



Serological Study of *Toxoplasmosis* in Slaughtered Animals in Mousl, Iraq

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Abstract. A study was conducted to elucidate the presence of *T.gondii* antibodies in the serum of slaughtered cattle, sheep & goats in Mosul abattoir. The study was carried out during the period from October 2002 to March 2003. For serological tests, 300 serum samples were collected from (100) of each slaughtered cattle, sheep & goats. Serum samples collected from slaughtered goats showed the highest percentage of infection using latex agglutination test and was (54%), followed by sheep (49%), while the lowest percentage (23%) was detected in cattle serum. To determine antibodies in positive serum samples, a modified latex agglutination test was used. The highest percentage of IgG was found in sheep (87.8%) followed by cattle (86.96%) and goats (83.3%). However, the highest percentage of IgM was found in goats (16.7%) followed by cattle (13.04%) and sheep (12.2%). A trial for preparing ELISA test manually was conducted in the laboratory of the Microbiology Branch/ College of Veterinary Medicine, University of Mosul, through the isolation of the parasite (antigen) from aborted sheep fetuses. The positive latex agglutination test, were confirmed by ELISA. Of these serum samples, (93.33%) were positive.

Keywords: *Toxoplasmosis*, Slaughtered Animals, Iraq, Serological Study.

Introduction

Toxoplasmosis is one of the most common parasitic diseases between humans and animals and is widespread worldwide (Lunden et al., 2002; de Barros et al., 2022; Brito et al., 2002). The disease is caused by the parasite *Toxoplasma gondii*, which is endemic within the cells of the host (Assmar et al., 1999; González-Parra et al., 2023). Cats and species of the feline family are the obligatory final hosts in the life cycle of the parasite (Dubey, 2003; Ferguson, 2022; James, 1998). For the intermediate hosts, the parasite can infect different types of animals such as rodents, birds, fish, farm animals and wild animals, in addition to humans (Jungersen et al, 2001; Wilson & McAuley, 1999). Toxoplasmosis is one of the diseases that has great economic importance resulting from the high cost required to establish

control and treatment strategies (Kamus et al., 2023; Freyre et al., 1999;), as well as its negative impact on the production of sheep, goats and pigs. It is one of the causes of abortion and stillbirth when the first infection occurs. The disease is a major cause of abortion in sheep and goats in Uruguay, New Zealand, the United Kingdom and Australia (Dubey, 2003; Ahaduzzaman & Hasan, 2022).

Congenital infection occurs in toxoplasmosis as a result of the initial infection with the disease, and the transmission of the infection from mother to fetus increases by (20-70%) with advancing gestational age (Wallon et al, 1990). The infection of the mother in the first three months of pregnancy leads to abortion, stillbirth, or the occurrence of congenital malformations in the fetus (Cisak, 1997). However, if the infection

occurred in the middle months of pregnancy, it leads to the occurrence of congenital malformations and the emergence of symptoms of jaundice, enlargement of the liver and spleen, and the predominant form is infection of the organ (Jungersen et al., 2001). The central nervous system of the fetus suffers from congenital mental retardation, vision loss, hearing loss, hydrocephalus, encephalitis, lymphadenopathy, and myocarditis (Dubey, 2003). However, the infection during the last three months of pregnancy is not accompanied by early clinical symptoms of the fetus, but it develops with the advancing age of the child, leading to choroidal retinitis and then infection with ocular toxoplasmosis and neurological diseases (Bowie et al., 1997).

Materials and Methods

1- Blood sampling

Blood samples were collected from sheep, goats, and cattle slaughtered in the Mosul abattoir. For serological tests, (100) blood samples were taken from sheep, (100) blood samples from goats, and (100) blood samples from cattle. The samples were brought to the laboratory of the Microbiology Branch / College of Veterinary Medicine, University of Mosul. Blood samples were collected from slaughtered animals at a volume of (5-10) ml per head in sterile test tubes and left for (1-2) hours at room temperature in order to obtain blood coagulation. The serum was separated using a centrifuge at a speed of (3000) revolutions / min for (10) minutes to sediment the blood cells and withdraw the serum by means of a Pasteur pipette, then, place it in small and sterile plastic test tubes. After labeling, they were kept in freezer at (-20 C°) until the necessary tests were performed.

1. Serological testes

a. Latex agglutination test (LAT)

The examination was carried out using a commercial kit called Toxocell-latex, produced by Bio kit (Spain). It is a qualitative and quantitative test to detect the antibodies present in the serum.

b. Modified latex agglutination test (2-ME)

The compound 2-ME has a chemical composition 2-Hydroxy ethylmercaptan, B-Mercaptoethanol. This compound is considered as a reducing agent for the disulfide bonds that binds the five units of the immunoglobulin IgM and then works to break

it down. Thus, when added to the serum, the IgM will be destroyed and the IgG will remain. As a result, the type of infection can be diagnosed (Desmonts & Remington, 1980). The compound was prepared in molarity (0.2) by taking (0.14) ml of it with a sterile pipette and completing the volume to (10) using a phosphate buffer solution with an acidity of (7.2) (Pumice 2001, Al-Dulaimi 2002). The test was completed by adding (0.1) ml of 2-ME solution to (0.1) ml of serum for each sample and placed in a clean and sterile test tube. The mixture was incubated at a temperature of (37°C°) for one hour and the quantitative examination was repeated.

c. ELISA assay

- Antigen preparation:

- 1- The tissue sacs isolated from the embryos were injected into the peritoneal cavity of (50) mice. Three days after the injection, the peritoneal exudate was withdrawn from the mice after injecting (2) ml of buffered phosphate at a concentration of (0.1) M, with a pH of (7.2).
- 2- The peritoneal aspirate, which was approximately (1) ml/mouse, was placed in a centrifuge at a speed of (2500) rpm for (10) minutes.
- 3- The filtrate was removed and the precipitate was suspended in (2) ml of buffer phosphate buffer, then passed through a medical syringe measuring (28) several times in order to destroy the phagocytes.
- 4- Expel the suspension in a refrigerated centrifuge at (4 o) m at a speed of (1350) rpm for (10) minutes.
- 5- The filtrate was centrifuged at a speed of (2500) rpm for 10 minutes at a temperature of (4 o) C, then the precipitate was washed in (50) ml of a buffer phosphate regulator three times.
- 6- The precipitate was suspended in (5) ml of buffer phosphate buffer and placed in an Ultra Sonic device (English MSE type) for (2) minutes at (4 o) C to obtain parasite antigen.
- 7- Re-centrifuge with a cooled centrifuge at (4 o) C for (2) hours at a speed of (2500) rpm (Fachado et al., 1997).
- 8- The antigen was lyophilized using a lyophilizer (Edwards, England) and the weight of the final antigen was (370) micrograms.

- Preparation of highly immunizing serum (positive control):

It was prepared according to the method of (Fachado et al.,1997) by taking (0.25) ml of (*Toxoplasma gondii* parasite antigen (1.3 mg / ml of protein) prepared as previously mentioned, and an equal volume of buffer phosphate was added to it. This diluted antigen was mixed with an equal volume of From Frond's Complete Assistant (DIFICO LABORATORIES Detroit Michigan USA) for a subcutaneous injection in a rabbit weighing (1.5) kg, the rabbit was injected four times, once a week for a month, and after (7) days had passed since the last injection, the blood was drawn from the rabbit and the serum was separated And kept under freezing (-20°C).

-Negative control:

Any serum free of specific antibodies against *Toxoplasma gondii*

Absorption	Negative control
0.055	1
0.060	2
0.062	3
0.177	Total

The mean of the negative control = the sum of the three readings / 3 = 0.177 / 3 = 0.059

Cut -off= mean of the negative control + 0.3

0.3+0.059 =

0.359 =

More reads than cut -off+

readings less than cut-off-

Readings equal to cut-off are repeated

Results

Three hundred serotypes of field animals slaughtered in the Mosul abattoir, including (100) sheep, (100) goats, and (100) cows, were examined to detect specialized antibodies against the *Toxoplasma gondii* parasite using the latex agglutination test (LAT) (Figure 1), and determine the type of antibodies (Figure 2) showed the number of cases that possess the (IgM) antibody in sheep slaughtered using the MLAT test is (6) cases, i.e. (12.2%), while the number of cases that possess the antibody (IgG) is (43) cases, i.e. (87.8%). At the same time, the number of cases that possess the (IgM) antibody in goats slaughtered using the MLAT test is (9) cases, i.e. (16.7%), while the number of cases that possess the (IgG) antibody is (45) cases (%83.3). Although the number of cases that possess the antibody (IgM) in cows slaughtered using the MLAT test is (3) cases, i.e. (13.04%), At the same time the

number of cases that possess the antibody (IgG) is (20) cases, i.e. a percentage.)%86.96(

We notice from Figure (1) when comparing the percentages of seropositive cases for the types of animals examined, that the highest percentage was in goats and formed (54%), followed by sheep (49%), then cows (23%).

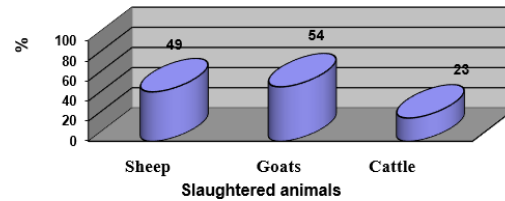


Figure 1. The percentage of seropositive cases in slaughtered animals in Mosul abattoir using LAT test.

When comparing the types of antibodies present for the types of animals examined, it was found that the antibody (IgG) is dominant, as shown in Figure (2), where the percentage of the presence of antibody (IgG) in sheep was (87.8%), followed by cows with a percentage of (86.96%), then goats with a percentage of (%83.3). As for the antibody (IgM), the highest percentage was found in goats, reaching (16.7%), followed by cows (13.04%), then sheep (12.2%).

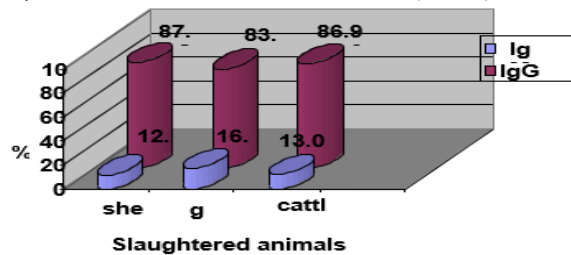


Figure 2. The percentage of IgG and IgM in slaughtered animals' serum in Mosul abattoir using MLAT test.

30 (serotypes of sheep that were positive for the latex aggregate test were examined, and the number of positive cases was (28) cases, i.e. (93.33%), as shown in Figure (3). This test was conducted to identify the accuracy of the latex aggregate test in Detection of antibodies to *Toxoplasma gondii*.

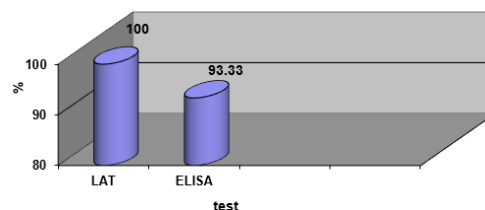


Figure 3. The percentage of positive cases LAT and ELISA.

Discussion

The meat of butchered animals, as indicated by many studies conducted in different regions of the world, is one of the important sources of transmission of infection with *T. gondii* to the consumer (Hill & Dubey, 2002). Our study was carried out to indicate that there are high infection rates in slaughtered animals in Nineveh Governorate. Where it turned out that the results of infection in slaughtered goats were higher than in sheep and cows through the percentage of positive cases using the LAT test, which amounted to (54%) as shown in figure (1). The highest positive cases were recorded at the criteria 128/1, 256/1, 1/512 and 1/1024, the least positive numbers were found at the standards 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64, but it must be emphasized that the presence of high levels in sera does not necessarily mean that the infection is severe, but the high levels can remain for several months (Jenum et al., 1997). This was reflected in the numbers and percentages of cases that carry the (IgM) and (IgG) antibody for goats slaughtered using MLAT, which are shown in figure (2). It reached (45) cases, constituting (83.3%). When making a comparison between the results we reached with those recorded in Iraq, it is noted that there is a great similarity to those recorded in the Baghdad abattoir, which amounted to (54.5%) (Rifaat & Jawdat, 1998).

But if our results were compared with neighboring countries and other countries of the world, the percentage was higher than what was recorded in the Jeddah abattoir, which amounted to (28%) (Amin & Morsy, 1997), and in Nigeria, which amounted to (4.5%) (Aganga et al., 1981). and in India (30.7%) (Chhabra et al., 1982).

What boost our results is that the goat is one of the most infected species of animals with *Toxoplasma gondii* (Dubey & Beatlie, 1988). Where it was recorded (Dubey & Livingston, 1986) that *Toxoplasma gondii* antibodies ranged between (21-50%) in the milking goats, and biological examinations of the tissues of infected goats naturally and experimentally showed that *Toxoplasma gondii* can remain in its tissues throughout the life of these animals. For example, it turned out that from Among (10) animals infected experimentally with *Toxoplasma gondii*, tissue cysts were observed in the thigh muscles of (10) of them, in the hearts of (6) of them, in the

diaphragm of (6) of them, and in the livers, kidneys and brains of (3) of them (Dubey, 1990a).

In addition, the reason for the high percentage of positive cases in goats may be due to the nature of the mountain breeding of goats. The high areas and the abundance of watery springs give nature higher humidity, which makes it a suitable atmosphere for sporulation of the egg oocyst (Plant et al., 1982).

It was also clear from the results of our study that the percentage of positive cases in slaughtered sheep in Nineveh Governorate amounted to (49%), as shown in figure (1), and that the highest numbers of positive cases were at the criteria 1/256, 1/512, and 1/1024, which reflects the presence of Cases of carrying antibody (IgM) using the MLAT test as shown in figure (2), where it reached (6) cases, which constituted (12.2%) of the total samples examined, while the number of cases carrying antibody (IgG) reached (43) cases, with a percentage of (87.8%), and this means that seropositive cases entered the slaughterhouses and their slaughtered without performing the required clinical examination before slaughter due to the difficulty of diagnosing these cases, which may not give distinct clinical symptoms that enable the veterinarian to isolate them.

When comparing our results with those recorded in Iraq, they were less than those recorded in the Dora and Shula abattoir, in which the percentage of positive cases in them using the IHAT test was (81.3%), while our results were higher than those recorded in the Baghdad abattoir using the IHAT and IFAT tests, which amounted to (26.2%).) and (18.2%), respectively (Rifaat & Jawdat, 1998), and if we noticed the results of examining slaughtered sheep in neighboring countries and other countries of the world, we would find that our results are higher than those reached by (Amin & Morsy, 1997) in Saudi Arabia, which amounted to (39%), and in Senegal (25.6%) (Vercruyssen, 1982), India (19.6%) (Chhabra et al., 1982), and Brazil (7.7%) (Silva & Langoni, 2001).

The reason for the high percentage of positive cases in the serums of slaughtered sheep in Nineveh Governorate may be due to the difference in the test used (Dubey et al, 1995), and to the difference in the surrounding environmental conditions. Also the animals are slaughtered directly upon their arrival to the slaughterhouse

without resting them in the shelters and not examining them before slaughter, in addition to the difficulty in diagnosing toxoplasmosis clinically in infected or carrier animals that are sent to the slaughterhouse, as well as the slaughtered of pregnant or aborted ewes in the slaughterhouse of the province, all of which would increase percentage of positive cases.

Our study confirms the high rates of positive cases in the sera of sheep, those studies were carried out by both Al-Maqdisi and Al-Samani in the Nineveh governorate. In fact, toxoplasmosis was a clear cause of abortions of ewes in the governorate, where Al-Maqdisi (2000) found that the percentage of positive cases in the serotypes of aborted ewes amounted to (% 48.16), and Al-Samani (2000) also found that the highest percentage in the sera of the examined ewes was in the aborted ewes, which amounted to (42.70%). As some serological surveys showed that the percentage of positive cases in the sera of sheep in general ranged between (4-100%) (Dubey, 1990a) and that (95%) of the aborted ewes were positive for *Toxoplasma gondii* (Dubey & Kirkbride, 1989). The reason may be due to the fact that the percentage of positive cases in sheep is less than it is in goats (Mumtaz *et al.*, 2022). Experiments show the percentage of tissue cysts of *Toxoplasma gondii* in the tissues of experimentally infected sheep.

As a result of the examination of the cattle sera, they showed little positive cases in comparison with sheep and goats. The percentage of positive cases was (23%), figer (1), and the highest positive numbers were at the standards 64/1, 128/1, and 512/1/. The number of cases that carry the (IgM) antibody was (3), which constituted (13.04%), while the cases that carried the (IgG) antibody amounted to (20) cases, which constituted .)%86.96(

When comparing our results, we find that they are similar to what was recorded in cattle slaughtered in Egypt, which amounted to (28.6%) (Rifaat *et al.*, 1978), in France (27.42%) (Dahan *et al.*, 1983), and in New Zealand (22%) (Knapen *et al.*, 1983). Higher than what was found in Czechoslovakia (14.7%) (Proseke *et al.*, 1980), and lower than what was found in Japan (33.9%) (Horio *et al.*, 2001), and in Spain (36%) (Garrido *et al.*, 1972), and in Germany (34%) (Janitschke *et al.*, 1967). The low percentage of positive numbers in the examined cows may

attributed to the fact that the oocyst of *Toxoplasma gondii* are often of moderate or weak infection. Furthermore, adult cows are often less susceptible to infection and they are not carriers of the parasite for a long time because of the rapid elimination of the parasite from their tissues (Radostitis *et al.*, 2000).

Results of latex test were confirmed by ELISA test. This was performed using (30) positive cases of the latex test. Among them, (28) samples were positive for the ELISA test, i.e. (93.33%). These results indicate that the latex test can be relied upon in conducting serological surveys to investigate *Toxoplasma gondii* antibodies for its ease of completion, its appropriate cost, and the lack of time and effort required to conduct it. These results are consistent with Pumice (2001), when comparing positive percentages in pregnant and non-pregnant women to detect *Toxoplasma gondii* antibodies using latex tests, ELISA and indirect antibody fluorescence. It was reported that the latex gave a rate of (86.6%), while the ELISA test gave a rate of (80%) and finally the indirect antibody fluorescence test, which recorded a rate of (70%). The results also agree with what the researchers reached (Rye *et al.*, 1996; Mazuwer *et al.* 1988), who indicated that the latex test is the best in conducting survey studies for the detection of antibodies to toxoplasmosis.

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