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RESEARCH ARTICLE

Seroprevalence and Risk Factors Associated with Sheep Neosporosis in Colima, Mexico

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ABSTRACT

The objective of this study was to determine the seroprevalence of *Neospora caninum*, and the effect of associated risk factors in ten sheep production flocks in the state of Colima, Mexico. A total of 350 blood serum samples were collected, processed via the ELISA technique, and risk factors were calculated using logistic regression analysis. The overall prevalence was 13.14%, and 80% of the flocks had at least one positive animal, with a variation from 5.71 to 45.71%. Prevalence was higher in males (18.75%) than in females (12.87%), in purebred animals (13.87%) than in crossbreed animals (11.42%), and in sheep with body condition less than two. Water source, flock size, and the presence of animals other than canines constituted the main risk factors associated with the presence of the disease ($P<0.05$). In addition, animals raised under an extensive production system tend to present a higher prevalence, while the presence of dogs did not appear to be a risk factor. Animals raised in flocks in which the managers received technical assistance and had more schooling showed 4.12- and 4.15-times higher probability of contracting the disease respectively. The presence of *N. caninum* in sheep and risk factor identification will allow the development and adoption of good husbandry, as well as proper sanitary and biosecurity practices in order to prevent the transmission of *Neospora caninum*.

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INTRODUCTION

Mexico has a sheep population of 8,725,882 heads, most of which are raised in production units characterized by showing poor infrastructure, zootechnical and health management, and biosecurity practices, all of which can promote the appearance of diseases in sheep, causing economic losses (SIAP, 2020).

Several definitive hosts of *N. caninum* have been identified so far. It mostly includes the members of the *Canidae* family, among them the first to be recognized was the dog, followed by the coyote (Gondim *et al.,* 2004), the Australian dingo (King *et al.,* 2010), and the gray wolf (Dubey *et al.,* 2011). The parasite has also been found in warm-blooded, vertebrates acting as the intermediate hosts, such as: horses, sheep, goats, alpacas, llamas (Abo-Shehada and Abu-Halaweh, 2010), birds (Romero *et al.,* 2016), water buffalo (Romero-Salas *et al.,* 2017), pigs (Snak *et al.,* 2019) and rodents (Medina-Esparza *et al.,* 2013). The definitive host (family Canidae) excretes unsporulated oocysts in their feces (sexual phase), which is ingested by the intermediate hosts such as sheep (asexual phase) through contaminated water or food. The sporozoites are released from the oocysts in the intestine of the intermediate host, converting into tachyzoites (acute infection), which lodge in the muscle, connective tissue and liver. In chronic infection, tissue cysts is formed in the central nervous system, peripheral nerves, retina, muscle and placenta. The definitive host becomes infected when it feeds on tissues with tissue cysts (Dubey, 2003, Dubey *et al.,* 2017).

According to a systematic review conducted on the seroprevalence of *N. caninum* during 2004 to 2016, the seroprevalence in sheep population ranges from 2% in Iran to 64% in Brazil (Romanelli *et al.,* 2021). The ovine neosporosis was studied by Castañeda-Hernández *et al.* (2014) and Romo-Gallegos *et al.* (2019) in the states of Aguascalientes and Jalisco, Mexico using ELISA and

PCR techniques, determining the prevalence rate of 5.5% and 13.5%, respectively. Furthermore, the effect of different associated risk factors had also been studied previously showing an impact on the disease prevelance. These risk factors include presence of dogs, flock size, production system, water source, sanitary conditions, adoption of good husbandry practices, and access to technical assistance (Abo-Shehada and Abu-Halaweh, 2010; Romanelli *et al.,* 2021).

As per significance of this parasitic infection across the world, the current study was designed to identify the seroprevalence of *N. caninum* in sheep production flocks at Colima, Mexico and to check the effect of associated risk factors on the occurrence of disease.

MATERIALS AND METHODS

Study Area: The study was carried out in the state of Colima, Mexico (18°41'-19°31' N - 103°29'-104°41' W) which has 492 sheep production flocks distributed in the ten municipalities, comprising of a total of 17,528 heads. For this study ten flocks from six municipalities (Coquimatlan (19°12′05″N 103°48′39″W), Comala (19°19′24″N 103°45′32″W), Colima (19°05 ′00″N 103°57′00″W) Cuauhtemoc (19°21′N 103°36′W), Tecoman (18°53′00″N 103°48′00″W) and Villa Alvarez (19°18 ′00″N 103°50′00″W) were selected. A large number of samples were selected from those areas which have the largest number of animals (INEGI, 2020) (Fig. 1). The study animals were comprising of 334 females and 16 males; 161 older and 189 younger than two years; 140 with a body condition greater than 2 and 210 with less than 2, and 245 purebred (Pelibuey, Katahdín, Dorper, Texcel, Pelifolk) and 105 crossbreed animals.

Samples: A total of 350 blood samples were collected from the jugular vein of sheep and placed in Vacutainer® tubes without anticoagulant. The samples were centrifuged at 3000 g for 5 minutes to obtain the serum, which was fractionated in 600 µL Eppendorf tubes and stored at -20°C until the serological analysis (ELISA) were performed.

ELISA Test: Serum samples were subjected to *enzyme-linked immunosorbent assay* (ELISA) for the identification of IgG antibodies against *N. caninum*. The commercial kit (ID Screen® *Neospora caninum* Competition by Innovative Diagnostics) was used for the serological analysis by using an optimized sensitivity and high specificity of 100% as per manufacturer instructions. The reading was performed by the optical density (OD) in a plate reader at 450 nm (ELIREAD, Kontrolab®). The sera were diluted at 1:10. The negative and positive controls (freeze-dried positive bovine serum and freeze-dried bovine serum negative for various diseases) were used as internal reference material for quality control (Alvarez-García *et al*., 2013). Two sample populations were established (one with low and one with high absorbance). From them, the cut-off point was established at >1.0.

Questionnaire: A questionnaire was designed to record all associated risk factors which may have an impact on disease occurrence. The risk factors included in the questionnaire were associated with the production units and animals' characteristics.

Statistical Analysis: Prevalence values were calculated and associations between the seropositivity of *N. caninum* and the variables studied as potential risk factors were evaluated by Chi-square test. For statistical significance ($P<0.05$) odds ratios (OR) were determined by Logistic Regression analysis at 5% level of significance. The analyses were performed using Statistix 9.0 statistical software.

RESULTS

The overall *N. caninum* seropositivity recorded was 13.14% (46/350), with a variation from 5.71% to 45.71%. In 8/10 (80%) flocks, at least one positive animal was identified (Table 1). The seropositivity of neosporosis was not found to be associated ($P>0.05$) with body condition, age, breed, or sex of the animal, nor with the presence of abortions in the flocks. Water source, flock size, and the presence of animals other than canines were significantly associated ($P<0.05$) with the presence of the disease, while the presence of dogs, production system, sanitation, relative humidity, and altitude of the production unit were not associated ($P>0.05$) with the disease. Sheep that consumed canal water and raised in flocks of less than 100 animals containing no cattle, poultry, felines, pigs, or goats, were more likely to contract the disease than animals that consumed well water and raised in flocks of more than 100 animals, coexisted with other species (Table 2). Access to technical assistance and a producer's schooling were significantly associated ($P<0.05$) with the presence of parasite. Sheep raised in flocks, where managers received technical assistance and had more schooling showed 4.12- and 4.15-times higher probability of contracting the disease, respectively (Table 3).

DISCUSSION

The seropositivity of *N. caninum* detected in the present study (13.14%) was higher than that found in Switzerland (0.8%) (Basso *et al*., 2022), Brazil (3.7%) (Gheller *et al.,* 2016) and Mexico (5.5%) (Castañeda-Hernández *et al.,* 2014). In contrast, higher prevalence has been reported in Argentina, Türkiye and Iraq with 17.2%, 20.6% and 21.05%, respectively (Novoa *et al*., 2023; Kılınç *et al.,* 2023; Alhamdany and Hussain, 2023). Similar seropositivity has been found by Guimarães *et al.* (2015) and Romo-Gallegos *et al.* (2019) in Tocantins, Brazil (13.7%) and Jalisco, Mexico (13.5%). Differences in seropositivity may be due to diagnostic technique, flock size, presence of dog or wild animals, lack of preventive medicine programs, and poor management practices (Romanelli *et al.,* 2021).

In the present study age and sex were not considered as a risk factor. In contrast, Selim *et al*. (2021) concluded that sheep older than 2 years old are 2.4 times more likely to get infected than sheep younger than 1 year-old and ewes are 3.3 times more likely to get infected than rams.

Body condition and production system tend to be associated with the presence of *N. caninum*, having higher prevalence in sheep with low body condition raised under

Table 1: Seropositivity of *Neospora caninum* antibodies in ten sheep production flocks at Colima State, Mexico.

Municipality	Sampled flocks	Sampled sheep	Positive sheep	Seropositivity (%)
Coquimatlán	1	35	0	0.00
Comala	1	35	5	14.28
Cuauhtémoc	1	35	5	14.28
Tecomán	3	105	25	23.80
Colima	2	70	5	7.14
Villa Álvarez	2	70	6	8.57
Total	10	350	46	13.14

Table 2: Risk analysis of neosporosis in sheep from Colima, México as per production units' characteristics.

Variable	Level	No. of Samples	Positive Samples	Seropositivity (%)	OR (95%CI)	P value
Water source	Deep well	304	26	8.56	0.46 (0.25-0.86)	0.00
	Canal	46	20	43.48		
Flock size	>100	175	15	8.57	0.44 (0.23-0.84)	0.03
	<100	175	31	17.71		
Presence of dogs	Yes	69	11	15.94		0.50
	No	291	35	12.46		
Presence of other animals	Yes	280	15	8.92	0.23 (0.12-0.44)	0.00
	No	70	21	30.00		
Relative humidity	>50%	245	36	14.70		0.25
	<50%	105	10	9.52		
Altitude	>500 m	140	15	10.71		0.33
	<500 m	210	31	14.73		
Production system	Intensive	210	33	15.71		0.12
	Extensive	140	13	9.28		
Sanity	Good	105	13	12.38		0.81
	Bad	245	33	13.46		

Table 3: Risk analysis of neosporosis in sheep from Colima, México as per producers schooling and their technical assistance access.

Variable	Level	No. of Samples	Positive Samples	Seropositivity (%)	OR (95%CI)	P value
Technical assistance	Yes	105	27	25.71	4.12 (2.17-7.81)	0.00
	No	245	19	7.76		
Schooling	Non-professional	140	32	8.57	4.15 (2.12-8.10)	0.00
	Professional	210	14	17.71		

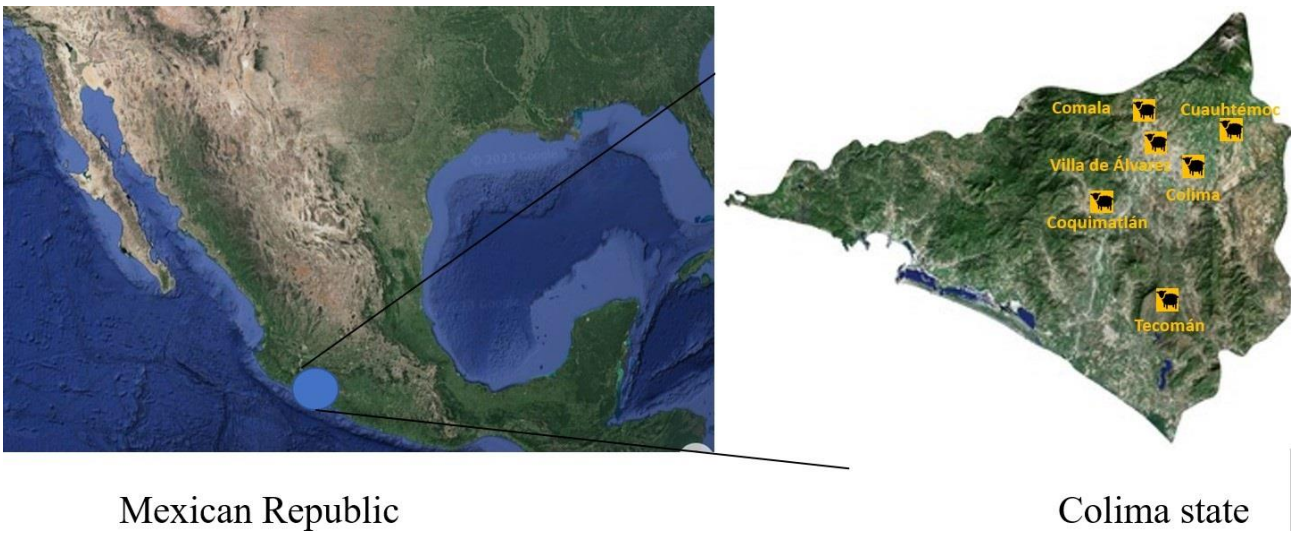

Fig. 1: Location of municipalities with the flocks selected for the study.

an extensive system. This is due to the fact that animals raised under this production system are fed only the scarce flora available for a period of only 6 hours per day, after which they are subsequently confined in pens with poor-quality supplementation. Similarly, Dubey and Schares (2011) found that in an extensive or semi-extensive system, animals are grazed and supplemented with silage or concentrate, which are stored in warehouses, allowing contact with the dogs and representing a risk of infection. In addition, in a semi-extensive system, animals are in high confinement with little space, as well as poor sanitation and feeding, which increases the possibility of infection.

There is a significant association between seroprevalence and several risk factors including water source, flock size, and the presence of animals other than canines. In the present study, a higher prevalence was found in animals that consumed water from canals than those that consumed water from wells. Alternatively, Faria *et al.* (2010) reported that the risk of acquiring neosporosis was 4.7 times higher when the water came from mixed sources (wells, canals, and public water supply). However, the water sources evaluation should be made to avoid contamination with *N. caninum* oocysts. Sheep raised in flocks with more than 100 animals have a significant association with occurrence of disease, because

as the density of animals increases, the possibility of these animals to come in to contact with vectors of infection and their transmission routes increases, as mentioned by Al-Majali *et al.* (2008). According to Dubey and Schares (2011), Dubey *et al*. (2017) and De Barros *et al.* (2018), the presence of equines, cattle, swine, goats, and birds can act as intermediate hosts of *N. caninum* and maintain the life cycle of parasite.

The presence of dogs did not represent a risk factor of disease, despite of the fact that all sheep production systems included in the study had at least one permanent dog. However, in present study, presence of dogs significantly affected the occurrence of disease having higher infection in flocks where dogs were present. Similarly, Al-Majali *et al.* (2008) reported that the presence of more than one dog on the property suggests horizontal transmission of the agent and increases the risk of infection by 2.4 times. Dubey and Schares (2011) also reported that the presence of dogs at farm is an important risk factor for *N. caninum* infection, especially if the animals are confined. Alternatively, Cerqueira-Cézar *et al.,* (2017) reported a non-significant association between presence of dogs and disease occurrence. The higher prevalence of disease in dogs' presence may be due to the fact that dogs act as the definitive host for *N. caninum* and play an important role in disease transmission. However, the risk can also be reduced by restricting the access of dogs to production areas in order to avoid the consumption placentas, and viscera, thus reducing the horizontal transmission and the elimination of oocysts into the environment.

Animals raised in flocks in which managers had more schooling and received technical assistance were more likely to contract the disease than those managed by owners with less schooling and no access to technical assistance. Similar findings were reported by Liu *et al*. (2015) and Arraes-Santos *et al*. (2016). This suggests that higher schooling and technical assistance in no way guarantees the implementation of good sanitary and productive management practices.

In conclusion, the prevalence of *N. caninum* in sheep at Colima state, Mexico was 13.14%. Source of water, flock size, and the presence of animals other than canines in the production unit, along with the access to technical assistance and schooling of the producer were identified as risk factors for the presence of disease. This knowledge will allow for the development and adoption of good husbandry, as well as proper sanitary and biosecurity practices, to prevent horizontal and vertical transmission of neosporosis.

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Authors Contribution: JLGV, LJGM, JARR conceived and designed the study, executed the experiment and analyzed the sera samples, interpreted the data. TLM. AJGC analyzed the sera samples, interpreted the data. RJMB analyzed and interpreted the data. MGGV apply the questionnaires and capture the information for further analysis. All authors revised and approved the final version of the manuscript.

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