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Original

Serological frequency of *Neospora caninum* in livestock production units in central Veracruz, Mexico

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ABSTRACT

Objective. To determine the frequency of *Neospora caninum* in two Livestock buffalo/bovine Production Units (LPU) in the central zone of the state of Veracruz, México. **Materials and methods.** The study was a cross-sectional study with non-probability convenience sampling. Two Livestock Production Units (LPUs), both with buffalo and bovine populations, located in the central zone of the state of Veracruz, Mexico, were included. The total populations of buffaloes, bovines, and canines were included. Blood samples were taken between February and March 2019 and processed with a commercial ELISA test (IDEXX®) and an Indirect Immunofluorescence Test (VMRD, Pullman, WA, USA) to determine *Neospora caninum* antibodies. In each LPU, a general and individual survey was applied. Data were analyzed with the STATA software version 14.0. **Results.** 68 out of 138 samples presented antibodies against *N. caninum*, with a frequency of 45.6% (95% CI, 37.1–54.3). The buffalo population had higher *N. caninum* antibody concentrations, 26/31 from the LPU of Cotaxtla with 83.8% (95% CI, 66.2–94.5) and 12/22 from the LPU of Medellín with 54.5% (95% CI, 32.2–75.6). Regarding the canines, 4/6 were positive (66.6%). Regarding the different animal species, 38/53 buffaloes presented the highest frequency with 71.6% (95% CI, 57.6–83.2). **Conclusions.** The presence of *N. caninum* antibodies in the bovine and buffalo populations of the studied LPUs was verified. There was a higher frequency of antibodies in water buffaloes when they cohabited with cattle and canines.

Keywords: Water buffalo; control; immunity; protozoan (*Source: MeSH*).

RESUMEN

Objetivo. Determinar la frecuencia de *Neospora caninum* en dos Unidades de Producción Pecuaria con sistema búfalo/bovino de la zona central del estado de Veracruz, México. **Materiales y Métodos.** Fue un estudio de tipo transversal con muestreo no probabilístico por conveniencia. Se incluyeron dos Unidades de Producción Pecuaria (UPP), ambas con población bufalina y bovina ubicadas en la

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zona central del estado de Veracruz, México. Se incluyó el total de la población bufalina, bovina y canina, tomando muestras sanguíneas entre febrero-marzo, 2019, las cuales fueron procesadas con la prueba comercial ELISA (IDEXX®) y la prueba de inmunofluorescencia indirecta (VMRD, Pullman, WA, USA). En cada UPP, se aplicó una encuesta general y una individual para la colecta de datos. Los resultados se analizaron con el software STATA versión 14.0. **Resultados.** Del total de la población bóvida analizada, 63/138 animales presentaron anticuerpos contra *N. caninum* con frecuencia de 45.6% ($IC_{95\%}$ 37.1-54.3). Con relación a las UPP, la población de búfalos presentó mayor presencia de anticuerpos contra *N. caninum*, 26/31 de la UPP Cotaxtla con 83.8% ($IC_{95\%}$ 66.2-94.5) y 12/22 de la UPP Medellín con 54.5% ($IC_{95\%}$ 32.2-75.6). Con relación a los perros, 4/6 resultaron positivos (66.6%). Se observó que de acuerdo con la especie animal 38/53 búfalos, presentaron la mayor frecuencia con 71.6% ($IC_{95\%}$ 57.6-83.2). **Conclusiones.** Fue comprobada la presencia de anticuerpos contra *N. caninum* en población bovina y bufalina de las UPP estudiadas. Se confirmó que existe una mayor frecuencia de anticuerpos en búfalos de agua cuando cohabitan con bovinos y caninos.

Palabras clave: Búfalo de agua; control; inmunidad; protozoario (*Fuente: MeSH*).

INTRODUCTION

Neospora caninum is a protozoan parasite that can infect a wide range of warm-blooded animals, causing reproductive disturbances in cattle, small ruminants, and some wild species, as well as serious neurological disorders in canines (1). Neosporosis represents the main cause of reproductive failure and abortion in susceptible bovine populations worldwide, causing great economic losses (2). In bovines, the infection is transmitted across the placenta (transplacental), in addition to reducing meat and dairy production (3).

The life cycle of *N. caninum* includes canines as definitive hosts and ruminants as intermediate hosts; a cycle between wild canids, such as coyotes and feral dogs, is believed to play an important role in the biology of *N. caninum* (4). Although exposure to *N. caninum* has been demonstrated in a wide variety of domestic and wild species, viable parasites have only been isolated from a limited number of these, including cattle, white-tailed deer, sheep, and water buffalo (2).

The water buffalo (*Bubalus bubalis)* is currently considered a booming animal throughout the world due to its adaptability and hardiness. Its economic importance lies in the fact that it is a triple-purpose animal since it can produce milk and meat and used as a traction animal (5). The number of water buffaloes in the world has increased rapidly in recent decades and, according to statistics from the Food and Agriculture Organization of the United Nations (FAO), there are currently around 195 million buffaloes in the world (6). These animals have a great capacity for adaptation and can subsist where other animals cannot, as they have managed to maintain themselves in environments as varied as those of Australia, Brazil, Russia, and Nepal (7), at temperatures ranging from 0 to 45°C, in flat or mountainous terrains, and in temperate, dry, and tropical climates (8).

Water buffaloes are susceptible to infection by *N. caninum*, either naturally or experimentally (9). Thanks to its economic importance in the international livestock industry, different studies have been conducted to determine the seroprevalence of *N. caninum* around the world, and due to the difficulties in diagnosing clinical neosporosis, serological tests are necessary and useful for the detection of antibodies (3).

Although disease has been widely studied internationally in buffalo and cattle separately, there are limited studies on the epidemiological situation when both species share the same space. In Mexico, few studies have been conducted to determine the epidemiological situation of *N. caninum* in buffaloes, and so far, there are no reports of this agent in Livestock Production Units (LPUs) where both species coexist. In this context, the objective of this study was to determine the frequency of *Neospora caninum* occurrence in two Livestock Production Units with a buffalo/bovine system in the central zone of the state of Veracruz, Mexico.

MATERIALS AND METHODS

Type of study. This study is a cross-sectional study with non-probability sampling for convenience.

Location. Two Livestock Production Units (LPUs) with bovine and buffalo populations were included. The LPUs were located in the central zone of the state of Veracruz, Mexico. The LPU1 was located in the municipality of Cotaxtla (18° 53'20"N 96° 09'29"W), at an elevation of 40 m above sea level, with a warm-dry-regular climate with an average temperature of 26°C and an average annual rainfall amount of 1,900 mm (10). The LPU2 was located in the municipality of Medellín (19°01'01"N 96°09'59"W) at 52 m above sea level, with a hot-humid-extreme climate with an average temperature of 25.3°C and an average annual rainfall amount of 1,417.8 mm (10).

Herd characteristics. In LPU1, cattle and water buffalo were kept in extensive pastures with Para grass (*Urochloa mutica*), Mombaza (*Panicum maximum*), and native grasses, with monthly rotation for cattle; buffaloes remained in the same paddock for periods of four to six months. Cattle were subjected to a vaccination program (twice a year, Bacterin 11) against *Clostridium chauvoei, Clostridium septicum, Clostridium novyi, Clostridium sordellii, Clostridium perfringens type C, Clostridium perfringens type D, Pasteurella multocida type A, Pasteurella multocida type D, Mannheimia haemolytica serotipo A1, Histophilus somni (Haemophilus somnus*), Vías, Bio Zoo, Jalisco, Mexico), and annual deworming (Ivermectin, Boehringer Ingelheim, Jalisco, Mexico; Albendazol, Zoetis, CDMX, Mexico).

In LPU2, bovids (cattle and water buffalo) were kept in extensive pastures with star grass (*Cynodon plectostachyus*) and native grass with monthly rotation; their diets were supplemented with corn waste (corn) and mineral salts. Only cattle underwent the vaccination and deworming program as described above.

Sampling. In both LPUs, the total buffalo, bovine, and canine population was included at the time of sampling. In LPU1, 31 buffaloes were included (3 males and 28 females aged 4 to 120 months, all of the Murrah phenotype), along with 50 cattle (4 males and 46 females aged 0.3 to 132 months, all of the cross phenotype) and four canines (2 females and 2 males from 3 to 36 months of the Creole phenotype). In LPU2, 22 buffaloes were sampled (8 males and 14 females aged three to 96 months, all of the Murrah phenotype), 35 bovines (7 males, 28 females, aged 1 to 121 months, all of the Beef master phenotype), and two canines (1 male, 1 female, aged 24 and 36 months, of the Creole phenotype). Blood samples were taken between February and March 2019 with vacuum equipment without anticoagulant by puncturing the jugular, caudal, or cephalic veins according to the species. For serum separation, the samples were centrifuged at 1,000 g for 10 minutes and stored at -20°C until further analysis.

Serological analysis. For the serological diagnosis in bovines and water buffaloes, we used the enzyme-linked immunosorbent assay (ELISA) for the identification of IgG antibodies against *N. caninum*, applying a commercial kit from the IDEXX®-Laboratories (sensitivity 98.6% and specificity 98.9%), following the manufacturer's specifications. The plates were read with a spectrophotometer of the Bio-Rad® model 680, and optical density and wavelength were measured with a 650-nm filter. In the case of canine sera, a commercial package of the indirect immunofluorescence test (IFA, VMRD, Pullman, WA, USA) was used following the manufacturer's recommendations.

Variables. In each LPU, a general survey and an individual survey were applied to collect data such as species, age, sex, body condition (where 1 is emaciated and 5 obese), born in the LPU, vaccination, water source (current or dam), and density (animals/hectare), among others. The variables taken into account have been mentioned as risk factors for the presence of antibodies anti-*N. caninum* (1,9,12).

Statistical analysis. The results were analyzed using the STATA software version 14.0. The frequency was calculated, and the association of infections with general data from LPU, bovines, and buffaloes was analyzed via X^2; in addition, the 95% CI confidence intervals were calculated. Values of $p< 0.05$ were considered statistically significant. A ranch was considered positive when at least one animal presented antibodies against *N. caninum*.

Ethical aspects. The work protocol was approved by the Bioethics and Animal Welfare Commission of the Faculty of Veterinary Medicine and Zootechnics of the Veracruzana University, in accordance with NOM -062-ZOO-1999 (11).

RESULTS

Antibodies were detected in both LPUs studied and in the three species analyzed. Table 1 shows that of the total population analyzed in both LPUs, 63/138 animals presented antibodies against *N. caninum*, with a frequency of 45.6% (95% CI, 37.1–54.3). In LPU1, located in Cotaxtla, 40/81 animals were seropositive, with 49.3% (95% CI, 38.1–60.6), and in LPU2, located in Medellin, 23/57 animals were seropositive, with 40.3% (95% CI, 27.8–54.1). The buffalo population showed the highest presence of antibodies against *N. caninum*, 26/31 from the LPU Cotaxtla with 83.8% (95% CI, 66.2–94.5) and 12/22 from the Medellin LPU with 54.5% (95% CI, 32.2–75.6).

Table 1. Frequency of *Neospora caninum* occurrence in cattle and water buffaloes for each Livestock Production Unit (LPU).

Variable	n	+	F%	*95% CI	X^2	P
LPU 1 Cotaxtla					23.8	0.00
Cattle	50	14	28.0	16.2–42.4		
Buffalo	31	26	83.8	66.2–94.5		
Subtotal	81	40	49.3	38.1–60.6		
LPU 2 Medellin					2.99	0.08
Cattle	35	11	31.4	16.8–49.2		
Buffalo	22	12	54.5	32.2–75.6		
Subtotal	57	23	40.3	27.8–54.1		
Total	**138**	**63**	**45.6**	**37.1–54.3**		

CI = Confidence Interval. +:Positives; F%:Frequency (%).

Regarding the canines, 4/6 were positive (66.6%) by the IFI test. Two puppies and one adult female presented antibodies within LPU1, while one adult male was positive to the same test in LPU2.

Table 2 shows that, according to the animal species, 38/53 buffaloes presented the highest frequency, with 71.6% (95% CI, 57.6–83.2). Animals at ages of 1–24 and 73–96 months showed a high presence of antibodies against *N. caninum*, namely 52.5% (95% CI, 36.1–68.4) and 50% (95% CI, 28.2–71.7), respectively. Regarding the sex of the animals, even when only males, such as breeding bulls, were sampled, 7/13 were seropositive, with 53.8% (95% CI, 25.1-80.7). For females, 56/118 were responsive, with 47.4% (95% CI, 38.1–56.8). According to the Body Condition Score (BCS) of the animals, the frequency ranged from BCS1 to BCS3 for 12.5, 50.0, and 42.1%, respectively.

Table 2. Frequency of *Neospora caninum* occurrence in cattle and water buffaloes according to species, age, sex, and body condition score.

Variable	n	+	F%	*95% CI	X^2	P
Species					23.5	0.00
Buffalo	53	38	71.6	57.6–83.2		
Cattle	85	25	29.4	20.0–40.2		
Age (months)					3.1	0.67
1–24	40	21	52.5	36.1–68.4		
25–48	28	11	39.2	21.5–59.4		
49–72	35	15	42.8	26.3–60.6		
73–96	22	11	50.0	28.2–71.7		
97–120	11	5	45.4	16.7–76.6		
>121	2	0	0	0–84.1		
Sex					1	0.3
Male	13	7	53.8	25.1–80.7		
Female	118	56	47.4	38.1–56.8		
Body Condition Score (BCS)					4.4	0.1
2	8	1	12.5	0.3–52.6		
3	92	46	50.0	39.3–60.6		
4	38	16	42.1	26.3–59.1		
Total	**138**	**63**	**45.6**	**37.1–54.3**		

CI = Confidence Interval. +:Positives; F%:Frequency (%).

Table 3 shows the results in relation to whether the animals were born in the LPUs or not; the highest frequency was presented in those born in the LPUs 44/86, with 51.1% (95% CI, 40.1–62.1). Animals that were not vaccinated (41/61) had the highest frequency of *N. caninum*, namely 67.2% (95% CI, 53.9–78.6). According to whether the animals frequent and / or drink dam water, 38/53 animals do so, representing 71.6% (95% CI, 57.6–83.2). Finally, the animals that live with a density of 1.2 animals per hectare presented the highest seropositivity, with the 83.8% (95% CI, 66.2–94.5).

Table 3. Frequency of *Neospora caninum* occurrence in cattle and water buffaloes according to birth place, vaccination, water source, and density.

Variable	n	+	F%	*95% CI	X^2	P
Born in the LPU					2.7	0.0
Yes	86	44	51.1	40.1–62.1		
No	52	19	36.5	23.6–51.0		
Vaccination					20.4	0.0
Yes	77	22	28.5	18.4–40.0		
No	61	41	67.2	53.9–78.6		
Water source					23.5	0.0
Dam	53	38	71.6	57.6–83.2		
Stream	85	25	29.4	20.0–40.2		
Density (animal/hectare)					25.1	0.0
1.2	31	26	83.8	66.2–94.5		
3	57	23	40.3	27.5–54.1		
4	50	14	28.0	16.2–42.2		
Total	**138**	**63**	**45.65**	**37.1–54.3**		

CI = Confidence Interval. +:Positives; F%:Frequency (%).

DISCUSSION

Based on previous studies, buffaloes show a higher *N. caninum* prevalence than cattle, mainly when both species coexist and/or interact frequently (9,12). This is consistent with the frequencies found here and the management system of both LPUs considered in this study. The frequency for LPU1 is comparable to the highest reported one (Australia, 88.3% of seropositive animals) in a study in which both species were included (13). However, our values are well above those reported for other parts of the world, such as China (0%), the Philippines (3.8%) (9), or Vietnam (1.5%) (9).

For Mexico, so far, there is only one report of serum prevalence in buffaloes, with 24.3% in the state of Veracruz (14). With respect to cattle, the seroprevalence found in both LPUs was higher than that reported for Mexico since few studies have been carried out under similar conditions. Among them, a 26% general prevalence has been reported for the state of Veracruz, including dairy cattle, beef cattle, and their crosses (15), and 20.8% in dual-purpose cattle (16). Likewise, in the southeast, a percentage of 11.6% has been reported for beef cattle (17). When analyzing the afore mentioned studies from Mexico, it is possible to consider neosporosis as an endemic issue, at least in cattle.

However, the different seroprevalences, seropositivity, or frequencies reported, whether referring to cattle, buffalo, any host of *N. caninum*, or another etiological agent, should be carefully reviewed and compared since many of the indirect tests used have not been standardized. For example, breakpoints are arbitrarily chosen, have been validated in different hosts, are based on the use of recombinant proteins, or different tests have been used (9). The only studies that should be compared are those that show high similarity in the variables studied, as well as homogeneity in the population and in the techniques used (18).

If these issues are taken into account, the present study has many variables similar to those of the 2017 study of buffaloes in Mexico (14), among the most noteworthy are the diagnostic technique used and the chosen population. In both studies, the ELISA test was chosen and animals of all ages were included, which can reduce bias since in some studies, only adult animals have been considered (12). The probability of presenting antibodies against *N. caninum* increases over time (9), and at an older age, the possibility of infection, reinfection, or reactivation increases (18). The difference between the seroprevalences described may be related to horizontal transmission due to the consumption of contaminated pastures and water mentioned here (9). Another reason for these differences might be that in our study, the total number of animals within the LPUs was considered, whereas in the other study, only a sample from each heard was used. In addition, in our study, cattle coexisted closely with buffaloes in both LPUs, which has a positive influence on the presence of antibodies.

Few studies have jointly included buffaloes and cattle. The similarities with the Australian study (13) are also noteworthy since in Mexico, it is a common practice to keep both species on the same pastures due to difficulties in keeping them separate. The described seroprevalence values of 88.3 and 31.8% in Australian buffaloes and cattle, respectively, are close to those found here in both LPUs using the same serological test, although it differs greatly in the number of herds and animals sampled.

In this study, we included canines and their serological diagnosis since they are host of *N.*

caninum (1,4), which should be considered in any study regarding neosporosis and *N. caninum* in cattle. Most of the previous studies only mention the presence of canines within the farms and, although this has been referred to as a risk factor (19), it contributes to the spread of infection or disease (1) and actively participates in domestic and wild cycles (4); however, serological analysis of canines is generally omitted.

The frequencies of 75 and 50% in the canines belonging to LPU1 and LPU2, respectively, indicate contact with the agent. The young age of the puppies present in LPU1 suggests a vertical infection; however, the detection of antibodies is not indicative of active infection at the time of sample collection, and the detection of the agent or its DNA is necessary to demonstrate the infection (20). Considering the difficulties in controlling feral or wild canines within the pastures and the high seropositivity in buffaloes and bovines, the existence of a wild cycle in this production system can be suggested (13). On the other hand, the feeding practices of canines in both LPUs is closely related to the possibility of seropositivity within the herd because of the possible consumption of aborted fetuses and/or placentas (18), to which canines have free access in this area.

The climatological conditions characteristic of the area can also contribute to seropositivity. High temperatures and rainfall seem to have a beneficial effect on the sporulation of *N. caninum* oocysts and their dissemination. In addition, these conditions promote the proliferation of fungi and bacteria, which affect livestock and weaken the immune system, which can lead to a reactivation of the infection in chronically infected animals (18).

Another risk of infection repeatedly identified in serological studies is sharing drinking fountains with other species as well as the origin of the drinking water (21), which may be related to horizontal transmission when water contaminated with sporulated oocysts of *N. caninum*, excreted by domestic or wild canines, is consumed (13,21).

The preference of buffaloes for flood areas means that oocysts can be carried away by water currents and are concentrated in resting and bathing sites, multiplying the risk of consuming them with water (13). On the other hand, buffaloes have a high possibility of presenting antibodies anti-*N. caninum* 3–7 times higher than bovines, and they are resistant to neosporosis, with fewer clinical signs of the disease (9). This phenomenon may be related to the hardiness of the buffalo, its immune system, age, route of infection, and external factors such as feeding and handling as well as infecting dose or pathogenicity (1). Although buffaloes have co-evolved with the parasite and have developed a certain tolerance (13), however, despite their apparent resistance to the disease, it has been possible to experimentally infect females, causing abortion in them (22,23), and the DNA of the parasite was detected in buffalo fetuses (24).

It has been repeatedly described that *N. caninum* can be transmitted across the placenta, vertically, or congenitally from an infected mother to her fetus throughout pregnancy, which is the main route of transmission of infection. This protozoan parasite is one of the organisms with the highest capacity for vertical transmission in cattle; up to 95% of calves can be born vertically infected in the same herd, and the parasite can pass from mother to fetus over several generations. Infected females will be positive throughout their lives, being more likely to have abortions when they become adults; abortions and infection can be repeated in the same animal, and a high percentage of calves born from seropositive females is clinically normal but may present antibodies against *N. caninum* before taking colostrum (1,18). The high serological frequency in buffalo cattle increased the probability of horizontal transmission within both LPUs, which is another reason why the frequency of antibodies is higher in buffalo cattle in this type of livestock production system. Since dairy cattle are generally kept at high population densities, prevalence and seroprevalences higher than 80% have been reported (26). Ranches with a high number of cattle can use feeding systems that increase the risk of horizontal transmission, such as grazing, through the consumption of food and water contaminated by definitive hosts in regions with a high presence of wild canines (27).

The findings described here provide relevant information on the high detection of antibodies in buffaloes when they coexist closely with bovines and canines, which implies the existence of a wild cycle in this production system. However, it is necessary to expand the number of animals and LPUs included to affirm if such a cycle exists in this production system where the three species coexist in the central zone of the state of Veracruz, Mexico.

In conclusion, the presence of *N. caninum* antibodies was verified in the bovine, buffalo, and canine population of the LPUs studied. We found a higher frequency of antibodies in water buffaloes when they coexisted with cattle and canines, indicating the existence of a wild cycle in this extensive production system.

Conflict of interests

The authors declare that they have no conflicts of interest.

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