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SEROPREVALENCE OF *TOXOPLASMA GONDII* AND *BRUCELLA ABORTUS* IN DAIRY ANIMALS FROM THE SUDAN: SPECIAL EMPHASIS TO THEIR SEROLOGICAL CO-EXISTENCE

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ABSTRACT

Toxoplasmosis and brucellosis are the most common zoonotic food borne diseases worldwide. The abortion caused by these agents is one of the major causes of socio-economic losses in human and livestock. We thus conducted a cross-sectional study during October 2012 to April 2014 to determine the seroprevalence and the co-existence of *Toxoplasma gondii* and *Brucella abortus* in dairy cattle and the co-herded camels, sheep and goats in the Khartoum State using different serological techniques. The study revealed over all seroprevalence of 94.9% at herd level and 63.2% at individual level. *T. gondii* and *B. abortus* seroprevalence were 46.2% and 22.7% respectively. Seroprevalence of these zoonosis showed highly statistically significant ($p<0.01$) differences among different localities and different animals species. Antibody to *T. gondii* was found to be more prevalent in Sheep, goats and camels respectively while antibody against *B. abortus* was more prevalent in cattle and camels. Mixed antibodies against the two zoonotic agents were detected in almost all seropositive herds (99.1%). Only one seropositive herd (0.9%) revealed a single *Brucella abortus* infection. Mix-infection was observed in 16.2% out of all seropositive animals. The univariate analysis of different localities and different animal species showed significant ($p<0.01$) association with mix-infection seropositivity. Increasing odds ratios without significant ($p>0.05$) associations were observed in the multivariate analysis of districts and animals species (odds=2.588, CI 95%) for cattle and (odds=2.200, CI 95%) for camels compared to goats as reference. Generally, the present results were the first comprehensive data explaining the serological co-existence of *T. gondii* and *B. abortus* as zoonotic organisms in Sudanese food animals. We came to conclude that, the exposure to these organisms as well as their co-infection may play a significant role in the economic losses in dairy farm industry in the country. Moreover, the great public health importance of *T. gondii, B. abortus* and their co-existence could not be neglected. Further studies to elucidate their socio-economic consequences in man and his livestock are recommended.

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INTRODUCTION

Food security is one of the major global challenges we face. *Brucella abortus (B. abortus)* is an important zoonotic abortifacient agent among dairy animals. However, nowadays the most common zoonosis, *Toxoplasma gondii (T. gondii)* is steadily reported as parasitic abortifacient from farm animals **(Seri *et al.*, 2003; Figliuolo *et al.*, 2004; Ortega-Mora *et al.*, 2007; Weiss and Kim 2007)**. Reports on Co-existence of *T. gondii* with *Neospora caninum* was more available worldwide **(Dubey *et al.*, 1998; Huong *et al.*, 1998; Hilali *et al.*, 1998; Helmick *et al.*, 2002).** However, studies on *T. gondii* and *B. abortus* co-existence are spares **(Yildiz *et al.*, 2009)**. Human and animal brucellosis was extremely investigated in the Sudan **(Angara 2005; Angara *et al.*, 2009)**. However, meager data on toxoplasmosis was available in the Sudanese people **(Abd Elhameed, 1991; Adnan, 1994; Elnahas *et al.*, 2003; Maha *et al.*, 2012; Khalil *et al.*, 2013; Abdel-Raouff and Elbasheir 2014)** and animals **(Seri *et al.*, 2003, Khalil and Elrayah 2011;**

Abdel Hafez 2013; Elfahal *et al.*, 2013; Ibrahim *et al.*, 2014a). Toxoplasmosis is an opportunistic infection. Antibodies to *T. gondii* are detected more frequently in sick (Immunosuppressed) animals and any complications of primary disease **(Svoboda *et al.*, 1988; Svoboda *et al.*, 1998)**.

Toxoplasma *gondii* infection has become a major public health concern in recent years due to the ravaging HIV/AIDS pandemic **(Dubey 2004; Lindstrom *et al.*, 2006; Negash *et al.*, 2008).** Both, *T. gondii* and *B. abortus* are transmitted to human through eating or drinking raw/undercooked or unpasteurized milk of infected animals **(Tenter 2009; Tenter *et al.*, 2000).** Thus we aimed to determine the seroprevalence, mix-infection and to interrogate the association of *T. gondii* and *B. abortus* seropositivity in dairy cows and the co-herded camels, sheep and goats in dairy farms from the Sudan.

MATERIALS AND METHODS

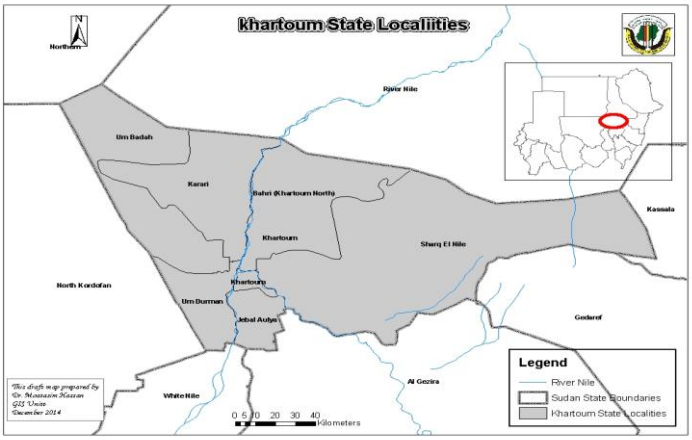
Study Area

Khartoum State (fig. 1), the capital State of the Sudan is the smallest state by area (22,142 km^2), but it is the most populous (5,274,321 in 2008 census). The Sudan dairy industries of different production systems are concentrated in this State (MLFR 2014).

Study Population

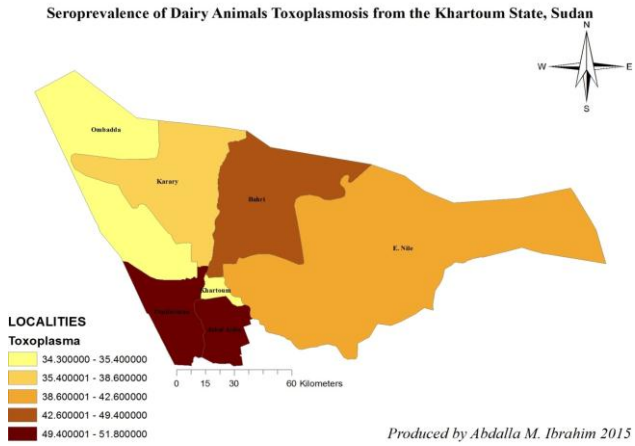
The study involved a total of 1447 adult animals, including 1190 heads of dairy cows, 60 she-camels, 97 sheep and 100 goats from 177 dairy herds selected at random from various dairy clusters in the seven localities of the State (fig.1).

Fig. 1: The Sudan map showing the Khartoum State Localities (the area of the study in red colour).



Samples

Taking the advantage of blood drawing for research project on Brucellosis 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 -20ºC until examined.



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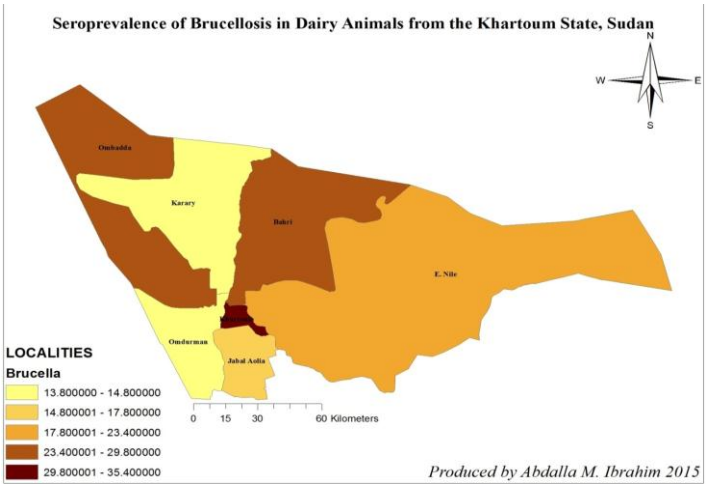

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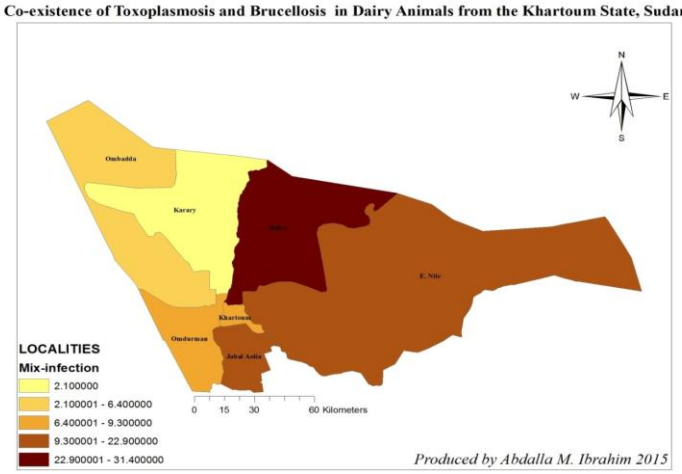

Serology

Toxoplasma ***gondii*:** The presence of *T. gondii* specific antibody was screened by Latex Agglutination Test (LAT). Generally, serum with antibody titration of ≥1:2 considered positive and a herd considered positive when one sample from the herd reacted positive. Dairy cows sera showed LAT positive were

re-examined for confirmation using ELISA. Details of these methods were described in our recent reports **(Ibrahim *et al.*, 2014a and b).**

Brucella ***abortus*:** The presence of *B. abortus* specific antibody was screened by Rosebengal Plate Agglutination Test (RBPT). Sera showed (Agglutination) positive reaction were re-examined for confirmation using cELISA. These methods were described in details elsewhere **(Angara 2005; Angara *et al.*, 2009).**

Statistical analysis

Differences in the seroprevalence and the co-existence of these agents among the investigated areas and animal species were analyzed by Chi-square test. The differences were considered statistically significant when ($p \leq 0.05$). The same test was used to analyze the association between their seropositivity (Univariate analysis) before multivariate analysis was used (Logistic Regression). The overall fit of the logistic regression models was assessed using Hosmer-Lemeshow goodness of fit statistics. All statistics were performed using SPSS version 17.0 statistical package.

Mapping

Maps were produced using *Arc GIS version 10.2.2 (ESRI, Redlands, California)* to show the study area and the distribution of the prevalence rate in different localities of the State.

RESULTS

The overall seroprevalence of *T. gondii* or/and *B. abortus* infections at herd level was 94.9% (Table 1). Antibody against *T. gondii* and *B. abortus* was detected in 92.7% and 65.6% of the tested herds respectively. The minimum and the maximum within herd prevalence rate were presented in table (2). The majority of the herds that infected with *T. gondii* recorded more than 50% prevalence rate, while the herds having *B. abortus* recorded less than 50% prevalence rate (Table 3). At individual level, the overall seroprevalence was 63.2% (Table 3).

As presented in table (4), *T. gondii* and *B. abortus* seroprevalence were 46.2% and 22.7 respectively. Highly statistically significant ($p<0.01$) differences were reported among animal species. Mix-infection was significantly higher ($p<0.05$) in cattle and camels followed by goats and sheep (Table 4). The details of co-existence of *T. gondii* and *B. abortus* was presented in table (5), where mix-infection was detected in 140 (16.2%) out of 865 seropositive animals.

As presented in table 6, the univariate analysis showed highly significant ($p<0.01$) association between the mix-infection of *T. gondii* and *B. abortus* seropositivity and the different localities ($p=0.000$) and different animals species ($p=0.000$). In the multivariate analysis, increasing odds ratio were observed in Khartoum (odds=1.444, 95% CI=0.566 – 3.689) and Khartoum North (odds=1.576, 95% CI= 0.718 – 3.459) districts, but without significant association ($p=0.052$). Cattle (odds=2.588, 95% CI=0.848 – 7.901) and camels (odds=2.200, 95% CI= 0.204 – 23.736) are more susceptible to the mix-infection than sheep and goats (Table 7).

Table 1: Overall Seroprevalence of *T. gondii* or/and *B. abortus* in dairy farms from the Khartoum State, Sudan.

Tested Samples for antibody against *Toxoplasma gondii* or/and *Brucella abortus*				
Results	N of Herds	%	N of Animals	%
Seropositive	168	94.9	865	63.2
Clean herds	9	5.1	503	36.8
Total	177		1368	

Table 2: Seroprevalence of *T. gondii* and *B. abortus* in dairy herds from the Khartoum State, Sudan.

Antibody against	N Tested	N P+ve	Prevalence%	Range %	Mean±SD
Toxoplasma gondii	177	164	92.7	8 - 100	51.27±24.34
Brucella abortus	163	107	65.6	4 - 100	30.64±18.06

Table 3: Frequency distributions of prevalence rates of *T. gondii* and *B. abortus* in dairy herds from the Khartoum State, Sudan.

Infection	1-24%	25-49%	50-74%	75-100%	Total herds P+ve
	Number of herds (%)				
Toxoplasma gondii	21(12.8)	57(34.8)	54(32.9)	32(19.5)	164
Brucella abortus	47 (43.9)	42 (39.3)	15 (14.0)	3 (2.8)	107

Table 4: Seroprevalence of *Toxoplasma gondii* and *Brucella abortus* in different dairy animals from the Khartoum tate, Sudan.

Animal / Antibody	Cattle		Sheep		Goats		Camels		p-value	Total	
	N	P+ve (%)	N	P+ve (%)	N	P+ve (%)	N	P+ve (%)		N	P+ve (%)
Toxoplasma	1190	497(41.8)	97	75(77.3)	100	64(64.0)	60	33(55.0)	0.000	1447	669(46.2)
Brucella	1190	301(25.3)	86	4(4.7)	75	5(6.7)	17	1(5.9)	0.000	1398	311(22.7)
Mix-infection	742	132(17.8)	69	3(4.3)	48	4(8.3)	6	1(16.7)	0.047	865	140(16.2)

Table 5: Distribution of Mix-infection among over all seropositive dairy animals from the Khartoum state, Sudan.

Mix Infection	N of Animals	Percent	p value
Single Toxoplasma infection	554	64.0	0.000
Single Brucella infection	171	19.8	
Brucella with toxoplasma	140	16.2	
Total Seropositive animals	865 (63.2%)		

Table 6: Results of Univariate association of Risk factors with *T. gondii* and *B. abortus* Mix-infection in Dairy animals from the Khartoum state, Sudan.

Risk Factors		**Seroprevalence (%)**	**Mix-infection**	**Percent**	***p-value***
Districts	Khartoum	311 (36.0)	45	32.1	0.052
	Kh. North	358 (41.4)	71	50.7	
	Omdurman	196 (22.7)	24	17.1	
Localities	Khartoum	62 (7.2)	13	9.3	0.000
	Jabal Aolia	249 (28.8)	32	22.9	
	Eastern Nile	162 (18.7)	27	19.3	
	Bahri	196 (22.7)	44	31.4	
	Omdurman	97 (11.2)	12	8.6	
	Karary	41(4.7)	3	2.1	
	Ombadda	58 (6.7)	9	6.4	
Animal sp.	Cattle	742 (85.8)	132	94.3	0.000
	Camels	6 (0.7)	1	0.7	
	Sheep	69 (8.0)	3	2.1	
	Goats	48 (5.5)	4	2.9	
Total		**865**	**140**	**10**	

Table 7: Results of Multivariate association of Risk factors with *T. gondii* and *B. abortus* Mix-infection in Dairy animals from the Khartoum State, Sudan.

Risk factors		N of P+ve (%)	Mix-infection	Wald (L.R)	p-value	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Districts	Omdurman	196 (22.7)	24 (17.1)	Reference				
	Khartoum	311 (36.0)	45 (32.1)	0.591	0.442	**1.444**	0.566	3.689
	Kh. North	358 (41.4)	71 (50.7)	1.287	0.257	**1.576**	0.718	3.459
Localities	Khartoum	62 (7.2)	13 (9.3)	Reference				
	Jabal Aolya	249 (28.8)	32 (22.9)	0.097	0.756	0.887	0.416	1.889
	Eastern Nile	162 (18.7)	27 (19.3)	1.854	0.173	0.691	0.406	1.176
	Bahri	196 (22.7)	44 (31.4)	0.306	0.580	0.769	0.302	1.954
	Omdurman	97 (11.2)	12 (8.6)	1.452	0.228	0.430	0.109	1.698
Animal sp.	Goats	48 (5.5)	4 (2.9)	Reference				
	Cattle	742 (85.8)	132 (94.3)	2.790	0.095	**2.588**	0.848	7.901
	Camels	6 (0.7)	1 (0.7)	0.422	0.516	**2.200**	0.204	23.736
	Sheep	69 (8.0)	3 (2.1)	0.773	0.379	0.500	0.107	2.343

DISCUSSION

Efforts aimed at reducing the impact of zoonotic diseases in human and his livestock will be vital to improve people livelihood and production efficiency. The majority of abortion cases in dairy farms originate from infectious agents including *T. gondii* and *B. abortus* **(Ortega-Mora *et al.*, 2007).** The present study revealed that dairy animals in the Sudan were widely exposed to *T. gondii* and *B. abortus*. Similar results were reported by **Yildiz *et al.*, (2009).** In agreement with the later authors, *T. gondii* was found to be more prevalent than *B. abortus*. Among all seropositive animals 16.2% were co-infected with both *T. gondii* and *B. abortus*. Lower results were reported (13.33% and 10% respectively) in Turkey **(Yildiz *et al.*, 2009).**

Our results (Toxoplasma seroprevalence and mix-infection) were also higher compared to that reported in Vietnam **(Huong *et al.*, 1998).** The univariate analysis revealed significant ($p<0.01$) association between mix-infection with different localities and different animal's species. However, the outcome of the multivariate analysis showed insignificant association ($p>0.05$) with an increasing odd ratio of cattle and camels being about three times more susceptible to mix-infection (odds=2.588, 2.200) compared to goats. In agreement with several authors **(Seri *et al.*, 2003; Wiss and Kim 2007; Taylor *et al.*, 2007; Ortega-Mora *et al.*, 2007; Innes 2011)** the present study revealed that, *T. gondii* seropositivity was significantly higher in sheep, goats and camels while *Brucella abortus* seropositivity was significantly higher in cattle.

Because of the contradiction reports in cattle toxoplasmosis **(Dubey 1995)** our cattle samples were retested using ELISA to support the results of the screening test (LAT). These results together with the known feeding habits in the Sudan (eating and drinking raw meat and unpasteurized milk), put people under high risk of getting infected with these serious zoonotic diseases. In our recent report **(Ibrahim *et al.*, 2014a**), it was stated that dairy animals with reproductive problems were usually sent to slaughter house for human consumption. Generally, the authors believe that the results of this study may provide the first comprehensive data explaining the co-existence of *T. gondii* and *B. abortus* in dairy industry in the Sudan for their economic and public health concern.

CONCLUSION

In conclusion**,** Co-existence of *T. gondii* and *B. abortus* is frequently occurring in food animals and their public health importance could not be neglected. Further in-depth study on Sudanese people and their livestock is recommended.

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