

Isolation *Toxoplasma gondii* from aborted fetuses and Sheep then study of the prevalence in aborted women in Karbala governorate

Asaad Khalaf Talal Al-Shuwaili¹, Ihab G. Al-Shemmari², Islam Jawad Al-khafaji³
^{1,2,3}Internal and preventive medicine/College of veterinary medicine / University of
 Kerbala/ Iraq.

Email: asaadkh89@yahoo.com

Abstract

The aim of this study is to isolation *Toxoplasma gondii* from sheep and its fetus's. Then the study conducted to determine the distribution of the parasite in women who have experienced abortion and threats in the province of Karbala and the surrounding areas. The zoonotic aspect investigated the different sources for infection with toxoplasmosis ,generally our results proved that ovine meats is an important sources for transmission of toxoplasmosis; the percentage of positive result with tachyzoites and tissue cysts was 80 % after pepsin digestion .When the comparative study of the different organs of aborted fetus, we found that the brain was the more sensitive organ and considered predilection site for Toxoplasma infection, other organs such as liver, kidney, lung, heart , muscles showing less tendency for this infection. The severity of infection with *Toxoplasma* was classified *Toxoplasma* infection in aborted fetus to sever, moderate and mild infection according to the number of tissue cysts in brain. The cases of severe infection were predominated when compared with mild and moderate infection. The immunological aspect of this study showed the presence of immunoglobulin G in the placenta 22 % on using modified latex agglutination test with 2-ME and 3 % on using indirect ELISA. The prevalence of the parasite *Toxoplasma* condi in women who have undergone abortions and threatened abortion in the city of Karbala, whose ages ranged between 25-34 years. Direct agglutination method and Elisa technique have been diagnosed serologically for 90 blood samples, the result showed that the infection rate was 18.88% by agglutination test and 13.33 by Elisa test. Infection rate was 10.71%,33.21.05% , 33.33% for threatened abortion , first time , % for threatened abortion ,one time and two times aborted women respectively by Elisa technique. Rate of measured IgM was 1.23, 1.55 and 1.44 for one time, two times and three times aborted women respectively.

Introduction

Toxoplasmosis is caused by the intracellular parasite *Toxoplasma gondii* (1) can infect many types of warm-blooded hosts, including humans and animals can infect many intermediate hosts such as rodents, birds, farm and wild animals (2). Forced cells. Feline, especially cats, are important final hosts of the sexual phase. This parasite is characterized by the presence of three different stages, each of which has the ability to cause infection in humans and farm animals. These stages include Sporulated oocysts and rapidly multiplying oocysts (3), but the parasite (Tachyzoites) and the slow-reproducing oocytes (Bradyzoites) contained within tissue cysts (4).

The infection is transmitted by the *Toxoplasma* parasite in several ways, including drinking water and eating vegetables and fruits contaminated with eggs of the parasite, as well as dealing with soil contaminated with infected cat feces. Infection may occur as a result of drinking unpasteurized milk.also important modes of transmission is transplacental infection (5). Finally, infection with the parasite is transmitted through the respiratory tract by inhaling the sacs of sporulated eggs (6)However, there are reports that indicate other uncommon methods of transmission of infection, including blood transfusions, as the parasite can remain in the blood stored at a degree of (4C°) for more than (50

days)(7). The infection can also be transmitted during organ transplantation (8).

Clinical signs in the case of toxoplasmosis infection vary according to the type of host. The clinical signs in cats may appear through dyspnea, tachypnea (Polypnea), jaundice (Icterus) and some abdominal disorder,Abdominal discomfort(9). However, these signs differ in the sheep, as miscarriage or still birth is observed (10).

Congenital toxoplasmosis in humans leads to the emergence of many different clinical signs, including miscarriage or mental retardation, blindness and hydrocephalus, as well as some congenital anomalies in children (11)

In humans, approximately one third of all humanity has been exposed to this parasite. Clinically , the disease is usually asymptomatic in immunocompetent adults,but it can cause a severe illness and even death in immunocompromised patients (12) .If the infection acquired during pregnancy, it may cause habitual abortion and various congenital anomalies in the fetus (13) .The most common manifestations of congenital toxoplasmosis is retinochoroiditis , psychomotor retardation , hydrocephalus, intracerebral calcification and secondary eye lesions leading to sever impairments (14) .Acquisition of toxoplasmosis before pregnancy is of least risk to the fetus , but , the greatest risk of congenital toxoplasmosis occurs

during the trimesters of pregnancy. However, it is during the third trimester that the highest level of transmission occurs (15).

Sampling

Collected (50) samples of aborted lamb fetuses collected during the second trimester of pregnancy, while the third section aborted in the last trimester, in addition to the birth of some lambs that died immediately after their birth Also, (50) blood samples were collected from slaughtered lambs, as well as collected (50) blood samples from these sheep in order to investigate the antibodies to Toxoplasmosis.

Where the cases were divided into severe infection cases when there are (1000) tissue cysts or more in the fingerprints of brain tissue located along the glass slide whose dimensions are (7.5 x 2.5) cm. As well as moderate and simple infections when there are less than (1,000) tissue bags in the brain tissue to prepare tissue printing slides used methods (16).

Serum samples from Women's

One hundred and seventy-two serum samples were collected from pregnant women at the first trimester of pregnancy at an age range of 25-34 years. These samples were collected from different region from Karbala .All samples were screened for the presence of ant- toxoplasma IgM and IgG antibodies separately using Plate III-The use Toxo cell latex of the product kit by the company Bio kit is as follows a-A drop of blood serum is taken and placed on a

slide, then a drop of the kit solution is placed b-It is tested on strength (10X) to note the degree of agglomeration or agglomeration, where it is zero as a negative result when there is no agglutination, and its opposite is positive and degree 1/80 or 1/160 or 1/320 positive.

The Elisa method used several types of Toxo IgM product by a company

Isolate the parasite

Isolation of the parasite from fetuses of aborted lambs we are made collected 50 samples of aborted lamb fetuses and made tissue fingerprints from brain, liver, kidney, lung, heart and skeletal muscles. Develop criteria to estimate the severity of infection.

Results

1- Examinations of aborted fetus

Examination of aborted fetuses, which their brain, lung, liver, heart, kidney and skeletal muscles (thigh) tissues have been smear, showed that there were (20) positive cases of toxoplasmosis infection out of (50) examined cases and the percentage of infection was 40% .

The most affected organs have been studied to identify toxoplasmosis infection by counting the cyst of different tissues.

Brain range (1397±275), liver (723±141), Kidney (718±133), lung (570±105), hear (477±96) and skeletal muscle (thigh) (218±36) as shown in the table No.1.

Table (1) Distribution of tissue cysts in different organs of aborted fetuses

Brain	Liver	Kidney	Lung	Heart	Skeletal Muscle (Thigh)
1397±275	723±141	718 ± 133	570 ± 105	477 ± 96	218±36

The values represent the mean ± standard error.

Brain tissue has been selected to estimate the severity of infection by toxoplasmosis.

Toxoout of 20 cases with 60%

plasmosis infection cases in aborted fetuses have been classified to sever, moderate and simple infection depending on brain tissue cysts number.

Around 1000 tissue of smeared brain cysts have been considered as severe infection of toxoplasmosis which was while less than 1000 cysts have been considered as moderate which was 8 out of 20 cases with percentage of (40%) as shown in table No. 2 .

Table (2) Classification of severe, moderate and simple toxoplasmosis infections in the brains of aborted fetuses based on the number of tissue sacs in them.

No.	Sever infection	Moderate and Mild infection
1	1976	343
2	5400	488
3	1204	316
4	1397	772
5	1512	764
6	1368	192
7	2400	984
8	1012	724
9	2800	
10	1872	
11	1316	
12	1096	
	1946±384	88±573

The values represent the mean ± standard error

2- 50 samples of skeletal muscle have been collected and examined by making swabs of digestive with pepsin.

Acidic pepsin digestion results for sheep showed that the number of positive cases were 40 out of 50

cases with a percentage of 80%. Slaughter sheep serum has been taken from kerbala slaughterhouse to investigate toxoplasmosis antibodies using latex particles agglutination test. Positive results have been showed by examination 43 out of 50 cases and the percentage was 86% IgG percentage 95.34% and IgM percentage 4.6% as shown in table No.3.

Table (3) Types of antibodies, numbers of positive cases and their percentages in sheep for Toxoplasmosis

Positive case	Positive	%	Type Ab	Positive	%
50	43	86	IgM	2	4.06
			IgG	41	95.34
			Total	43	100

Different criterias of Toxoplasmosis antibody have been examined and the results were varied greatly between (1:2- 1:512), high standards of antibodies show the highest number of positive cases ; 8 cases of criteria on (1:512),cases of criterion (1:256), 6

cases of criterion (1:16) and (1:8), 5 cases of criteria on (1:128), 4 cases of criterion (1:64) , 3 cases of criterion (1:32) and 2 cases of criterion (1:4) and (1:2) as shown in table No.4. while its average in negative cases is 0.55.

Table (4) Preparing different cases of antibody criteria in sheep sera

Titration Ab	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Number of cases	2	2	6	6	3	4	5	7	8

3- Positive and negative cases by Eliza and agglutination methods

The numbers and percentages of positive and negative cases by agglutination and lysing methods for women threatened with abortion

are shown in table 5. The average amount of IgM antibody type in positive cases is (10.71%) and Elisa method is (3.57%) , the average amount of IgM antibody type in positive cases is 1.61 while its average in negative cases is 0.55±0.19.

Table (5) shows the numbers and percentages of positive and negative samples by agglutination and lysing methods in women threatened with abortion.

Total	- agglutination		+ agglutination		-Elisa		IgM	+Elisa		IgM
	No.	%	No.	%	No.	%		No.	%	
3	2	66.66	1	33.33	2	66.66	0.55±0.19	1	33.33	1.39±0.00

Single abortion women are shown in table 6. As can be seen the number of examined samples were 38 cases, the percentage of positive cases which are examined by stacking method and Elisa method was 78.94%. The average amount of IgM antibodies in positive cases was 1.33± 0.28, while

the average amount of IgM antibodies in negative cases was 0.55 ± 0.20; therefore no difference in positive cases between the two methods. The numbers and percentages of positive and negative cases by agglutination and lysing methods.

Table (6) shows the numbers and percentages of positive and negative samples by the methods of agglutination and lysing among women subjected to a single abortion.

Total	- agglutination		+ agglutination		-Elisa		IgM	+Elisa		IgM
	number	%	number	%	number	%		number	%	
38	30	78.94	8	21.05	30	78.94	0.55±0.20	8	21.05	1.23±0.28

Two abortion women are shown in table 7 ,the percentage of positive cases by agglutination method is 77.77%, the percentage of positive cases by Elisa method is 11.11% the average

amount of IgM antibodies in positive cases was 1.55±0.46 and its average in negative cases was 0.59±0.18 and its average in negative cases was 0.59±0.18.

Table (7) shows the numbers and percentages of positive and negative samples by agglutination and lysing methods in women undergoing two abortions.

Total	- agglutination		+ agglutination		-Elisa		IgM	+Elisa		IgM
	No.	%	No.	%	No.	%		No.	%	
18	1430	77.77	416	22.22	1630	88.88	0.59±0.18	2	11.11	1.55±0.46

Women who subjected to three abortions, the percentage of positive cases by agglutination

method was 33.33% compared to Elisa method, as shown in table 8.

Table (8) shows the numbers and percentages of positive and negative samples by the methods of agglutination and lysing among women subjected to three abortions.

Total	- agglutination		+ agglutination		-Elisa		IgM	+Elisa		IgM
	No.	%	No.	%	No.	%		No.	%	
28	25	89.28	3	10.71	27	69.42	0.57±0.19	3.57	1	1.66±0.00

Discussion

Toxoplasma parasite transmitted to human and animals in different ways such as; food, estrus, organ transplants, blood transfusions (17,18,19) and transmission from mother to fetus during pregnancy (20). *Toxoplasma* parasite tissue cysts have been examined in aborted lamb fetuses by examining different organs tissue such as; brain, heart, lung, kidney, liver and skeletal muscles. The number of positive cases of the presence cysts is 20 out of 50 cases and the percentage more than 40%. It indicates that congenital transmission infection of toxoplasmosis condensate occurred by transmitting through the placenta from the mother to fetus during the pregnancy (21).

Increasing rate of toxoplasmosis infection because of infected cats shed egg sacs of the parasite which plays an important role in an infection spreading by contamination pregnant ewes' food. Infection could be occurred by contacting with newborn lamb, non-infected ewes to both placenta and infected fetal fluids and milk to ewes infected with *Toxoplasma gondii* (22). These results are in similar studies regarding infection rates, which recorded the seropositivity rate of abortion in pregnant ewes because of toxoplasmosis. It is 42.7% the agglutination of latex particles and 52% to test the indirect hematopoietic for abortion (23). It is also agree with other results which indicate the percentage 48.16% of seropositivity of abortion in pregnant ewes (24).

Globally, the percentage of lambs that died after birth was 36.66% in south Dakota (25), however, the percentage of lambs born from ewes which infected with toxoplasmosis infection and seropositive is (30.7%)(26). The percentage of stillbirth and abortion which happened together in ewes at the university of Connecticut in the united states of America is 25%(27). Investigation the toxoplasmosis condylome antibodies shown that the percentage of anti-toxoplasmosis antibodies for lambs which slaughtered in four different slaughterhouses in the north eastern united states of America were 42%,47%,58.94% and 64%(28).

The percentage of abortion for infected cows sporangia of *Toxoplasma* parasite is 45% (29). o-oocysts and the percentage oocysts parasite may suffer from abortion due to infection with sporangia of *Toxoplasma* parasite is 45%(29).

Dogs may suffer from abortion due to infection with oocysts parasite and the percentage is 33.33%

(30).

In addition, rats could be infected with *Toxoplasma* parasite and the infection is transmitted congenitally to their young, the transmission percentage is 44%(31).

This results didn't agree with the studies that indicate the percentage of *Toxoplasma gondii* antibodies seropositivity 11.4% in lamb flocks when abortion occurred using the latex agglutination test. Infection rates indicate that the percentage of abortion in ewes which caused by toxoplasmosis in northern and central united states of America within seven seasonal years were 10.75%,20%,17.2%,16.16%,17.64% and 7.92% in 1983,1984,1988,1985,1987,1987,1988 and 1989 respectively (32,33). In addition high rates of infection with toxoplasmosis has been indicated and the percentage of aborted ewes at Oregon state university in united states of America was 88.23%(34).

Congenital transmission of toxoplasmosis could be occurred in mice which born from chronically infected mothers with a percentage of 27%, while the percentage of infection increases in mice born from mothers infected during the pregnancy is 79.3%.

One of the most important methods to diagnose toxoplasmosis parasite infection is tissue impression which depends on the used organ tissue such as, brain, heart, lung and kidney. Liver and skeletal muscles tissue cysts have been examined and shown that brain and it is sensitive to infection. The difference of tissue cysts numbers in different organs tissue depends on the host physiology which indicated that brain is the most affected organs by *Toxoplasma* parasite because of brain histological structure which is neurons and rich in fatty substances. There most congenital malformation could be occurred in the brain such as, hydrocephalus inflammation of the head. Brain calcification, choroidal retinitis, mental retardation cases, Mongolianism, epilepsy and head-to-earth rebound in children as a result of seropositivity with toxoplasmosis (36), which confirmed that brain is the best organ used to detect the infection with toxoplasmosis and parasite isolation (37). That results are in agreement with studies which indicate that brain is the best organ to detect *Toxoplasma* parasite in adult sheep that infected with 10^5 *Toxoplasma* sporophyte egg sacs (38). The results are also in agreement with studies mentioned that brain is the most susceptible organ to infect with *Toxoplasma*

parasite whether in aborted fetuses or in lambs born from ewes infected with the parasite and it is also the best organ used for diagnostic purposes with toxoplasmosis (39). Therefore, brain has been identified to estimate toxoplasmosis infection severity because it was the most affected organ by infection.

This study results agree with the study which conducted on serea of arms slaughtered in the Al-Dora and Al-Shulathouse slaughter slaughter house in the province of Baghdad (40). Used indirect lactase agglutination assay and the percentage was 81.3%, while the percentage of toxoplasma parasite presence in digestion sediments pepsin was 37.28%. Some studies showed lower percentages than those has been obtain Abdullah (2004) recorded in this study a percentage of 49% by using latex particles agglutination test in Nineveh governrate. Another study showed that toxoplasmosis serum positivity percentage was 26.6% by using complement fixation test and 18.2% by using fluorescence test in sheep slaughterhouse in Baghdad governorate (41).

Globally, serum positivity rate of toxoplasmosis in Tehran abortion was 61.24% by using direct agglutination test 42.43. The percentage of serum positive in ewes was 59% which indicate a study to investigate serum positive rate of toxoplasmosis in five sheep slaughter houses in northeastern united states of America where the serum positively percentage in these slaughterhouses was 80%, 82%, 27%, 65% and 87.5% (44).

The results of this study didn't agree with the study that indicate the percentage of serum positive which 34% when using indirect blood agglutination test to detect toxoplasma antibodies in goat serum as the antibody parameters ranged between (1:4:1024) (45).

Pregnant women abortion occurred due to toxoplasmosis parasite ability to penetrate placental tissue to get the fetus which leads to an immune response that cause the spontaneous abortion, and fever due to the infection (46,47). These results indicated that the infection rate in single abortion was 21.05% by both diagnosis methods which agree with the percentage of 22% single abortion case agree with percentage northern of government of Iraq infection rate 15-26% and agree with Ramagi city infection rate which was 5.8 when measuring IgG antibodies (28,49,50).

Thses results agreed with IgM measurement which is consistent with Baghdad city infection rate that found the percentage of aborted rate is 34.7% two aborted women 22.22% by agglutination method and 11.11% by Elisa method (50,51). Therefore had an effect on the serum positivity of aborted women which is 31.25% of two aborted women (52). Infection rate in Basra Governorate was 37% as abortion threatened women and the percentage was 10.71% by agglutination method and 3.57% by Elisa method (53) which agreed with the percentage of infection rate 18% in Baghdad city for three percentage of

times aborted women (54) rate is 33.33%. By Elisa and adjuvant methods which agreed with the percentage 6.25% for multiple abortion women (48).

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