Area-wide Detection of Anti-*Toxoplasma gondii* Antibodies in Dairy Animals from the Khartoum State, Sudan

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Abstract: Toxoplasmosis is an important cause of reproductive failure in man and farm animals resulting in significant socio-economic losses worldwide. The aim of this study was to avail large scale data-regarding the sero-prevalence of *T. gondii* infection in dairy animals from the Khartoum State, Sudan using the commercially available Latex Agglutination Diagnostic kits. The study revealed that the overall seroprevalence of *T. gondii* infection at herd level of dairy animals in the State was 92.7%. The within herd seroprevalence was ranging from 8% up to 100% with mean of 51.3% in different herds of different dairy animals species in the State. Only 6 herds, 3 herds, 3 herds and 1 herd of cattle, camel sheep and goat respectively were found to be clean from anti-*T. gondii* antibody in the present study. Interestingly, most of the clean herds (12 herds) are located in the Khartoum district. The differences between the three districts, the seven localities and the four animal species were statistically highly significant ($P < 0.01$). The overall sero-prevalence of *T. gondii* infection in dairy animals—at individual level—was 45.3%. Sheep scored the highest seroprevalence rate (75.0%) followed by goats (64.0%), camels (54.1%) and cattle (40.9%) with high statistically significant differences ($P < 0.01$). The highest level of antibody titration was reported in sheep and goats. This is the first area-wide and large scale report on seroprevalence of *T. gondii* infection in dairy animals from the Sudan. It was concluded that dairy animals in the Sudan are widely exposed to *T. gondii* and people in the Sudan should be aware of the possibility of hyper-prevalence of human toxoplasmosis through these food animals. Research on clinical toxoplasmosis in dairy animals and human in the Sudan is recommended to evaluate the role *T. gondii* infection in economical losses in dairy farm industry and for building strategy of sustainable toxoplasmosis control.

Key words: Seroprevalence, *T. gondii*, LAT, dairy animals, Sudan.

1. Introduction

Farms ruminant industry suffers major economic losses from reproductive failure result in not only loss of offspring and an increase in calving or (lambing/kidding) intervals, but in other factors such as increased culling, reduced milk production as well as reduced value of breeding stock [1, 2]. Routine healthy production is very important to sustain the

economy of dairy farms. Many studies on dairy animal's reproduction stated that reproductive disturbances are the main cause of infertility [3, 4]. *Toxoplasma gondii* (*T. gondii*) is one of the most important causes of protozoal parasitic infectious abortion in livestock worldwide [5, 6]. The parasite is widely prevalent in humans and animals on all continents [7-9]. However, very few data is available about the prevalence of *T. gondii* infection in Sudanese man [10-12] and animal [13-17]. About 50% of human toxoplasmosis cases were related to food

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borne infection [18, 19] during consumption of raw or undercooked meat or other edible parts of meat producing animals. Tachyzoites of *T. gondii* have been detected in body fluids, including saliva, sputum, urine, tears, semen and milk of several intermediate hosts, such as sheep, goats, cows and she-camel [19-21]. Consumption of unpasteurized goat's milk has been associated with acquired clinical toxoplasmosis in humans [19]. Though intake of milk or milk by-products containing tachyzoites may cause the infection in animals and humans [22, 23]. On view of the importance of *T. gondii* as a possible cause of reproductive failure and in worldwide public health, farm animals breeding, the lack of recent data on its large scale prevalence in the Sudan, this work aimed to estimate the *T. gondii* seroprevalence in dairy cattle, sheep, goats and camel herds by specific antibody search using the commercially available Latex Agglutination Test (LAT) as a diagnostic kit in the Sudan.

2. Materials and Methods

2.1 The Study Area

Khartoum State—the capital of the Sudan is one of the 17 states of the Sudan. The State is located in the central region of the country. It has an area of 22,122 km². Human population is estimated to be 3,512,144 with growth rate of 8.9% (The Economic Review, 2002). The River Nile, Blue Nile and White Nile divide the state into three districts (towns) namely, Khartoum, Khartoum north and Omdurman. The State has seven localities, two in Khartoum (Khartoum and Jabal Aolia), two of them in Khartoum North (Bahri and Eastern Nile) and three localities in Omdurman (Omdurman, Karary and Ombadda). Sudan dairy farms industries of different production systems are concentrated in this State.

2.2 Animals

The study population encompassed 177 herds including 132 herds of cattle (1,216 dairy cows), 16

herds of sheep (100 head), 16 herds of goats (100 head) and 13 herds of camel (61 head). Only adult female dairy animals (cattle and co-herded camel, sheep and goats) were tested (≥ 2 years for cattle and camel and ≥ 1 year for shoats).

2.3 Samples

Blood for serum was collected from the jugular vein of the above mentioned animals during October 2012 to April 2014. Their sera were separated and cryo-preserved in -20 °C until needed for examination.

2.4 Latex Agglutination Test (LAT)

The presence of *T. gondii* antibodies was investigated using Toxo-Latex slide agglutination test (*Spinreact, S.A./S.A.U, Spain*). These diagnostic kits were purchased from *Shifak Company, Khartoum, Sudan*.

d.1. Screening test: Initially, all sera were examined for anti-*T. gondii* antibodies qualitatively in the LAT based on the manufacturer's instructions. Briefly, measured 50 μ L of the test serum, a drop of (50 μ L) the positive control serum and the negative control serum were separately placed on the slide and a drop of the antigen (25 μ L) was added. After mixing the antigen and the serum samples (test and controls) by special sticks, and mixed thoroughly by shaking for ≤ 4 min, and then examined for agglutination. The samples showed clear agglutination were considered positive. The antibody titration of the positive samples group was determined in the next step (Antibody titration).

d.2. The level of Antibody Titration: For titration of positive serum, serial double dilutions of 1:2 up to 1:128 were prepared from serum using normal saline and examined by the same method mentioned above. The last titer with positive agglutination was recorded as the titer of the serum samples. Sera having titer of $\geq 1:2$ were considered positive for antibodies to *T. gondii*. The herd considered positive when at least one animal was found positive.

2.5 Data Analysis

Data were entered in to Microsoft Excel spread sheet, labeled 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 *T. gondii* and other variables studied. The differences were considered statistically significant when $P \leq 0.05$.

3. Results

3.1 Ser-prevalence of *T. gondii* Infection in Dairy Farms from the Khartoum State at Herd Level

The overall seroprevalence of *T. gondii* infection in dairy herds from the Khartoum state was 92.7%. The within herd seroprevalence was ranging from 8% up to 100% with mean of 51.3% in different herds of different dairy animals species in the State (Table 1). The majority of examined herds (86, 52.4%) recorded more than 50% seroprevalence rate with highly statistically significant differences at $P \leq 0.05$ (Table 2).

The highest seroprevalence rate was reported in

herds from Omdurman District (100%) followed by Khartoum North which recorded 98.2% seroprevalence (Table 3). The differences between the three districts revealed high statistically significant ($P < 0.01$). Similar statistically significant differences were reported within localities (Table 4).

The differences in the frequency distribution of seroprevalence rate of the examined herds revealed insignificant differences ($P > 0.05$) at both districts (Table 5) and locality level (Table 6).

With statistically significant ($P < 0.05$) differences among different animal species herds in the State, camel and cattle herds reported the highest seroprevalence rate followed by goats and sheep respectively (Table 7).

The differences in the frequency distribution of seroprevalence rate of *T. gondii* infection in the different dairy animals herd was highly statistically significant ($P < 0.01$), when most of sheep herds (76.9%) reported the heist range (75-100%) of herd prevalence rate followed by goat (46.9) and camel herds (40.0). while the lowest seroprevalence rate (8.7%) for the same range was reported in cattle herds (Table 8).

Table 1 Sero-prevalence of *T. gondii* infection in dairy herds from the Khartoum State.

LAT	N of herds	Percent	Prevalence Range%	Mean $\pm$ SD
Positive	164	92.7	8-100	51.3 $\pm$ 24.3
Negative	13	7.3		
Total	177	100		

Table 2 Frequency distribution of sero-prevalence rate of *T. gondii* infection in the State.

Herd Prevalence Rate	N of positive Herds	Percent	<i>P value</i>
1-24%	21	12.8	0.000
25-49%	57	34.8	
50-74%	54	32.9	
75-100%	32	19.5	
Total*	164	100	

Table 3 Prevalence of antibodies to *T. gondii* in dairy herds from the three districts of the State.

District	Herds examined	Positive (%)	Negative (%)	<i>P value</i>
Khartoum	70	58 (82.9)	12 (17.1)	0.000
Khartoum North	57	56 (98.2)	1 (1.8)	
Omdurman	50	50 (100)	0 (0)	
Total	177	164 (92.7)	13 (7.3)	

**Area-wide Detection of Anti-*Toxoplasma gondii* Antibodies in Dairy Animals
from the Khartoum State, Sudan**

Table 4 Sero-prevalence of *T. gondii* infection in dairy herds from the seven localities of the State.

Locality	Herds examined	Positive (%)	Negative (%)	<i>P</i> value
Khartoum	12	11 (91.7)	1 (8.3)	0.005
Jabal Aolia	58	47 (81)	11 (19)	
Eastern Nile	28	27 (96.4)	1 (3.6)	
Bahri	29	29 (100)	0 (0.0)	
Omdurman	32	32 (100)	0 (0.0)	
Karary	7	7 (100)	0 (0.0)	
Ombadda	11	11 (100)	0 (0.0)	
Total	177	164 (92.7)	13 (7.3)	

Table 5 Frequency distribution of prevalence rate of *T. gondii* infection in herds of the three districts of the State.

District	Total Positive	Herd Prevalence rate				<i>P</i> value
		1-24%	25-49%	50-74%	75-100%	
Khartoum	58	8 (13.8)	19 (32.8)	14 (24.1)	17 (29.3)	0.34
Kh. North	56	7 (12.5)	20 (35.7)	21 (37.5)	8 (14.3)	
Omdurman	50	6 (12)	18 (36)	19 (38)	7 (14)	
Total	164	21 (12.8)	57 (34.8)	54 (32.9)	32 (19.5)	

Table 6 Frequency distribution of prevalence rate of *T. gondii* infection in herds of the seven localities of the State.

Locality	Total Positive	Herd Prevalence Rate Range (%)				<i>P</i> value
		1-24%	25-49%	50-74%	75-100%	
Khartoum	11	2 (18.2)	6 (54.5)	3 (27.3)	0 (0.0)	0.06
Jabal Aolia	47	6 (12.8)	13 (27.7)	11 (23.4)	17 (36.2)	
Eastern Nile	27	5 (18.5)	8 (29.6)	11 (40.7)	3 (11.1)	
Bahri	29	2 (6.9)	12 (41.4)	10 (34.5)	5 (17.2)	
Omdurman	32	4 (12.5)	7 (21.9)	15 (46.9)	6 (18.8)	
Karary	7	0 (0.0)	5 (71.4)	1 (14.3)	1 (14.3)	
Ombadda	11	2 (18.2)	6 (54.5)	3 (27.3)	0 (0.0)	
Total	164	21 (12.8)	54 (34.8)	57 (32.9)	32 (19.5)	

Table 7 Sero-prevalence of *T. gondii* infection in dairy herds of different animals in the State.

Animal Species	Herds examined	Seropositive (%)	Seronegative (%)	<i>P</i> value
Cattle	132	126 (95.5)	6 (4.5)	0.025
Camel	13	10 (96.9)	3 (3.1)	
Sheep	16	13 (81.3)	3 (18.8)	
Goat	16	15 (93.8)	1 (6.3)	
Total	177	164 (92.7)	13 (7.3)	

Sero-prevalence of *T. gondii* infection in the dairy farms at individual level:

The overall Sero-prevalence of *T. gondii* in dairy animals in the State was 45.3% (Table 9).

There were no significant differences ($P > 0.05$) in the sero-prevalence among the three districts of the State. However, there were statistically significant variations in the distribution of antibody titrations of their positive sera (Table 10).

The examined localities showed highly ($P < 0.01$)

significant differences in both, the sero-prevalence and the distribution of antibody titration (Table 11).

As shown in Table 12, sheep were the superior in the sero-prevalence of *T. gondii* infection (75.0%). Cattle revealed the lowest sero-prevalence (40.9%). With highly statistically significant differences ($P < 0.01$), sheep and goat recorded the highest level of antibody titration (1:128) compare to cattle and camel who did not exceed 1:32 dilution (Table 12).

Table 8 Frequency distribution of prevalence rate of *T. gondii* infection in herds of dairy animal in the State.

Herd	Total Positive	Herd Prevalence rate				P value
		8-24%	25-49%	50-74%	75-100%	
Cattle	126	21 (16.7)	51 (40.5)	43 (34.1)	11 (8.7)	0.00
Camel	10	0 (0.0)	1 (10.0)	5 (50.0)	4 (40.0)	
Sheep	13	0 (0.0)	1 (7.7)	2 (15.4)	10 (76.9)	
Goat	15	0 (0.0)	4 (26.7)	4 (26.7)	7 (46.7)	
Total	164	21 (12.8)	57 (34.8)	54 (32.9)	32 (19.5)	

Table 9 Sero-prevalence of *T. gondii* infection in dairy animals from the Khartoum State.

	Tested samples for antibody against <i>T. gondii</i> using LAT	Percent
Positive	669	45.3
Negative	808	54.7
Total	1477	100.0

Table 10 Sero-prevalence of *T. gondii* infection in dairy animals from the three districts of the State.

District	Tested	*P + ve (%)	**Distribution of specific antibody titers to <i>T. gondii</i> positive reaction (%)							Negative (%)
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	
Khartoum	474	226 (47.7)	28 (12.4)	53 (23.5)	60 (26.5)	36 (15.9)	42 (18.6)	4 (1.8)	3 (1.3)	248 (52.3)
Khartoum North	629	282 (44.8)	30 (10.6)	54 (19.1)	79 (28.0)	43 (15.2)	74 (26.2)	1 (0.4)	1 (0.4)	347 (55.2)
Omdurman	374	161 (43.0)	20 (12.4)	33 (20.5)	58 (36.0)	6 (3.7)	44 (27.3)	0 (0.0)	0 (0.0)	213 (57.0)
Total	1477	669 (45.3)	78 (11.7)	140 (20.9)	197 (29.4)	85 (12.7)	160 (23.9)	5 (0.7)	4 (0.6)	808 (54.7)

*Insignificant ($P = 0.3$) at $P \leq 0.05$. **Significant ($P = 0.002$) at $P \leq 0.05$.

Table 11 Sero-prevalence of *T. gondii* infection in dairy animals from the seven localities of the State.

Locality	Tested	*P + ve (%)	**Distribution of specific antibody titers to <i>T. gondii</i> positive reaction (%)							Negative (%)
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	
Khartoum	96	34 (35.4)	1 (2.9)	1 (2.9)	14 (41.2)	3 (8.8)	15 (44.1)	0 (0.0)	0 (0.0)	62 (64.6)
Jabal Aolia	378	192 (50.8)	27 (14.1)	52 (27.1)	46 (24.0)	33 (17.2)	27 (14.1)	4 (2.1)	3 (1.6)	186 (49.2)
Eastern Nile	301	123 (40.9)	12 (9.8)	25 (20.3)	33 (26.8)	19 (15.4)	34 (27.6)	0 (0.0)	0 (0.0)	178 (59.1)
Bahri	328	159 (48.5)	18 (11.3)	29 (18.2)	46 (28.9)	24 (15.1)	40 (25.2)	1 (0.6)	1 (0.6)	169 (51.5)
Omdurman	186	93 (50.0)	13 (14.0)	19 (20.4)	33 (35.5)	5 (5.4)	23 (24.7)	0 (0.0)	0 (0.0)	93 (50.0)
Karary	83	32 (38.6)	1 (3.1)	7 (21.9)	11 (34.4)	1 (3.1)	12 (37.5)	0 (0.0)	0 (0.0)	51 (61.4)
Ombadda	105	36 (34.3)	6 (16.7)	7 (19.4)	14 (38.9)	0 (0.0)	9 (25.0)	0 (0.0)	0 (0.0)	69 (65.7)
Total	1477	669 (45.3)	78 (11.7)	140 (20.9)	197 (29.4)	85 (12.7)	160 (23.9)	5 (0.7)	4 (0.6)	808 (54.7)

*Significant ($P = 0.002$) at $P \leq 0.05$. **Significant ($P = 0.001$) at $P \leq 0.05$.

Table 12 Sero-prevalence of *T. gondii* infection in different dairy animal species from the State.

Animals	Tested	*P + ve (%)	**Distribution of specific antibody titers to <i>T. gondii</i> positive reaction (%)							Negative (%)
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	
Cattle	1216	497 (40.9)	56 (11.3)	101 (20.3)	152 (30.6)	46 (9.3)	142 (28.6)	0 (0.0)	0 (0.0)	719 (59.1)
Camels	61	33 (54.1)	7 (21.2)	10 (30.6)	5 (15.2)	7 (21.2)	4 (12.1)	0 (0.0)	0 (0.0)	28 (45.9)
Sheep	100	75 (75.0)	10 (13.3)	16 (21.3)	19 (25.3)	21 (28.0)	6 (8.0)	2 (2.7)	1 (1.3)	25 (25.0)
Goats	100	64 (64.0)	5 (7.8)	13 (20.3)	21 (32.8)	11 (17.2)	8 (12.5)	3 (4.7)	3 (4.7)	36 (36.0)
Total	1477	669 (45.3)	78 (11.7)	140 (20.9)	197 (29.4)	85 (12.7)	160 (23.9)	5 (0.7)	4 (0.6)	808 (54.7)

*Significant ($P = 0.00$) at $P \leq 0.05$. **Significant ($P = 0.00$) at $P \leq 0.05$.

4. Discussion

In the present study, the overall flock and individual sero-prevalence of *T. gondii* infection (92.7% and 45.3%) were higher than that (70.5% and 31.6%) reported in Ethiopia [24]. In this study, the sero-prevalence of *T. gondii* infection in dairy cattle at herd and individual level (95.5% and 40.9%) were found to be higher than that (87.5% and 7.8%) of French beef cattle [25]. These results were also higher compared to that (50% and 12.7%) reported by [16], although the number of herds and animals tested in our study was extremely higher (132 herds and 1216 cow) than theirs (29 herds and 134 cattle). That is may be attributed to the different serological tests used, or may be because we examined adult female animals only, but their samples included different ages and sexes. The result of the study on dairy cattle toxoplasmosis was approximately similar to that reported from slaughtered cattle in the Sudan (40%) and Turkey (41.6%) by [13] and [26] respectively. In agreement with many authors [1, 2, 8, 9], the present work also stated that, sheep and goats were more susceptible to *T. gondii* infection than any other farm animals when they recorded the highest antibody titration (1:128). In the present study, the sero-prevalence rate of *T. gondii* infection in sheep (75.0%), goats (64.0%) and cattle (40.9%) were higher than the world average (30%, 15%, and 9%) respectively [7, 27]. The result of the study in sheep was within the range (64%-88%) that reported in adult sheep from Gabon [28], Turkey [29], USA [30] and Iran [31]. The results of the study were higher than the results obtained from slaughtered animals in different abattoirs in the Sudan [13, 15, 17], Egypt [32-34] and South Africa [35]. The results of the study result on sero-prevalence of *T. gondii* infection in dairy camels (54.1%) was closely similar to that reported in slaughtered camels (54%) by [13], higher than [36] and lower to that (67% and 61.7%) reported by [37] and [20]. This is may be because of the differences in

the management system of the examined camels. Generally, this is the first area wide and large scale study on sero-prevalence of *T. gondii* infection in different farm animal species co-herded in dairy farms in the Sudan. It can be concluded that, dairy animals including cattle, camel sheep and goats are widely exposed to *T. gondii* infection and the role of toxoplasmosis in the economic losses affecting dairy farm industry due to abortion and reproduction failure could not be neglected or excluded in the Sudan. Moreover, the role of dairy animals in the epidemiology of human toxoplasmosis is strongly suspected, particularly when we know that, during this study, most of the dairy animals with infertility problems or reproduction failure were sent to slaughter houses. Further studies on clinical toxoplasmosis in dairy animals from the Sudan as well as the role of milk in the epidemiology of human toxoplasmosis are recommended. Unfortunately, although eating and drinking raw meat and milk of the present investigated animals is a common behaviour in the Sudan, there is no consistent and scientific medical data on human toxoplasmosis in the country.

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