Original Article

Seroprevalance of Toxoplasmosis in human: Iraq/Sulaimani

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Summary:

Background: Toxoplasma gondii infection in humans is widespread through out the world, approximately half a billion humans have antibody to T. gondii.

Patients and Methods: Blood samples were collected from (186) persons, of different sexes and ages. Tow differ serological tests , ELISA and LAT for qualitative determination of T. gondii antibody titer in serum samples.

Results: Out of the 186 human sera, the seropositivity for *T. gondii* IgG anti-body by ELISA 70 (37.63 %) and 108 (58.06 %) by LAT. The prevalence of toxoplasmosis was highest in age groups 35-44 (48.5%) and 45-54 (52.o%) in human, in comparism with other age groups.

Conclusion: Statistical results show no significant differences between both tests (ELISA &LAT) at ($P \geq 0.05$).The prevalence of toxoplasmosis was increased proportionally with the age of individuals, while gender has no effect on the prevalent rate.

Keywords: Toxoplasma gondii; seroprevalence;ELISA;LAT; Sulaimani

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Introduction:

Toxoplasmosis is an important zoonosis that causes abortion in human and congenital abnormality in children , it also causes economic losses in animal herds due to abortion and stillbirth as well as changes in the reproductive and neural system of susceptible animals (1)._Toxoplasmosis is a world wide distribution, approximately half a billion humans have antibody to T. gondii (2), occurring naturally in man, domesticated, wild animals and birds and a high incidence may occur in particular areas (3). Intermediate hosts become infected by ing-estion of sporulated oocysts- contaminated meats, contact with free tachyzoites ,or congenitally via placenta (4), acquired infections in immuno-competent individuals are generally asymptomatic or associated with lymph-adenopathy and a flue-like illness (5). During the acute stage of infection, tachyzoites invade all organs especially the muscles including the heart, liver, spleen, lymph nodes and CNS. During latent infection bradyzoites are present in tissue cysts, and the sporozoites are found in environmentally resistant oocysts formed after the sexual stage of the life cycle (6). Serodiagnosis for antibody detection has been a more full and adequate tool to diagnose Toxoplasma infection in both human and animals, such as the latex agglutination test(7), and Enzyme Linked ImmunoSorbent Assay (ELISA) (8) (9)(10). The incidence of infection in humans and animals may vary in different parts of a country (11), toxoplasmosis is more prevalent in worm, moist areas of the world than in cold or hot dry areas (12). In Iraq many previous studies revealed different rates of infection according to different tests used , the percent of positive cases for IgM against T.gondii by ELISA test in the 120 cases of miscarriage was found to be 19.17%, while the percent of positive cases of Toxoplasma antigen by immunohistochemical analysis in the 120 cases was found to be 21.67%.(8).On the other hand, (9) in a study conducted in Basra/Iraq , using LAT test he founded that the rate was 41.1 % in city center and 52.1 % and 44.9 % in semi-rural and rural areas respectively.,and 22.06% of the total population is infected with T.gondii (13).Toxoplasmosis in countries surrounding Iraq was studied , (14) reported the rate of 49.6 % in healthy persons in compared to 72.3 % in suspected patients by IFAT in Khoozestan province of Iran. , and in Saudi Arabia the incidence of human infection ranges between 21% and 49.3% (15). The aims of the present study is to determin the prevalence of T. gondii in human of both genders and different ages in Sulaimani province by using two serological tests (ELISA and LAT).

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Materials and Methods:

Blood Samples : Blood samples were collected from 186 persons randomly, from October to December of 2006 in central laboratory in Sulaimani city. The study population involves both genders: of these 105 were females and 81 were males, and their ages were ranged from (5 - 54) years, which divided in to five age groups.

Serological Tests

Latex Agglutination Test (LAT) was performed by using a comer tail kit received from (Bio kit, S.A.) Spain and used for diagnosing all the serum samples. A qualitative test performed on a undiluted serum sample can be used to estimate the immunologic response of the individual. A negative reaction:- it means the absence of Toxoplasma antibodies or titers lower than 10 IU/ml. A positive reaction :- it means presence of Toxoplasma antibodies which may reflect chronic or an acute Toxoplasma infection.

ELISA:Two types of ELISA kits were used for antibody detection in this study:-

1 – The MONO BIND, INC. Costa Mesa, CA 92627 (USA), kit for qualitative determination of Toxoplasma gondii IgG antibody in human serum samples. The IgG value of anti-Toxo in each patient is obtained as follows:-

$$\text{EU/ml of Sample} = \frac{\text{O. D. of Samples}}{\text{O. D. of Calibrator}} \times \text{EU /ml of Calibrator}$$

According to the manufacturers instruction, results were reported in (IU); and samples contain anti-toxo IgG less than 10 IU/ml were considered negative, samples contain anti-toxo IgG between 10 - 20 IU/ml were considered equivocal and samples contain anti-toxo IgG above 20 IU/ml were considered positive.

2 – The Evlonline Toxoplasma test kit, by B.V. EUROPEAN VETERINARY LABORATORY. (B.V. EVL), Cat. no. B 1011 - AB 01, for detecting of total antibodies against Toxoplasma gondii infections in serum and plasma samples. Test samples are considered Toxoplasma positive if the absorbency is above two times the absorbency of negative control.

Statistical Analysis:The chi-square test (X^2) was used for the analytic assessment between proportions, the differences were considered to be statistically significant when the P value less than (0.05) .

Results:

Seroprevalence of Toxoplasmosis by ELISA and LAT : Out of the 186 sera, 70 (37.63 %) were seropositive for T. gondii IgG anti-body by ELISA and 27 (14.52 %) were equivocal, while 108 (58.06 %) were seropositive by LAT for the detection of T. gondii IgG and IgM antibodies, According to the gender the seropositivity were 34 (41.98 %) and 50 (61.73 %) in males, 36 (34.29 %) and 58 (55.24 %) in females by ELISA and LAT respectively. And 12 (14.81 %) in males, and 15 (14.28 %) in female were equivocal to IgG antibody by ELISA. Statistical results show no significant differences between both tests and between genders at ($P \geq 0.05$) Table (1).

The seropositivity of toxoplasmosis in different ages was illustrated in Table (2). The highest rate 25 (52.08 %) was recorded in the age group (45 - 54) years and the lowest rate was 2 (15.39 %) in the age group (5 - 14) years.

While the rates were 3 (20 %), 7 (16.67 %) and 33 (48.53 %) in the age groups (15 - 24), (25 - 34) and (35 - 44) years respectively by ELISA test. Also the highest rate 34 (70.83 %) was recorded in the age group (45 - 54) years, and the lowest rate 4 (30.77 %) was recorded in the age group (5 - 14) years. While the rates were 5 (33.33 %), 18 (42.86 %) and 47 (69.12 %) in the age group (15 - 24), (25 - 34) and (35 - 44) years respectively by LAT, with higher significant differences between age groups by using both tests at ($P \geq 0.05$). The amount of antibody titers of T. gondii in human sera were estimated by semiquantitative method of LAT . The highest titer 1: 16 was prevalent in 14 (12.96 %) sera and the lowest titer 1: 2 was recorded in 40 (37.04 %) sera.While titers 1: 4 and 1: 8 were recorded in 28 (25.93 %) and 26 (24.07 %) sera respectively . According to the gender ,the titer 1: 2 was the more prevalent in females than in males, 24 (60 %) and 16 (40 %) for females and males respectively. Table (3) . Antibody titer according to age groups illustrated in Table (4) revealed that the titer 1: 2 was a prevalent one, and 1: 16 was the least one. In the age group (14 - 25) years the titer 1: 2 was the prevalent (80%). About the age group (25 - 34) years, the prevalent titer was 1: 4 (44%). And in the age group (35 - 44) years the prevalent titer was 1: 2, (44%). The last age group was (45 - 54) years, and the prevalent titer was 1: 8 (32%)

Discussion:

Seroprevalence of toxoplasmosis: The result of serological tests in present study was shown in Table (1) revealed an over all prevalence of toxoplasmosis 37.63 % by ELISA and 58.06 % by LAT, as the ELISA technique in this study detected only IgG antibody to *T. gondii*, while LAT gave positive results in the presence of both IgG and IgM antibodies. Although the detection of specific IgG antibodies useful to establish whether the patient has been exposed to *T. gondii* , the detection of *T. gondii* specific IgM antibodies is suggestive of recent exposure or ongoing active infection (16)(17). The present study shows no significant differences in the results of both serological tests ($P \geq 0.05$). But the variation between percentage of positive cases may be related to many factors, for example as reported in used IgG ELISA kit that, the result usually be negative in infected persons during the latent period (2 - 3 weeks after infection). Also (18) reported that relatively low IgG ELISA values could often be obtained with specimens from patients with recent infection, even when the dye test titers were high. So some of LAT positive cases may have been infected recently with toxoplasmosis in which only IgM antibody may be found and the levels of IgG

antibody do not reach the readable positive value by ELISA techniques are regarded as equivocal or negative cases, also (19) recommended that negative or low IgG ELISA results could occurs with sera containing high titers of *Toxoplasma* -specifi IgM antibody. But in this study results some what differ from this as the observed antibody titers by LAT were with low value. Also the evidence proved that the IgM cases detected by LAT were having lower titers (1: 2, 1: 4 & 1: 8) (20), or the reason for this variation between the results of both tests may be due to the persistence of IgM for a long time after primary infection or presence of natural IgM antibodies (21). These antibodies not related to *Toxoplasma* infection, and cross reaction of *Toxoplasma* antibody with rheumatoid factor or other auto antibodies , which react with Fc portion of antibody give non- specific agglutination not related to *Toxoplasma* infection (22) by LAT. The presence of IgG antibody only means exposure because asymptomatic human can develop high *T. gondii* antibody titers and remain elevated for several years or even whole life if repeated exposure are encountered although an eight fold rise in antibody titer taken two weeks apart is indicative of a recent infection (23). Compared to IgG antibody, IgM antibody is short-lived and they appear before IgG antibody (24)(25). In spite of these, the antibodies response varies consi-derably among individuals, in patients with acute infection and during very active synthesis of specific IgG, the IgM antibody may be depressed, while in other patients IgM antibody may persist for months or years (22). In the present study the recorded prevalence rate were higher than those reported by ,(8)who founded that the percent of positive cases for IgM against *T.gondii* by ELISA test in the 120 cases of miscarriage was found to be 19.17%, while the percent of positive cases of *Toxoplasma* antigen by immunohistochemical analysis in the 120 cases was found to be 21.67%.(9) in Basra, Iraq founded that the rate was 41.1 % in city center and 52.1 % and 44.9 % in semi-rural and rural areas respectively by LAT. (14) reported the rate of 49.6 % in healthy persons in compared to 72.3 % in suspected patients by IFAT in Khoozestan province of Iran. Also the overall prevalence of infection was 69 % in Serbia, as the specific anti-*Toxoplasma* antibodies were detected by the reference of Sabin-Feldman dye test (26), and (27) reported that the seroprevalence of specific IgG anti- *T. gondii* was 52.4 % among Swiss population was higher than reported result of the present study. Also (28) reported the rate of 36 % of IgG antibody among the healthy volunteers in Kayseri, Turkey, and (29) reported that the sero- prevalence of the anti- *T. gondii* IgG and IgM antibodies were respectively 20.25 % and 2.33 % by ELISA among the Turkish blood donors. Also (30) reported that inactive toxoplasmosis (IgG levels) in the eastern regions of Saudi Arabia was 25 % and active toxoplasmosis (IgM levels) was 5 %. Similar to LAT results in this study (31) reported 8 % in Indonesia. It was evident from the results in Table (1) that there were no significant differences between genders by using both tests, although higher prevalence rate was observed in males, which were 41.98 % and 61.73 % by ELISA and LAT respectively. In compared to the prevalence rate in females were 34.29 % by ELISA and 55.24 % by LAT. Similar results were reported by (Konishi *et al.*, 2000) which were 63 % in males and 52 % in females, and by (32) in Nigeria, while (33) in Sudan (34) in India, (35) in Malaysia, South east Asia and (36) in Saudi Arabia reported higher prevalence rate in females than in males in their studies. Table (2) showed the significantly higher seropositivity of *Toxoplasma* ($P \leq 0.01$) among the participant age groups by both tests, which means that there was an increase in seropositivity with ages. Similar results were reported by (35) in Malaysia, South east Asia, (36) in Saudi Arabia and (9) in Basra, Iraq. Also (37) and (22) reported that increasing seroprevalence with age was a predictable result because of the increasing time of exposure, and a decline in both humoral and cell mediated immune function has been observed to occur with increasing in age. In this study the results with the increase in titers reciprocal the prevalence rate of seropositivity decrease (table-3), indicated that acute (active) toxoplasmosis was not a common finding among Sulaimani province population, and all reported cases were asymptomatic (inactive) as their sera had low antibody titers by LAT. These suggested that *Toxoplasma* infection had been probably acquired more than one year ago, also it was reported that only the high titers are important clinically as they indicate a recent infection (50). While (35) reported that the titers remain elevated for several years or even for whole life if repeated exposure are encountered

Table (1) Prevalence of toxoplasmosis in both gender of human by ELISA and LAT.

Gender	ELISA							LAT			
	No.of tested	No.of – ve	(%)	No.of Eq.	(%)	No.of + ve	(%)	No.of – ve	(%)	No.of + ve	(%)
Female	105	54	51.43	15	14.28	36	34.29	47	44.76	58	55.24
Male	81	35	43.21	12	14.81	34	41.98	31	38.27	50	61.73
Total	186	89	47.85	27	14.52	70	37.63	78	41.94	108	58.06

Table (2) Prevalence of toxoplasmosis among different ages of human by ELISA and LAT.

Age (y)	Total tested No.	ELISA						LAT			
		No.of – ve	(%)	No.of Eq.	(%)	No.of + ve	(%)	No.of – ve	(%)	No.of + ve	(%)
5-14	13	10	76.92	1	7.69	2	15.39	9	69.23	4	30.77
15-24	15	9	60	3	20	3	20	10	66.67	5	33.33
25-34	42	28	66.67	7	16.67	7	16.67	24	57.14	18	42.86
35-44	68	25	36.76	10	14.71	33	48.53	21	30.88	47	69.12
45-54	48	17	35.42	6	12.5	25	52.08	14	29.17	34	70.83
Total	186	89	47.85	27	14.52	70	37.63	78	41.94	108	58.06

Table (3) Antibody titers of T. gondii quantities by LAT in human sera between gender.

Gender	Total Sera	No. of – ve	(%)	No. of + ve	(%)	Antibody titers			
						1:2	1:4	1:8	1:16
Female	105	47	44.76	58	55.24	24 (60%)	14 (50 %)	13 (50 %)	7 (50 %)
Male	81	31	38.27	50	61.73	16 (40 %)	14 (50 %)	13 (50 %)	7 (50 %)
Total	186	78	41.94	108	58.06	40	28	26	14

Table (4) Quantities of T. gondii antibody titers by LAT in human sera of different ages.

Age (y.)	Total Sera	No. of – ve	(%)	No. of + ve	(%)	Antibody titers			
						1:2	1:4	1:8	1:16
5 - 14	13	9	69.23	4	30.77	1 25%	1 25%	1 25%	1 25%
15 - 24	15	10	66.67	5	33.33	4 80%	1 20%	1 20%	1 20%
25 - 34	42	24	57.14	18	42.86	7 39%	8 44%	2 11%	2 11%
35 - 44	68	21	30.88	47	69.12	21 44%	9 19%	11 23%	2 4%
45 - 54	48	14	29.17	34	70.83	7 21%	9 26%	11 32%	7 21%
Total	186	78	41.94	108	58.06	40 37%	28 26%	26 24%	14 13%

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