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Epidemiology of Human Toxoplasmosis at Erbil Governorate /Kurdistan Region / Iraq

Mustafa Khalid salih
Knowledge University College of science Department of pathological analysis

Mustafa Riyadh Ayyed Taha
Knowledge University College of Sciences Department of pathological analysis

Abdul Sattar Khalil Azzawi Jassim
Samarra University College of Applied Sciences Pathological Analysis Department

Amara Hameed Jassim Mohammed
University of Samarra College of Applied Sciences Pathological Analysis Department

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Abstract: Human toxoplasmosis is a worldwide significant food-borne zoonotic disease that results from the infection with T. gondii ,(Toxoplasma gondii)which is a ubiquitous, single-celled, obligate intracellular protozoan parasite of warm-blooded vertebrates, including humans, land and sea mammals, and various bird species.

The aim of the present study was to analyses the diagnostic yield of the Latex and Biozek techniques for the rapid diagnosis of human toxoplasmosis in Erbil Governorate.

The research project included 120 human blood samples. Latex and Biozek were positive for 25/120 (20.8%) individuals with toxoplasmosis, The infection in female was higher 15/59(25.4%), while the rate of infection in male was lower 10/61(16.4%).

After analysing the models and made the statistics of the most age infected individuals with the disease are those aged between 31-

40 years (36.7%), also we confirmed that the frequency of infection with this disease in urban and rural areas were (21.3%) and (20.0%). Regarding the rate of infection during the period of study, we noticed that in November 15/62 (24.2%), then in December 5/26 (19.2%), while the lowest rate was found in February 1/11(9.1%). We concluded that the prevalence of T.gondii among human in Erbil Governorate was high, and the infection occurred at different stages of life. The significance of public health hazards was discussed.

Introduction

Human toxoplasmosis is a worldwide significant food-borne zoonotic disease that results from the infection with Toxoplasma gondii , which is a ubiquitous, single-celled, obligate intracellular protozoan parasite of warm-blooded vertebrates, including humans, land and sea mammals, and various bird species (1,2).

Toxoplasmosis is serious public health trouble creating a wide range of clinical syndromes in humans, because *T.* gondii(toxoplasma gondii) can persist for long periods in the human body, probably even for a lifetime. Of those who are infected, however, very few have symptoms because the immune system of a healthy person normally keeps the protozoa from causing disease. However, pregnant women and immunosuppressed individuals should be cautious of them (3,4).

Till the starting of the 21st century, latent toxoplasmosis, the lifelong occurrence of gradually dividing bradyzoites encysted in different tissues of infected hosts, had typically been believed harmless and asymptomatic in immunocompetent subjects.

On the other hand, within the past 20 years, several studies have revealed that latent toxoplasmosis associated with an increased risk of many neurological and psychiatric disorders, such as Alzheimer disease, autism, bipolar disorder, schizophrenia, recurrent migraines, personality disorder, and even brain tumors . However, infection by the parasite may cause cerebral and ocular damage and even death, especially in immunodeficient patients [5,6).

Further, latent toxoplasmosis increases the incidence of liver cirrhosis; inflammatory bowel disease; diabetes mellitus types 1 and 2 ; in the infected individuals; and chronic heart failure, myocarditis, arrhythmia (7,8).

Approximately one-third of the human population has been exposed to T. gondii. The seroprevalence studies show a drastic global variation, with as low as 4% in Korea to a very high of 78% in Nigeria. Even within Europe, it varies from 11% in Norway to 63% in Germany. Generally, T. gondii has been recovered from all regions through the world, with seropositivity rates range from less than 10% to over 90%, except Antarctica, and is called a ‘Silent threat’ in most of the Asian countries (9,10).

Felidae (domestic and wild cats) are the only definitive hosts that defecate million environmentally resistant oocysts of T. gondii in their feces , and thus pollute the environment, so it plays a significant role in the epidemiology of T. gondii (11,12).

T. gondii has a compound life cycle that comprises of two stages; sexual and asexual stages. Sexual reproduction occurs in cats where oocysts are formed and defecated with feces. Meiosis of oocysts in the environment leads to the creation of sporozoites that are infective to the intermediate hosts, which include livestock animals and rodents. In the intermediate hosts, quickly duplicating tachyzoites are spread throughout the body creating tissue cysts containing bradyzoites (13,14).

Consumption of tissue cysts by carnivorous or omnivorous animals leads to transmission to other middle hosts or cats regenerating the sexual phase of the life cycle. The asexual phase happens in the middle hosts where rapid intracellular growth of the parasite as tachyziote takes place. The tachyziotes are spread in every part of the body leading to the progress of tachyzoites to cysts particularly in muscular and neural tissues Which can persist for a long time (15,16).

Humans usually become infected with T. gondii by consumption of insufficiently cooked or raw red and white meat comprising the tissue cysts, polluted meat or shellfish after handling it and not washing hands carefully (T.gondii cannot be absorbed through intact skin); eating food that was contaminated by cutting boards, utensils, or other foods that had contact with raw, polluted meat or shellfish; and drinking unpasteurized milk (tachyzoites)(17,18).

Toxoplasmosis is a major public health problem producing a wide range of clinical syndromes in humans, particularly pregnant women and immunosuppressed individuals, land and sea mammals, and various bird species . T. gondii has been recovered from all locations throughout the world, except Antarctica, and is described as a ‘Silent threat’ in most of the Asian countries.

Therefore, the objectives of this research were to:-

1. Monitoring the human Toxoplasmosis according to gender, age, and residence.
2. Application of Latex and Rapid Kit to detect T.gondii antibodies in human.
3. 3-Study association between occurrence of toxoplasmosis in humans with months.
4. High light on the hazard of toxoplasmosis and how to avoid this prevalent disease.

2-1- Study Design and Sampling

One hundred and twenty (120) blood samples, aged between (10 to 70 (years, were randomly collected from the individuals who attended to the Rezgary hospital and other private medical laboratories in Erbil governorate , during the period from November 2020 to february2021. Five ml blood sample were collected in vacutainer tube without anticoagulant. Samples were labelled kept in a standard ice-packed storage box and were transported to the Biochemistry lab, Coollege of Science, Knowledge University (KNU) for analysis. (19).

2-2-Personal information

A structured questionnaire was used to collect information on gender, age, andresidence site.

2.3. In laboratory

the detection of T.gondii antibodies in serum was done by using Toxoplasmosis Latex Kit and ToxoIgG/IgM combo Rapid Test Cassette .

2.3.1. Detection of T.gondii antibodies in Blood by using Latex Test

Total IgM and Iggy antibodies were examined with latex agglutination test kit (Plasmatic Laboratory Products, Lab 21 Healthcare Ltd.U.K.) according to the manufacturer's instructions (20). Briefly :-

1. Allow each component to reach room temperature.
2. Gently shake the latex reagent to disperse the particles.
3. Place a drop of undiluted serum onto a circle of a test slide.
4. Add 25µl of the latex reagent next to the drop of Serum.
5. Spread the reagent and serum sample over the entire area of the test circle using a separate stirrer for each sample.
6. Gently tilt the test slide backwards and forwards approximately once every two seconds for four minutes.

NOTE: Positive and negative controls should be included at regular intervals. Both are ready for use and do not require further dilution. At the end Of the test rinse the test slide with distilled water and dry . Normal laboratory precautions should be maintained while handling patient's samples.

INTERPRETATION OF RESULTS

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator table.

The presence of agglutination indicates an antibody concentration equal or greater than 4 IU/ml.

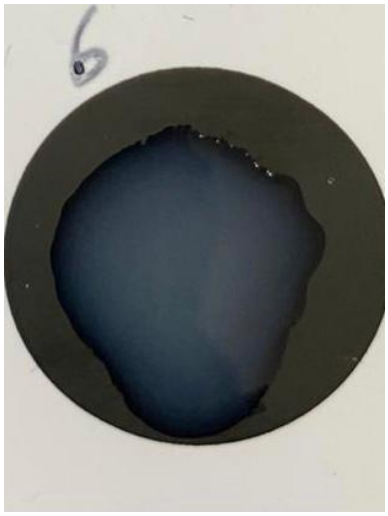

figure 1. Negative Result

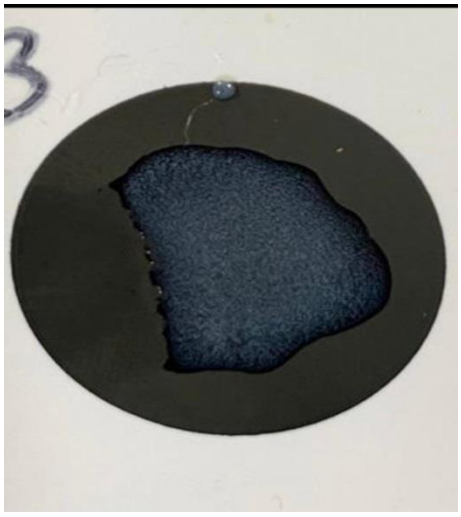

Figure 2. Positive Result

2.3.2. Detection of T.gondii antibodies in Blood by using Biozek

The kit for anti – Toxoplasma IgG and IgM detection test (Medical-Biozec kit) was obtained from purchase sources (Biozek- Medical Vissenstraat 32, 7324AL Apeldoorn- Netherlands) (wwwbiozek.com). Serum were tested for existence of specific anti-T. gondii IgM and IgG antibodies via (Medical-Biozec kit) according to the manufacturer's instructions.

DIRECTIONS FOR USE

Allow the test ,specimen , buffer and/or controls to reach room temperature (15-30°C) prior to testing .

1. Remove the test cassette from the sealed pouch and use it within one hour. Best results will be obtained if the assay is performed as soon as possible.
2. Place the test cassette on a clean and level surface . Hold the dropper vertically ; draw the specimen about 1cm above the upper end of the nozzle as shown in illustration below.

   Transfer 1 full drop (approx.20µl) of specimen to each sample well,then add 2 drops of buffer (approximately 80µl) to each sample well and start the timer . See the illustration below.

3. Wait for the colored line (s) to appear . The result should be read at 15 minutes . Do not interpret results after 20 minutes .

INTERPRETATION OF RESULTS

POSITIVE: Two colored lines appear . One colored line should always appear in the control line region (C) and another line should be in the test line region .

NEGATIVE: One colored line appears in the control line region (C) . No line appears in the test line region.

3 Results

3.1. Prevalence of T.gondii antibodies in human according to gender

The total prevalence of T.gondii in human blood was 25/120 (20.8%)(Table 1), also from this table, we noticed that the female is more exposed 15/59 (25.4%) to infection with T.gondii, compared with male infection rate 10/61 (16.4%).

Table (1): -Prevalence of T.gondiiantibodies for human according to gender.

Gender	No. samples	Positive samples No.	%	Negative samples No.	%
Male	61	10	16.4	51	83.6
Female	59	15	25.4	44	74.6
Total	120	25	20.8	95	79.2

3.2. Prevalence of T. gondii antibodies in human according to age

This study showed that the prevalence rate of T.gondii antibodies was high (36.7%) in the age group between 31 - 40 years, followed by the group with age between 51-60 years (27.8%) (Table 2).

Table (2): -Prevalence of T.gondii antibodies for Human according to Age

Age Group (Years)	No. Examined	Positive samples No.	%	Negative samples No.	%
10-20	8	0	0.0	8	100.0
21-30	48	8	16.7	40	83.3
31-40	30	11	36.7	19	63.3
41-50	12	1	8.3	11	91.7
51-60	18	5	27.8	13	72.2
61- 70	4	0	0.0	4	100.0
Total	120	25	20.8	95	79.2

3.3. Prevalence of T.gondii antibodies in human according to residence site

According to the residence site of the subjects, the frequency of T.gondii antibodies in urban and rural area was 21.3% ,while the frequencies of T.gondii antibodies detection was 20.0% for rural area (Table 3).

Table (3):- Seroprevalence of Human Toxoplasmosis in relation to residence site

Habitation site	No. of samples Examined	Positive samples		Negative samples	
		No.	%	No.	%
Urban	75	16	21.3	59	78.7
Rural	45	9	20.0	36	80.0
Total	120	25	20.8	95	79.2

3.4.The relationship between Months and prevalence of T.gondii antibodies during the period of Study

Table 4 points up the relationship between months and prevalence of *T.gondii* antibodies in human blood during the period of study. From this table we indicated that the highest rate of prevalence of T.gondii antibodies was found in November 15/62 (24.2%), then in December 5/26 (19.2%), while the lowest rate was found in February 1/11(9.1%).

Table (4): -Relationship between Months and prevalence of T.gondii antibodies during the period of Study

Month	No. examined	Positive samples		Negative samples	
		No.	%	No.	%
November 2020	62	15	24.2	47	75.8
December 2020	26	5	19.2	21	80.8
January	21	4	19.1	17	80.9
February 2021	11	1	9.1	10	90.9
Total	120	25	20.8	95	79.2

4 Discussion

Toxoplasmosis is a neglected tropical disease of poverty caused by the obligated intracellular protozoan parasite, so the infection by T.gondii remains one of the most significant and prevalent protozoal diseases globally with more predominance in developing countries (21,22).

Our results show that the rate of T. gondii infection among human is 20.8% had a positive result for latex and Biozek kit (Table 1).

These findings are consistent with previous study in Czech Republic conducted by (Kolbekova et al., 2007 (23) whom clarified that the seroprevalence of toxoplasmosis was (23.0%). in Palestine (17.6%) [24]; in Saudi Arabia (24.1%) [25]. Abdullah and Mahmood, 2017, in Erbil City/ Kurdistan Region/ Iraq whom reported that from two hundred sixty-three serum samples were tested, 92/263 (34.8%) of them had IgG antibodies and 34/263 (12.93%) were positive for IgM antibodies against Toxoplasma gondii [3] .

Al-Daoody, 2012.,in Erbil, Iraq, confirmed that the overall prevalence was 29.19% (94/322) using LAT [8]. Al-Adhroey et al., 2019 (26), in Yemen, registered that the overall seroprevalence of anti-T. gondii antibodies (IgG and/or IgM) among the participants was (21.2%).

However, higher prevalence was reported in Iraq [6], the overall prevalence of T.gondii IgG by ELISA and the rapid test was (40.65%and 12.8%), respectively; Ethiopia (67.8%) (Mulugeta et al., 2020 (27); Iran(33.8%) [28] ; Iraq, Duhok province((35.61) [9]; Malaysia ((59.7%)(Wana et al., 2020 (29). Generally speaking, prevalence rates can vary depending largely on local environmental factors, especially the region of study, on temperature and moisture, kitchen habits, hygienic standards, socio-economic status, and method of detection [3, 17].

According to the residence site of the subjects, the frequency of T.gondii antibodies in urban and rural area was 16/75(21.3%) and 9/45 (20.0%)respectively.

However ,Munoz-Zanzi et al., 2016 (30) ,illustrated that the seroprevalence of infection was high in both rural and urban slum communities with unique risk factor profiles for each community type. Findings highlight the role of the household and the community environment as influential factors in the epidemiology of the infection.

According to Aguirre et al.,2019 (31) and Wana et al., 2020 (29) , highest priority should be given to prevent and reduce risk of toxoplasmosis from food and from the Environment,which includes , avoid raw meat and cured meat; wash all vegetables and fruit carefully before eating and cooking to get rid of all traces of soil; avoid unpasteurised milk and dairy products made from it; wash hands, chopping boards and utensils perfectly after preparing raw meat; cover children's sandpits to prevent cats using them as litter boxes, wear gloves when gardening and wash hands and gloves afterwards;remove faeces from cat litter tray every day wearing rubber gloves, scald trays regularly with boiling water; avoid drinking untreated water ; teach children the significance of washing hands to prevent infection, and Keep outdoor sandboxes covered.

5 Conclusions

1. The total percentage of prevalence of T.gondii antibody in human blood was (20.8 %).
2. Our results indicate that food especially meat may be a vehicle for transmission of T.gondii to human.
3. November (15/62 (24.2%) and December 5/26 (19.2%), showed the highest prevalence with T.gondii in human compare with other months .
4. The prevalence of T.gondii infection in Erbil Governorate seems to be high and acquired in different stage of life.
5. The high prevalence of T.gondii infection because poor hygienic and poor information about the methods of transmission, that can increase the risk of infection.

Recommendations

Due to the importance of this research project and according to the results which were established, we recommended the following:

1. Further studies should be intended on T.gondii due to limitation of data about the incidence of T.gondii.
2. Pay attention to cleanliness:

- Wash hands with soap after going to the toilet.
- Wash hands with soap after handling bit animals , reptiles or animal faeces.

3. More studies are necessary to find methods for transmission of T.gondii to the human.
4. We recommend establishing a national center for Toxoplasma researches and diagnosis for different purposes.
5. High light on the hazard of toxoplasmosis and how to avoid this prevalent disease.

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