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Cross-sectional study of seroprevalence and risk factors of *Toxoplasma gondii* and *Neospora caninum* in dromedary camels in two border areas of Egypt

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ABSTRACT

Tissue protozoa play a significant role as a cause of economic losses in reproductive and productive aspects in camels beside the zoonotic importance. A Cross-sectional study was designed to estimate the seroprevalence and risk factors of *Toxoplasma gondii* and *Neospora caninum* in dromedary camels in Matrouh and Aswan border governorates of Egypt. One hundred and eighty-two serum samples from apparently healthy dromedary camels and tested serologically for neosporosis and toxoplasmosis. The prevalence of possible related risk factors was investigated from December 2020 to November 2021. The serological testing of 182 camel serum samples revealed a prevalence (15.93%, 29/182) for *Neospora caninum*, (58.24%, 106/182) for *Toxoplasma gondii* and (9.34%, 17/182) for both infections together. The total seroprevalence rate was (64.84%, 118/182). Camels in Matrouh governorate were at high risk ($p < 0.05$) of infection with tissue protozoa 3.74 times more than camels in Aswan governorate. Maghrabi camels were found more significant ($p < 0.05$) prevalent for T. gondii infection (62/78, 79.49%) by 5.28 times than Sudani camels, Otherwise, in Sudi camels, *N. caninum* is almost 2.70 times Maghrabi camels. The results showed that age and sex were mostly significant for *N. caninum* and *T. gondii*. This study revealed that camels are a possible source of infection for the studied tissue protozoa, some of which are significant for public health. Further research is needed to describe their true situation and epidemiology in dromedary camels.

Introduction

Camelus dromedarius (one-hump dromedary), *Camelus bactrianus* and *Camelus bactrianus ferus* (two-hump bactrian camel are the three species included in the genus Camelus (Kadim *et al*., 2014; Saeed *et al*., 2018). The one-humped camel accounts for around 95 % of Camels from the Old World is found in forty seven countries, mainly in Africa and Asia (FAOSTAT, 2020). Camels are well adapted anatomically and physiologically to thrive in extremely arid conditions where forages and water are rarely found (Parsani *et al*., 2008). In Egypt, camels are implemented on a small scale, while increasing demand for dromedaries meat and milk to overcome shortage in food production with growing population as well as their hide and hair for manufacturing so the country's burdens are fulfilled by importation primarily from Sudan (Badawi, 2018; Ahmed *et al*., 2020).

Camels is known to be affected with many infectious diseases that causes economic losses as bovine ephemeral (Zaghawa *et al*., 2016; Zaghawa *et al*., 2017). On the other hand, paratuberculosis is a significant infectious disease affecting camels causing severe economic losses with intermittent diarrhea which can be confused with tissue protozoa (Housawi *et al*., 2015; Salem *et al*., 2019)

Neospora caninum (*N. caninum*) and *Toxoplasma gondii* (*T. gondii*) are structurally and biologically similar intracellular coccidian parasite (Dubey *et al*., 2017). Severe economic losses caused by tissue protozoa in the dairy industry especially by *N. caninum* worldwide (Nazir *et al*., 2017). They make sever economic losses in the production of livestock through reproductive problems that result in embryonic reabsorption, mummification, abortion, stillbirth, and neonatal losses (Dubey 1999, 2009; Reichel *et al*., 2013; Semango *et al*., 2019). Beside they has public health importance as in *T. gondii* zoonotic transmission accelerated by eating meat or milk (Mosa *et al*., 2015).

N. caninum is an intracellular coccidian parasite that has unknown life cycle but unlike *T. gondii* , the dog played an important role in the life cycle of *N. caninum* (Dubey and Lindsay, 1996). Hilali *et al*. (1998) firstly found *N. caninum* antibodies in camels in Egypt that extends geographic range and host for it, its life cycle includes 3 infectious phases (tachyzoites, tissue cysts, and oocysts), and the intracellular stages observed in the intermediate hosts are tachyzoites and tissue cysts that also contain bradyzoites. Tissue cyst is round or oval in the central nervous system (CNS) and also reported that it found in muscles of cattle and dogs naturally infected and intramuscular tissue cysts have not been yet found in experimentally infected animals (Dubey, 2003).

In Egypt, *T. gondii* infection had higher prevalence in both humans and animals through many serological surveys with the lack of specific clinical signs of disease (Abbas *et al*., 2019). In order to diagnose toxoplasmosis, it is necessary to show either the organism or its antibodies (Manal, 2003). If infection occur in healthy animals; no signs appear (Dubey, 2009). Abortion, and neurological symptoms are mostly observed in intermediate hosts of *T. gondii* (Dubey, 2010). In camels majority of infections is subclinical infection, if clinical signs are present become nonspecific as period of respiratory disorder, fever anorexia and diarrhea (Hanon, 2017).

Enzyme Linked Immunosorbent assay (ELISA) is most commonly used for serodiagnosis of *N. caninum* (Nazir *et al*., 2017) and *T. gondii* (Fatima *et al*., 2019) infections in camels which are naturally infected with both protozoan parasites (Al-Anazi, 2011). ELISA is widely used in screening for clinical and epidemiological surveys of tissue protozoa in domestic animals (Gamble *et al*., 2005; Shaapan *et al*., 2008). The test is effective diagnostic tool in detection and selective diagnosis of them (Mosa *et al*., 2015). Moreover, it is a useful tool for epidemiological studies of tissue protozoa infection in animals and humans (Shaapan *et al*., 2008; Sroka *et al*., 2011). IgM ELISA has been established to detect recent infection but IgG for the old infection (Uroquhart *et al*., 1996). To enable testing of multiple species (cows, camels, sheep and goats), IgG ELISA employed

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protein G horseradish peroxidase conjugate rather as the kit conjugate (Björck and Kronvall, 1984; Abu-Zeid, 2002a; Werre *et al*., 2002; Zhang *et al*., 2010; Schaefer *et al*., 2011).

Some serological assays were applied for identifying *N. caninum* and/or *T. gondii* antibodies in Egypt by using different serological assays in camels, as adopted by several authors (Hilali *et al*., 1998; Toaleb *et al*., 2013; Kuraa and Malek 2016; Ahmed *et al*., 2017; Selim and Abdelhady 2020). Our present study aimed to evaluate *Neospora caninum* and *Toxoplasma gondii* seroprevalence affecting Sudani (imported) and maghrabi (native) camels to know real situation of tissue protozoa affecting camels in Egypt. A cross-sectional study will be conducted to investigate this and identify the pertinent risk variables influencing the seroprevalence of these tissue protozoa in camels.

Materials and methods

Description of the study area

This study was conducted in two border governorates (Matrouh and Aswan) in Egypt (Figure 1). Matrouh governorate is in the northern west (latitude: 31.352778°N and longitude: 27.236111°E), while Aswan governorate in the southern part of the country (latitude: 24.088938°N and longitude: 32.899830°E). Their climate according to Köppen climate classification have a desert condition, with extremely hot and dry summer, warm winter, and rare annual rainfall. In addition, Aswan has the hottest summer days of any city in Egypt. These governorates are considered from the main sources and gates of dromedary camels in Egypt. All investigated live camels in Matrouh governorate were maghrabi camels and living in semi-intensive system (grazing and feeding), While in camels Aswan governorate are Sudani and grazing camels were transported from Sudan.

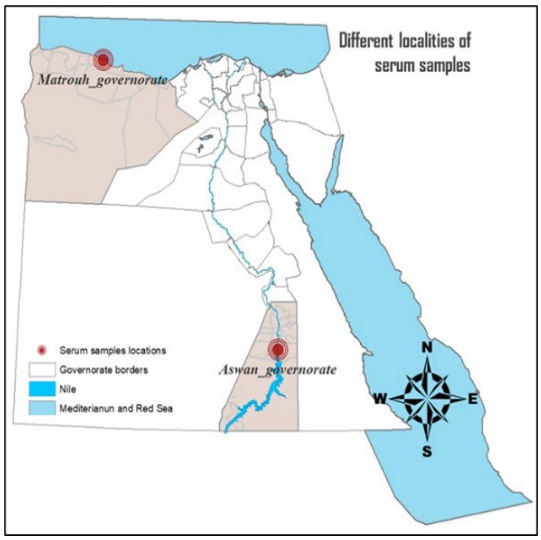

Fig. 1. Collection of serum samples from Matrouh governorate and Aswan governorate for studying tissue protozoa affecting camels.

Ethics approval

All studies have been conducted as per the guidelines of the Institutional Animal Ethics Committee, Department of Animal Medicine and Infectious Diseases, University of Sadat City, Egypt. However, camel species used in this study is reared by farmers. Therefore, use of this animal in research does not require ethical clearance. We have obtained permission from the IACUC, Faculty of Veterinary Medicine, University of Sadat City, Egypt (approval nu/ VUSC-026-1-22).

Animals

A total of 182 apparently healthy dromedary camels of both sexes (71 males and 111 females) and different ages (1- 10 years) were used to collect serum samples during winter and summer seasons. Data collected from owners during sampling included age, sex, breed, grazing and veterinary care for each camel.

Study design and sample size determination

A cross-sectional study was conducted from December 2019 to November 2021in the above-mentioned two governorates, The sample size was calculated using the formula suggested by Thrusfield (2018). n= 1.962 pexp (1−pexp)/d2. where (n) is the number of needed samples and (pexp) is a predicted prevalence of 12.60% (Abdallah *et al*., 2020; Selim and Abdelhady 2020), and (d) margin of error at 95% confidence level of 5% (standard value of 0.05).

Specimen collection and laboratory analysis

One hundred eighty-two blood samples were drawn from the jugular vein and placed in plain vacutainer tubes, then labeled and transferred as fast as possible to laboratory for serum separation. *N. caninum* and *T. gondii* were detected in serum by using camel *N. caninum* ELISA Kit® (Yuhang District, Hangzhou, Zhejiang, China), (Sunlong Biotech Co., Ltd, Catalog No. Sl0037Cm, Lot: 23082152) and camel TOXO-IgG ELISA Kit® (Yuhang District, Hangzhou, Zhejiang, China), (Sunlong Biotech Co., Ltd, Catalog No. Sl0035Cm, Lot: 23082153), respectively.

Data analysis

Data of each camel including locality, season, age, sex, breed, health condition, grazing and veterinary care were received during blood sampling. The relationship between positive samples and various animal traits was determined using a univariate logistic regression analysis model in IBM SPSS statistics for Windows version 21.0. IBM SPSS Inc, Armonk, New York. The relationship between animal characteristics (locality, season, age and sex) and seropositivity to *N. caninum*, *T. gondii* and total infection were examined using a multivariate logistic regression model. Initially, a univariate logistic regression model was constructed to examine the relationship between each animal feature and *N. caninum*, *T. gondii*, and total infection status. The Phi correlation coefficient was calculated to measure the collinear relationship between two attributes. The significance of this collinear association among two of attributes was examined using chi square in the multivariate analysis; $P < 0.05$ was used, and the variable considered most biologically believable was kept. A binary logistic regression model was constructed using all variables that passed the first two steps. In order to keep only variables with $P < 0.05$ in the final model, a manual backward stepwise selection strategy was utilized for the selection of variables in that model. We evaluated each two-way interaction between variables that was kept in the model. By monitoring the change in logit of components after removing a suspected factor from the model, confounder was tested.

Results

Seroprevalence of tissue protozoa in apparently healthy camels

The results showed that, *N. caninum*, T. gondii and both mixed infections were revealed percentages of (29/182, 15.93%), (106/182, 58.24%) and (17/ 182, 9.34%) respectively with total seroprevalence rate (118/182, 64.84%) as mentioned in Table 1.

The effect of locality on the prevalence of *N. caninum* and T. gondii from camels is investigated. The low significant ($p < 0.03$) rate of infection with *N. caninum* was founded in Matrouh governorate (7/78, 8.97%) by 0.37 times (OR= 0.37) (means that prevalence of *N. caninum* are almost in Aswan governorate 2.70 times Matrouh governorate). In contrast the

Table 1. Seroprevalence of tissue protozoa affecting dromedary camels in Matrouh and Aswan governorates.

	N. caninum					*T. gondii*					Mixed				Total			
	+ ve					+ ve					+ ve				+ ve			
	No.	%	P Value	OR	95% CI	No.	%	P Value	OR	95% CI	No.	%	P Value	OR	95% CI	No.	%	P Value	OR	95% CI
Matrouh governorate (78)	7	8.97	0.03	0.37	0.15-0.91	62	79.49	0.05	5.28	2.69-10.36	6	7.69	0.51	0.7	0.25-1.99	63	80.77	0.05	3.74	1.89-7.40
Aswan governorate (104)	22	21.15	-	-	-	44	42.31	-	-	-	11	10.58	-	-	-	55	52.88	-	-	-
Overall (182)	29	15.93	0.01	-	-	106	58.24	0.05	-	-	17	9.34	0.33	-	-	118	64.84	0.05	-	-

N. caninum No.: no. of *N. caninum* only; *T. gondii* No.: no. of *T. gondii* only; Mixed No.: no. of *N. caninum* + no. of *T. gondii* in the same sample; Total No.: no. of *N. caninum* only + no. of *T. gondii* only + Mixed No.
Variables with statistical significance in the univariable analysis (P < 0.05)

high significant (p < 0.05) rate of infection with *T. gondii* was recorded in Matrouh governorate (62/ 78, 79.49%) by 5.28 times (OR= 5.28) compared with Aswan governorate (44/104, 42.31%) as shown in Table 1.

Camels in Matrouh governorate (63/78, 80.77%) were at high risk (p < 0.05) of infection with tissue protozoa 3.74 times (OR= 3.74) more than camels in Aswan governorate (55/ 104, 52.88%) as listed in Table 1.

Risk factors of N. caninum and T. gondii infection in apparently healthy camels

A high significant (p < 0.05) seroprevalence was recorded during summer in *T. gondii* infection (62/64, 96.88%) (OR=52.14), on the other hand, *N. caninum* infection was non-significant highly recorded during winter (22/118, 18.64%). Total seroprevalence of tissue protozoal infection in apparently healthy camels was highly significant (p < 0.05) recorded in summer (63/64, 98.44%) (OR=72.16). All samples collected from camels of different age divided into three groups, first age from one to five years, second group with age more than five years till ten years and third group more than ten years. The results revealed that, the third group in total seroprevalence was most prevalent (37/45, 82.22%) followed by the second group (72/101, 71.29%), then the first group (9/36, 25%). There was a clear positive association between camel age and seroprevalence of tissue protozoa as in first age group was significant (p < 0.05) lower than third age group by 0.07 times (OR=0.07) (means that seroprevalence of total tissue protozoa in third group is almost 14.28 times first age group).

The effect of sex on the seroprevalence of *N. caninum* and *T. gondii* seroprevalence in camels is studied. The female infection rate was significant (p < 0.05) high in T. gondii and total infection (85/111, 76.58%) and (86/111, 77.48%) by (7.78 and 4.20 times) (OR=7.78 and 4.20), respectively. Also, the rate of infection in female was non-significant high in mixed infection (17/111, 15.31%). While the infection rate of male was non-significant high in *N. caninum* infection (11/71, 15.50%).

In this study, Maghrabi camels were found more significant (p < 0.05) prevalent for T. gondii and total infection (62/78, 79.49%) and (63/78, 80.77%) by (5.28 and 3.74 times) (OR=5.28 and 3.74), respectively than Sudani camels. *N. caninum* in Maghrabi camels were lower significant (p < 0.03) infection (7/78, 8.98%) by 0.37 times (OR=0.37) than Sudani camels (means that seroprevalence of *N. caninum* in Sudani camels is almost 2.70 times Maghrabi camels). Sudani camels were non-significant high in mixed infection (11/104, 10.58%). All the previously mentioned results are shown in table (2a and b).

The final model in *N. caninum*, *T. gondii* and total infection was made using 4 variables locality, season, age and sex. The multivariable analysis was shown in Table (3). It is clear that age and sex were mostly significant for *N. caninum*, *T. gondii* and total infection.

N. caninum infection in second age group (p < 0.06) were 3.19 times (OR=3.19) third age group and in females (p < 0.05) were 4.28 times (OR=4.28) males the risk of getting infection.

T. gondii infection in first age group (p < 0.05) are 0.01 times (OR=0.01) third age group (means that seroprevalence of *T. gondii* in third age group is almost 100 times first age group) and in females (p < 0.05) are 22.61 times (OR=22.61) males the risk of getting infection.

Total infection in first age group (p < 0.05) are 0.01 times (OR=0.01) third age group (means that seroprevalence of total infection in third age group is almost 100 times first age group) and in females (p < 0.05) are 24.58 times (OR=24.58) males the risk of getting infection

Discussion

Even in times of drought when other animals are barely surviving, camels can survive, reproduce, produce milk and meat, and work due to their special physiological and anatomical characteristics (Abdalla *et al*., 2018; Abd El-Hamid, 2021).Therefore, this work was designed to study serological analysis of tissue protozoa affecting this neglected animal

Table 2a. Impact of several risk factors on tissue protozoal infection in dromedary camels.

Factor	Variable		*N. caninum*					*T. gondii*				
			+ ve					+ ve				
			No.	%	P Value	OR	95% CI	No.	%	P Value	OR	95% CI
Season	Summer	64	7	10.94	0.18	0.54	0.21-1.33	62	96.88	0.05	52.14	12.15-223.75
	Winter	118	22	18.64	-	-	-	44	37.29	-	-	-
	Total	182	29	15.93	0.01	-	-	106	58.24	0.05	-	-
Age	Young (1- 5 y)	36	1	2.78	0.28	0.29	0.03-2.74	9	25	0.05	0.07	0.02-0.21
	Middle (5 – 10 y)	101	24	23.76	0.04	3.19	1.04-9.83	60	59.4	0.01	0.32	0.13-0.75
	Old (> 10 y)	45	4	8.89	-	-	-	37	82.22	-	-	-
	Total	182	29	15.93	0.01	-	-	106	58.24	0.05	-	-
Sex	Male	71	11	15.5	-	-	-	21	29.58	-	-	-
	Female	111	18	16.22	0.9	1.06	0.47-2.40	85	76.58	0.05	7.78	3.98-15.25
	Total	182	29	15.93	0.01	-	-	106	58.24	0.05	-	-
Breed	Maghrabi	78	7	8.98	0.03	0.37	0.15-0.91	62	79.49	0.05	5.28	2.70-10.36
	Sudani	104	22	21.15	-	-	-	44	42.31	-	-	-
	Total	182	29	15.93	0.01	-	-	106	58.24	0.05	-	-

N. caninum No.: no. of *N. caninum* only; *T. gondii* No.: no. of *T. gondii* only; Mixed No.: no. of *N. caninum* + no. of *T. gondii* in the same sample; Total No.: no. of *N. caninum* only + no. of *T. gondii* only + Mixed No.

Variables with statistical significance in the univariable analysis (P < 0.05)

Table 2b. Impact of several risk factors on tissue protozoal infection in dromedary camels

Factor	Variable		Mixed					Total				
			+ ve					+ ve				
			No.	%	P Value	OR	95% CI	No.	%	P Value	OR	95% CI
Season	Summer	64	6	9.38	0.99	1.01	0.35-2.86	63	98.44	0.05	72.16	9.69-537.66
	Winter	118	11	9.32	-	-	-	55	46.61	-	-	-
	Total	182	17	9.34	0.33	-	-	118	64.84	0.05	-	-
Age	Young (1- 5 y)	36	1	2.78	0.28	0.29	0.03-2.74	9	25	0.05	0.07	0.02-0.21
	Middle (5 – 10 y)	101	12	11.89	0.6	1.38	0.42-4.55	72	71.29	0.16	0.54	0.22-1.30
	Old (> 10 y)	45	4	8.89	-	-	-	37	82.22	-	-	-
	Total	182	17	9.34	0.33	-	-	118	64.84	0.05	-	-
Sex	Male	71	0	0	-	-	-	32	45.07	-	-	-
	Female	111	17	15.31	0.99	$29*10^7$	0	86	77.48	0.05	4.2	2.20-7.99
	Total	182	17	9.34	0.33	-	-	118	64.84	0.05	-	-
Breed	Maghrabi	78	6	7.69	0.51	0.7	0.25-1.99	63	80.77	0.05	3.74	1.89-7.40
	Sudani	104	11	10.58	-	-	-	55	52.88	-	-	-
	Total	182	17	9.34	0.33	-	-	118	64.84	0.05	-	-

N. caninum No.: no. of *N. caninum* only; *T. gondii* No.: no. of *T. gondii* only; Mixed No.: no. of *N. caninum* + no. of *T. gondii* in the same sample; Total No.: no. of *N. caninum* only + no. of *T. gondii* only + Mixed No.

Variables with statistical significance in the univariable analysis (P < 0.05)

Table 3. Results of multivariate logistic regression analysis for identification of risk factors for *N. caninum*, *T. gondii* and total infection.

	Variable	*N. caninum*			*T. gondii*			Total infection		
		P Value	OR	95% CI	P Value	OR	95% CI	P Value	OR	95% CI
Locality	Matrouh governorate	0.99	0	0	0.99	0	0	0.99	0	0
	Aswan governorate	-	-	-	-	-	-	-	-	-
Season	Summer	0.99	$19*10^7$	0	0.99	2.50E+11	0	0.99	2.50E+12	0
	Winter	-	-	-	-	-	-	-	-	-
Age	Young (1- 5 y)	0.18	0.21	0.02-2.08	0.05	0.01	0.00-1.39	0.05	0.01	0.00-0.13
	Middle (5 – 10 y)	0.06	3.19	0.95-10.73	0.98	0.98	0.22-4.20	0.23	2.55	0.55-11.77
	Old (> 10 y)	-	-	-	-	-	-	-	-	-
Sex	Male	-	-	-	-	-	-	-	-	-
	Female	0.05	4.28	1.47-12.41	0.05	22.61	4.7- 107.94	0.05	24.58	2.90-207.95

species and have a zoonotic and economic impact on human from the point of one health. Seroprevalence of *N. caninum* in apparently healthy camels was (29/182, 15.93%) and this percentage agreed with Wernery *et al*. (2008) in United Arab Emirates, Nazir *et al*. (2017) in Pakistan and Mohammed *et al*. (2020) in Saudi Arabia at percentages of 13.70%, 11.10% and 16.60%, respectively. Lower percentages were recorded by Hilali *et al*. (1998) in Egypt, Sadrebazzaz *et al*. (2006); Hosseininejad *et al*. (2009); Hamidinejat *et al*. (2013) in Iran as 3.80%, 5.80%, 3.20% and 3.90%, respectively. Other research showed higher seroprevalence as Mentaberre *et al*. (2013) in Canary Islands in Spain, Ibrahim *et al*. (2014) in Sudan, Namavari *et al*. (2017) in Iran and Selim and Abdelhady (2020) in Egypt at percentages of 86%, 38.50%, 27% and 30.60%, respectively. The variety in seroprevalence rates in different studies based on the serological test, the first serum dilution used, environmental patterns or geographical factors and applied hygienic measures (Hamidinejat *et al*., 2013; Fereig *et al*., 2016; Selim and Ali 2020). Many animal species can be infected by *Neospora caninum* (camels from various global regions) and presence of antibodies against it in camels, suggesting that the parasite has a wider geographic range than previously thought (Hilali *et al*., 1998).

T. gondii Seroprevalence in apparently healthy camels was (106/182, 58.24%) which is nearly similar with Selim *et al*. (2018) in Egypt 46.90%, Ahmed *et al*. (2017) in Kalubiya governorate in Egypt 52.60%. Other research showed higher seroprevalence as Toaleb *et al*. (2013) in Egypt and Kuraa and Malek (2016) in Assiut governorate in Egypt at percentage of 66.70% and 96.40%, respectively. On the other hand lower percentages recorded by Mosa *et al*. (2015) in Najran region in Saudi Arabia, Hanon (2017) in Waist province in Iraq, Tilahun *et al*. (2018) in Oromia Region in Ethiopia, Mohammed *et al*. (2020) in different cities in Saudi Arabia and Abu-Zeid (2002b) in United Arab Emirates at percentages of 21.10%, 23.62%, 26.50%, 14.38%, 34.20% and 22.40%, respectively. The variation between the mentioned seroprevalences can be related to the size of sample (Khalil and Elrayah, 2011), The limitations and sensitivity of serological testing (Dubey 2010; Al-Anazi 2011), Distribution of cats and wild felines (Gebremedhin *et al*., 2014), Stressful factors, shortages of food, and some infections as trypanosomiasis are also issues (Kassa *et al*., 2011), the difference in weather, types of soil (Dubey, 2010) and Livestock management practices could also be considered as mentioned by Dubey and Jones (2008) and Gebremedhin *et al*. (2014). The finding of camel *T. gondii* antibodies reveals that consumption of raw, inadequately cooked meat or raw, unboiling milk from dromedaries may increase the risk of human infection with the *Toxoplasma*. Due to the significant significance of domestic ruminants in parasite transmission, whether via direct contact or consumption of the animal meat products, *T. gondii* infection in camels has grown recently (Abdallah *et al*., 2020).

Higher significant in seroprevalence of *N. caninum* (22/104, 21.15%) was recorded in Aswan governorate than Matrouh governorate (7/78, 8.97%) by 2.70 times. that is related to feeding style of animal, which are free grazing, with repeat exposure of animals with parasite oocyst excreted by infected dogs (Nazir *et al*., 2017). This will increase their susceptibility for infection and also little is known about the frequency of oocyst shedding, the ability of oocysts to survive in the environment or whether other canids serve as permanent hosts for *N. caninum* (Dubey, 2003). Matrouh governorate had a significantly greater seroprevalence of *T. gondii* (62/78, 79.49%) than Aswan governorate (44/104, 42.31%) by 5.28 times. This may be related to the stray cats that entered their environment making them coming into touch with oocysts of *T. gondii* (Lundén *et al*., 1994; Mosa *et al*., 2015; Mohammed *et al*., 2020). Ingestion of contaminated food and water, or inhalation of oocysts produced by infected cats in the environment, could result in exposure. The longer an animal lives, the greater the probability of *Toxoplasma* infection by animals (Hanon 2017).

Camels in Matrouh governorate were at high risk of infection with tissue protozoa 3.74 times more than camels in Aswan governorate. Camel contact with small ruminants is more likely in the Matrouh governorate, which could explain the much higher seroprevalence in Matrouh. This might be explained by the fact that *T. gondii* is most prevalent in small ruminants and keep its bradyzoite for the rest of their lives (Abdallah *et al*., 2020). Again seroprevalence of tissue parasite depend on different animal conditions, such as environmental contributions, management techniques and husbandry factors, expose a variable infection percentage (Hamidinejat *et al*., 2013).

High seroprevalence of *T. gondii* (106/182, 58.24%) compared to *N. caninum* (29/182, 15.93%) in this study may be related to use IgG ELISA for *T. gondii* in which positive IgG indicates infection at least 1 year previously (Mosa *et al*., 2015).

Increased *T. gondii* seroprevalence (106/182, 58.24%) than *N. caninum* (29/182, 15.93%) in this study may be related to use IgG ELISA for *T. gondii* in which positive IgG indicates infection at least 1 year previously (Mosa *et al*., 2015).

A higher significant prevalence was recorded during summer (63/64, 98.44%) than winter (55/118, 46.61%) by 72.16 times. Seasonal variation as temperature and humidity beside contact with animals (infected or healthy) are all possible factors effect on seroprevalence of these protozoa. Jung *et al*. (2014) found that possible decrease of *N. caninum* and *T. gondii* frequency, during cold seasons is due to the less viable oocysts are present in the environment. This result is supported by Ibrahim *et al*. (2021) who said that in cold temperature *N. caninum* and *T. gondii* has lower prevalence and this could be attributed to the temperature's stressful effect on the animals (Selim *et al*., 2018). Moreover Abdollahzadeh *et al*. (2022) reported that seasonal variation of *N. caninum* and *T. gondii* could be linked with environmental factors that have a greater impact on the viability and persistence of oocysts in the environment. Clearly adult camels showed increased significantly seropositive (Nazir *et al*., 2017) that support our finding in this study as high non-significant total seroprevalence in older camels (> 10years old) in total seroprevalence was recorded in percentage of (37/45, 82.22%). Other studies found no relation between camel age and prevalence of tissue protozoa (Hamidinejat *et al*., 2013; Aljumaah *et al*., 2018).

Repeated infection exposure during life may have an impact on how quickly an illness spreads horizontally (Okumu *et al*., 2016; Elhaig *et al*., 2018).

Higher significant seroprevalence of *T. gondii* (37/45, 73.82.22%) in animals > 10 years than in animals from 1-5 years by 14.28 times, that could be attributed to the cumulative effect of camel age (Tenter, 2009; Rouatbi *et al*., 2019). This is due to the lack of consistent culling programs, insufficient veterinary facilities, local owned ground changes and cat keeping to control rats (Fatima *et al*., 2019). Other factor related to age is the immune status of animal which is low in old camels (Zarnke *et al*., 2000). These results corresponds to those of Gebremedhin *et al*. (2014) in Ethiopia and (Hussein *et al*., 1988; Elamin *et al*., 1992) in Kingdom Saudi Arabia, as they reported greater seroprevalence in adult camels compared to young ones. However, these findings contradict those of Elamin *et al*. (1992) and Chaudhry (2014), as they found low seroprevalence in elderly animals compared to small age animal

The effect of sex on the total seroprevalence of tissue protozoa in camels showed higher significant seroprevalence in female (86/111, 77.48%) than male (32/71, 45.07%) by 4.20 times, that agree with Sadrebazzaz *et al*. (2006); Al-Khatib (2011); Mentaberre *et al*. (2013) and Bártová *et al*. (2017). Other researchers found that no significant difference in both sexes of camels as Elamin *et al*. (1992); Hamidinejat *et al*. (2013) and Khamesipour *et al*. (2014). However, male camels had a greater seropositive rate than female camels (Abu-Zeid 2002b). Female susceptibility might be related with decreased immunologic resistance at certain periods of their lives due to female used for dairy production and fertility. Moreover male animals are slaughtered earlier than female so no long exposure to infection (Guimarães *et al*., 2013; Maspi *et al*., 2021) and innate immune responses are higher in males (Fatima *et al*., 2019).

In this study Sudani camels compared with Maghrabi camels for detection of tissue protozoa infection and noted that, Maghrabi (63/78, 80.77%) is significantly higher than Sudani (55/104, 52.88%) by 3.74 times. The high percentage may be related to Maghrabi camels in this work are female used for productive and reproductive propose and these parasites were vertically transmitted to calves and have immunological response with or without clinical signs (Mohammed *et al*., 2020). In addition, they live in semi-intensive system so, several stray dogs and cats capable of contaminating the pipe water source with infective oocysts. Few dogs and cats are suitable to contaminate a wide area in short time, since one infected dog or cat sheds millions of oocysts. Watering source and the storage of animal feeds stored outdoor which are easily to dogs and cats contamination, points that cannot be ignored (Ahmed *et al*., 2017; Tilahun *et al*., 2018).

Conclusion and Recommendation

Antibody prevalence against both *N. caninum* and *T. gondii* in the serum of apparently healthy camels indicated that they are continuous natural infection with these protozoa so further clinical and molecular studies are needed to know the part that may be enacted by camels in the epidemiology of these diseases. It is important to pay attention to camel meat intake, which could be a source of infection for people. As a result, camel meat and other edible portions should be fully prepared before consumption. Owing to the financial losses affecting the camel industry owing to abortion and reproduction failure as well as the public health implications of camel toxoplasmosis, further control programs for camel neosporosis and toxoplasmosis are required.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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