

Seroprevalence of *Neospora caninum* in Local Breed chickens in AL-Fallujah District, Iraq.**Ayoub Ibrahim Ali Al-Jumaily^{1*} and Haider Mohammed -Ali Al-Rubaie²**¹Department of Parasitology, College of Veterinary Medicine, University of Fallujah, Al-Anbar, Iraq.²Department of Parasitology, College of Veterinary Medicine , University of Baghdad ,Baghdad ,Iraq.*Corresponding author; Ayoub119688@gail.com. Mobile:07802610330Doi: <https://doi.org/10.37940/AJVS.2019.12.2.12>**Abstract**

This study was conducted to study the prevalence of *Neospora caninum* in the local breed chickens in Al-Fallujah district, Iraq. One hundred and eighty (91 males and 89 females) were purchased from four regions (Al-Fallujah Center, Al-Shehabi, Al-Saglawia and Al-Karma) for study the effects of sex, and months in the infection rate. Blood samples collected and sera were isolated for (c-ELISA) during the period from December 2018 to September 2019 . The total infection rate was 9.4% and there was highly significant ($P \leq 0.01$) difference between the females 11.2% (10/89) and males 7.6% (7/91) infections. Regions of Al- Fallujah district showed highly significant ($P \leq 0.01$) difference in the infection rates that were ranged between 4.5% (2/44) in Al-Fallujah Center to 14.5% (7/48) in Al-Shehabi. The highest infection rate was demonstrated in May 25% (6/24) compared with December, July and August that were recorded 5% (1/20) with significant ($P \leq 0.01$) difference ,and no infection rate was recorded in the January and February months. In conclusion (it is the first study that described the infection rate of *N. caninum* in the local breed chickens) in Al- Fallujah district, Iraq.

Keywords: *Neospora caninum*, Seroprevalence, Domestic chickens, Gullas , ELISA.**الانتشار المصلي لطيفيلي *Neospora caninum* في الدجاج المحلي في مدينة الفلوجة. العراق****الخلاصة**

أجريت هذه الدراسة لتحديد مدى انتشار طيفيلي *Neospora caninum* في الدجاج المحلي في مدينة الفلوجة ، العراق. تم شراء 180 طيرا (91 ذكور و89 أنثى) من اربعة مناطق لمدينة الفلوجة (مركز الفلوجة , الشهابي , الصقلاوية والكرمة) لدراسة تأثير الجنس , مناطق الدراسة والاشهر الدراسة في معدل الإصابة. تم جمع عينات الدم وعزل المصل لاستخدامه في فحص مقايضة الممدص المناعي المرتبط بالأنزيم التنافسي (c-ELISA). خلال الفترة من شهر كانون الاول 2018 ولغاية شهر ايلول 2019. بلغ معدل الإصابة الكلي 9.4%. وكانت نسبة الإصابة في الإناث أعلى من الذكور بفارق معنوي كبير في المستوى $P \leq 0.01$ حيث بلغت نسبة الإصابة في الإناث 11.2% (89/10) وفي الذكور 7.6% (91/7). وبينت الدراسة وجود فرق معنوي كبير $P \leq 0.01$ بين مناطق الدراسة حيث سجلت أعلى نسبة إصابة في منطقة الشهابي 14.5% (7/48) وأقل نسبة إصابة سجلت في مركز الفلوجة 4.5% (2/44). ووفقا لأشهر الدراسة فقد بينت النتائج وجود فرق معنوي كبير عند المستوى $P \leq 0.01$ وكانت أعلى نسبة إصابة في شهر ايار 25% (6/24) بالمقارنة مع الاشهر كانون الاول وتموز واب حيث بلغت نسبة الإصابة 5% (1/20). في حين لم تسجل اية إصابة في شهري كانون الثاني وشباط. وحسب معلوماتنا فان هذه الدراسة هي الاولى في العراق التي وصفت انتشار طيفيلي *Neospora caninum* في الدجاج المحلي ، العراق.

Introduction

Neospora caninum is a cyst-forming parasite belong to apicomplexan with a wide range of animals species such as cattle, water buffalo, sheep and birds served as intermediate hosts (1, 2 and 3). Dogs (*Canis familiaris*) is the definitive host of the parasite, sexual phase occurs in their intestine, and discharged oocysts with its feces (4). Also the Australian dingo (*Canis lupus dingo*) (5), coyote (*Canis latrans*) (4) and gray wolf (*Canis lupus*) (6) are served as definitive hosts and can release oocysts in their feces. The presence of dogs and poultry together increases the risk factors for neosporosis (7). They get the food from the soil and become more acquired for infection therefore these birds could serve as good indicator for environmental pollution with oocysts (8). *Neospora caninum* can infect several birds like, chickens (8), magpies (*Pica pica*) sparrows (*Passer domesticus*) (9), buzzard (*Buteo buteo*) (10) and Psittaciformes (11) without showed signs of the disease. Experimentally, chickens and embryonated eggs infected by parasite and transmission of this infection to dogs was confirmed. (12).

Serological testing such as indirect fluorescent antibody test (IFAT), agglutination test and (ELISA) is important techniques for disclose *N. caninum* infection (1, 13, 14 and 15).

In Iraq, there are few reports conducted with this parasite in the livestock animals such as cattle (16), water buffaloes (*Bubalus bubalis*) (17), goats (18) and sheep (19).

However, there is a few data about prevalence of *N. caninum* infection in bird worldwide. There are no any researches before about neosporosis in chickens in Iraq, for that this study was aimed to determine the prevalence of *N. caninum* infection in local domesticated breed chickens (*Gallus gullas domesticus*) in AL - Fallujah District, Iraq.

Materials and methods

Sample collection and area of study

One hundred and eighty wing venous blood samples were collected randomly from the different ages and both sexes of the local breed chickens during the period from December 2018 to September 2019 from different areas (Al-Fallujah Center, Al-Shehabi, Al-Saglawia and Al-Karma) in Al- Fallujah District, Iraq.

The blood samples (about 5 ml each) were collected after cleaning and disinfectant the area by using ethyl alcohol (70%) from the brachial wing vein in plane tube without anticoagulant additive. The samples were transported on cold box to the parasitology laboratory, College of Veterinary Medicine, Baghdad University. placed at room temperature for 1 h, and centrifuged of the clotted blood at 3000 rpm for 10 min. in order to obtain the sera that were stored at -20°C until used for ELISA (20).

“**ELISA:** Sera were examined by c-ELISA according to (*Neospora caninum* competition, ID.VET –France) kit procedure as follows:

1. All the reagents were allowed to come to room temperature ($21 \pm 5^{\circ}\text{C}$) at least 30 minutes before use. And homogenized by inversion.
2. prepare wash solution(1x) by diluting the wash concentrate (20x) to 1/20 in distilled water.
3. 50 μl of dilution buffer¹⁴ was added to each micro well.
4. 50 μl of the positive control was added to wells A1and B1.
- 5.50 μl of the negative control was added to wells C1 and D1.
- 6.50 μl of each sample to be tested was added to the remaining wells.
7. The plate was mixed gently and covered with a cover and incubated for 45 minute (± 4) at 37°C ($\pm 3^{\circ}\text{C}$).

8. The cover was removed and empty the wells. Wash each well 3 times at least 300µl of the wash solution (1x) and avoid drying the wells between washes.
9. Prepare the conjugate 1x by diluting the concentrated conjugate 10x to 1/10 in dilution buffer 12.
10. 100 µl of the conjugate 1x was added to each well.
11. cover the plate and incubate for 30 minute $\pm$ 3 at 21°C $\pm$ 5°C.
12. The cover was removed and empty the wells. Wash each well 3 times at least 300µl of the wash solution (1x) and avoid drying the wells between washes.
13. 100 µl of substrate solution was added to each well, then mixed gently.
14. Cover The plate and incubate 15 $\pm$ 2 minutes at 21 $\pm$ 5 °C in the dark.
15. 100 µl of stop solution was added into each well in order to stop the reaction.
16. Finally, the plate was read and record the optical densities at 450 nm

Calculations:

For each sample calculate the competition percentage (S/N%) by:

$$S/N\% = \frac{OD \text{ sample}}{OD \text{ negative control}} \times 100$$

This formula was applied to obtain the interpretation of the results. Sample presenting a S/N %: Less than or equal to 50% are considered as positive.

- Greater than 50% and less than or equal to 60% are considered as doubtful.

- Greater than 60% are considered negative.

Calculation of results:

Results	Status
$S/N \leq 50\%$	Positive
$50\% < S/N \leq 60\%$	Doubtful
$S/N > 60\%$	Negative

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study percentage. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study (21)”

Results and discussion

Total infection rate

The overall seropositivity for *Neospora caninum* by ELISA was 9.4% (Table, 1, Figure, 1).

Table (1) : Total infection rate of *N. caninum* in local breed chickens by ELISA .

No. of Samples Examined	Positive	Doubtful	Negative
180	17 (9.4 %)	28 (15.5 %)	135 (75 %)

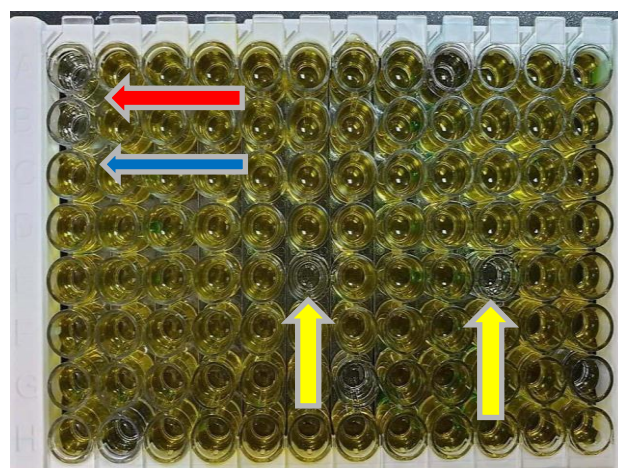


Figure (1): The positive results of sera by ELISA.

Red arrow refers to positive control. yellow arrow refer to positive result Blue arrow refer to negative control.

Several immunological examinations can be used to assess the effectiveness of the immune system in epidemiological studies (22). ELISA test is one of the most used tests to measure the

percentage of immunoglobulin in the serum of birds (23) and has become commonly used for the serological study of *N. caninum*. (24).

Numerous serological study of *N. caninum* prevalence in different livestock have been carried out by using various techniques in numerous areas of the world and very few studies have conducted to estimate the infections by *N. caninum* parasite in Iraq. (16) recorded 17.5% in cattle serum samples in Al-Muthana and Al-Nasseria provinces. Overall infection rate of 20% analyzed by ELISA recorded in water buffaloes (*Bubalus bubalis*) in Baghdad city by (17). In goats (18) reported overall prevalence as 5.6% (6/106) in four different areas of Wasit Province, while in sheep the infection rate was 3.91%, (19). In the results of our study the total infection rate was 9.4 %, it is higher than that recorded in USA 7.2% (25) but lower than that in the Egypt 21.43% (20), in Iran 17.33 % (26) ,in Brazil 23.5% (8), in Mexico 18.5% , in Venezuela 21.7% ,while the infection rate was 58.1% , 62.3% in Argentina and Chile respectively (25). These variations may be due to difference in the activity of the serological tests used, breeds of chickens and the weather factors of each area (27). Also (28) was recorded the presence of more than one dog in the herd as an effect factor for disease. (29) found an association between feeding on moldy maize-silage and the presence of dogs and poultry in the farm result in neosporosis abortion outbreaks in the Netherlands . In Italy (7) showed that the presence of high number of dogs and poultry on farms result in a higher seropositivity in cattle. For that ,specific *N. caninum* antibodies were 21.43% in free-range chickens but were 11.11% in caged chickens this means that free range chickens have more vulnerable to infected by parasite due to contamination the soil with oocysts because off the free range chickens get food from the ground (20) .In Iran (30) has been recorded highly prevalence of infection in pigeons (30.39%) may be related to the pigeons

feed directly from the ground and have more chance to exposed to the parasite oocysts from the soil. On the same hand, wild birds contributed in the prevalence of parasite particularly in field conditions, high seroprevalence levels of antibodies (35.8%) was recorded in common raven (*Corvus corax*) captured from farms (31) and the effect of wild birds as intermediate hosts confirmed by existence of antibodies and DNA in the house sparrow (9). Birds that get the food directly from the soil and water, such as the water birds are capable to eating oocysts discarding from final hosts, and play important role in the transmission of parasite in sylvatic cycle, and the antibody was recorded in (34.3%) in sera of waterfowl (32).

Infection rate according to areas of study.

Results were showed that a significant ($P \leq 0.05$). difference between areas of the study in the infection rate of *N. caninum*. The high infection rate was recorded in Al-Shehabi (14.5%) and the low infection rate was found in the AL-Fallujah Center 4.5% (Table,2).

Table (2): Infection rate of *N. caninum* in local breed chickens by ELISA according to areas of the study.

Areas	No. of samples examined	Positive	Percentage (%)
AL-Fallujah center	44	2	4.5
Al-Karmah	46	3	6.5
AL-Saglawia	42	5	11.9
AL- Shehabi	48	7	14.5
Total	180	17	9.4
Chi-Square (χ^2)	---	---	4.372 *
* ($P \leq 0.05$).			

The study showed a significant difference of prevalence *N. caninum* among different areas in AL-Fallujah district, that was agreed with the previous studies in chickens from Delta region of Egypt Provinces, the prevalence was high (34%) in Qalyoubiya, in Minufiya (17.39%), in Kafr EL-Shaykh (14.75%), and on the other hand, the seroprevalence was very low in Beheira 2% (20) and high in Brazil 36.5% (33). Also the infection rate that recorded by (34) in the sera of eared doves (*Zenaida auriculata*) by ELISA test was 31.7%, with variation in the infection rates due to the presence of definitive hosts in the different areas. (35) mention that the variation in the infection rates in different areas may be due to variation in the areas of the studies and different climatic and geographical conditions or characteristics (Sensitivity and Specificity) of the tests that used. Climatological differences between geographical areas maybe affects the oocysts survival and subsequently the prevalence of disease (36, 37). The variation in the infection rates in different areas in the present study may be due to the methods of chicken rearing and management (free range or closely) and the number of dogs that contamination the water, food or soil by oocysts; also, insect may be contributed in the mechanical spread of these oocysts.

Infection rate according to sex: -

Sex was showed non-significant differences between male and female chickens. A high infection rate (11.2%) was found in females, than males 7.6% (Table, 3).

Table (3): Infection rate of *N. caninum* in local breed chickens by ELISA according to sex.

Sex	No. of samples examined	Positive	Percentage (%)
Male	91	7	7.6
Female	89	10	11.2
Total	180	17	9.4
Chi-Square (χ^2)	---	---	1.744 NS
NS: Non-Significant.			

According to sex, the study showed non-significant difference between females and males in the infection rate. The females showed numerically higher than males which recorded 10 (11.2 %) and 7 (7.6 %) respectively, that is agreed with (20) who showed that an increase in the infection rate in females 284 (16.55 %) than males 77 (11.69 %) in Delta of Egypt. and they disagree with (34) which recorded increase prevalence of parasite in males 33.3% (38/114) than females 30.3% (41/135) in eared Doves in Brazil, the reason for these differences is due to the chickens behavior for males and females or differences in the management.

Our study was showed that both sexes infected with *N. caninum* with variation in the infection rates this may be due to the females has a higher chance for get the infection because of its reared for a longer period, which need more nutrient supplements for eggs producing than males, as well as due to their physiological and immunological status compared to males.

Infection rate according to months: -

The infection rate according to months was showed the highest rate in May (25%) followed by April (20%), March (11.1%), June (9.2%) and in December, July and August had the same

infection rate (5%), but there is no infection rate was recorded in January and February with significant ($P \leq 0.01$) difference (Table,4) .

Table (4): Infection rate of *N. caninum* in local breed chickens by ELISA according to months of the study.

Months	N0. of samples examined	Positive	Percentage (%)
December	20	1	5
January	20	0	0
February	17	0	0
March	18	2	11.1
April	20	4	20
May	24	6	25
June	21	2	9.2
July	20	1	5
August	20	1	5
Total	180	17	9.4
Chi-Square (χ^2)	---	---	9.026 **
** ($P \leq 0.01$).			

Months of the study showed a significant difference in the infection rates of *N. caninum*, the highest rate was recorded in May (25%) followed by April (20%) and March(11.1%), lower infection rate recorded during the December, July and August which had the same infection rate (5%) ,but in January and February there is no infection rate was recorded, these results disagree with (20) who recorded in chicken from Egypt by ELISA test high infection rate in the Spring (22.62%), followed by Autumn (21.28%), Summer (17.54%), and 9.83% in Winter. Similar results were obtained by (38) who demonstrated that the prevalence of parasite in dairy buffaloes (*Bubalus bubalis*) in Pakistan, was closely associated with the seasons as reflected by the highest infection rate in Summer and the lowest in Winter.

The differences in the infection rates in the present study may be related to effects of some environmental conditions (humidity and

temperatures), which are considered an important factor for increase resistant of oocysts in the soil or may be due to the increase the spread of insects, that play an important role as mechanical transmitter vectors for oocysts. Also, it was found that the infection influence by the differences between day and night temperature (39).

Conclusion

In conclusion: Our study recorded for the first time antibodies against *Neospora caninum* in local breed chickens at Al-Fallujah district, with a moderate infection rate that significantly affected by sex, months, and areas.

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