Determination and correlation of anti-*Neospora caninum* antibodies in dogs and cattle from Mexico

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Abstract

The aim of the present study was to determine and to compare through an indirect enzyme linked immunosorbent assay (ELISA) test, the presence of anti-*Neospora caninum* antibodies in city and farm dogs, as well as in farm cows, and the relationship among them. The correlation between anti-*N. caninum* antibodies in farm dogs and cattle was also assessed. The research was conducted in the dairy region of Tizayuca, Hidalgo, Mexico. The frequency of anti-*N. caninum* antibodies was significantly higher in farm dogs ($n = 14$) (51%) when compared to those from the city ($n = 6$) (20%) ($P < 0.05$), suggesting that farm dogs have a higher risk of exposure to the parasite. There was no significant difference in seropositivity between males ($n = 11$) (39%) and females ($n = 9$) (33%) ($P > 0.05$). The frequency of anti-*N. caninum* antibodies in farm cattle was significantly higher in farms with dogs ($n = 158$) (58%) when compared to those with no dogs ($n = 43$) (35%) ($P < 0.05$). These results suggest the possible transmission of the parasite from dogs to cattle.

Résumé

*Cette étude avait pour but de déterminer et de comparer au moyen d'une épreuve immuno-enzymatique indirecte (ELISA) la présence d'anticorps anti-*Neospora caninum *chez des chiens de ville et des chiens de ferme, de même que chez des vaches, afin d'évaluer la relation entre eux. La corrélation entre les anticorps anti-*N. caninum *chez les chiens de ferme et les bovins fut aussi évalué. Cette étude fut effectuée au Mexique dans la région de Tizayuca, Hidalgo. La fréquence des anticorps anti-*N. caninum *était significativement plus élevée ($P < 0,05$) chez les chiens de ferme (51 % des chiens) comparativement aux chiens de ville (20 % des chiens), ce qui suggère que les chiens de ferme ont un risque plus grand d'exposition au parasite. Aucune différence significative ne fut observée dans la séro-positivité des mâles (39 %) et celle des femelles (33 %) ($P > 0,05$). La fréquence des anticorps anti-*N. caninum *chez les bovins était significativement plus élevée ($P < 0,05$) dans les fermes où l'on retrouvaient également des chiens (58 %) comparativement à celles sans chien (35 %). Ces résultats suggèrent la transmission possible du parasite des chiens aux bovins.*

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Neosporosis has been considered an important disease in cattle and dogs over the last years (1). Recently, dogs proved to be a definitive host of *N. caninum* (2). The oocysts are excreted through dog feces and then, after they are consumed by an intermediary host (ruminants, horses, and wild animals), the sporozoites are liberated and infect the small intestine. They divide rapidly by endodyogeny and are converted into tachyzoites, which can infect both skeletal and cardiac muscles, connective tissue and liver, where they multiply. With time, cysts containing bradyzoites are developed. These show affinity for the central nervous system, peripheral nerves, and retina (2–4).

The most frequently infected intermediary host is cattle, in which abortion is the most common clinical sign. Morales et al (5) conducted a seroepidemiologic study in dairy cattle, proving that *N. caninum* infection is widely distributed among Mexican dairy herds. Although there is limited information concerning prevalence of *N. caninum* infection in dogs, cases of neosporosis have been reported from most of the developed countries of the world; including, North America, Europe, South Africa, Japan, Australia, and Costa Rica (6–8).

In Mexico there have been no studies on the prevalence of neosporosis in dogs, or its association with infection in dairy cattle. Because the dog is a definitive host of *N. caninum* (2), it is plausible that cattle are infected by exposure to canine oocysts, although this infection route remains to be proven. Thus, it is important to assess the prevalence of neosporosis in dogs and its correlation with infection in cattle. The objectives of the present study were to determine and to compare the presence of anti-*N. caninum* antibodies between city and dairy farm dogs, as well as to determine their correlation through an analysis of the risk factors involved in the transmission of the disease. For this purpose, an indirect enzyme linked immunosorbent assay (ELISA) test was used and a questionnaire was applied only to those farms that had dogs.

The study was conducted in the dairy region of Tizayuca, located at the Industrial Agricultural Center of Tizayuca, Hidalgo, Mexico. Serum samples from 57 dogs were taken. Twenty-seven belonged to 9 different farms and the remaining samples were obtained from dogs living in Mexico City, chosen by quota, non-probabilistic sampling which describes general characteristics from the population

to study (9). In order to be included in the study, the dogs should have an owner and no previous contact with cattle.

For the serologic evaluation of cattle, 390 serum samples from 13 different farms were analyzed. Dogs were present at 9 of these farms. From each farm, samples from 30 cows were obtained through quota, non-probabilistic sampling. These farms were selected on the basis of identified neosporosis in herds from previous studies through serology in the mothers, and histopathology and immunohistochemistry in the fetus (5).

Sera were obtained by collecting 2 mL of blood from each animal, using sterile tubes without anticoagulant. The samples of blood from the cattle were taken from the coccigeal vein, while blood obtained from the dogs was taken from the cephalic vein.

The detection of anti-*N. caninum* antibodies in cattle, was assessed by an indirect ELISA test using both reagents and the method described by Herdchek anti-*Neospora* (sensitivity 100% and specificity 98.9%; IDEXX Laboratories Inc., Westbrook, Maine USA), with a 0.50 cut point (CP).

For the detection of antibodies to *N. caninum*, an ELISA test that had been developed for bovine sera was used. When used on canine sera, the anti-bovine immunoglobulin conjugate was replaced by an anti-canine IgG conjugate (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Maryland, USA). A CP of 0.47 was chosen, based on the frequency distribution of the extinction values of all 57 canine sera (10). All samples were used at a 1:100 dilution. Although *N. caninum* is closely related to *Toxoplasma gondii*, *Sarcocystis* sp., and other apicomplexans, serologic cross-reactivity has not been a major issue in animals (11,12).

An identification of the risk factors in cattle was carried out using an analysis of the following variables: the dog's access to the food, water, or both destined to be consumed by cattle; the presence of dogs on the premises during the last 5 y; the dog's access to placentas, aborted fetuses, or both; and the presence of other animals species on the farms.

The frequency of sera seropositive to anti-*N. caninum* antibodies from both cattle and dogs was determined. In the former, seropositivity was evaluated taking into account the presence or absence of dogs in the farms. In the latter, positive cases were compared by their origin and sex. For dogs, positivity by Chi-squared test, the probability index (Odds ratio = OR), and their confidence intervals were assessed (13,14). The data obtained from the questionnaire of the farms for each of the different variables was coded into categories. These were evaluated using logistic regression, where the dependent variable was seropositivity for *N. caninum* (15). The variable selection was carried out by means of forward selection method, in which the only variables admitted were those with the likelihood ratio X^2 of $P \leq 0.10$ to enter and a $P \leq 0.05$ to stay in the model.

Based on the CP used (OD = 0.47), 14 out of 27 farm dogs (51%) and 6 out of 30 city dogs (20%) were positive to anti-*N. caninum* antibodies. The difference between the positivity of farm and city dogs was statistically significant ($P < 0.05$), with an OR = 4.31 and a 1.17 to 16.57 confidence interval (Table I). When comparing the positivity between male ($n = 11$) (39%) and female ($n = 9$) (33%) dogs, no significant difference was found ($P > 0.05$) (Table II).

Even though the logistic regression model included the 2 variables; presence of dogs and dog's access to the food, water, or both destined

Table I. Prevalence of anti-*Neospora caninum* antibodies in farm and city dogs

	Positive	Negative	Total	%
Farm dogs	14	13	27	51
City dogs	6	24	30	20
Total	20	37	57	35

Likelihood ratio (X^2) = 6.48
Probability (P) $<$ 0.05
Odds ratio (OR) = 4.31
Confidence interval (CI) = 1.17 to 16.57
Cut point (CP) = 0.47

Table II. Prevalence of anti-*Neospora caninum* antibodies in dogs by sex

	Positive	Negative	Total	%
Male	11	17	28	39
Female	9	20	29	31
Total	20	37	57	35

Likelyhood ratio (X^2) = 0.42
Probability (P) $>$ 0.05
Cut point (CP) = 0.47

to be consumed by cattle, only the first variable remained in the model (Table III). The seroprevalence in the seropositive cows was significantly higher ($P < 0.05$), when compared to the positive cows from farms with no dogs, OR = 2.52 and a confidence interval of 1.61 to 3.94.

The prevalence of anti-*N. caninum* antibodies shown in the present study was significantly higher in farm dogs when compared with that from city dogs. This agrees with research conducted by Sawada et al (16) in Japan, with a 31% prevalence for farm dogs and 7% for city dogs. Also, Wouda et al (10) found similar results in Holland, 23.6% of farm dogs were positive, but only 5% of the sera from city dogs were positive. The high frequency of anti-*N. caninum* antibodies in farm dogs could be attributed to the consumption of fetuses or placentas infected with tachyzoites or cysts of *N. caninum*. Also, dogs could go from one farm to another and increase the probability of infection. Thus, some authors recommend not to feed dogs fetuses, fetal membranes, or dead calves, which could contain *N. caninum* cysts.

Another possible explanation for the high prevalence of anti-*N. caninum* antibodies in farm dogs, is contact with wild animals (coyotes and foxes) and other domestic species (horses, sheep, and goats). Evidence of infection by *N. caninum* in these species has been previously stated (17–20).

The presence of anti-*N. caninum* antibodies in city dogs could be associated with subclinical transplacental transmission through several generations. To a lesser extent, dogs could become infected by the horizontal route if they consume sporulated oocysts of *N. caninum*. Nevertheless, both modes of transmission could also be applicable to farm dogs (1,2).

The lack of a significant difference between male and female dogs to anti-*N. caninum* antibodies agrees with the studies conducted by Sawada et al and Barber et al (16,21), but differs from the findings

Table III. Model for the identification of risk factors related to seropositivity of *Neospora caninum* in cattle

Risk factor	Coefficient		OR	CI	P
	α	β			
Presence of dogs	−0.5825	0.9266	2.52	1.61 − 3.94	< 0.05
Dog's access to food, water, or both destined to be consumed by cattle			0.82	0.66 − 1.01	0.08
Presence of dogs on the premises in the last 5 years			1.19	0.82 − 1.73	0.315
Dog's access to placentas, aborted fetuses, or both			1.19	0.82 − 1.73	0.315
Presence of other animals species on the farm			1.07	0.88 − 1.31	0.4898

OR — Odds ratio; CI — Confidence interval

of Wouda et al (10), where a higher frequency in females was found. This implies the need to perform more studies with larger sample sizes in order to establish a real sex predisposition.

A higher seroprevalence of anti-*N. caninum* antibodies was found in cattle from farms with dogs in comparison with those with no dogs. This is similar to that observed by Paré et al (22) in Québec, where a 22% seropositivity was observed in cattle from herds with dogs and a 7.5% seropositivity in cattle from herds with no dog. Moreover, Wouda et al (10) also states that the seroprevalence of anti-*N. caninum* antibodies in cattle was significantly less ($P = 0.05$) in those farms with no dogs. These results suggest a strong association between the seroprevalence to *N. caninum* in cattle and the presence of dogs, suggesting that dogs may be involved in the transmission of *N. caninum* to cattle as a definitive host (1). The elimination of oocysts of *N. caninum* through dog feces could contaminate the food and water that are then consumed by cattle, thus infecting them (3).

Although the seropositivity was lower in farms with no dogs, finding a seroprevalence of up to 46% could be associated with transient dogs. Another possibility is that seropositive cows in these farms were already infected when they were purchased. Many animals from countries with a high seroprevalence of bovine neosporosis are imported into the dairy region of Tizayuca, Hidalgo.

Vertical transmission is an important mode of infection which contributes significantly to the persistence of the infection within a herd. Several studies indicate that 80% or more seropositive cows may transmit the infection to their offspring. However horizontal transmission has also been identified (23–25).

The present study brings to light information concerning the relevance of banning dogs on dairy farms if neosporosis needs to be controlled or eradicated. If dogs are to be present, specific areas for their housing should be established, thus diminishing the presence of dog feces in the cattle's food that could contain *N. caninum* oocysts.

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