Vet.Med.J.,Giza. Vol.50, No.3. (2002) : 357-375.

TOXOPLASMA INFECTION AMONG GOATS WITH SPECIAL REFERENCE TO ITS EFFECT ON LIVER AND KIDNEY FUNCTION

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Received: 23. 1. 2002

Accepted:17. 3. 2002

SUMMARY

96 (ninety-six) goats housed in farm-Al-Amiria-Alexandria govenerate, were investigated for *Toxoplasma gondii* (*T. gondii*). Serum samples were analyzed for serological and biochemical studies to detect the liver and kidney alterations. The distribution of titer of Indirect heamagglutination Test (IHAT), Indirect Fluorescent antibody Test (IFAT) and Enzyme linked imuno-assay (ELISA) was determined. The percentage of samples as test sensitive for each of these tests were 18.751, 28.12% and 31.25% for IHAT, IFAT and ELISA respectively. 66 goats (68.75%) were sero-negative by ELISA test and 30(31.25%) was sero-positive. The positive were classified into low, medium and high titer on the basis of severity of infection and titer reaction.

The results indicated that lower titer of Toxoplasma antibody demonstrated highly significant increase of serum bilirubin, aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and lower significant increase of urea with low significant decrease of serum total protein and alanine aminotransferase (ALT). The moderate titer of *T. gondii* characterized by an highly significant increase of serum total bilirubin, ALP and uric acid and moderate significant increase of AST, and urea and significant decrease of serum total protein and ALT. It were also detected high significant decrease of total protein with significant elevation of serum bilirubin, AST, ALP, urea was accompanied with high Toxoplasma titer. Changes in the protein profile especially albumin and β-globulin were observed.

The conclusion of this study revealed that ELISA is the most sensitive serological tool. Liver and kidney function are altered by *T.gondii*, the functions greatly impaired especially with the presence of heavy infection which appears as a high antibody titer.

INTRODUCTION

The genus Toxoplasma has a single species of Toxoplasma gondii which having heteroxenaus life cycle. Cats act as a final host while all other animals act as intermediate host. (Dubey, 1976). Infections by Toxoplasma gondii are widespread in many species of warm blooded animals (Dubey & Beattie, 1988). The life cycle includes a facultative system phase which is an important cause of abortion in sheep and may also cause a zoonosis in human. Undoubtedly the most important role of toxoplamsosis in ruminant is associated with abortion in ewe and prenatal mortality in lambs. Dubey, (1980) isolated *T. gondii* from meat of goat. Human became infected by ingesting tissue cysts of *T. gondii* containing bradyzoites of uncooked meat, water or food contaminated with cysts of affected goats. (Dubey & Beattie. 1988) . These infected goats were older when sold and are made into processed meats, which are usually highly seasoned, smoked, heated or frozen. The market weight animals are sold as commercial cuts of goat meat and *T. gondii* has been found in these goat tissues used for human consumption. Serological surveys suggest Toxoplasma infection to be common in goat. (Cross et al, 1976; Tizard et al, 1977; Ruppanner et al, 1978 and Al-Khalidi & Dubey, 1979). Toxoplasma gondii may found in the goat milk (Tizard et al, 1977) and Toxoplasma tachyzaites passed in the milk of infected does, so there is a risk to children and pregnant women. (Smith & Sherman, 1994).

Although Jacobs et al, (1999) evaluated ELISA test as sensitive tools for the diagnosis of acute and chronic acquired infection with *T. gondii*. They measured the sensitivity of ELISA for Ig-G to be 81% for acute phase samples and for routine screening sera. For chronic-phase sera, the sensitivity was 79%. Diagnosis of *T. gondii* depends on serological test results to identify the infected goats.

In this paper, we compared between three serologic tests: IHAT, and IFAT,the last one is perfect tool as it does not require live organisms. More recently, an ELISA test has been developed which is capable of detecting a recent infection by the estimation of Ig-M, as compared to Ig-G antibody. (Urquhart et al, 1988). Presence of little evidence or information supports either relation or affect of infection by *T. gondii* or its titer has effect on liver or kidney and its function on the Egyptian goat. On the other hand there are no data on the specificity and sensitivity of various serologic tests for detection of latent *T. gondii* infection in caprine. The objective of this study was, to assess the sero-prevalence of *T. gondii* among goat population. Secondly, to compare the sensitivity of various serologic tests for diagnosis of acute *T. gondii* infection in goat naturally exposed to *T. gondii*. Thirdly, the relationships between the Toxoplasma titer and the liver and kidney statues with special view of protein profile as the main product to the liver tissue was also studied. Finally, evaluation the both liver and kid-

ney function test as tools in prognosis of *T. gondii* was also conducted.

MATERIALS AND METHODS

Blood samples were obtained from the 96 goats from jugular vein housed in farm -Al-Amiria - Alexandria govenerate in March 2001. Serum samples were separated and stored at -20°C. and were frozen until be analyzed for serological and biochemical studies.

Serologic examination for *Toxoplasma gondii* :
Serum samples were frozen and thawed several times during the process of examination by the various serologic assays: IHAT, ELISA and IFAT tests.

1. Indirect heamagglutination Test (IHAT):
The IHAT was preformed by using commercial kits and following the manufacture instructions. (Sigma Co.-USA). Sera were diluted twofolds, from 1: 32 to 1: 512. A titer more than 1:64 was considered as a positive result in the IHAT. (Patton et al., 1990 and Dubey et al, 1996).

2. Enzyme linked imuno assay (ELISA):
Ninety-sex (96) well microtitration plates U-shape were coated with *T. gondii* Tachyzaite laysate (9.2 µgm of protein/ml corresponding to the equivalent of $5X10^8$ tachyzaites/ml diluted in phosphate buffer saline (pH 7.2) by inoculating 1100 µl/well overnight at 4°C, after each step, microtitration plates were washed 5 times with 0.01 M phosphate-buffer, (pH 7.4) containing 0.5 M NaCl and 1% Tween 20 (PBST) using ELISA washer. Each assay included 5 positive-control sera and 1-negative control one along with the test sample. To perform the ELISA, coated plates were washed and sera diluted were 1:400 with PBST and incubated for one hour at room temperature. The plates were washed; coated with rabbit anti-goat Ig-G conjugated to horse radish peroxides diluted 1: 2.000 in BPST, 1% normal bovine serum albumin was added. After incubation at room temperature, the plates were washed and substrate was added, the substrate consists of 2mg O-phenylenylenediamine dihydrochloride in 12 ml citrate buffer (pH 5) with 5 M 30% hydrogen peroxide. The substrate was incubated for 10 minutes; the reaction was terminated by adding 100 µl 0.5 M sulfuric acid. The plates were read by measuring O.D. at 940 nm on a dual-beam V-max ELISA spectrophotometer automatically subtracting reference absorbency at 690 nm. O.D. more than 0.360 gave a significantly positive for *T. gondii*. (Dubey, et al., 1996).

3- Indirect Fluorescent antibody Test (IFAT):
Multi-spot slides layered with air-dried Rh strain *T.gondii* tecyzoites. The RH strain *T. gondii* tachzaites and rabbit anti-goat Ig-G flouorescein conjugate were used, serum were

diluted 1:20 with phosphate buffer saline (PBS), (pH 7.2) and conjugate was diluted 1:100 and the test was performed. (Sigma Co. USA). Slides were mountained at - 20°C until used. Staphylococcal protein fluorescing isothiocyanate (Fitc) was used as the conjugate (0.25 µg of T.C/mg protein) and the test was preformed as described by Johnson et al., (1987) and Literak et al., 1997). A titer of 1:32 or more considered as positive for *T. gondii* According to Michael et al., (1985) .

B. Biochemical analysis:

Serum samples were analyzed for the following enzymes activites: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), (Reitman & Frankel, 1957) and alkaline phosphatase (ALP), (Belfield & Goldberg,1971). Biochemical parameters preformed as methods described by Henry, (1975);Doumas et al, (1973); Patton & Crouch,(1977); Young et al, (1975); and Faulkner & King, (1976) for total protein, total bilirubin, urea, uric acid and creatinine respectiviely. Electrophoritic set was assembled according to the procedures described by Bio Rad manual. (Ologohobo et al., 1995).

3.Statistical analysis:

The positive results of a titer 1: 64, or at O.D. 0.360 or 1: 32 or higher for IHAT, ELISA and IFAT respectively were considered confirmatory for presence of *T.gondii* antibody. The agreement among serologic test was evaluated by calculating the Kappa statistic and its 95% confidence interval. (Fleis, 1981).

According to ELISA results, we classified the sera samples into three categories: Low T.gondii titer, serum contains Ig-G with less than 1 O.D. (0.360-0.999), Moderate T.gondii titer, serum contains Ig-G between than 1 and less than 2 O.D. (1.000- 1.999), while *T.gondii* high titer, serum contains Ig-G more than 2 O.D. (2.000 and more) and compared with control goat serum from the same farm.

The statistical analysis of the results was preformed using T-test, ANOVA test (one way Analysis of variance) and correlation values using Pearson correlation with P-value. (Farver, 1989).

RESULTS

Our Data demonstrated that the incidence of T.gondii in the acute stage of infection in goat was 31.25%, 28.12% and 18.75% using Elisa, IFAT and IHAT respectively. The titre incidence of *T. gondii* using ELISA method was 33.33, 43.34 and 23.33% in low, medium and high infection titer respectively. The results reflected the immuo-statues of the goat against the infection and the sensitivity of the tests.

Serological results are summarized in table (1-5). 30 goats from 96 goats were serologically sero-

sitive by ELISA. Results obtained by IHAT nd IFAT were less constant and lower than those btained by Elisa test (table 1) indirect heamag-lutination test (table 1) found that titer < 64 in 78 1.25%) goat had a sero-negative. The reaming 8 (18.75%) goats had sero-positive, therefore 9 50%) had titer 1:64, and the strongest response were obtained from 6 (33.33%) and 3 (16.66%) goats gad titer > 128 and > 512 respectively.

For IFAT titer < 32 in 69 (71.87%) of animals were sero-negative, where 27 goats (28.12%) had sero-positive titer. However 3(11.11%) had a titer > 32, 12 (44.44%) had titer > 64 3, 3 (11.11%) had titer > 128, 4 (14.81%) had titer of > 256 and 5 (18.52%) animals had a maximal titer > 512.

66 goats (68.75%) were sero-negative by ELISA test, the remaining 30(31.25%) was sero-positive, we were classified into low, medium and high titer on the basis of severity of infection and titer reaction, were 10 (33.33%), 13 (43.34%) and 7 (23.33%) goats with O.D. > 0.360, >1.000 and >2.000 respectively.

Table (1) Distribution of antibody titer of *T. gondii* by the use of 3 serologic tests.

IHAT			IFAT			ELISA		
Titer	Ereq.	%	Titer	Ereq.	%	Titer	Ereq.	%
0-<32	66	68.75	0	27	28.12	0-350	66	68.75
<32	5	5.26	2-8	28	29.16	Low	10	10.42
32	7	7.29	16	14	14.58	Med.	13	13.54
64	9	9.28	32	3	4.35	High	7	7.29
128	6	6.25	64	12	12.5			
>512	3	3.17	128	3	4.35			
			256	4	4.30			
			512	5	5.21			
Total	18/96	18.75	Total	27/96	28.12	Total	30/96	31.25

The distribution of titer of IHAT, IFAT and ELISA was determined (table1). The percentage of samples as test sensitive for each of these tests were 18.751, 28.12% and 31.25% for IHAT, IFAT and ELISA respectively.

Table (2): Comparison of *T. gondii* antibody titer in goat by use of IHAT & IFAT tests.

IFAT	IHAT						Total
	0	<32	32	64	128	>512	
0	27	0	0	0	0	0	27
2-8	28	0	0	0	0	0	28
16	11	0	0	3	0	0	14
32	0	3	0	0	0	0	3
64	0	2	5	3	2	0	12
128	0	0	0	1	1	1	3
256	0	2	0	2	1	1	4
512	0	0	2	0	2	1	5
Total	66	5	7	9	6	3	96

The strongest responds was obtained from 3 goats had titer > 512 by IHAT in comparison with 5 goats had titer > 512 by IFAT.

Table 3: Comparison of *T.gondii* antibody titers in goat by use of IHAT and ELISA tests.

ELISA	IHAT						Total
	0	<32	32	64	128	>512	
0	66	0	0	0	0	0	66
Low	0	5	5	0	0	0	10
Medium	0	0	0	8	3	2	13
High	0	0	2	1	3	1	7
Total	66	5	7	9	6	3	96

The highest titer showed by ELISA in 7 goats in contrast with 3 goats by IHAT.

Table 4: Comparison of *T.gondii* antibody titers in goat by the use of IFAT and ELISA tests.

ELISA	IFAT								Total
	0	2-8	16	32	64	128	256	256	
0-360	66	0	0	0	0	0	0	0	66
Low	0	0	3	3	3	0	0	1	10
Medium	0	0	0	0	8	1	3	1	13
High	0	0	0	0	1	2	1	3	7
Total	27	28	14	3	12	3	4	5	96

Comparison *T. gondii* antibody titer revealed the strongest reaction in 5 goats at titer > 512 by IFAT corresponding to 7 goats at high O.D. (2.000 or more) by ELISA test.

Table 5: correlation table between IHAT, IFAT and ELISA as tools of detection *T. gondii* antibody titers in goat

	ELISA	IHAT
IIHAT	0.514 0.004	---
IFAT	0.489 0.006	0.294 0.115

The correlation table indicated the presence of moderate positive correlation between ELISA and IHAT than between ELISA and IFAT. (Pearson correlation, P- values).The result in table (5) indicated medium positive correlation between the result ELISA and IHAT and with less degree the correlation of the result of ELISA and IFAT.

Table 6: Biochemical changes in serum of toxoplasmaosis infected goat.

Case		Total Protein	Bilirubin	ALT	AST	ALP	UREA	Uric acid	ACreatinine
		g/dl	mg/dl	U/L	U/L	U/dL	mg/dl	mg/dl	mg/dl
Control		7.520 ±0.80	0.747 ±0.05	20.76 ±1.09	48.64 ±0.21	64.02 ±0.77	7.543 ±0.78	49.74 ±2.57	1.014 ±0.07
ELISA Titer	Low	7.236 ±0.90*	0.856 ±0.02***	20.54 ±0.43*	50.62 ±0.38***	161 ±10.8***	11.39 ±0.52*	48.75 ±1.94*	0.939 ±0.06*
	Med.	6.873 ±0.50**	0.932 ±0.05***	20.16 ±0.30**	49.75 ±0.52**	163.2 ±15.8***	10.69 ±0.61*	50.36 ±1.61*	1.014 ±0.05
	High	6.334 ±0.23***	0.881 ±0.06***	20.50 ±0.30*	50.29 +0.57***	185.1 +23.4***	13.77 ±1.69**	47.98 ±0.93**	1.072 ±0.05

Results of table (6) (using student T-test) indicated that lower titer of toxoplasmosis demonstrated highly significant increase of serum bilirubin, AST and ALP, lower significant increase of urea with low significant decrease of serum total protein, ALT, uric acid and creatinine. The moderate titer of *T. gondii* characterized by an highly significant increase of serum total bilirubin, ALP and moderate significant increase of AST, and urea and uric acid. Significant decrease of serum total protein and ALT. The highly significant decrease of total protein, ALT and uric acid with significant elevation of serum bilirubin, AST, ALP, urea, and accompanied with high Toxoplasma titer.

Table 7: Serum Protein profile changes in toxoplasmosis infected goat (gm / dL).

	Control	Toxoplasmosis (Elisa titer)		
		Low	Medium	High
Albumin	4.388 ± 0.226	3.821 ± 0.18**	3.934 ± 0.19**	3.411 ± 0.17
α- 1 globulin	1.214 ± 0.06	1.418 ± 0.07***	1.246 ± 0.06	0.509 ± 0.03***
α- 2 globulin	0.391 ± 0.02	0.305 ± 0.02*	0.287 ± 0.01**	0.411 ± 0.02**
β- 1 globulin	0.485 ± 0.03	0.587 ± 0.03***	0.288 ± 0.01***	0.999 ± 0.05***
β- 2 globulin	0.163 ± 0.01	0.335 ± 0.02***	0.351 ± 0.02***	0.321 ± 0.02***
γ- 1 globulin	0.297 ± 0.02	0.365 ± 0.02*	0.303 ± 0.02	0.336 ± 0.06***
γ- 2 globulin	0.581 ± 0.03	0.406 ± 0.02***	0.462 ± 0.01***	0.346 ± 0.02***
A/G	1.401 ± 0.07	1.119 ± 0.05***	1.339 ± 0.06***	1.167 ± 0.08***

Using ANOVA test (one way ANOVA) represented that there was highly significant decrease in albumin reach to 22.26% in the high titer of toxoplasmosis goat and start with 10.35% in the low titer infected goats. The α-1 globulin (acute phase protein) increased in case of low titer toxoplasmosis infected goat but significantly decreased in high titer toxoplasmosis infected goat. But the α-2 globulin significantly decreased in both low and medium titer of toxoplasmosis-infected goats but significant increased in high titer of toxoplasmosis infected goats. The β-1 globulin (mainly to kidney function test) increased in both low and high titer of toxoplasmosis infected goats but significant decreased in medium titer of toxoplasmosis infected goats. The β-2 globulin significantly increased (three times) in low, medium and high titer of infected goat. Although γ-1 globulin significantly increased in low, medium and high titer of toxoplasmosis infected goats, γ-2 globulin significantly decreased in low and high titer of toxoplasmosis infected goats. The A/G ratio is expressed the change of decreased albumin and increased globulin in low,

medium and high titer of toxoplasmosis by significant decrease in A/G ratio. It was clearly obvious demonstrate that the percentages change in the serum protein fraction in relation to total protein is changing in the percentage itself not change in the total protein amount only. The albumin level significant decreased about 4-10%. The α-1 globulin significant increased about 11-21% increased in moderate and low titer of toxoplasmosis - infected goats and significant decreased about 50% in high titer. The α-2 globulin decreased in both low and moderate titer of infected goat but increased in high level titer. The β-1 globulin increased about 144.61% in high titer toxoplasmosis infected goat, while significactly decrease in medium infected goat. The γ-1 increased significantly in low, moderate and high titer of toxoplasmosis infected goats. the level of γ-2 significantly decreased in low, moderated and high titer level of toxoplasmosis. The change in A/G ratio expressed in the change of decteased albamin and increased globulin.

DISCUSSION

Results of these investigation indicated that 31.25% of 96 clinically normal goat in a farm in AL-America, Alex. governorate had been exposed to *T. gondii*. The high prevalence of *T. gondii* specific antibody detected in this study indicated heavy environmental contamination with *T. gondii* in these desert part of Alexandria governorate and also highly susceptibility of goat to *T. gondii*. These results are not similar to the ... logical prevalence of *T. gondii* in different ... mals at different localities. Weinmam & Chandler (1954 and 1956) noticed increased infection rate (48%) in farms where used uncooked garbage was fed to pigs and rats were abundant in the swine houses. Sibalic, (1966) found 10.10% over all prevalence of *T. gondii* in pigs. Matsui et al (1967) observed that *T. gondii* infection rates varies in different animals from low 0 to high 30%, the over all prevalence was 10.30%. Ohshima et al, (1971) demonstrated the infection rate to be 11.4% in breeding sows. Dubey et al, (1991) found that antibodies to *T. gondii* were found in 23% of marked fed pigs slaughtered in USA from 1983 to 1984. In other research report, Dubey et al, (1995) mentioned that 17% from 1000 saw were naturally infected with *T. gondii*.

Whatever, This difference was related to either to the locality or to the demographics of goat population samples (the goats in the present study were in an open farm, state wide and natural ecology than surrounding private lands and farm, and there were a lot of wild cats) or to the methods of husbandry rather than the of ecosystem, which play a greater role in *T. gondii* infection in goats.

The present results revealed that goats could develop *T. gondii* antibody titer without clinical signs.

Our tabulated data declared that revealed an in-

crease incidence of infection of goats, 31% ($P<0.05$) compared with other animals. The overall prevalence of *T. gondii* antibody in these goat is 31.25%, 28.12% and 18.75% by ELISA, IFAT and IHAT respectively. The variability of antibody response of goats is in part related to antigen and the type of reaction in three serological tests used in the present study. Chaudhary et al, (1996) observed an 18% prevalence of toxoplasmosis, diagnosed by the indirect latex agglutination test, was observed in 100 racing camels. The present data revealed that high incidence of goat susptability to toxoplasmosis compared with camels.

The data of present study showed that strongest response was obtained from 3, 5 and 7 goats had titer >512, >512 and O.D >2.000 or more by IHAT, IFAT and ELISA respectively. This declared that ELISA test is useful for serological diagnosis of *T. gondii* infection in goats. This observation was supported by Dubey et al, (1996) who mentioned that ELISA are useful tools in diagnosis of latent toxoplasmosis in pigs. Another reasons that ELISA can detect a recent infection in goats because antibodies against tachyzaites stages of the parasite developed early.

Among the examined serological test, the result demonstrated that ELISA had a high sensitivity (30/96) (31.25%) (titer >0.360 O.D.) compared with IFAT and IHAT. However, the ELISA had a higher false-positive rates (estimated 3.13-12.5%) than other tests (IFAT and IHAT). There is little known to actual infection level in Egyptian goats. ELISA appears to be the most practical assay for toxoplasmosis but will need refinement in procedures and standardization of antigen used. The sensitivity and specificity of ELISA compare favorably and be useful as a screening test with that of Dye test (DT). Waltman et al, (1984).

The IHAT has the lowest prevalence and sensitivity (table 1-5), estimating using manufactureís recommended cut-off titers. The IHAT has low percentage because a higher cut-off titer 1: 64 compared with that of other tests. Specificity at a cut-off of titer 1: 32 was reduced to 66.66% in IHAT. So that IHAT is the least sensitivie test for diagnosis of *T. gondii*. In agreement with our result, Dubey et al, (1995) concluded that IHAT is the least sensitive test for diagnosis of *T. gondii* infection in pigs.

Little is known about specificity and sensitivity of IHAT in goat. Durfee et al, (1974) mentioned that *T. gondii* was isolated from 9 of 31 pigs in Indonesia including 7 pigs with IHAT titer less than 1:64. The commercial IHAT has sensitivity of 29.4% at titer of 1:64 and 53.5% at titer of 1:32. We suppose that for the different techniques which were used to prepare the soluble antigen for *T. gondii* used in IHAT in different laboratories and there are no data comparing sensitivity of various IHAT used. Dubey & Beattie, (1988) considered that the manufacture recommendation cut-off titer of 1:64 for determination of positive re-

sults. The IHAT had the lowest sensitivity among the tests compared here; it also had poorest agreement with the other serological survey in *T. gondii* survey. IHAT was used with horse sera, poor sensitivity was observed by Al-Khalidi & Dubey, (1979). Also finding of, Sato et al, (1962); Hanaki et al, (1963) and Ohshima et al, (1971) were supported our observation in this study by using IHAT serological diagnosis of *T. gondii*.

Although the D.T test was not used in our study, IFAT titer generally parallel Dye test titers. (Ishizuka, 1978) . Our result declared that the IFAT (28.12%) is less sensitive than ELISA but more sensitive than IHAT. Omata et al, (1994) isolated *T. gondii* from pigs diaphragm, in Brazil, of 5 of 12 with IFAT titer (1:1.024 or greater); 6 of 57 with IFAT titer (between 1:64 and 1:256) and 3 of 40 with IFAT titer (1.16 or less a negative result for *T. gondii* infection in swine. Venturinin et al, (1999) found 15 of 738 samples in pigs by IFAT. On the other hands, our observation is in agreement with Shirahata & Shimizu, (1974) and Ishizuka, (1978) used IFAT.

With respect to table (5), the presence of moderate positive correlation between the results obtained from ELISA and that obtained from IHAT and between ELISA and IFAT, these results are agreement with observation of Landi & Kock, (1977) who reported good correlation between ELISA and indirect fluorescent antibody (IFA) titer, but not IHAT titer in 100 serum from naturally exposed swine. Takahashi & Konishi, (1986) found poor correlation between ELISA and latex agglutination test (LAT) in swine. Waltman et al, (1984) and Prickett et al, (1985) compared the sensitivity 2 types of ELISA in pigs inculcated with 10.000 *T. gondii* oocystes, they reported 97.4% (sensitivity) using IFAT and end titer ELISA; 88.2% for percentage ELISA and 81.6% for IHAT. This result suggest higher sensitivity for the Elisa than for IHAT, which in consistent with results of our study.

Our data revealed that, *T. gondii* was isolated from 30 goats by using ELISA. The rest of goat (66) did not detect antibody by ELISA. (seronegative). It indicated that either those goats were recently infected and had not yet developed *T. gondii* antibodies or that the antibodies titers had decreased to undetectable values, or that these goats not exposed to infection with *T. gondii*.

Determination of *T. gondii* antibodies in serum of goats is useful, economical and rapid for the diagnosis of *T. gondii* induced abortion in goats. Species of intermediate susceptibility includes sheep, goats, where toxoplasma induced abortion can constitute sever problems, (Buxton 1990 and Kirkbride, 1993) .

Toxoplasma organisms spread via the blood stream to other tissue including muscle, brain and liver. (Smith & Sherman, 1994). Although Kidney rare mentioned as affected organ. We used

the antibody titer as indirect way to study the effect of *T. gondii* or it's Tachyzoites. (Dzierszinsk et al, 1999) mentioned that an increasing titer would at least indicate recent infection *T. gondii* encoding two glycolytic enzymes (glucose 6-phosphatase isomerase and endolase). Results of table (6) indicated that goats with lower titer of toxoplasmosis demonstrated highly significant increase of serum bilirubin, AST and ALP, lower significant increase of urea. The significant increase of bilirubin, AST, ALP, urea and uric acid and significant decrease of serum total protein and ALT accompanied with goats with medium Toxoplasma titer. The significant decrease of total protein uric acid and ALT with the significant elevation of bilirubin, AST, ALP and urea accompanied goats with high Toxoplasma titer. Our result is contrary to the result of Chaudhary et al, (1996) who observed that the blood chemistry (ALP, ALT urea, total protein and albumin) did not differ significantly between the control and toxoplasmosis infected camel. But we can attribute the result into the difference in the animal species and the incidence of infection.

The ALP activity increased from 2.5-3 times in low, medium and high titer of toxoplasmosis infected goat, which may be attributed into the hepatic dysfunction, and cholestasis, Olsson et al, (1982) evaluated cholestasis by ALP. They mentioned that elevation of serum ALP is associated with irritation or destruction of biliary epithelium and can occur in obstructive condition of the biliary system. This supported by the highly significant elevation of serum total bilirubin. Although Wasfi and Adam, (1976) mentioned that the dramatic increases in serum or plasma bilirubin in the goat are due to hemolytic crises rather than liver dysfunction.

Although ALT is low significant decrease in our research which indicated the disruption of hepatocytes and can lead to measurable increased in serum ALT levels as intracellular hepatic enzymes in goats but we cannot considered him as only monitor of hepatic parenchmal diseases. In agreement with us, Kanecko, (1990) who mentioned that ALT is used as indicator of hepatic disease in cat but not in ruminant because livers of cattle and sheep contains low levels of ALT. This also true in goats. Low changes in serum ALT concentration in response to hepatic injury are support to this theory.

As the increase in AST observed high activity may damage is not liver specific and a result from muscle damage. Smith & Sherman, (1994) supported our present observation of AST elevation especially in low and high titer of toxoplasmosis, we can suggest the AST measurement to reveal the degree of muscle affection as predilection site of *T. gondii*.

There was highly significant decrease in albumin reach to 22.26% in the high titer of toxoplasmosis goat and start with 12.92% in the low titer

infected goats. The liver is the main primary site of albumin production and hepatic insufficiency leads to hypoalbuminemia. Kanecko, (1990) reported that serum albumin may diminish in acute liver disease but the level rarely fall to pathological low values. This notes are supported by Yoda et al., (1990) who reported taht the serum albumin decreased 37% to 40%, 12 h later and returned to 40% by 48 h after injection of Toxoplasma laysate antigen in Toxoplasma-immune cattle. Data in Table (7) revealed that thae α -1 globulin (acute phase protein) increased in case of low titer toxoplasmosis infected goat but significantly decreased in high titer of toxoplasmosis infected goat. The α-2 globulin significantly decreased in both low and medium titer of toxoplasmosis-infected goats but significantly increased in high titer of toxoplasmosis infected goats. Th β-1 globulin (affected mainly to kidney function) increased in both low and high titer of toxoplasmosis infectedgoats but significant decreased in medium titer of toxoplasmosis infected goats. The β-2 globulin significantly increased three times in low, medium and high titer of infected goat. The γ-1 globulin significantly increased in low, medium and high titer of toxoplasmosis infected gaots, This may due to the acute infection of toxoplasmosis because this area of globulin contains the I-g G and Ig-M. Supported of this finding, the presence of necrotic foci surrounded by inflammatory cells and focal leucomaloci in histopathological examination of aborted fetal tissues (brain, lung and liver) or due to presence of Dense granule antigen (GRA6) of *T. gondii* which plays an important role of antiginicity and pathogenicity of *T. gondii*, (Fazaeli et al., 2000). The γ-2 globulin significantly decreased in low, medium and high titer of toxoplasmosis infected goats. This was supported by observation of Chaudhary et al., (1996) who observed a significant ($P < 0.05$) decrease in the white blood cell count and an increase in esoinophile counts were observed in camels positive for toxoplasmosis. The A/G ratio expressed the change of decreased albumin and increased globulin in low, medium and high titer of toxoplasmosis by significant decrease in A/G ratio.

The effect of *T. gondii* on liver as the main protein manufacture which clearly obvious demonstrated the percentages of serum proteins. The albumin level decreased about 4-10%, which in concern with other result declared that the ability of infected hepatocytes to synthesize protein is greatly impaired. The α-1 globulin significant increased about 11-21% increased in moderate and low titer of toxoplasmosis-infected goats and significant decreased about 50% in high titer. The α-2 globulin decreased in both low and moderated titer of infected goat but increased in high level titer. Faulkner, & King, (1976) reported that elevation of the α-2 globulin region proteins are seen in conjugation with a depression of albumin, but Kanecko, (1990) reported that in acute inflammatory disease and in the nephrotic syndrome, α-2 globulin increase due to partialy increase of the

α-2 macroglobulin, (It's decay, kidney origin). The β-1 globulin increased about 144.61% in high titer toxoplasmosis infected goat. This region in the polyacrylamid electrophoresis contains β-2 globulin, transferrin, ferritin and hemopexin. Kanecko, (1990). Faulkner & King, (1976) mentioned that elevation of protein in this region occurs in liver diseases and compensatory increases are seen in sever albumin loss (nephrosis). Kanecko, (1990) reported that β-globulin increased in association only with active liver disease and in nephorotic syndrome. Although creatinine is not significanlty increase in serum, increase in the β-globulin support our observaiton that kidney start to affected especially with high *T. gondii* infection. The γ-1 increased significantly in low, moderate and high titer of toxoplasmosis infected goats. Ig-A, Ig-M and Ig-E are found in the γ-1 region, while Ig-G is found primarily in the γ-2 region. The increased level may due to protein C which will recognized by serum Ig-G antibodies from *T. gondii* - infected humans and *T. gondii* infected sheep. Mevelec et al., (1998).

The level of γ-2 significantly decreased in low, moderated and high titer level of toxoplasmosis. This result declared, the association of results of serology tests with that obtained of electrophoresis and second that this infection is recent infection. The change in the protein profile is strong suggestion of the selective effect of *T. gondii*. on the liver.

Our control tabulated data are in the range data results of Hasim & Braun, (1989) who ranged serum urea, uric acid and creatinine as 10-48, 0.9-1.8 and 0.33-1.0 mg/dl, respectively. The elevation of serum uric acid in toxoplasmosis infected goats may be attributed to presence of evidence of activity of hypoxanthine guanine phosphriboxyl transferees and xanthsine 5 monophosphatase of the *T. gondii*, (Heroux et al., 1999). The non-significant elevation of creatinine appears to be attributed to creatinine clearance. It was depend on existence of a tubular secretary mechanism for creatinine excretion in goat. Smith & Sherman, (1994) mentioned that creatinine clearance was determined not to be a reliable measure of glomerulare filtration rate (GRF) in goat. Although it is well known that Bedouin goat possessed a superior capacity to reduce glomerulare filtration rate, thus lowering the amount of urea filtrated by the kidney. Urea thus is considered available to the rumen microflora. (Silanikove, 1984). The significant elevation of urea indicated both affection to the liver and kidney. It may attribute to either disturbance in hepatic protein metabolism, or alteration in urea transport to kidney This hypothesis is supported by elevation of β-1 globulin especially in the high titer of toxoplasmosis.

All the evidences connected between the presence of toxoplasmosis and elevation of antibody titer and the liver and kidney functioning in goat especially the reduction of albumin production and elevation of serum β-1 globulin level. These may

due to presence of proteins inhibitor of *T. gondii* techyzoite (Serine proteins inhibitor (Serpin). Pszenny et al, (2000).

The conclusion of this study revealted that ELISA is the most sensitive serological tool. Liver and kidney function are altered by *T. gondii*, the functions greatly impaired especially with the presence of high infection which appears as a high antibody titer.

REFERENCES

Al-Khalidi, N. W. and Dubey, J.P. (1979): Prevalence of *T. gondii* infection in horses. J. Parasitology. 65 : 331-334.

Belfield, A. and Goldberg, D.M. (1971): Revised assay from serum phenyl phosphatase activity using 4- Amino- antipyrine. Enzyme, 12: 561-573.

Buxton, D.(1990): Ovine toxoplasmosis. J. Res.Soc. Med 1990, 83: 509-511.

Chaudhary Z.I.; Iqbal. J. Raza, M. and Kandeal, M. I. (1996): Hematological and biochemical studies on toxoplasmosis in Racing camels. J. of camel Practice and Research, 1996, 3 :1, 1-7.

Doumas. B.T. ; Perry, B. and Sasse, E. (1973): Clinical Chem. 19: 984 - 993.

Dubey, J.P.(1976): Reshedding of Toxoplasma oocystes by chronically infected cats. Nature, 262:312-214.

Dubey, J.P.(1980)[10]: Mouse pathogenisity of *T.gondii* isolated from goat. Am. J. Vet. Res. 41 (3) :247-249.

Dubey, J.P. & Beattie, C.P.(1988) : Toxoplasmosis of animals and Man. CRC press, Florida. P220.

Dubey, J.P.; Lighty,J.C. and Beal, V.C.(1991): National sero-positive of *Toxoplasma gondiii* in pigs. J. Parasi. 77: 517-521.

Dubey, J. P.; Thulliez, Z. P.; Weigel, R. M.; Andrews, C.D.; Lind, P. And Powell, E. C.(1995): Sensitivity and specificity of various serologic tests for detection of *T. gondii* infection in naturally infected sows. Am. J. vet. Res 56, :1030-1036.

Dubey, J.; Andrews, C.; Lind, P.; Howeck, O. and Thuliez, P. (1996): Antibodies responses measured by various serologic test in pigs Orally inoculated with low numbered of *T. gondii* oocysts. Am. J. Vet. Resc. 1996, 57 (12): 1733-1737.

Durfee, P.T.; Leksana,S.B.;Joseph,S.W.and Koesharjono, C. (1974): The relation ship between heamagglutinating antibody titers and Infection with *Toxoplasma gondii* in swine. Sourhest Asian J. Trop.Med. Puplic. Health., 5:4-8.

Dzierszinsk F.; Popescu, O. Toursel, C. and Tomavos, H. (1999): The protozoan parasite toxoplasma gondii expresses to Functional enzyme-like glycolytic enzymes. J. biol. Chemis. 1999, 274,47: 35, 24888- 24895.

Farver, B.T.(1989): Concepts of normality in clinical biochemistry. In clinical Biochemistry of domestic animals, Kaneko, edit. P2-18. Academic Press. New York.

Faulkner, W.R. & King, J.W. (1976): In fundamentals of clinical chemistry, 2nd ed. Tietz, W. edits. P975. Sandures Puplisher, Philadelphia.

Fazaeli, A. ; Carter, P.E.; Parade M. L. and Penniugton. T.H. (2000): Molecular typing of *T.gondii* strains by GRA6 gene Sequences analysis. Inter. J. of Parasitology, 2000, 30: 5, 637-642.

Fleis, J.L.(1981): Statistical methods for rates and proportions.2nd edi.P 13-15, 217-219. John Wiley & Sons, New York.

Hanaki, T.; Sato, U.; Nishimura, Y. and Nobuto,K. (1963): Relationship between skin test and presence of Toxoplasma in a Symptomatic infection in swine and the proparties of Toxoplasma Isolated. Annu. Rep. Nat.Vet. Assay Lab. 3 :69-70.

Hasim, M. and Braun, J.D. (1989) : The diagnostic value of GGT in ruminant. Point Vet. 21:169-174.

Henry, R.J. (1975): Clinical cehmistry, P181. Harper & Row Publishers, New York.

Heroux, A.; White, E.L.; Davis, R.L. and Borhan, D.W. (1999): Crystal structure of *T. gondii* hypoxanthine-guanine Phospho riboxylransferase with XMP pyrophosphate, and two Mg 2+ ions bound. Insight into catalytic mechanisms. Biochemistry 1999, 38 :14495- 14506.

Ishizuka, M.M.(1978): Estudo comparative entre as provas de sabin feldman e de Immunofluorescencia indireta para a determinacao de anticorpos anti- toxoplasma em soeos de suinos. Rev.Fac. Med. Zootec. Univ. Sao. Paulo; 15 : 45 49.

Jacobs, D.; Vercammen, M. and Saman, E. (1999): Evaluation of recombinant dense granule antigen 7(GRA7) of *T.gondii* .for Detection of Ig-G antibodies & analysis of a major antigenic domin. Clinical & Diagnostic Lab. Immuno. 6(1), 24 -29.

Johnson, G.D.; Halborow, E.W. and Darlings, H.(1987): Immunoflourescence and immunoenzyme techniques "in Weir, P15-16. D.M., ed." Hand book of expermintal immunology" Oxoford, England. Black well sci. pupli. England.

Kaneeko, J J. (1990) : Clinical biochemisitry of domestic animals. 4th ed. P185-160. Academic press. New York.

Kirkbride, C.D. (1993) : Diagnosis in 1984, ovine abortion and still births diseases. J. Vet. Diagn. Invest. 5 : 328-402.

Landi, R.J and Kock, G.G. (1977): The measurement of observer agreement for categorical data. Biometrics. 33: 159-174.

Lingna, A. ; Zufferey, R.; Lingnan, M. and Russell, D.G. (1999): Characterization of tGLP-1, a Golgi and lysosme-associated, Transmembrane glycooprotein of African trpanosomes. J. of Cell Science 1999, 112:18. 3061-3070.

Literak, I.; Pinowski,J.; Anger, M.; Juricova, Z.; Kguhwang, H. and Romanowski, J. (1997): Toxoplasma gondii antibodies in house sparrows(passer Domesticus) and tree sparrows(p. montanus) Avian. Pathology. 26: 823-827.

Matsui, T.; Tokutoml, G.; Onda, H.; Kanbayashi, M.; Okabe, S.; Ito, K.; Noubuto, T.;Hanaki, K.; Matsuno, T. (1967): Serologic survey of Toxoplasmossia in pigs at 230 slaughter Houses in Japan. Bull. Inst. Pupli. Health. 16, 112-119.

Mevelec, M. N.; Mercereau, P. O.; Buzoni, G. D.; Bourguin, I.; Charades, T. and Dubremetz, J.F. (1998): Mapping of B epitopes in GRA4, a dense granule of *T. gondii* and protection studies using recombinant proteins adminestrated by oral routs. Parasite Imunology 1998, 20 : 4, 183 - 195.

Michael, D.; David, D.; Waltiman, D.; Jack, L. and John, B. (1985): Correlation of tissue infection and serologic finding in pigs fed *T. gondii* oocysts. Am. J. Vet. Res. 46(5) 110-115.

Ohshima, S.; Inami, Y.; Tanaka, H.; Yazawa, M.; Kobaya-

shi, A. And Humada, M. (1971): Prevented effect of SDDS against Toxoplasma in swine with . Repeated inoculates of Toxoplasma. Japn. J. Parasito. 20 :6 -21.

Ologohobo, A. D.; Apatai, D.F. and Oyejite, A.(1995): A comparison of protein fractions preparation from lima beans (Phasoeolus luntus) in starter diets for broiler chicks. J. Applied Animal Research, 4: 13 -30.Olsson, K. Beulmilh, S. and Orberg, D.(1982): A serial study of fluid balance during pregnancy, lactation and An estrus in goats. Acta Physil. Scand. 115: 39-45.

Omata, Y.; Dilorenzo, C. and Venturini, C.(1994): Correlation between antibody levels in *T. gondii* infected Pigs and Pathologenicity of isolated parasite. Vet. Parasitology. 51 :205-210.

Oshhima, S.; Tusbota, N. and Hiraoka, K.(1981): Latex agglutination microtitration test for diagnosis of Toxoplasma. Infection in animals. Zentralbl. Bakterial. Parasitenkd.. Infektionskr. Hyg. Abs. 1, 250:376-382.

Patton, C.J. and Crouch, S.R. (1977): Analytical chemistry 49: 464-469.

Patton. S. ; Johanson, S.S. and Puckelt K. (1990): Prevalence of toxoplasmosis gondii antibodies in nine population of dairy goat. Compare titer using modified direct agglutination and indirect heamagglutinaationn. J. Parasite. 76:74-77.

Prickett, M.;Dresen, D. and Waltman, W.(1985): Corrleation of tissue infection and serologic finding in pigs fed Toxoplasma gondii oocystes. Am.J. Vet. Res. 46 :1130-1132.

Pszenny V Angel S.O Duschak V G. Paulino M Ledsma B anf Yabó M (2000): Molecular coloring, sequencing and expression of a Seiren Proteins inhibitor gene from *T. gondii*. Mol. & Bioch. Parasitology. 200, 107: 2, 241-249.

Reitman, S. and Frankel, S. (1957): Am. J. Clin. Path. 28 : 56-66.

Ruppanner, R.; Riemann, HP. And Forver, T.B.(1978): Prevalence of Coxiella burneti (Q fever) and *T. gondii* among dairy goats in California. Am. J.Vet. Res. 39: 867-870.

Sato, U.; Hanaki, T. and Nobuto, K. (1962): Heamagglutination test a swine toxoplasmosis with TSC antigen. (Antigen for intra dermal test). J. Jpn. Vet. Med. Assoc. 15:273-278.

Shirahata, T., and Shimizu, K.(1974): Immunoglobulin responses in pigs inoculated with live and killed. Trophozoites of *T. gondii*. Res. Bull. Obihiro. Univ., 9 : 35-47.

Sibalic, D. (1966): Prilog pozavanju ucestalosti infekcije sa *T. gondii* kod svinja. Acta Vet. Beoger. 16 :193-196.

Silanikove N. (1984): Renal excretion of urea in response to changes in nitrogen intake in desert(black Bedouin) and non-desert (Swiss saanen) goats. Comp. Biochem. Phusiol.79 A: 651-654.

Smith, M.C. & Sherman, M.D. (1994): 49 "Goat medicine" P.125.

Lea & Frbiger Press, Tokoyo. Takahashi, J. and Konishi, E. (1986): Quantitiation of antibodies for *T. gondii* in swine sera by Enzyme linked immunosorbent assay. J. Immunoassay, 7:257 -272.

Tizard, I.R.; Carrington, M.and Lai, CH. (1977): Toxoplasma in goats in southern Ontarion-Aublic health hazard. Can.Vet.J. 18 :274-277.

Urquhart,G.; Armour, J.; Duncan, J.; Dum, A. and Jennings, F. (1988): Veterinary parasitology. Hand book. 2nd ed. Living stone Inc., new York.

Venturinin, M. C.; Bachigalupe, D.; Venturini, L.; Machu-

ca, M.; Perfuma, C. J. and Dubey, J.P. (1999): Detection of antibodies to *T. gondii* in still born piglets in Argentina. Vet. Parasitology. 89: 331-334.

Waltman, W.;Dreesen, D.; Prickett, M.; Blue, J. and Oliver, G.(1984): Enzyme linked immuno-asorbent assay for the detection of toxoplasma in swine. Interpreting assay results and comparing other serological test. Am.J. Vet. Res. 46: 1719-1725.

Wasfi, I.A. and Adam S.E. (1976): The effect of I.V. inj. Of small amount of Cu-sulphate in Nubian goats. J.comp. Path. 86: 387-391.

Weinman, D & Chandler, A.H. (1956): Toxoplasmosis in man and swine, an investigation of the possible Relationships. J. Am. Med. Assoc. 161 :229-232.

Weinman, D. and Chandler, R.H.(1954): Toxoplasmosis in swine and rodents, reciprocal oral infection and Potential human hazard. Proc. Soc. Exp. Biol. Med. 37: 211-216.

Yoda, T.; Igarashi, H.; Omata, Y.; Saito, A. and Suzuki, N. (1990): Changes in immune serum of *T. gondii* following administration of *T. gondii* lysate antigen. Research Bulletin of Obihiro Univ. 1990, 4 : 215-224.

Young, D.S.; Verca, M. and Samoil, M. (1975): Clinical chem. 27: 1, 246-249.