

Seroprevalence of human and ewes Toxoplasmosis in Baghdad city

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Abstract

This study was conducted to determine the prevalence of *Toxoplasma gondii* in human and sheep during the period from November/ 2012 -March/ 2013 in Baghdad city. Two hundred and forty two blood samples were collected 100 blood samples collected from women who had abortion. They were referred to the Al-Zahraa hospital and many private laboratories in Baghdad according to the physician's reports, indicating the possibility of them having toxoplasmosis, 92 Blood samples of ewes collected from pregnant ewes from many regions in Baghdad (College of Veterinary Medicine, Abu-Graib, Al-Shulla, Sabaa Al-Boor and Al-Yousefia), And 25 blood samples as control groups from both women and ewes were examined by using indirect enzyme linked immunosorbent assay (ELISA). In women, the result showed that total infection rate was 55%, the higher infection rate 62.5% was recorded in the age group between 25-35 years old, while the lowest rate 44% was recorded in the age group under 25 years old. In ewes the result showed that total infection rate was 23.9%, the higher infection rate 30% was recorded in the age group between 3-6 years old, while the lowest rate 12% was recorded in the age group under 3 years old.

الانتشار المصلي لداء المقوسات *Toxoplasmosis* في النساء والنعاج في مدينة بغداد

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الخلاصة

هدفت هذه الدراسة الى تحديد الانتشار المصلي لطفيلي *Toxoplasma gondii* في النساء والنعاج في مدينة بغداد للفترة 2012/11 - 2013/2 من خلال فحص 242 عينة دم (100 عينة دم نساء مجهضات ومرسلات من قبل الطبيب المختص لفحص الاضداد للطفيلي المسبب لداء المقوسات و92 عينة دم نعاج حوامل من مناطق مختلفة في محافظة بغداد) بالإضافة الى 25 عينة دم من كلا المجموعتين كمجاميع سيطرة باستعمال مقايسة الممدص المناعي المرتبط بالأنزيم (الايزا) غير المباشرة. بلغت نسبة الإصابة الكلية بالنساء 55% سجلت اعلى نسبة اصابة 62.5% في الفئة العمرية ما بين 25-35 سنة و اقل نسبة اصابة 44% في الفئة العمرية اقل من 25 سنة. اما في النعاج اظهرت النتائج ان نسبة الاصابة الكلية كانت 23.9% سجلت اعلى نسبة للإصابة 30% في النعاج التي تتراوح اعمارها ما بين 3-6 سنوات، و اقل نسبة اصابة 12% في النعاج التي قلت اعمارها عن 3 سنوات.

Introduction

Toxoplasma gondii is an intracellular coccidian parasite and causes the most common protozoal disease of animals and human (1). The definitive host is the house cat and certain other Felidae (2). The infection has a worldwide distribution. Toxoplasmosis occurs in wide range of prevalence and this variability is related to

various factors such as, age, sociocultural, nutritional habits, contact with domestic cats, climatic and geographical conditions. Approximately one-third of all humanity has been exposed to this parasite; this proves the importance of toxoplasmosis as zoonotic diseases (3). In small ruminant (sheep and goats), *T. gondii* infection has considerable economic importance as it causes abortion, stillbirth and neonatal loss, especially in sheep, or the birth of weak lambs, kids, which may be accompanied by a mummified fetus (4). In humans investigated evidence of infection have been found in all population groups during the first few weeks post-exposure, the infection typically causes a mild flu-like illness or no illness. Thereafter, the parasite rarely causes any symptoms in otherwise healthy adults. However, those with a weakened immune system, such as AIDS patients, organ transplant, malignancy patients, and pregnant women, it can lead to severe or even lethal damage as an opportunistic parasite (5, 6). Moreover, congenital toxoplasmosis is of great clinical importance. It occurs as acute maternal infection during pregnancy which affects the fetus, resulting in retinochoroiditis, intracranial calcifications, hydrocephalus, mental retardation and even spontaneous abortion and neonatal death (7). *Toxoplasma gondii* may be transmitted horizontally through ingesting sporulated oocytes or tissue cysts of infected animal or vertically by Tachyzoites to the fetus via placenta. Therefore toxoplasmosis was significantly associated with the presence of cats roaming in the farms (8). *T.gondii* infection in human and animal can be detected by numerous serologic methods: indirect haemagglutination test (IHAT), indirect immunofluorescence antibody test (IFAT), Latex agglutination test (LAT), Sabin-Feldman Dye Test (DT), direct agglutination test (DAT), Modified agglutination test (MAT), Complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) which is widely used in epidemiological surveys to detect the infection in different countries (1, 9, 10). Due to the importance of disease in human and animals this study was conducted.

Materials and Methods

- **Collection of blood samples:** A total of 242 blood samples were collected from humans and sheep during December/ 2012-march/ 2013 in Baghdad province. Divided into: 100 blood samples of human, collected from women who had abortion. They were referred to the Al-Zahraa hospital and many private laboratories in Baghdad according to the physician's reports, indicating the possibility of them having toxoplasmosis. The woman ages ranged between 20-40 years old. 92 blood samples of sheep (ewes), collected from pregnant ewes from many regions in Baghdad (College of Veterinary Medicine, Abu-Graib, Al-Shulla, Sabaa Al-Boor and Al-Yousefia). And 25 blood samples as control groups from both women and ewes. Five milliliter of blood samples was collected from median cubital vein of human and from jugulars vein of sheep (ewes) after antiseptic the site of collection by ethanol.
- **Serum collection:** The blood samples was collected in sterile evacuated tube, to complete clotting then put in a cold container and transferred to a laboratory, the samples placed in refrigerator at 4-8°C for the next day serum were separated from clot by using centrifuge at 3000 rpm for 5 Minutes; the samples will be placed in appendorf and marked by number and stored at -18°C the information about samples include the case history of abortion, physiological state, health state. All these will be documented in a special notebook for this purpose.
- **Serological tests:**
- **Enzyme Linked Immunosorbent Assay (ELISA):** This commercial kit produced under the authority of the France Laboratories HUMAN® for human, and for sheep IDScreen® they were performed according to manufacturer's instructions. These

kits used the whole parasite protein of cell membrane as antigen and determined the antibodies in sera of humans and sheep, and stored at $\pm 4^{\circ}$ C until used.

Results and Discussion

- **Toxoplasma infection Percentage in human (women):** According to the result; Out of 100 samples sera 55 were positive by indirect ELISA IgG with an infection rate 55%, while negative in control group. Table (1).

Table (1) Percentage of human Toxoplasmosis by using indirect ELISA test

Test	No. of samples	Toxoplasma positive	Percentage%
ELISA IgG	100	55	55
Contorol	25	-	-

- **Toxoplasma infection rate in women according to age:** The infection rate of Toxoplasmosis according to age by using indirect ELISA test showed the highest infection rate was 62.5% in the age group 25-35 years old, while the lowest infection rate was recorded in the age group under 25 years old, were 44%. Table (2).

Table (2) Relationships between the age and women Toxoplasmosis

Age group of woman	No. of women examined	No. of women positive	Prevalence%
<25	25	11	44
25-35	56	35	62.5
>35	19	9	47.36
Total	100	55	55

$X^2= 2.92$

Our finding determined that the high rate of toxoplasmosis in women 55% by ELISA (IgG class) results assign that these women were infected at some time in their life and they are now immune to the disease, These findings are in agreement with the study of (11) which demonstrated the percentage of pregnant women with acute toxoplasmosis (with IgM class) was also found to be relatively high 35.55% and those with past or chronic toxoplasmosis (with IgG class) the rate was higher 50%. However, when IgG were present in the blood, they indicated that toxoplasmosis cysts were already present in the tissues (12). Immunocompetent mothers infected before pregnancy do not pass the parasite to their off spring in uterus, even if re-exposure to the parasite occurs during the critical period of the pregnancy (13). On the other hand, the results of the 25 apparently healthy pregnant women with no case of abortion (control group) indicated that 25 100% women were negative for anti-*T. gondii* antibodies. Negative results which appeared in control group indicate that women have not been infected with *T. gondii*, or sometimes they may be tested early in the course of the disease before their body had a chance to produce antibodies. If such women acquire primary infection during gestation or the results are not actually correct they are at the risk of transmitting the infection to their fetuses, so they will need to be tested in 2-3 weeks (14). It is acknowledged that seroprevalence increase with age, as seen in studies conducted in various countries (15). Result of antibody detection according to the age of patients was shown in (Table 2). It mentioned that the age group 25- 35 year which is 62.5% was the most frequent one. The age 25-35 year was the most frequent age for marriage and the parity period in women in Iraq and most of Arabic countries. The present result was in agreement with (16, 17, 18, 19 and 20) in Iraq ,also conceit with the data of (21) in Palestine. It must be mentioned that the cause of high percentage of IgG *Toxoplasma* antibodies in women infected with *T.gondii* in the present study, is due to the high numbers of stray cats in crowded-public area of Baghdad city (where the samples were collected), because the close contact with cats, the habits of eating unwashed vegetables and fruits that contaminated with soil and to the direct contact

with meat that may be contaminated with oocyst of *T.gondii* (22). *Toxoplasma gondii* has been found in almost every country of the world in many species of carnivores, insectivores, rodents, pigs, herbivores, Primates, and other mammals as well as birds. The importance of the organism as a human pathogen has stimulated a huge amount of researches in recent years, since toxoplasmosis is a major congenital abnormalities in the united states than rubella, herpes and syphilis combined (23). Generally, up to one-third of the world's population had evidence of exposure to achronic infection. Prevalence of human toxoplasmosis ranged from 7.5-95% worldwide (24); 37% in Jordan (25); 95.5% in Kuwait (26) 37.4% in Saudi Arabia (27); 37.5% in Libya (28). Acute primary maternal toxoplasmosis if acquired during the first trimester of pregnancy, can cause significant morbidity and mortality in developing fetus (29, 30). The principle modes of transmission are from mother to fetus, through food or water contaminated with cat feces or by eating undercooked meat of infected animals (29, 31, 32). Traditional manual laboratory methods have been replaced with automated or semi-automated procedures involving little more pupation and risk of human error. For years, the serological profile defend by experienced investigators has been accepted in the routine laboratory diagnosis (33). Toxoplasmosis is usually diagnosed by using serological testes with increase in specific IgG and IgM antibodies (34). Differential methods can be used for the diagnosis of *T.gondii* including isolation of the parasite and several serological tests that are available for the detection of *T.gondii* antibodies. Since *Toxoplasma* serologic profile that include (Sabin-Feldman dye test, IgM ELISA, IgA, IgE, agglutination test (32, 35). Enzyme linked immunosorbent assay (ELISA-IgG) was used to determine the prevalence of toxoplasmosis), because of the ELISA is of a great sensitivity, objective, quantitative and may be automatically adopted, although it needs a refinement in the procedures (36).

- ***Toxoplasma* infection rate in ewes:**

Table (3) *Toxoplasma* infection rate in ewes according to area

Area	No. of samples	<i>Toxoplasma</i> positive	Percentage%
Collage of Vet. Medicine	5	5	100
Abu-Graib	20	0	-
AL-Shulla	20	0	-
AL-Yousefia	31	12	38.70
Sabaa AL-Boor	16	5	31.25
Total	92	22	23.9

$\chi^2 = 32.59$

Out of 92 serum samples detected by ELISA (IgG class) according to the different area in Baghdad province were 23.9, the results of the present investigation affirmed the presence of antibodies against *T. gondii* in the tested ewes sera in different regions of the Baghdad province. The highest percentage of *T.gondii* antibodies was seen in group taken from Collage of Vet. Medicine 100%, 38.70% in Al-Yousefia and 31.25% in Sabaa Al-Boor respectively, but there was no infection recorded in Abu-Graib and Al-Shulla city. The results showed the percentage of *T. gondii* antibodies for sheep 23.9% by indirect ELISA IgG test.

- ***Toxoplasma* infection Percentage in ewes:** Out of 92 ewes samples 22 sera were positive by indirect ELISA with an infection rate 23.9%, while no infection recorded in control group. Table (4).

Table (4) Percentage of ewes *Toxoplasmosis* by using indirect ELISA test

Test	No. of samples	<i>Toxoplasma</i> positive	Percentage%
ELISA IgG	92	22	23.9
Contorol	25	-	-

- **Toxoplasma infection rate in ewes according to age:** The infection rate of *Toxoplasma* in the present result showed that the highest infection rate was 30% in the age group 3-6 years old, while the lowest infection rate was 20% recorded in the age group under 3 years old. Table (5).

Table (5) Relationships between the age and Toxoplasmosis in sheep (ewes)

Age group of ewes	Ewes examined	Ewes positive	Prevalence%
<3	25	3	12
3-6	40	12	30
>6	27	7	25.9
Total	92	22	23.9

$\chi^2= 2.81$

Age is an important factor; older sheep have higher prevalence of toxoplasmosis than younger sheep (37). The results of the current study are accepted by (38) in Pakistan who found that relation between age and sheep toxoplasmosis in a 518 sheep surveyed serum samples by ELISA was the highest 38.88% in age group of 16-28 month and lowest 8.51% in age group of 68-80 months, In Iraq (39) found that the highest infection rate of goat toxoplasmosis was 44.28% in age group more than 5 years old, while the lowest infection rate was 33.33% in age group under 2 years old. Different finding were reported by (40) who noticed that sheep and goats, more than 4 years old, were negative to ELISA (IgM and IgG) when investigating toxoplasmosis in Al- Ramadi city. Other studies found the incidence of toxoplasmosis in sheep in Iraq 29.1% by LAT and 37.85% by IHAT, in Nineveh province, there recorded by (41) and (42) showed the prevalence in Mosul was 49% by LAT and 87.8% by MAT. (43) who found that Toxoplasmosis infection In Baghdad province were 30.04% by LAT and 16.66% by ELISA IgG test and showed that there are significant differences ($p<0.05$) between the regions of Iraq, and this agrees with a previous study by (44) in Baghdad were recorded the prevalence of toxoplasmosis among sheep and goats was 26.6% by complement fixation test (CFT) and 18.2% by IFAT. (45) Was showed that the prevalence of toxoplasmosis in the Diyala province was 32.8% by direct agglutination test (DAT) and 19.6% by ELISA IgG test. In Mesan 25%, Thyqar 12.71%, Muthanna 16.06% by using ELISA test (46) While, (47) recorded in Mosul, Iraq that 79% was sero-positive. Also, in a previous study, (48) reported that 42.03% of sheep flocks in the Baghdad province were infected with toxoplasmosis by using both indirect heamagglutination test (IHAT) and indirect immunofluorescent antibody test (IFAT). Some studies In the other countries, they found lower prevalence were reported in sheep in Iran, 35% were recorded by using IFAT (49), in Egypt (50) who found out of 200 sera samples of sheep were infected with toxoplasmosis 16.5% and 20% by using Modified Agglutination Test (MAT) and ELISA Test, respectively. However, the differences between the obtained results of serological tests during the present study and those reported by other investigators and the low percentages of ovine toxoplasmosis in Baghdad province might be attributed to several factor like the host-parasite relationship depends upon the virulence of *T.gondii* strains; the immune status of the different infected sheep, the age and the management of sheep in different localities; the time of exposure to infection and biology of the parasite or the system of breeding play an important role in epidemiology and prevalence of the disease (51). This agreed with most researchers in other countries such as (52) who detect that the frequency of the infection is extremely variable in the different regions of the world and infection is more common in warm climates and mountainous region, also (53) who suggested that infection by toxoplasmosis differs from area to area and year to year according to feeding, movement, type of grazing, (54) showed that there are significant differences in seroprevalence between locations that were evident. The presence of domestic cats and

other Felidae family and farms would eventually contaminate the environment with *T.gondii* oocysts (55), these elements give a high prevalence and a high resistance for a long time, of oocysts. Enzyme linked immunosorbent assay (ELISA-IgG) was used for detecting the prevalence of toxoplasmosis in sheep (ewes), because ELISA is of a great sensitivity, quality, objectivity procedures (56), In addition, it can be used successfully in screening and epidemiological survey (57).

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