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Sero-prevalence of Toxoplasmosis in patients attending to Kassala Hospital, Kassala State 2016

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Abstract:

Toxoplasmosis is intracellular pathogen, caused by the protozoan parasite, belong to the phylum Apicomplexa**.** The present research aimed to determine the sero-prevalence of *Toxoplasma gondii* among patients attending in Kassala hospital. Blood samples were collected in blood container by using sterile syringes (300), 5 ml of venous blood was drawn and required for the laboratory examination for Latex agglutination and ELISA techniques. high prevalence of *T. gondii* recorded (56.7%) for Latex Agglutination technique. Age-groups (18-40) showed higher rate of infection 62.2%. Statistical analysis verified no variation according to the gender and contact with cats ($P > 0.05$). high prevalence calculated, for those eating undercooked meat, drinking row milk, 67.1%, 65.5% respectively. Fainaly the present study recommended to Implantation of health education program, Toxoplasmosis should be checked before donating blood and Improvement of the standard of hygienic, sanitary and disease control.

Keywords: Sero, Prevalence, Toxoplasmosis, Kassala

Introduction:

Nicolle and Mancaeux (1909), initially first described the organism, after they observed the parasites in the blood, spleen, and liver of a North African rodent, and after the rodent. Toxoplasmosis is a disease caused by the protozoan parasite, (Simpore *et al.* 2006,). It is obligate intracellular pathogen within the phylum Apicomplexa, which is found in humans and in many species of animals, (Dardé et *al.*, 2011, Ryan &Ray, 2004). The parasite infects most genera of

warm-blooded animals, including humans, but the definitive host is the domestic cats and other Felines, Felid (cats) family. Humans and other mammals as well as birds are intermediate hosts, (Montoya, *et al*., 2010). Cats are the primary source of infection to human hosts, Fecal contamination of hands is a significant risk factor, (Torda, 2001). However, the seroprevalence of *T. gondii* in human population varies greatly among different countries, geographical areas within the same country, and among the ethnic groups living in the same area (Falusi *et al*., 2002).

Approximately one-half of women infected with toxoplasmosis can transmit the infection across the placenta to their unborn babies. Infection early in the pregnancy is less likely to be transmitted to the baby than infection later in the pregnancy. Early infection results in more severe symptoms in the baby than a later one. Most babies infected during pregnancy show no sign of toxoplasmosis when they are born, but they may develop learning, visual, and hearing disabilities later in life, (Nijem & Amleh, 2009).

In Sudan, toxoplasmosis in human was identified when Carter and Fleck in 1966 used the Dye test (DT) and reported the prevalence of 61% in four different states in the country. Another study done in Khartoum State using ELISA IgG recorded 34.1% (Khalil *et al*., 2009). Abdel-Hameed in 1991 in Geizera area in the central of Sudan (about 200 km distance from to capital) reported the prevalence of 41.7% using the Latex agglutination (LAT). Also, there was study done in North Geizera in childbearing age women and the prevalence was 73, 1%, (Khalil *et al*., 2009). The prevalence of infection via toxoplasmosis among the pregnant woman in Kassala, Sudan was 70% (Khadega, 2016).

Transmission of *T. gondii* accidentally by ingesting oocysts passed in cat feces through contaminated soil or handling of cat litter, (Jones & Dubey 2012). Also, ingesting tissue cysts within raw or undercooked meat (lamb, pork and beef), Drinking unpasteurized milk, contaminated water, or unwashed fruits or vegetables, (CDC, 2013). Jones *et al.*, 2001, reported the direct transmission of tachyzoites, from mother to fetus through the placenta (congenital infection).

Approximately one-half of women infected with toxoplasmosis can transmit the infection across the placenta to their unborn baby; (Nagaty *et al*., 2009).

Several serological procedures are available for the detection of *T. gondii* antibody, which may aid diagnosis; these include the Sabin-Feldman dye test (DT), the indirect Haemagglutination assay, the indirect fluorescent antibody assay (IFA), the direct agglutination test, the latex agglutination test (LAT), the enzyme-linked immunosorbent assay (ELISA), and the immunosorbent agglutination assay test (IAAT). The IFA, IAAT and ELISA have been modified to detect immunoglobulin M (IgM) antibodies, (Hill and Dubey, 2002). Important Justifications of this

Study, globally *T. gondii* remains a major health problem and the prevalence of infections increase in Sudan including Kasala State.

The present investigation aims to determine the effect of some factors in the prevalence of toxoplasmosis, also to estimate the sensitivity of LAT and ELISA techniques in detecting of T. gondii infection.

Materials and Methods:

Study Area:

Kassala State is the one of twenty-six districts of Sudan which includes five Localities: Kassala, State, Hamashkorib, ElGash and Nahr Atbra. It is mostly inhibited by Bija tribes including Halanga, Bani-Amir and Hadandawa tribes. Besides there are some tribes from the North of Sudan. The study was carried out at Kassala Teaching Hospitals in the center of the town.

Sample size: Study populations consist of males and females, adults and children. 300 blood samples were collected randomly from patients attending to the hospitals. Cross-sectional, hospital-based study. The sample size calculated according to the following formula:

$$N = n / 1 + n * (e)^2$$

N = Sample size

n = number of populations in Kassala

e = margin of error

Ethical consideration:

Permission to carry out the study was taken from Kassala hospital**.** The people were informed for purpose of the study before collection of the specimens and verbal consent was taken**.**

Blood Samples Collection:

Blood samples were collected from each patient in blood container by using sterile syringes; 5 ml of venous blood was drawn and required for the laboratory examination for ELISA and Latex agglutination test.

Latex agglutination test (LAT):

Qualitative determination of anti- *T. gondii* antibodies IVD

The latex agglutination test will be done to screen *T.gondii* antibody in serum by commercially available kits.

Principle of the (LAT) method:

The Toxo- Latex is a slide agglutination test for the qualitative and semi quantitative detection of anti- *T. gondii* antibodies

Latex practice coated with soluble *T.*gondii antigens will agglutinate when mixed with serum containing antibodies.

Procedure (LAT):

1- Reagents and samples were allowed to reach room temperature.

2- 50ul of each serum sample and one drop were done to each positive and negative control into separate circles on the slide test.

3- The Toxo-latex reagent was swirled gently before using and 25ul was added to the reagent next samples to be tested.

4- Drop was mixed with a stirrer spreading them over the entire surface of the circle; different stirrer was used for each sample.

5- The slide placed on mechanical rotator at 80-100 r.p.m for 4 minutes.

6- False positive results could appear if the test was read later than four minutes.

***Toxoplasma* IgM ELISA kit:**

Qualitative assay for anti- *T. gondii* IgM antibodies product code GD81:

The ELISA technique used to detect both *T. gondii* IgG and IgM antibodies in serum, using microtitre plates coated with inactivated *T. gondii* antigens.

Principle of the *Toxoplasma* IgM ELISA kit:

Test sera are diluted (1:100) with the sample diluents provided. Anti- human IgG is added to the sample diluents to eliminate the possibility of interference by antigen –specific IgG and rheumatoid factor if present. Diluted serum or plasma specimens were incubated for 20 minutes to allow specific antibodies to *T. gondii* to bind to antigen –coated wells. After washing away unbound antibodies and other serum constituents *T. gondii* specific IgM was detected used rabbit ant- human IgM conjugated to horseradish peroxides. After 20 minutes incubated unbound conjugate is removed by washed and TMB/enzyme substrate was added for 10 minutes. A blue color develops if antibodies to *T. gondii* were present Addition of stop solution gives yellow color and the optical density of controls,10 u/ml stander and samples were measured used a micro plate reader.

Materials included in the kit:

- Micro plate 96 wells in 12 x 8 break-apart strips, pre- coated with *T. gondii* purified membrane antigen.
- IgG absorbent: Anti-human IgG, 3x3. 5ml
- Reagent (1): Sample diluents, 46 ml.
- Reagent (2): Wash buffer PH 7.2
- Reagent (3) Conjugate (peroxides conjugated rabbit ant- human IgM 12ml, green ready to use)
- Reagent (4): TMB subsrate, 12 ml. ready to use.
- Reagent (5): Stop solution 12 ml ready to use.
- Positive control: (red) 1 ml ready to use.
- 10 u/ml standred: (yellow), I ml ready to use.
- Negative control: (green), 1 ml ready to use.
- Incubation bag.

Other equipment required:

- 10x 60 tubes for dilution, pipettes 10ul, 100ul, and 1000ul: repeating dispenser 100ul, micro plate reader with 45nm filter micro plate washing device. Distilled or de- ionized water, general laboratory apparatus.

- Samples: Only freshly drawn and properly refrigerated sera or plasma used in this assay. Avoid heamolysed, lipemic or bacterial contaminated sera. Sera should be stored at 2-8c for no longer than 5 days. If delay in testing is anticipated, store test sera at -20c. Avoid multiple freezes.

Methods:

Ensure that all materials are at room temperature before beginning the procedure. The 10 u/ml standard and the controls are always run in duplicate. Samples may be run singly or in duplicate.

1- The numbers of strips were assembled required for the assay.

2- Sufficient IgG – absorbent was prepared by containing sample diluents for the number of samples tested. One-part IgG absorbent was done to 4 parts of reagent and mixed thoroughly. Any unused of IgG – absorbent containing diluents was discarded.

3- Samples were diluents 1:100(e.g. 5ul serum plus 0.5 ml diluents).It was important to dispense all samples and controls into the wells without delayed.

4- 100ul of the negative control were dispense, the 10 u/ml standard, the positive control and diluted patient's samples into the wells.

5- The strips were placed into the incubation bag provided and incubate for 20 minutes at room temperature. During all incubations, avoided direct sunlight and close proximity to any heat sources.

6- Wash buffer (Reagent 2) was diluted 1:9 in distilled water to make sufficient buffer for the assay run e.g. 50 ml wash buffer concentrate was added to 450 ml water. The diluted wash was stable for two months at 2-8C^{o}.

7- After 20 minutes the wells contents were decanted or aspirated, and washed three times used an automatic plate washer. The wells were blotted on absorbent paper before preceded.

8- 100ul of conjugate (Reagent3) was dispense into each wells, reagent was colored coded green, all pipettes were kept and other equipment used for conjugate completely separate for the TMB substrate reagent. The wells incubated for 20 minutes in the incubation bag at room temperature.

9- The wells contents were discarded after 20 minutes and carefully washed four times with wash buffer. The wells insured were completely washed. The micro plate was blotted on absorbent paper to removed final drops of wash fluid.

10- Repeated dispenser was used, rapidly dispensed 100ul of TMB substrate (Reagent 4) into each well. The plates were incubated for 10 minutes.

11- 100ul of stop solution (Reagent 5) was added to each well, to allowed equal reaction times, stop solution was added to the wells in the same order as the TMB substrate.

12- The optical density at 450nm was readied in micro plate reader with 10 minutes.

***Toxoplasma* IgG ELISA kit:**

- Materials included in the kit resample IgM except IgG absorbent: Anti-human IgG, 3x3. 5ml.

Methods:

1- The numbers of strips were assembled required for the assay.

2- The samples were diluents x10 concentrate contains 0.09% sodium azide was preservative. Prepare sufficient working strength diluent for the assay run. However, if the working sodium azide (0.9 g/L). Store unused sample diluent concentrate and diluent sample diluent at 2-8c. Diluent the sample diluent (Reagent1) 1:9 in distilled water to make sufficient buffer for the assay run e.g. add 10 ml sample diluent concentrate to 90 ml water.

3- Samples were diluents 1:100(e.g. 5ul serum plus 0.5 ml diluents).It was important to dispense all samples and controls into the wells without delayed.

4- For qualitative determination dispense 100ul of the negative control the 15 IU/ml stander, the positive control and the diluent patient sample into the wells. For semi quantitative determination, use sample diluent as 0 IU/ml and additionally dispense the 50 IU/ml and 150 IU/ml stander.

5- The strips were placed into the incubation bag provided and incubate for 20 minutes at room temperature. During all incubations, avoided direct sunlight and close proximity to any heat sources.

6- Wash buffer (Reagent 2) was diluted 1:9 in distilled water to make sufficient buffer for the assay run e.g. 50 ml wash buffer concentrate was added to 450 ml water. The diluted wash was stable for two months at 2-8C^{o}.

7- After 20 minutes the wells contents were decanted or aspirated, and washed three times used an automatic plate washer. The wells were blotted on absorbent paper before preceded.

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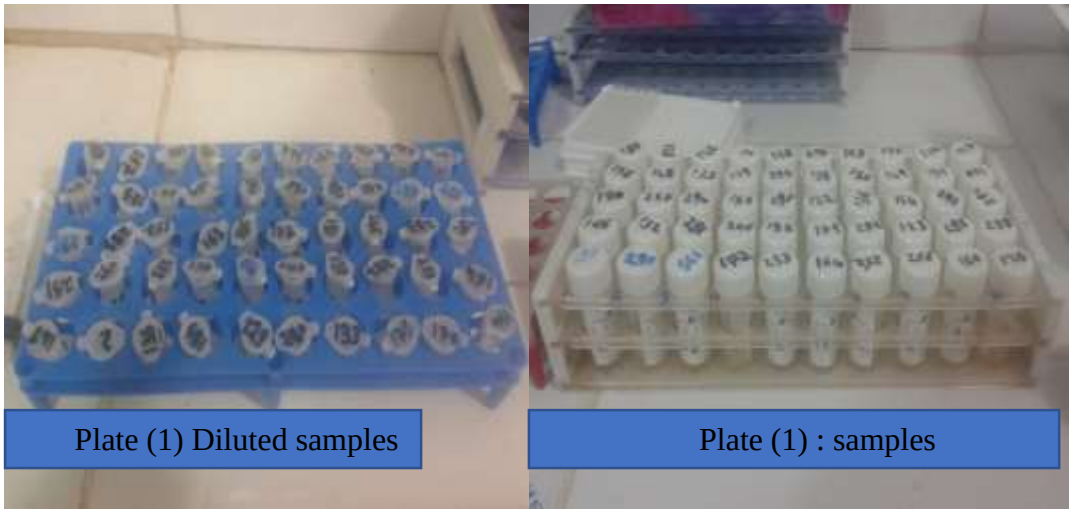

Plate (1) Diluted samples

Plate (1) : samples

Statistical analysis:

Statistical analyses were performed with the SPSS (version16.0) package. Data were expressed as Frequencies and percentages. The effect of different factors on the levels of tested Igs was

determine using chi square with confidence intervals of 95%, which are interpreted as the relative risk for increasing Igs level. I other words, the level of significance in this study was set to be taken if *P. value* is ≤ 0.05.

Results:

Overall prevalence of T. *gondii* infection by using the both techniques:

figure (1), verify that the high prevalence of *T. gondii* infection recorded for (56.7%) Latex Agglutination technique (LAT). while the prevalence according to ELISA technique was 32.3% IgG and (26.7%), IgM.

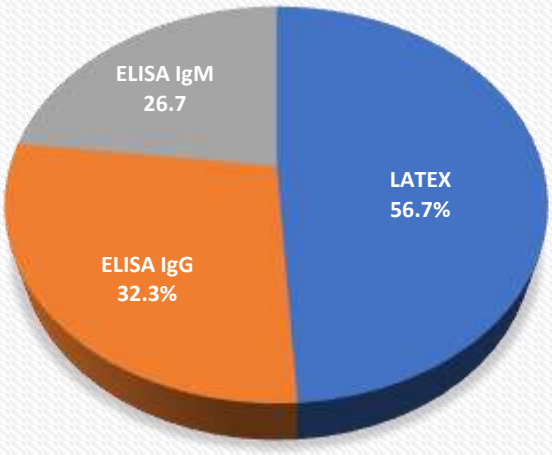

Figure (1): Overall prevalence of T. *gondii* infection by using the both technique

Prevalence of *T. gondii* using the both techniques according to the age-groups:

Table (1) shows the prevalence of *T. gondii* infection depend on the age group. The high prevalence observed among age-groups (18-40), 62.2% and the lower prevalence in age-groups less than 5 years old 35.7%. Statistical analysis verified significant variation between the LAT and ELISA techniques, (P <0.05).

Table (1): Prevalence of *T. gondii* using the both techniques according to the age-groups				
Age	**Number examined (Frequency %)**	**LAT Prevalence (%)**	**ELISA Prevalence (%)**	
			IgG	**IgM**
Less than 5	14 (4.7)	5(35.7)	2(14.3)	3(21.4)
5-18	68 (22.7)	34(50)	17(25.0)	14(20.6)
19-40	193 (64.3)	120(62.2)	73(37.8)	58(30.1)
More than 40	25 (8.3)	11(44.0)	5(20.0)	5(20.0)
Total	**300 (100)**	**170 (56.7)**	**97 (32.3)**	**80 (26.7)**
Statistical significance		**P < 0.05**	**P < 0.05**	**P > 0.05**

Prevalence of *T. gondii* using the both techniques against the gender:

Table (2) verify the prevalence of *T. gondii* infection according to gender, the prevalence by Latex Agglutination Test (LAT) was higher in females (61.5%) than males (49.6%), and similar result observed in case of ELISA technique. Statistical analysis verified significant variation (P < 0.05).

Table (2): Prevalence of *T. gondii* using the both techniques against the gender				
Gender	**Number examined Frequency (%)**	**LAT Prevalence (%)**	**ELISA Prevalence (%)**	
			IgG	**IgM**
Males	121(40.3)	60(49.6)	34(28.1)	23(19.0)
Females	179(59.7)	110(61.5)	63(35.2)	57(31.8)
Total	**300(100)**	**170(56.7)**	**97(32.3)**	**80(26.7)**
statistical significance		**P > 0.05**	**P > 0.05**	**P < 0.05**

Prevalence of *T. gondii* according depending on contact with cats:

Table (3) show the prevalence of *T. gondii* among the residents had contact with cats, the high rate of prevalence observed when using Latex Agglutination technique, LAT, (57.3%). Statistical analysis showed no significant difference *via* LAT and ELISA IgG ($P > 0.05$), while verified significant variation in ELISA IgM and, ($p< 0.05$).

Table (3): Prevalence of *T. gondii* according depending on contact with cats

Contact with Cats	Number examined Frequency (%)	LAT Prevalence (%)	ELISA Prevalence (%)	
			IgG	IgM
Yes	47 (15.7)	25(53.2%)	14(29.8%)	7(14.9%)
No	253 (84.3)	145(57.3%)	83(32.8%)	73(28.9%)
Total	**300(100)**	**170(56.7)**	**97(32.3)**	**80(26.7)**
Statistical significance		**P > 0.05**	**P > 0.05**	**P < 0.05**

Prevalence of *T. gondii* according to eating undercooked meat

Table (4) illustrate the prevalence of *T. gondii* for those eating undercooked meat, the high prevalence recorded for Latex Agglutination technique (LAT) was (67.1%) in peoples whom eating undercooked meat, ELISA IgG (39.7%) and ELISA IgM (30.7%). Statistical analysis verified significant variation in *T. gondii* IgG and IgM antibodies, ($P< 0.05$).

Table: (4) Prevalence of *T. gondii* according to eating undercooked meat

Eats undercooked meat	Number examined Frequency (%)	LAT Prevalence (%)	ELISA Prevalence (%)	
			IgG	IgM
Yes	121 (40.3)	55(67.1%)	30(36.6%)	25(20.7%)
No	179 (59.7)	115(52.8%)	67 (30.7%)	55 (30.7%)
Total	**300(100)**	**170(56.7)**	**97(32.3)**	**80(26.7)**
Statistical significance		**P> 0.05**	**P< 0.05**	**P < 0.05**

Prevalence of *T. gondii* depend on drinking raw milk

Table (5), clarify the prevalence of *T. gondii* in people according to drinking raw milk, the high prevalence rate by using Latex Agglutination Test (LAT) was (65.5%) in people who consumption raw milk. Statistical analysis verified significant variation in Latex Agglutination Test (LAT), (P< 0.05).

Table (5): Prevalence of *T. gondii* by drinking of raw milk

Drinking raw milk	Number examined Frequency (%)	LAT Prevalence (%)	ELISA Prevalence (%)	
			IgG	IgM
Yes	113 (37.7)	74(65.5%)	42(37.2%)	32 (28.3%)
No	187 (62.3)	96(51.3%)	55 (29.4%)	48 (25.7%)
Total	**300(100)**	**170(56.7)**	**97(32.3)**	**80(26.7)**
Statistical significance		**P < 0.05**	**P > 0.05**	**P > 0.05**

Discussion:

The results of the present investigation showed that the sero- prevalence of *T. gondii* infection by latex agglutination test (LAT) was (56.7%). These finding are in agreement with study done by Abdel-Hameed, (1991) used LAT in the Gezira area (41.7%) and Khalil *et al., (*2012) in North Geizera in childbearing age women the prevalence was 73, 1%. The prevalence is higher than studies done in Korea 3, 4% (Han *et al.*, 2008), and in Philippines recorded 27, 1 % (Salibay *et al.*, 2008).

However, seroprevalence estimate for human populations vary greatly among different countries among different geographical areas within one country and among different ethnic group living in the same area.

The present study verified that the high prevalence observed among age-group 18-40 years, which contrast with finding recorded commonly in Europe (Dueby and Jones, 2008). Also, findings in Sudan, reported that age group between 20-49 years as high prevalence in Gezira (Khalil *et al.*, 2012). The variance of the result might be due to the target groups which most of them in age

between 15-45years which known as reproductive age. This finding reflects the important of *T. gondii* infection which targets high productive women.

The prevalence among females was significantly high than the males according to the present investigation. This similar to Khalil *et al.,* (2012) in Khartoum state which may be duo to that the women in Sudan are always in contact with source of raw meat or sometimes eat undercooked meat. This finding is in synchrony will the results obtained by Adnan (1994) who found that the prevalence of females was almost double that in males.

Contact with cat and presence cat at home has been considered as major risk factors for acquisition of infections. However, while several studies have concluded that contact with cats increased the risk of *T. gondii* seropositivity (Khalil *et al.,* 2012 and Khadega.,2016)

Although other studies have identified an association between raw milk, meat and *T. gondii* seropositivity (Buffolano *et al.,* 1996). This study is the initial investigation of the role of the raw meat in transmission of *T. gondii* in Kassala state, marara (sheep) and um fitfit, local name in Sudan of lung, liver and stomach of sheep or cow which people use as raw after washing by water and cutting to small pieces, (this is famous traditional food in Sudan). the present study confirms the previous studies, the prevalence of *T. gondii* was (67.1%) and (65.5%) for those eating undercooked meat and drinking row milk respectively.

Recommendations:

- Implantation of health education program.
- Toxoplasmosis should be checked before donating blood.
- Improvement of the standard of hygienic and sanitary.
- Implantation of control program for toxoplasmosis.

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