

Original

Prevalence of *Neospora caninum* bovine serum antibodies in the Central and Southern Gulf of Mexico regions

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ABSTRACT

Objective. To determine the prevalence of antibodies against *Neospora caninum* (PSA) in beef and dual-purpose cattle from the Central and Southern Gulf of Mexico regions, as well as to evaluate its association with the reproductive status (RS) of the cow (pregnant, not pregnant). **Materials and methods.** Blood samples were taken from 422 cows. Antibodies were detected with a commercial ELISA kit. PSA was analyzed with a logistic regression model that included state, township nested within state, and ranch nested within township. **Results.** The states of Tabasco, Puebla and Veracruz had similar PSA ($p>0.05$). The mean PSA for the three states was 24.0%. The PSA varied from 7.8 to 43.3% across townships. In Puebla, the PSA in cattle from San José Acateno township was more than two-fold greater than that in cattle from Hueytamalco and Nauzontla townships ($p<0.05$). In Tabasco, Cunduacán, Huimanguillo and Ranchería El Puente townships showed similar ($p>0.05$) PSA. In Veracruz, cows from Cotaxtla and Medellín de Bravo townships presented higher ($p<0.05$) PSA than those from San Rafael township. The RS of seropositive females was similar ($p>0.05$) to that of seronegative females. **Conclusions.** The three states had similar PSA. All the herds had antibodies against *N. caninum*, suggesting that this parasite is amply distributed in the three states. In addition, great variation existed among herds and among townships; finally, the presence of *N. caninum* antibodies was not associated with the RS of the cow.

Keywords: Cows; frequency; Neosporosis; serum antibodies; reproductive status; tropical climate (*Source: CAB*).

RESUMEN

Objetivo. Determinar la prevalencia de anticuerpos contra *Neospora caninum* (PAS) en bovinos carne y doble propósito de las zonas Central y Sur del Golfo de México, así como evaluar su asociación con el estatus reproductivo (ER) de la vaca (gestante, no gestante). **Materiales y métodos.** Se colectaron

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muestras de sangre de 422 vacas. Los anticuerpos se detectaron con una prueba comercial de ELISA. La PAS se analizó con un modelo de regresión logística que incluyó estado, municipio anidado en estado, y rancho anidado en municipio. **Resultados.** Los estados de Tabasco, Puebla y Veracruz tuvieron similar PAS ($p>0.05$). La PAS promedio de los tres estados fue 24.0%. La PAS varió de 7.8 a 43.3% entre municipios. En Puebla, la PAS en los bovinos del municipio de San José Acateno fue más de dos veces mayor que en los bovinos de los municipios de Hueytamalco y Nauzontla ($p<0.05$). En Tabasco, los municipios de Cunduacán, Huimanguillo y Ranchería El Puente mostraron similar ($p>0.05$) PAS. En Veracruz, las vacas de los municipios de Cotaxtla y Medellín de Bravo presentaron mayor ($p<0.05$) PAS que las del municipio de San Rafael. El ER de las hembras seropositivas fue similar ($p>0.05$) al de las seronegativas. **Conclusiones.** Los tres estados tuvieron similar PAS. Todos los hatos tuvieron anticuerpos contra *N. caninum*, sugiriendo que este parásito está ampliamente distribuido en los tres estados. Además, existió gran variación entre hatos y entre municipios; finalmente, la presencia de anticuerpos contra *N. caninum* no estuvo asociada al ER de la vaca.

Palabras clave: Anticuerpos séricos; clima tropical; estatus reproductivo; frecuencia; Neosporosis; vacas (*Fuente: CAB*).

INTRODUCTION

As in other countries, it has been recently reported that *Neospora caninum* is an important pathogenic agent that is present in dairy (1,2), beef (3,4) and dual-purpose herds (5,6) in the Mexican territory. Even though the entire pool of Mexican studies available in the literature includes cattle from only half of the states in Mexico, it has revealed important aspects regarding this coccidian parasite.

The scientific literature indicates that in the Mexican States of Hidalgo (1,7,8), Guanajuato (8), Estado de México (1,9) and Querétaro (1,8) *N. caninum* is abundantly spread and that these states have considerable levels of prevalence of bovine serum antibodies against this obligate intracellular parasite, with mean values between 53 and 60%.

Another important aspect is that seropositivity to *N. caninum* has been found to be associated with cow abortion in many Mexican herds. For instance, a strong correlation between *N. caninum* seropositivity and abortion was observed in 50 Mexican cattle herds in various states in Mexico (1). In a study carried out in Aguascalientes, Mexico, the seroprevalence in cows with abortion was 21% higher than in cows without abortion (2). In a more recent study, Mexican researchers found *N. caninum* DNA in one aborted fetus (9).

However, most Mexican studies on the prevalence of serum antibodies against *N. caninum* and its association with reproductive failure have been conducted with Holstein cows from dairy herds

in the Mexican Plateau (1,2,7,9,10,11,12,13). In contrast, Mexican studies related to the prevalence of serum antibodies against *N. caninum* in beef and dual-purpose cattle raised under tropical and/or subtropical conditions of Mexico are scarce (5,6).

Based on the above-mentioned information, the aim of the present investigation was to determine the prevalence of bovine serum antibodies against *N. caninum* in beef and dual-purpose cattle from the Central and Southern Gulf of Mexico regions, as well as to evaluate its association with the reproductive status of the cow.

MATERIALS AND METHODS

Location. The study was carried out from March 2011 to December 2015 in 24 ranches. Seven ranches were located in three townships in the state of Veracruz (Medellín de Bravo, Cotaxtla, and San Rafael); eleven ranches were located in five townships in the state of Puebla (Nauzontla, Ayotoxco de Guerrero, San José Acateno, Hueytamalco and Xochitlán); and six ranches were located in three townships in the state of Tabasco (Huimanguillo, Cunduacán, and Ranchería el Puente). Eleven ranches were dedicated to the production of milk and calves (dual-purpose system) and thirteen ranches were dedicated to the production of calves (cow-calf system).

Animals. The study included pure Brahman (N=20) and *Bos taurus* x *Bos indicus* crossbred (N=402) cows with one or more calvings.

Because of economic restrictions and the fact that cows present higher prevalence of *N. caninum* serum antibodies compared to calves and heifers, the present study only included adult cows. Cows remain more time in the herd; therefore, they have a higher probability of being infected with diverse pathogenic agents. Not a single cow presented clinical signs of illness during the course of the study.

General management of herds. Cows were maintained in a rotational grazing system. In both dual-purpose and cow-calf systems, calves were weaned between 7 and 9 months of age. The herds were officially free of *Brucella abortus* (brucellosis) and *Mycobacterium bovis* (tuberculosis). The reproductive status (pregnant, not pregnant) of each female was diagnosed, by rectal palpation, the same day blood samples were taken; however, it was not possible to perform such reproductive diagnosis in the State of Tabasco herds.

Selection of ranches and sample size. The ranches were selected based on a convenience non-probabilistic sampling, in accordance with cattlemen's interest of participating in the current study. On the other hand, sample size was calculated in accordance with the budget of the study, therefore, not all cows from each ranch were sampled; however, at least 12 cows were selected in each ranch. Within each ranch, cows were selected by simple random selection.

Blood sampling and serum collection. Blood sampling (around 10 ml per cow) was performed by puncture of the coccygeal vein using vacutainer tubes without anticoagulant. Blood samples were immediately transported to the laboratory in iceboxes. After clot formation, sera were obtained by centrifuging the whole blood at 1000 g for 15 minutes. Finally, sera were preserved in polystyrene plastic tubes at -20°C until subsequent detection of *N. caninum* antibodies was performed.

Serological test. *N. caninum* serum antibodies were detected by indirect ELISA, using the CIVTEST BOVIS NEOSPORA commercial kit (HIPRA Laboratories; Girona, Spain), following the manufacturer's specifications. The optical density value of each assayed serum sample was determined with a spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) at 405 nanometers.

Variables. Two response variables were analyzed: prevalence of serum antibodies

against *N. caninum* and reproductive status of the cow. Both were considered binary variables. The first one was recorded as 1 when *N. caninum* antibodies were detected; otherwise, it was recorded as 0. The second binary variable was recorded as 1 when a cow was diagnosed pregnant; otherwise (cow not pregnant), it was recorded as 0.

Statistical Analyses. The two dependent variables were analyzed with the GENMOD procedure of SAS (14). For prevalence of serum antibodies against *N. caninum*, the logistic regression model included the effects of state, township nested within state, and ranch nested within township. For reproductive status of the cow, the statistical model included sanitary status of the cow (seropositive or seronegative), state, and township nested within state. The sanitary status of the cow denoted whether a cow had serum antibodies against *N. caninum* or not. In the model statement of the GENMOD procedure, a binomial distribution and a logit link function were declared. In both logistic regression analyses, the convergence criterion was set to 10^{-8} . Differences among least square means were tested with the PDIF option of the previously mentioned procedure.

RESULTS

Probability levels of fixed effects for prevalence of antibodies against *N. caninum* are presented in Table 1. State showed no significant effect ($p > 0.05$), indicating that the States of Tabasco, Puebla and Veracruz had similar prevalences; in contrast, township nested within state, and ranch nested within township were highly significant sources of variation ($p > 0.01$) for prevalence of *N. caninum* serum antibodies.

Table 1. Degrees of freedom (DF), Chi-square values, and probability levels of effects included in the statistical model to analyze prevalence of bovine serum antibodies against *N. caninum*.

| Effect | DF | Chi-Square | Probability |
|------------------------------|----|------------|-------------|
| State | 2 | 0.75 | 0.6864 |
| Township nested within state | 8 | 32.59 | <0.0001 |
| Ranch nested within township | 13 | 31.57 | 0.0028 |

Table 2 shows least squares means, standard errors and 95% confidence intervals for prevalence of serum antibodies against *N.*

caninum, by State. The mean prevalence of serum antibodies for the three States evaluated was 24.0%.

Table 2. Least squares means, standard errors and 95% confidence intervals for prevalence (%) of bovine serum antibodies against *N. caninum*, by state.

| State | Mean | Lower limit | Upper limit |
|----------|-------------------------|-------------|-------------|
| Puebla | 26.0 ± 4.9 ^a | 17.5 | 36.8 |
| Tabasco | 23.8 ± 6.9 ^a | 12.8 | 39.8 |
| Veracruz | 22.3 ± 4.0 ^a | 15.4 | 31.1 |

^aMeans with the same letter are not different ($p > 0.05$).

Least squares means, standard errors and 95% confidence intervals for prevalence of *N. caninum* serum antibodies, by township, are presented in Table 3. The prevalence of *N. caninum* serum antibodies varied from 7.8 (San Rafael township) to 43.3% (San José Acateno township) among townships.

Table 3. Least squares means, standard errors and 95% confidence intervals for prevalence (%) of bovine serum antibodies against *N. caninum*, by township.

| Township | Mean | Lower limit | Upper limit |
|--------------------------|----------------------------|-------------|-------------|
| State of Puebla | | | |
| Ayotoxco de Guerrero | 20.0 ± 8.9 ^{abc} | 7.7 | 42.8 |
| Hueytamalco | 17.1 ± 4.3 ^{bc} | 10.2 | 27.2 |
| Nauzontla | 15.4 ± 10.0 ^{bc} | 3.9 | 45.1 |
| San José Acateno | 43.3 ± 6.4 ^a | 3.1 | 56.0 |
| Xochitlán | 42.9 ± 18.7 ^{ab} | 14.4 | 77.0 |
| State of Tabasco | | | |
| Cunduacán | 16.7 ± 10.8 ^{bc} | 4.2 | 47.7 |
| Huimanguillo | 40.6 ± 8.0 ^{ab} | 26.3 | 56.7 |
| Ranchería El Puente | 18.2 ± 11.6 ^{abc} | 4.6 | 50.7 |
| State of Veracruz | | | |
| Cotaxtla | 32.2 ± 7.9 ^{ab} | 18.9 | 49.1 |
| Medellín de Bravo | 36.8 ± 7.9 ^{ab} | 23.1 | 53.1 |
| San Rafael | 7.8 ± 3.5 ^c | 3.2 | 18.2 |

^{a,b,c}Means with different letter are different ($p < 0.05$).

In the State of Puebla, the prevalence of *N. caninum* serum antibodies in cattle from San José Acateno township was more than two-fold greater than that in cattle from Hueytamalco and Nauzontla townships ($p < 0.05$), but had similar prevalence to cattle from Ayotoxco de Guerrero and Xochitlán townships ($p > 0.05$). Cattle from Hueytamalco, Ayotoxco de Guerrero, Xochitlán and Nauzontla townships did not differ ($p > 0.05$) in prevalence of *N. caninum* serum antibodies.

In the State of Tabasco, cattle from Huimanguillo, Cunduacán and Ranchería El Puente townships did not differ ($p > 0.05$) in the prevalence of serum antibodies against *N. caninum*.

In the State of Veracruz, cattle from Medellín de Bravo and Cotaxtla townships presented higher ($p < 0.05$) prevalence of serum antibodies against *N. caninum* than cattle from San Rafael township. Cattle from Medellín de Bravo and Cotaxtla townships did not differ ($p > 0.05$) in the prevalence of serum antibodies against *N. caninum*.

The frequencies of bovine serum antibodies by ranch suggest that *N. caninum* was present in all 24 herds, and that a significant variation existed among them, from 4.8 (San Ignacio ranch) to 75.0% (San Pedrito ranch) (Table 4).

Table 4. Least squares means, standard errors and 95% confidence intervals for prevalence (%) of bovine serum antibodies against *N. caninum*, by ranch.

| Ranch | Mean | Lower limit | Upper limit |
|----------------------|-----------------------------|-------------|-------------|
| Colozapa | 20.0 ± 8.9 ^{cde} | 7.7 | 42.8 |
| Cuaxocota | 30.0 ± 10.3 ^{bcde} | 14.1 | 52.7 |
| El Canelo | 30.0 ± 10.3 ^{bcde} | 14.1 | 52.7 |
| Las Margaritas | 8.7 ± 5.9 ^{de} | 2.2 | 28.9 |
| Maquiquila | 28.6 ± 9.9 ^{bcde} | 13.4 | 50.8 |
| Valle Roncal | 5.0 ± 4.9 ^e | 0.7 | 28.2 |
| Cirillogco | 15.4 ± 10.0 ^{cde} | 3.9 | 45.1 |
| Ayoteaca | 40.0 ± 11.0 ^{abcd} | 2.1 | 62.0 |
| El Cedral | 40.0 ± 11.0 ^{abcd} | 2.1 | 62.0 |
| San Andrés | 50.0 ± 11.2 ^{abc} | 2.9 | 70.6 |
| San Bernardo | 42.9 ± 18.7 ^{abcd} | 14.4 | 77.0 |
| El Arenal | 16.7 ± 10.8 ^{cde} | 4.2 | 47.7 |
| Lomas de San Antonio | 57.1 ± 13.2 ^{ab} | 31.6 | 79.4 |
| Los Palomos | 21.4 ± 11.0 ^{cde} | 7.1 | 49.4 |
| San Felipe | 16.7 ± 10.8 ^{cde} | 4.2 | 47.7 |
| San Pedrito | 75.0 ± 12.5 ^a | 44.8 | 91.7 |
| Cristina | 18.2 ± 11.6 ^{cde} | 4.6 | 50.7 |
| El Copite | 20.0 ± 8.9 ^{cde} | 7.7 | 42.8 |
| Los Rivera | 47.4 ± 11.5 ^{abc} | 26.8 | 68.9 |
| El Rosario | 21.7 ± 8.6 ^{cde} | 9.3 | 42.8 |
| San Ramón | 55.0 ± 11.1 ^{ab} | 33.6 | 74.7 |
| Jaral | 10.0 ± 6.7 ^{de} | 2.5 | 32.4 |
| San Ignacio | 4.8 ± 4.6 ^e | 0.7 | 27.1 |
| Tres Bocas | 10.0 ± 6.7 ^{de} | 2.5 | 32.4 |

^{a,b,c,d,e}Means with different letter are different ($p < 0.05$).

Probability levels of fixed effects for reproductive status of the cow are presented in Table 5. Sanitary status did not account for variation in reproductive status of the cow ($p > 0.05$), which means that the reproductive status of seropositive cows was similar to that of seronegative cows

(58.2 vs 55.6%; Table 6). State and township nested within state were not significant sources of variation for cow's reproductive status either ($p > 0.05$; Table 5).

Table 5. Degrees of freedom (DF), Chi-square values, and probability levels of effects included in the statistical model to analyze reproductive status of the cow.

| Effect | DF | Chi-Square | Probability |
|---|----|------------|-------------|
| Sanitary status of the cow ^a | 1 | 0.15 | 0.7009 |
| State | 1 | 0.02 | 0.8783 |
| Township nested within state | 6 | 6.27 | 0.3931 |

^aSeronegative or seropositive.

Table 6. Least squares means, standard errors and confidence intervals (95%) for reproductive status of the cow (%), by sanitary status of the cow, State and township.

| | Mean | Lower limit | Upper limit |
|-----------------------------------|------------------------|-------------|-------------|
| Sanitary status of the cow | | | |
| Seronegative ^a | 55.6±4.7 ^c | 46.4 | 64.5 |
| Seropositive ^b | 58.2±6.2 ^c | 45.8 | 69.7 |
| State | | | |
| Puebla | 56.3±7.0 ^c | 42.5 | 69.2 |
| Veracruz | 57.6±4.8 ^c | 48.0 | 66.7 |
| Township | | | |
| Ayotoxco de Guerrero | 50.6±11.9 ^c | 28.7 | 72.2 |
| Hueytamalco | 54.7±5.6 ^c | 43.6 | 65.3 |
| Nauzontla | 42.7±14.6 ^c | 18.8 | 70.6 |
| San José Acateno | 48.8±7.3 ^c | 35.0 | 62.8 |
| Xochitlán | 80.2±17.8 ^c | 31.1 | 97.3 |
| Cotaxtla | 50.1±8.8 ^c | 33.4 | 66.8 |
| Medellín de Bravo | 69.8±7.7 ^c | 53.1 | 82.5 |
| San Rafael | 51.9±7.2 ^c | 38.1 | 65.4 |

^aSeronegative= absence of *N. caninum* serum antibodies.

^bSeropositive= presence of *N. caninum* serum antibodies.

^cMeans with the same letter are not different ($p > 0.05$).

DISCUSSION

The prevalence of bovine serum antibodies against *N. caninum* for the State of Veracruz reported in this study is similar to the prevalence reported in two previous studies carried out in Northern Veracruz State (6) and in the Medellín de Bravo township in the State of Veracruz (5), with values of 20.8 and 22.6%, respectively. However, other researchers (3) found no serum antibodies against *N. caninum* in cattle of Paso de Ovejas and Medellín de Bravo townships, also in the State of Veracruz.

The seroprevalence of *N. caninum* bovine antibodies for the State of Puebla obtained in the

current investigation (26%) is somewhat lower than that (39%) previously reported for the State of Puebla (1). The difference (13%) could be caused by differences in the type of cattle; females included in the present study came from beef and dual-purpose herds; however, females of the previous report (1) came from dairy herds.

No previous journal articles on the seroprevalence of *N. caninum* bovine antibodies in the State of Tabasco were found; therefore, it seems that the current prevalence of antibodies against this obligate intracellular protozoan in cattle from the State of Tabasco ($23.8 \pm 6.9\%$; Table 2) is the first estimate available in the formal scientific literature. Knowledge of the prevalence of pathogenic agents in a state, municipality and/or locality, among other factors, is essential to understand the epidemiology of diseases and focus interest on control strategies.

The mean prevalence of bovine serum antibodies against *N. caninum* for Tabasco, Puebla and Veracruz found in the present study (24%) is lower than the corresponding prevalence reported for dairy herds of the States of Hidalgo (7), Chihuahua (8), Guanajuato (8), Querétaro (8) and Estado de México (9). This difference is not surprising, since cattle included in the present study grazed under extensive conditions; however, cattle from previous studies were raised under intensive conditions with higher population density that enables the viability and transmission of *N. caninum* oocysts. Other authors (3,15) have also reported low prevalence of bovine serum antibodies in Chiapas (15.0%), Yucatán (11.3%) and Tamaulipas (11.9%), Mexican states with tropical climate and cattle raised in extensive conditions. One study revealed that production system was a significant risk factor for the prevalence of bovine serum antibodies against *N. caninum*; the prevalence in the intensive system was 22% higher than in the dual-purpose system (8).

The range of the prevalence of serum antibodies by municipality found in the present study (7.8 to 43.3%) is similar to the range (0 to 45%) observed in a study conducted in the States of Yucatán, Veracruz and Chiapas (3), despite the fact that the present and the previous studies included different states (except Veracruz) and, in consequence, different townships and localities.

The variation of the prevalence of serum antibodies across ranches (4.8 to 75.0%) found

in the present investigation could be caused by differences in the existence and abundance of dogs, poultry and coyotes, and/or in the origin (same farm, different farm) of replacement females among herds. In a study conducted in central Mexico, the presence of coyotes and domestic poultry in dairy herds was identified as potential risk factors associated with the seroprevalence of *N. caninum* antibodies (13).

The presence of serum antibodies against *N. caninum* in the cows did not affect their reproductive status. This finding is in accordance with the results of previous studies conducted with Holstein cows in the States of Querétaro (10) and Aguascalientes (11), Mexico, where there was no difference in the titer of antibodies against *N. caninum* between cows that aborted and those that did not. No previous studies

related to the effect of the presence of bovine serum antibodies against *N. caninum* on cows' reproductive status were found in the scientific literature.

In conclusion, the States of Veracruz, Tabasco and Puebla showed similar prevalence of *N. caninum* bovine serum antibodies. All the herds had serum antibodies against *N. caninum*, suggesting that this obligate intracellular parasite is abundantly dispersed in the three states. In addition, a great variation existed among herds and among townships. Finally, no association between *N. caninum* bovine serum antibodies and cows' reproductive status was found.

Conflict of interest

The authors declare no conflicts of interest.

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