Using of Serological and Molecular Technique for Toxoplasma Gondii Diagnosis in Goat and Cow in Some Regions of Baghdad Governorate

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Abstract
Toxoplasmosis is aworldwidezoonosis common disease, caused by Toxoplasma gondii. Goats and cows are important sources of infection for humans,which caused public health problems in many countriesdue to reproductive and economic result from abortion and neonatal mortality in goats, so the aim of this study was to conduct a Seroprevalence of Toxoplasmosis in Baghdad governorate to determine the incidence of infection and link it to risk factors. Serum samples were collected randomly from 50cows and 104 goats, from different areas of northeast of Baghdad in Iraq and T. gondiiantibodiestiter were detected by ELISA technique using kit from ID vet company (French origin) in Central Veterinary Laboratory. The results of the serological examination showed that the infection rate in cows was 18% andin goats 60% .There was clear effect for gender, age, food and water source, and region on the infection rate.The results of the molecular examination, when it was conducted on samples positive for the ELISA test, showed that the rate of infection incows was 33.33% (3/9)and ingoats 45% (18/40) positivefor PCR technique, by targeting a specific part of the B1 gene.
Keywords: T.gondii,Goats, Cows , ELISA, PCR,Risk factors, Baghdad.

1. Introduction

Toxoplasmosis is a commonParasitic disease. The disease isworldwide and widespread caused by asingle-celled species protozoa called Toxoplasma gondii [1], an obligate intracellular host, belonging to the phylum Apicomplexa Class Sprozoasida [2].The disease was first discovered in Gondi Rats in Tunisia in 1908.Toxoplasma infects humans and many warm-blooded animals such as cows, buffaloes, goats, birds and others, which is the intermediate host. The final host is the domestic and wild Felidae family, which is the main source of the spread of the disease in clinical and subclinical forms.

T. gondii has three different phases that cause infection ,first the fast-reproducing (vegetative) phase called the tachyzoite phase .Second the slow-reproducing phase is called the Bradyzoites, which are located inside the tissue sacs .The last is sporozoite phase which is located inside the oocyst sacs [3].T. gondii has a sexual cycle in Felidae and a two-stage asexual cycle in all other warm blooded animals. Domestic cats and other felids in which sexual and asexual reproduction occurs, while all warm-blooded organisms in which the parasite reproduces only asexually [4]

ion of Toxoplasmato farm animals by Ingestion undercooked meatcontaining Toxoplasma cysts, and water contaminated with soil containing infected cat feces [5]. or congenitaltransmission throughplacenta from mother to fetus [6].

Sheep, cows and goats in Iraq are the main source of meat and milk and play an important role in the Iraqi agriculture economy and the income of breeders. Toxoplasmosis Infection of farm animals(intermediate host) during pregnancy leads to great economic losses represented by abortion, death of newborns and congenital malformations [7], The disease also leads to milk drying and temporary or permanent sterility [8] in addition to the risk of animal-to-human transmission through eating undercooked meat which is the main sources of human infection with Toxoplasma gondii [9], drinking unpasteurized milk, or directly handling raw meat [10].

Because of the great threat of the disease onpublic health, this study was which aimed to:

Investigate the infection of Toxoplasmosis inGoats andCows herdsfrom different areas of northeast of Baghdadgovernorate in Iraq using the enzyme-linked immunosorbent assay(ELISA) technique to measure the levels of IgG antibodies and study some factors likegender, age, food ,water source, and region on the infection rate.

Molecular detection of the Toxoplasma parasite in the blood samples of the animals under studyusing the Polymerase Chain Reaction (PCR) technique, by targeting a specific part of the B1 gene.

2. Materials and Methods

2-1. Study Area

The current study included the areas located in the north-east of Baghdad governorate, namely Al-Jaza'ir, Al-Tha'alba, Bob Al-Sham, Al-Maskar, Al-Zohour (Al-Hussainiya), Kazem Al-Hassan Village, Hajj Darwish Village, Al-Saridat, Saad Talib, Al-Bayariq, Al-Boudali, Al-Jala'a, Al-Intisar, Badran, Hadlan The ceremony, the endowment, the village of Ali Al-Fayyad, Zubair Jaata, Al-Nour, Abdullah Al-Fayyad, the village of Sheikh Deraa.

doi.org/10.31838/hiv22.02.280 Received: 28.04.22, Revised: 29.05.22, Accepted: 18.08.22

2.2. Sample Collection

The samples of the current study were collected from the beginning of November 2020 until April 2021 and included 154blood samples, 50 cows and104 goats,labeled with information about each animal such as sex, age, water source, type of nutrition , region and presence of cats was recorded in a form designed for this purpose as shown .

Blood was drawn from the jugular vein at a rate of 10 ml using disposable syringes, 3 ml of blood was placed into plastic tubes containing the anticoagulant Ethylene Diamine Tetra Actic (EDTA Tube). The tubes were kept at -20°C until they were used for DNA extraction for PCR technique. The remaining part of the blood was placed into plastic tubes without anticoagulant for ELISA technique and left for an hour at room temperature 21 °C and then centrifuged at 4000 rpm for 10 minutes to get serum which preserved in eppendrof tubes and kept at -20 °C until the testwascarried out.

2.3. Serological Test (ELISA).

We used indirect ELISA technique multispecies diagnostic kit from IDvet company (France origin). The reagents and the procedure of ELISA technique were performed on the instructions' of the kit at Serology unit - Department of Veterinary Laboratories and Research –Veterinary directorate Baghdad -Al-Nahda .

2-4.Polymerasechain reaction (PCR)

2-4-1. DNA Extraction

Genomic DNA was extracted from blood samples using a commercially available kit, QIAamp DNA, blood kit (Promega, USA). DNA extraction and purification protocol was recommended by the manufacturer.

Amplification was conducted in a total volume of 25 µl. The reaction mixture contained 12.5 ul Master mix (Go Taq ® G2 Green master mix ; USA). The reaction mixture contained 1 ul of each forward and reverse primers, 3ul DNA template, and 7.5 ul Nuclease free Water to a total volume of 25 ul.

2-4-2.The primers used in PCR Reaction

The polymerase chain reaction was carried out using specific primers, as a pair of pairs of primers for the B1 gene (399 bp) of the Toxoplasma parasite were used, which were adopted by Khlaty and Aiz (2015) and prepared by the Korean company Macrogen whose nucleotide sequences are shown in the table (1).

Table (1): T.gondii primers used in the study with their nucleotide sequence .

Primer	Sequence		product size
Toxoplasma gondii B1 gene	Forward	5-GAACCACCAAAAATCGGAGA¯3	399bp
	Reverse	GATCCTTTTGCACGGTTGTT¯53-	

2.4.3. PCR Program and Electrophoresis

The amplification was carried out in a thermal cycler (Ependrof, Germany) according to the following program: an initial denaturation step at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 30 sec, annealing, primer at 52°C for 30see, extension step at 72°C for 1min. and a final extension step at 72°C for 10 min. Amplified PCR products were the PCR products examined by electrophoresis in 1.5% agarose gel, stained with ethidium bromide solution, visualized under UV transilluminator and photographed.

3. Statistical Analysis

The statistical program System -SAS (2012) was used in data analysis to study the effect of different factors and the significant differences were compared with a Chi-Square- -χ2

4. Results and Discussion

3-1.Serological Test for Toxoplasmosis

ELISA technique results shown that were high significant differences at the level of significance P≤0.01 in the rates of toxoplasmosis infection in both cows and goats, which amounted to 18% and 60%, respectively as shown inTable (2). The high rate of infection in goatmay be due to nature of feeding .

Some studies dealt with the investigation of Toxoplasmosis in Cows using the ELISA test, Akbar et al. (2014) in Iraq recorded an infection rate of 14%, Gharekhani [11] in western Iran, an infection rate of 2.3%, and Tilahun et al. [5] in Ethiopia. 10.7%, while the incidence of Toxoplasmosis in cattle of Sudan, northern Punjab, Pakistan, Algeria and India was 89.3%, 19.75%, 28.7% and 61.5%, respectively [12-15].

As for goats, some local studies recorded infection with toxoplasmosis, as Altaie and Hashim et al. [16] recorded in Sulaymaniyah governorate, the rate of infection with toxoplasmosis reached 54%, and in Babylon governorate it amounted to 19.79% [17] as well as the study of Al Hamada et al. 2019) in Dohuk, where the infection rate was 36.1%. while the rates of Toxoplasmosis infection in goats from northern Pakistan, Ethiopia, Egypt, China, Algeria and Mongolia were42.8%, 27.6%, 39.2%, 42%, 28.7% and 32%, respectively [5, 14, 18-20].

The difference in the current study from local and international studies is due to many factors play an important role in infection like animal breed, sex, farm size, environmental and climatic conditions surrounding the animal, nature of feeding , management andpresence cats withfarm animals [21].

Table 2: The results of the ELISA test and the polymerase chain reaction test for animal species, positive for the ELISA test .

Enzyme Linked Immunosorbent Assay (ELISA)				Polymerase chain Reaction (PCR)		
Species	N	No. Positive	%	No. Positive (ELISA)	No. Positive (PCR)	%
Cows	50	9	18	9	3	33.3
Goats	104	62	60	40	18	45

Total	154	71	46	49	21	42.82
Chi-square		13.071**		4.372*		

3-2. Risk Factor

3-2-1. Six

The table(3,4) shows that females was higher than males with a high significant difference in cows with a significance level of P≤0.01, and in goats with a level of significance P≤ 0.05 .

The current research results are consistent with the results of a series of local and global studies, which show that women are more sensitive to Toxoplasma gondii infection than men. [5, 14, 22] .Femalesusceptibilityto Toxoplasma gondiidue toimmunosuppressionduring life cycle, such as pregnancy and lactation. [14].In addition, the difference between male and female hormone system may play an important role in determining the sensitivity of animals to Toxoplasma gondii infection, because estradiol and progesterone affect the immunogenicity of Toxoplasma gondii.There are anther factors may play an important role immunosuppression of female like sex hormones, nutrition, age [23, 24].

3-2-2. Age

The table (3,4) shows the infection rate of Toxoplasma gondii by age groups in light of the ELISA. The results showed that there is a clear effect of the age of the animals on the rates of the presence of the specific IgG antibody to Toxoplasma parasite, as it was observed that with increasing age the infection rate increased. In cows, the highest infection rate was recorded by 30% in the age group (4-7) years, while goats recorded the highest percentage 73% were injured in the age group (>5) years, and the statistical analysis showed the presence of high significant differences at the level of significance P≤0.01.

The current research results are different from the results of a series of studies involving age factors and their relationship with Toxoplasma gondii infection. Abdelbaset et al. [25] has the highest infection rate. 2.1 years old was 66.7%.Lashari et al. [24] recorded a minimum age infection rate of 38.88% (28-16 months), and the results of this study were consistent with Chikweto et al. [26]: in sheep, goats and goats Cows under the age of 5 had the highest infection rate, 83.3%, 100% and 18.2%, respectively. Lahmar et al. [27] showed that cows under the age of 3 had the highest infection rate, 70.84%. Abdallah et al. [14] showed the highest infection rate. The infection rates of cattle, sheep and goats over 5 years old were the highest, 52.43%, 31.1% and 31.48% respectively.

The incidence of toxoplasmosis increases with the age of animals, which may be due to the increase of oocysts exposed to the environment Andrade et al. [28]. In addition, the level of IgG antibody formed after the first infection The incidence of toxoplasmosis decreases with the age of animals, so it is possible to relapse [29].

3-2-3.Water Source

The table (3,4) showed that the highest percentage of infection with Toxoplasmosis was in cows, the highest percentage that depended on multiple water sources was 20%, while in Goats had the highest percentage of infection that depended on multiple water sources amounted to 70%, and the statistical analysis showed the presence of high significant differences at the level of significance P≤0.01.

The study of Al Hamada et al. (2019) in northern Iraq recorded similar results with the results of the current study, as the rate of infection with Toxoplasmosis in goats that take from the river as a source of drinking reached 71%, while it differed with the study of Tegegne et al. [30] in Ethiopia, where it reached the highest infection rate In ruminants that depend on the tap, it reached 40.35%, followed by the animals that depend on the river as drinking water with a rate of 33.33% and the study of Tilahun et al. [5], where the highest infection rate in ruminants that depend on tap water reached 54%, followed by those animals that depend on multiple sources with a rate of 34 As for the study of Abdallah et al. [14] in Algeria, the highest infection rate was in the herds that depend on well water by 30%.

This difference in the sources of drinking herds as a result of the geographical difference and environmental conditions between countries or regions within the same country [31], may represent the water contaminated with Oocyst egg sacs of the Toxoplasma conidial parasite by cats in barns or stray cats in farms and residential areas Cenci-Goga et al. [32] the source of infection, which explains the high rates of infection in herds that depend on river water and multiple sources [33]. In addition, the bodies of dogs contaminated with egg sacs may play a role in spreading the parasite to streams, ponds and sewage water, many of which take them as swimming places or as a source of drinking [34].

3-2-4.Food source

The table (3,4) shows the distribution of the types of animals under study and the rate of infection with Toxoplasma gondii according to the type of food source in light of the results of the ELISA test. The results showed that the highest rate of infection with Toxoplasmosis was in goats that use waste as a food source, reaching 84.61, while in cows, the highest infection rate was in those that take from green pastures as a food source, amounting to 28.57%. The statistical analysis showed that there was a significant significant difference at the level of significance P≤0.01.

The high rates of infection in goats can be explained , as many breeders resort to grazing in landfills and damaged foods that cats shelter in, which contributes to the permanence and continuity of the parasite's life cycle, as cats are an important factor in the epidemiology of toxoplasmosis [35] As for the reason for the high rate of infection in cows that take from green pastures as places for grazing and a source of food, as rain and others spread eggs and thus pollution includes large areas of farms [36], at the same time vegetation cover (weeds) provides the appropriate conditions for the survival of egg bags For a

longer period, the more likely farm animals will be infected [37].

3-2-5. Presence of Cat

The results showed a clear effect of cats on the rates of IgG antibody presence, as it was noted that the rates of infection in animals that have cats in their barns and grazing places are higher compared to animals whose barns are devoid of cats. The incidence of infection in sheep that are near cats was 60% compared to 35% without cats, in cows it was 23% versus 0%, and in goats 68% versus 35.71%, and the statistical analysis showed high significant differences at the level of significance P≤0.01.

The results of a group of local and international studies agreed with the results of the current study, which indicated that the presence of cats is an effective contributor to the spread of Toxoplasmosis in sheep, cattle and goats [5, 25, 38, 39].

The high incidence of toxoplasmosis in animals and herds with close contact with cats leads to the fact that the presence of cats is a very important factor in the epidemiology of toxoplasmosis, as cats throw a large number of egg sacks into the environment, which leads to the pollution of large areas of fields, farms and animal pens Which can be transmitted to animals when they are fed on herbs and drinking water [11], in addition, their easy access to irrigation ponds, streams and places for storing ruminant feed [5].

3-2-6.Region

The table(3,4) shows: the distribution of cows and goats and their percentages according to the geographical areas under study in the light of the results of the ELISA test. . In Cow, Albu Dali region had the highest infection rate of Toxoplasma gondii, which amounted to 40%, while the village of Abdullah Al-Fayyad recorded the lowest infection rate, which amounted to 0%. In goats, Al-Zohour District (Al-Husseiniya) occupied the highest rate of infection with Toxoplasmosis, reaching 78%, while the lowest infection rate was in the village of Sheikh Zubeir Jaata, which amounted to 0%.

The difference in the incidence of Toxoplasmosis in cattle between regions may be attributed to the difference in environmental and health conditions, the method of breeding, the density of cats, the age of the animal, and the host's immunity [11]. To the presence of cats and rats, the area has an urban, rural character suitable for the presence of cats that are easy to access to grazing areas, animal pens and fodder stores, in addition to the presence of puncture channels that may be a haven for rodents and ratsAs for the decrease in infection in the village of Abdullah Al-Fayyad, it may be due to the lack of cats and the availability of healthy environmental conditions for animals.

To get rid of aborted fetuses and other pollutants [40]. As for the reason for the low incidence of infection in the Zubair Jaata area, it may be attributed to the good health conditions and the lack of cats in breeding fields and pastures because the area is agricultural with dense orchards, and this is consistent with what Bisson et al. [41] found, which found that goats raised in residential (urban) areas are more susceptible to infection. Of the goats that are raised in agricultural (rural) areas and the reason is that the final host (cats) and rodents increase in density in urban areas due to the availability of appropriate conditions, and thus the rate of infection of intermediate hosts increases .

5. Molecular Diagnosis

The results of the conventional polymerase chain reaction (PCR) test used to detect a specific part of the B1 gene of Toxoplasma parasite (399 bp) of the animal species under study were conducted on 71 positive samples for ELISA examination with the highest absorption of 9 cows, 40 goats, while cows recorded 33.33% and goats. 45% is positive for this test, and the statistical analysis showed a significant difference at the level of significance P≤0.05 (Fig.1).

The results of the current study agreed with the results of a number of studies that examined the DNA of Toxoplasma parasite, which confirmed the accuracy and sensitivity of the PCR test in diagnosing toxoplasmosis in the animals under study [42-46].

The causes of low molecular diagnostic rate were compared by ELISA. Conclusion the level of serum IgG antibody in patients with chronic hepatitis B is high, the number of nutrients and tachyzoites in blood is small, the level of serum IgG antibody is low, Another body tissue, such as the brain and eyes, is a saccular body [47].

Table 3: Risk factors analysis of T.gondii infection in goats.

Variables		Total N0.	No. of Positive	Percentage %	Chi-square	P-value
Sex	Male	22	11	50	4.947	P≤ 0.05
	Famale	82	51	62		
Age (years)	<1	11	4	36	10.185**	P≤ 0.01
	1-2	18	7	39		
	2-3	22	14	64		
	3-4	30	21	70		
	4-5	12	8	67		
	> 5	11	8	73		
Water Source	Tap	22	12	54.5	11.391**	P≤ 0.01
	River	21	7	33		
	Well	0	0	0		
	Mixed	62	43	70		
Food Source	Green pastures	20	4	20	13.68**	P≤ 0.01

	Refuse	39	33	84.61		
	Mixed	45	25	55.55		
Presence of cat	Yes	76	52	68	9.811**	P≤ 0.01
	N0	28	10	35.71		
Region	Al-Zohour	18	15	78	14.504**	P≤ 0.01
	Al-Intisar	10	7	70		
	Hajj Darwish	16	11	69		
	Kazem Al-Hassan	12	8	67		
	Al-Maskar	12	7	58		
	Al-Jaza'ir	12	6	50		
	Hadlan	9	4	44		
	Sheikh Deraa	9	4	44		
	Zubair Jaata	6	0	0		

Table 4: Risk factors analysis of *T.gondii* infection in Cow .

Variables		Total N0.	No. of Positive	Percentage %	Chi-square	P-value
Sex	Male	17	1	6	7.821**	P≤ 0.01
	Female	33	8	24		
Age (years)	< 2	17	1	6	8.772**	P≤ 0.01
	2-4	13	2	15		
	4-7	13	4	64		
	>7	7	2	73		
Water Source	Tap	26	4	15	7.407**	P≤ 0.01
	River	0	0	0		
	Well	0	0	0		
	Mixed	24	5	20		
Food Source	Green pastures	20	4	20	5.17*	P≤ 0.05
	Refuse	39	33	84.61		
	Mixed	45	25	55.55		
Presence of cat	Yes	39	9	23	9.811**	P≤ 0.01
	N0	11	0	0		
Region	Al-Boudali	5	2	40	11.752**	P≤ 0.01
	Almurasama	7	2	28		
	Al-Zohour	5	1	20		
	Saad Talib	6	1	17		
	Badran	7	1	14		
	Al-Saridat	8	1	12.5		
	Al-Intisar	8	1	12.5		
	Abdullah Al-Fayyad	4	0	0		

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