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SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH *Neospora caninum* IN GOATS FROM MUNICIPALITIES OF THE CENTRAL REGION OF VERACRUZ

[SEROPREVALENCIA Y FACTORES DE RIESGO ASOCIADOS CON *Neospora caninum* EN CAPRINOS DE MUNICIPIOS DEL CENTRO DE VERACRUZ]

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SUMMARY

The objective of the study was to determine the seroprevalence and risk factors associated with caprine neosporosis in five municipalities of the central region of the state of Veracruz, Mexico, through a multistage and stratified study. A total of 182 animals from 26 production units (PU) were included, with a sampling fraction of six animals per PU. The PU were selected by clusters according to the tables by Cannon and Roe. Neosporosis was diagnosed through the ELISA test. Seroprevalence was determined by VassarStats® and the risk factors by odds ratio (OR). Overall seroprevalence was 3.8 %, by affected municipalities 60 %, and by PU 15.4 %. The municipality of Coacoatzintla was a risk factor for the infection (OR = 5.95; $CI_{95\%}$: 1.27 – 27.94), whereas Coatepec and Chiconquiaco, as well as the bucks, were protective factors (OR = 0; $CI_{95\%}$: 0 - 0). In conclusion, neosporosis in goats had a low seroprevalence but a medium distribution within municipalities and PU. Goats in Coacoatzintla were 5.9 times more likely to become infected with *Neospora caninum*, whereas goats from Coatepec, Chiconquiaco and the bucks, were protective factors.

Key words: Seroprevalence; neosporosis; risk factor; protective factor.

RESUMEN

El objetivo del estudio fue determinar la seroprevalencia y los factores de riesgo asociados con neosporosis caprina en cinco municipios del centro del estado de Veracruz, México, mediante un estudio polietápico y estratificado. Se incluyeron 182 animales de 26 unidades de producción (PU), con fracción de muestreo de seis animales por PU. Las PU fueron seleccionadas por conglomerados según las tablas de valores de Cannon y Roe. El diagnóstico de neosporosis fue mediante prueba de ELISA. La seroprevalencia se determinó con el programa VassarStats® y los factores de riesgo por razón de momios (OR). La seroprevalencia general fue 3.8 %, por municipios afectados 60 % y por PU 15.4 %. El municipio de Coacoatzintla fue factor de riesgo para la infección (OR = 5.95; $CI_{95\%}$: 1.27 – 27.94), mientras que Coatepec y Chiconquiaco, así como los sementales, fueron factores protectores (OR = 0; $CI_{95\%}$: 0 - 0). En conclusión, la neosporosis caprina tuvo seroprevalencia baja, pero con distribución media

en los municipios y PU. Los caprinos de Coacoatzintla tuvieron 5.9 más veces riesgo de infectarse con *Neospora caninum*, mientras que los de Coatepec, Chiconquiaco y los sementales, fueron factores protectores.

Palabras clave: Seroprevalencia; neosporosis; factor de riesgo; factor protector.

INTRODUCTION

One of the parasitic diseases that seriously affects the economy of livestock production worldwide is neosporosis, caused by *Neospora caninum* (Thilsted and Dubey, 1989). In the Netherlands, Bartels *et al.* (2006) found that in 24% of 108 dairy herds the economic losses to neosporosis accounted for €2000 a year. *Neospora caninum* was first reported in Norway in 1984 as an unidentified protozoan very similar to *Toxoplasma gondii* (Bjerkas *et al*., 1984), with the dog being the definitive host (McAllister *et al*., 1998). The *Neospora* genus belongs to the Phylum *Apicomplexa* and the Family *Sarcocystidae*, which shares with the genera *Toxoplasma* and *Sarcocystis*, and includes two species: *Neospora caninum* and *Neospora hughesi* (Ortega-Mora *et al*., 2003).

Neospora caninum was first recognized as a cause of nervous problems in canids (McAllister *et al*., 1998), and was linked by the first time to the occurrence of one abortion in a dairy herd in New Mexico (Thilsted and Dubey, 1989). In dairy and beef cattle, abortion problems and congenital infections related to *N. caninum* have been reported (Anderson *et al*., 2000), and the presence of *N. caninum* has also been associated with pathologies that lead to abortion during the fourth to seventh month of gestation, with no previous clinical signs, and its has been observed that infected calves can be born with a normal clinical appearance (McAllister *et al*., 1998).

The infection by *N. caninum* can occur by two vias: vertical transmission (from mother to offspring) and horizontal transmission (by direct contact), and it has even been related to clinical infection in horses, goats, sheep and deer (Dubey, 2003). The presence of antibodies against *N. caninum* has been found in wild canids (coyote and fox), buffalo, camels, cats, mice, pigs, primates, horses, deer, moose and wild felines, which shows that animal pets and wildlife play an important role in the epidemiology of this disease (Barr *et al*., 1995; Dubey and Lindsay, 1996; Gondim *et al*., 2004).

The dog is the definitive host of this parasite, so the presence of dogs in the PU has been identified as risk factor for the transmission of the disease (McAllister *et al*., 1998; Paré *et al*., 1998; Mainar-Jaime *et al*., 1999; Schares *et al*., 2004; von Blumröder *et al*., 2004). Likewise, the presence of dog feces in the food dispensers, grass or silage, is the factor with more evidence of causing postnatal infections (Dijkstra *et al*., 2002).

Worldwide, there are few studies available on neosporosis in goats. To this respect, in France a prevalence of 8.9 % has been reported (Chartier *et al.*, 2000), which differs to the prevalence obtained in Brazil of 42 % in Sao Paulo and 93 % in Mossoro, Rio Grande, in PU considered as infected (Figliuolo *et al.*, 1998; Chartier *et al.*, 2000; Ribeiro *et al.*, 2008).

In Mexico, literature available on neosporosis in goats is scarce. The present study was carried out in the state of Veracruz, since it is one of the main producers of goats in Mexico. In Veracruz, 90 % of the goats are concentrated in its central region in small communities with low economical resources, which, however, have a major contribution to the production of goat's milk in the state. For this reason, goats' sanitary status concerning many diseases, particularly those of zoonotic importance, is unknown. Therefore, the objective of this study was to determine the seroprevalence of caprine neosporosis in five municipalities of the central region of Veracruz, Mexico, as well as the risk factors associated with this disease.

MATERIALS AND METHODS

Location

The study was conducted from March to June 2010 in the municipalities of Chiconquiaco, Coacoatzintla, Coatepec, Tlacolulan and Yecuatla, all located in the central region of the state of Veracruz, Mexico (Lat. 19° 27' to 19° 52' N and Long. 96º 47' to 97° 00' W, at 420 to 2040 m of altitude), with climates varying from tropical to temperate, and annual temperatures ranging between 12.5 and 22.5 °C (García, 1988).

Study design

The study was cross-sectional, multistage and stratified, where the flocks were randomly selected from clusters (Daniel, 2008). The sample size was calculated through the Win Episcope Ver. 2.0 program

proposed by the University of Zaragoza, Spain (Thrusfield *et al.*, 2001), under the category "estimate proportions", for an estimated prevalence of 50 %, with 5 % error and 95 % confidence, thus, sample size resulted in 182 animals included in the study. The proportional number of PU was calculated according to the table of values proposed by Cannon and Roe (1982), that provides the number of PU that are necessary to know the presence of the disease and the number of animals that have to be considered in each PU from a general sample. According to this, of 95 that was the total number of PU in the municipalities selected, only 26 PU were considered, and six animals in each of them.

The criteria of inclusion considered for this study were female goats older than 3-months-old and the bucks. The criteria of exclusion were the males that were not used for breeding and the females younger than 3-months-old.

Two questionnaires were applied, one general in each PU included, and other individual for each animal sampled. The variables of interest for this study were: type of PU, other PU near those studied, other domestic and wild animal species found inside or near the PU, type of feeding, cleanness of the water and food dispensers, water sources available for the animals, deworming schedule, excreta management, carcasses disposition, mobilization of animals, and abortions.

Sampling

Blood samples obtained for the serological tests were collected via jugular venipuncture using Vacutainer® tubes without additives. Samples were transported to the laboratory at 4 °C, and centrifuged for 15 min at 1000 x *g* to separate the serum, which was stored at -20 °C until analyzed.

Diagnostic tests

The ELISA test was used to identify anti-*N. caninum* IgG antibodies using a commercial kit (sensibility 100 % and specificity 98.9 %; IDEXX® Laboratories, Inc., Westbrook, Maine, USA) (Bartels *et al*., 2005). The samples were diluted at 1:100 with phosphate buffered saline, at pH 7.4, with 0.05 % Tween 20. The washing solution was prepared at temperature of 20 to 25 °C by stirring up until diluting the salts that tend to precipitate. Positive and negative control sera were used. The reading was made using an ELISA reader with a filter with optical density of 650 nm. The positive or negative result of the test was calculated with the X-Check (IDEXX® Laboratories, Westbrook, Maine, USA) program.

Data analysis

Seroprevalence was determined through the VassarStats® program to estimate proportions and confidence intervals as proposed by Thrusfield (2005); the association among the study variables and the seropositivity was calculated by odds ratio with the Win Episcope Ver. 2.0 program (Thrusfield *et al*., 2001).

RESULTS AND DISCUSSION

Overall seroprevalence of the study

Overall seroprevalence of neosporosis in goats from the municipalities included in the study (Table 1) was 3.8 % ($CI_{95\%}$: 1.7 - 8.09), which was similar to the 6.4 % reported by Figliuolo *et al*. (1998) in Sao Paulo Brazil when the $CI_{95\%}$ is considered; however, it was higher than the 1.4 % obtained in France by Chartier *et al*. (2000), and than the 1.05 % reported by Ribeiro *et al.* (2008) in Mossoro, Rio Grande, Brazil.

Nowadays, the real scenario of caprine neosporosis worldwide is still unknown, which is in contrast with the large number of studies on this disease carried out in cattle. In reference to this, Silva *et al.* (2002) studied the seroprevalence of *N. caninum* in dairy cattle from the Lima valley; likewise, Lozada (2004) determined the presence of antibodies against *N. caninum* in dairy herds from northern Ecuador. In Mexico, specifically in the state of Veracruz, Montiel *et al.* (2010) found that seroprevalence of bovine neosporosis in the municipalities of Tierra Blanca, Tres Valles and Juan Rodríguez Clara was 15.5 %, whereas Ling *et al.* (2008) in the same state, but in the municipalities of Paso de Ovejas, Veracruz, Jamapa, Manlio Fabio Altamirano and Medellín, obtained a seroprevalence of 8.6 %.

Overall seroprevalence by municipalities

Table 1 shows the prevalence by municipality. To this respect, Coacoatzintla had the highest seroprevalence with 11.1 % ($CI_{95\%}$: 3.62 – 27), whereas Coatepec and Chiconquiaco were negative. The goats from Coacoatzintla were 5.9 times more likely to become infected with *N. caninum*, which indicates that this municipality represents a risk factor for neosporosis, in a situation that is apparently similar to that in Yecuatla and Tlacolulan, where there were positive animals too. These municipalities have similar climatic conditions,

with average temperature of 18° C and temperate-wet climate. Ribeiro *et al.* (2008) proposed that caprine neosporosis can have different seroprevalence according to the different climatic conditions where the PU are located, whereas Montiel *et al*. (2010) indicated that *N. caninum* is present in cattle from regions with temperate to extreme climates, with average temperature of 22.8 °C, which allows to suggest that this parasite may be found under these circumstances. On the other hand, Coatepec and Chiconquiaco turned out to be protective factors in this study (OR = 0; $CI_{95\%}$: 0 – 0). The ecological characteristics of these municipalities, as well as the production systems and the absence of other species that might act as carriers, such as dog, coyote, cattle, horse and the Mexican opossum, prevent the presence of the parasite (Montiel *et al.*, 2010), although the absence of this protozoan could also be due to the fact that the seroprevalence is very low; however, although the sample size was apparently adequate, it might be necessary to have a larger sample size, since it was assumed that seroprevalence was 50 % because there were no data on this respect (Thrusfield, 2005).

Table 1. Seroprevalence of antibodies against *Neospora caninum* in goats from five municipalities in the central region of the state of Veracruz, Mexico.

Municipality	No. animals sampled	No. positive animals	Prevalence %	CI95 %	OR	CI95 %
Coatepec	41	0	0	0 - 10.67	0	0
Chiconquiaco	32	0	0	0 - 13.34	0	0
Yecuatla	34	2	5.8	1.02 - 21.05	1.78	0.33 - 9.63
Coacoatzintla	36	4	11.1	3.62 – 27	5.95	1.27 - 27.94
Tlacolulan	39	1	2.6	0.13 - 15.07	0.6	0.07 - 5.14
Total	182	7	3.8	1.7 - 8.09		

OR = odds ratio, CI = confidence interval.

Seroprevalence in caprine PU by municipality

Table 2 shows the seroprevalence found in the 26 PU (15.4 %; $CI_{95\%}$: 5.04 – 35.72). Again, the municipality of Coacoatzintla had the highest seroprevalence with 40 % ($CI_{95\%}$: 7.26 – 82.96), whereas the lowest seroprevalence was obtained in Coatepec and Chiconquiaco (0 %; $CI_{95\%}$: 0 – 53.71).

Bovine neosporosis is a widely distributed disease in the state of Veracruz in PU located in regions where climatic conditions are favorable to the presence of the protozoan (Montiel *et al*., 2010), which can be higher than 90 %. Ribeiro *et al.* (2008) reported that 93 % of caprine PU selected in Mossoro, Rio Grande, Brazil, were infected. Likewise, Figliuolo *et al.* (1998) indicated that 42 % of caprine PU were affected, and Chartier *et al*. (2000) found that 3 to 19 % of caprine PU in western France were infected with neosporosis. This last datum, unlike the three previous studies carried out in Mexico and Brazil, is very similar to that found in the five municipalities (15.4 %; $CI_{95\%}$: 5.04 - 35.72) in the present study, which means that the distribution by PU in these locations is medium, except in the case of Coacoatzintla, where the distribution is high (40 %; $CI_{95\%}$: 7.26 – 82.96). Thus, since the climatic conditions in the municipalities affected are similar, the seroprevalence could increase over time if adequate preventive measures are not taken to control the disease (Montiel *et al*., 2010). The PU located in Coatepec and Chiconquiaco turned out to be protective factors in this study, since none of the goats from these PU showed evidence of the presence of antibodies against *N. caninum* (OR = 0; $CI_{95\%}$: 0 – 0) (Table 1).

Seroprevalence of caprine neosporosis according to the productive status of the goat

The pregnant does were the group with the highest seroprevalence (9.1 %; $CI_{95\%}$: 1.59 - 30.62), and the lowest seroprevalence was observed in the groups of bucks, weaned and dry does (Table 3).

In general, pregnant does can have immunosuppression periods due, among other factors, to a poor body condition and to the presence of hormones of her own and of their fetuses, so they are more susceptible to become infected (Hafez and Hafez, 2000; Tizard, 2008), situation that is coincident with that observed in the present study. Haddad *et al.* (2005) indicated that the economic losses due to

neosporosis are related to reproductive problems, stillbirths, abortions, embryo death and resorption, increase in days open or infertility, and costs by veterinary services and treatment as consequence of abortions.

On the other hand, Thurmond and Hietela (1996) and Dubey (1999) have established that the seropositive animals produce less milk and are more likely to be culled before the end of their productive life; this could be applied to the group of lactating does in the present study, in which although the seroprevalence was low (5.7 %; $CI_{95\%}$: 0.99 - 20.52), the milk production below the flock average could be a reason to cull a doe. In this study, the bucks turned out to be more resistant to the infection (OR = 0; $CI_{95\%}$: 0 - 0), and were considered as protective factors. No literature available was found that mentions that bucks are less prone to become infected by *N. caninum*. Moreover, the weaned and the dry does were also considered as protective factors (OR = 0; $CI_{95\%}$:0 - 0), and no literature was found on studies related with these factors either.

Table 2. Seroprevalence of *Neospora caninum* in caprine production units (PU) by municipality, in the central region of Veracruz, Mexico.

Municipality	PU sampled	Positive PU	Prevalence %	CI95 %	OR	CI95 %
Coatepec	5	0	0	0 - 53.71	0	0
Chiconquiaco	5	0	0	0 - 53.71	0	0
Yecuatla	5	1	20	1.5 - 70.12	1.5	0.12 - 18.44
Coacoatzintla	5	2	40	7.26 - 82.96	6.33	0.63 - 63.64
Tlacolulan	6	1	16.6	0.88 - 63.52	6.33	0.63 - 63.64
Total	26	4	15.4	5.04 - 35.72		

OR = odds ratio, CI = confidence interval.

Table 3. Seroprevalence of *Neospora caninum* in goats from municipalities of the Central region of Veracruz, Mexico, according to their productive status.

Productive status	No. animals sampled	No. positive animals	Prevalence %	CI95 %	OR	CI95 %
Doeling	24	1	4.2	0.22 - 23.12	1.1	0.12 - 9.57
Pregnant doe	22	2	9.1	1.59 - 30.62	3.1	0.56 - 17.05
Lactating doe	35	2	5.7	0.99 - 20.52	1.72	0.32 - 9.26
Buck	26	0	0	0 - 16.02	0	0
Weaned doe	2	0	0	0 - 80.21	0	0
Dry doe	4	0	0	0 - 60.42	0	0
Doe in production	69	2	2.9	0.5 - 11.01	0.64	0.12 - 3.41
Total	182	7	3.1	1.7 - 8.09		

OR = odds ratio, CI = confidence interval.

Seroprevalence in does according to their history of abortions

Table 4 shows the seroprevalence of neosporosis in does with a history of abortions (0.64 %; $CI_{95\%}$: 0.03 - 4.06) and in does with no history of abortions (3.85 %; $CI_{95\%}$: 1.58 – 8.56).

Chartier *et al.* (2000) pointed out that neosporosis does not seem to be a major factor causing abortions in goats, which might be coincident with the findings of the present study, since the owners of the goats mentioned that only few of the females selected had a history of abortions; however, this information can not be confirmed because of the lack of productive records of the goats at the PU. Nonetheless, due to the low seroprevalence (4.5 %; $CI_{95\%}$: 1.98 - 9.39), abortions might be unlikely. This is opposite to what was indicated by McAllister *et al*. (1998), that the disease causes abortions during the fourth to seventh month of

gestation in cattle; this would suggest that the goats are rather a carrier of the protozoan, and that their coexistence with cattle is a risk factor for the occurrence of abortions.

Table 4. Seroprevalence of neosporosis in does with or without history of abortions in municipalities from the central region of Veracruz, Mexico.

	No. animals sampled	No. positive animals	Prevalence %	CI95 %	OR	CI95 %
History of abortion	7	1	0.6	0.03 - 4.06	8.11	0.73 - 89.5
No history of abortion	175	6	3.8	1.58 - 8.56	0.12	0.64 - 1.18
Total	182	7	4.5	1.98 - 9.39		

OR = odds ratio, CI = confidence interval.

Seroprevalence by goat breed

The French Alpine breed had the highest prevalence (7 %; $CI_{95\%}$: 2.27 - 17.83), and the lowest prevalence was observed in the Toggenburg breed (4 %; $CI_{95\%}$: 0.21 – 22.32) (Table 5).

In cattle there is evidence that some breeds are more susceptible to become infected with neosporosis (Montiel *et al*., 2010); however, since the information on goats is scarce, no literature was found that relates with this condition. In the present study, the French Alpine breed was apparently more susceptible than the Saanen and Toggenburg breeds, since the seroprevalence was almost twofold; nonetheless, no association of neosporosis with the breed was observed, which suggests that any breed is equally susceptible to get the infection, because besides finding no association (OR = 3.069; $CI_{95\%}$: 0.664 - 14.19) in the $CI_{95\%}$ of the seroprevalence for the three breeds studied, the mean value of the seroprevalence of the French Alpine breed was within the values for the Saanen and Toggenburg breeds.

Table 5. Seroprevalence of *Neospora caninum* in goats from municipalities in the central region of Veracruz, Mexico, according to their breed.

Breed	No. animals sampled	No. positive animals	Prevalence %	CI95 %	OR	CI95 %
French Alpine	57	4	7	2.27 - 17.83	3.06	0.66 -14.19
Toggenburg	25	1	4	0.21 - 22.32	1.04	0.12 - 9.09
Saanen	49	2	4.1	0.71 - 15.14	1.08	0.20 - 5.80
Total	131	7	5.3	2.36 - 11.1		

OR = odds ratio, CI = confidence interval.

Seroprevalence of neosporosis in caprine PU depending on the presence of dogs

Table 6 shows the seroprevalence of neosporosis in goats that live together with dogs (11.5 %; $CI_{95\%}$: 3.03 - 31.28) and in goats that do not live with dogs (3.8 %, $CI_{95\%:}$ 1.58 – 8.56).

The infection caused by *N. caninum* is closely related to the presence of dogs, because these animals are the definitive hosts of the parasite, as indicated by McAllister *et al*. (1998) and Corbellini *et al*. (2006), who mentioned that the presence of dogs in the PU is a risk factor for seropositivity, based on the reports of the last ten years in the United States. In the present study, a seroprevalence of 11.5 % ($CI_{95\%:}$ 3.03 - 31.28) was observed in the PU that had dogs; however, although the seroprevalence was higher than in the PU that had no dogs (3.8 %; $CI_{95\%}$: 0.2 - 21.59), no association was observed between seroprevalence and the presence of dogs (OR = 0.143; $CI_{95\%}$: 0.007 - 2.94). In addition, Dijkstra *et al*. (2002) indicated that the presence of dog feces in the goat feed dispensers, grass or silage was the factor in the UP with more evidence of being the cause of postnatal infections in goats in the USA.

Seroprevalence of neosporosis in caprine PU depending on the presence of cattle

Seroprevalence of neosporosis in caprine PU that either have or do not have cattle was 7.7 % ($CI_{95\%}$: 1.34 - 26.6) for both, because half of the affected PU had cattle and the other half did not (Table 7).

Chartier *et al.* (2000) have suggested the possibility that the goats are carriers or *N. caninum*, and therefore their coexistence with cattle represent a risk for the transmission of the infection and for the occurrence of abortions in this species. However, in the PU of the selected municipalities it was not observed that cattle represented a risk for the goats (OR = 0.467; $CI_{95\%}$: 0.54 - 4.029), although the seroprevalence was 7.7 % ($CI_{95\%}$: 1.34 – 26.6). Several authors have indicated the ability of *N. caninum* to affect different animal species such as cattle, goat, sheep, horse, mouse, deer, water buffalo, coyote, red fox and camel (Barr *et al*., 1993; Dubey and Lindsay, 1996; Dubey, 1999; Jensen *et al*., 1999), as well as to experimentally infect animals such as cats, jerboas, non human primates and pigs.

Table 6. Seroprevalence of neosporosis in caprine production units that either have or do not have dogs, in municipalities from the central region of Veracruz, Mexico.

Presence of dogs	No. animals sampled	No. positive animals	Prevalence %	CI95 %	OR	CI95 %
With dogs	166	6	11.5	3.03 - 31.28	0.14	0.00 - 2.94
Without dogs	16	1	3.8	0.2 - 21.59	78.42	0.10 - 5.66
Total	182	7	15.4	5.04 - 35.72		

OR = odds ratio, CI = confidence interval.

Table 7. Seroprevalence of neosporosis in caprine production units that either have or do not have cattle, in municipalities from the central region of Veracruz, Mexico.

Presence of cattle	No. animals sampled	No. positive animals	Prevalence %	CI95 %	OR	CI95 %
With cattle	110	3	7.7	1.34 - 26.6	0.46	0.05 - 4.02
Without cattle	72	4	7.7	1.34 - 26.6	2.14	0.24 - 18.5
Total	182	7	15.4	5.04 - 35.72		

OR = odds ratio, CI = confidence interval.

CONCLUSION

Overall seroprevalence of neosporosis in goats from the municipalities studied was low; however, the disease had a medium distribution within these locations. Seroprevalence by PU was at a medium level. Goats from Coacoatzintla had a higher risk of become infected than goats from the other municipalities. The pregnant does had the highest seroprevalence. The French Alpine breed showed a higher seroprevalence than Toggenburg and Saanen breeds. The municipalities of Coatepec and Chiconquiaco were protective factors for neosporosis. The weaned and dry does were protective factors, and the bucks turned out to be more resistant to the infection by *N. caninum*.

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