



Seroprevalence of *Neospora caninum* infections in cattle in Kirkuk province

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Abstract

Neospora caninum was identified as a coccidian parasite as in 1988, before that date it was misclassified as *Toxoplasma gondii* based on structural similarities. The aim of this study was to determine the seroprevalence of *N. caninum* in cattle in Kirkuk Province. A total of 185 cattle serum samples were collected randomly. Data about ages, gender and abortion history of females were recorded. Serum samples were examined by using ELISA as a screening test for the detection of anti- *N. caninum* IgG antibodies of past infection. Results showed that 53 (28.6%) cattle were seropositive infected, with significantly higher seropositive infection of age group >2 years in comparison to other age groups 94.3%. The distribution of *N. caninum* of total infected cattle showed that the seropositive infection in females were 47(88.7%), which was more than males 6 (11.3%) with a highly significant difference. There were 22(48.8%) aborted cattle out of 47 infected cattle with *N. caninum* with no significant difference in comparison with the healthy group.

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Introduction

Neospora caninum is a protozoa coccidian parasite, obligatory intracellularly (order Eucoccidiorida, suborder Eimeriorina, and family Sarcocystidae), identified by Bjerkas at 1984 and causes abortion in cattle and dogs with neuromuscular disorder, and cattle are its major intermediate host (1-3). In both milk and beef cattle, *N. caninum* is a cause of abortion. Cows of any age will abort their pregnancy from 3 months of gestation to term and most induced neosporosis abortions occur at a gestation of 5-6 months. Fetuses may die in utero and resorbed, stillborn, wrinkled, autolysis, born alive with clinical signs or born clinically healthy but chronically infected. Epidemic or endemic abortions may occur in neosporosis (4). Neosporosis is the leading cause of reproductive failure in many countries with significant economic losses (5). Neospora congenital transmissions can often happen in the same animal. In livestock, fetal deaths in utero, mummification and birth of infected calves can occur with or without clinical signs. Dairy and beef cattle are also

reported reductions in milk yield and underweight calves (6). Infected dogs in their feces excrete oocysts that are then ingested by intermediate hosts such as horses, goats, sheep and water buffaloes. (7).

There is no data available on prevalence of *N. caninum* in cattle in Kirkuk province. The aim of this study was to determine the seroprevalence of *N. caninum* infections in Kirkuk at the north part of Iraq.

Materials and methods

This study was conducted from 1st September 2018 to 15th October 2019 on 185 randomly chosen cattle from which blood samples were collected by means of disposable syringes. Data about the ages, sex and history of abortion in females were recorded. All specimens were taken to the laboratory immediately. Serum was separated by centrifugation in 1000xg for 10 min. All serum samples were equally divided into two micro-tubes and stored before laboratory testing at -20 °C.

Serum samples were tested by using ELISA (Bio-X Diagnostics) for detection of anti *N. caninum* (IgG) antibodies. Absorbance values above the cut-off level of 0.50 were considered as a positive result. This ELISA test was recorded on sensitivity and specificity 100% and 93%, respectively. The predictive values of the test were 93.75% positive and 100% negative according to the instructions of the manufacturer (Bio-X Diagnostics).

Statistical analysis

Data analysis was done using SPSS Vr.24 program, and t-test and Mont Carlo test (MCP) were used at 5% and 1% levels of significance.

Table 1: Allocation of *N. caninum* IgG in examined groups according to age

Age group (year)	No. of examined cattle		<i>N. caninum</i> -IgG positive		P value
	Count	%	Count	%	
<1	19	10.3	1	1.9	0.02 Significant P value<0.05
1-2	22	11.9	2	3.8	
>2	144	77.8	50	94.3	
Total	185	100	53	100	

Table 2: incidence of *N. caninum* IgG in examined groups according to the sex

Gender	No. of examined cattle		<i>N. caninum</i> -IgG positive		<i>N. caninum</i> -IgG negative		P value
	Count	%	Count	%	Count	%	
Male	16	8.6	6	11.3	10	7.6	0.001 Highly significant P value<0.05
Female	169	91.4	47	88.7	122	92.4	
Total	185	100	53	100	132	100	

Table 3: The aborted cattle among the positive females infected with *N. caninum*

Anti- <i>Neospora</i> Antibodies IgG		Studied groups			Chi-Square (P-value)
		Control	Abortion	Non abortion	
Negative	N	122	0	0	0.1 Non-significant
	%	100.0%	0.00%	0.00%	
Positive	N	0	22	25	
	%	0.00%	48.8%	51.2%	
Total	N	122	22	25	
	%	100.0%	48.8%	51.2%	

Discussion

Neospora caninum is seen as one of the world's most significant causes of abortion in the cattle. There are reports of infection in other animals including sheep, goat, deer and horse (8). Yearly, economic loss due to neosporosis in Australia in beef and dairy cattle is considered 25 and 85 million respectively. The estimation for New Zealand in dairy cattle is 17.8 million. According to Dubey, the loss for California is 35 million but the real economic loss should be more than this estimation (9,10). A study in Iraq on Buffaloes was performed and found about 20 % prevalence

Results

The number of examined age group >2 years was more (144) and significantly higher seropositive than others age groups (94.3%) (Table 1). The distribution of *Neospora* infections according to sex demonstrated that the seropositivity in females 47 (88.7%) were more than males 6 (11.3%), with a highly significant difference (Table 2). There were 22(48.8%) aborted cattle out of 47 seropositive cattle with *Neospora caninum* with no significant difference in comparison with healthy control (Table 3).

of *N. caninum* (7). The prevalence of *N. caninum* was 17.5% in dairy and beef cattle in other Iraqi provinces (8). This result was the first investigation of *N. caninum* among Iraqi cattle in Kirkuk, which indicated that the parasite is present, and at a high level with percentage of 28.6%. The seroprevalence of 28.6% is the highest compared to international studies which reported the seroprevalences of *N. caninum* in cattle between 2.8-50% (11-17). However, found the lowest level in cattle between 32-44.4% (10,18), while in another study, the overall prevalence of *N. caninum* infection was 46.9% (19). Perhaps the difference is due to the geographical area or the surrounding environment and

topographical reasons that affect the development and survival of oocysts (20). Many factors may affect the seroprevalence of parasites including sample size, environmental differences age and breed of cattle (21,22). Infectious materials, like fetuses prone to miscarriage, dead calves and fetal membranes can be essential sources of dogs infected. Thus, the high seroprevalence of neosporosis could be linked to the appearance on the dairy farm of many stray and domestic dogs noticed at the time of the study in Kirkuk city, management systems are also noted to affect breed seroprevalence more than breed related susceptibility to infection (23). The results revealed that females cattle were most infected than males with *N. caninum*, this is what was explained by Olmo *et al.* (24), who reported that females were most infected dairy cattle than males. Also about 22 (48.8%) of infected cattle out of 45 have positive cases with *N. caninum*. Another study reported the most of the injuries that occur in cattle are in females because, tachyziote can penetrate the placenta and cause necrosis of the placental membrane of the cow and causes early miscarriage (25).

Conclusions

According to this study, there are highly *N. caninum* infections among cattle in Kirkuk province and the females are more susceptible to infection than the males. Further studies are warranted to investigate the distribution of *N. caninum* including high female and male cattle number. The study also revealed that the abortion in the cows is a major consequence of *N. caninum* infections which need more investigation of this criterion.

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Conflict of interest

The authors declared that there is no conflict of interest.

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الانتشار المصلي لعدوى *Neospora caninum* في الأبقار في محافظة كركوك

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الخلاصة

تم تصنيف البوغية الكلبية *N. caninum* من ضمن الطفيليات الكوكسيدية سنة ١٩٨٨، حيث أنها كانت تعتبر قبل هذه الفترة ضمن المقوسات الكوندية بسبب التشابه الهيكلي مع المقوسات الكوندية. الهدف من الدراسة هو الكشف المصلي لطفيلي *Neospora caninum* في الأبقار في محافظة كركوك. تم جمع ١٨٥ عينة مصل دم من الأبقار بشكل عشوائي، وتم تسجيل جميع المعلومات حول جنس وأعمار الأبقار وكذلك حالات الإجهاض السابقة في الإناث. فحصت عينات المصل باستخدام تقنية الإنزيم المناعي المتمز للكشف عن الأجسام المضادة (IgG) لـ *Neospora caninum*. أظهرت النتائج أن ٥٣ (٢٨,٦٪) من الأبقار التي تم فحصها هي موجبة الإصابة للطفيلي وكانت الإصابة في الأعمار التي هي أكبر من سنتين أكثر مقارنة مع الأعمار التي هي أقل من السنتين ٩٤,٣٪، كانت نسبة انتشار الإصابة في الإناث ٤٧ (٨٨,٧٪) أكثر من الذكور ٦ (١١,٣٪) مع وجود فرق معنوي كبير. كما أظهرت الدراسة وجود الإجهاض في ٢٢ (٤٨,٨٪) من أصل ٤٧ من الأبقار المصابة بالطفيلي مع عدم وجود فرق معنوي مقارنة مع الأبقار التي لم تسجل فيها الإصابة.