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Toxoplasmosis in Women, Men, Infants and Animals in Jazan District

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This study determines the immune status against *Toxoplasma gondii* in human and animal sera in Jazan district using different techniques. Sera from 124 women, 50 men, 16 infants, randomly were obtained from king Fahd central hospital outpatients' clinics, 30 sheep 31 goat were obtained from animals attending the veterinary clinics for any reasons. Sera were employed for *Toxoplasma gondii latex agglutination (LA), Indirec hemagglutination (IHA) & Enzyme-Linked Immunosorbent Assay [ELISA (IgG & IgM)].*

The results revealed anti-toxoplasma by *LA, IHA & ELISA (IgG & IgM)* as follow 28.22%, 33.87%, 41.9 and %5.65% in women; 20%, 28 %, and 38%, 0% in men; 6.25%, 12.5%, 25% and, 0% in infants and 33.33%, 46.66% in sheep and 29%, 14, 16% in goats by LA and IHA respectively. ELISA showed highest sensitivity, specificity, and highest accuracy. Hygienic conditions must be applied in case of active toxoplasmosis to minimize transmission of the disease. Women should be routinely tested for *Toxoplasma* antibodies before and after pregnancy to avoid severe or fatal consequences.

Key word: *Toxoplasma gondii*, Human, Animal, Anti-toxoplasma, Jazan District.

Toxoplasmosis is a worldwide zoonosis of increasing concern in medicine. The disease caused by the obligate intracellular protozoan *Toxoplasma gondii*. In most adults it does not cause serious illness, but in some cases it can cause blindness and mental retardation. In congenitally infected children, blindness may occur as a result of acute or reactivated chronic infection after birth. Devastating disease or death may occur in immunocompromised individuals[22], patients those given immunosuppressive therapy for organ transplants or malignancies[7,16].

Economic losses associated with toxoplasmosis in humans are high, and include educational and residential care costs of mentally retarded people, as well as deaths due to encephalitis and blindness[25,26]. Toxoplasma invades various organs such as brain, liver, eye, placenta, and skeletal muscles[7]. Various mammals and birds can be infected with *T.gondii*, but only cats play a major role in transmission of disease, because they harbor the reproductive forms of the parasite[21,19].

Consumption of raw or undercooked meat products containing *T. gondii* tissue cysts (bradyzoites) or drinking contaminated water with infectious oocysts (sporozoites) from cat faeces or blood transfusion are risk factors associated with *T. gondii* infection[5,9].

The prevalence of human toxoplasmosis ranged from 1% to 90% relay on the predominant risk factors[22]. The diagnosis of toxoplasmosis depends on the detection of the type of antibody against toxoplasma[13].

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The reference dye test is a serological test for the diagnosis of toxoplasma infection which was introduced by Sabin and Feldman in 1948 has biohazard which limits the availability of the test[28]. Alternative tests which are equally effective but less exacting than enzyme linked immunoabsorbent assays (ELISA) that are the most sensitive (indirect immunofluorescence Latex agglutination, indirect hemagglutination, and others were used).

Due to the role of *Toxoplasma* in causing dangerous cases among populations, this study was aimed to detect the prevalence of *Toxoplasma gondii* in human and animal sera in Jazan district, as well as comparison of sensitivities, specificities of latex agglutination test (LAT) and indirect haemagglutination (IHA) assays regarding enzyme linked immunoabsorbent was done.

MATERIALSANDMETHODS

Population

Sera from 124 women, 50 men, 16 infants, randomly obtained from king Fahd central hospital outpatients clinics who had came to laboratory to investigate one or more analyses. Sera from 30 sheep and 31 goats were obtained from animals attending the veterinary clinics for any reasons. The sera were kept in deep freezer at -20^{o}C till testing. Sera were employed for anti-*Toxoplasma* anti-bodies by using latex agglutination (LA), indirect haemagglutination (IHA) and enzyme-linked immunosorbent assay [ELISA (IgG & IgM)].

Latex agglutination test (LA)

This was carried out by using a kit (Biokit, S.A. Barcelona, Spain). The test was performed according to the manufacturer's instructions.

INDIRECT HAEMAGGLUTINATION TEST (IHAT)

The Indirect haemagglutination test (IHAT): This was carried out by using a kit supplied by Dade Behring Marburg GmbH Germany. The test was performed according to the manufacturer's instructions.

Qualitative Test (screening): The procedure briefly, 50µl serum buffer was dispensed to wells needed for samples and controls. 2µl from serum and controls was added to the appropriate wells and 50µl Toxoplasmosis IHA reagent was added to the wells. The microtitre plate was shaken for 15-20 seconds, covered, and incubated at room temperature for 3-24 hours. The plate was then read by looking at the agglutination occured. Each serum was also tested with un-sensitized cells to check for non-specific reactions. Complete agglutination of the cells (carpet formation), or agglutination with light sediment is positive for *Toxoplasma* antibodies, sediment of the cells (button formation) is negative for *Toxoplasma* antibodies.

Qualitative Test

A 75µl serum buffer was added in A1-H1 wells and 50µl in the remaining wells. A 25µl was added to samples and controls in wells A1, B1, C1 and mixed. A 50µL was transferred from (1/8 dil.) A1, B1, C1 (samples and controls) to well 2, mixed, and continued to make serial dilution. A 50µl was discarded from the last well. A 50µl of Toxoplasmosis IHA reagent was added to the wells. The microtitre plate was shaken for 15-20 seconds, covered, and incubated at room temperature for 3-24 hours. The titer of antibodies in tested sample is the highest dilution which show agglutination[12].

Enzyme linked immunoabsorbent assay (ELISA)

Serum IgG antibodies to *T. gondii* were determined by ELISA using a validated commercial ELISA kit (HUMAN TOXO IgG Human Gesllschaft fur Biochemical und Diagnostica mbH Wiesbaden. Germany). Serum IgM antibodies to *T. gondii* were determined by ELISA using a validated commercial ELISA kit (HUMAN TOXO IgM Human Gesllschaft fur Biochemical und Diagnostica mbH Wiesbaden. Germany)[8].

RESULTS

124 blood samples randomly collected from females in Jazan District was tested for *Toxoplasma gondii* by using different methods. 35 out of 124 (28.22%) samples were detected by latex agglutination test while 42 out of 124 (33.87%) samples were detected by indirect haemagglutination test. In ELISA test, 52 out of 124 (41.9%) samples were IgG positive while 7 out of 124 (5.65%) samples were IgM positive. The results are shown in table 1.

50 blood samples randomly collected from males in Jazan District was tested for *Toxoplasma gondii* by using different methods. 10 out of 50 (20%) samples were detected by latex agglutination test while 14 out of50 (28%) samples were detected

by indirect haemagglutination test. In ELISA test, 1 out of 50 (38%) samples were IgG positive while 0 out of 50 (0%) samples were IgM positive The results are shown in table 2.

Table 1. Frequency of Detection of *Toxoplasma gondii* among Females in Jazan District using Different methods (n=124)

Positive (%)	Frequency	Test method
28.22	35/124	latex agglutination
33.87	42/124	indirect haemagglutination
41.9	52/124	ELISA (IgG)
5.65	7/124	ELISA (IgM)

Table 2. Frequency of Detection of *Toxoplasma gondii* among Males in Jazan District using Different methods (n=50)

Positive (%)	Frequency	Test method
20	10/50	latex agglutination
28	14/50	indirect haemagglutination
38	19/50	ELISA (IgG)
0	0/50	ELISA (IgM)

Frequency of Detection of *Toxoplasma gondii* among Infants in Jazan District using Different methods 16 blood samples randomly collected from infants in Jazan District was tested for *Toxoplasma gondii* by using different methods. 1 out of 16 (6.25%) samples were detected by latex agglutination test while 2 out of 16 (12.5%) samples were detected by indirect haemagglutination test. In ELISA test, 4out of 16 (25%) samples were IgG positive while 0 out of 16 (0%) samples were IgM positive. The results are shown in table 3.

Table 3. Frequency of Detection of *Toxoplasma gondii* among Infants in Jazan District using Different methods (n=16)

Positive (%)	Frequency	Test method
6.25	1/16	Latex agglutination
12.5	2/16	Indirect haemagglutination
25	4/16	ELISA (IgG)
0	0/16	ELISA (IgM)

Table 4. Frequency of Detection of *Toxoplasma gondii* among Sheep and Goats in Jazan District using Latex Agglutination Indirect Haemagglutination methods

Frequency in goats (n=31)	Frequency in sheep (n=30)	Test method
9/31 (29%)	10/30 (33.33%)	Latex agglutination
14/31 (45.16%)	14/30 (46.66%)	Indirect haemagglutination

Frequency of Detection of *Toxoplasma gondii* among Sheep and Goats in Jazan District using Different methods 30 blood samples were randomly collected from sheep and goats in Jazan District and tested for *Toxoplasma gondii* by using different methods. 10 out of 30 (33.33%) samples from sheep were detected by latex agglutination test while 14 out of 30 (46.66%) samples were detected by indirect haemagglutination test. For goats, 9 out of 31 (29%) samples from sheep were

Table 5. Sensitivity, Specifity, PPV and NPV of Latex Agglutination and Indirect of *Toxoplasma* antibodies

Total	L A		Total	IHA		ELISA
	-ve	+ve		-ve	+ve	
75	26	49	75	17	58	+ve
115	111	4	115	112	3	-ve
190	137	53	190	129	61	Total

detected by latex agglutination test while 14 out of 31 (45.16%) samples were detected by indirect haemagglutination test .The results are shown in table 4.

Sensitivity of *Toxoplasma* Ab by IHA 77.33%, specifity 97.39%, PPV (positive predictive value) 95.1% and NPV (negative predictive value) 86.82% Sensitivity of *Toxoplasma* antibodies by LA 65.33%, specifity 96.52%, PPV (positive predictive value) 92.45% and NPV (negative predictive value) 81%.

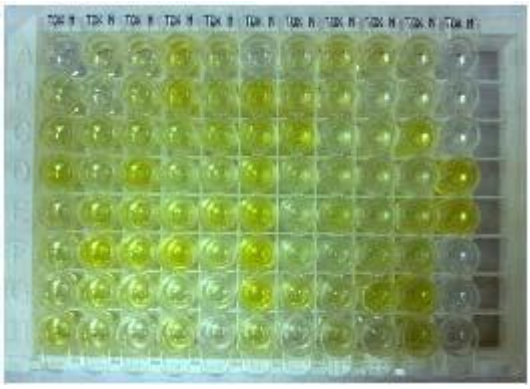

Fig. 1. Positive and negative ELISA IgM test

Fig. 2. Positive and negative ELISA IgG test

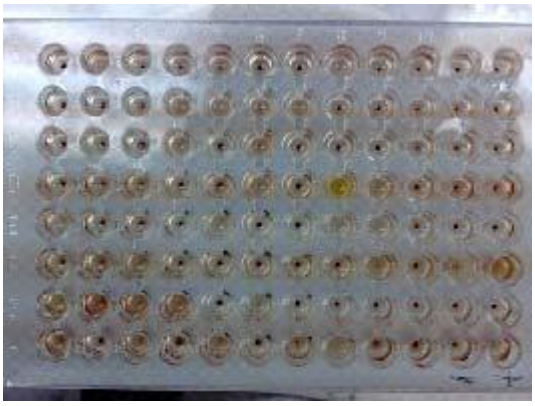

Fig. 3. Positiveand negative
Indirrect Hemagglutination test

Fig. 4. Positive and negative Latex Agglutination test

DISCUSSION

The best of the choice test for the detection antibodies of *Toxoplasma gondii* is Sabin-Feldman dye test is based on complement-mediated cytolysis of antibody-coated live *T. gondii* tachypzoites indicated by their inability to take up methylene blue. It is highly specific and sensitive, but , it cause biohazard ,so,it is not available at any laboratory[24,23].

ELISA had the higher sensitivity and specificity and high level of equivalency to Sabin-Feldman dye test[18,31] , safer. So, it consider the superior methods used in determination of the seropositivity to *T. gondii*. Also, in evaluation other tests

The frequency of infection is extremely variable in the different regions of the world. seroprevalence in the human population ranges from 0 to 90% and infection is more common in warm climates[7]. In our study, the overall prevalence of antibodies in collected sera from human in Jazan was 24.2% ,30.52%,39.47%,3.6% by using latex agglutination(LA), indirect haemagglutination (IHA) & enzyme-linked immunosorbent assay [ELISA (IgG & IgM)] techniques respectively , this

within the range of results of the former authors, but this disagree with where they found over-all prevalence was 9·8% in randomly selected Chinese inhabitants of Hong Kong[27] , the distinctly high prevalence in sera in Jazan can probably be attributed to the presence of cats in most domestic households , Also there was significant difference in prevalence between female and male population table (1)and (2)in comparing with no significant difference in prevalence between the male and female population[27] .While in Somalia the overall prevalence of antibodies was 56% in the village and 40% in Mogadishu. However, the soil in Somalia is heavily contaminated with cat faeces and the humid climate in the southern part of the country may contribute to long survival of oocysts. In the villages all household activities are performed on the ground and in Mogadishu children play mainly outdoors on the ground[10,32].

The presence of IgG against *Toxoplasma gondii* which determined by ELISA among female in this study was lower [52/ 124(41.9%)] than that observed by Tonkal (2008) in Jeddah patients with different clinical features were tested[27], 43/70 (61.4%) , meanwhile only 16/ 43 (37.2%) were approved to be positive by PCR. So, Tox-IgG indicated catching *Toxoplasma* infection but not enough with immune modulation , that is in agree with female sera 7/124 (5.65%) sera have Toxo-IgM in our work indicated catching *Toxoplasma* acute infection (active disease),and that is very important to rapid manage of this patient group. We don't find IgM antibodies in male and infant sera indicating the high risk of infection in female.

In table (2) female sera seropositivity showed 5/124 (28.22%), 42/124 (33.87%) 52/124 (41.9%),7/124 (5.65%) by latexagglutination (LA), indirect haemagglutination (IHA) & enzyme-linked immunosorbent assay [ELISA (IgG & IgM)] that is nearly similar of finging by 6(Dawoud et al.2009) *where sera* was positive in 51/186 (27.4 %) by LA, and 42/186 (22.6%) were positive by IFA, thus, ELISA is the more sensitive than IFA.

Four samples were LA-positive for *Toxoplasma* but not by ELISA . Only 111 samples were negative for *Toxoplasma* antibody by LA, compared with 137 samples by ELISA. Sensitivity of *Toxoplasma* Ab by LA 65.33%,specifity 96.52%,PPV (positive predictive value)92.45%. Three samples were IHA positive for *Toxoplasma* but not by ELISA Only 112 samples were negative for *Toxoplasma* antibody by LA, compared with 129 samples by ELISA, Sensitivity of *Toxoplasma* Ab by IHA 77.33%,specifity 97.39%,PPV (positive predictive value)95.1% and NPV (negative predictive value)86.82%, So the priority to use ELISA then IHA lastly LA in detecting seropositivity of toxoplasma, A high sensitivity (97.0%) was recorded and a high specificity (99.8%) as well[17].

In the present study, seroprevalence of *Toxoplasma* was 24.2% ,30.52%,39.47%,3.6% by using latex agglutination (LA), indirect haemagglutination (IHA) & enzyme-linked immunosorbent assay [ELISA (IgG & IgM) respectively . The results were closely matching the seroprevalence in Al-Hassa area of the Eastern region[33], and lower than that from Asir[2]. This difference could be attributed to difference in cultural or feeding habits

Prevalence of human infection ranged from 7.5-95% worldwide: 7.5% in Scotland[14], 37.4% in Saudi Arabia[1], 50% in USA[30], 54.0% in Ken-ya[10], 37.5% in Libya[15], 47% in Nigeria[15], 37% in Jordan[20], and 95.5% in Kuwait[20]. Alonso *et al.* (1984) stated that patients with AIDS developed up to 50% cerebral toxoplasmosis[3] and Bernstein *et al.* (1999) *in-vivo* reported acute toxoplasmosis after blood transfusion[4].

As widespread in humans and other animal species, having already been reported in many countries and different climates , seroprevalence of Toxoplasmosis was done in sheep and goats sera resulting a 10/30 (33.3%),9/ 31 (29%). by LA and 14/30 (46.66%) ,14/31(45.16%) by IHA respectively this results more or less similar to findings in Pakistan where they found positivity of (11.2%), (25.4%)in sheep and goats[11], and the result of Sanad and Al-Ghabban were 23.4 and 41.8 % in sheep and 19.3 and 40.7 in goats by LA and IHA tests[29].

CONCLUSION

These results declare that the highest seropositivity was in women in Jazan ,therefore, great attention must be paid before entering the childbearing period by proper planning to raise

the knowledge of risk factors ,appropriate counseling and serological screening in order to minimize severe or fatal consequences.

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