [OR = 7.34; 95% confidence interval ($IC_{95\%}$) = 2.35–22.92; P < 0.05] and replacement cattle originating from another Mexican state [OR = 2.23; $IC_{95\%}$ = 1.13–4.39; P < 0.05] were significant risk factors associated with Neospora caninum seropositivity.

Figure 4

Table 2. Comparison of the frequency of seropositive cattle with the risk factors for neosporosis.

Factor (variable)		Positive animals	Negative animals	Odds ratios (OR)	Confidence interval (CI)	P
Presence of canids						
Dog	yes	71	267	1.4313	.91–2.24	NS
	no	34	183			
Alien dog	yes	98	409	1.4034	.61–3.22	NS
	no	7	41			
Coyote	yes	86	341	1.4468	.84–2.48	NS
	no	19	109			
Fox	yes	21	75	1.25	.72–2.14	NS
	no	84	375			
Presence of abortions/ neonatal deaths						
Cow abortion	yes	8	5	7.3402	2.35–22.92	< 0.05
	no	97	445			
Background of abortions on farm	yes	80	335	1.0985	.66–1.8	NS
	no	25	115			
Dead calf	yes	2	3	2.8932	.47–17.53	NS
	no	103	447			
Origin of replacements						
Local origin	yes	28	99	1.2893	.79–2.09	NS
	no	77	351			
State origin	yes	57	200	1.4844	.96–2.27	NS
	no	48	250			
National origin	yes	14	29	2.2334	1.13–4.39	< 0.05
	no	91	421			
Feeding						
Rented pasture	yes	10	67	0.6017	.29–1.21	NS
	no	95	383			
Commercial feed	yes	45	176	1.1676	.75–1.79	NS
	no	60	274			
Vaccination programs						
Brucellosis	yes	21	66	1.4545	.84–2.5	NS
	no	78	384			
***IBR/BVD**	yes	27	102	1.181	.72–1.92	NS
	no	78	348			
Leptospirosis	yes	27	102	1.181	.72–1.92	NS
	no	78	348			

*IBR = infectious bovine rhinotracheitis; BVD = bovine viral diarrhea

* NS = Not significant

Further logistic regression analysis of the variables indicated that the risk factors associated with cows that have presented abortion and farms that obtain cattle replacements from another Mexican state were statistically significant (P ≤ 0.05) (Table 3).

Figure 5

Table 3. Logistic regression analysis of risk factors with P ≤ 0.05.

Risk factor	Coefficient	95% Confidence interval (CI)	Z-statistic	P
Cow abortion	2.0172	2.3927–23.6105	3.4539	< 0.01
Origin of replacements	0.8256	1.1508–4.5303	2.3618	< 0.05
Constant	−1.6031		−13.4028	< 0.01

DISCUSSION

Although seroepidemiological studies performed in Mexico have demonstrated the presence of neosporosis and its association with abortions in dairy cattle (Morales et al.

2001; Garcia-Vázquez et al. 2002, 2005; Sanchez et al. 2003; Gutiérrez et al. 2007), there is not much information with regard to dual-purpose and beef cattle. A study performed in the state of Nuevo Leon on beef cattle indicated that 10% of cattle in Linares (3/29 animals from two herds) and Pesqueira (3/30 animals from one herd) were seropositive for Neospora caninum (Salinas et al. 2005). In a study of 596 beef cattle from the states of Chiapas, Yucatan, and Veracruz, the prevalence of neosporosis was 15% (30/200), 11.3% (21/186), and 8.6% (18/210), respectively (Garcia-Vázquez et al. 2009). There have been only a few similar studies examining dual-purpose cattle in Mexico. This is likely due to the extensive and semi-housed production systems practiced in the south of Mexico that make it difficult to identify and record abortions. Therefore, there is no knowledge of the impact of this disease on dual-purpose cattle as compared to dairy cattle.

The frequency of Neospora caninum seropositivity (18.91%) in dual-purpose cattle determined in the present study was slightly higher than the frequencies reported for beef cattle from Nuevo Leon (10%), Chiapas (15%), and Yucatan (11.3%) (Salinas et al. 2005; Garcia-Vazquez et al. 2009). With regard to Veracruz, the seropositivity was higher than that observed by Garcia-Vazquez et al. (2009) (8.6%), but lower than that observed by Romero-Salas et al. (2009) (24.6%). On the other hand, these frequencies are similar to or lower than those observed in South American beef cattle. For example, in Argentina, seroprevalences of 18.9% to 20.3% have been recorded (Moore et al. 2002, 2003; Moore, 2005), and in Brazil the reported seroprevalence ranged from 6.7% to 29.9% (Ragozo et al. 2003). Seroprevalences of 26.6%, 13.9%, and 11.5% have been reported in Paraguay, Uruguay, and Venezuela, respectively (Osawa et al. 2002; Bañales et al. 2006; Lista-Alves et al. 2006). However, as reported in Mexico and in other parts of the world, the Neospora caninum-seroprevalences in dual-purpose and beef cattle, were lower than in dairy cattle (von Blumröder et al. 2004; Bartels et al. 2006; Dubey et al. 2007).

With respect to the risk factors analyzed in the present study, the statistical significance of a history of abortion [OR = 7.34; $IC_{95\%}$ = 2.35–22.92; $P < 0.05$] suggests that cattle could be previously infected with neosporosis (6 times more likely to be seropositive for Neospora caninum than are cattle with no abortion history). This result coincides with other epidemiological studies in which cows seropositive for neosporosis have a two- to four-fold greater risk of abortion than do seronegative cattle (Paré et al. 1997; Pfeiffer et al. 2002; Dubey and Schares, 2006).

The other factor that was significant in this study was obtaining replacement animals from other Mexican states [OR = 2.23; $IC_{95\%}$ = 1.13–4.39; $P < 0.05$]. Cattle from farms that obtained replacements from another state were 1.2 times more likely to be seropositive than were cattle from farms that did not buy heifers from other states. This result suggests that the replacement cattle from other states or regions of high Neospora caninum prevalence pose a risk of bringing the infection to the farm. Cattle are moved to introduce specialized cattle to a region for genetic improvement of the resident herds. Such cattle are frequently obtained from the Central Mexican Plateau where neosporosis has been identified (Morales et al. 2001). It is recommended that producers develop a replacement program that uses offspring from seronegative cows. However, in studies of dairy and beef cattle, the replacement of cows with heifers from the same herd may be a significant risk factor when the herd has a high prevalence of disease, owing to the efficiency of endogenous transplacental transmission (Barling et al. 2001; Dubey et al. 2007). However, in studies performed by Sanderson et al. (2000) on beef cattle from northeastern United States of America and by Gutiérrez et al. (2007) on dairy cattle in Aguascalientes, Mexico, there were no associations between seroprevalence and the origin of the replacement animals from the same or different herds.

With regard to the other factors that were evaluated in the present study, there was no association with seropositivity for the presence of owned or alien dogs or wild canids. Although there are no epidemiological studies in these canids of this region, this result suggests that the prevalence of neosporosis in these animals is low. This finding was similar to those of Fischer et al. (2003) and Hobson et al. (2005). Additionally, there was no association between seropositivity and the types of feeding and vaccination programs. These findings were different than those reported for dairy cattle, which could be attributable to the different production systems used. Dual-purpose cattle are generally managed in extensive systems where the animals are fed mainly with green forage rather than stored feed, which may decrease the intake of food contaminated with the feces of dogs or wild canids. There are few reports of naturally-infected dogs that eliminate oocysts. However, some epidemiological studies have indicated that the presence of dogs on dairy farms is a risk factor for neosporosis in cattle, and this is still an economically-important transmission route (Bartels et al. 1999; Dijkstra et al. 2002; Sanchez et al. 2003). Infected cattle may be present even when there are no dogs on a production unit. Therefore, endogenous transplacental transmission is a very effective way for the parasite to be maintained within a herd (Basso et al. 2001; McGarry et al. 2003; Schares et al. 2005; Cedillo et al. 2008).

Currently, there is no worldwide general strategy or program for the control of bovine neosporosis because there are great differences in the epidemiology of the infection and regional differences in zoosanitary procedures. Therefore, for the development of a prevention and control program, it is important to determine the current state of the infections at a regional and local level, or at least at the level of the production unit (Dubey et al. 2007).

CONCLUSIONS

Dual-purpose cattle from the municipalities of Jamapa and Veracruz, Veracruz, Mexico, have been exposed to Neospora caninum because 23 out of 28 farms examined (82%) had at least one animal that was seropositive. The presence of abortions and herd replacements from different Mexican states were the only factors that were associated with Neospora caninum seropositivity. The association between the abortions and Neospora caninum seropositivity suggests that cattle could be previously infected with the parasite. Due to there was not a correlation between the presence of canids and Neospora caninum seropositivity, the main route of transmission must be between cattle (endogenous and exogenous). Therefore, serological test for neosporosis is important for preventing the spread of the infection

Finally, to identify other risk factors that have not been examined, additional epidemiological studies are needed within municipalities near the municipalities examined here, as well as municipalities in other Mexican states with similar geographic conditions, types of animals, and management systems.

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