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Determination of Toxoplasma Gondii Genotypes from Mice Inoculation as an Iraqi Strains (REVIEW)

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ABSTRACT

In this study, (50) mice were inoculated by Toxoplasma gondii Tachyzoite taken from Amniotic Fluids and partially extracted placental in Alzahraa Hospital/ Diyala Province, during the period from February to October 2024. The results showed that The prevalence rate of Toxoplasmosis inoculated in mice according to the age 2 weeks was 14 (28.0%), 3 weeks was 17 (34.0%), and the prevalence rate 4 weeks, 5 weeks and 6 weeks was 4 (8.0%) respectively. Also, the distribution of Toxoplasmosis according to sex showed that prevalence rate in male was 30 (60%) and in female 20 (40%). The mean ±SD of IgM was (0.97±0.22) and mean ±SD of IgG was (1.30±0.24). The Mean±SD of IgM concentration in male was (0.89±0.29) and in female was (1.09±0.29) with significant variation (P=0.019). Also, the Mean ±SD of IgG concentration was (1.40±0.31) in male and the Mean ±SD of IgG concentration was (1.20±0.32) in female with significant variation (P=0.024). The mean of IgM in the age 2 weeks was 14 (1.00±0.25 B), and in the age 3 weeks was 17 (0.89±0.22 B), and in the age 4 weeks was 9 (0.94±0.18 B), in the age 5 weeks was 6(1.01±0.15 AB), in the age (6) weeks was (1.21±0.06 A), with significant variation (P=0.043). A clonal (Type II) was identified in 48 (93.3%), and type I was identified in 9 (15.25%), while type III was identified in 2 (3.38%), and these findings are considered entero-zoonotic parasite and this case recorded in Iraq for the first time.

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1- INTRODUCTION

Toxoplasmosis is a universal zoonotic parasitic infection, caused by Toxoplasma gondii, which is an obligate intracellular protozoal parasite. The definitive hosts are felids, whereas the intermediate hosts are the other warm blooded animals. Following ingestion of environmental oocysts by cats contaminated feces forms, humans, mammals and birds will develop dormant tissue cyst [1]. Raw and uncooked meat of animals that harbor the infective tissue cyst are other infection sources for humans and other meat-consuming animals. Within domestic environments, rodents are thought to be the main intermediate host in *T. gondii* life cycle since they are often the main prey species for a domestic cat [2]. Nevertheless, while some rodents show resistance to some virulent strains and infected with chronic toxoplasmosis, other rodents develop high susceptibility to the majority of *T. gondii* strains and quickly die due to acute infection with toxoplasma [3]. Thus, the genetic resistance patterns specific to local rodent species may determine the environmental transmission of *T. gondii* strains, and such mechanism can powerfully figure the structure of various worldwide *T. gondii* populations [4]. Strains which can cause persistent infection to local rodents in accordance with their patterns of respective resistance may represent, at least partially, the strains which contribute to local transmissions to cats, environmental contamination and human's

infections [5]. Human infections in Africa, mostly occur from local sources usually because of contacts with contaminated soils. Hence, assessment of *T. gondii* circulations in rodents and describing the strains that cause chronic toxoplasmosis in intermediate hosts may be essential for determination of the species which act as a local reservoir of potent pathogenic strain [6]. It is useful to screening the rodents for *T. gondii* to determine the toxoplasmosis prevalence, and to diagnose the persons with chronic infections prior to perform a bioassay and isolate the infection-causing strains or to directly genotype them. For rodents, serological examinations have been commonly used, primarily within the prevalence study frameworks [7]. Nevertheless, serological methods have never been confirmed by a 'gold standard' procedure, that brings into questions their dependability to identify infections. The modified agglutination tests (MATs) are most frequently utilized serological tests. Nonetheless, these tests do not give trustworthy results in all species [8].

The possible variations in the establishment of domestic cat history according to region, as well as the climate geographical variabilities (that affect oocyst viability in the environments), may expose such intermediate hosts to various environmental contamination levels with *T. gondii* [9]. Such putative variations in the evolutionary history of invasive native small mammals might result in distinctive host-parasite co-adaptation. Therefore, we assume that native and invasive species may show various immune response patterns to *toxoplasmosis* related to variable innate susceptibilities to different strains of *T. gondii* .

2- MATERIAL AND METHOD

In this study, (50) mice were inoculated by Toxoplasma gondii Tachyzoite taken from Amniotic Fluids and partially extracted placental in Alzahraa Hospital/ Diala Province, during the period from February to October 2024. They were obtained from the peritoneal exudates of previously inoculated mice aged between 2-8 weeks. The inoculation period was 7-10 days earlier during *T. gondii* strain maintenance or taken from mice inoculated with amniotic fluids and partially extracted placental tissues, after 7-10 days after inoculation. Tachyzoites were detected inside leukocytes (macrophage and lymphocyte) of mice or freely found in peritoneal exudate following leukocyte rupture. The isolated tachyzoites were found in freshly-obtained exudates of unstained peritoneum in College of Veterinary Medicine in Diyala Province**.** Samples were treated with special materials in the toxoplasma kit for DNA extraction to do PCR technology. Serological tests were used for detection of Toxoplasma IgM and IgG of peritoneal fluid.

DNA (Taq) polymerase enzyme: (500 U / µL).

Table (1): Primers specialized to amplify the B1 gene

Forward (R) and Reverse primers sequences	
B1 (F1)	GGAACTGCATCCGTTCATGAG
B1(R1)	TCTTTAAAGCGTTCGTGGTC
B1 (F2)	TGCATTAGGTTGCAGTCACTG
B1(R2)	GGCGACCAATCTGCGAATACACC

The amplification was done by the use of F3 & R3 starters warmly at 46 ° C localizations, but for second amplification, 3 µl of PCR products were used from the first amplifications as templates for the second rounds of the same condition interactions but with the exclusion of F2 & R primers (table 2). It was used electrically on 2% agarose gel [11].

Table (20): prefixes specialized to amplify the ends - 3 and 5 - the site SAG2

Forward (R) and Reverse primers sequences	
F4	GCTACCTCGAACAGGAACAC
R4	GCATCAACAGTCTTCGTTGC
F	GAAATGTTTCAGGTTGCTGC
R2	GCAAGAGCGAACTTGAACAC
F3	TCTGTTCTCCGAAGTGACTCC
R3	TCAAAGCGTGCATTATCGC
F2	ATTCTCATGCCTCCGCTTC
R	AACGTTTCACGAAGGCACAC

Statistical analysis

Analysis of data was carried out using available statistical IBM SPSS-22, Chicago, USA), including the Mean ±SD and t test.

3- RESULTS AND DISCUSSIONS

The results showed that the prevalence rate of Toxoplasmosis inoculated in mice according to the age 2 weeks was 14 (28.0%), the age 3 weeks was 17 (34.0%), and the prevalence rate 4 weeks, 5 weeks and 6 weeks was 4 (8.0%) respectively. Also, the distribution of Toxoplasmosis according to sex showed that prevalence rate in males was 30 (60%) and in females 20 (40%). The mean ±SD of IgM was (0.97±0.22) and mean±SD of IgG was (1.30±0.24), as shown in table (3).

Table (3): Base line characteristics of the study groups

Characteristic		**Study groups (n=50)**
Age groups, No (%)	2 weeks	14 (28.0)
	3 weeks	17 (34.0)
	4 weeks	9 (18.0)
	5 weeks	6 (12.0)
	6 weeks	4 (8.0)
Sex, No (%)	Male	30 (60)
	Female	20 (40)
IgM (ng/ml), Mean ±SD		0.97±0.22
IgG (ng /ml), Mean ±SD		1.30±0.24

Our results agreed with (Shen,et al., 2023) who showed a highly significant anti-*T. gondii* IgG levels observed in vaccinated mice, and there was a remarkable increase in the titer of IgG ($P < 0.05$) following each vaccination as shown in figure (4), suggesting an induction of robust humoral responses. The IgG subclass levels (IgG1 & IgG2a) were examined for the characterization of the types of immune responses. There was a significant increase in the IgG1 & IgG2a levels ($P < 0.05$) in RHΔ*ompdc*Δ*uprt*-vaccinated mice when compared with the control group mice. Moreover, there was a notable increased IgG2a level when compared with IgG1 levels, suggesting that Th1/Th2 mixed and Th1-biased immune vaccinations were elicited by RHΔ*ompdc*Δ*uprt* in mice [12].

The Mean±SD of IgM concentration in male was (0.89±0.29) and in female was (1.09±0.29) with significant variation (P=0.019). Also, the Mean±SD of IgG concentration was (1.40±0.31) in male and the Mean ±SD of IgG concentration was (1.20±0.32) in female with significant variation (P=0.024), as shows in table 2, Figure 1 and Figure2.

Table (4): Distribution of IgM and IgG according to sex

Parameter	Sex group	Mean ±SD	P-value
IgM	Male	0.89±0.29	0.019 (S)
	Female	1.09±0.29	
IgG	Male	1.40±0.31	0.024 (S)
	Female	1.20±0.32	

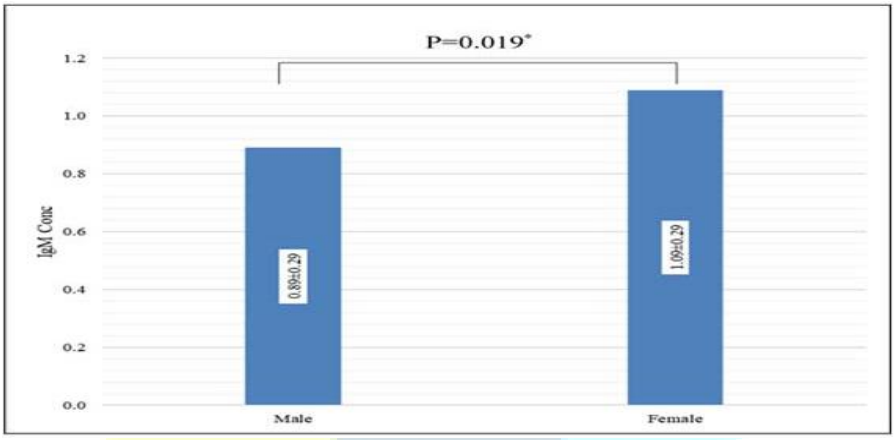

Figure (1): Distribution of IgM according to sex

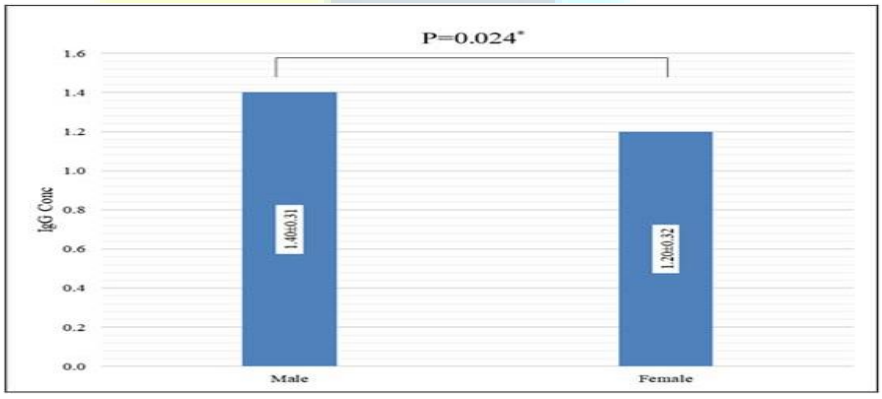

Figure (2): Distribution of IgG according to sex

Simanjuntak, et al.,(2017), reported that there was a significant increase in the levels of anti-toxoplasma IgM antibodies following 24 hours of tachyzoite injection in all doses which remained high until day 21. Following 72 hrs, injection, there was a significant increase in the levels of anti-toxoplasma antibody IgG, which remained high until day 21. The abortion incidence was 100% in mice injected with tachyzoites of 1×10^3 and 1×10^4 levels, and abortion incidence occurred in 2 - 4 days after injection. 100% of mice injected with tachyzoites 1×10^1 & 1×10^2 had labor at term. Physical anomalies were detected in baby mice which were injected with tachyzoite 1×10^2 [13].

The mean of IgM in the age 2 weeks was 14 (1.00±0.25 B), and in the age 3 weeks was 17 (0.89±0.22 B), and in the age 4weeks was 9 (0.94±0.18 B), in the age group (5-6) weeks was (1.01±0.15 AB), in the age 6 weeks was 4(1.21±0.06 A), with significant variation (P=0.043), as shown in table 5.

Table (5): Distribution of IgM according to Mice Age group

Parameter	Age group	N	Mean	P-value
IgM	2 weeks	14	1.00±0.25 ^{B}	<0.01 (S)*
	3 weeks	17	0.89±0.22 ^{B}	
	4 weeks	9	0.94±0.18 ^{B}	
	5 weeks	6	1.01±0.15 ^{AB}	
	6 weeks	4	1.21±0.06 ^{A}	
	Total	50	0.97±0.22	

*different letters mean statistically significance <0.05

The prevalence of IgG antibodies according to the age group, The age 2 weeks 14 with mean (1.22±0.23 ^{B}), The age 3 weeks 17 with mean (1.27±0.28 ^{B}), The age 4 weeks 9 with mean (1.31±0.14 ^{AB}), The age 5 weeks 6 with mean (1.39±0.25 ^{AB}) and the age 6 weeks 4 with mean (1.39±0.25 ^{AB}) were significant variation P=0.043. As shows in table 6.

Table (4): Distribution of IgG according to age group

Parameter	Age group	N	Mean	P-value
IgG	2 weeks	14	1.22±0.23 ^{B}	0.043 (S)*
	3 weeks	17	1.27±0.28 ^{B}	
	4 weeks	9	1.31±0.14 ^{AB}	
	5 weeks	6	1.39±0.25 ^{AB}	
	6 weeks	4	1.56±0.15 ^{A}	
	Total	50	1.30±0.24	

*different letters mean statistically significance <0.05

Previous studies showed that there was a tendency of IgG seroprevalence to be increased with age from 35.44% (95%CI: 29.89–41.30) in the age group 2–3 weeks to 62.85% (95%CI: 56.57–68.82) in the age group 4–5 weeks,

revealing significantly increased age-association ($p < 0.001$). Detectable T. gondii IgG antibodies were observed. IgM antibodies of toxoplasma were shown in 8.90% (95%CI: 6.88–11.43) IgA of T. gondii was shown in 1.65% (95%CI: 0.90–3.01), while both T. gondii IgM and IgA were detected in 0.99% (95%CI: 0.45–2.14). There was a tendency of IgA antibodies to be decreased with avidity increase from 75% (95%CI: 19.41–99.37) in low avidity samples to 11.76% (95%CI: 4.44–23.87) in high avidity samples ($p = 0.01$). in the samples which were positive for both T. gondii IgM and IgA antibodies, 66.67% showed decreased results of equivocal IgG avidity in comparison with 6.25% of mice which showed positive IgM.

Determination of the genotypes of T. gondii strains.

Samples were taken from peritoneal fluid from inoculated mice. The nested PCR method was used to analyze the samples at the *SAG2* locus, where locus 5' and 3' ends were amplified separately (R)**.**

To separately amplify the 5′ and 3′ ends of the SAG2 locus, the primers were selected.. The splatted fragment size was 241 bp and 221 bp respectively. Two endonuclease enzyme Sau3A1 types were applied to perform the splitting process, in which the 3rdallele (Type III) at 5′ end is digested. Meanwhile, the 2nd allele (Type II) is digested by Hhal enzyme where the splitting occurs at 3′. When splittings or fragmentations don't take place by these two enzymes, it will referred to existence of Type I strains.

The clonal (Type II) was identified in 48 (93.3%), and 9 cases of type I [9(15.25%)] were detected, while [2(3.38)] of Type III was detected. This case which was recorded for the first time in Iraq, is considered as an entero-zoonotic parasite (Figures 3, 4, 5,6 and 7).

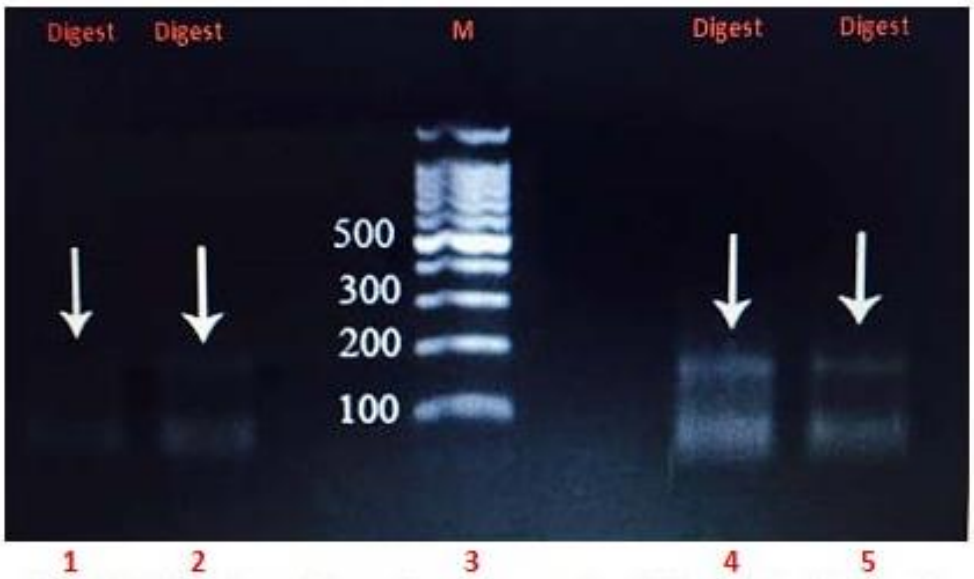

Figure3: Molecular weight markers correspond to 100 bp ladder (fermintus).
1,2,4 and 5 The digested 3' end of SAG2 with HhaI
At 2.5% agarose gel with Ethidium bromide (0.5µg/ml)

Figure 4: 1-Molecular weight markers correspond to 100 bp ladder (fermintus). 3 and5, The digested 3' end of SAG2 with Hhal. At 2.5% agarose gel with Ethidium bromide (0.5 µg/ml)

Figure 5:1- Molecular weight markers correspond to 100 bp ladder (fermintus). 2 & 3. The digested 3' end of SAG2 with Hhal. 5 & 6 The 3' end of SAG2 digested with Hhal. 7 & 8 The undigested 5' end of SAG2. 9 & 10The 5' end of SAG2 digested with Sau3AI. At 2.5% agarose gel with Ethidium bromide (0.5µg/ml)

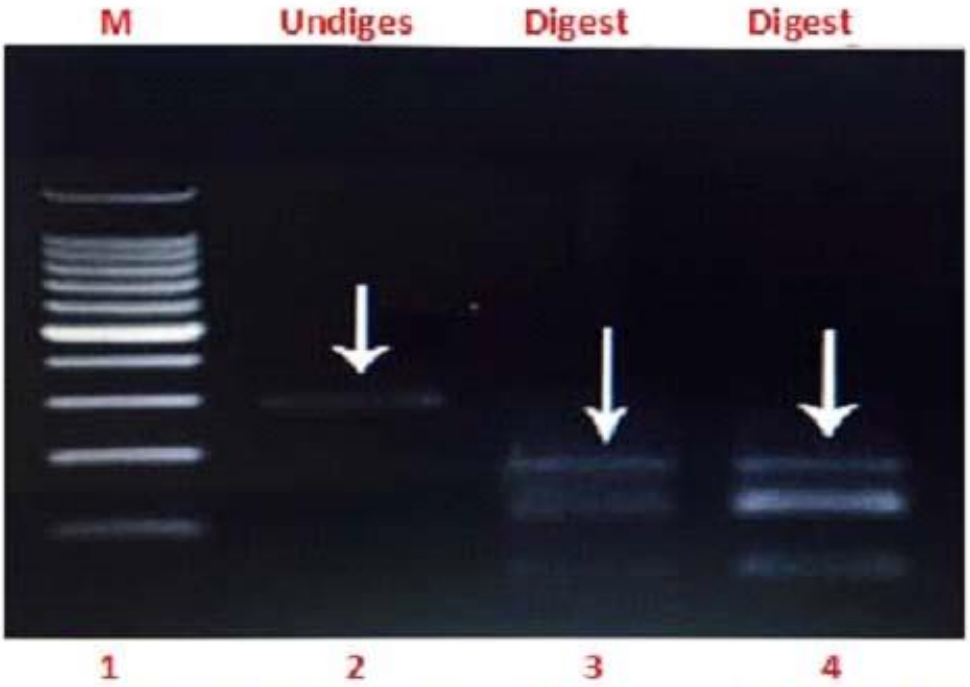

Figure 6: 1- Molecular weight markers corresponding to 100 ladder (fremitus) 2,3 and 4 digested 3' end of SAG2 with Hhal. At 2.5% agarose gel with Ethedium bromide (0.5µg/ml)

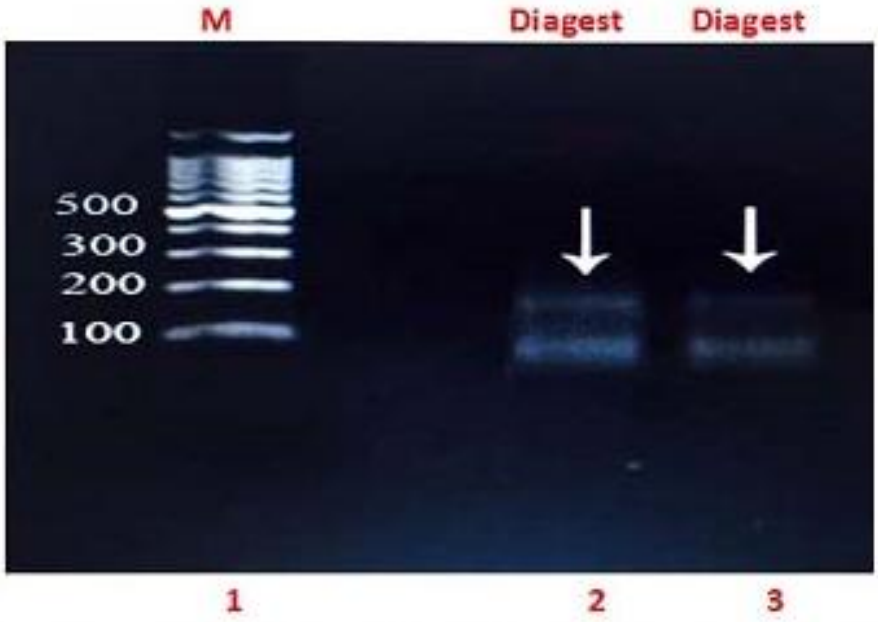

Figure 7: 1- Molecular weight markers corresponding to 100 ladder (fremitus) 2,3 and 4 digested 3' end of SAG2 with Hhal. At 2.5% agarose gel with Ethedium bromide (0.5µg/ml)

The findings in our study are in line with those in other countries, that showed that there is an association usually between toxoplasmosis and second type II strains and it predominant in Iraqi females (Salaah Aldeen and Ibraim, 2023) [15]. It was suggested by some studies that there is a usually a link between first Type I strain among congenital toxoplasmosis patients (that agrees with these results) and toxoplasmosis networks according to the site SAG2 (Bollani, L. et al., 2022)[16].

Therefore, the most deadly type is the first genotype I indicating an increase in the risks of going through the placenta and producing severe symptoms to the newborns and fetuses [17]. This indication is considered true because it clarifies the dead birth cases and birth defects which develop among embryos from women included in our study; although their frequency was lower when compared with type II. This result totally agrees with previous studies which revealed that the rates of type II are most prevalent in humans and animals and can be the highest spread rate of strains in human toxoplasmosis and simply reflecting the strain sources which result in human infections (Ţarcă, et al., 2021) [18]. The prenatal diagnosis of Congenital Toxoplasmosis depends usually upon molecular-based detections of the DNA in *Toxoplasma gondii*. In this case, we reported a serious type of congenital toxoplasmosis that leads to pregnancy termination. There was a similarity between the isolate and type I *Toxoplasma gondii*. In Europe, type II genotypes circulate in human-beings, which is associated with congenital and acquired toxoplasmosis (Maldonado, Y. et al., 2017) [19]. Genotype of 86 *Toxplasma gondii* isolates obtained from congenital toxoplasmosis cases and their relationship with the clinical findings have been assessed. Our study results showed a highly predominance of type II isolates and the isolates of type I and atypical isolates were not detected in benign or asymptomatic toxoplasmosis (Barbosa,I. et al., 2023). PCR was used in the current study to detect the DNA of *Toxoplasma* in peritoneal fluids in mice. This method is relatively highly sensitive with about 50% specificity (S. Romand *et al.,* 2004). Our results assured the peritoneal fluid infections in mice (He, Xi. et al., 2021) [21].

According to an epidemiological study of the disease, the sero-prevalence of antibodies specialized against toxoplasmosis of 97.7% among housewives and this high percentage may be due to the consumption of fruits and vegetables un washed or peeled or neither eating undercooked meat nor presence of cats in or around the house. This case which was recorded for the first time in Iraq, is considered as an entero-zoonotic parasite (Figures 3, 4, 5,6 and 7).

4- CONCLUSION

To detect Genotypes of Toxoplasma gondii by PCR technique, it was noticed that most the isolates were of (Type II) and very lesser of type I .While the minority of the third type (Type III) was considered as an entero-zoonotic parasite and this case was reported in Iraq for the first time.

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تحديد جينات طفيليات المقوسات الكوندية من تطعيم الفئران كسلالات عراقية

الخلاصة

في هذه الدراسة تم تلقيح (50) فأراً بطفيليات المقوسات الكوندية المأخوذ من السائل الأمينوتي المستخرج جزئياً من المشيمة في مستشفى الزهراء/ محافظة ديالى خلال الفترة من شباط إلى تشرين الأول 2024. أظهرت النتائج أن معدل انتشار داء المقوسات في الفئران الملقحة حسب الفئة العمرية 2 أسبوع كان 14 (28.0%)، 3 أسابيع 17 (34.0%)، ومعدل الانتشار 4 أسابيع، 5 أسابيع و6 أسابيع 4 (8.0%) على التوالي. كما أظهر توزيع داء المقوسات حسب الجنس أن معدل الانتشار عند الذكور كان 30 (60%) وعند الإناث 20 (40%). وكان متوسط ± الانحراف المعياري لـ IgM (0.97±0.22) وكان متوسط ± الانحراف المعياري لـ IgG (1.30±0.24). بلغ متوسط ± الانحراف المعياري لتركيز IgM في الذكور (0.89±0.29) وفي الإناث (1.09±0.29) مع تباين كبير (P=0.019). كما بلغ متوسط ± الانحراف المعياري لتركيز IgG في الذكور (1.40±0.31) وبلغ متوسط ± الانحراف المعياري لتركيز IgG في الإناث (1.20±0.32) مع تباين كبير (P=0.024). بلغ متوسط IgM في الفئة العمرية 2 أسبوعاً 14 (1.00±0.25 B)، وفي الفئة العمرية 3 أسبوعاً17 (0.89±0.22 B)، وفي الفئة العمرية 4 أسابيع 9(0.94±0.18 B)، وفي الفئة العمرية 5 أسابيع 6(1.01±0.15 AB)، وفي الفئة العمرية (6) أسابيع (1.21±0.06 A)، مع وجود تباين معنوي (P=0.043). بلغ متوسط مستوى الأجسام المضادة لـ IgG في الفئة العمرية (2-14) أسبوعًا (1.22±0.23 B)، وفي الفئة العمرية (3-17) أسبوعًا (1.27±0.28 B)، وفي الفئة العمرية (4-9) أسابيع (1.31±0.14 AB)، وفي الفئة العمرية (5-6) أسابيع (1.39±0.25 AB) وفي الفئة العمرية (6) أسابيع 4 (1.39±0.25 AB) مع تباين كبير (P=0.043). تم تحديد المستنسخ (النوع الثاني) في 48 (93.3٪)، وتم تحديد النوع الأول في 9 (15.25٪)، بينما تم تحديد النوع الثالث في 2 (3.38٪)، وتعتبر هذه النتائج طفيليات معوية حيوانية المنشأ وهذه الحالة سجلت لأول مرة في العراق.