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Seroprevalence of *Neospora caninum* in rural and urban dogs from southeast Mexico State

Seroprevalencia de *Neospora caninum* en perros rurales y urbanos del suroriente del Estado de México

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ABSTRACT

*Neospora caninum*ç is the etiological agent of neosporosis in cattle and dogs. It is considered one of the leading causes of abortion in cows. Dogs are considered the definitive host; in intrauterine infected and immunosuppressed dogs, it causes multisystemic lesions. The aim of this study was to determine the seroprevalence against *Neospora caninum* in urban and rural dogs from the southeast of Mexico State and its association with risk factors for these species. Through a non-probabilistic convenience sampling, 70 serum samples were obtained from dogs from an urban area and 76 from a rural one. Information on age, gender, diet and environment was obtained through a questionnaire. The statistical analysis was applied by means of Chi square, as well as the determination of odds ratio (OR) and discriminant multiple linear regression. The general seropositivity to *Neospora caninum* by IFAT was 51.3% (75/146), in urban dogs, it was 48.6%, and 53.9% in rural dogs (p=0.314). As for the age risk factor, seropositivity was higher as it increased; the consumption of placentas was recognized as a risk factor. A wide distribution of *Neospora caninum* was identified in dogs as well as constant exposure to it in the area studied.
Keywords: dogs, *Neospora caninum*, Mexico.

RESUMEN

Neospora caninum es el agente etiológico de la neosporosis en bovinos y perros, es una de las principales causas de aborto en vacas; en perros infectados intrauterinamente e inmunodeprimidos, ocasiona lesiones multisistémicas y son considerados los hospederos definitivos. El objetivo de este estudio fue determinar la seroprevalencia contra *Neospora caninum* en perros urbanos y rurales del sur oriente del Estado de México y su asociación a factores de riesgo para esta especie. Mediante un muestreo no probabilístico por conveniencia se obtuvieron 70 muestras de suero de perros de una zona urbana y 76 de zona rural. Información sobre edad, género, alimentación y medio ambiente se obtuvo mediante un cuestionario. El análisis estadístico fue por medio de Ji cuadrada, la determinación de la razón de probabilidades (OR) y regresión lineal múltiple discriminante. La seropositividad general a *Neospora caninum* mediante IFI, fue 51.3% (75/146), en perros urbanos de 48.6% y en rurales de 53.9%, (p=0.314). Para el factor edad, la seropositividad fue mayor a medida que incrementó la misma; el consumo de placentas se reconoció como

factor de riesgo. Se identifica una amplia distribución de *Neospora caninum* en perros, así como una constante exposición al mismo en el área de estudio.
Palabras clave: perros, *Neospora caninum*, México

INTRODUCTION

Neosporosis caused by *Neospora caninum*, an Apicomplexa protozoan, has been considered a major cause of epidemic and endemic abortion with great economic impact on the livestock industry worldwide (Dubey, 2003), in addition to causing multisystemic lesions in immunosuppressed or intrauterine infected dogs (Lindsay & Dubey, 2000). *Neospora caninum* is maintained in the environment by a heteroxenous life cycle involving the dog as the definitive host and a wide range of intermediate hosts (Dijkstra *et al.*, 2001). Immunocompetent adult dogs are asymptomatic (Kul *et al.,* 2015; Silva & Machado, 2016), and can serve as definitive and intermediate hosts by excreting oocysts, which upon sporulating in the environment, can be ingested by intermediate hosts causing horizontal transmission in these animals (McAllister *et al*., 1998; Lindsay *et al.,* 1999; Dubey, 2003). Dogs acquire infection by ingestion of organs, tissues, fetuses or fetal membranes with parasitic cysts present in intermediate hosts, by ingestion of sporulated oocysts found in the environment and by vertical transplacental transmission (Dubey *et al.*, 2007). This parasite also maintains a sylvatic cycle among wild canids and wild herbivores (Almeira, 2013; Donahoe *et al.,* 2015). In several countries, anti-*Neospora caninum* antibodies have been detected in dogs using different diagnostic techniques and different risk factors have been associated for its presentation in these populations (Anvari *et al.,* 2020). In Mexico, small-scale milk production systems (SPLPE), have played an important role in national production (López *et al.,* 2008); and according to what reported by Montiel-Olguin *et al.,* 2019, they identified high seroprevalence of neosporosis as a risk factor associated with low reproductive performance in these production units; for their part, the presence of *Neospora caninum* and its association as a cause of abortion in SPLPE units in the southeastern region of Mexico State, has been confirmed (Ojeda *et al.,* 2016; Reyes-Sandoval *et al.,* 2017); however, the serological status in dogs residing in these production units, as well as in surrounding urban areas, that would allow quantitative estimation of its possible distribution is unknown. Therefore, the aim of this study was to determine the seroprevalence of *Neospora caninum* in dogs from a rural and an urban area in the southeast of Mexico State and its association with possible risk factors for this species.

MATERIAL AND METHODS

Geographic area

The municipalities of Amecameca, Tlalmanalco and Ayapango are considered a rural area, between the coordinates 19°07'-19°80' north latitude and 98°45'-98°51' west longitude, in the southeastern part of Mexico State, at an average altitude of 2420 m a.s.l. The climate is temperate sub-humid with summer rainfall Cb (w2), average temperature of 12 to 18 °C and rainfall of 935 mm per year. For the urban zone, Ciudad Nezahualcóyotl, Mexico State at 2240 m a.s.l, the climate is semi-dry temperate BS(1k) with summer rains, temperature between 20-32 °C and an average rainfall of 775 mm.

Animals under study

Seventy-six blood samples were collected from dogs in the rural zone, coming from production units of milk-producing cattle in which there is a history of abortion, where other species such as sheep, goats, pigs and horses cohabit. For the urban zone, 70 dogs visited a veterinary clinic for preventive treatment or care of pathologies and diseases not related to neosporosis. For both zones, the dogs were included in the study after the owners signed a consent form for this purpose and answered a semi-structured questionnaire to obtain information to identify possible risk factors. The inclusion criteria were: dogs of any age and gender, non-pregnant females and in good general health. Samples were processed at the the National Center for Disciplinary Research in Animal Health and Safety of the National Institute of Forestry, Agriculture and Livestock Research (Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad del Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias,CENID-SAI del INIFAP), Jiutepec, Morelos.

Sampling and sample collection

The type of sampling was non-probabilistic by convenience. Blood samples were obtained by puncture of the cephalic vein in vacutainer tubes without anticoagulant, identified and kept at room temperature until serum exudation, separated by centrifugation at 1500 xg for 10 minutes and stored in polypropylene vials at -20 °C until analysis. All dogs were handled in strict accordance with the accepted guidelines for ethical use, care and animal welfare established by the Research Ethics and Bioethics Committee of the Amecameca University Center of the Autonomous University of Mexico State, with approval and act number 08/2021.

Serological study

The sera were thawed and processed in a 1:50 dilution, and analyzed by the Indirect Immunofluorescence Test (IFI), using the method described by (Dubey *et al.,* 2007); having commercial slides with *Neospora caninum* tachyzoites, cultured in Vero cells, fixed and unstained (Catalog No., SLD-IFA-NC, VMRD Inc., Washington, USA); a fluorescein-conjugated canine anti-IgG of goat origin (Catalog No., CJ-F-CANG VMRD Inc., Washington, USA) was used as secondary antibody. For each slide, *Neospora caninum* positive and negative dog sera were used as test controls (Catalog No., 211-P-NC-CAN and 211-N-NC-CAN, VMRD Inc., Washington, USA); slides were observed under an epifluorescence microscope. Those sera showing a number ≥ 20 tachyzoites fluorescing per optical field were considered positive (Dubey & Lindsay, 1996, 1996). Titration was performed with serial double dilutions starting from an initial 1:50 dilution until the endpoint was determined using the methodology described above.

Statistical analysis

Prevalence and possible risk factors such as: age, gender, cohabitation with other dogs and/or other species, as well as consumption of aborted fetuses, placentas, or bovine tissue offal were considered as dichotomous variables; except for type of food administered in which a description of the variable is made. Initially, they were compared using Chi-square with the Yates correction and in those in which the risk factor was significant ($p < 0.05$) the odds ratio or odds ratio (OR) was estimated, where values greater than one indicated association, using the OpenEpi version 3.01 program.

On the other hand, the relationship between seropositivity and potential risk factors was determined by means of a multiple linear discriminant regression analysis using the Statsgraphics Centurion XVIII program.

RESULTS

From 146 sera analyzed, 75 showed positive reaction to *Neospora caninum*, so the identification of specific antibodies in the dogs under study was 51.3%, the seroprevalence in dogs from the urban area was 48.6% (34/70) and for the rural area 53.9% (41/76), finding no statistical difference (p=0.314). Anti-*Neospora caninum* antibody titers were: 66.7% (50/75) with 1:50, 10.7% (8/75) with 1:200, 9.3% (7/75) with 1:400 and 13.3% (10/75) with 1:800. Although a higher seroprevalence was identified in males 55. 9% (47/84) compared to females 45.1% (28/62), there was no significant statistical difference between urban and rural genders (p=0.131) Table 1.

For the risk factor age, the seroprevalence of *Neospora caninum* was higher as the age of the dogs increased (Table 2); significant difference was only found in the stratum of 5 to 10 years, for the two zones (p=0.02697), with an OR=11 (95% CI: 1.137-106.4).

Table 1. *Neospora caninum* seroprevalence by gender in dogs from the southeast of Mexico State

	Gender	Number of sera	IFAT positive sera	Seroprevalence (%)
Urban area	Females	34	15	44.1
	Males	36	19	52.7
Rural area	Females	28	13	46.4
	Males	48	28	58.3
General	Females	62	28	45.1
	Males	84	47	55.9

Table 2. *Neospora caninum* seroprevalence by age in dogs from the southeast of Mexico State

Age (Years)	Urban area		Rural area		General	
		Seroprevalence (%)		Seroprevalence (%)		Seroprevalence (%)
≤ 1	3/10	30.0	14/38	36.8	17/48	35.4
1 – 5	17/35	48.5	16/26	61.5	33/61	54.1
5 -10	8/19	42.1	8/9	88.9	16/28	57.1
≥ 10	6/6	100.0	3/3	100.0	9/9	100.0
Total	34/70	48.6	41/76	53.9	75/146	51.3

In seropositive dogs in the urban area and for the factor type of food, the following were identified: 2.94% (1/34), consumed chicken; 8.7% (3/34), kibble/chicken; 8.7% (3/34), kibble/moist food; 73.5% (25/34), kibble; 2.94% (1/34), beef waste and 2. 94% (1/34), chicken/kibble/feed waste; for this and the risk factors cohabitation with other species, cohabitation with other dogs, consumption of bovine tissues, it was not possible to demonstrate an association with seroprevalence. This contrasts with the discriminant multiple linear regression analysis in which seropositivity and possible risk factors were considered; placenta consumption was identified as a factor with significance (p=0.0146) which is related to age and the equation of the adjusted model is:

$$seropositive = 0.209517 + 0.485705 * placenta\ comsumption + 0.138303 * age$$

DISCUSSION

The overall seroprevalence to *Neospora caninum* determined in this study in the southeast of Mexico State, using the IFA test was high, with 51.3% of dogs, being higher than the 35% obtained for Tizayuca city, Hidalgo, Mexico; reported by Sánchez *et al.,* 2003 and the reported by Cruz-Vázquez *et al*., 2008 of 32% in Aguascalientes, Mexico, both studies employing the ELISA test; so the same trend is observed comparing it with more recent studies in other countries such as China, of 20% (Gao & Wang, 2019); Brazil, in Paraná State, of 19.6% (Spiti *et al.,* 2018); and in Sao Paulo, where the seroprevalence in three consecutive years was 7.8%, 4.8% and 6.8% (Sevá *et al.,* 2020). In this research, a seroprevalence of 53.9% was determined in dogs in rural areas, similar to the 51.3% reported by (Sánchez *et al.,* 2003); however, for this study a smaller number of farm dogs (14) were used in Tizayuca, Hidalgo; but it contrasts with what was reported by Cruz-Vázquez *et al.*, 2008 with 41% for dogs that had close contact with dairy cattle in which 152 dogs were sampled. In the study area there is recent knowledge of the high seroprevalence existing in dairy herds of the small-scale production system 51.7% (Ojeda *et al.,* 2016); However, there are no previous reports on the seroepidemiological situation of dogs, whose importance lies in the fact that they are considered the definitive hosts in the cycle of this parasite and in the exogenous transmission to cattle, without leaving aside the perpetuation of the infection through endogenous transmission in cattle. Considering that it is a common practice to allow the consumption of placentas, fetuses and other tissues by dogs cohabiting in livestock production units, which is identified by the multiple linear discriminant regression analysis used in this study and is related to the age of the dog; Around the world, parasitosis has been reported to be present in a range of 12 to 42% in aborted bovine fetuses (Lefkaditis *et al.,* 2020). Although the parasite can lodge in any tissue, it has been identified mainly in the central nervous system, skeletal and cardiac muscle and liver (Cedillo *et al.,* 2008; Cavalcante *et al.,* 2011).

For the urban area, a seroprevalence of 48.6% was estimated; higher than the 20% reported by Sánchez *et al.,* 2003 and Cruz-Vázquez *et al.*, 2008; with a sampling of 6 and 116 dogs, respectively. It is considered that infection by the parasite in dogs occurs after consumption of tissues with the presence of cysts, which raises a question about the possible mechanisms of transmission in urban dogs. Reported seroprevalences vary widely around the world, which can be attributed to geographical, topographical, climatic factors, type of dog population (domestic, street, shelter, farm), dog keeping conditions, type of feeding; as well as the different sampling and diagnostic methods employed (Dwinata *et al.,* 2018; Anvari *et al.,* 2020); it is identified in this study a high number of seropositive dogs whose main feeding is with kibble, being that they do not live or cohabit with other dogs or other production species; therefore, the presence of anti-*Neospora caninum* antibodies indicates the exposure of these to the parasite without having direct

contact with farm animals, which may be associated with vertical transmission from mother to fetuses for several generations or post-weaning horizontal transmission (Sloan *et al.,* 2017) and consumption of water and feed contaminated with sporulated oocysts (McAllister, *et al.,* 1998; Langoni *et al.,* 2013).

For the risk factor age, the seroprevalence was higher as dog age increased, which coincides with what is reported by Cruz-Vázquez *et al*. 2008 in Mexico with 67% for dogs between 11 and 15 years, Ferroglio *et al.,* 2007, in Italy; Collantes-Fernández *et al.,* 2008, in Spain and Wang *et al.,* 2016 in China, suggesting a longer postnatal exposure time to infection by direct or indirect horizontal transmission of the parasite. The result obtained in this research may be due to the fact that in the habitat where dogs live in both rural and urban areas, the parasite cycle is ensured, having high possibility that seroconversion is greater the longer the dog lives (Dijkstra *et al.,* 2001; Bandini *et al.,* 2011). However, Sloan *et al.,* 2017, found higher seroprevalence in dogs one year old or younger particularly for that study they attribute it to increased seropositivity in pregnant bitches and vertical transmission of the parasite in Australia.

The antibody titers found in this work were from 1:50 to 1:800, in this regard, dede Souza *et al.,* en 2002 obtained similar titers, other authors have found lower titers from 1:25 or higher up to 1:1600 (Langoni *et al.,* 2013; Spiti, *et al.,* 2018). Bandini *et al.,* en 2011 report titers from 1:800 to 1:1160 in sera of dogs experimentally infected with high doses of oocysts, being this a reference of the degree of exposure of dogs in the study area of this work.

CONCLUSION

The seroprevalences of *Neospora caninum* found in this study determine a wide distribution of this protozoan in dogs in the southeastern region of Mexico State, both in rural and urban areas; likewise, the antibody titers demonstrate the constant exposure of dogs to the parasite in both areas. The consumption of placentas was identified as a risk factor for dogs living in the rural area, which may be related to the high prevalence of the parasite in cattle in the area under study.

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Errata Erratum

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