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Analysis of Risk Factors Associated with Seroprevalence of *Neospora caninum* in Dairy Animals from Khartoum State, Sudan

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ABSTRACT

A cross-sectional study was conducted between October 2012 and April 2014 to determine the sero-prevalence and the main risk factors associated with the *N. caninum* infection in dairy cattle and co-herded camels, sheep and goats in dairy farms from the Khartoum State. A total of 906 serum samples from 645 dairy cows, 61 camels, 100 sheep and 100 goats were examined for specific *N. caninum* IgG antibodies using Competitive Enzyme Linked Immunosorbent Assay (cELISA) Diagnostic kits (VMRD). The overall seroprevalence of *N. caninum* at herd and individual levels were 32.2% (56/174) and 8.8% (80/906) respectively. The highest percent inhibition (pi) recorded was 93% with mean of 38.7±12.3 and 39.3±13.9 at herd and individual level respectively. Possible association of the *N. caninum* infection with the investigated risk factors was analyzed by univariate analysis and variables significant at $p \leq 0.25$ were included in multivariate logistic regression models. The results showed that, There were no significant ($p>0.05$) differences in the seroprevalence of *N. caninum* among the regions and the different animal species. The univariate analysis included production system, source of concentrate, keeping dogs, other diseases (Toxoplasmosis) as risk factors associated with cELISA positive status of *N. caninum* infection. Region, herd size, herd type, source of fodder, brucellosis and stray dogs were not significant risk factors ($p>0.05$). The multivariate analysis indicated only production system (Intensive, $p=0.019$) and source of concentrate (Readymade, $p=0.007$) as the statistically significant ($p<0.05$) risk factors of being Neospora cELISA positive. The study also reported occurrence of abortion (72.4%), repeat breeding (76.3%), and stillbirth (18.4%) during interview with the owners of the investigated dairy herds. Interestingly, 18.2% of the aborted dams have repeated abortions. In conclusion, the authors believe that, the results of this study may provide a comprehensive data explaining the association of the main possible risk factors with *N. caninum* seropositivity in dairy animals. Research on abortifacients agents such as Neospora, Brucella, Toxoplasma and Listeria collectively is recommended to evaluate their individual role in the severe economical losses in dairy farm industry and for control strategy.

INTRODUCTION

Neospora caninum (*N. caninum*) is one of the most important causes of protozoal parasitic infectious abortion in mammals particularly cattle (Figliuolo *et al.,* 2004; Ortega-Mora *et al.,* 2007; Dubey and Schares 2011). The structure and the life cycle of *N. caninum* resemble *Toxoplasma gondii* (Taylor *et al.,* 2007; Dubey *et al.,* 1988) with the exception of dogs as final host and neosporosis is not considered zoonotic (Dubey and Lindsay, 1996). The disease can cause abortion, fetal death, mummification and birth of infected calves with or without clinical signs. Reduction in milk yield and underweight calves has also been reported in dairy and beef cattle (Dubey and Lindsay, 1996). The worldwide seroprevalence of *N. caninum* varies from 0.7 - 80.9% in cattle, 0 - 67% in dogs, 0.6 - 30.8% in sheep, 2 - 23% in goats and 3.7 - 13.7% in camels (Dubey and Lindsay, 1996; Hilali *et al.,* 1998; Dubey and Schares, 2011). Neosporosis is an important cause of abortion in farm animals resulting in significant economic losses worldwide. In the Sudan, epidemiological data on *N. caninum* infection in dairy animals is not available. Industrial and rural areas, gestation number, abortion number and age of cattle were found to have effect on *N. caninum* infection in Iran (Youssefi *et al.,* 2010; Youssefi *et al.,* 2009). Information on *N. caninum* infection in domestic animals in the Sudan is scarce (Amira *et al.,* 2012; Hussein *et al.,* 2012; Manal *et al.,* 2013; Ibrahim *et al.,* 2014b). The economical importance of *N. caninum* infection and the lack of its epidemiological data in the Sudan, prompted us to conduct a cross-sectional survey to investigate the main possible risk factors associated with the *N. caninum* seroprevalence in dairy cows and the co-herded camels, sheep and goats in dairy farms by specific antibody search using VMRD Competitive ELISA kits.

MATERIALS AND METHODS

The Study Area: Khartoum State is one of the eighteen states of the Sudan (Figure 1). The State has seven localities, two (Khartoum and Jabal Aolya) in Khartoum district, two (Bahri and eastern Nile) in Khartoum North and three (Karary, Ombadda and Omdurman) in Omdurman district (MLFR 2014).

Study Population: the study involved a total of 906 adult female animals, including 645 heads of dairy cows, 61 she-camels, 100 sheep and 100 goats from dairy farms (174 herds) selected at random from various dairy clusters in the different regions (7 localities) of the Khartoum State.

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 at -20ºC until needed.

Serological examination: The presence of *N. caninum* specific IgG antibodies was examined by cELISA as described in our recent report (Ibrahim *et al.,* 2014b). 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.

The Results of other diseases: data on Brucella and Toxoplasma seropositivity in these farms were available since these animals were included in the national brucellosis and toxoplasmosis research projects. The details of which were reported elsewhere (Angara *et al.,* 2014; Ibrahim *et al.,* 2014).

Collection of Epizootiological data: An Interview was made among farm personnel (owner, farm manager or worker) at the time of blood collection. The questionnaire included data on farm type, herd size (small≤100, Large>100 heads), production system, feeds and water, reproductive

problems and intervention as well as presence of dogs and cats in the farm.

Statistical analysis:

Differences in the seroprevalence 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 influence of all examined factors on *N. caninum* seroprevalence (Univariate analysis). Variables significant at $p \leq 0.25$ at 95% confidence Intervals (95% CI) level were selected for inclusion in multivariate analysis. The overall fit of the logistic regression models was assessed using Hosmer-Lemeshow goodness of fit statistics. Results are presented as odds ratios (OR) with 95% CI. The level of significance was 5% ($p \leq 0.05$). All statistics were performed using SPSS version 17.0 statistical package.

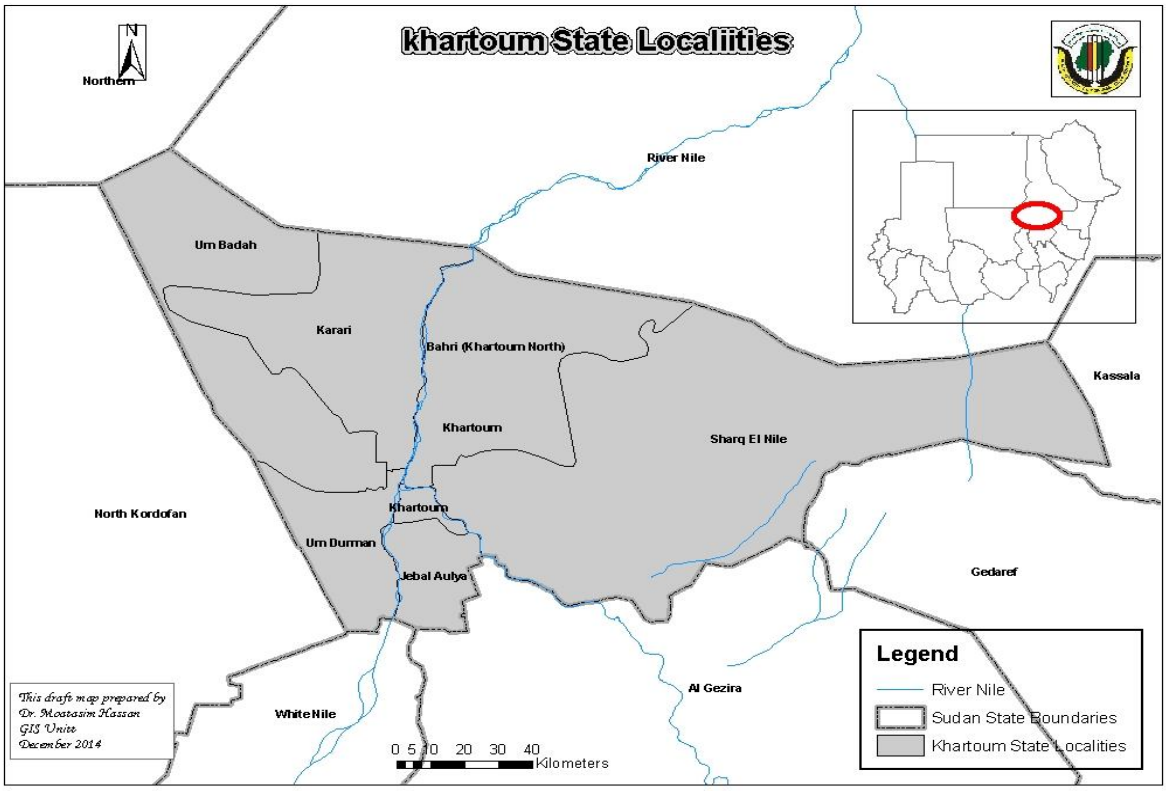

Figure 1: The Sudan map showing Khartoum State (the area of the study in red colour)

RESULTS

Zoographic and farm management characteristics of the investigated population are presented in Tables (1 and 2). Antibodies were detected in 80 out of 906 (8.8%) dairy animals examined distributed in 56 out of 174 (32.2%) dairy herds. The seropositive rates of cattle, camels, goats and sheep were 9.9%, 9.8%, 6.0% and 4.0% respectively, with no statistically significant ($p>0.05$) differences among districts, localities and animal's species (Table 1). Univariate analysis of risk factors showed that production system, source of concentrate, other diseases (Toxoplasma) and keep dogs in farms were significantly ($p<0.05$) associated with the seropositivity of *N. caninum*. Regions, animal species, herd size,

herd type, source of fodder, Source of water, brucellosis and presence of stray dogs were not significant ($p>0.05$) risk factors (Tables 1 and 2). The results of multivariate analysis revealed only production system (Intensive $p=0.019$) and source of concentrate (readymade, $p=0.007$) as statistically significant risk factors associated with Neospora cELISA seropositive at ($p<0.05$). Animal species (sheep), keeping dogs, stray dogs, keeping both dogs and cats and presence of both stray dogs and cats were the factors showed increasing odds of being cELISA positive (Table 3) and found not to be significantly associated ($p>0.05$) with *N. caninum* infection. The summary of response of the farm owners was presented in Tables (4 and 5). The occurrence of reproductive problems such as abortion (72.4%), repeat breeding (76.3%), still-birth (18.4%) and repeated abortions (18.2%) were reported from the questionnaire. Most of the aborted calves (75.7%) age were less than 7 months, and 45.9% of the aborted cows age were less than 6 years old while the rest (54.1%) were older (more than 6 years old) when they aborted. A number of 24 owners out of 76 (31.6%) know causes of abortion. Out of these 24 owners, 10 (41.7%) mentioned *Brucella* as the cause of abortion while the majority 14(58.3%) mentioned other causes including FMD, young age of pregnant cow or young bull, concentrates and out grazing. Almost, all of the visited dairy farms suffer from neonatal deaths.

Table 1: Estimated Seroprevalence of *N. caninum* infection in Dairy animals from Khartoum State and Univariate analysis for the associated Risk factors using Chi square (October 2012 to April 2014)

Risk Factors		No. of examine Animals	No. of +ve	Seroprevalence (%)	*p-value*
Districts	Khartoum	369	30	8.1	
	Kh. North	335	32	9.6	0.801
	Omdurman	202	18	8.9	
Localities	Khartoum	46	1	2.2	
	Jabal Aolia	323	29	9.0	
	Eastern Nile	207	20	9.7	
	Bahri	128	12	9.4	0.517
	Omdurman	151	15	9.9	
	Karary	22	0	0.0	
	Ombadda	29	3	10.3	
Animal sp.	Cattle	645	64	9.9	
	Camels	61	6	9.8	0.177
	Sheep	100	4	4.0	
	Goats	100	6	6.0	
Prod. system	Intensive	98	19	19.4	0.000
	Semi-intensive	808	61	7.5	
Total		**906**	**80**	**8.8**	

Table 2: Results of univariate association of Risk factors with cELISA Neospora seropositivity in dairy animals from Khartoum state using Chi square (October 2012 to April 2014)

Risk factors		N of Animals examined	N of P+ve	P+ve %	p-value
Herd size	Large	658	55	8.4	0.415
	Small	248	25	10.1	
Herd type	One species	668	57	8.5	0.597
	Multi-species	238	23	9.7	
Source of concentrate	Ready made	333	45	13.5	0.000
	Prepared in farm	573	35	6.1	
Source of fodder	Buy from market	521	41	7.9	0.236
	Cut from farm	385	39	10.1	
Source of water	Tap water/well	704	65	9.2	0.425
	Common canals	202	15	7.4	
*Other Diseases	Brucella P+ve	155	17	11.0	0.328
	Brucella N-ve	672	57	8.5	
	Toxoplasma P+ve	506	36	7.1	0.041
	Toxoplasma N-ve	400	44	11.0	
Keep dogs	Yes	326	20	6.1	0.032
	No	580	60	10.3	
Stray dogs	Yes	644	52	8.1	0.209
	No	262	28	10.7	
Keep dogs and cats	Yes	177	10	5.6	0.096
	No	729	70	9.6	
Stray dogs and cats	Yes	387	27	7.0	0.090
	No	519	53	10.2	
Total		**906**	**80**	**8.8**	

*Total animals examined for Brucella were 827.

Table 3: Results of multivariate association of Risk factors with cELISA Neospora seropositivity in dairy animals from Khartoum state (October 2012 to April 2014)

Risk factors		No. of examined Animals	No. of +ve (%)	Wald (L.R)	p-value	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Animal sp.	Sheep	100	4(4.0)	Ref.				
	Cattle	645	64(9.9)	2.721	0.099	0.270	0.057	1.279
	Camels	61	6(9.8)	0.780	0.377	0.493	0.102	2.369
	Goats	100	6(6.0)	0.149	0.699	0.766	0.199	2.957
Prod. System	Semi-intensive	808	61(7.5)	Ref.				
	Intensive	98	19(19.4)	5.500	0.019	0.385	0.174	0.855
Other diseases	Toxoplasma P+ve	506	36(7.1)	Ref.				
	Toxoplasma N-ve	400	44(11.0)	1.284	0.257	0.753	0.460	1.230
Keep dogs	Yes	326	20(6.1)	Ref.				
	No	580	60(10.3)	0.033	0.856	1.083	0.458	2.560
Keep dogs and cats	Yes	177	10(5.6)	Ref				
	No	729	70(9.6)	0.530	0.467	1.684	0.414	6.852
Stray dogs	Yes	644	52(8.1)	Ref.				
	No	262	28(10.7)	1.865	0.172	1.566	0.823	1.980
Stray dogs and cats	Yes	387	27(7.0)	Ref.				
	No	519	53(10.2)	0.161	0.689	1.158	0.564	2.378
Source of concentrate	Prepared in farm	573	35(6.1)	Ref.				
	Ready made	333	45(13.5)	7.182	0.007	0.374	0.182	0.768
Source of fodder	By from market	521	41(7.9)	Ref.				
	Cut from farm	385	39(10.1)	0.494	0.482	0.789	0.408	1.527

Table 4: Results of owner's interview on occurrence of abortion, repeat breeding and still-birth among the dairy herds in the Khartoum State.

Reproductive problem	No. of Herds	No. of Yes	Percent
Occurrence of abortion	76	55	72.4
Occurrence of repeated abortion	55	10	18.2
Occurrence of repeat breeding	76	58	76.3
Occurrence of still-birth	76	14	18.4

Table 5: Results of owner's awareness on causes of abortion and toxoplasmosis

Awareness	No. of Owner's interviewed	Yes (%)	No (%)
Know cause of abortion	76	24(31.6)	52(68.4)
Know Toxoplasmosis	76	4(5.3)	72(94.7)
Know Neospora	76	3(3.9)	73(96.1)
Intervention	65	15(23.1)	50(76.9)
Culling (Slaughtering)	76	47(61.8)	29(38.2)

DISCUSSION

Neosporosis is primarily a disease of cattle and dogs. The present results on seroprevalence of *N. caninum* in camels (9.8%), goats (6.0%) and sheep (4.0%) were to be one of the very few internationally available data, and it is within the worldwide average (Dubey and Lindsay, 1996; Hilali *et al.,* 1998; Figliuolo *et al.,* 2004; Dubey and Schares, 2011). However, these results were the first data to be reported in the Sudan. The study revealed (9.9%) seroprevalence in dairy cows. Lower result (9.0%) was reported in the State by Amira *et al.,* (2012) although her dairy cattle examined were fewer (262 heads). Higher prevalence rate (15.9%) was reported by Hussein *et al.,* (2012) outside Khartoum State. The difference may be just because most of their animals examined were from extensive production system, because animal breed has no significant effect on *N. caninum* infection (Yildiz *et al.,* 2009). Moreover, they examined very few number of cows (276 heads) compared to our examined population (645 adult cross breed dams). Based on these results, the economical value of *N. caninum* infection should be considered in dairy farm industry in the Sudan. In the present study, with the exception of intensive production system ($p=0.019$) and source of concentrate ($p=0.007$), the majority of the possible risk factors investigated were not significantly ($p>0.05$) found to be associated with Neospora cELISA seropositivity. It is Anderson *et al.,* (1991, 1995) who stated that, *N. caninum* is cited as the cause of abortion in many countries and regions with intensive dairy industry. An increasing odds of infection without statistically significant association ($p>0.05$) was found in risk factors like presence of dogs and animal species. That is may be because stray dogs were observed on or near all farm premises, even when farm personnel/owners denied their presence. Similar observation was reported by (Figliuolo *et al.,* 2004). Based on these

results, unlike *Toxoplasma gondii* infection (Villena *et al.,* 2004; Vesco *et al.,* 2007; Ibrahim *et al.,* 2014), the environmental factors does not play an effective role on *N. caninum* infection in dairy animals. We thus found our results to be in agreement with many authors (Dubey and Lindsay 1996; Figliuolo *et al.,* 2004; Ortega-Mora *et al.,* 2007; Dubey and Schares, 2011) who believe that the main route of transmission of *N. caninum* is vertical. On the other hand, this study stated that causes of abortion other than *Brucella* in the Sudan are neglected, although 75.7% of the abortions occur in the first or the second trimester of the pregnancy. The study revealed high prevalence of reproductive failure problems including neonatal deaths, abortions and repeated abortions, where *N. caninum* infection suggested (Ortega-Mora *et al.,* 2007). Moreover, in agreement with many reports (Anderson *et al.,* 1995; Ortega-Mora *et al.,* 2007; Pabon *et al.,* 2007), a number of repeated abortions and stillbirth were reported in this study. In conclusion, in addition to providing large scale data on *N. caninum* infection in dairy animals as well as its possible economical impact in the Sudan, this is the first report on analysis of potential risk factors associated with *N. caninum* infection in the Sudan. Raising the awareness of farmers on causes of abortion may serve in control measures. Moreover, the laboratory diagnosis of animal's abortions should not be restricted to common bacterial abortifacients.

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