



Neosporosis prevalence, distribution and risk factors in goats in central Veracruz state, Mexico



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Abstract:

Caprine neosporosis can cause abortion and generate economic losses in goat production systems. A study was done to estimate neosporosis seroprevalence, distribution and associated risk factors using a stratified multistage cross-sectional study in fourteen municipalities in central Veracruz, Mexico. Six animals were sampled at each of 81 farms. Sampled animals were males and females from 3 mo-old on. Diagnosis was done by a commercial indirect enzyme-linked immunosorbent assay (ELISA) kit. Risk factors were identified using two questionnaires: a general one for each herd and an individual one for

each selected animal. Descriptive statistics were run, an odds ratio was used to identify associations between variables, and logistic regression was applied to search for relationships to risk factors. Overall seroprevalence was 3.6 % based on a total of 550 samples. Prevalence among the municipalities was 64.2 %, and 17.2 % among the farms. Risk factors included the interaction between animal movement and provenance from other states ($P<0.05$). Seropositive females were more susceptible to abortions than the seronegative. Overall neosporosis seroprevalence in goats in central Veracruz is low although *N. caninum* is widely distributed among the sampled municipalities and farms. Several risk factors contribute to its presence.

Keywords: Animal diseases, Goats, Latin America, *Neospora caninum*, Risk factors.

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Neospora caninum is a protozoan which can cause abortion and death in cattle, sheep, goats, dogs and horses, as well as nervous system disorders in dogs and cattle. Its definitive hosts are domestic and wild canines, although antibodies for it have been found in coyotes, foxes, buffaloes, camels, cats, mice, pigs, primates and horses; clearly, pets as well as wildlife play important roles in *N. caninum* epidemiology^(1,2).

As is common worldwide, goat production in Mexico largely occurs in highly marginal, economically poor areas. The lack of formal education and economic solvency frequent in these areas often prevent producers from acquiring the knowledge and technology needed to implement preventive and biosecurity measures that can keep livestock healthy and avoid potential zoonoses⁽³⁾. Goat production is a minor livestock activity in the state of Veracruz, Mexico, ranking 27th among the country's 32 states. It occurs mostly in the state's central zone, and production conditions are similar to those reported in the rest of Mexico⁽⁴⁾.

Bovine neosporosis seroprevalence, distribution and risk factors have been studied in Veracruz^(5,6). However, data for goats in Veracruz is limited⁽⁷⁾, especially in central Veracruz where most goat farms are located. Dogs are the definitive hosts of *Neospora caninum*, and, in Mexico, are often present in goat production - during grazing, animal handling and in facilities. This clearly increases contagion risk. However, neosporosis is widely distributed in cattle both in Mexico and worldwide⁽⁶⁾, suggesting there may be other vectors. Quantifying neosporosis presence and risk factors in goats in central Veracruz, Mexico, is important for supporting this productive activity in the region where it is most concentrated. The study

objective was to quantify neosporosis prevalence, and identify its distribution and risk factors among goat producers in central Veracruz.

The study was cross-sectional. The study area encompassed fourteen municipalities in the state of Veracruz, Mexico: Chiconquiaco, Coacoatzintla, Coatepec, Emiliano Zapata, Ixhuacán de los Reyes, Jalacingo, Las Minas, Las Vigas de Ramírez, Perote, Tatatila, Yecuatla, Tlacolulan, Villa Aldama, and Xico.

The number of sampled animals per farm was estimated using the Win Episcopo 2.0 program with an assumed seroprevalence of 50 %, 5 % error and 95 % confidence. Estimated minimum sample size was 385 animals, with six animals sampled per farm. At each farm, male and female goats 3 mo of age or older were randomly selected for sampling.

Blood samples were collected from each animal using jugular puncture. Sampling tubes contained no anticoagulant were processed using a commercial indirect Enzyme Linked Immune Sorbent Assay (ELISA) kit (sensitivity 100%, specificity 98.9%, IDEXX Laboratories™ Inc., Westbrook, Maine, USA) at the Diagnostic Unit, Faculty of Veterinary Medicine and Animal Science of the Universidad Veracruzana.

Data on risk factors was collected via two questionnaires. One was general and applied at every farm included in the study. It contained general questions about farm data, facilities, and genetic, health, feeding, and reproductive animal management, among other items. The other was animal-specific, and included items on age, stage, health background, and neosporosis-related clinical signs. All collected data was entered into spreadsheets for processing (Microsoft Excel™). Descriptive statistics to identify *N. caninum* risk factors for the evaluated animal- and farm-level explanatory variables were developed using the VassarStats online program. Seroprevalence calculation and descriptive epidemiology were also done with this program. Potential risk factors were identified based on responses considering variables such as age, and were analyzed for association by odds ratio (OR). Confidence intervals (95%CI) were estimated by logarithmic approximation. No logistic regression was run because the evaluated variables were not significant⁽⁸⁾.

A total of 550 blood samples were collected from 81 farms in fourteen municipalities. A total of 20 animals were positive, resulting in a 3.6 % overall seroprevalence (95%CI: 2.28-5.63). Seroprevalence was highest in the municipalities of Emiliano Zapata and Coacoatzintla. No seropositive goats were identified in five municipalities: Chiconquiaco, Coatepec, Ixhuacán, Las Minas and Xico (Table 1). Among all the municipalities, positive frequency was 64.29 % (95%CI: 35.63-86.02), because nine of the fourteen municipalities had at least one seropositive animal.

Table 1: Overall and municipality-specific seroprevalence of *Neospora caninum* in goats in central Veracruz, Mexico

| Municipality | Sampled animals (N) | Seropositive animals, N (%) | 95%CI | OR | OR 95%CI |
|-----------------|---------------------|-----------------------------|--------------|------|-------------|
| Emiliano Zapata | 34 | 4 (11.76) | 3.83 – 28.39 | 5.1 | 0.5 – 47.7 |
| Coacoatzintla | 36 | 4 (11.11) | 3.62 – 27 | 4.75 | 0.5 – 44.7 |
| Villa Aldama | 50 | 3 (6) | 1.56 – 17.54 | 2.43 | 0.2 – 24.3 |
| Yecuatla | 34 | 2 (5.88) | 1.02 – 21.05 | 2.29 | 0.2 – 27.4 |
| Las Vigas | 41 | 2 (4.88) | 0.85 – 17.81 | 1.9 | 0.1 – 22.4 |
| Jalacingo | 32 | 1 (3.13) | 0.16 – 18.01 | 1.22 | 0.07 – 20.4 |
| Perote | 67 | 2 (2.99) | 0.52 – 11.32 | 1.16 | 0.1 – 13.3 |
| Tatatila | 39 | 1 (2.56) | 0.13 – 15.07 | 1.00 | - |
| Tlacolulan | 39 | 1 (2.56) | 0.13 – 15.07 | 1.00 | - |
| Chiconquiaco | 32 | 0 (0) | 0 – 13.34 | ND | ND |
| Coatepec | 41 | 0 (0) | 0 – 10.67 | ND | ND |
| Ixhuacán | 25 | 0 (0) | 0 – 13.32 | ND | ND |
| Las Minas | 33 | 0 (0) | 0 – 12.98 | ND | ND |
| Xico | 49 | 0 (0) | 0 – 9.06 | ND | ND |
| Total | 550 | 20 (3.62) | 2.28 – 5.63 | | |

95%CI = 95% confidence interval; OR= odds ratio; OR 95%CI = 95% confidence interval for the odds ratio; ND = not determined.

Fourteen of the 81 sampled goat farms harbored seropositive animals (Table 2), resulting in a 17.28 % frequency among the farms (95%CI: 10.10-27.64). Farms in the municipalities of Villa Aldama, Emiliano Zapata, and Coacoatzintla were the most affected, while those in Chiconquiaco, Coatepec, Ixhuacan, Las Minas and Xico had no seropositive animals.

Table 2: *Neospora caninum* seroprevalence by farm in goats in central Veracruz, Mexico

| Municipality | Herds (N) | Positive herds, N (%) | 95%CI |
|-----------------|-----------|-----------------------|---------------|
| Villa Aldama | 7 | 3 (42.86) | 11.81 – 79.76 |
| Coacoatzintla | 5 | 2 (40.00) | 7.26 – 82.96 |
| Emiliano Zapata | 5 | 2 (40.00) | 7.26 – 82.96 |
| Perote | 10 | 1(20.00) | 1.05 – 70.12 |
| Jalacingo | 5 | 1(20.00) | 1.05 – 70.12 |
| Yecuatla | 5 | 2(20.00) | 3.54 – 55.78 |
| Las Vigas | 6 | 1(16.67) | 0.88 – 63.52 |
| Tatatila | 6 | 1(16.67) | 0.88 – 63.52 |
| Tlacolulan | 6 | 1(16.67) | 0.88 – 63.52 |
| Chiconquiaco | 5 | 0 (0) | 0 – 53.71 |
| Coatepec | 5 | 0 (0) | 0 – 53.71 |
| Ixhuacán | 4 | 0 (0) | 0 – 60.42 |
| Las Minas | 5 | 0 (0) | 0 – 53.71 |
| Xico | 7 | 0 (0) | 0 - 53.71 |
| Total | 81 | 14(17.28) | 10.10 – 27.64 |

95%CI = 95% confidence interval.

The highest numbers of positive animals were identified in the municipalities of Coacoatzintla (OR: 3.89, 95%CI: 1.22-12.32) and Emiliano Zapata (OR: 4.16, 95%CI: 1.31-13.24) (Table 1). Of the age categories, goats 12 mo-old (OR: 0.2, 95%CI: 0.) and 42 mo-old (OR: 13.8, 95%CI: 1.2-160) were the main ages affected (Table 3).

Table 3: *Neospora caninum* seroprevalence by age in goats in central Veracruz

| Age, months | Goats, N | Positives, N (%) | 95%CI | OR | OR 95%CI |
|-------------|----------|------------------|--------------|------|--------------|
| <12 | 22 | 2 (9.09) | 1.59 – 30.62 | 6.7 | 0.58 – 77.8 |
| 12 | 55 | 5 (9) | 3.3 – 20.7 | 6.7 | 0.76 – 59.2 |
| 18 | 19 | 2 (10.5) | 1.8 – 34.5 | 7.8 | 0.67 – 92.2 |
| 24 | 51 | 2 (3.9) | 0.6 – 14.5 | 2.7 | 0.24 – 31.0 |
| 30 | 70 | 2 (2.8) | 0.5 – 10.8 | 1.9 | 0.17 – 22.3 |
| 36 | 3 | 1 (33.3) | 1.7 – 87.4 | 33.5 | 1.5 – 749.8 |
| 42 | 162 | 4 (2.4) | 0.7 – 6.6 | 1.69 | 0.19 – 15.5 |
| 48 | 68 | 1(1.4) | 0.08 – 9 | 1.0 | - |
| 60 | 11 | 1(9) | 0.4 – 42.8 | 6.7 | 0.39 – 115.9 |

95%CI = 95% confidence interval; OR= odds ratio; OR 95%CI = 95% confidence interval for the odds ratio.

Goats that coexisted with dogs were more susceptible to *Neospora caninum* infection (OR: 1.6; 95%CI: 0.36 – 7.02). Seropositive females were more likely to have had abortions (OR: 21.75; 95%CI: 6.35-74.38) (Table 4).

Table 4: *Neospora caninum* seroprevalence by coexistence with dogs and by abortions in goats in central Veracruz

| | | Positive (n) | Prevalence (%) | 95%CI | OR | OR 95%CI |
|--------------------|-----|-----------------|-------------------|-------------|------|--------------|
| Coexist with dogs: | | | | | | |
| Yes | 468 | 18 | 3.8 | 2.4 – 6.1 | 1.6 | 0.36 – 7.02 |
| No | 82 | 2 | 2.4 | 0.4 – 9.4 | 1.0 | - |
| Abortions: | | | | | | |
| Yes | 13 | 5 | 38.4 | 15.1 – 67.7 | 21.7 | 6.35 – 74.38 |
| No | 537 | 15 | 2.7 | 1.6 – 4.7 | 1.0 | - |

95%CI = 95% confidence interval; OR= odds ratio; OR 95%CI = 95% confidence interval for the odds ratio.

The overall 3.62 % (95%CI: 2.28-5.63) seroprevalence identified here is lower than the 8.9 % reported in goats from Sao Paulo, Brazil⁽⁹⁾. The 64.29 % (95%CI: 35.63-86.02) frequency by municipality was notably high because seropositive animals were identified in nine out of the fourteen studied municipalities. A similar phenomenon was also reported in two surveys done on 56 dairy goat herds in western France in which seroprevalence at the herd level ranged from 3 to 19 % despite an overall 1-2 % rate in both surveys⁽¹⁰⁾. Again, in a sample of 381 goats from fourteen farms in the municipality of Mossoró, Rio Grande do Norte, Brazil, prevalence among farms was 28.6 % while overall seroprevalence was only 1.05 %⁽³⁾. Notably higher seroprevalences have been reported previously. In one study in Brazil, overall seroprevalence was 10.7 % in 401 goats from eleven farms in southern Minas Gerais state⁽¹¹⁾. In another study in Brazil, seroprevalence was 17.39 % in 46 goats from five herds in the districts of Amarante do Maranhão and Buritirana, Imperatriz microregion, in western Maranhão State⁽¹²⁾. Finally, a 10.7 % seroprevalence at the individual animal level and one of 44 % at the herd level were reported for cattle in a sample of 262 animals from 25 herds⁽¹³⁾.

The presence of goats was a neosporosis risk factor in the municipalities of Coacoatzintla (OR: 3.89, 95%CI: 1.22-12.32) and Emiliano Zapata (OR: 4.16, 95%CI: 1.31-13.24). Both municipalities had relatively high seroprevalences and share similar climate conditions. How much climate contributes to *N. caninum* seroprevalence is unclear. Some propose that neosporosis prevalences in goats can vary in response to weather conditions⁽³⁾, and that prevalences in cattle are higher in warm climates⁽⁶⁾. In contrast, studies done in Brazil⁽⁹⁾ and France⁽¹⁰⁾ have reported seropositive goats in both tropical and cold climates, suggesting that environmental temperature may not be a factor in neosporosis infection. This is supported by

the present results since the varying climates at the sampled municipalities (average annual temperatures ranging from 12.5 to 22.5 °C) had no apparent effect on seroprevalence.

Goats in the 12 mo-old (OR: 3.2, 95%CI: 1.1-9.1) and 42 mo-old (OR: 13.8, 95%CI: 1.2-160) age groups were also identified as risk factors. This agrees with other studies stating that seropositivity increases with age^(14,15,16), and with results showing significant differences in seropositivity between age groups, with the highest levels in 3+ yr-old goats⁽¹¹⁾. In contrast, other reports state that animal age is unrelated to seropositivity status or increased risk of infection^(5,17). For example, a study in Rio Grande do Norte, Brazil, found no statistical association between seropositivity agents and sex, reproductive problems and the presence of dogs and/or cats on farms⁽³⁾. In a separate study, presence of domestic cats and/or dogs on a farm was also found to have no effect on neosporosis seropositivity and veterinary care, although food supplementation did represent a significant risk factor ($P<0.05$)⁽¹²⁾. One factor not analyzed here that may affect seropositivity is goat breed; higher seroprevalence has been reported in the Alpine breed (24 %) versus the Saanen (14 %) and Nubian (3 %) breeds⁽¹⁷⁾.

Logistic regression identified the introduction of new animals ($P=0.002$, OR: 5.59, 95%CI: 1.84-16.95) and their provenance ($P=0.021$, OR: 2.99, 95%CI: 1.18-7.59) as significant risk factors. These two factors interacted with all the analyzed variables and seem to be highly related to breeding stock acquisition. Introduction of goats from other states without prior testing for *N. caninum* clearly represents a risk for disease spread⁽¹⁸⁾.

In the present results, *Neospora caninum* seroprevalence was notably higher (38.4 %) in does which had aborted in the past. Various studies coincide with these results. For example, one study found *Neospora caninum* infection to be a major cause of abortion in does (OR: 21.75, 95%CI 6.36-74.38), whereas seronegative does were less susceptible to abort (OR: 0.04, 95%CI: 0.01-0.15)⁽¹⁰⁾. Another study did not identify a positive correlation between *N. caninum* prevalence and reproductive problems in herds, but farms with a history of reproductive problems were 18.64 % positive on average whereas farms with no such history were 13.97 % positive⁽¹¹⁾; after separating reproductive problems by category, the relationship between abortion and *N. caninum* seroprevalence was significant ($P<0.05$). An analysis of seroprevalence at the individual level found it to be significantly higher ($P<0.05$) in animals with a history of abortion (12.8 %) compared to apparently healthy animals (11.3 %), and to does with a record of infertility (8.1 %), or neonatal death (4.3 %); but, no significant differences in seroprevalence were observed due to locality, animal breed, sex, or age ($P>0.05$)⁽¹³⁾. Finally, *Neospora caninum* is known to cause abortion in cattle⁽¹⁹⁾.

Overall neosporosis seroprevalence in goats in the state of Veracruz, Mexico, was found to be low, but high prevalences among municipalities and herds highlighted the existence of risk factors contributing to its presence: animals in the municipalities of Emiliano Zapata and

Coacoatzintla; goats in the 12- and 42 mo-old age groups, animal provenance, introduction of new animals to a farm, and a history of previous abortions at a farm.

Institutional Review Committee Statement

The study was carried out in accordance with the guidelines of the Declaration of Helsinki and was approved by the Bioethics Committee of the Faculty of Veterinary Medicine and Animal Science of the University of Veracruz.

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Conflicts of interest

The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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