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Seroprevalence of *Neospora caninum* of domestic and stray dogs in Baghdad city

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Abstract

The purpose of this study was N. caninum detection in dogs and determine the effects of sex, age, months and areas on the infection rate in Baghdad by using (Indirect Enzyme-Linked Immunosorbent Assay -iELISA) from January to October (2021).Ninety one blood samples were collected from the stray dogs (50 samples) and domesticate dogs (41 samples). The result of IELISA was recorded overall infection rate 24.17% (22/91). The higher infection rate 34.00% (17/50) was recorded in stray dogs than domestic dogs 12.19% (5/41) and males 29.16% (14/48) than females 18.60% (8/43). A higher infection rate in domestic dogs was recorded in the age group more than 5 years (18.18%), while in the age less than 6 months, there was no infection rate. In stray dogs and domestic dogs a higher infection rate (40.00%) was found in April and May; there was no infection rate (0.00%) in January and February. Between areas, there was a significant ($P \leq 0.05$) difference with Al-Shalaa (36.84%) having the highest infection rate and Plalestine Street has the lowest infection rate (8.33%). Antibodies to N. caninum have been found in the blood of stray and domestic dogs in Baghdad, Iraq.

Keywords: Neospora, dogs, indirect ELISA, Baghdad city.

Introduction

Neospora caninum is an obligate intracellular protozoan parasite that causes illness in puppies; the most common symptom is hind limb paralysis (1). It is a heteroxenous parasite that requires multiple hosts to complete its life cycle, with sexual replication taking place in definitive canid hosts and asexual replication taking place in intermediate hosts. Dogs have been recognized as definitive and intermediate hosts for the parasite; following the intake of parasite-infected tissues, they shed oocysts in their feces and Although herbivores can be infected by ingesting oocysts and ascending transmission can be seen through the placenta, herbivores can also be intermediate hosts -Transplacental transmission (2, 3). Epidemiology of *N. caninum* is poorly understood (4). Neosporosis has been reported in Brazil (5), Costa Rica (6), Mexico (7), Thailand (8), Iran (9) and Iraq (10). Many serological techniques are used for *N. caninum* like IFAT (Indirect Fluorescent Antibody Test), NAT (Neospora Agglutination Test), and ELISA (Enzyme-Linked Immunosorbent Assay) (11). The purpose of this study was to estimate the seroprevalence of the parasite in Baghdad due to a scarcity of knowledge on *N. caninum* serological detection in dogs by iELISA and the influence of several risk factors on the infection rate.

Materials and Methods

Collection of samples:

Ninety one blood samples (5 ml) were collected of different ages and sexes of domestic and stray dogs from different areas

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(Al-Shalaa, Abu Ghraib, Aljihad, Plalestine Street, Algzalia and Al-Salam) in Baghdad city and transported to Baghdad Veterinary Hospital during the period from 1/ January / 2021 until 31/ October / 2021. Blood were collected in sterile tubes without anticoagulant for adequate sera which allowed to clot for about 1-2 hrs. After that, it was centrifuged for 10 minutes at 3000 rpm. The separated sera were stored in 1.5 ml Eppendorf tubes at -20°C until they were used (12).

Indirect ELISA:

A commercially indirect ELISA kit (SUNLONG BIOTECH / China) for the detection of anti-*N. caninum* antibodies, the serum was used as directed by the manufacturer.

Statistical Analysis:

The Chi-square test was used to analyses the data to evaluate the different risk factors, with P≤0.05 and P≤0.01 being considered statistically significant **(13).**

Results

ELISA detected N. caninum IgG antibodies in 24.17% (22/91) of the dogs. The seropositive of *N. caninum* in stray dogs was 34.00% (17/50) and that was greater than in domestic dogs 12.19% (5/41), with a significant difference (*P*≤0.01). (Table,1). Between male and female dogs, there was a significant (*P*≤ 0.01) difference in *N. caninum* rates of infection. A higher infection rate 29.16% (14/48) is recorded in males and the lower infection rate is found in females 18.60% (8/43). (Table,2). In domestic dogs, there was a significant (*P*≤0.05) difference in infection rates between age groups. The age group older than 5 years had the highest infection rate (18.18%), followed by the age groups between 2-5 years (16.16%) and 6 months - 2 year (11.11%), while in the age group under 6 months, there was no infection rate (0.00%) (Table,3). *N. caninum* infection rates were greater in April and May (40.00%) followed by March and June (30.00%), July, August and September (25.00%) and October (11.11%), while there was no infection rate (0.00%) in January and February. (Table,4). A significant (*P*≤ 0.05) difference was observed of *N. caninum* infection rates which differed between study areas. Infection rate was found to be greater in Al-Shalaa (36.84%), while the lower infection rate (8.33%) was found in Plalestine Street. (Table, 5).

Table (1): The percentage of dogs infected with *Neospora caninum*.

Dogs	Number of samples tested	Positive	Percentage (%)
Stray	50	17	34.00
Domestic	41	5	12.19
Total	91	22	24.17
Chi-Square (χ^2)	11.97*		

*** *P* ≤ 0.01**

Table (2) : Infection rates of *Neospora caninum* in dogs by sex.

Sex	Number of samples tested	Positive	Percentage (%)
Males	**48**	**14**	**29.16**
Females	**43**	**8**	**18.60**
Chi-Square (χ^2)	**12.21***		

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* $P \leq 0.01$

Table (3) : Total *Neospora caninum* infection rate in domestic dogs according to age.

Age	Number of samples tested	Positive	Percentage (%)
< 6 months	9	0	0.00
6 months - 2 years	9	1	11.11
> 2-5 years	12	2	16.66
> 5 years	11	2	18.18
Total	41	5	12.19
Chi-Square (χ^2)	12.66*		

* $P \leq 0.05$

Table (4) : *Neospora caninum* infection rate in dogs, month by month

Months	Number of samples tested	Positive	Percentage (%)
January	9	0	0.00
February	9	0	0.00
March	10	3	30.00
April	10	4	40.00
May	10	4	40.00
June	10	3	30.00
July	8	2	25.00
August	8	2	25.00
September	8	2	25.00
October	9	1	11.11
Chi-Square (χ^2)	15.25*		

***NS = Non significant**

Table (5): *Neospora caninum* infection rates in dogs according to areas.

Areas	Number of samples tested	Positive	Percentage (%)
Al-Shalaa	19	7	36.84
Abu Ghraib	18	6	33.33
Al-Salam	17	4	23.52
AlJihad	12	2	16.66
Plalestine Street	12	1	8.33
AlGzalia	13	2	15.38
Chi-Square (χ^2)	12.94*		

* $P \leq 0.05$

Discussion

The result of the present study is showed that the overall seroprevalence for *N. caninum* infection in dogs 24.17% (22/91), which it is higher than that infection rates observed in

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German 7.33% (14), China 15.00% (1), Indonesia 3.40% (15), and 2.22% (16), and 4.86% Iran (9), while it is lower than that recorded in Portugal 32.50% (17), and Italy 32.00% (18), but it is closer to the infection rates in the rural dogs in Korea 21.60% (19) and in Poland 21.70% (20). Many *N. caninum* serological studies have been conducted in other species of animals have been undertaken using various methods and places around the world, but few research have looked into the parasite's spread in Iraq, (10) reported 17.50% of sera were collected from cattle positive in the Al-Muthana and Dhi-Qar Provinces, south Iraq; and the infection rate in sheep was 3.91% in Al-Fallujah District (22); in goats (24) reported an overall prevalence 5.60% in various regions of Wasit Province; in Baghdad city 20.00% of buffaloes were positive by using ELISA test (23); In addition, seropositivity in dogs was largely used as a marker for previous or current parasite contact. Differential seroprevalence in different areas and countries could be due to geographical and climatic circumstances, or (Sensitivity and Specificity) test features, and because different tests and cut-off values were utilized, prevalence based on serological tests could not be compared across nations. (26, 27, 28). The high prevalence, on the other hand, could be explained by the presence of many dogs, which were regarded as the farm's definitive hosts where the sample was taken because of their crucial involvement in the spread and maintenance of the disease in the herds (29). The results of the positive samples of *N. caninum* infection in stray dogs 34.00% (17/50), which is higher than of the domestic dogs 12.19% (5/41) with significant ($P \leq 0.0$) difference, this results are close agreement with (1), who recorded a significantly higher infection rate of *N. caninum* in rural dogs 18.17%, compared of dogs raised in urban areas (11.33%). This intriguing observation could be due to feeding patterns such as eating raw meat carrying parasite cysts, as well as variances in welfare and living conditions. (1). Dogs in both urban and rural settings are likely to become infected by eating raw or badly cooked beef, and vertical transmission from bitches to subsequent litters has also been seen. (35). Dogs in rural / nature conservation regions, on the other hand, are more likely to predate possibly infected small mammals and birds, and they may have access to aborted fetal tissues from cattle, wild ruminants, and wild animal carcasses. (17). The present results shows a significant ($P \leq 0.01$) difference between male and female dogs in the case of *N. caninum* infection, a high infection rate was recorded in males than females with, that results are agreement with the study in China by (1, 9), while in Iraq, the higher infection rate was showed in females than males but without significant difference in water buffaloes (23); Also, disagree with (15) in Indonesia, and in Poland (20). In both horses and camels, the percentage of females infected with Toxoplasma gondii was higher than that of males 21.1 and 20.2%, respectively (21). The more male dogs than female dogs were infected may be related to the roaming of male dogs rather than to a susceptibility differences due to size , sex or hair length (22). The difference in the infection rates between males and females may be related to the levels of sex hormones between both sexes (31). A significant ($P \leq 0.05$) difference in the prevalence of *N. caninum* infection among different age groups. The age group with the highest infection rate was over 5 years, whereas the age group with the lowest infection rate was less than 6 months. The current serological data showed that there is a relationship between serological status and age groups in dogs. The positive seroprevalence of increasing in age above 5 years and this is agreement with results of (1) who recorded the prevalence of infection in dogs increased significantly ($P \leq 0.05$) with increasing the age

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of animal; the highest infection was detected in the age group six-year-old or older dogs, followed in the age group 3–6 year, while the lower infection rate found in dogs less than 3 years. Also, in the other study that conducted in dogs in central Poland by (20), who found the prevalence of infection increase with age, less than 1 year old, 1- 4 years, 5-10 years, and the highest infection rate in the age group more than 10 years. (32) The parasite seroprevalence was found to be substantially greater in sheep and goats beyond the age of 4 years than in younger animals. (10) Suggested that may be due to more ingestion of oocysts (horizontal transmission) that shedding by the final host. According to months, the prevalence of *N. caninum* was higher in April and May followed by March and June, while in January and February, no infection rate was detected. This result close agree with (33) who recorded in the local bread chickens different infection rates in different months which recorded an infection rates in May (25%) followed by April (20%), March (11.1%) and during the December, July and August a same infection rate (5%), in June the infection rate was 9.2%, but there was no infection rate was recorded in January and February. The difference between the present study and the previously reported may be related to the difference in the temperature and humidity. (34) Suggested that an increase in the infection rates in some months or seasons than others due to the horizontal transmission for both intermediate and final hosts. Our study was manifested a significant ($P \leq 0.05$) variation between areas of study. The higher infection rate of parasite is registered in Al-Shalaa area while the lower infection rate (8.33%) was found in Plalestine Street. This difference in the prevalence of *N. caninum* among areas in this study may be related to that the dogs living near or with the domestic animals such as cattle and sheep. In addition, some areas are near to the animal slaughterhouses that agree with (22) who mention that an increase in the infection rate in these areas. The diversity may be due to the difference in the environmental conditions in the areas of the studies, or quality of the tests (sensitivity and specificity) that used for detection of the parasite (35).

Conclusion:

N. caninum is considered spread in Baghdad city in high manner in stray dogs, females, some months, areas and synchronizing with increasing the age of dogs.

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