Seroprevalence of *Toxoplasma gondii* in Cattle in Gezira and Khartoum States: A Comparison between ELISA and Latex Agglutination Tests

Elfahal[1], A.M., Amira M. Elhassan[2,*], Hussein[1], M.O., Enan[1], K.A., Azza B. Musa[1] and El Hussien[2], A.M.

(1) Central Laboratory, P. O. Box 7099, Khartoum, Sudan.
(2)Veterinary Research Institute, P. O. Box 8067, Khartoum, Sudan.
* Corresponding author
amirahassan98@hotmail.com

ملخص البحث

أُجريت هذه الدراسة لمقارنة فعالية إختباري تراص اللاتكس (LAT) و المقايسة المناعية المرتبطة بالإنزيم غير المباشرة (iELISA) في تحديد الإنتشار المصلي للمقوسة القوندية في أبقار اللبن في ولايتي الخرطوم والجزيرة. تم جمع وفحص عينات دم من 181 من الأبقار في الولايتين لتحديد مدي التطابق بين الإختبارين. كان الإنتشار المصلي على مستوى القطيع 44.8٪ بإستخدام كلا الإختبارين و كانت هذه النسبة 50% في ولاية الخرطوم بينما في ولاية الجزيرة 33.3٪. و على المستوى الفردي كانت نسبة الإنتشار الكلية 13.3٪ (24/181) و21.50% (39/181) باستخدام فحص التراص (LAT)و المقايسة (iELISA) على التوالي. كشف تحليل النتائج المتحصل عليها أن الإختبارين يتطابقان بنسبة 70٪ (126/181). في الختام، فانه من الواضح صلاحية إختبار فحص التراص (LAT) كفحص سريع لداء المقوسات في الأبقار علي مستوي القطيع وليس علي المستوي الفردي.

Summary

This Study was conducted to compare efficacy of latex agglutination test (LAT) and indirect enzyme linked immunosorbent assay (iELISA) in determination of *Toxoplasma gondii* seroprevalence in dairy cattle in Khartoum and Gezira States. Blood samples were collected from 181 dairy cattle in the two states and serologically examined to determine the two tests concordance. Seroprevalence of *T. gondii* at the herd level was 44.8% (13/29) using both LAT and iELISA. The infection rates at the herd level in Khartoum State was 50% whereas 33.3% in Gezira State. The overall seroprevalence of *T. gondii* at the individual level in both states attained 13.30% (24/181) and 21.5% (39/181) using iELISA and LAT, respectively. However, analysis of the obtained results revealed that the two serological tests had 93% (27/29; Kappa 0.86) and 70% (126/181; Kappa -0.048) concordance at the herd and individual levels, respectively. In conclusion, the results herein reported suggest that LAT is a simple and reliable test for quick screening of toxoplasmosis in dairy cattle at the herd level but not at the individual level.

Introduction

Toxoplasmosis, a zoonotic disease, is caused by the obligatory intracellular protozoan parasite *Toxoplasma gondii*, which infects all warm-blooded animals worldwide (Fayer, 1981; Chang, 1996). The definitive host is the domestic cat as well as wild felids.

Transmission of *T. gondii* occurs by ingestion of sporulated oocysts or bradyzoites in tissues of food-producing animals. It also occurs transplacentaly, by blood transfusion or aerosols (Dubey, 1994; Esteban-Redondo *et al.,* 1999; Tenter *et al.,* 2000). Seroprevalence in human populations ranged between 0 to 90% (Dubey and Beattie, 1988) and infection is more common in areas with warm climate and low lands than in mountainous regions with temperate climate, where environmental condition for development, sporulation and survival of oocysts are less conducive (Desmonts, 1961; Aitken, 2007).

Meagre data on prevalence of toxoplasmosis in livestock is available in Sudan (El Bedawi *et al*, 1984; Ishag, 2003; Shamoun, 2013). Camel toxoplasmosis was first reported in Sudan by El Din *et al* (1985) who reported an infection rate of 54%. Thereafter, Bornstein and Musa (1987) reported 22.5% in Sudanese she-camels. More recent study reported seroprevalence rates of 20%, 32% and 57.5% in camels, cattle and sheep, respectively (Khalil and Intisar, 2011).

The present study was carried out to determine the seroprevalence in dairy cattle herds suffering from infectious reproductive failures using LAT and iELISA tests. The study also aimed at assessing the suitability of LAT as a screening test for bovine toxoplasmosis using undiluted serum samples as used for human toxoplasmosis screening. LAT is easier to perform and would be more effective for large scale screening purposes and sero-surveys.

Materials and Methods

Sample collection

A total of 181 blood samples (168 from females and 13 from males) were collected from dairy cattle herds with infectious reproductive failures, *viz* abortion, infertility and stillbirth. The herds were raised under different management systems in farms around Khartoum (Khartoum, Khartoum North and Omdurman) and Gezira (Al Kamleen and Wad Medani) States. Blood was collected per vein puncture of the jugular vein. Following centrifugation at room temperature and 1500 rpm for 20 minutes, sera were harvested, labelled, and kept at -20°C until tested.

Serological examination

All sera were tested by LAT and iELISA for the detection of *T. gondii* antibodies. A herd was considered positive for *T. gondii* if only one animal was seropositive.

Latex Agglutination test (LAT)

Commercial serum agglutination kits (Toxo-Latex) for *Toxoplasma* antibodies detection using undiluted blood serum samples, were purchased from Coromatest (Barcelona, Spain). Any visible degree of sample agglutination is considered positive; while a smooth suspension is considered negative. Sensitivity of the test is 3-7 IU/ml, normal levels in adults are significantly less.

Indirect enzyme linked immunosobent assay (iELISA)

Commercial iELISA kits (Ruminant Serum Toxoplasmosis) for detection of *T. gondii* antibodies, were purchased from Lsivet (Nouzilly, France). Positive serum samples will present yellow colour. The colour visualized in each well is proportional to the titre of *T. gondii* specific antibody present in the diluted sample (1/400). All serum samples with an antibody titre of ≥ 20 are considered positive.

Results

Infection at the herd level

Out of 29 herds tested at both Khartoum and Gezira States, 13 (44.80%) were seropositive for *T. gondii* antibodies using either ELISA or LAT. Ten out of twenty (50%) herds in Khartoum State and three out of nine herds (33.3%) in Gezira State were positive (Table 1). Analysis of the obtained results revealed that the two serological tests had a 93% concordance (27/29; Kappa 0.86) (Table 2).

Infection at the individual level

Seroprevalence of *T. gondii* at the individual level in both states amounted to 13.30% (24/181) and 21.5% (39/181) using ELISA and LAT, respectively. When iELISA was used a seroprevalence of 12.7% (17/134) was reported in Khartoum State, and areawise, was as follows: 12.9% (8/62), 14% (6/43) and 10.3% (3/29) in Khartoum, Khartoum North and Omdurman, respectively. However, in Gezira State it was 14.9% (7/47); positive samples were detected in Alkamleen [25% (2/8)] and Wad Medani [12.8% (5/39)] (Table 3).

Table 1: Seropositivity for *T. gondii* at dairy cattle herd level in Khartoum and Gezira States using LAT and ELISA test.

States	Location	No. Herds tested	ELISA		LAT	
			+Ve Herds	+Ve%	+Ve Herds	+Ve%
Khartoum	Khartoum	8	4	50	4	50
	Khartoum North	7	4	57.1	4	57.1
	Omdurman	5	2	40	2	40
Subtotal		20	10	50%	10	50%
Gezira	Alkamleen	1	1	100	0	0.00
	Wad Medani	8	2	25	3	37.5
Subtotal		9	3	33.3	3	33.3
Total		29	13	44.8	13	44.8

Table 2: Concordance of the results obtained by LAT and iELISA at dairy cattle herd level in Khartoum and Gezira States.

	LAT(+)	LAT (–)	Total	Concordance%
ELISA (+)	12	1	13	
ELISA (–)	1	15	16	93%
Total	13	16	29	

Kappa= 0.86

Table 3: Seropositivity for *T. gondii* of dairy cattle in different locations in Khartoum and Gezira States using LAT and ELISA test

States	Location	No Samples tested	LAT		ELISA	
			No. positive	Positive%	No. positive	Positive%
Khartoum	Khartoum	62	19	30.6	8	12.9
	Khartoum North	43	11	25.6	6	14
	Omdurman	29	5	17.2	3	10.3
	Subtotal	134	35	26.1	17	12.7
Gezira	Alkamleen	8	0	0.00	2	25
	Wad Medani	39	4	10.3	5	12.8
	Subtotal	47	4	8.5	7	14.9
	Grand total	**181**	**39**	**21.5**	**24**	**13.3**

Using LAT, the overall seroprevalence in Khartoum State was 26.1% (35/134) and according to areas, it reached 30.6% (19/62) in Khartoum, 25.6% (11/43) in Khartoum North and 17.2% (5/29) in Omdurman whereas in Gezira State it was 8.5 (4/47) with 0% (0/8) and 10.3% (4/39) detected seroprevalence in Alkamleen and Wad Medani, respectively (Table 3). With regard to Gezira State, it was 10.3% (4/39) in Wad Medani with no positive serum samples in Alkamleen (Table 3).

Upon comparison of the results obtained by the two tests, 39 serum samples were positive by LAT, 4 were positive by both LAT and ELISA, 20 by iELISA only and 122 were negative by both tests. This revealed an overall concordance of 70% (126/181; Kappa -0.048; Table 4).

Table 4: Concordance of the results obtained by LAT and iELISA in serum samples of 181dairy cattle in Khartoum and Gezira States.

	LAT(+)	LAT (–)	Total	Concordance%
ELISA (+)	4	20	24	
ELISA (–)	35	122	157	70%
Total	**39**	**142**	**181**	

Kappa= -0.048

Discussion

The results show an almost complete agreement between the two tests in detecting *Toxoplasma* infection at the herd level. However, LAT detected more positive samples at the individual level (Table 1;3).

In the current study, *T. gondii* antibodies were prevalent both at the herd and at the individual dairy cow level using both LAT and iELISA tests. Nine out of twenty herds (45%) tested in Khartoum State were seropositive. In Gezira State only three out of nine dairy herds (33.3%) were seropositive. The highest herdwise seropositivity (57.10%) is reported in Khartoum North in Khartoum State. It is interesting to note that this high seroprevalence (4/7) can explain the high abortion rates reported among dairy cattle kept in farms of this particular area where an earlier bovine toxoplasmosis suspect cases had been reported in 2005 (O.M. Ahmed, personal communication).

Seroprevalence of *T. gondii* reported in the current study in Khartoum State attains 13.3% and 26.1% using ELISA and LAT respectively. More recently, Khalil and Intisar (2011) reported 32% (16/50) seroprevalence of *T. gondii* in cattle in Khartoum State using LAT test. The discrepancy between the results of the two studies is difficult to explain but it may be due to the small number of cattle investigated by Khalil and Intisar (2011). The relatively low concordance (70%) observed between the two tests, in the present study, may be due to the fact that ELISA is more specific than LAT test. The low Kappa value (-0.048) indicates that LAT test using undiluted serum should not be used for toxoplasmosis detection in individual animals. However, the high value of Kappa (0.85) obtained from comparing the test data at herd level suggests its usefulness as screening tool for infection at herd level.

Acknowledgements

The authors are deeply grateful to the Director/ Veterinary Research Institute and Director General/ Animal Resources Research Corporation for permission to publish this article. Thanks are also extended to Prof. Ali Yousif for corrections of manuscript.

References

Aitken, I.D. (2007). *Diseases of sheep*. 4th edn. Blackwell, Oxford. Pp. 137-142.

Bornstein, S. and Musa, B.E. (1987). *J. Vet. Med B*., **34**: 364–370.

Chang, H.R. (1996). *Inter. J. STD and AIDS.,* **7**: 18–22.

Desmonts, G. (1961). *Annales de Biologie Clinique,* **19**: 13–28.

Dubey, J.P. (1994). *J. Am. Vet. Med. Assoc.,* **205**: 1593-1598.

Dubey, J.P. and Beattie, C.P. (1988). *Toxoplasmosis of Animals and Man*. 2nd edn. CRC Press, Boca Raton, USA, pp. 200-02.

El Bedawi, K.S.; Michael, S.A. and Morsy, T.A. (1984). *J. Egypt. Soc. Parasitol.,* **14**: 289 – 293.

El Din, E.A.Z.; El Khawad, S. and Kheir, S.M. (1985). *Rev.'Elev. Méd. Vét. Pays. Trop*., **38**: 247-249.

Esteban-Redondo, I.; Maley, S.W.; Thomson, K.; Nicoll, S.; Wright, S.; Buxton, D. and Innes, B.A. (1999). *Vet. Parasitol.*, **86**: 155–171.

Fayer, R. (1981). *Canadian Vet. J*., **22**:344-352.

Ishag, Manal Y. (2003). *Studies* on *Toxoplasma and Sarcocystis* from *camels (Camelus dromedarius)* in the *Sudan* Ph.D. Thesis, University of Khartoum, Sudan.

Khalil, M.K. and Intisar, E. (2011). *J. Vet. Med. Anim. Hlth.*, **3**: 36-39.

Shamoun, Awadia A. (2013). *Prevalence of Toxoplasma gondii slaughtered in Port Sudan and Tokar slaughter house, Red Sea State.* Ph.D. Thesis, University of Khartoum, Sudan.

Tenter, A.M.; Heckeroth, A.R. and Weiss, L.M. (2000). *Int. J. Parasitol.*, **30**: 1217–1258.