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A Molecular and Histological study of Turkey birds infected with Toxoplasmosis

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Abstract. *Toxoplasma gondii* parasite has three contagious stages: the oocytes, which is introduced into the external environment with the feces of the infected cats, which later form the spores. The current study depended on detection of *T. gondii* parasite used to Polymerase chain reaction , as well as the lack of time and effort required to perform them, the result of the test, 10 samples were selected for dissection and investigation of B1 (399bp) in their organs (liver, eye and intestine) using the normal polymerase chain reaction technique. There were (4) samples (40%) of the birds containing the tissues on the gene B1 (399bp) and recorded the highest incidence of the gene in the samples of the liver, where 30% (three samples) and one sample in the small intestine by 10% B1 in the eye samples, the results of the statistical analysis indicated that there were significant differences in the ratio of TWA D gene B1 in the tissues of the bird members under the level of probability of P≤0.05 , In the study of histopathological changes in *turkey birds* members, the presence of Lesions lesions associated with infection in both the liver and the small intestine was characterized by the presence of congestion in the blood vessels, as well as the expansion of the jibaniat with the loss of the engineering structure of the liver. In the tissue with a degenerative degeneration.

Key word: Toxoplasmosis, PCR , Al-Basrah.

Introduction

A parasite is a single-cell, microscopic or multicellular organism such as flatworms, capillaries, and nematodes that settle for some time of its life cycle. The body of a larger organism known as the host or host may be a human being or an animal that guarantees food, protection, and shelter. The relation between the parasite and the parasite is known as parasitism. This relationship involves two extremes, one of which is called parasite and the other is affected. This is called Host. This relationship does not depend on food and shelter, but rather for other purposes such as growth, sexual maturity, and reproduction (Hassan, 2016; Al-Abodi, 2018).

Toxoplasma gondii parasite has three contagious stages: the oocytes, which is introduced into the external environment with the feces of the infected cats, which later form the spores. Tachyzoite, which is rapidly divided into all the host and intermediate host cells, is sometimes surrounded by an

irregularly shaped bag The thin layer of the wall is known as the pseudocyst, and the slow-growing Bradyzoite, which proliferates slowly in a thick wall sac known as the tissue cyst, is formed within the different organs of the host's body (Al-abodi, 2017 ; Al-Ammash *et al*., 2018) . This sac varies in size and shape depending on age and The injury occurs as it has an elongated shape in the muscles and is circular or oval in the rest of the organs (Foulon *et al*., 1999).

As a result of the infection, the lesions of the lesions of the lesions are accompanied by an inflammatory reaction and Necrosis in the organs, leading to symptoms such as anemia, headache, fever, and pain in the muscles. The disease can cause complications that result in the destruction of cells in the various organs of the host's body such as the brain, Liver and heart, and sometimes the central nervous system. The disease may become chronic, accompanied by the rapid multiplication of fast-paced hippocampal tissues and the formation of tissue cysts containing the slow-growing molds that remain in the tissues for several years without any clinical symptoms (Simpson, 2002).

Materials and Methods

Collection of blood samples Collection of blood samples

80 Blood samples were collected from intravenous vesicles under the wing of birds by syringes of 5cc, the sample brought to a laboratory parasites in College of Veterinary/University of Al-Basrah, the serum extract by using centrifuge through 3000cycles/min at 5 minutes, then kept at -20 °C until the diagnostic parasite.

Diagnosis Methods Polymerase chain reaction (PCR) polymerase chain reaction

This test was performed using its own number and equipped by BIONEER. This test is based on extracting the DNA from the tissue samples and then multiplying the oxygen-deficient DNA by using the primers of the *T. gondii* and finally determining the multiplication results on the karose gel.

.1Extraction from tissue samples Extraction from tissue samples

After the dissection of the birds that were given positive results for the rapid test cassette, especially chronic cases of infection (IgG, IgG + IgM), pieces of liver tissue, eye and small intestine were divided into two parts, part placed in formalin solution at 10% For the purpose of fixation and study Histopathological changes, and the second part was used to extract the DNA from those tissues and according to the method described in the test kit processed by the company and explained as follows (here you cannot mention the steps below in detail and only in accordance with the method Are described in the test kit from the company processed

1- mg of tissue was taken and transferred to 1.5 ml Eppendorff tubes and, in the case of liver, size reduced to 10 mg.

2- The tissues placed in the tubes were crushed by micro pestle rods fitted with the kit

3-Add 200 microliters of GT solution to the contents of the tubes and continue the crushing process until the samples are homogenized.

4- .Add 20 µl proteinase k to the contents and mix with the Vortex rotor.

5. Incubate the tubes in a 60-mm water bath for 30 minutes until the samples dissolve and incubate the tubes every 5 minutes.

5- .Add 200 µl of the regulated GBT solution and mix the samples with rotor for 5 seconds. Then incubate the tubes at 60 ° C for 20 minutes. To confirm the analysis of the samples, turn the tubes in

the incubation every 5 minutes. At this time, Elution Buffer solution (200µl / sample)) And then incubate this solution at 60 m until it is used in step 18.

6- .Add 200 microliters of ethanol ethanol to the contents of the tubes and mix the contents by rotor for 10 seconds.

7- .Place the small tubes containing the Filter filter in the tubes collection 2 ml.

8- .Transfer the whole mixture, including the deposit to the small tubes containing the filter.

9- The tubes were inserted into the refrigerated centrifuge and expelled at speeds of 14,000-16,000 rpm for two minutes.

10- .The small tubes and the DNA container were transferred to other 2 ml tubes and the old tubes containing the leachate were discarded.

11- Then 400 microliters of controlled W1 solution were added to the small tubes.

12- .The tubes were centrifuged at a speed of 14,000-16,000 cycles / min for 30 seconds. The liquid was discharged into the tube and the small tubes containing the filter were returned to the old tubes.

13- .Add 600 microliters of the Wash Buffer solution added to the small tubes.

14 -The tubes were expelled at a speed of 14,000-16,000 cycles / min for 30 seconds and the liquid was discharged and the small tubes were returned to the old collection tubes.

15- .The tubes were then expelled at a speed of 14,000-16,000 rpm for 3 minutes to drain the filter tubes.

16- .The small dried tubes were transported to new, clean 1.5 ml Eppendorff tubes.

17- .Add 100-50 microliters of the Elution Buffer solution to the tubes containing the filter.

18- .Leave the tube stationary for at least 5 minutes to ensure that the Elution Buffer solution is absorbed by the filter.

19- .The tubes were then expelled at a speed of 14,000-16,000 cycles / min for 30 seconds to concentrate the DNA in the Eppendorff tube and then to store the DNA solution at -20 ° C until use.

After extraction from all samples, the polymerization chain was reacted using specialized primers. A pair of primers of the diagnostic gene B1 (399bp) of the conical curved parasite were used for conventional-PCR reaction. These primers were designed using (No.AF179871 1. NCB1 Gene-Bank Primer3 is equipped by BIONEER Company, Korea.

.**Method of testing the polymerase chain reaction**

Prepare the reaction mixture using the Accu Power-PCR Premix kit supplied by the Korean BBIZ Company.

1 .The test tubes were used to test the polymerase chain reaction (PCR Mix Tubes) and the container on polymerase chain reaction components and other components of the reaction mixture

2 After completing the preparation of the reaction mixture, close the tubes and carefully mix the Vortex carburetor for 5 seconds.

3 .Transfer all the PCR test tubes containing the reaction mixture and placed in the thermal cycler

4 .The sample is positive when the package (399bp) specialized in B1 of the T. rhondii has been shown for the polymerase chain reaction.study histopathological changes study

-Preparation of Alcroz Gel Preparation of Agarose- gel

Prepare the karose gel according to the method described by Sambrook et al. (1989) by dissolving 1.5 g of alkaloose powder in 100 ml of the TBE solution on a hot plate at 100 ° C and then adding the radioactive atomide bromide (0.3 µl / 100 ml)) After cooling the gel to a temperature of 50 m.

Next, pour the gel into the backing plate after the comb is installed 1 cm away from the front edge of the plate. Leave the gel to harden for 20 minutes, then carefully lift the comb leaving Wells in the gel where the DNA samples will be loaded.

Loading DNA samples of DNA Samples

DNA samples were extracted as follows:

The samples were removed from the thermal cycler. Take 10 microliters from each specimen. Carefully take each sample into the hole in the hardened alkaloise gel from hole 2, and hole 1 is loaded with 8 microliters of the Ladder solution and equipped with the test kit by Buster.,Immerse the karose gel completely with the TBE solution and then insert the Tray plate into the electrical relay cell.

Electrophoresis

Use the Power Supplier to equip the electric relay cell with 40 volts for 1-3 hours at room temperature. After complete use, use the UV-Transilluminator as a 256-nanometer UV wavelength to examine the locations of chromosome and plasmid DNA bands (Mary, 1998).

Histopathological changes were studied using the histological segments presented by the sectioning section described by Bancroft & Stevens (1982).

. **Preparation of histological slides**

A-Washing Washing
B- Anacaz & Thrilling Clearing & Dehydration
C-impregnation impregnation
D-Landfill Embedding
E-trimming Trimming
F-pigmentation and loading Staining & Mounting
.Statistical analysis

The data were analyzed using SPSS version 10.5 software, as mentioned in Niazi (2001).

Results and Discussion

Molecular detection using polymerase chain reaction technique

Polymerase chain reaction technique was used as a diagnostic method to confirm the results of the serological test represented by the rapid cassette test. This technique has a high sensitivity and specificity when used for the detection of conical arc parasites in different biogeographic samples (Ho-Yen, 1992).

Based on the results of the rapid cassette test, which was used to determine antibody antibodies in IgG (IgG + IgM), 10 samples were selected for dissection and investigation of B1 (399bp) in their organs (liver, eye and intestine) using the normal polymerase chain reaction

technique. There were (4) samples (40%) of the birds containing the tissues on the gene B1 (399bp) and recorded the highest incidence of the gene in the samples of the liver, where 30% (three samples) and one sample in the small intestine by 10% B1 in the eye samples, the results of the statistical analysis indicated that there were significant differences in the ratio of TWA D gene B1 in the tissues of the bird members under the level of probability of P≤0.05 as shown in Table 3.

The percentage recorded in bird ewe is higher than the percentage mentioned in (2012) in the local chicken liver and household bath and the peritoneal bath, which reached 19%, 28%, 20.6% and in the heart was 3%, 2%, 2% and in the brain was 4% ,% 2,% 3 on the sequence.

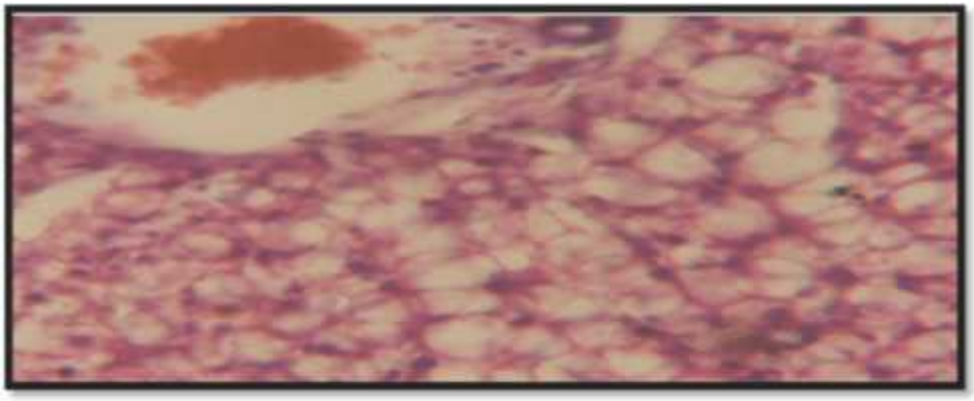
The reason for the high incidence of the liver is that the liver is considered the first candidate in which the blood is filtered and removed from the foreign components as well as being rich in nutrients (Gyton & Hull, 1995). The presence of parasitic arcoid parasites in domestic and wild poultry It is an important source of human infection with the parasite as a result of feeding on the meat of these birds, especially the livers that may be eaten by some semi-raw and may be due to non-presence of parasite in the tissues of the eye to the absence of parasite in that part of the tissue (30 mg) or perhaps Because the parasite did not reach those tissues This is consistent with what was pointed out (1995b) Dubey et al. In his study, which included the examination of 22 birds not having the DNA of parasitic arcoid parasite in the tissues of those birds using the interaction of polymerization chain normal.

Histopathological changes

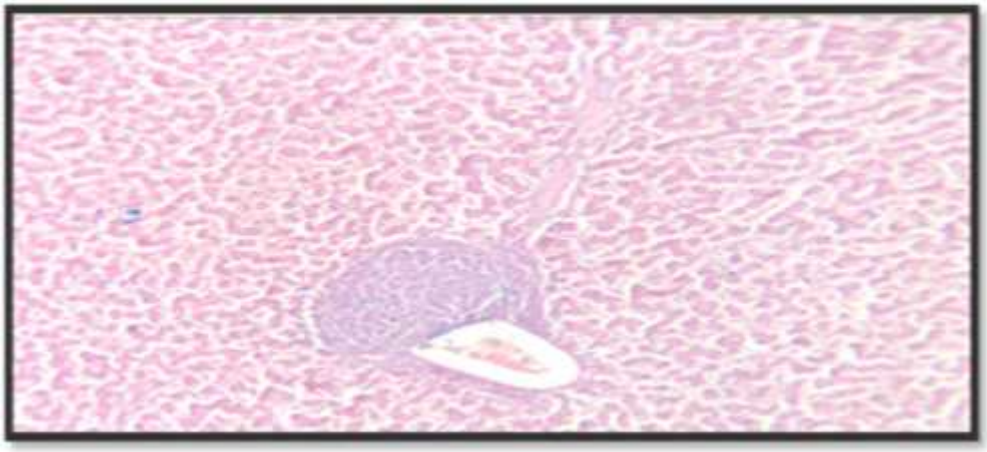
In the study of histopathological changes in the cyclic sphincter members, the presence of Lesions lesions associated with infection in both the liver and the small intestine was characterized by the presence of congestion in the blood vessels, as well as the expansion of the jibaniat with the loss of the engineering structure of the liver. In the tissue with a degenerative degeneration. It was also noticed that there was a tissue bag for the parasite located near the blood vessel. This is consistent with the findings of all of Al-Kanani et al. (2006) and Amin et al. (2012) and Alkhaled (2012) Hyperplasia of liver cel, It is believed that the reason for the emergence of congestion in the blood vessels is the ability of the colonic parasitic parasite to move and spread through the blood in addition to the ability to release some protein substances that stimulate the platelets and lining cells to the secretion of some chemical media, which helps to adhesion and aggregation of blood platelets and blood components on the walls Blood vessels also work to increase the vascular permeability and thus enter the tissue and stability in the organ (Burney et al., 1999).

While the histological changes in the small intestine were the presence of hyperplasia in the vesicles. This may be due to the infiltration mechanism of the parasite of the intestinal cells leading to apoptosis of the cells in order to get rid of the parasite in addition to the ability of these cells to regenerate quickly, Signs of regeneration in irregularly shaped cells are sizes and shapes represented by hyperplasia (Liesenfeld, 2002).

Figure (1): (11-14) columns represent the blood of pigeons samples birds cationic polymerization reaction of the normal chain, where B1 with a molecular weight of the private 399bp parasite *Toxoplasma gondii* in four samples of the gene appears, while valuable columns represent (1-10) negative samples of the reaction, the column represents M the Ladder with a molecular weight 100-1500bp.



Figure(2): A section in the fly liver infected with conical parasitic parasites is observed at 40X.H & E.



Figure(3): Clip in the infected bird it is noted that there is congestion of the blood vessel with a tissue cysts

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