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Serological Detection of *Neospora caninum* in One-Humped Camels of Al-Qadisiyah Province, Iraq

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Abstract

Background: *Neospora caninum* is a protozoan parasite of major concern in the veterinary community due to its significant impact on the health and productivity of livestock.

Aims: The current study insights the first serological detection of *N. caninum* in camels of Al-Qadisiyah province using of indirect ELISA, with estimation the severity of infection and the association of infection to some animal risk factors (age and sex).

Materials and methods: Totally, 184 dromedary camels of different ages and sexes were selected from various areas in Al-Qadisiyah province (Iraq) during April to July (2024), and subjected to sampling of venous blood. After centrifugation, the obtained sera were tested qualitatively using of ELISA.

Results: Totally, 17.93% of study camels were shown a positive reactivity to anti-*N. caninum* antibody. Regarding severity of infection, significant variation was recorded in numerical and quantitative distribution of positive infections. Numerically, significant increases ($p \leq 0.0247$) were shown in mild infection (57.58%) while significant reduction was seen in severe infection (15.15%) when compared to moderate infection (27.27%). Based on the quantity of ODs in positive samples, the findings of mild, moderate and severe infections were 0.282 ± 0.006, 0.407 ± 0.006, and 0.555 ± 0.018, respectively. Concerning the risk factors, the findings of age groups detected that significant highest values of seropositive neosporosis were seen in groups of <1 year (21.43%) and 1-<4 years old (20.25%); while, the lowest value was observed in a camel group of 4-<8 years (13.79%). However, no positive camels (0%) to *N. caninum* infection were identified in a group of ≥ 8 years old. In addition, the findings of Odds Ratio and Relative Risk were increased significantly in <1 year (1.341 and 1.268, respectively) and 1-<4 years (1.316 and 1.254, respectively); while, the lowest was detected in camel groups of 4-<8 years (0.645 and 0.695, respectively) and ≥ 8 years (0 and 0, respectively). The association of sex factor to seropositive values identified that the prevalence rate of *N. caninum* infection is differed insignificantly ($p \leq 0.0998$) between female camels (18.01%) and males (17.39%). However, the results of Odds Ratio and Relative Risk reported that the female camels were significantly at higher risk of *N. caninum* infection (1.044 and 1.350, respectively) than males (0.958 and 0.966, respectively).

Conclusion: This study confirms the occurrence of *N. caninum* in camels of Al-Qadisiyah province (Iraq). However, little attention toward *N. caninum* infection suggesting the greater

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importance of furthermore of serological and / or molecular studies for understanding the epidemiology and possible implication of parasite in health status of different animals, and the role of camels in transmission of infection to other domestic animals.

Keywords: Neosporosis, *Camelus dromedaries*, Dromedary camels, Coccidian parasites, Enzyme-linked immunosorbent assay (ELISA)

Introduction

Neospora caninum is an apicomplexan parasite classified under the Sarcocystidae family. The parasite was discovered firstly by Bjerkas et al. (1984) in dogs having neurological complications and misclassified as *Toxoplasma gondii* due to structural similarities. In 1988, Dubey et al. was the first described *N. caninum* as a species from experimentally infected dogs. Then, *N. caninum* become of major concern in the veterinary community due to its significant impact on the health and productivity of livestock (Al-Gharban et al., 2017; Al-Shaeli et al., 2020). Researchers have been actively investigated the epidemiology of *N. caninum* including its distribution, transmission dynamics, and association of risk factors to infection (Gazzonis et al., 2019; Reichel et al., 2020; Gharban et al., 2022). One of the key areas of focus in the epidemiology is the host range and seroprevalence in different animal populations in addition to genetic diversity and biological properties of different isolates (Anvari et al., 2020; Calarco and Ellis, 2020). Also, molecular epidemiological studies have identified numerous genotypes of the parasite with some genotypes being more commonly associated with particular host species or geographical regions (Khan et al., 2020).

The parasite has a complex lifecycle involves both definitive and intermediate hosts. The definitive host of *N. caninum* is the dogs and the wild canids, in which, the parasite undergoes sexual reproduction and occurs when the dogs ingest the infected tissues that containing the parasite. After ingestion, the parasite reproduces sexually in the dog's intestine, leading to the production of oocysts that are shed in feces. The intermediate hosts include a wide range of domestic mammals such as camel, cattle, goats, sheep, and horses; in which, the parasite undergoes asexual reproduction. Once an intermediate hosts ingest the oocysts, the parasite sporozoites are released and penetrated the intestinal wall to enter the blood streams, and developed rapidly into tachyzoites that transported through the host's body and established an infection in various organs (King et al., 2011; Al-Shaeli et al., 2020; Nazari et al., 2023). The tachyzoites can then transform into slow-growing bradyzoites, which form tissue cysts primarily in the central nervous system and muscle tissues (Jaiswala et al., 2023).

Worldwide, diagnostic approaches of *N. caninum* are necessary for confirmation of infection, effective management and potential control strategies (Reichel et al., 2014). Commercially available serologic techniques as ELISA and immunofluorescence antibody test (IFAT), are generally considered the gold standard, and titers give indication of the likelihood of *Neospora* being responsible for disease rather than simply exposure to the parasite (Chatzis et al., 2014; Campero et al., 2018). ELISA is widespread applied for epidemiological studying of various infections including neosporosis as it commercially existed, rapid, easy to perform, and providing the results automatically within hours. Also, ELISA has a higher level of

specificity and sensitivity when compared to various serologic tools (Lasri et al., 2004; Von Blumröder et al., 2004; Wei et al., 2022). In Iraq, only a recent study has been conducted in camels to detect the prevalence of neosporosis in Wasit province (Gharban et al., 2022). Hence, the current study insights the first serological detection of *N. caninum* in camels of Al-Qadisiyah province using of indirect ELISA, with estimation the severity of infection and the association of infection to some animal risk factors (age and sex).

Materials and methods

Ethical approval

This study was licensed by the Scientific Committee of the Animal Production Department in the College of Agriculture (University of Al-Qadisiyah).

Samples

Totally, 184 dromedary camels of different ages and sexes were selected from various areas in Al-Qadisiyah province (Iraq) during April to July (2024). Each study camel was subjected to collection 5ml of jugular venous blood using a disposable syringe into free-anticoagulant glass-gel tube that transferred cooled in vertical position. At laboratory, all blood tubes were centrifuged (5000rpm, 5 min), and the obtained sera were transferred into 1.5 ml Eppendorf tubes that kept frozen (4°C) until be tested qualitatively by ELISA (Razooqi et al., 2022).

Serological examination

According to manufacturer instructions (SunLong Biotech, China) of the Camel *Neospora caninum* ELISA Kit (Catalogue No: SL0037Cm), the solutions of the kit and the sera were prepared at room temperature, processed step by step, and the optical density (OD) was read at absorbance of 450nm using the Automated Microplate Reader (Agilent Technologies, USA). The test effectiveness critical value (Cutoff) was determined, and the samples were considered positive if their ODs ≥ Cutoff. Also, the positive ODs were divided into three levels according to their severity (Mohammad et al., 2022).

Statistical analysis

One-Way ANOVA, Odds Ratio and Relative Risk in GraphPad Prism Software (version 8.0.1) were served to evaluate link between the prevalence rates of neosporosis and the risk factors (age and sex). Values were reported as either percentage (%) or Mean ± Standard Error (M±SE), and difference between values is considered significant at $p<0.05$ (Gharban and Yousif, 2021).

Results

Among totally 184 animals, the findings of ELISA revealed that 33 (17.93%) were seropositives to *N. caninum*; while, 151 (82.07%) were seronegative (Figure 1). Regarding severity of infection, significant variation was recorded in numerical and quantitative distribution of positive infections. Numerically, significant increases ($p\leq0.0247$) were shown in mild infection [57.58% (19/33)] while significant reduction was seen in severe infection [15.15% (5/33)] when compared to moderate infection [27.27% (9/33)], (Figure 2). Based on the quantity of ODs in positive samples, the findings of mild, moderate and severe infections were 0.282 ± 0.006, 0.407 ± 0.006, and 0.555 ± 0.018, respectively (Figure 3).

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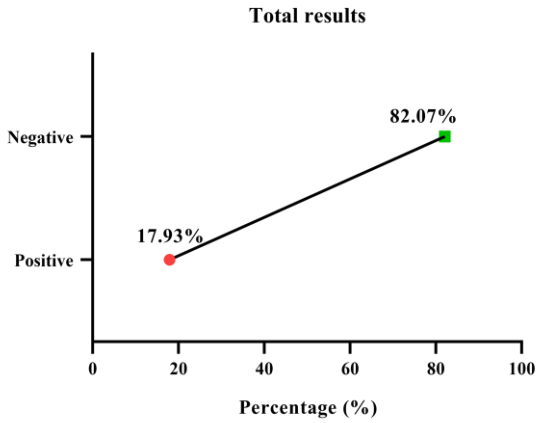

Figure (1): Total results for testing of totally 184 camel's sera by ELISA

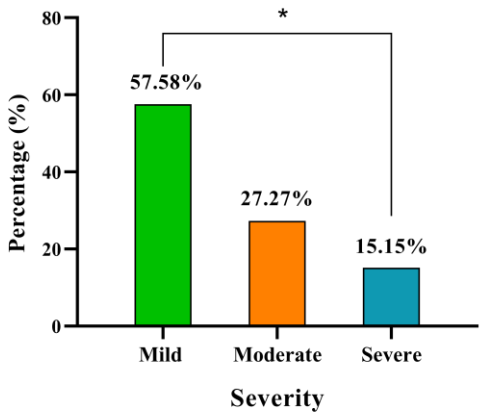

Figure (2): Numerical classification of positive ODs according to severity of infection

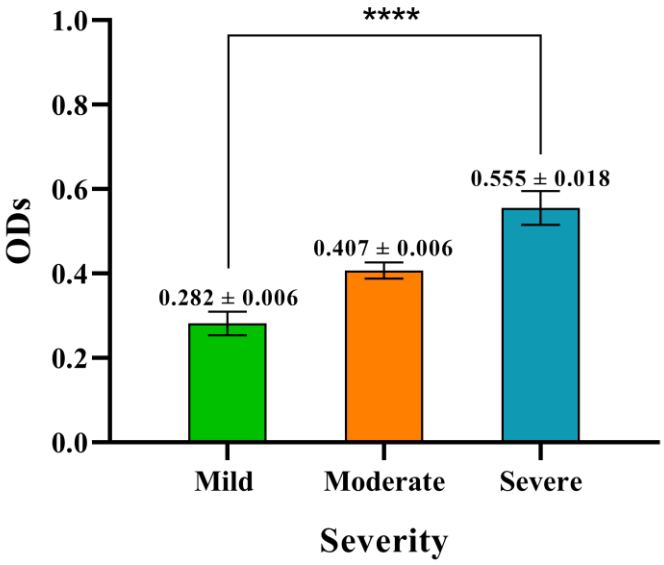

Figure (3): Quantitative classification of positive ODs according to severity of infection

Concerning the age and sex factors, the findings of related groups to each factor were

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recorded a significant variation ($p<0.05$) in their values. In age factor, significant highest values of seropositive neosporosis were seen in groups of <1 year [21.43% (9/42)] and 1-<4 years old [20.25% (16/79)]; while, the lowest value was observed in a camel group of 4-<8 years [13.79% (8/58)]. However, no positive sera to *N. caninum* infection were identified in camels of ≥ 8 years old [0% (0/5)], (Figure 4).

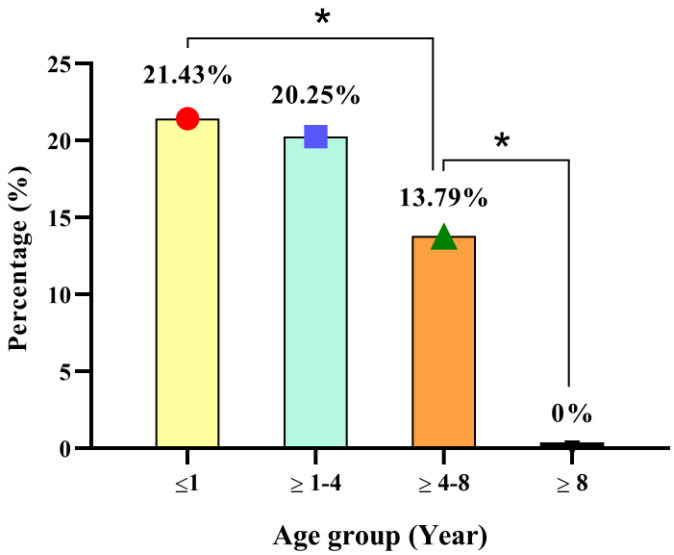

Figure (4): Distribution of positive *N. caninum* infection among the age groups of study camels

Statistical analysis of positive results reported significantly ($p\leq0.0001$) that highest values of Odds Ratio were found in <1 year (1.341) and 1-<4 years (1.316); while, the lowest was detected in camel groups of 4-<8 years (0.645) and ≥ 8 years (0), (Figure 5). Also, the findings of Relative Risk confirmed significantly ($p\leq0.0007$) that age groups of <1 year (1.268) and 1-<4 years (1.254) were at higher risk of *N. caninum* infection than those of 4-<8 years (0.695) and ≥ 8 years (0), (Figure 6).

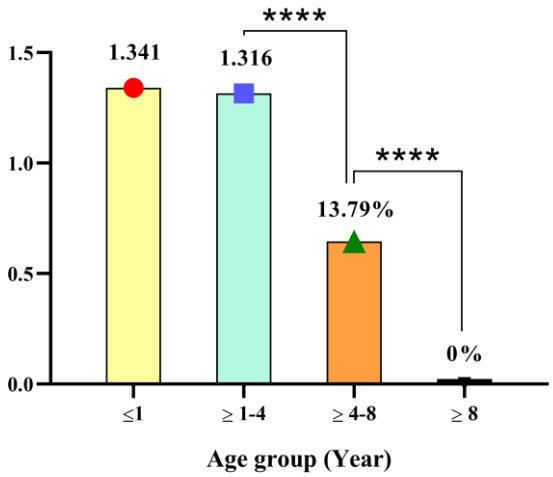

Figure (5): Results of Odds Ratio among the age groups of study camels

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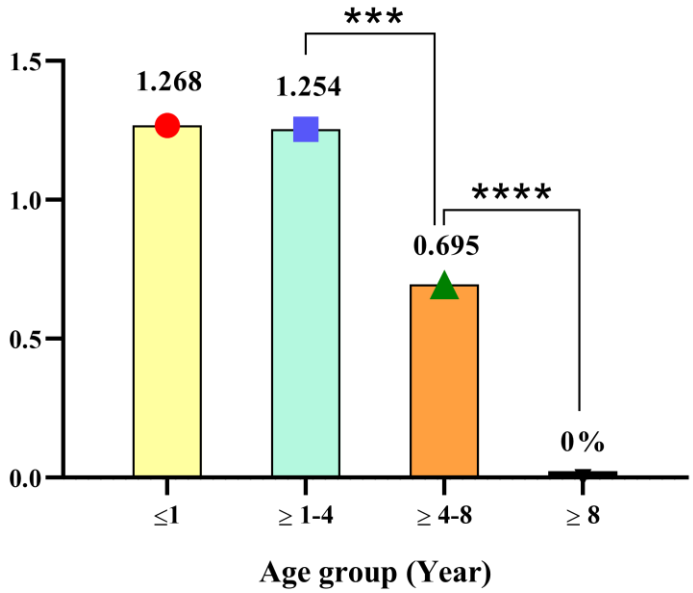

Figure (5): Results of Relative Risk among the age groups of study camels

The association of sex factor to seropositive values identified that the prevalence rate of *N. caninum* infection is differed insignificantly (p≤0.0998) between female camels [18.01% (29/161)] and males [17.39% (4/23)], (Figure 6). However, the results of Odds Ratio and Relative Risk reported that the female camels were significantly (p≤0.0273 and p≤0.008, respectively) at higher risk of *N. caninum* infection (1.044 and 1.350, respectively) than males (0.958 and 0.966, respectively), (Figures 7, 8).

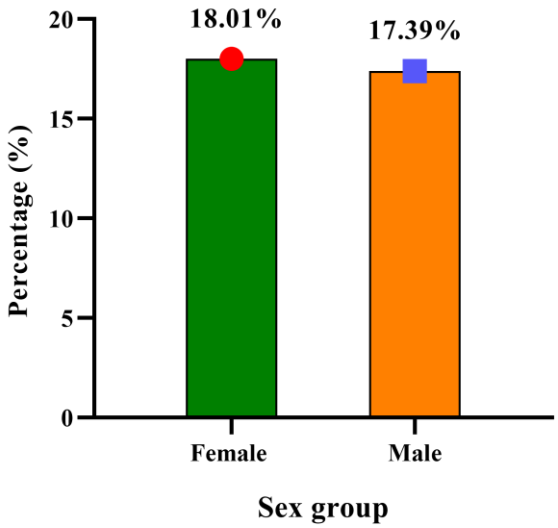

Figure (6): Distribution of positive *N. caninum* infection among the sex groups of study camels

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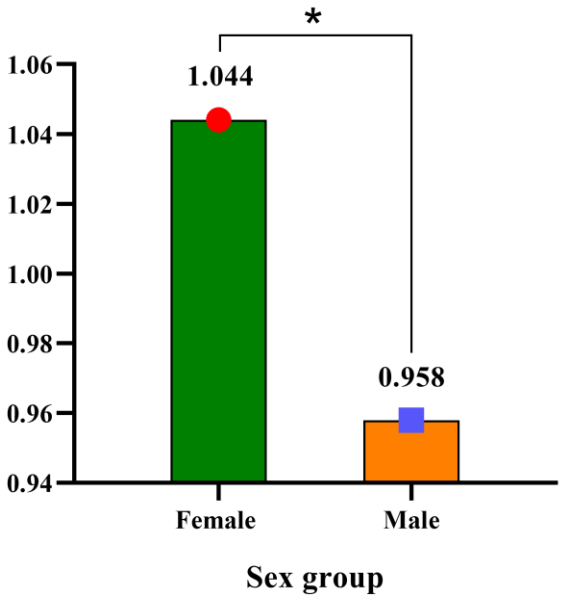

Figure (7): Results of Odds Ratio among the sex groups of study camels

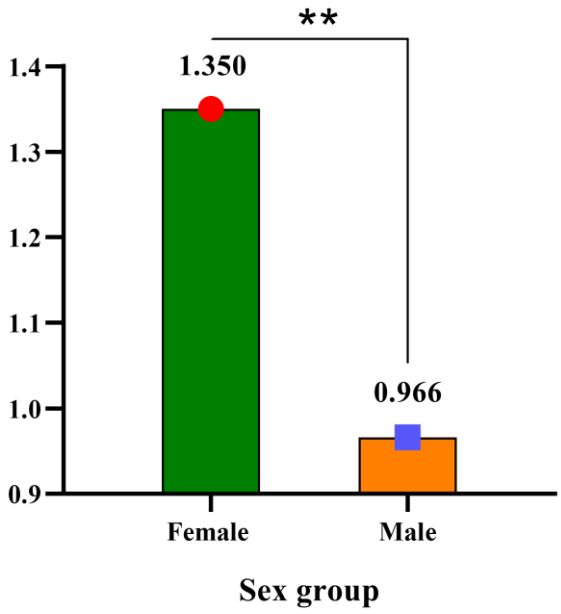

Figure (8): Results of Relative Risk among the sex groups of study camels

Discussion

As with cattle, *Neospora* infection in other animals including camels is usually subclinical (Mahmoud et al., 2024). Also, naturally occurring *Neospora* parasitism, but mostly without reports of disease, have been detected in adult aborted domestic herbivores such as goats, sheep, llama, alpaca, and other several species suggesting that these animals may serve as efficient intermediate hosts of the parasite (Hassan et al., 2021). Our findings showed revealed that 17.93% of study camels were infected with *N. caninum*; in which, mild infection (57.58%) was prevalent significantly while severe infection was reduced (15.15%) in

comparison with the moderate infection (27.27%). The Iraqi researchers found the prevalence rate of *N. caninum* infection in various wild and domestic animals such as dogs (Mallah et al., 2012), buffaloes (Al-Farwachi and Al-Badrani, 2013), horses (Al-Obaidii and Al-Kennany, 2014), cattle (Al-Gharban et al., 2017), goats (Faraj and Ghattof, 2018), sheep (Al-Shaeli et al., 2020), and camels (Gharban et al., 2022). Internationally, *N. caninum* infection in camels was 38.5% in Sudan (Ibrahim et al., 2015), 21.99% in Saudi Arabia (Mohammed et al., 2020), 10.99% in Egypt (Selim and Abdelhady, 2020), and 36% in Iran (Karimi et al., 2023). Although the above mentioned findings weren't comparable due to differences in served serological assays and variation in their Cutoff values; however, result of serological tests provides evidences that several animal species were exposed to *N. caninum* infection. Additionally, differences in study design, analytical methodology, parasite variability, and regional management schemes may play a role in these variations discussed above.

Due to the great effect of risk factors on prevalence rate of neosporosis among various animals existed at several areas and management systems, control planes must always be aimed, identified and take into account parameters. In this study, the findings of risk factors showed that *N. caninum* is significantly prevalent and more risk in camels having <1 and 1-<4 years old which could be attributed to increasing of immunity as a result of the long exposure to infections and the cumulative effect of camel age. Our findings were agreed Chaudhry et al. (2014) in Pakistan but in contrast with those obtained in Egypt by Mahmoud et al. (2024) who reported neosporosis in elderly camels more than small age ones.

For sex factor, although positive values of female and male camels were differed insignificantly, female camels were appeared significantly at higher risk of *N. caninum* infection than male camels. Absence of significance between female and male camels was recorded by Hamidinejat et al. (2013); whereas, Mahmoud et al. (2024) confirmed that female camels were having a greater seropositive rate of neosporosis than males. However, the increasing susceptibility of females to various infections including *N. caninum* might be associated with hormonal changes and immunological reduction at certain periods of their lives in particular at milk production and gestation.

Conclusion

This study confirms the occurrence of *N. caninum* in camels of Al-Qadisiyah province (Iraq). However, little attention toward *N. caninum* infection suggesting the greater importance of furthermore of serological and / or molecular studies for understanding the epidemiology and possible implication of parasite in health status of different animals, and the role of camels in transmission of infection to other domestic animals.

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Conflict of interest

No.

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