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Molecular and Serological Detection of *Toxoplasma gondii* in Random Sample of pregnant and aborted women of Nineveh

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Abstract

The present study is to identify the rate of parasitic disease of toxoplasmosis among among females from 120 pregnant and aborted women attending to the Department of Obstetrics and Gynecology of the AL-Kansaa, AL- Salaam Hospital and 2 private clinics during (September 2019-febrauary2020). Three methods of tests to search for toxoplasmosis latex test, combo IgG and IgM rapid cassette and finally by PCR. Questionnaire is applying by pregnant and aborted patients and according to the information the females was divided to groups involved age, career, gestational period, housing cat or not in living zone ,number of abortion ,blood group and according to residence. The results show big rate of toxoplasmosis by latex test the positive cases 62(62%).the result re-tested by another serological method combo IgG and IgM rapid cassette and give the result 44 (73.33%) and 6 (9.09%) for IgG and IgM respectively. The result was confirmed by third test using molecular test conventional PCR to show the result 32 (51.61%). We used EDTA tube for whole blood for PCR, gel tube for serological testes. Three methods of tests to search for toxoplasmosis latex test, combo IgG and IgM rapid cassette and finally by PCR.

Keyword: Molecular detection, *Toxoplasma gondii*, pregnant and aborted, serological tests, latex test, rapid test, IgG and IgM.

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الكشف الجزيئي والسيرولوجي في التوكسوبلازما جونيدي في عينة عشوائية من النساء الحوامل والمجهضات في محافظة نينوى

علياء حسين علي **نجاح صبحي نايف**

جامعة الموصل/ كلية العلوم/ قسم علوم الحياة

الخلاصة

اجريت الدراسة الحالية لتبين نسبة انتشار داء المقوسات الكوندية للمريضات من الحوامل والمجهضات الوافدات الى مستشفى الخنساء للحمل والولادة ومستشفى السلام التعليمي واثنين من العيادات الخاصة خلال الفترة الزمنية من سبتمبر 2019الى فبراير 2020، ثلاثة طرق من طرق الفحص استخدمت للتحري عن المقوسات الكوندية تضمنت اختبارين مصليين باستخدام فحص التلازن اللاتكس وشريط الفحص السريع لفحص الاجسامIgM, IgG وتمت اعادة النتائج باستخدام طريقة جزيئية وهو فحص التفاعل المتسلسل PCR.

وبعد توزيع استبيان للمريضات يتضمن العمر ومستوى التعليم والمهنة ووجود قطة او حيوان اليف في مكان السكن كما تم الاستعلام عن فصيلة الدم.

أظهرت النتائج ان نسبة كبيرة من المصابين بداء القط بواسطة فحص اللاتكس هم 62(62%) وتم أعادة النتائج باستخدام فحص مصلي آخر هو شريط الـ IgG وIgM للفحص السريع حيث أظهرت النتائج 44 (73.33%) و6(9.09%) لـ IgG وIgM على التوالي.

تم تأكيد النتائج بطريقة جزيئية ثالثة وهو فحس الـ PCR حيث أظهرت النتائج 32(51.61%).

في هذا البحث تم استخدام نوعين من انابيب الاختبار الاول انبوب الحاوي على مانع التخثر edta لجمع الدم الكامل وهذا الانبوب يستخدم للغرض حفظ العينات التي تستخدم التفاعل المتسلسل الانبوب الثاني انبوب الهلام والذي استخدم للفحوصات المصلية.

الكلمات المتفاحية: الفحص الجزيئي، داء القط، حوامل ومجهضات، طرق مصلية، فحص التلازن اللاتكس، الفحص السريع IgG، IgM

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INTRODUCTION

Toxoplasmosis is a zoonotic protozoan illness produced by the parasite Toxoplasma gondii(Dardé and Ajzenberg) Toxoplasma is a parasite is spreading in hot and humid countries capable to progress in a wide range of vertebrate hosts. (Liu and other ,2012)

Cats and of Felidae family members are the definitive hosts (Knoll and other ,2019) whereas wide range of vertebrate include animals and humans act as intermediate hosts (Roberts and Janovy ,2005). Parental infections account for (2% to 3%) of all congenital Toxoplasma defects (Ocak and other, 2007).

The ways of transmission of the parasite to human is either by eating under cooked raw of meat (Dubey and Beattie ,1988) the second way of transmission by the blood transfusion and transplantation of organ , oocysts ingestion from dirty food or water (Dubey and Jones,2008) Fecal contamination of hands is a significant risk factor and (Torrey, 2007).

During pregnancy the Primary maternal T. gondii infection is often related with its transmission to the fetus (Pappas, 2009).

The rate of transmission of maternal infection to the fetus is valued to be about 45% of these 60% are subclinical infections ,9% causing in fetus death and 30% have big harms such as intracerebral calcification, hydrocephalus, chorioretinitis and mental delay (Montoya and Remington , 2008]. In most cases the laboratory diagnosis of acute and latent toxoplasmosis include detection of IgG and IgM antibodies.

Serological tests of Toxoplasmagondii include the LATEX agglutination test, indirect fluorescence antibody test (IFA),ELISA(Hajsoleimani ,2012) and hemagglutination test have been used for the detection of antibodies against T. gondii in pregnant women (Kadir ,2012).

Toxoplasma gondii levels high on the list of diseases which lead to death in patients with (AIDS) acquired immunodeficiency syndrome (Luft and Remington ,1992)

MATERIAL AND METHOD

A total of 120 pregnant and aborted women attending to the Department of Obstetrics and Gynecology of the AL-Kansaa , AL-Salaam teaching Hospital and 2 private clinics during (September 2019-febrauary2020) .A questionnaire form was applied by each female which included: age, address, blood group, number of abortion, cats at residence and carrier.

Blood samples were collected and serum was separated for the estimation of antibodies against *T. gondii* infection.

Specimen collected from Venous blood (5ml) was drawn carefully and transferred into EDTA tube, the specimen was left for 15-30 min. then centrifuged at 300 rpm for 5min. to separate clear serum, the sera were tested for the estimation of antibodies against *T. gondii* infection .

First method: Latex Agglutination Test

Latex Agglutination Test is use according to manufactured instruction of plasmatic com. U.K. one-step rapid LATEX particle agglutination test on slide for qualitative and quantitative determination of Toxoplasma antibodies in serum. Latex test use as primary method of tests for indicated the positive ceases by notice agglutination drops on the slide.

Second method: cassette IgG\IgM combo rapid test

A rapid one step test for the simultaneous detection and differentiation of IgG and IgM anti-*Toxoplasma gondii* in human serum, plasma or whole blood.

Third method: Poly chain reaction (PCR)

Third method : In this study we use the PCR in different female samples as following:

PCR that technique used to rapidly increase the number of copies of specific region of DNA.

DNA Purification from whole blood according to (QIAgen commercial mini kit).

To prepare Agarouse gel with concentration of 1% needed to:

0.5 gm of Agarouse powder, 50ml of X1TBE and 3ml of red safe With stirring to reach boiling by use heating supply and let it to cool at room temperature (25-38) c.

Pours the gel solution in l tray in electrophoreses after fixing the special comb to form wells in the edges of the gel .

Adjust the concentration of DNA in all this study samples by dilution with TE buffer solution to make PCR reaction and it is (50) Nano g/µL.

For each sample, prepare the Master reaction mixture for every PCR reaction that by DNA sample with the specific gene primer with master-mix in side eppendrof 0.2ml (Biolaps co.)

Mix the mixture in microfuge for (5-3) second and enter the reaction tubes to thermocycler to make the polymerase reaction by use the specific program for each reaction.

Carried sample in Agarous gel wells that prepare previously with concentration 1%. And add the ladder DNA in one well.

Detection of *Toxoplasma gondii* B1gene in sample of blood of pregnant and aborted woman amplification was performed using two primers with the following sequences:

Table 1. Sequences of primers

Forward	TTTTGACTCGGGCCCAGC
Revers	GTCCAAGCCTCCGACTCT

Put the tubes in the Thermocycler machine to make the reaction according to its specific program as following:

Table 2. Thyermocycler program

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	90	7 min.	1
2.	denaturation	90	50 sec.	35
3.	Annealing	58	1 min.	
4.	Extension	73	1 min.	
5.	Final extension	73	5 min.	1
6.	Initial denaturation	95	6 min.	1

This study include 120 women, 20 cases were neglected because some information was missing.

At first we use the serological method include Latex test and combo rapid IgG and IgM cassette the results show the following information:

High levels of infection by Latex 62% the positive results of latex test re-tested by IgG and IgM to give 73.33% and 9.09% respectively by using rapid test cassette.

Second step we use the conventional PCR the results was re-tested to give 51.61% as positive result.

Result and discussion

Table (3) the rates of positive Toxoplasmosis, %, in relation to different laboratory techniques in pregnant and aborted women

Lab. Tech.	no. +=62	% +
Latex	**62**	**62%**
IgG	**44**	**73.33%b**
IgM	**6**	**9.09%a**
PCR	**32**	**51.61db**

By SPSS program version no.26 for statistic there is a statistically significant difference between serological and molecular method that we use,***a-d***, proportions within column with different lettered superscripts are significantly different (p<.o5).

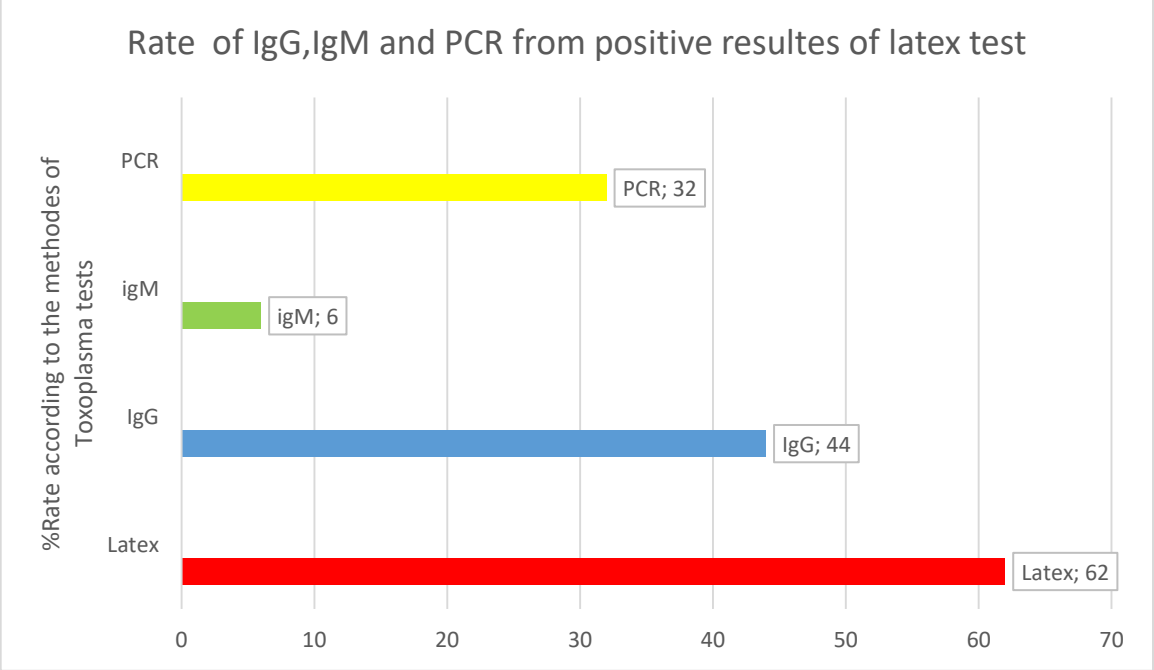

Figure (1): Analysis distributed according to different laboratory techniques in pregnant women

Positive infection with latex test
Positive IgG infection with rapid cassette
Conventional PCR
Positive IgM infection with rapid cassette

The results showed 44(70.96%) for IgG and 6(9.67 %) IgM this results because the body first produced measurable IgM antibodies in the

blood one to two weeks after infection , a few months later the IgM became un detectable and is replaced by IgG antibodies that will be present for longer time.

The IgM antibodies may re-appear if the infection is reactivated or the infection is chronic. Detection of toxoplasmosis is particularly important in pregnancy time because of the high risk (30-40) %of transmitting the infection to the fetuses causes which numerous complication or may lead to death. This study results were in agreement with a survey done in New Zealand in which 500 aborted women were tested using ELISA. They found that 2.5% and 33% of 500 women were seropositive for IgM and IgG anti-toxoplasma antibodies respectively. (Morris , Croxson 2004). This study results were in agreement in the relation of IgG antibodies with the results prepared in Cameroon in which 100 pregnant women were tested by using ELISA and 70% of them had positive IgG which demonstrated great rates of past infection. This showed that great rate of *Toxoplasma* infection might also be because of the geographical location, low hygienic ,low education levels, large increasing of stray cats ,and low socioeconomic status (Hassani and Zghair, 2010) (Al-Taie, 2011) .

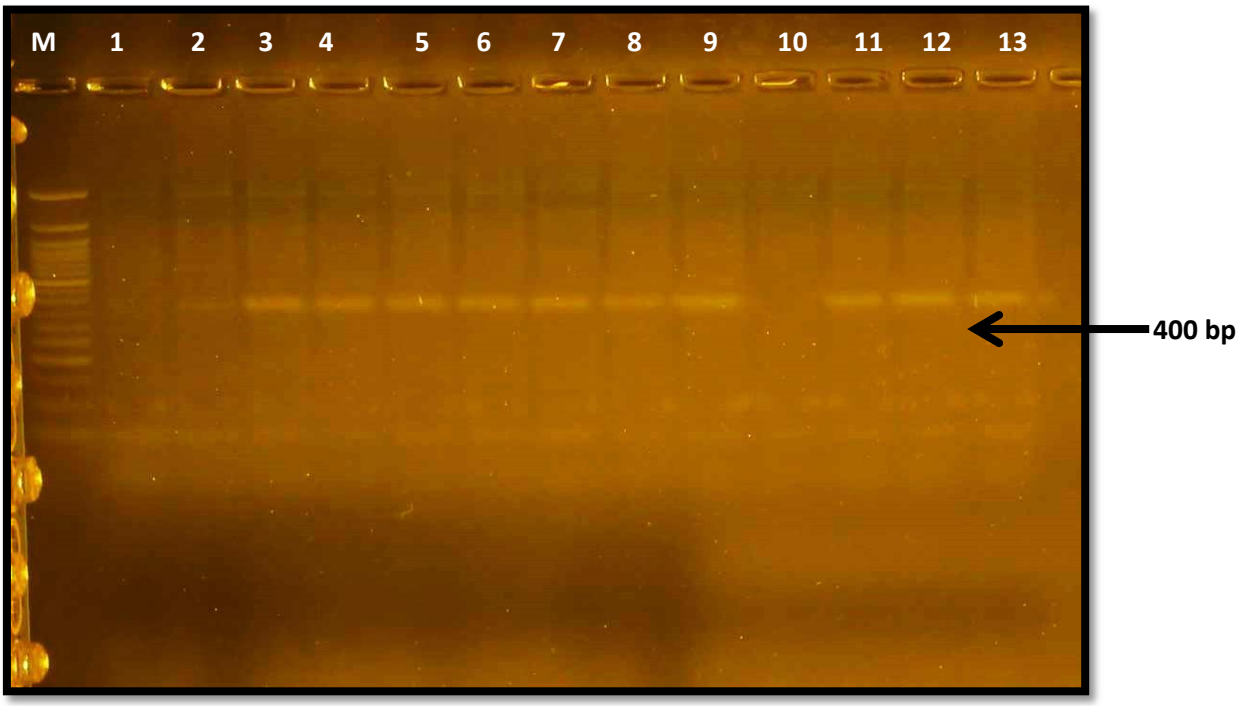

Figure (2) the result of PCR reaction of toxoplasma B1gene in pregnant and aborted blood in result of reaction 400pb

The results clearly indicated that the molecular method characterized by high sensitivity and specificity with no opportunity for false positive and false negative results because it deals with specific genomic DNA of Toxoplasma.

Therefore PCR test is considered as a gold standard test for diagnosis, since out of 100 pregnant and aborted women examined in the present study 32 cases were PCR positive while 44 cases were IgG positive this is a high rate of antibodies which might be due to false positive results and cross-reactivity.

The results of the current study were in disagreement with the results of a study that was carried out in Saudi Arabia in which out of 137 pregnant and aborted women with BOH (bad obstetric history) were tested by using PCR. They showed that 41% of the cases were PCR positive. (Dajem and Al-Mushait, 2012) while in this research show 22.22% were PCR positive.

Regarding PCR test this study shows a disagreement with the results of a study in Shiraz/Iran (Asgari and other,2013), In which a total of 542 of pregnant and aborted women were tested by PCR found14.4% had PCR positive while in the current study showed that 22.22% of pregnant and aborted women were PCR positive.

Table (4) the general characteristics of the total of 100 sample from pregnant and aborted women that included in this study.

characters	Carrier	House wife	Student	Employee	
Samples numbers	N=100	32	9	3	
percentage		72.72%	20.45%	6.81%	
characters	House animal	Cat exposure	Having domestic animal	Have no animal	
Samples numbers	N=100	20	10	14	
percentage		45.45%	22.72%	31.81%	
characters	age	(15-20)	(21-26)	(27-32)	33>
Samples numbers	N=100	9	12	18	5
percentage		20.45%%	27.27%	40.90%	11.36%
characters	Gestational period	First trimester	Second trimester	Third trimester	
Samples numbers	N=100	27	10	7	
percentage		61.36%	22.72%	15.90%	
characters	Number of abortion	First time	Second time	Third time	Forth time and more
Samples numbers	N=100	24	18	2	0
percentage		54.54%	40.90%	4.54%	0%
characters	Blood group	A	B	AB	O
Samples numbers	N=100	18	12	9	5
percentage		40.90%	27.27	20.45%	11.36%
characters	Residence	Rural	Urban		
Samples numbers	N=100	30	14		
percentage		68.18%	31.81%		

According to the information that obtained from the female patients they divided into groups as following in Table (2) that displays all (100) examined women were distributed as groups according occupation status the housewives 32(72.72%) employees, 3 (6.81%) and students 9 (20.45%). According to the history of abortion for the first time 24 (54.55%), with second time abortion 18(40.90%) and with triple abortion or more abortions 2(4.54%).

Age groups were 15_20 years=9 (20.45%), 21-26 years=12 (27.27%), 27-32years=18(40.90%), and 33>years=5 (11.36%).

Regarding the gestational period of first trimester cases is27 (61.63%) second trimester 10 (22.72%) and third trimester 7 (15.90%).

Blood group was used as following group: O 5(11.36%), group A 18(40.90%), group B 12 (27.27%) and group AB 9 (20.45%).

Regarding the housing of animal involved according to cat exposure 20(45.45%) having domestic animal 10(22.72%) having no animal 14(31.82%). Finally regarding to the living zone the cases were divided to rural 30(68.18%) and urban 14(31.81%).

Table (5) The rates of gondii antibodies,%, due to toxoplasmosis in relation to the pregnant women status and exposure to cast or domestic animals

Lab. Tech.	ni. +	% +
Exposure to cats	20	45.45a
Own domestic animals	10	22.72b
No house animals	14	31.82
Total	44	

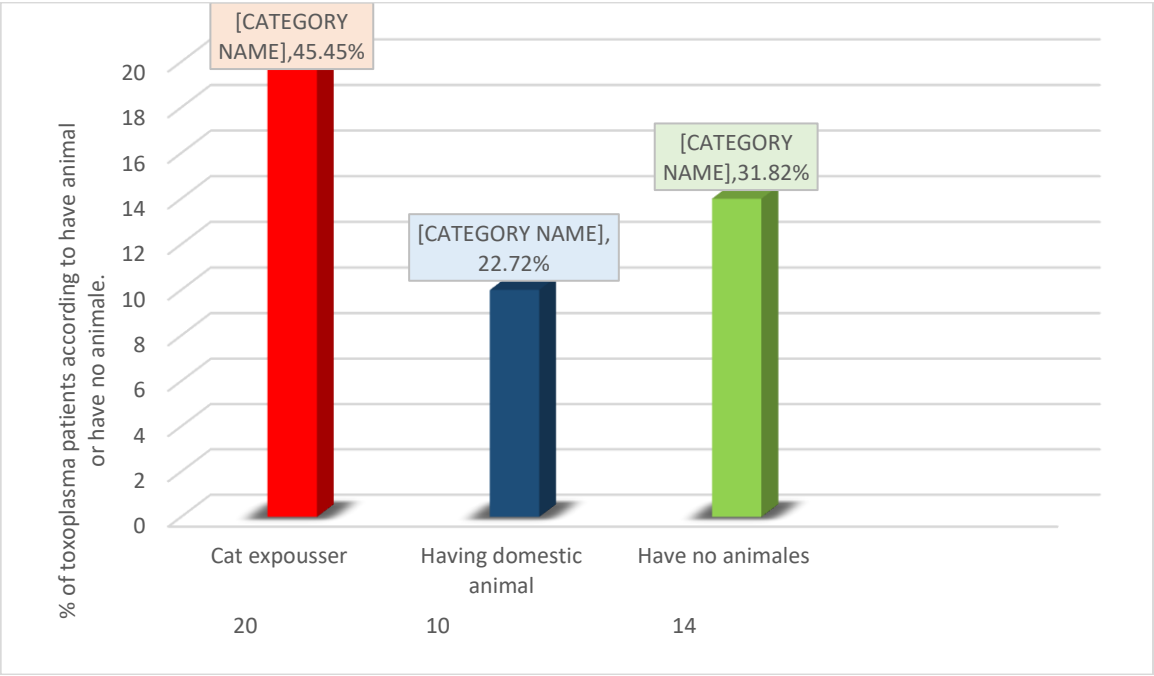

Figure (3) Analysis distributed according to exposure to cat, domestic animal and housing no cat and no domestic animal

Women that exposure to cat.
Woman housing domestic animal.
Women housing no cat and no domestic animal.

The oocyst takes a minimum of 24 hours in cat feces to sporulate and become infective thus, a frequent removal of feces from the litter box while not wearing gloves and not washing hands afterward increases the possibility of infection.

Exposed to the parasite by touching an infected cat because they rarely carry the parasite on their fur and also doubtful that infection through cat bites or scratches.

Table (6) The rates of gondii antibodies,%, due to toxoplasmosis according to different living zone

Living zone	no. positive	%, positive
Rural	30	68.18a
Urban	14	31.81b
Total	44	

***a-b*, proportions within column with different lettered superscripts are significantly different (p<.o5).**

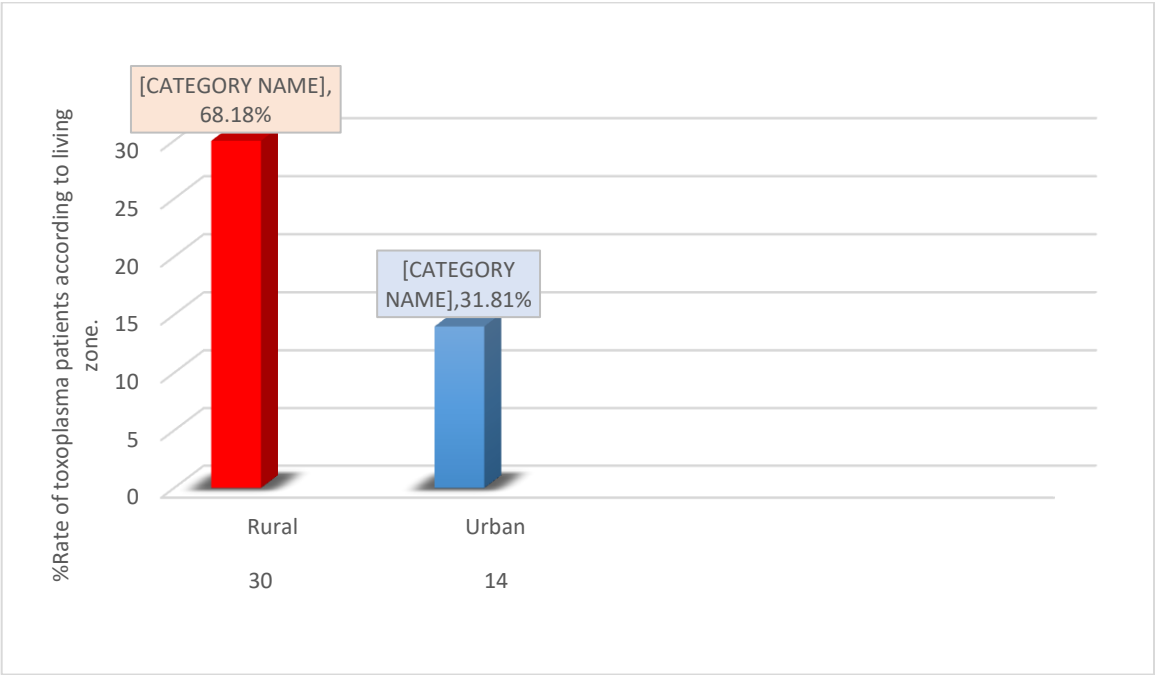

Figure (4) Analysis distributed according living zone.

Regarding the residency, this study showed that females living in farms had significantly greater prevalence of Toxoplasmosis compared to urban ones, There is a significant difference in the proportions of

individuals with the characteristic of interest within the two groups rural and urban (P = 0.010)

This result may be due to the consumption of raw vegetable and high usage of water that might have been contaminated with *T. gondii* oocyst.

Lack of knowledge increased the risk of infection, for instance, lack about the disease and its relation with undercooked meat and as being the source of infection. Additionally, lack of knowledge about the risk of meat undercooked or tasting it during cooking. This unawareness was estimated as a high risk factor, association of *T. gondii* infection with soil, unwashed vegetables and fruits all at risk of *T. gondii* on fetus.

Table (7) the rates of gondii antibodies, %, due to toxoplasmosis, in relation to different women ages

Age group	ni	%,+
15.20	9	20.45%a
21.26	12	27.27%a
27-32	18	40.90%a
33>	5	11.36%ac
Total	44	

a-c proportions within column with different lettered superscripts are significantly different. (p<.o5).

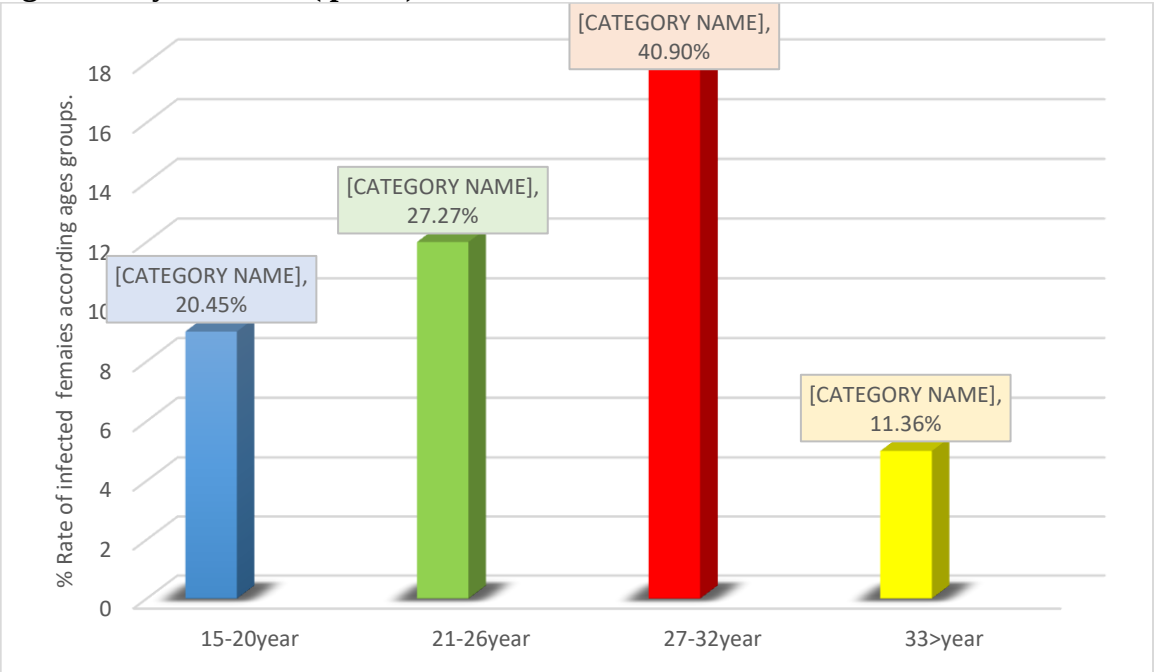

Figure (5) Analysis distributed according to age groups of pregnant and aborted women

(15-20) year

(21-26) year
(27-32) year
33> years

Table (8) the rates abortion cases, %, due to toxoplasmosis in relation to different stages of trimester

Stage of trimester	no.+	abortion ,%
1st trimester	27	= 61.36 a
2nd trimester	10	= 22.72 ab
3rd trimester	7	=15.90 b
Total	44	

***a-b*, proportions within column with common lettered superscripts are significantly similar(p<.o5)**

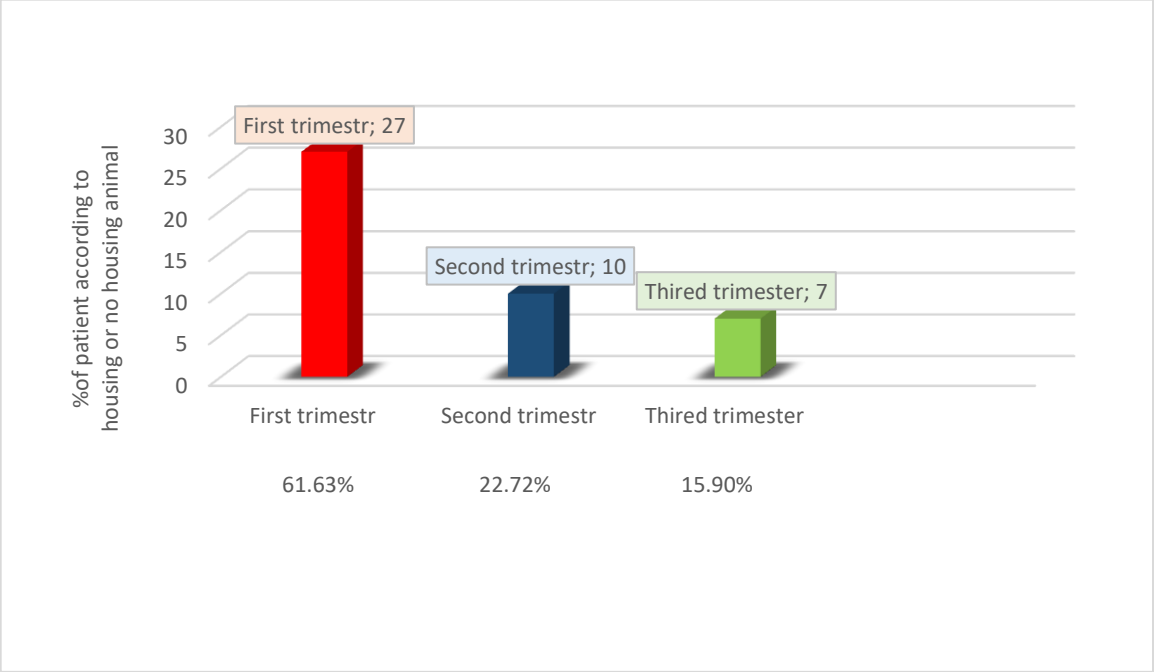

Figure (6): Analysis distributed according to pregnancy trimester

First trimester
Second trimester
Third trimester

Women in the first trimester 61.36% were more infected than second 22.72% and third 15.90%. In this study we agree with Khalil (2017) who has found in his study the infection of *Toxoplasma* is 48.2% in first trimester and 37.3%, 14.3% in second and third trimester respectively, This study also agree with AL-Hindi and Lbbad (2009) in Gaza and also agrees with Diana ((2001) in France.

Table (9) the frequency of abortion due an infliction with toxoplasmosis

Frequency	no. of aborted women	% of abortion
1st time	24	54.54a
2nd time	18	40.91a
3rd time and more	2	4.55b
Total	44	

***a-b*, proportions within column with different lettered superscripts are significantly different (p<.o5).**

The prevalence of *Toxoplasma gondii* infection in the first, second-trimester abortion and third-trimester are compared. The distribution and prevalence of IgG, IgM, and PCR are shown in Table (4.6).

The results show 27(61.36%) in first trimester, 10(22.72%) and third trimester 7(15.90%) there was a significant difference in the infection by *Toxoplasma gondii* between 1st (first) and 2nd (second) trimester, third trimester of abortion. This result may be because the parasite are transmitted to the fetus in early pregnancy months.

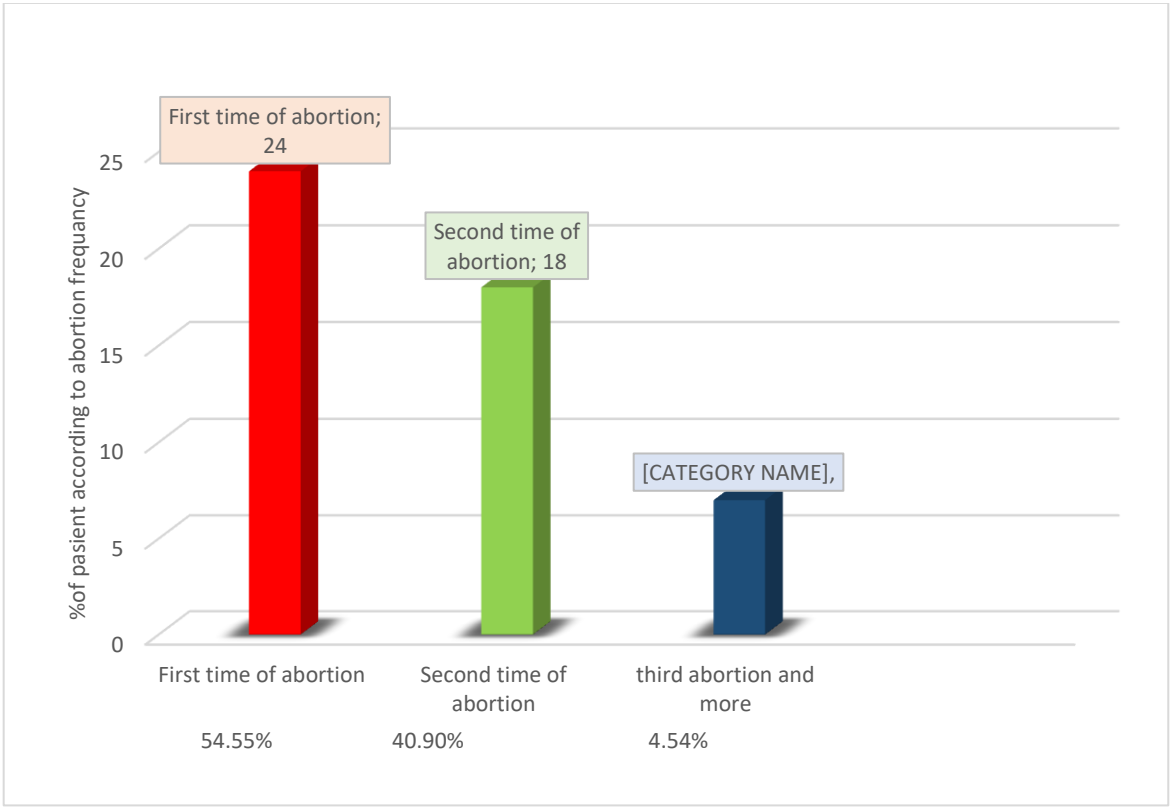

Figure (7) Analysis distributed according frequency of abortion in women infected with toxoplasmosis

1st time of abortion

2nd time of abortion
3rd time of abortion and more

According to the number of abortions, it is found that (52.55%) of single abortion have toxoplasmosis. Besides (32.35%) and (15.44%) cases of women with two, three and more abortions have toxoplasmosis, respectively, as shown in Table

Table (10) the rates of gondii antibodies, %, due to toxoplasmosis, in relation to occupation of women

Status.	ni +	%+
House wife	32	64.71a
Students	9	26.47b
Employees	3	8.82b
Total	44	

***a-b*, proportions within column with different lettered superscripts are significantly different (p<.o5).**

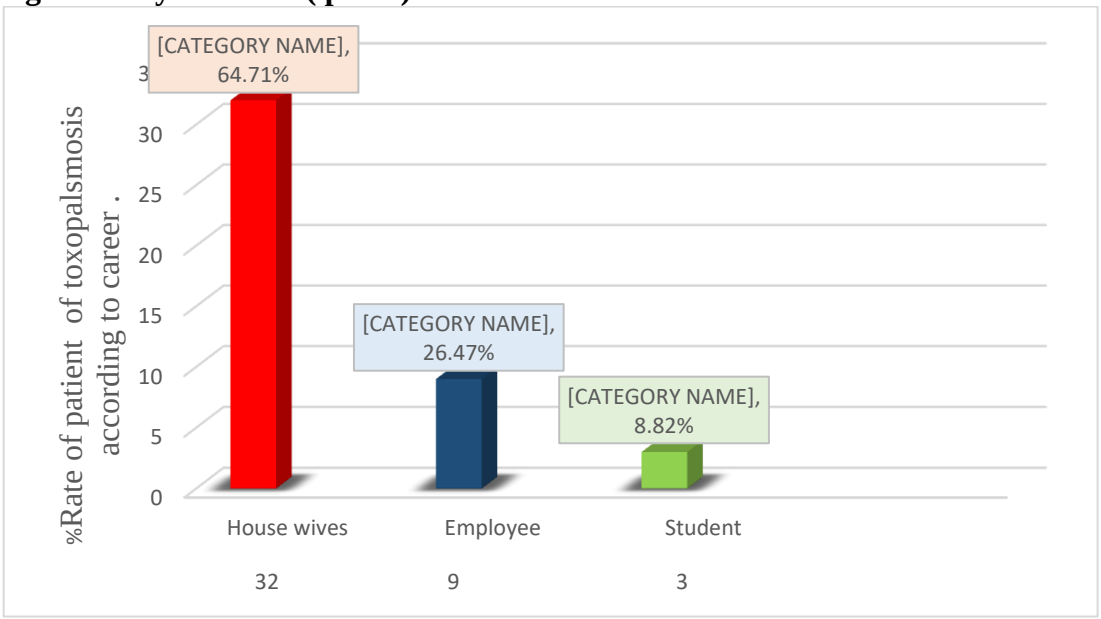

Figure (9) Analysis distributed according frequency of abortion in women infected with toxoplasmosis

House wives
Student
Employee

Table (11) The rates of abortion cases,%, in women infected with toxoplasmosis gondii , according to their blood groups

Blood group	ni +	abortion %
A	18	40.90a
B	12	27.27ab
AB	9	20.45ab
O	5	11.36b
Total	44	

a-b proportions within column with similar lettered superscripts are significantly similar (p<.05).

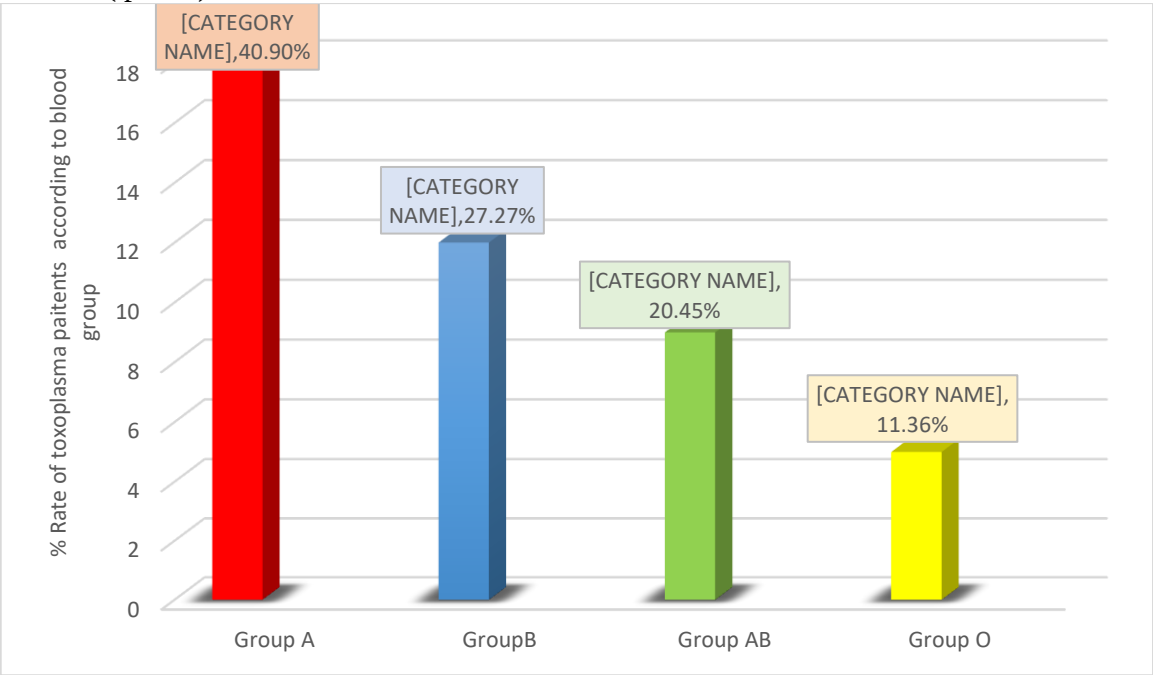

Figure (9) Analysis distributed according blood groups of pregnant and aborted women

Group A
Group AB
Group B
Group O

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