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Seroprevalence of *Neospora caninum* antibodies in dogs, cows, and humans in Assiut province, Egypt: a pilot study

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Abstract

Neospora caninum is an intracellular coccidian parasite infecting a broad range of hosts globally. Despite the existence of several epidemiological studies on neosporosis, there is a limited knowledge regarding the prevalence of *N. caninum* infection among dogs, cows, and humans in Egypt. To address this knowledge gap, we conducted an epidemiological investigation in the Assiut province of Egypt to determine the seropositivity of *N. caninum* infection among cows, dogs, and pregnant women, as well as the associated risk factors. By employing an indirect enzyme linked immunosorbent assay, we found specific *N. caninum* IgG antibodies in 6% (6 of 100) and 2.33% (1 of 43) of cows and dogs' sera, respectively. However, we are unable to detect antibodies in the 48 tested human sera. Moreover, no statistical significance was observed among the analyzed risk factors associated with seropositive cows and dogs. Our study highlights the presence of *N. caninum* in cows and dogs in the Assiut province, Egypt. Further, it underscores the need for improved comprehensive surveillance of *N. caninum* infection in a large population of cattle across various locations to obtain a better understanding of the economic burden associated with the disease.

Graphical abstract

Anti- *Neospora caninum* antibodies in Assiut, Egypt

6% (6/100) — cow
2.33% (1/43) — dog
0% (0/48) — human
ELISA
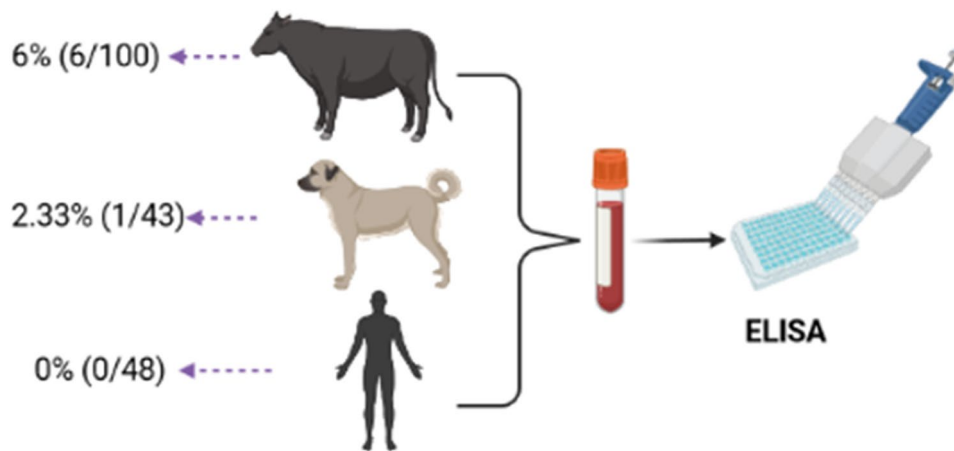
Keywords *Neospora caninum* · Cows · Seroprevalence · Dogs · Egypt

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Introduction

Neospora caninum (*N. caninum*) is a cyst-forming obligate intracellular coccidian parasite closely related to *Toxoplasma gondii* (Dubey and Schares 2011; Abdelbaset et al. 2022). This parasite can infect canids, which serve as both an intermediate and definitive hosts, and natural intermediate hosts such as cattle, horses, water buffalo, bison, goats, and sheep (McAllister et al. 1998; Dubey 2003). Infections can occur through horizontal transmission via consumption of food or water containing sporulated *N. caninum* oocysts, vertical transmission from mother to fetus during pregnancy or via ingestion of tissues containing *N. caninum* cysts (Tranas et al. 1999; Trees and Williams 2005).

Neospora caninum is distributed globally and can cause neuromuscular disorders in young dogs as well as significant economic losses in cattle, due to epidemic abortions, stillbirths, neonatal mortality, and early embryonic losses (Lindsay and Dubey 2020). In fact, neosporosis in cattle has an annual economic impact exceeding 1 billion USD in various countries, including Australia, Argentina, Brazil, Canada, Spain, New Zealand, the United Kingdom, the USA, and Mexico (Reichel et al. 2013).

The epidemiological and zoonotic aspects of *N. caninum* infection in human remain largely unknown, primarily due to the absence of viable *N. caninum* isolated from human tissues. Nevertheless, given that this parasite has a wide variety of intermediate hosts, the possible human infection with this parasite should not be excluded. In contrast to previous reports that showed lack of evidence of human infection (McCann et al. 2008; Robert-Gangneux and Klein 2009; Calero-Bernal et al. 2019), others demonstrated a serological and/or molecular evidence of human infection with *N. caninum* with seropositivity rates of 24.3% (49/201), 8.2% (21/256), 26.1% (81/310) and 6.7% (69/1029), respectively, among the tested population (Tranas et al. 1999; Lobato et al. 2006; Oshiro et al. 2015; Duarte et al. 2020).

In Egypt, two studies surveyed *N. caninum* infection in dogs found that the seropositivity rates were 27% (9/27), and 5.8% (10/172), respectively (El Ghaysh et al. 2003; Salama et al. 2022). Studies on neosporosis in cows have shown a seroprevalence of *N. caninum* infection as 18.9%, 14.89%, 20.34%, and 38.04% (Ibrahim et al. 2009, 2021; Fereig et al. 2016; Gaber et al. 2021), with a single report detecting antibodies to *N. caninum* in 7.92% of pregnant women (Ibrahim et al. 2009). However, the epidemiological status of this parasite in several provinces in Egypt, such as Assiut province, is lacking and requires further investigation. The overall economic burden associated with neosporosis in domestic livestock in Egypt is unknown. Therefore, the current study aimed to identify *N. caninum* specific antibodies in cows, dogs, and pregnant women and investigate the possible risk factors associated with *N. caninum* infection in Assiut.

Materials and methods

Ethics statement

This study was conducted in compliance with the ethical standards of Assiut University institutional ethical committee. The study was approved by the Research Committee of Ethics of Assiut University (Permit number: 6-2023-006). All pregnant women were provided with information about the study objectives, methodology, voluntary participation, and confidentiality of personal information. Due to the cultural context in Egypt, the pregnant women were not able to provide written consent as they were not literate enough to do so. Therefore, verbal consents were taken from all pregnant women prior to the participation. All animals were handled in accordance with the regulatory rules for animal research and informed consent was obtained from cows owners.

Animals and sampling

The present study was conducted between 2019 and 2021 in Assiut province, Egypt. A total of 100 serum samples were obtained from female cows (mean age 5.43 ± 2.1 years), from different villages of Assiut province, living in close contact with dogs. Blood samples were collected by jugular vein puncture using sterile needles and vacutainer tubes. Sample identity and relevant data, such as age, pregnancy status, and abortion history were recorded. Forty-three serum samples were obtained from stray, mostly native dogs from the same area. The age of these dogs was determined by dentition. The relevant data including age, breed, and sex of dogs were recorded. Anesthesia gun was used to anesthetize stray dogs. These guns fire a hypodermic needle, filled with a dose of anesthetic solution (1 mg/kg xylazine 2% (Xyla-Ject, ADWIA Co., Egypt) and 2 mg/kg ketamine 5% (Ketamine, sigma-tec pharmaceutical industries, Egypt) in one syringe, which once injected will temporarily impair the animals' physical functions to a safe level that allows them to be approached without resistance. Blood samples were collected by cephalic vein puncture. Centrifugation of samples were performed at 3000 rpm for 10 min and sera were collected in clean and dry Eppendorf tubes, labeled, and stored at $-20\,^\circ\text{C}$ until use.

Human samples

A total of 48 pregnant women (mean age 25.4 ± 5.6 years) were included in this study. Using a sterile syringe and

needle, 5 ml of blood was taken by an experienced laboratory technician from each study participant. Blood samples were properly handled as described earlier. Participant data including name, age, locality, pregnancy, parity and contact with dogs for each participant were collected. Among those participants, 23 were pregnant women in either the first or second trimester, while 25 were non-pregnant. Furthermore, out of the total participants, 32 women had previous pregnancies and deliveries, whereas 16 had no prior deliveries. A history of abortion was reported in 16 cases. All the cases included individuals who owned pet dogs primarily for the purpose of guarding against intruders.

Serological examination

All sera were tested for antibodies against *N. caninum* by using a commercially available ELISA kit (ID Vet, France, Catalogue no. NCC2P) coated with *N. caninum* antigen. 50 µl of each serum sample as well as the provided positive and negative controls was diluted in 50 µl of sample diluent. The microtiter plate was incubated at 37 °C for 45 min. After three washing steps, 100 µl of the multi-species conjugate was added to each well and incubated under the same conditions. The washing steps were repeated and 100 µl of substrate was added into each well and incubated at 21 °C for 15 min. The reaction was stopped by adding 100 µl stopping solution. The microtiter plate was read at a wavelength of 450 nm. The results were interpreted according to the producer equation:

$$S/N\% = 100 \times \frac{\text{Sample OD}}{\text{Negative control OD}}$$

where OD is the optical density of samples.

Sample of S/P % ≥ 50% is considered a positive sample to *N. caninum* infection.

Statistical analysis

To evaluate the influence of individual factors on the incidence of disease in cows (Age, pregnancy, and abortion history) and dogs (age, sex, and breed), we measured the odds ratio and 95% confidence intervals using GraphPad Prism 5.0 software (La Jolla, CA, USA). We considered *P*-Value of less than 0.05 to be statistically significant.

Results

Screening of sera by ELISA test detected a *N. caninum* antibodies in 6% (6 of 100) and 2.33% (1 of 43) in cows and dogs respectively, while we couldn't detect any antibodies in 48 tested human sera. There was no significant effect ($P > 0.05$) observed for age, sex, and breed of the examined dogs (Table 1). Likewise, the analyzed variables including age, abortion history and pregnancy status of the tested cows were statistically insignificant ($P > 0.05$) (Table 2).

Discussion

Given the scarcity of reports about *N. caninum* in southern parts of Egypt, it is worthwhile to do relevant research to better understand the epidemiological situation regarding the parasite of interest. Our findings revealed a higher seroprevalence in cows compared to the definitive host, dogs (6% vs. 2.33%). Such varied seropositivity rate observed in the same survey area might be attributed to the uncontrolled movement of cows across different Egyptian provinces. The seropositivity rate of neosporosis in cows and dogs detected in our survey is the lowest compared to those reported previously by other Egyptian researchers. For instance,

Table 1 Seroprevalence of *N. caninum* antibodies and the impact of different risk factors in dogs

Factor	No. tested	Seroprevalence		Odds ratio (95% CI)	*P*-value
		Positive no. (%)	Negative no. (%)		
Age					
< 3 years	28	1 (3.57)	27 (96.43)	1.69 (0.06–44.11)	1.00
> 3 years	15	0 (0)	15 (100)	0.59 (0.02–15.43)	
Total	43	1 (2.33)	42 (97.67)		
Sex					
Male	21	0 (0)	21 (100)	0.33 (0.01–8.66)	1.00
Female	22	1 (4.55)	21 (95.45)	3.00 (0.12–77.90)	
Total	43	1 (2.33)	42 (97.67)		
Breed					
Native	39	1 (2.56)	38 (97.44)	0.35 (0.01–9.97)	1.00
Other	4	0 (0)	4 (100)	2.85 (0.10–81.05)	
Total	43	1 (2.33)	42 (97.67)		

Table 2 Seroprevalence of *N. caninum* antibodies and the impact of different of risk factors in cows

Factor	No. tested	Seroprevalence		Odds ratio (95% CI)	*P-value*
		Positive no. (%)	Negative no. (%)		
Age					
1–3 year	13	2 (15.38)	11 (84.62)	Reference	
> 3–6 year	68	2 (2.94)	66 (97.06)	0.17 (0.02–1.31)	0.12
> 6 year	19	2 (10.53)	17 (89.47)	0.65 (0.08–5.30)	1.00
Total	100	6 (6.00)	94 (94.00)		
Pregnancy					
Pregnant	46	4 (8.70)	42 (91.30)	2.48 (0.43–14.19)	0.41
Non-pregnant	54	2 (3.70)	52 (96.30)	0.40 (0.07–2.31)	
Total	100	6 (6.00)	94 (94.00)		
Abortion history					
No abortion	84	5 (5.95)	79 (94.05)	Reference	
Once	12	1 (8.33)	11 (91.67)	1.44 (0.15–13.47)	0.56
Twice	4	0	4 (100)	1.61 (0.08–33.83)	1.00
Total	100	6 (6.00)	94 (94.00)		

Ibrahim et al. (2009) in Northern Egypt found 20.43% in cows, Gaber et al. (2021) in Kafrelsheikh province, Egypt detected 38.04% among aborted cows and 28% among heifers born from seropositive aborted cows, Ibrahim et al. (2021) in Menoufia province (Egypt) detected seropositivity accounted for 12.21% (IgM) and (14.89%) for IgG in examined cows. Additionally, the prevalence rate of *N. caninum* in dogs in the current study was lower than the values (27% and 5.8%) obtained by earlier researchers (El Ghaysh et al. 2003; Salama et al. 2022).

In other countries, *N. caninum* seroprevalence was 36.2% of examined cows in Northeast Algeria (Abdeltif et al. 2022), 37.8% of examined dogs from Argentina (Basso et al. 2001), 8.3% of examined dogs in Brazil (Cañón-Franco et al. 2003), 20% of examined dogs in China (Gao and Wang 2019), 5% of cattle in Southern Ethiopia (Tache et al. 2022), 17.4% and 27% of cattle and dogs, respectively in West of Iran (Gharekhani et al. 2020). The disparity in prevalence rates between studies may be due to variations in sampling time, geographic location, sample size, species examined, and diagnostic test.

In relation to age, no significant relationship was detected between the age of animals (dogs and cows) and *N. caninum* infection rate indicating that all ages were relatively at equal risk of acquiring infection. This result was consistent with many authors (Beck et al. 2010; Gao and Wang 2019; Ibrahim et al. 2021; Tache et al. 2022), however, other studies found that the age of examined dogs and cows has significantly impacted the frequency of *N. caninum* occurrence (de Souza et al. 2002; Haddadzadeh et al. 2007; Abdeltif et al. 2022).

Concerning gender, many previous investigations stated that seropositivity of *N. caninum* was higher among examined female than male animals (de Souza et al. 2002; Fard et al. 2008; Gao and Wang 2019; Gharekhani et al. 2020). Likewise, the present study exhibited a similar result.

In addition, there was no detected significant difference between *N. caninum* infection and breed of dogs. This result agrees with Gao and Wang 2019, in contrast Gharekhani et al. (2020) recorded a significant relation between infection and breed of dogs.

In the present study, a higher seroprevalence was noticed among pregnant cows than non-pregnant, with a higher odds of exposure in pregnant cows. This result approves our statement that neosporosis is an emerging disease and the infected cows either pregnant or non-pregnant act as possible zoonotic risk for humans. The obtained result agrees with Tache et al. (2022).

In our study, four pregnant cows tested positive for *N. caninum* infection; two were in the first trimester of pregnancy, while the other two were in the mid-gestational period. Although seropositive cows have been reported to have twice the risk of abortion compared to seronegative cows during pregnancy, the occurrence of abortion is multi-causal and greatly influenced by the exposure to *N. caninum* and the maternal immune response (Paré et al. 1997).

According to this study, there was non-significant association between seroprevalence and abortion. The attained result consistent with Abdeltif et al. (2022). On contrary, other authors found significant correlation between seroprevalence and abortion (Meenakshi et al. 2007; Beck et al. 2010; Tache et al. 2022). According to this study, only one seropositive cow had a history of abortion, with the hypothesis that *N. caninum* was the absolute cause. Also, our finding informed that most of the seropositive animals were sub-clinically infected and the majority of infections with *N. caninum* among cows were inapparent.

Contradictory data of human infection with *Neospora* are reported in literature. While previous studies demonstrated evidence of human infection with *Neospora* (Tranas et al. 1999; Lobato et al. 2006; Duarte et al. 2020), others detected no evidence (McCann et al. 2008; Robert-Gangneux and Klein 2009; Calero-Bernal et al. 2019). Such controversial reports might be mainly attributed to differences in diagnostic tests and type of population recruited to each of the survey studies. In agreement with the latter studies, importantly, we have not observed any serological evidence of neosporosis in pregnant women. Lack of seropositivity in such a high-risk population group, pregnant women, indicates that human infection with neosporosis is unlikely to happen.

Study limitations

While the current investigation represents the initial exploration of the epidemiological status of *N. caninum* infection in Assiut province across various hosts, the limited number of samples analyzed is inadequate for providing a high-resolution understanding of the epidemiological profile of *N. caninum* infection.

Conclusion

In conclusion, our data provide evidence for the circulation of *N. caninum* among cows and dogs in Assiut province, Egypt. However, the low seroprevalence of *N. caninum* infection detected in the sampled dogs and cows indicates that the risk of infection may be relatively low in this region. Nevertheless, improved comprehensive surveillance and epidemiological studies that investigate a large population of cows at different localities in Egypt are required to better assess the disease burden associated with *N. caninum* infection.

Declarations

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