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Seroprevalence of *Neospora caninum* Antibodies in Cattle in Veracruz, Mexico

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Abstract: The objective of this study was to determine the seroprevalence of *Neospora caninum* antibodies in 863 female cattle 1 to ≥6 years old from 78 herds of dairy, beef and crossbred cattle in Veracruz, Mexico. Serum samples were tested for the presence of *N. caninum* antibodies using an ELISA assay. Overall seroprevalence of *N. caninum* antibodies was 26%. By municipality, the highest prevalence was 47.4% and the lowest 6% ($p<0.05$). By age, the highest prevalence was found in 5 year olds (28%) and the lowest (11.1%) in 1 year olds ($p<0.05$). Regarding the breed, the highest seroprevalence was observed in crossbred females ($p<0.05$). Considering the reproductive status, the highest seroprevalence was in cows with >5 calvings (32.1%) and the lowest was in pubertal heifers (11.1%; $p<0.05$). Seroprevalence was 26.2% in homebred and 23% in purchased animals ($p>0.05$). Prevalence in females with a history of abortions was 40 and 26.2% in females with no records of previous abortions ($p<0.05$). The results show that bovine neosporosis is widespread in the State of Veracruz, Mexico, as in other regions of the country.

Key words: Cattle, *Neospora caninum*, neosporosis, seroprevalence, Mexico

INTRODUCTION

Neospora caninum is a heteroxenous cyst-forming apicomplexan protozoan which is considered a major cause of infectious bovine abortion worldwide and has been associated with endemic, epidemic and sporadic abortions (Hall *et al*., 2005; Dubey *et al*., 2007; Kul *et al*., 2009). The infection causes important economical losses to the cattle industry due to reproductive failure associated with abortion and mortality in congenitally infected calves (Moskwa and Cabaj, 2003; Dubey *et al*., 2007). *Neospora caninum* infection has been reported in dairy cattle herds on all continents (Dubey *et al*., 2007; Yildiz *et al*., 2009).

In the *N. caninum* life cycle, dogs (McAllister *et al*., 1998) and coyotes (Gondim *et al*., 2004) are the definitive hosts, whereas cattle and other mammals act as natural intermediate hosts (Chávez-Velásquez *et al*., 2004; Rodrigues *et al*., 2004). In cattle, *N. caninum* infection may occur by horizontal transmission due to ingestion of sporulated oocysts shed by the definitive host (Dijkstra *et al*., 2001; Frössling *et al*., 2005). However, vertical transmission is the predominant route of infection (Frössling *et al*., 2005; Hall *et al*., 2005). Vertical transmission occurs when tachyzoites cross the placenta of a persistently infected dam and infect the foetus (Trees and Williams, 2005). Transplacental transmission can occur in consecutive pregnancies in the same cow and so the infection can persist in cattle herds through many generations (Innes *et al*., 2005; Dubey *et al*., 2006). The infection usually has a chronic course and persists throughout the life of an infected animal (Björkman *et al*., 1996). *Neospora caninum* DNA has been reported in fresh and frozen semen from naturally infected bulls (Caetano-da-Silva *et al*., 2004; Ferre *et al*., 2005) and the possibility of venereal transmission in bovine neosporosis has been suggested (Garcia-Vazquez *et al*., 2009).

Nonpregnant adult cattle that are infected with *N. caninum* do not show any signs of disease but infection in a pregnant animal may result in abortion, or in the birth of weak, diseased, sub-clinically infected or healthy calves (Dubey *et al*., 2007; García-Melo *et al*., 2009). Abortions may occur at any stage of pregnancy

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and irrespective of whether the infection in the cow is recent, chronic, or congenital (Wouda *et al*., 1998; Dubey *et al*., 2007). Diagnosis of the infection in live animals can be achieved by detection of anti-*N. caninum* specific antibodies by serological tests (González-Warleta *et al*., 2008). The presence of antibodies to *N. caninum* in the serum of an individual indicates that it is, or has been, infected with the parasite (Stenlund *et al*., 2003), although the absence of antibodies does not rule out neosporosis (Ghanem *et al*., 2009).

In Mexico, bovine neosporosis was first detected in 1997 in an aborted five-months male Holstein fetus (Morales *et al*., 1997). It is believed that this disease might be widely distributed in the dairy cattle herds in Mexico (Morales *et al*., 2001), since in recent years neosporosis has been reported in dairy and beef cattle in several states (Salinas *et al*., 2005; Garcia-Vazquez *et al*., 2009). The aim of the present study was to determine the seroprevalence of *N. caninum* antibodies in cattle in different municipalities of the State of Veracruz, Mexico.

MATERIALS AND METHODS

Characteristics of the study and experimental animals: A cross-sectional study was carried out from September 2007 to March 2009 to determine the seroprevalence of *N. caninum* antibodies in 78 herds of dairy (Brown Swiss, Holstein), beef (Simmental, Zebu) and crossbred (Brown Swiss x Zebu, Holstein x Zebu) cattle from 15 municipalities of the State of Veracruz, Mexico, in the Central Coast of the Gulf of Mexico. A total of 863 female cattle 1 to ≥6 years-old were tested for the presence of anti-*N. caninum* antibodies in blood serum. A random sample of animals was selected from each herd assuring that the number was sufficient to estimate the herd prevalence with 10% error and 95% Confidence Interval (CI), conservatively assuming the variance to be 25% and seroprevalence 50%. The variables studied were age, genotype, reproductive status, origin of cattle and abortion history.

Blood sampling: From each of the 863 females, blood samples were collected into sterile plain vacutainers tubes via coccygeal or jugular veinpuncture and transported in ice to the laboratory for centrifugation at 1000× g for 15 min to separate the serum, which was stored at -20°C until used for antibody determination.

Serological tests: Serum samples were assayed using an enzyme-linked immunosorbent assay (ELISA; IDEXX, Laboratories Inc., Westbrook, Maine, USA) for detection of antibodies against *N. caninum*. Each sample was tested in duplicate. Sera with absorbance values above the cut-off level of 0.50 were considered to be positive, in accordance with the manufacturer's instructions. The sensitivity and specificity of the ELISA test was 100 and 98.9%, respectively. The predictive values of the test were 93.75% positive and 100% negative (Wapenaar *et al*., 2007).

Management practices in the farms: At the start of the study, a questionnaire was completed during a personal interview with the owner or the manager of each herd. Animal-level information included age and number of abortions during the last 3 years.

Statistical analyses: Seropositive rates for *N. caninum* antibodies were analyzed in relation to the animal data, such as age, genotype, reproductive status, origin and abortion history using a two-sided chi-square test or the Fisher exact test. $p<0.05$ were considered as statistically significant. The tests used were from SAS (Cody and Smith, 1991).

RESULTS

The overall seroprevalence of *N. caninum* antibodies determined in the study was 26%. By municipality, the highest prevalence was 47.4% and the lowest was 6% ($p<0.05$; Table 1).

By age, the highest prevalence was found in females 5 year olds (28%) and the lowest prevalence (11.1%) was in 1 year olds ($p<0.05$; Table 2).

Regarding the breed, the highest seroprevalence of *N. caninum* antibodies was observed in the crossbred (Brown Swiss x Zebu, Holstein x Zebu) females, compared to the purebred animals ($p<0.05$; Table 3).

Considering the reproductive status, the highest seroprevalence was in cows with >5 calvings (32.1%) and the lowest was in pubertal heifers (11.1%; $p<0.05$; Table 4).

Concerning the origin of cattle, of 789 homebred animals 207 were seropositive, for a prevalence of 26.2% and of the 74 animals that were purchased and introduced into the herds 17 were seropositive, for a prevalence of 23% ($p>0.05$).

The number of females with a history of abortions was 25, of which 10 were seropositive to anti-*N. caninum* antibodies, for a prevalence of 40%, whereas of 793 females with no records of previous abortions 218 were seropositive, for a prevalence of 26.2% ($p<0.05$).

Table 1: Prevalence of anti-*Neospora caninum* antibodies in bovine females located in several municipalities of the State of Veracruz, Mexico

Municipality	No. of herds	No. of animals	No. of positives	Prevalence (%)
Acajete	4	52	12	23.1
Alvarado	5	46	9	19.6
Alto Lucero	5	52	16	30.8
Coacoatzintla	3	28	8	28.6
Cuitlahuac	4	45	11	24.4
Huatusco	4	44	15	34.1
Ignacio de la Llave	4	38	11	28.9
Juan Rodríguez Clara	4	49	7	14.3
Medellin	4	53	12	22.6
Manlio F. Altamirano	4	47	5	10.6
Nautla	5	50	3	6.0
Playa Vicente	13	171	81	47.4
Tierra Blanca	8	96	19	19.8
Tlalixcoyan	5	46	9	19.6
Tres Valles	6	46	6	13.0
Total	78	863	224	26.0

Table 2: Prevalence by age of anti-*Neospora caninum* antibodies in bovine females from several municipalities of the State of Veracruz, Mexico

Age (years)	No. of animals	No. of positives	Prevalence (%)
1	18	1	11.1
2	64	14	21.9
3	134	29	21.6
4	141	39	27.7
5	175	49	28.0
≥6	331	91	27.5
Total	863	224	26.0

Table 3: Prevalence by genotype of anti-*Neospora caninum* antibodies in bovine females from several municipalities of the State of Veracruz, Mexico

Breed	No. of animals	No. of positives	Prevalence (%)
Zebu	72	15	20.8
Crossbred	547	150	27.4
Holstein	100	24	24.0
Simmental	9	2	22.2
Brown Swiss	135	33	24.4
Total	863	224	26.0

Table 4: Prevalence by reproductive status of anti-*Neospora caninum* antibodies in bovine females from several municipalities of the State of Veracruz, Mexico

Reproductive status	No. of animals	No. of positives	Prevalence (%)
Pubertal heifers	27	3	11.1
Pregnant heifers	34	4	11.8
1st-calving cows	192	45	23.4
2nd-calving cows	186	55	29.6
3rd-to-5th calving cows	371	100	26.9
Cows with >5 calvings	53	17	32.1
Total	863	224	26.0

DISCUSSION

The presence of *N. caninum* antibodies has been demonstrated in cattle populations worldwide but the estimated prevalence of cattle infected with *N. caninum* varies considerably among herds, regions and countries (Frössling *et al*., 2008). Overall seroprevalence of anti-*N. caninum* antibodies found in the present study was lower than those obtained in other studies carried out in Mexico. To this respect, in the central and northern regions of Mexico, 56% of the cows from 50 dairy herds were positive to *N. caninum*, with seroprevalence of 72% in cows that had aborted recently and 36% in cows that had abortion rates of up to 12% annually over the last 3 years (Morales *et al*., 2001). In 44 beef and dairy cattle herds from the northeastern region of Mexico, overall prevalence was 36% (range 16-45%; Salinas *et al*., 2005). In cattle from 20 dairy herds, Garcia-Vazquez *et al*. (2005) estimated a prevalence of infection of 42% and it was higher among cows raised on-farm (41%), than among replacement cattle purchased outside the farm (28%), contrary to the results, with similar prevalence among raised-on farm and purchased cattle which might suggest that infected replacement heifers purchased outside the farms have been introduced into the herds, leading to the presence of persistently infected animals. Seroprevalence found in the present study was higher than that reported in southern states of Mexico where overall prevalence in 31 beef cattle herds was 11.6% (range 8.6-15%), with overall herd-prevalence of 70.9% (range 50-90%) and with the highest age-prevalences found in animals 1 (19.4%) and 4 years old (19.6%) and the lowest in 3 year olds (6.2%), suggesting that the major transmission route of *N. caninum* may be transplacental (Garcia-Vazquez *et al*., 2009). This is contrary to the findings of the present study, since the highest prevalence was in animals 5 years old and the lowest in 1 year old, which might indicate that horizontal transmission of the infection could have been occurring. In the present study, the higher prevalence of *N. caninum* antibodies in females with a history of abortions, compared to those with no abortions, could indicate that this parasite plays an important role in the occurrence of pregnancy loss in cattle, as was previously mentioned by Garcia-Vazquez *et al*. (2005).

Worldwide, prevalences have been estimated at between 14.1 and 40.4% in America (Chi *et al*., 2002; Moore, 2005), 5.7-35.6% in Asia (Koiwai *et al*., 2005) and 6-21.1% in Oceania (Reichel, 1998; Hall *et al*., 2006), comparable to the findings of the present study.

In Argentina, prevalence of *N. caninum* antibodies in dairy cattle was reported in 64.5% (Venturini *et al*., 1999). In Canada, *N. caninum* infection can be found in a large number of herds. In dairy cattle, cow-level prevalences range from 5.6-25.5%, depending on the region (Keefe and VanLeeuwen, 2000). In female cattle over 6 months of age from 23 dairy herds, Bergeron *et al*. (2000) indicated an overall prevalence of seropositive animals of 21.9% (range 4.3-61.8%); VanLeeuwen *et al*. (2005) reported 5.6% of cows testing positive from 51 dairy herds while VanLeeuwen *et al*. (2006) indicated that 8.3 and 9.1% of dairy and beef cows, respectively, were

seropositive. In beef cows, 9% of animals from 174 herds were positive and 36% of herds had at least one positive cow (Waldner *et al*., 2001).

In a European study by Bartels *et al*. (2006), 49, 63 and 76% of dairy herds in Germany, Spain and The Netherlands, respectively, had at least one seropositive cow. In Ireland, McNamee *et al*. (1996) reported seroprevalence of 3.0%. In Italy, seroprevalence in dairy herds ranged from 40-65% (Varcasia *et al*., 2006). In Galicia, Spain, 79.3% of 385 dairy herds and 15.7% of 5,196 cows and heifers were found to be seropositive (González-Warleta *et al*., 2008). In Sweden, Frössling *et al*. (2008) reported that 8.3% of 2,978 dairy herds were positive whereas Stenlund *et al*. (2003) in one dairy herd found 63-87% seropositive cows and heifers (4 months to ≥4 years old) during six consecutive years and a large number of young animals tested seropositive although their dams were seronegative, indicating that a transmission of the parasite other than the vertical had also occurred, which could be compared to the findings of the present study. In a study carried out during five consecutive years in England, Woodbine *et al*. (2008) reported that 94% of 115 dairy herds with female cattle ≥2 years of age had at least one seropositive cow, 12.9% of adult cattle had at least one seropositive test and approximately 90% of herds were seropositive at all visits, with a mean seroprevalence of 10% in positive herds (range 0.4-58.8%) and a positive association between the serostatus of offspring and dams that were ever seropositive. These findings are similar to those of the present study, since 100% of the herds sampled had at least three seropositive animals.

In Dakar, Senegal, West Africa, seroprevalence of *N. caninum* antibodies in dairy cattle from four farms was 17.9%, with all the farms having seropositive animals; in these farms, seroprevalence was 53.3% in local breeds (Gobra breed), 25% in crossbreeds and 13.4% in exotic breeds, although these latter accounted for the highest number of animals; however, there was no difference in the seroprevalence of *Neospora* infection among age groups (Kamga-Waladjo *et al*., 2009). In the present study, breed-seroprevalence was highest in the crossbred females, which could be explained because these cattle were predominant in all the herds included in the study. Although there are indications from several countries that seroprevalence of *N. caninum* differs according to the breed of cattle (Bartels *et al*., 2006), the differences observed might be caused by differences in the management systems used for the different breeds and not by differences in breed-related susceptibility to infection (Dubey *et al*., 2007). In Turkey, Aktaş *et al*. (2005) reported seroprevalence of 4-15% (average 7%) in cattle from four provinces and 3% of aborting cows were seropositive against *N. caninum* while Içа *et al*. (2006) indicated seroprevalence of 7% in the region of Kayseri, with 33.3% of aborting cows found to be seropositive, this latter percentage compared to that from the present study.

In Bali (*Bos javanicus*) cattle, the predominant beef cattle in the island of Bali, in Indonesia, seroprevalence of antibodies to *N. caninum* in female and male cattle was 5.5%, with a likely effective transplacental transmission from the infected dam to her calf; it was the first serological evidence for the presence of natural *N. caninum* infection in this genotype, extending the spectrum of bovine hosts of this parasite; this study also revealed, for the first time, the occurrence of *N. caninum* infection in Indonesia (Made-Damriyasa *et al*., 2010). This finding completed the list of Southeast Asian countries where *N. caninum* had been already reported to affect dairy cattle or water buffaloes: Vietnam (Huong *et al*., 1998; Duong *et al*., 2008), Thailand (Chanlun *et al*., 2006), Malaysia (Cheah *et al*., 2004) and the Philippines (Konnai *et al*., 2008). In Korea, Bae *et al*. (2000) indicated 23% of cattle positive to *N. caninum* whereas Kim *et al*. (2002) reported seropositivity of 4.1% in Korean native beef cattle from nine provinces.

The reason for the regional differences in seroprevalence to *N. caninum* antibodies is partially known, but dog density, presence of coyotes and climatic factors have been suggested as explanations (Rinaldi *et al*., 2005; Dubey *et al*., 2007). The putative ways by which dogs may pose an infection risk to cattle have been reported such as defecation by farm dogs on grass and density of the dogs on the farm location, similar to what might happen with the presence of coyotes (Dubey *et al*., 2007). González-Warleta *et al*. (2008) reported a higher herd-prevalence of neosporosis in farms that had dogs and mention that in order to enable reasonable control measures to be established, it is essential to identify the risk factors associated with the epidemiology of this parasitic infection in different areas. Even though the major transmission route of *N. caninum* may be transplacental, horizontal transmission could be also an important route since it is the via the dams get infected, thus, reducing the risk of introduction of the parasite into the herd will contribute to reduce the risk of both vertical and horizontal transmission. Although in the present study dog presence in the farms was not studied in relation with the prevalence of *N. caninum* infection, it can not be eliminated as a main contributing factor for the seroprevalence of the infection in the cattle herds. More study is required to clarify the transmission routes of *N. caninum* infection among cattle.

CONCLUSION

This study demonstrated that *N. caninum* is widespread in dairy, beef and crossbred cattle herds of Veracruz, Mexico. The highest prevalence in 5 year olds females and the lowest in 1 year old, could suggest that horizontal transmission of the infection could have been occurring in addition to transplacental transmission. Moreover, the high prevalence of *N. caninum* antibodies in females with a history of abortions could confirm that this parasite plays an important role in the occurrence of pregnancy loss in cattle.

Since cattle industry is one of the key industries in the region studied, it is essential to identify the risk factors associated with the epidemiology of *N. caninum* infection to control the spread of this parasitic disease in order to contribute to reduce the incidence of pregnancy losses in cattle.

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