



Detection of Antibodies against *Toxoplasma gondii* and *Neospora caninum* in Dairy Camels from the Khartoum State, Sudan

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ABSTRACT

Toxoplasmosis and neosporosis are important causes of reproductive failure in humans and animals resulting in significant socio-economic losses worldwide. In addition to its importance as food animals for most of the rural areas in the Sudan, camels are recently introduced as dairy animals in some semi-intensive farms in the Khartoum State. The aim of this study was to avail data-regarding the sero-prevalence of *T. gondii* and *N. caninum* infection in camels raised for milk production in the Khartoum State. Two large dairy camel's farms in Eastern Nile and Bahri localities were sampled as well as the co-herded camels with dairy cows in dairy cattle farms in the State. A total of 61 dairy she-camels sera were collected from 13 dairy herds and examined for the evidence of anti-*T. gondii* and anti-*N. caninum* antibodies using the commercially available Latex Agglutination Diagnostic kits (LAT) and competitive Enzyme Linked Immunosorbent assay (cELISA). The study revealed that, the overall seroprevalence of *T. gondii* and *N. caninum* infection in dairy camels at herd level was 76.9% (10/13 herds) and 38.5% (5/13 herds) respectively. Their seroprevalence at individual level was 54.1% (33/61 She-camels) and 9.8% (6/61 She-camels). Interestingly, 9.09% (3/33) of the *T. gondii* seropositive camels showed mix-infection with *N. caninum*. Approximately, 50% of the seropositive camels (16/33) reported an anti-*T. gondii* antibody titration of more than 1:8, and the highest level of antibody titration reported in the present study was 1:32. Interestingly, camels were found to be relatively similar to cattle in the occurrence of *N. caninum* infection in this study, with percent inhibition ranging from 30% to 74%. Generally, this is the first serological evidence of *N. caninum* in Sudanese camels. The study concluded that camels in the Sudan are widely exposed to *T. gondii* and *N. caninum*. Moreover, it is the first report on mix-infection of the two parasites in camels. Thus, based on the Sudanese feeding habitat of camel products, people in the Sudan should be aware of the possibility of hyper-prevalence of human toxoplasmosis through this important food animal. Research on clinical toxoplasmosis and neosporosis in Sudanese camels is recommended to evaluate the role of these parasitic abortifacients in the economical losses in camels industry and for building strategy of sustainable camel management and control.

Keywords: Seroprevalence, Toxoplasmosis, Neosporosis, Camels, Sudan.

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INTRODUCTION

Dairy farms industry suffers major economic losses from reproductive failure. Routine healthy production is very important to sustain the economy of dairy farms (Ortega-Mora *et al.* 2007; Weiss and Kim 2007). Camels are recently introduced as dairy animals in some semi-intensive farms in the city centers of the Khartoum State. Many studies on dairy animal's reproduction stated that, reproductive disturbances are the main cause of infertility (Ortega-Mora *et al.* 2007; Taylor *et al.* 2007). *Toxoplasma gondii* (*T. gondii*) and *Neospora caninum* (*N. caninum*) are morphologically and biologically related parasites. They are the most important causes of protozoal parasitic infectious abortion in livestock worldwide (Dubey and Lindsay 1996; Huong *et al.* 1998; Ortega-Mora *et al.* 2007; Taylor *et al.* 2007; Weiss and Kim 2007; Innes 2011). Until 1988 (Dubey and Beattie 1988) *N. caninum* was misidentified as *T. gondii* (Dubey and Schares 2011; Dubey *et al.* 2002; Dubey and Lindsay 1996). Unlike *T. gondii*, its zoonotic potential is unknown (Dubey and Lindsay 1996) and it can cause repeated abortion (Pabon *et al.* 2007). Animals may acquire infection of *N. caninum* and *T. gondii* through the ingestion of oocysts that are shed in the faeces of infected dogs and cats respectively, or by congenital infection where the parasites pass from mother to foetus via the placenta (Dubey and Schares 2011; Weiss and Kim 2007; Taylor *et al.* 2007; Dubey and Lindsay 1996; Anderson *et al.*, 1997). These parasites were reported to cause abortion, stillbirth and neonatal mortality in farm animals of many countries (Dubey and Lindsay 1996; Hilali *et al.* 1998; Weiss and Kim

2007; Taylor *et al.* 2007; Bishop *et al.* 2010; Manal *et al.* 2013). Seroprevalence of *T. gondii* infection in the worldwide camel's zone is extremely variable (Hussein *et al.* 1988; Hilali *et al.* 1998; Abu-Zaid 2002; and Abu-Zaid *et al.* 2006; Shaapan and fathia 2005; Shaapan and fathia 2008) using various serological tests. However, to our knowledge, the only available serologic reference on camel neosporosis was that of Hilali *et al.* (1998).

Most of the studies on animal toxoplasmosis in the Sudan was conducted in camels (Seri *et al.* 2003; Manal 2003; Manal and Majid 2008; Khalil and Elrayah 2011) using different serological techniques. There is no data available on prevalence of *N. caninum* in Sudanese camels. The first bioassay and molecular study in dogs and mice of *N. caninum* using Sudanese camel's tissues was recently reported by Manal *et al.* (2013). Therefore, the present study was planned to assess the sero-prevalence of *N. caninum* and *T. gondii* infection in dairy camels from the Khartoum State using Competitive Enzyme Linked Immunosorbent assay (cELISA) and Latex agglutination Test (LAT) respectively.

MATERIALS AND METHODS

The study area: Khartoum State -the capital State of the Sudan- is one of the 18 states of the Sudan (Figure 1). The State is located in the central region of the country. It has three districts including 7 localities with an area of 22,122 km². Human population is estimated to be 3512144 with growth rate of 8.9% (The economic Review, 2002). Sudan dairy farms industries of different production systems are concentrated in this State.

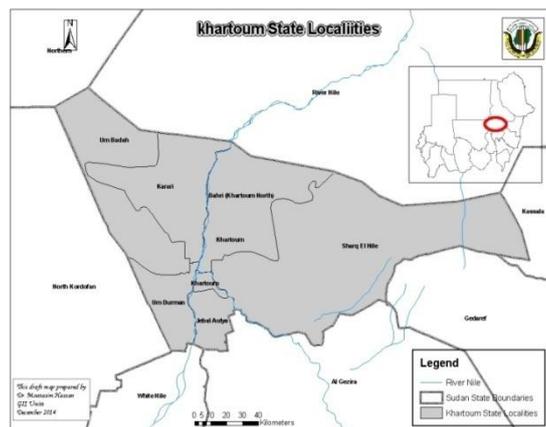


Figure 1: Khartoum State

Camel dairy farms were recently introduced in the State. Out of the 7 localities of the State, three localities (Eastern Nile, Bahri and Jabal Aolya)

in two out of the three districts of the States (Khartoum North and Khartoum) were surveyed in the present study (Figure 2).

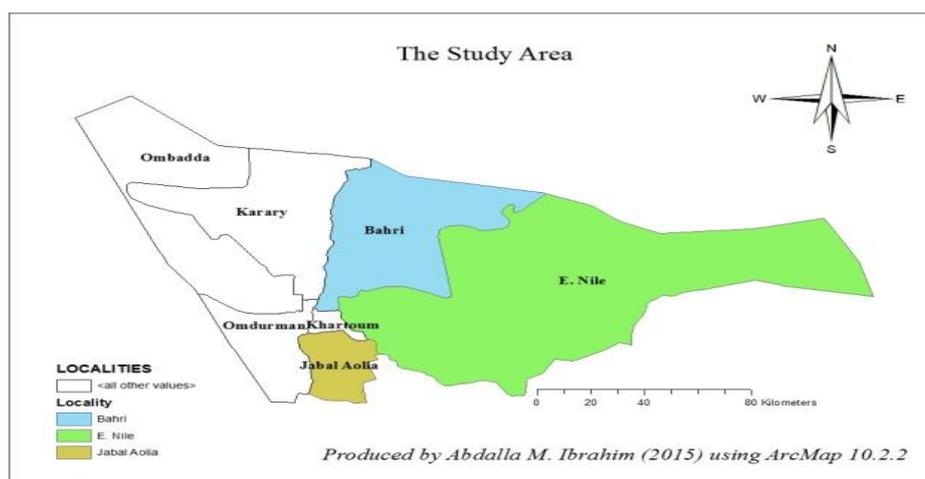


Figure 2: The study area

Animals: The study population encompassed 61 heads of adult she-camel from 13 herds of camels raised for milk production in the State.

Samples: Blood for serum was collected from the jugular vein of these animals during December 2013 to June 2014. Sera were separated and preserved cryotubes as two aliquots in -20°C until needed for examination.

Detection of antibodies against *N. caninum*: The level of antibodies directed to *N. caninum* in the sera of investigated animals was determined using a commercially available cELISA kits for detection of anti-*N.*

caninum IgG. The details of this technique were available in our reports elsewhere (Ibrahim *et al.* 2014). Generally, serum with 30% percent inhibition (pi) considered positive and a herd considered positive when at least one serum sample from the herd reacted positive.

Detection of antibodies against *T. gondii*: The presence of *T. gondii* specific antibodies was examined by Latex Agglutination Test as described in our recent report (Ibrahim *et al.* 2014). Based on the manufacturer construction, after the qualitative screening, the level of *T. gondii*

antibodies in the positive sera was determined using serial double dilution (titration) starting from 1:2. A herd considered positive when at least one serum sample from the herd reacted positive.

Data analysis: Data were entered in to Microsoft Excel spread sheet, labeled (coded) and then transferred to computer program for statistical analysis using the software (SPSS version 17). The results obtained for serum evaluation were analyzed statistically via the chi-square test (χ^2) of independence to determine significant associations or differences between infection by *N. caninum* or/and *T. gondii* and other variables studied. The differences were considered statistically significant when $p \leq 0.05$.

Maps were produced using *Arc GIS 2014 version 10.2.2 (ESRI, Redlands, California)*.

Table 1: Descriptive statistics of seroprevalence rate of *T. gondii* and *N. caninum* in dairy camels herds from Khartoum State

Parasite	N Herds	Prevalence (%)	Min%	Max%	Mean±SD%
<i>T. gondii</i>	13	10 (76.9)	34	100	68.80±24.36
<i>N. caninum</i>	13	5 (38.5)	10	34	26.40±10.99

Ser-prevalence of *T. gondii* in dairy camels from the Khartoum State:

Antibody to *T. gondii* was detected in 54.1% (33/61) she-camels. Almost 50% of seropositive camels revealed antibody titration of $\geq 1:8$. There was significant differences ($p=0.05$) in the seroprevalence of camel toxoplasmosis between the two districts of the State. However, there were no statistically significant variations ($p=0.475$) in the distribution of antibody titrations of their positive sera (table 2). Khartoum North recorded higher seroprevalence

RESULTS

Overall Seroprevalence:

The overall seroprevalence of both *T. gondii* or/and *N. caninum* among the investigated camel herds and individual she-camels was 84.6% (11/13) and 60.7% (37/61) respectively.

Antibody against *T. gondii* was detected in 10 herds out of the 13 herds (76.9%), while *N. caninum* seroprevalence was 38.5% (5/13) without any statistically significant differences ($p > 0.05$) between the two districts and among the three localities (table 1). Four herds (40%) have serologic mix infection of *T. gondii* with *N. caninum*. Mix infection of *T. gondii* with *N. caninum* was observed in 9.09% (3/33) of the *T. gondii* seropositive camels.

of *T. gondii* (62.8%) than Khartoum district (33.3%). Similarly, it reported higher level of antibody titration compared to Khartoum district.

As presented in Table (3), the examined localities showed no significant differences ($p > 0.05$) in both, the seroprevalence ($p=0.071$) and the distribution of antibody titration ($p=0.703$). Eastern Nile reported higher seroprevalence (66.7%) of *T. gondii* and higher level of antibody titration, followed by Bahri locality (50%).

Table 2: Sero-prevalence of *T. gondii* infection in dairy camels from two districts of the State

District	Tested	P+ve (%)	Distribution of specific antibody titers to <i>T. gondii</i> positive reaction (%)					N-ve (%)
			1:2	1:4	1:8	1:16	1:32	
Khartoum	18	6 (33.3)	2 (33.3)	3 (50.0)	0 (0.0)	1 (16.7)	0(0.0)	12 (66.7)
Kh. North	43	27 (62.8)	5 (18.5)	7 (25.9)	5 (18.5)	6 (22.2)	4 (14.8)	16 (37.2)
Total	61	33 (54.1)	7 (21.2)	10 (30.3)	5 (15.2)	7 (21.2)	4 (12.1)	28 (45.9)
P value		0.050			0.475			

Table 3: Sero-prevalence of *T. gondii* infection in dairy animals from three localities of the State

District	Tested	P+ve (%)	Distribution of specific antibody titers to <i>T. gondii</i> positive reaction (%)					N-ve (%)
			1:2	1:4	1:8	1:16	1:32	
J. Aolya	18	6 (33.3)	2 (33.3)	3 (50.0)	0 (0.0)	1 (16.7)	0(0.0)	12 (66.7)
E. Nile	33	22 (66.7)	4 (12.1)	5 (22.7)	4 (18.2)	6 (27.3)	3 (13.6)	11 (33.3)
Bahri	10	5 (50)	1 (20)	2 (40)	1 (20)	0 (0)	1 (20)	5 (50)
Total	61	33 (54.1)	7 (21.2)	10 (30.3)	5 (15.2)	7 (21.2)	4 (12.1)	28 (45.9)
P value		0.071			0.703			

Ser-prevalence of *N. caninum* in dairy camels from the Khartoum State:

IgG antibodies directed against *N. caninum* were observed in 9.8% (6/61) she-camels. There were no significant differences ($p>0.05$) in the occurrence of *N. caninum* and the frequency distribution of pi at both district and

locality level (table 4). The maximum pi reported in camel was 71% with mean $40.17\pm 17.09\%$. One third of the Neospora seropositive camels revealed pi of more than 50% (table 4). Higher seroprevalence and higher pi of *N. caninum* was recorded in Khartoum North district compared to Khartoum.

Table 4: Seroprevalence of *N. caninum* in dairy camels from Khartoum State

District	Locality	Tested	P+ve (%)	Percent Inhibition		Total (%)
				30-49	50-74	
Khartoum	J. Aolya	18	1 (5.6)	0 (0)	1 (100)	1 (5.6)
Kh. North	E. Nile	33	3 (9.1)	3 (100)	0 (0)	5 (11.6)
	Bahri	10	2 (20)	1 (50)	1 (50)	
Total		61	6 (9.8)	4 (66.7)	2 (33.3)	6 (9.8)
	P value		0.459		0.153	0.468

DISCUSSION

Sudan is one the most important countries in food producing animals production in the world. These animals are now facing several health problems including reproductive failure and infertility. There is meager information about the frequency and etiological factors of abortion in dairy animals in the Sudan other than *Brucella* and none is known about other causes such as *T. gondii* and *N. caninum*. The present study, Revealed 60.7% as overall seroprevalence of *T. gondii*

or/and *N. caninum* in dairy camels. Mix-infection of *T. gondii* with *N. caninum* was reported in different livestock worldwide (Huong *et al.* 1998; Hassan *et al.* 2000; Helmick *et al.* 2002; Figliuolo *et al.* 2004; Yildiz *et al.* 2009; Al-Qassab *et al.* 2009b; Mbiye *et al.* 2013). The present study revealed 9.09% mix-infection of *T. gondii* with *N. caninum* and this is may be the first report concerning the co-existence of these two related parasites in camels, because, all the Neospora-seropositive Egyptian camels (6, 3.7%)

were found to be seronegative for *T. gondii* (Hilali *et al.* 1998). Our result on seroprevalence of *T. gondii* infection in dairy camels (54.1%) was closely similar to that reported in slaughtered camels (54%) by Zein Eldin (1985), higher than Manal and Majid (2008) and lower to that (67% and 61.7%) reported by Elamin *et al.* (1992) and Manal (2003). This is may be because of the differences in the management system of the examined camels.

There are very few data on seroprevalence of *N. caninum* in animals other than cattle worldwide (Dubey and Schares 2011). The present data was the first serological evidence of *N. caninum* infection in camels from the Sudan. The result of this study (9.8%) was higher than that (3.7%) reported by Hilali *et al.* (1998) in Egypt using Neospora Agglutination test (NAT). That is may be because of the different serological test used. The serological evidence of *N. caninum* infection in camel in this study may confirm the report of Manal *et al.* (2013) who succeed to isolate the parasite from Sudanese camel tissues using Bio assay and molecular technique. The absence of significant differences in *N. caninum* infection among the studied areas in the present study was consistent with various studies worldwide (Nourollahi *et al.* 2008; Youssefi *et al.* 2009; Dubey and Schares 2011) including the Sudan (Amira *et al.* 2012; Hussein *et al.* 2012). This may support that, the usual method of *N. caninum* transmission is transplacental (Dubey and Lindsay 1996; Taylor *et al.* 2007; Dubey and Schares 2011). Based on our recent report (Ibrahim *et al.* 2014), seroprevalence of *N. caninum* in camels (9.8) was comparable to that (9.9%) of dairy cattle in the Khartoum State.

Generally, in addition to toxoplasmosis, camels are widely exposed to *N. caninum*. The role of Neosporosis in the economic losses affecting camels industry due to abortion and reproduction failure as well as the public health importance of camel toxoplasmosis could not be neglected. Further studies on clinical neosporosis together with toxoplasmosis and other abortifacients in camels is recommended.

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