REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.12.e1777
ISSN 2007-3380

Original article/Artículo original

Neosporosis prevalence and semen quality in cattle in the Costa Chica region of Guerrero, Mexico

Prevalencia de neosporosis y calidad seminal en ganado bovino en la Costa Chica de Guerrero

Bottini Luzardo, M. B.[1], *Núñez Martínez, G.*[2], *Sánchez Santillán, P.*[1], *Saavedra Jiménez, L. A.*[1*]

[1] *Facultad de Medicina Veterinaria y Zootecnia No. 2. Universidad Autónoma de Guerrero. Km. 197 Carretera Acapulco – Pinotepa Nacional. C.P. 41940. Cuajinicuilapa, Guerrero, México.*
[2] *Universidad Popular Autónoma del Estado de Puebla. Calle 21 sur 1103, Barrio de Santiago, Heroica Ciudad de Puebla de Zaragoza. C.P. 72410. Puebla, Puebla, México.*

Please cite this article as/Como citar este artículo: Bottini Luzardo, M. B., Núñez Martínez, G., Sánchez Santillán, P., Saavedra Jiménez, L. A. (2025). Neosporosis prevalence and semen quality in cattle in the Costa Chica region of Guerrero, Mexico. *Revista Bio Ciencias*, 12, e1777. https://doi.org/10.15741/revbio.12.e1777

Article Info/Información del artículo
Received/Recibido: October 16th 2024.
Accepted/Aceptado: December 30th 2024.
Available on line/Publicado: March 04th 2025.

ABSTRACT

Bovine neosporosis can have negative reproductive consequences and economic impacts. Developing effective control strategies requires extensive data on its presence and prevalence in cattle herds. The present study objective was to quantify neosporosis prevalence in cattle and sperm quality in *Neospora caninum*-positive bulls in the municipalities of Cuajinicuilapa and Ometepec, in the Costa Chica region of the Guerrero state, Mexico. Blood samples (10 mL) were collected from 300 cows and 100 bulls of reproductive age from 38 production units. Samples were taken from the coccygeal vein and centrifuged at 3500 rpm for 15 min to separate the plasma, which was placed in 1.5 mL Eppendorf vials and stored at -20 ºC until analysis. Antibodies against *N. caninum* were identified with the ID Screen® *Neospora caninum* Competition kit for ELISA. Sperm individual motility, concentration, and morphology were evaluated. Overall N. caninum prevalence was 15.3 % (58/378), 17.5 % (50/286) in cows, and 8.7 % (8/92) in bulls. Bulls positive for N. caninum exhibited sperm concentrations slightly lower than levels deemed fertile. The observed N. caninum prevalence in the sampled animals is low, although its presence in bulls may cause lower sperm concentrations.

KEY WORDS: Bulls, cows, *Neospora caninum*, risk factors, southwest Mexico.

***Corresponding Author:**

Luis Antonio Saavedra Jiménez. Facultad de Medicina Veterinaria y Zootecnia No. 2. Universidad Autónoma de Guerrero. Km. 197 Carretera Acapulco – Pinotepa Nacional. C.P. 49140. Cuajinicuilapa, Guerrero, México. Teléfono: (741) 414 0783.
E-mail: 19188@uagro.mx

RESUMEN

La neosporosis bovina es una enfermedad con consecuencias reproductivas e impacto económico negativo. El objetivo fue determinar la prevalencia de neosporosis en bovinos y la calidad espermática de sementales positivos a *Neospora caninum* en Cuajinicuilapa y Ometepec, municipios de la Costa Chica de Guerrero, México. Se visitaron 38 unidades de producción en las cuales se muestrearon 300 vacas y 100 sementales en edad reproductiva. Se tomaron 10 mL de sangre de la vena coccígea. Las muestras fueron centrifugadas a 3500 rpm por 15 min para obtener el plasma, el cual fue depositado en viales tipo Eppendorf de 1.5 mL y mantenidos a –20 ºC hasta su análisis. Los anticuerpos contra *N. caninum* se determinaron utilizando el kit ID Screen® *Neospora caninum* Competition para ELISA. Las características espermáticas evaluadas fueron motilidad individual, concentración y morfología. La prevalencia de *N. caninum* en general fue de 15.3 % (58/378), en hembras fue 17.5 % (50/286) y en el caso de los sementales fue de 8.7 % (8/92). Los machos positivos a *N. caninum* pueden presentar problemas de fertilidad debido a que la concentración espermática se vio disminuida. En concusión, la prevalencia de anticuerpos en los municipios evaluados puede considerarse baja y su presencia puede disminuir la concentración espermática.

PALABRAS CLAVE: Factores de riesgo, *Neospora caninum*, suroeste mexicano, vacas, toros.

Introduction

Neosporosis in cattle is an important disease worldwide with negative reproductive effects (van Velsen, 2021). It is responsible for losses in cattle production of an estimated 1.298 billion dollars year^{-1} (Reichel *et al*., 2013). In Mexico, its presence has been documented in the states of Chihuahua, Durango, Coahuila, Aguascalientes, Hidalgo, Querétaro, Jalisco (García-Vázquez *et al*., 2009), Nuevo León, Tamaulipas, Chiapas, Veracruz, Yucatán (Salinas *et al*., 2005; Romero-Salas *et al*., 2010), and recently in the Guerrero state (Ascencio-Díaz *et al*., 2020). In the latter, cattle production is a vital agricultural activity, supporting 45 % of the state's population (Periódico Oficial del Estado de Guerrero, 2023).

Bovine neosporosis is caused by the protozoan *Neospora caninum* (Marugan-Hernandez, 2017). Several mammals (goats, sheep, deer, horses, and cattle) can function as intermediate hosts, and canids (fox, coyote, and dog) are the definitive hosts (Gondim *et al*., 2004). Transmission of *N. caninum* to intermediate hosts can occur horizontally, by oocyst ingestion, or vertically, through

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transplacental transmission. Vertical transmission can be either endogenous or exogenous. Endogenous transmission is caused by the reactivation of tissue cysts in an infected animal and is associated with a pattern of reproductive failures. Exogenous transmission occurs after a primary horizontal infection via oocyst ingestion by a pregnant animal and is associated with an epidemic pattern of abortions (Marugan-Hernandez, 2017).

In cattle, the most important clinical sign of neosporosis is abortion and infertility in females (Reichel *et al*., 2020), although cases of embryonic or neonatal death may occur (Marugan-Hernandez, 2017). Abortions are caused by parasite damage in placental tissue, which triggers inflammatory processes and failures in blood flow (Cantón *et al*., 2014). In bull sires, *N. caninum* has been reported in semen (Jafari *et al*., 2012) and the epididymides (Gharekhani *et al*., 2023). This implies parasite presence in the testicles, which can cause inflammatory processes that affect semen quality.

In the tropics of Mexico, a principal problem affecting cow reproductive efficiency is that they do not produce one calf cow^{-1} year^{-1}. This is likely due to multiple factors, and lack of knowledge about any possible cause hinders the development of strategies aimed at improving reproductive performance. In Guerrero, *N. caninum* has been identified in cattle in the municipalities of Ometepec and Cuajinicuilapa (Ascencio-Díaz *et al*., 2020). Because it can negatively affect herd reproductive results by causing abortions, it is vital to determine its presence in a herd and what percentage of the herd is affected. The present study aimed to identify the prevalence of neosporosis in cattle in these two municipalities of the Costa Chica region of Guerrero and evaluate sperm quality in sires positive for *N. caninum*.

Material and Methods

Study area

The study area consists of three locations in the Cuajinicuilapa municipality and five in the Ometepec municipality (Figure 1). Sampling was done at 38 randomly selected cattle production units in the same area sampled by Ascencio-Díaz *et al*. (2020). Samples were collected from February 2019 to December 2021.

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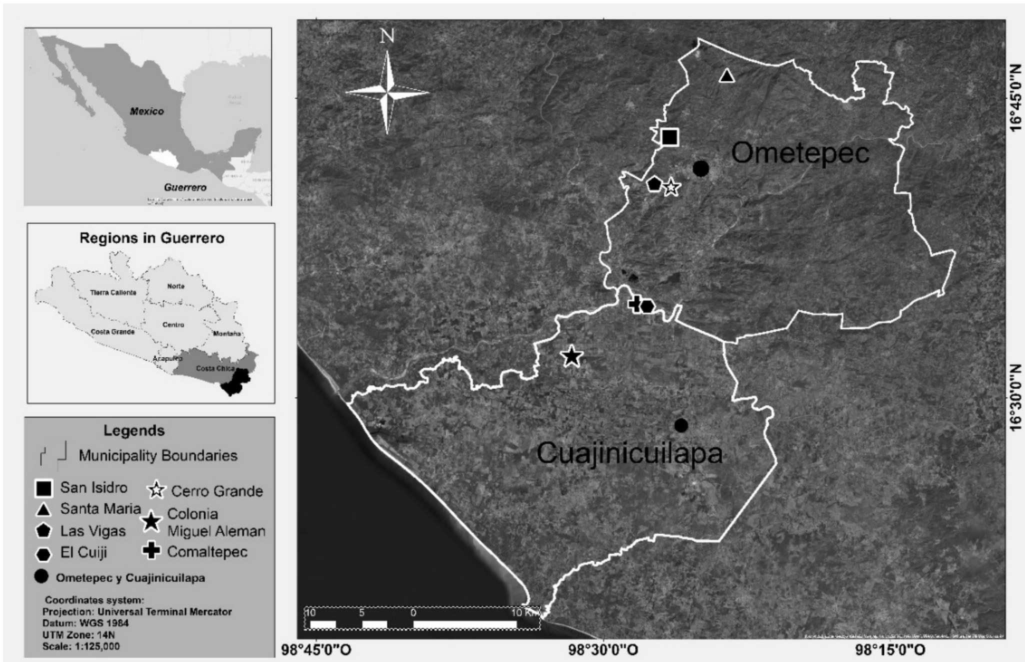

Figure 1. Sampling area in the municipalities of Cuajinicuilapa and Ometepec, Guerrero.

Source: Own elaboration based on INEGI (2019) data.

Animals

The sampled animals were of reproductive age. One hundred sires (average age = 6 ± 2 yrs) were evaluated: 77 *Bos indicus*, 13 *Bos taurus*, and 10 *Bos indicus* x *Bos taurus* crosses. Three hundred cows (average age = 5 ± 3 yrs), all *Bos indicus* x *Bos taurus* crosses, were also sampled. All animals were in extensive or semi-extensive production systems with mineral supplementation and free access to water.

Production and risk factor records

Questionnaires were applied to the producers to collect data on reproductive parameters: 1) age at first parturition; 2) parturition interval; 3) abortions; and 4) stillborn or weak calves. Data was also collected on the presence of dogs in the production unit and any parasite treatments applied to them, as well as any sightings of wild fauna such as foxes. Responses were recorded by production unit.

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Sample collection and processing

Blood samples (10 mL) were collected from the coccygeal vein after disinfection of the area along the medial line of the tail. Collection was done using Vacutainer® tubes containing EDTA anticoagulant. Samples were centrifuged at 3500 rpm for 15 min to separate the plasma, which was placed in 1.5 mL Eppendorf tubes and stored at -20 °C until analysis.

Neospora caninum antibody

Identification of the *Neospora caninum* antibody was done by indirect ELISA, using a commercial kit (ID Screen *Neospora caninum* Indirect Kit; ID, Vet. Grabels, France), with 100 % specificity and 100 % sensitivity. Results were read with the proprietary software (Grabels, France).

Testicle and semen analyses

Bulls in production units specializing in sire breeding and sales were evaluated. The main inclusion criteria were ≥ 16 months old, no apparent defects or pathologies in the reproductive organs, and good general health (e.g. no fevers or infections in three months prior to evaluation).

Scrotal circumference was measured using a testicular tape at the testicular equator, after completely lowering them into the scrotal sac (Koziol & Amstrong, 2018).

Verification of a normal echogenic pattern in the testicular parenchyma was done by ultrasound evaluation of the testicles on dorsoventral and mediolateral axes, as described by Momont & Checura (2015). The evaluation was done with an ultrasound machine (Chison Eco 5, China), with a 7.5 MHz linear transducer.

Semen was collected using an electro-ejaculator (Standard Precision USA). Individual sperm motility was quantified following the technique and parameters of Koziol & Armstrong (2018). Briefly, a drop of semen was placed on a slide at 37 °C, a coverslip placed over it, the sample observed at 40X under a microscope (Model i4 Infinity, LW Scientific, USA), and the percentage of sperm with uniform rectilinear movement estimated. Sperm morphology was evaluated with a smear and eosin-nigrosin staining. The sample was observed at 100X, 100 sperm counted, and the percentage of those sperm with primary and secondary abnormalities quantified. Sperm concentration was quantified by counting in a Neubauer Bright line chamber following an established technique (WHO, 2010).

Experimental design and statistical analyses

Data dispersion was determined using descriptive statistics. Point (p) prevalence was calculated with a 95 % confidence interval ($CI_{95\%}$) with the formulas:

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$$p = \frac{Total\ number\ of\ existing\ cases\ at\ time\ t}{Total\ evaluated\ population\ at\ time\ t} * 100$$

$$CI_{95\%} = p \pm 1.96\frac{p(1-p)}{\sqrt{n}}$$

Results and Discussion

Reproductive and risk factor records

None of the surveyed producers kept any written reproductive records for their herds. They did mention, however, the sporadic occurrence of abortions and parturition intervals greater than one year. In addition, they observed that cows had first parturition at 4 years of age.

The presence of dogs was confirmed in all production units, 98% of which belonged to the unit and 2% were owned by workers; the latter dogs travel with their owners. Forty-five percent of the dogs present in the production units were only treated for ticks and fleas, but not for other parasites. Only 20% of owners reported having seen foxes in the pastures, but never near the house or pens. The presence of dogs in cattle production units can increase the risk of *N. caninum* in herds (Pereyra et al., 2021), particularly in extensive systems. Both factors are prevalent in the study area,suggesting a high probability of *N. caninum* exposure among cattle.

The lack of written records precludes any quantification, but the occurrence of abortions and long parturition intervals suggests *N. caninum* may be affecting reproductive health in the sampled herds. In cows in the tropics, both abortions and long parturition intervals have been attributed mainly to nutritional factors. It is also possible that *N. caninum* could be contributing to the relatively late onset of cow reproductive life in the study area (Pulido *et al*., 2017).

Neospora caninum antibody

The overall prevalence of *N. caninum antibodies* in the studied animals was 15.3 % ($CI_{95\%}$: 14.0 – 16.7 %). Cows exhibited a higher prevalence than bulls (Table 1). Of the sampled cows, 95.3 % could be analyzed, of which 50 were positive, resulting in a 17.5 % prevalence ($CI_{95\%}$: 15.8 – 19.2 %). Of the bulls, 92 % were analyzed, of which 8 were positive, resulting in an estimated prevalence of 8.7 % ($CI_{95\%}$: 7.10 – 10.3 %). The inter-assay CV was 8 % and the intra-assay was 7 %.

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Table 1. *Neospora caninum* antibody prevalence by sex in cattle from two municipalities in the Costa Chica region of Guerrero.

Sex	Animals			Prevalence	$CI_{95\%}$
	Sampled	Analyzed	Positive		
Female	300	286	50	0.175	0.158 – 0.192
Male	100	92	8	0.087	0.071 – 0.103
Total	400	378	58	0.153	0.140 – 0.167

$CI_{95\%}$: 95 % confidence Interval.

The present *N. caninum* prevalence results are similar to those reported in studies under tropical conditions: 11.6 % in beef cattle (García-Vázquez *et al*., 2009); and 20.8 to 26 % in dual-purpose cattle (Romero-Salas *et al*., 2010; Montiel-Peña *et al*., 2011; Zárate-Martínez *et al*., 2021). This similarity could be explained by the match in feeding (predominantly grazing), management, and climate conditions in cattle production systems in tropical areas, including the presence of dogs at most production units. In contrast, the present prevalence values are notably lower than reported for production units in dry, temperate, or cold climates, be they dairy, beef, or dual-purpose systems. Prevalences of 36 to 80 % have been reported in dairy cattle (Salinas *et al*., 2005; Medina *et al*., 2006; Ojeda-Carrasco *et al*., 2016), while 36 % has been reported in beef cattle (Garcia-Vázquez *et al*., 2005; Salinas *et al*., 2005; Mondragón-Zavala *et al*., 2011) and dual-purpose systems (Zárate-Martínez *et al*., 2021). In addition to the differences inherent to different production systems, the methodology used to identify *N. caninum* antibodies in various studies may be responsible for some of the discrepancies between them.

Data for *N. caninum* antibody prevalence is particularly scarce for bulls. In beef cattle in Mexico, prevalences of 5.6 % (Garcia-Vazquez *et al*., 2009) and 30 % (Mondragón-Zavala *et al*., 2011) have been reported. In Belgium, a 9.2 % prevalence was reported in Belgian Blue sires (Kemel *et al*., 2022). The 8.7 % observed in the present results is within the range for beef cattle.

Testicle and semen analyses

In bulls positive for *N. caninum* antibodies, the average scrotal circumference was 34.5 cm, while in negative bulls, it was 36 cm. All exhibited normal healthy parenchyma, without degeneration, tumors, or other disorders (Figure 2).

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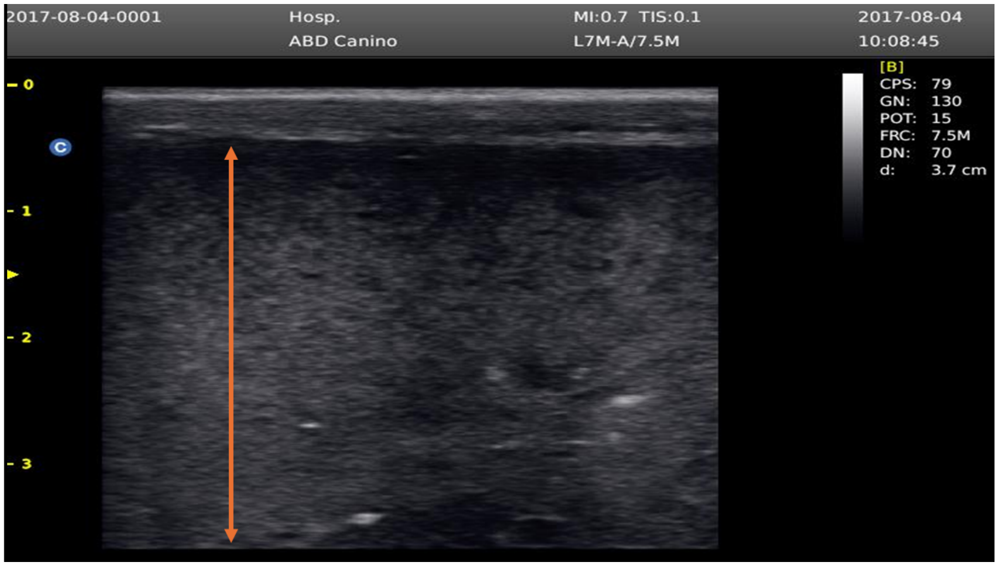

Figure 2. Ultrasound image of testicular parenchyma of a *Neospora caninum*-positive bull.

Source: Present results.

In both positive and negative bulls, semen motility and morphology (Table 2) were within acceptable parameters (Koziol & Armstrong, 2018), although values were slightly lower in positive bulls. This differs from studies in which semen motility and morphology were significantly lower in neosporosis-positive bulls with acute infections, with accompanying inflammation and tissue damage in the testicular parenchyma, the probable cause of the lower values (Bahrami *et al*., 2018, 2019). The present results include the identification of *N. caninum* antibodies but not the infection phase. The normal testicular parenchyma ultrasound images and lack of signs of inflammation in positive bulls in the present results suggest they had chronic infections in which semen motility and morphology were not severely affected.

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Table 2. Semen motility, concentration, and morphology in *Neospora caninum*-positive and -negative sires and prospective sires in two municipalities in the Costa Chica region of Guerrero.

Variable	Negative	Positive
Individual motility (%)	61	53
Concentration (Millions of spermatozoids mL^{-1})	308	207
Morphology (% normal spermatozoids)	87	84

Source: Present results.

Sperm concentration was higher in the negative bulls than in the positive ones (Table 2). The 207 x 10^6 spermatozoa mL^{-1} concentration in the positive bulls was below the 250 x 10^6 spermatozoa mL^{-1} minimum for bulls to be considered fertile (Koziol & Armstrong, 2018). These results coincide with previous reports that the presence of *N. caninum* in the testicles decreases sperm concentration by depressing levels of testosterone and FSH, the hormone responsible for initiating spermatogenesis (Bahrami *et al*., 2018, 2019; van Velsen, 2021). These same studies state that further research is needed because infected bulls did not exhibit fertility problems. The small positive males number identified in the present study prevented running a correlation analysis with semen quality.

The present semen quality results in the negative bulls only indicate that sperm concentration was below that accepted in a fertile bull. Also, sperm concentration directly indicates seminiferous tubule functionality for sperm development, which can also be affected by hormonal deficiencies (Momont & Checura, 2015). Testosterone and thyroid hormone levels do decline in response to *N. caninum* infection (Bahrami *et al*., 2018), which could explain the lower sperm concentrations observed here.

Data on the effects of *N. caninum* infection in sires is scarce worldwide (Polo *et al*., 2023), highlighting the need for further research. The present results and the literature suggest that bulls, which can be asymptomatic, play a fundamental role in the transmission of infectious pathogens, which can affect cattle herd fertility rates. Future research should address the relationship between *N. caninum* infection and fertility problems in cattle and consider parasite prevalence and associated risk factors in both sexes. In the Guerrero state, this is the first study of neosporosis prevalence in cattle and its association with semen quality in *N. caninum*-positive bulls.

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Conclusions

Neospora caninum antibody prevalence in the sampled cattle from the municipalities of Cuajinicuilapa and Ometepec in the Costa Chica region of Guerrero is low, with higher levels in cows than in bulls. Infected bulls exhibited lower spermatozoa concentrations, with possible consequences for fertility. Due to a small sample size, further data is needed to confirm any relationship between *N. caninum* infection and reproductive parameters in bulls.

Author contributions

Study conceptualization and methodology development: MBBL, GNM; Software and data management, manuscript writing and preparation: MBBL, LASJ; data analysis, writing, revision and edition: MBBL, PSS, LASJ.

All authors have read and accepted the published version of the manuscript.

Financing

The research reported herein was financed by the authors.

Conflicts of interest

The authors declare no conflict of interest.

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