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Prevalence of Camels Toxoplasmosis in Gedarif State Eastern Sudan

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Abstract: The current study was conducted to evaluate the seroprevalence of toxoplasmosis in camels Gedarif State Eastern Sudan. Also compare efficacy of latex agglutination test (LAT) and indirect enzyme linked immunosorbent assay (iELISA) in determination of Toxoplasma gondiisero prevalence, Latex Agglutination Test(LAT) was applied to screen all serum samples for Toxoplasmosis while iELISA was also used to confirm the positive result obtained by LAT. A total of 300 serum samples from camels were tested, the result of LAT test show 149 camels 49.7%,infection, 78 females 52.3% and 71 males 47.7%.There were statistically significant differences in the ser-prevalence among the locations ($P<0.05$) , The positive samples in LAT were Confirmed by(ELISA) found that 44 camels 29.9% were positive . In conclusions of this study the result obtained that to confirm the wide spread of T. gondii in camels in Gedarif State, during the 2015-2016.
Keywords: seroprevalence, T. gondii, iELISA, camels, Gedarif

INTRODUCTION

Toxoplasmosis is one of the most significant animal zoonosis, distributed worldwide and affecting almost all warm-blooded animal species , and especially humans [1]. However, very few data is available about the prevalence of human [2-4] and animal toxoplasmosis in the Sudan including camel [5-6]. The vast majority of natural T, gondii infections in domestic animals are subclinical, Clinical signs, when present, are generally vague and non-specific and may include a period of fever, anorexia, respiratory distress and sometimes diarrhea. Central nervous system disorders are rarely reported T. gondii infection, however, is the major cause of abortion and prenatal mortality in sheep and goats [7], this study was aiming to estimating the prevalence of anti-toxopalsma antibodies in camel in El-Gadarif state and investigate individual animal risk factors.

MATERIAL AND METHODS

Study Location: Gedarif state It lies between latitude 12o 45 N and 14o 15 N and longitude 34o E and 37o E, and have borders with Sinar, Aljezera Kassala, Khartoum and Nile state. With Ethiopia in frontiers and Eriteria to the east. Gedarif state is an area of 71,621 km square. The study also included part of Butana area which is one of the most important grazing areas and is situated in the north part of Gedarif state .

Samples collection: The required sample size was determined to be 300 animals(represent 161 males &139 females) were collected by jugular vein puncture in sterile tubes without anti-coagulant and labeled samples were kept at−20∘C and stored for further analysis. Serum samples collected from different localities in Gedarif state (60 samples wasat AL-Gedarif-60 samples AL- shwak-100 samples AL-Rahad and 80 samples AL- Butana) serum samples were collected during the period from 2015-2016.

Laboratory procedures

Latex agglutination test

The Toxo-latex agglutination test kit was obtained from the Spinreact, S.A./S.A.U., Ctra, Santa Coloma, (GI), Spain. The Toxo-latex agglutination test was carried out as described by the manufacturer.

Enzyme-linked immunosorbent assay iELISA

The iELISA kit was obtained from the IDvet Innovative Diagnostics, rue Louis Pasteur, Grabeis, France. The kit components were reconstituted as directed by the manufacturer. These included concentrated conjugate (10X), positive and negative control sera, dilution buffer 2 and 3, concentrated wash solution (20X), substrate solution, and stop solution (0.5 M). The test procedure was carried out as per the manufacturer's protocol. A positive/negative cut-off was calculated as S/P% of ≥ 30%.

Statistical Analysis

The collected data were analyzed using the descriptive statistic of the Statistical Package for Social Sciences (SPSS) version 21.

RESULTS

Serum samples from 300 camels were tested from different localities in Gedarif state (Wasat AL-Gedarif, AL-Shwak, AL-Rahad and Al-Butana) using different serological tests, latex agglutination test for toxoplasma gondii and iELISA kits, the result of LAT test in camels show a seroreactivity correlated with significance between the surveyed locations (P<0.05). 49.7%(149camels)and 52.3% (78 female)and 47.7% (71 male)were detected to be postive. in table (1)

Table 1: Sero prevalence of Toxoplasma gondii in different Sex sera by Latex agglutination test

			Sex		Total
			Female	Male	
LAT-TEST	Positive	Count	78	71	149
		% within LAT-TEST	52.3%	47.7%	100.0%
		% within Sex	56.1%	44.1%	49.7%
	Negative	Count	61	90	151
		% within LAT-TEST	40.4%	59.6%	100.0%
		% within Sex	43.9%	55.9%	50.3%
Total		Count	139	161	300
		% within LAT-TEST	46.3%	53.7%	100.0%
		% within Sex	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.308a	1	.038		
Continuity Correctionb	3.841	1	.050		
Likelihood Ratio	4.318	1	.038		
Fisher's Exact Test				.049	.025
Linear-by-Linear Association	4.294	1	.038		
N of Valid Cases	300				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 69.04.

b. Computed only for a 2x2 table

Seroprevalence of Toxoplasma gondii antibodies in study localities by Latex agglutination test

In the300 sera samples collected from camels at different localities follows:(60 sample from AL-Shwak,80 from AL-Butana, 60 sample from Wasat AL-Gedarif and 100 sample from AL-Rahad. The positive sample with LAT test was149 - 25(16.8%) AL-Shwak,48(32.2%), AL-Butana,10(6.7%) Wasat AL-Gedarif and 66(44.3%)AL-Rahad).

Sero prevalence of Toxoplasma gondii in different Sex sera by Latex agglutination test

In the all serum samples300 ,number of females139 and males 161,positive sample was78 in females(52.3%)and in males 71 (47.7%). ser-prevalence between males and females were significantly different ,females were showing a higher prevalence 52.3% and in males were showing a lower prevalence of 47.7% as presented in table (1)

Seroprevalence of Toxoplasma gondii in different Age sera by Latex agglutination test

There were no statistically significant differences in the ser-prevalences among the age groups**.** Serum sample from camels age follows:(1-2) years142, (3-4) years78, (5-6) years 48, (7-8) years 23, (9-10) years 9. Positive sample from camels at different age follows:73(49%), 36(24.2%), 24(16.1%), 13(8.7%), 3(2%) respectively In table (2).

Table 2: Sero prevalence of Toxoplasma gondii in camels at different Age by using Latex agglutination test

			LAT-TEST		Total
			Positive	Negative	
Age sets	1-2	Count	73	69	142
		% within Age sets	51.4%	48.6%	100.0%
		% within LAT-TEST	49.0%	45.7%	47.3%
	3-4	Count	36	42	78
		% within Age sets	46.2%	53.8%	100.0%
		% within LAT-TEST	24.2%	27.8%	26.0%
	5-6	Count	24	24	48
		% within Age sets	50.0%	50.0%	100.0%
		% within LAT-TEST	16.1%	15.9%	16.0%
	7-8	Count	13	10	23
		% within Age sets	56.5%	43.5%	100.0%
		% within LAT-TEST	8.7%	6.6%	7.7%
	9-10	Count	3	6	9
		% within Age sets	33.3%	66.7%	100.0%
		% within LAT-TEST	2.0%	4.0%	3.0%
Total		Count	149	151	300
		% within Age sets	49.7%	50.3%	100.0%
		% within LAT-TEST	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.952[a]	4	.745
Likelihood Ratio	1.973	4	.741
Linear-by-Linear Association	.141	1	.708
N of Valid Cases	300		

a. 2 cells (20%) have expected count less than 5. The minimum expected count is 4.47.

ELISA Test

ELISA was used to test or confirm the positive reactors for LAT which detects T.gondii, the result of ELISA revealed 44 (29.9%) positive cases from (149) LAT positive cases table (3).

Table 3: ELISA-TEST

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	44	29.5	29.9	29.9
	Negative	103	69.1	70.1	100.0
	Total	147	98.7	100.0	
Missing	System	2	1.3		
Total		149	100.0		

Toxoplasma gondii prevalence in camels by using ELISA test in localities from positive in LAT

A total of 44 positive serum sample from different localities confirmed by ELISA from 149 positive by LAT, this result follows: (Wasat- AL-Gedarif 5 (50%), AL-Shwak 12(48%), Al-Rahad18(28.1%) and Al-Butana 9(18.8%)in table (4)**.**

Sero prevalence of Toxoplasma gondii in different Age sera by ELISA test:

Out of 300 samples tested, 149 turned out to be positive by LAT in camels age groups follows: (1-2)years72, (3-4) years 36, (5-6)years 24, (7-8) years 12, (9-10) years 3. And out of 149 Positive sample with LAT test, 44turned out to be positive when confirmed by ELISA test in camels age groups follows: 23(31.9%), 12(33.3%), 5(20.8%), 2(16.7%), 2(66.7%) respectively. There were no statistically significant differences in the ser-prevalences among the age groups**.** in table (5).

Sero-prevalence of Toxoplasma gondii in different Sex sera by ELISA test

A Total of 44 positive serum sample with ELISA test were, females 23(52.3%)and in males 21(47.7%). no Significantly (P< 0.05) sero-prevalence of T. gondii.

Table 4: Toxoplasma gondii prevalence in camels by using ELISA test in localities from positive in LAT

			ELISA-TEST		Total
			Positive	Negative	
Localities	AL-Shwak	Count	12	13	25
		% within Localities	48.0%	52.0%	100.0%
	AL-Butana	Count	9	39	48
		% within localities	18.8%	81.3%	100.0%
	Wasat AL-Gadaref	Count	5	5	10
		% within Localities	50.0%	50.0%	100.0%
	AL-Rahad	Count	18	46	64
		% within Localities	28.1%	71.9%	100.0%
Total		Count	44	103	147
		% within Localities	29.9%	70.1%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.773[a]	3	.032
Likelihood Ratio	8.568	3	.036
Linear-by-Linear Association	.540	1	.463
N of Valid Cases	147		

a. 1 cells (12.5%) have expected count less than 5. The minimum expected count is 2.99

Table 5: T. gondii prevalence in camels by using ELISA test in different Age from positive in LAT

			ELISA-TEST		Total
			Positive	Negative	
Age sets	1-2	Count	23	49	72
		% within Age sets	31.9%	68.1%	100.0%
	3-4	Count	12	24	36
		% within Age sets	33.3%	66.7%	100.0%
	5-6	Count	5	19	24
		% within Age sets	20.8%	79.2%	100.0%
	7-8	Count	2	10	12
		% within Age sets	16.7%	83.3%	100.0%
	9-10	Count	2	1	3
		% within Age sets	66.7%	33.3%	100.0%
Total		Count	44	103	147
		% within Age sets	29.9%	70.1%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.222[a]	4	.377
Likelihood Ratio	4.190	4	.381
Linear-by-Linear Association	.344	1	.557
N of Valid Cases	147		

a. 3 cells (30.0%) have expected count less than 5. The mini expected count is .90.

Table 6: Toxoplasma gondii prevelance in camels by using ELISA test in different Sex from positive in LAT

			ELISA-TEST		Total
			Positive	Negative	
Sex	Female	Count	23	54	77
		% within Sex	29.9%	70.1%	100.0%
		% within ELISA-TEST	52.3%	52.4%	52.4%
	Male	Count	21	49	70
		% within Sex	30.0%	70.0%	100.0%
		% within ELISA-TEST	47.7%	47.6%	47.6%
Total		Count	44	103	147
		% within Sex	29.9%	70.1%	100.0%
		% within ELISA-TEST	100.0%	100.0%	100.0%

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.000a	1	.986		
Continuity Correctionb	.000	1	1.000		
Likelihood Ratio	.000	1	.986		
Fisher's Exact Test				1.000	.564
Linear-by-Linear Association	.000	1	.986		
N of Valid Cases	147				
a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 20.95. b. Computed only for a 2x2 table					

Comparison of latex agglutination test (LAT) & ELISA test for detection of positive serum samples from camels

In this study, 149 (49.7%) out of 300 camels showed positive results in serum by LAT test. Moreover, 44 (29.9%) out of those 149 seropositive camels, a Toxoplasma. gondii infection was also confirmed by the commercial ELISA. The result indicated that significantly between the two test in positive result. There were no statistically significant differences in the ser-prevalence among the age groups show in table(2)and table (5). Comparison of LAT results with results obtained by ELISA in infection females and males 52.3%and 47.7% respectively are displayed in Table (1) and table (6). There was no significant difference in sex between latex agglutination test (LAT)and ELISA. infection in male and female by two tests was same result, females and males 52.3%and 47.7% respectively show in table (1) and table (6). There were significant statistical differences in the sero-prevalences of the surveyed localities between two test table in (4). In different localities the positive sample with LAT test was149 serum samples 25(16.8%) AL-Shwak,48(32.2%)AL-Butana,10(6.7%) Was at AL-Gedarif and 66(44.29%)AL-Rahad). And 44 positive serum sample by ELISA from positive result in LAT test were detected(Wasat AL-Gedarif 5 (50%), AL-Shwak 12(48%), AL- Rahad18(27.3%) and AL-Butana 9(18.8%) in table (7) .

Table 7: comparison between positive result in LAT test when confirmed ELISA test in localities

	Tested sample	**LAT-Test**	**ELISA-Test**	
		Positive	Positive	Percent %
AL-Shwak	60	25	12	48
AL-Butana	80	48	9	18.8
Wasat AL-Gadaref	60	10	5	50
AL-Rahad	100	66	18	27.3
Total	300	149	44	29.5

DISCUSSION

In the current study, T. gondii antibodies prevalent in Gedarif state in camels by using LAT was 49.7% and iELISA test 29.9% from positive result in LAT. The prevalence of this study is similarly to 44% from positive in camels on Tumbool-Sudan slaughterhouse using LAT [8], and also slaughtered animals (44.1%) in Tanta abattoir [9]. The prevalence of this study was lower than this reported in the Butana plains, mid –eastern Sudan 67% [5] by LAT, another high prevalence for Toxoplasma Gondii seropositivity was detect in Sudan using the LAT(61.7%) [6], and higher than reported 20% in camels from El-Kadaro area -Sudan using the same technique [10]. And lower when using LAT, over all prevalence of 51.3% of anti-T. gondii antibodies from sera of calf-camels with diarrhoea from different parts of the Sudan [11]. Also higher than reported in Egypt 30.7% prevalence rate in camel [12]. The difference is may be among the present study and the other reports might be due to the difference type of camel management system and number of samples taken using different serological techniques used. The results show an almost no agreement between the two tests in detecting Toxoplasma infection in camel in gedarif state. However, LAT detected more positive samples at the individual level.

CONCLUSION

From this study it clear that camel toxoplasmosis is widespread in Gedrif state. The contamination factors such as source of water and source feeds played an effective role on T. gondii infection in camels. Also positive result in LAT test should be confirmed by ELISA test.

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