RESEARCH PAPER | Veterinary | Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

Evaluation of Screening, Confirmative and Reference of Different Tests for Diagnosis of Toxoplasmosis in Goats

KEYWORDS: Toxoplasmosis, Goat, abortion, serological tests, LAT, ELIS, IFAT

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ABSTRACT *Toxoplasmosis is an important zoonotic disease caused by the intracellular protozoan parasite Toxoplasma gondii, the disease relevant to both veterinary and human medicine. This study was designed to evaluate screening, confirmative and reference tests used for diagnosis of toxoplasma infection in goats. One hundred ninety three female goats of local breed in different status and age groups were randomly selected from five herds in Diyala province. These included 139 pregnant goats, 42 non pregnant and 14 aborted goats. Serum samples were collected and tests by latex agglutination test (LAT), enzyme linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT). Results: Higher percentage was recorded in aborted goats using three tests showed the LAT (41.66%), ELISA (25%) and IFAT (33.33%). While lower percentage was recorded in the pregnant goats by ELISA (10.79%) and IFAT (9.35%), both tests were similarly reacted in non pregnant goats. Conclusion: High correlation was observed between results of ELISA and IFAT in diagnosis of toxoplasmosis in goats.*

Introduction
Toxoplasmosis is an important zoonotic disease caused by the intracellular protozoan parasite *Toxoplasma gondii*. The disease is relevant to both Veterinary and Human Medicine (Bisson et al., 2000; Hill et al., 2005; Abu-Dalbou et al. 2010). The organism has a worldwide distribution and is a major cause of infertility, stillbirth, and abortion in animals and man (Aspinall et al., 2002; Dubey and Lindsay 2006; Dubey and Jones, 2008; OIE 2008). The positive diagnosis of toxoplasmosis requires isolation of the organism from the placenta or fetal organs (brain, lung or muscle). The preferred diagnostic procedure is to identify *T. gondii* antibodies in fetal fluid or pre suckling blood of newborn or infected animal (Tenter et al., 2000). Numerous serologic procedures are available for detection of humoral antibodies, including Sabin Feldman dye test, indirect heamagglutination test, direct agglutination test, latex agglutination test, indirect fluorescent antibody test, complement fixation test, and enzyme-linked immunosorbent assay (Al-Sim'ani, 2000). In Iraq, the toxoplasmosis was reported in different animal species with different percentages in northern, middle and southern provinces (Al-Sim'ani, 2000; AL-Husseiny; 2009; Khadi et al., 2009; Abou-Zeid et al. 2010). This study was designed to evaluate screening, confirmative and reference tests used for diagnosis of toxoplasma infection in local breed goats.

Materials and Methods
Blood samples were obtained from 193 female goats of local breed in different status and age groups, the animals were randomly selected from five herds in different regions of Diyala province (Table I). The samples were kept in cooling transport and sent to the laboratory, sera were separated, allotted in eppendorf tubes (0.5-1 ml each) and stored at -20ºC until used. Three serological tests were used for detection of toxoplasma antibodies of animals, these included, Latex agglutination test (LAT- Plasmatic Laboratory products -UK); Enzyme linked immunosorbent assay (ELISA-IgG- a commercial kit product of the European veterinary laboratories (IDEXX), Switzerland origin)) and Indirect fluorescent antibody test (IFAT- a commercial kit product of the VMRD in USA). These tests were performed according to the manufacturer's instructions. Data were analyzed by one way Analysis of Variance (ANOVA) continued with Least Significant Difference (LSD) and $p>0.05$ was considered to be significant.

Table I: Number of tested animals in different herds and pregnancy status of Diyala province

Herds	Pregnant	Non Pregnant	Aborted	Total
I	14	13	4	31
II	17	9	0	26
III	33	3	1	37
IV	30	5	0	35
V	45	12	7	64
Total	139	42	12	193

Results
The results of this study showed the overall of latex agglutination test was 34.72% of the total tested animals. Higher percentage (41.66%) was recorded in aborted goats, followed by pregnant (36.69%) and non pregnant goats (26.19%). There was a significant difference ($P<0.05$) between different animals pregnancy status. Enzyme linked immunosorbent assay displayed overall percentage of 12.44% in all tested animals. Higher percentage (25%) was recorded in aborted goats, followed by non pregnant (14.28 %) and pregnant (10.79%). There was no significant difference between animals' pregnancy groups. The overall percentage of toxoplasmosis displayed by indirect fluorescent antibody test was 11.92%. Higher percentage (33.33%) was recorded in an aborted goats and lower percentage (9.35%) was recorded in pregnant goats, while a percent of (14.28%) was displayed in non pregnant goats. There is no significant difference between animals' pregnancy groups (Table II).

Regarding age of animals group, the LAT showed revealed lower percentages 15.55% and 29.62% in the age groups of less than one year and 1-2 years respectively .While, higher percentages ranging of 40 to 47.82% were recorded in 3-6 years age groups. The results of ELISA and IFAT showed highest percentages in the age group of 3-4 years old (Table III).

Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

Table II: Positive cases Toxoplasmosis detected by LAT, ELISA and IFAT

Animal status	NO of tested animals	Positive Diagnostic Tests		
		LAT*	ELISA**	IFAT***
Pregnant	139	51(36.69%)	15 (10.79%)	13 (9.35%)
Non-pregnant	42	11(26.19%)	6 (14.28 %)	6 (14.28 %)
Aborted	12	5(41.66%) a	3(25%)	4(33.33%)
Total	193	67(34.72%)	24 (12.44%)	23 (11.92%)

*X^2=1.84, P=0.398, DF=2; **X^2= 2.21, P=0.33, DF=2; ***X^2=6.33, P=0.042, DF=2

Table III: Positive cases in different age groups

Age group	NO.	LAT	ELISA	IFAT
Up to 1 year	45	7(15.55%)	2(4.44%)	2(4.44%)
1-2 year	27	8(29.62%)	3(11.11%)	3(11.11%)
2-3 year	22	9(40.9%)	3(13.63%)	3(13. 63%)
3-4 year	25	11(44%)	7(28%)a	7(28%)a
4-5 year	30	12(40%)	5(16.66%)	4(13.33%)
5-6 year	23	11(47.82%)a	4(17.39%)	4(17.39%)
More than 6 years	21	9(42.85%)	0	0
Total	193	67(34.72%)	24(12.44%)	23(11.92%)

Discussion

The overall percentage displayed by LAT (34.72%) in this study was higher than those reported in previous study (Al-Sim'ani, 2000), while lower than those recorded in the previous studies (AL-Husseiny, 2009; Abou-Zeid et al., 2010). ELISA revealed 25% of aborted goats, this result was similar to those recorded by Abd-Al Hameed (2007), while the overall percentage (12.44%) was lower than those reported previously (Waltner-Toews et al., 1991; Hamidinejat et al., 2008 AL-Husseiny, 2009; Khadi et al., 2009) in different countries. IFAT displayed higher percentage in aborted goats (33.33%), similar findings were confirmed previously (Ahmed et al., 2008; Abid, 2010).

However, the cumulative percentage (11.92%) recorded by this test was lower than those observed in the previous studies (Bisson et al. 2000; Karaca et al., 2007; Prelezov et al., 2008), and higher than those reported (Nieto and Melendez, 1998; Sharma et al., 2003), although it was in accordance with those recorded (Masala et al., 2003). The higher percentage recorded in aborted goats by the three tests may be attributed to that, immunity in females can be broken down due to various factors example, nutrition, age, pregnancy and environment. The results of the diverse age groups were similar to those observed (Ramzan et al., 2009; Tasawar et al., 2011), in different world countries. Similar and diverse results were reported in Iraq in different animal species, ages and regions (AL-Husseiny, 2009; Abid, 2010). due to higher or lower degree of environmental contamination with cat fecal oocysts in different locations and /or different cut –off points in the serological tests used, initial serum dilution, virulence and type of *T. gondii* strains used in the antigen preparation, the immune status of the host, time of exposure, climatic conditions, difference in management methods and species susceptibility of investigated animals in different locations (Bisson et al., 2000).

Comparing the results of the three tests revealed that the overall percentage of LAT was higher than those of ELISA and IFAT. This may ascribed to LAT detects non specific antibodies, in addition to both IgM and IgG antibodies in animals experienced toxoplasmosis, whereas ELISA and IFAT are specific for detection of only IgG antibodies. Therefore, both tests do not detect the initial antibody response early infection, as well as infections during incubation periods and acutely infected cases. However, ELISA is usually used for confirming the results of LAT, as this test is more specific and sensitive for detecting ovine and caprine toxoplasmosis than LAT (Abd-Al Hameed, 2007; Karaca et al., 2007; Prelezov et al., 2008). Otherwise the correlation between the results of ELISA and IFAT has been observed (O'Donoghue et al., 1987; El-Ghaysh and Mansour, 1994; Karaca et al., 2007) in different serological surveys on toxoplasmosis. In conclusion, high correlation was observed between results of ELISA and IFAT in diagnosis of toxoplasmosis in goats.

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