Histopathological and diagnostic study of Toxoplasmosis in human and sheep by using ELISA in Kut city

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Summary

This study is designed to diagnosis blood Toxoplasmosis in human (women and men) and sheep (male and female) by using Elisa test; as well histopathological examination of heart, liver and lung of sheep were also done, internal organs were also obtained heart, liver and lung. Women diagnosis was performed by collection 96 samples from aborted women ageing from 15-45 years and divided into three groups; first group 15-25 years involved 50 sample, second group 25-35 years include 37 samples; and the third group 35-45 years included 9 samples. The results of Elisa test showed six positive of IgG, (two for each group) and one positive IgM for the second group, a significant ($P<0.05$) difference in the first and third groups between IgG and IgM was found. The total percentage of the infection 4, 8.1and 22.2 for the first, second and third groups respectively, and Total of 96 blood samples were collected from men with different chronic diseases and were divided into four groups, (first, second and third) each containing 25 samples and suffering from Thalasemia, renal failure and cancer respectively; of them 21 samples collected from normal men as fourth control group. The results of Elisa diagnostic test indicated 9, 9, 7 and 2 positive of IgG for first, second, third and fourth groups respectively, and 1, 2, 0 and 1 positive of IgM. The total percentage of Toxoplasmosis infection 38.4, 44, 28 and 14.28 for the first, second, third and fourth groups respectively, with a difference between them except the second group appeared significantly P(0.05). 92 samples of sheep blood were collected from male 46 and 46 female. The results of Toxoplasmosis showed one case positive IgG for female and one for male only. The histopathological examination of sheep positive case with chronic infection revealed pathological changes in the organs, the heart showed multiple variable sizes of parasite cysts embedded and scatter between cardiac muscle fibers, in addition to aggregation of inflammatory cells around the blood vessels forming nests of inflammatory cells with congestion of blood vessels in liver and lung. In conclusion that the infection with Toxoplasmosis in Kut city revealed the percentage of infection in men more than the aborted women, also it reported few cases of infection in sheep.

Keywords: Toxoplasmosis, ELISA, Aborted women, Sheep.

Introduction

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii* (1). Up to one third of the world's human population is estimated to carry a *T. gondii* infection (2). In general, *T. gondii* infections are asymptomatic and self–limiting, especially among healthy immunocompetent hosts, however the infection might be cause severe complications in pregnant women and immunocompromised patients (3 and 4).

Infection with *T. gondii* induces a cascade of immunological events that involve both the innate and adaptive immune responses. In recent years a major effort has been made toward improving the ability to diagnose the active disease, among these are measurement of serum IgG avidity test, that are proving to be of great value towards this end (5). Different aspects of the disease have been studied using different techniques including Indirect Heamagglutination Test (IHAT), Indirect Fluorescent Antibody Technique (IFAT), Enzyme linked immune -sorbent assay (ELISA) by (6). Toxoplasmosis also causes heavy economic losses to sheep industry worldwide (3). Toxoplasmosis was reported in other parts of Iraq, and in different animals, in sheep and goats (4), in cat (7). Infection generally occurs through ingestion of either oocysts shed in cat faeces, or viable tissue-cysts present in undercooked meat (6). Toxoplasmosis causes fetal resorption, abortion at any stage of pregnancy, fetal mummification, stillbirth or birth of weak live off spring in sheep (8). Therefore this study designed to diagnosis of toxoplasmosis in human and sheep in Kut city.

Materials and Methods

Samples were collected from different hospitals in Kut province, Al-Karamah's hospital, Kut teaching hospitals, Al-Suwaira hospitals, Kut Slaughterhouse. Blood samples 5 ml were drawn from each human (men and women) also collect blood 5ml from sheep heart slaughter. Each sample was put in two tubes: 5ml was placed in a sterilized plain tube and left to stand for 30 minutes at room temperature to clot, then centrifuged at 2000 rpm for 10 minutes for serum collection, dispensed into sterile tube and stored at -20°C until used for ELISA tests. Total of 96 of blood samples were collected from suddenly aborted women with age range from 15 to 45 years, for ELISA diagnosis were divided into three groups, first group (50) samples of 15-25 years, second group 37 samples 25-35 years old; while the third group 9 samples 35-45 years. Blood was collected from 96 men with age range 2 to 70 years divided in to four groups: First group include (25) men with cancer disease. Second group include (25) men with Thelassema disease and from patients of acquired immunodeficiency disease (AIDS). Third group include (25) men with renal failure. Fourth group as control (21) healthy men. The Toxo IgG ELISA is based on the classical ELISA technique the microtiter strip wells as solid are coat with Toxoplasma antigens (TOXO-Ag) prepared from sonicated whole *Toxoplasma gondii* parasites (Tachyziotes).

A total of 92 sheep samples blood were collected from (male and female) for ELISA diagnosis and biopsy from liver, heart and lung were obtained and fixed in buffer formalin for the histopathological examination.

Results and Discussion

Toxoplasmosis in women showed six positive results of IgG, (two for each group) and onlyone positive IgM of the second group. The percentage of the disease and the mean significant P<0.05 difference of the positive results between IgM and IgG present in (Table, 1). There was a significant differences in the first and third groups, while the second group revealed no difference between IgG and IgM. *T. gondii* IgM antibodies peaked at 3 weeks and precede IgG response (9). Experimentally inoculated intravenously with tachyzoites, IgM was detected by one month and persisted for three months (10).

The total percentage of the infection 4, 8.1and 22.2 for (the first, second and third groups) respectively, differences between proportions were not significant (Table, 1), and this result was agreed with, (11), that show the prevalence of infection increases with older age groups. This is due to the cumulative probability of a woman having contact with one of the several routes of infection. High values of prevalence for infection by *T. gondii* were found in young adult women, probably due to more frequent *toxoplasma* contact in childhood and adolescence. It is important to consider that the population in this study belongs to the middle or lower classes and is made up of a high number of women that work in activities that bring them in contact with soil. Thus it is probable that pregnant women in rural areas have more contact with sources of infection.

It was observed that the seropositive in this study was similar to that recorded by (12) who recorded a seropositive rate was 15.62% in the north of Iraq. Study by (13) the rate was (16.9%) in Baghdad, while (14) was found about 18.42% in Thi-Qar governorate, and the infection of Toxoplasmosis in this studies were decrease that recorded by (15) who demonstrated the seropositive rate was 2.5% in Baghdad.

Table, 1: Differences between proportions (IgG, IgM and total) of *Toxoplasmosis* in women.

Number	Age	Sample	Positive		Negative	Total %
			IgG	IgM		
1	15-25 years	50	*2	0	48	4
2	25-35 years	37	2	1	34	8.1
3	35-45 years	9	*2	0	7	22.2
(Chi-square value=3.806 P=0.149)						

(*) mean significant difference between means comparation with columns groups at levels (0.05). (n.s) mean non-significant difference between means.

The Elisa results showed 9 cases were IgG positive and one IgM positive, the first group, the second group showed 9 positive results of IgG and 2 positive of IgM, and third group had 7 positive results of IgG; while the fourth

group (control) showed 2 positive for IgG and 1 positive of IgM. The percentage of Toxoplasmosis and the mean significant difference of the results between IgM and IgG and a significant P<0.05 difference in all groups except the fourth group which raised no difference between IgG and IgM (16) Showed that diagnosis of *Toxoplasma* infection is complex in immunosuppressed patients. Because the immune response in these patients is low; clinical awareness of the possibility of infection was therefore important. As well as Toxoplasmosis can result in significant morbidity and mortality in immunosuppressed patients. The total percentage of Toxoplasmosis infection were 38.4, 44, 28 and 14.28 with no differences between total proportion of type of disease for the first, second, third and fourth groups respectively, except the second group appeared significant P<0.05 difference (Table, 2), and (17) suggested that the seroprevalence of latent Toxoplasma infection found in more than half of renal patients and 56.1-76.5% in Turkey a reported by (18 and 19). A higher rate of toxoplasmosis was found among older patients, which was not surprising due to the fact these patients usually have a well established socioeconomic status leading to greater risk of exposure to *Toxoplasma* than younger patients, and in HIV-infected patients. Toxoplasmosis has a high prevalence in immunosuppressed patients, such as cancer and AIDS patients (20). The risk for infection in individual patients depends on two factors: the prevalence of toxoplasmosis in the community and the degree and nature of immunosuppression (16). (21) Explain that Toxoplasmosis in immunocompromised individuals (i.e. persons with AIDS, transplant recipients, persons receiving immune -suppressive drugs) usually was due to reactivation of latent infection but can result from acute infection.

In Sheep Elisa test (IgG) showed two cases with positive result one for female and one for male serum sample for male, while the negative results appeared in 90 cases, 45 cases for female and 45 cases for male each one female and male (Table, 3). These results show that climate may influence on the spread of toxoplasmosis in sheep. Oocysts can survive in the environment for months, depending on moisture and temperature (22). Thus, low humidity and high temperatures are deleterious to oocysts. This fact suggests by (23) who explained that climate characteristics of dry regions were likely to decrease the chance of oocyst survival, generally resulting in a low prevalence of toxoplasmosis, and this result agree with our results.

Table, 2: Differences between proportions (IgG, IgM and total) of Toxoplasmosis in men suffering from different chronic diseases.

Number	Disease	Samples	Positive		Negative	Total %
			IgG	IgM		
1	Thalassemia	25	*9	1	16	38.4
2	Renal failure	25	*9	2	14	44*
3	Cancer	25	*7	0	18	28
4	Normal	21	2	1	18	14.28
(Chi-square value=5.369 P=0.14)						

(*) mean significant difference between means comparation with columns groups at levels at 0.05. (n.s) mean non-significant difference between means.

Table, 3: The positive and negative results of Toxoplasmosis in sheep (female and male) by using ELISA test (IgG).

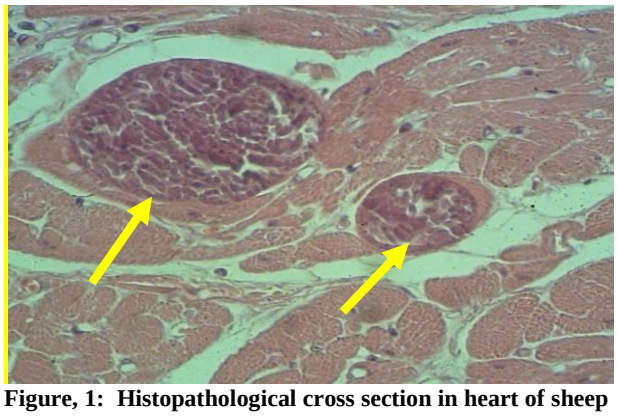
Test	Sex	Number	Result	
			Positive	Negative
ELISA	Female	46	1	45
	Male	46	1	45

Histopathological study, hearts Showed multiple variable sizes of parasite cysts embedded and scatter between cardiac muscle fibers without inflammatory cells response (Fig. 1). In sheep, the parasite was more frequently detected in the brain, and heart than in muscle samples (10). One distinct characteristic of *T. gondii* in immune -competent hosts, the tissue cysts were able to persist for several years (life of the host) after infection and the immunity does not eliminate an established infection (6). The formation of tissue cysts under certain circumstances is an important aspect of the pathogenesis of Toxoplasmosis. The cyst wall may considerably reduce the availability of exogenous materials to the bradyzoites. Thus, the switch from tachyzoites to bradyzoites was done (24). However, the parasite persists in its

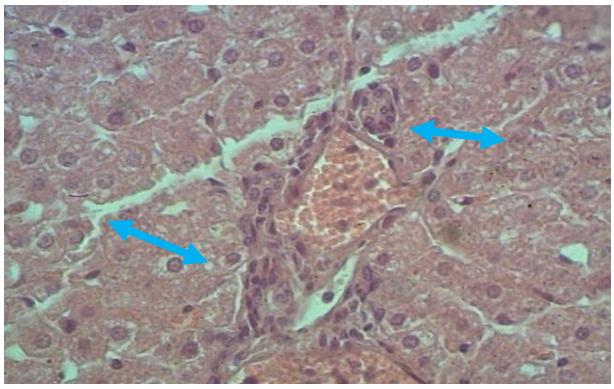
bradyzoite form, inside the intracellular cysts. The periodic rupture of these cysts was thought to be the origin of maintained immunity against Toxoplasma (25).

The microscopical examination of liver showed inflammatory cells aggregation around the B.V. forming nests of inflammatory cells with congestion of blood vessels (Fig. 2). although macrophages and monocytes possess phagocytic mechanisms in the resting state, these mechanisms can be enhanced, and new mechanisms can be expressed when they were activated (3). Activation can occur through exposure to microbial products (*Toxoplasm gondii* antigen), both innate and adaptive immune responses are activated following *T. gondii* infection. Mechanisms in the resting state while the high phagocytic activity represent the infection in male and female sheep, indicate chronic or carrier state (26).

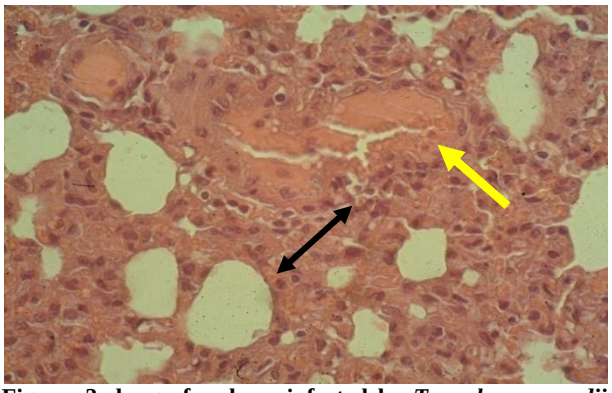
T. gondii is able to triggering the non-specific. This non-specific immune response reacts immediately after the first contact between the parasite and the host. It peaks at the end of the first week, and then slowly reduces until absent in the second the beginning of infection (25). Increased thickness of intra alveolar septa of the lung due to congested capillary blood vessels and inflammatory cells infiltration (Fig. 3). Protective immunity in sheep that infected with *T. gondii* is similar to the situation in other host species, involves cellular responses.



Figure, 1: Histopathological cross section in heart of sheep infected by chronic *Toxoplasma gondii* shows multiple variable size of parasite cyst (⟶) scatter in the cardiac muscle tissue without inflammatory cells response. (H and EX400).



Figure, 2: Histopathological section in liver of sheep infected by *Toxoplasma gondii* shows inflammatory cells aggregation around the blood vessels forming as a nest (⟷) with congestion of B.V (⟶). (H and EX400).



Figure, 3: lung of sheep infected by *Toxoplasma gondii* shows increased thickness of intra alveolar septa of the lung due to congested blood vessel (⟶) and inflammatory cells infiltration (⟷) (H and EX400).

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دراسة مرضية وتشخيصية لداء القطط في الإنسان والأغنام باستعمال فحص الألايزا في منطقة الكوت

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الخلاصة

صّممت هذه الدراسة لتشخيص مرض داء القطط في الإنسان (نساء ورجال) وفي الأغنام (ذ كور وأناث) باستعمال فحص الألايزا، فضلا عن دراسة التغيرات النسجية للقلب والكبد والرئة. أجري التشخيص بجمع (96) عينة دم من النساء اللاتي تراوحت أعمارهن من (15-45) سنة وقسمت على ثلاث مجاميع، المجموعة الاولى 15-25 سنة، جمعت (50) عينة، المجموعة الثانية 25- 35 سنة تضمنت 37 عينة، في حين اشتملت المجموعة الثالثة 35-45 سنة على 9 عينات. أظهرت نتائج فحص الألايزا ست حالات موجبة لـ IgG (حالتين لكل مجموعة)، وحالة موجبة واحدة لـ IgM للمجموعة الثانية، بوجود فرق معنوي في المجموعة الاولى والثالثة بين الـ IgG و IgM. وأظهر معدل النسب المئوية للإصابة 4 و8.1 و22.2 للمجاميع (الاولى والثانية والثالثة) على التوالي بعدم وجود فرق مهم بينها. جمعت 96 عينة دم من الرجال واللذين يعانون من أمراض مزمنة مختلفة حيث قسموا على ثلاث مجاميع، إذ احتوت كل مجموعة على 25 عينة فالمجموعة الأولى (مرضى الثلاسيميا) والمجموعة الثانية الذين يعانون من الفشل الكلوي، اما المجموعة الثالثة فالمرضى المصابون (بمرض السرطان)، في حين أنّ المجموعة الرابعة 21 عينة دم من أشخاص سليمين (مجموعة سيطرة). أظهرت نتائج فحص الألايزا 9 و9 و7 و2 حالات موجبة لـ IgG للمجاميع الاولى والثانية والثالثة والرابعة على التوالي: 1 و0 و2 و1 حالات موجبة لـ IgM للمجاميع الأولى والثانية والثالثة والرابعة على التوالي. وبينت نتائج فحص الألايزا لـ IgM ظهور1 و2 و0 و1 حالات موجبة للمجاميع الأولى والثانية والثالثة والرابعة على التوالي، أظهرت معدلات النسب المئوية للإصابة بداء القطط 38.4 و 44 و 28 و 14.28 للمجاميع الأولى والثانية والثالثة والرابعة على التوالي بعدم وجود فرق مهم بينها ما عدا المجموعة الثانية التي اظهرت وجود فرق $P< 0.05$ معنوي. جمعت 92 عينة دم من الاغنام 46 عينة للذكور و46 عينة للاناث، اظهرت حالة موجبة واحدة IgG للاناث وحالة موجبة واحدة للذكور. أوضحت الدراسة المرضية النسجية للحالات الموجبة والمصابة بطفيلي داء القطط في الأغنام بوجود تغيرات مرضية في الأعضاء التي جمعت، حيث أظهر القلب وجود اكياس للطفيلي متعددة ومتباينة الأحجام مطمورة ومتناثرة بين ألياف العضلة القلبية، فضلاً عن تجمع الخلايا الالتهابية حول الأوعية الدموية مكونة أعشاشاً من الخلايا الالتهابية مع إحتقان الأوعية الدموية. تستنتج من هذه الدراسة وجود الاصابة بمرض داء القطط في مدينة الكوت بان نسب الاصابة في الرجال اكثر من النساء المجهضة, كذلك سجلت حالات إصابة ضئيلة في الاغنام بهذا المرض.

الكلمات المفتاحية: داء القطط، فحص الألايزا، النساء المجهضة، الأغنام.