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RESEARCH ARTICLE

Molecular identification of *Toxoplasma gondii* from domestic cats in Diwanya city.

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Abstract

The aim of this study was to determine the occurrence *Toxoplasma gondii* infection in domestic cats using Immunoassay Rapid IgM /IgG test and polymerase chain reaction (PCR). The blood samples were collected from 96 cats from Diwanya city. Then all samples were investigated by Immunoassay to distinguish of chronic and acute or reactivated infections *Toxoplasma* infection and only positive IgM or IgG cases were subjected to polymerase chain reaction. PCR was based on used specific primers that amplification of 529bp fragment in DNA of *Toxoplasma gondii*. The serological survey results were show 58.3% (56/96) of IgG seropositive as chronic infection and 39.9% (38/96) IgM seropositive as reactivated chronic infection. PCR technique results show detection of *Toxoplasma gondii* in positive at (24%) and (23/96) respectively. We conclude that these study demonstrate that the direct Polymerase Chain Reaction (PCR) assay techniques is a simple, rapid and valuable tool for the detection of *T. gondii* in domestic cats, and these animals may be play important role that increase the risk of transmission of disease to human.

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Introduction

Toxoplasmosis is most important parasitic zoonotic diseases of worldwide distribution that caused by the intracellular protozoa *Toxoplasma gondii* (1). The domestic cats have essential role in distribution of the disease due to the sexual reproduction; they excrete a large number of infective oocysts which became more resistance to environmental conditions (2). *Toxoplasma gondii* infects most mammals including humans and most birds which are consider as intermediate hosts, most vertebrates, the food animals are particularly important due to they are proposed for human consumption (3,4). The domestic cats which become infected with parasite when they ingested the encysted tissue from the intermediate hosts such as rodents and birds whereas, the human was acquired infection when they ingested the sporulated oocysts from contaminated water sources, the soil, and tissue cysts from raw or from undercooked meat, and via transplacenta (5). The infected cats it frequently appears as asymptomatic disease and it is very rare to development clear clinical signs. Toxoplasmosis in cats is reported high seroprevalences rats from (60 to 90%) (6,7). There are numerous diagnostic methods for detection *T. gondii* infection. The most widely used serological test for detection of toxoplasmosis is the Rapid immunoassay (IgM and IgG). In addition, the molecular test such as polymerase chain reaction (PCR) which allows to detection of parasite DNA, this test is

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highly specific and sensitive and very useful together with serological tests to differentiation of chronic, acute or reactivated toxoplasmosis infection (8,9). Therefore our study was designed to determine the prevalence of *Toxoplasma gondii* chronic, acute or reactivated infection in domestic cats using Immunoassay Rapid IgM /IgG test and polymerase chain reaction (PCR).

Materials and Methods:-

Stool and Blood samples collection:-

A96 blood samples from domestic cats were collected from formal vein after cleaning, shaving, and disinfected the vein area using sterile syringe. The cats were collection from different areas in Diwanya city of Iraq. The collected blood samples were kept in clean test tubes and anticoagulant tubes for serum collection and DNA extraction. The samples transported into the laboratory. Serum was separated by centrifugation at 3000rpm for 10 minutes and stored freezer at -20°C . As well as 10gm feces samples were taken from rectum of cats, and placed in sterile plastic containers and transferred into parasitology laboratory in college of Science. Al-Qadissya university for tested the samples.

Toxoplasma IgG/IgM Rapid Test:-

The Serum samples were examined using flow chromatographic immunoassay (OnSite Toxo IgG/IgM Rapid Test. CTK Biotech, Inc.USA) for the simultaneous detection and differentiation of IgG and IgM anti-*Toxoplasma gondii* (T. gondii) in cat serum samples. and done according to kit instructions.

Stool Flotation method:-

The flotation method were done by using sheather's solution, 5gm feces samples were mixed with small amounts (10ml) of D.W. Than feces mixture were filtered by using sieve (40 ange) to discarded from large particles. After that, filtrates were collected in sterile plastic tubes and placed in centrifuge at 1000 rpm for 3 minutes. The supernatant was discarded and small amount of sheather's solution was added into precipitate and mixed well by using wood sticks. After that placed in centrifuge at 1000 rpm for 2 minutes. Plastic tests tubes were place on holder and stand vertical and drops of sheather's solution were added by pipetter until fill the tubes. Than glass cover slide was placed on up end of tubes for 2-5 minutes. The glass cover slide was lifted carefully and placed under at (10X, 40X, and 100X) Magnification power under microscope to observed the *Toxoplasma gondii* oocyst (10).

Genomic DNA extraction:-

Genomic DNA was extracted from feces samples by using (Stool DNA extraction Kit, Bioneer. Korea). The extraction was done according to company instructions by using stool lysis protocol method with Proteinase K. After that, the extracted gDNA was checked by Nanodrop spectrophotometer, and then stored at -20C at refrigerator until used in PCR amplification.

Polymerase Chain reaction (PCR):-

PCR assay was performed for detection of *Toxoplasma gondii* by using specific primer that amplification of repetitive DNA sequence of *Toxoplasma gondii* according to method described by Homan et al. (2000) (11). The primers TOX4 (5-CGCTGCAGGGAGGAAGACGAAAGTTG-3') and TOX5 (5'-CGCTGCAGACACAGTGCATCTG GATT-3') these primers were flanked a 529bp fragment of T. gondii DNA. The primers were provided by (Bioneer company . Korea). Then PCR master mix was prepared by using (AccuPower[®] PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250 μM , Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl₂ 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20 μl total volume by added 5 μl of purified genomic DNA and 1.5 μl of 10pmole of forward primer and 1.5 μl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20 μl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (Mygene, Bioneer. Korea) by set up the following thermocycler conditions; using the following cycling conditions: 7min at 94°C for denaturation in cycle one, followed by 33 cycles on 1minute at 94°C for denaturation, 1minute at 55°C for annealing and 1minute at 72°C for extension, followed by a final extension of 10 minute at 72°C . The PCR products (529bp) were examined by electrophoresis in a 1% agarose gel, stained with ethidium bromide dye, and visualized under UV transilluminator.

Results and Discussion:-

The microscopic examination of flotation wet mount stool samples was appeared good identified *Toxoplasma* oocysts that show (11-13 μ m) in diameter in size and some of them sporulated and take oval to circle in shape as shown in figure (1). The *Toxoplasma* IgG/IgM Rapid Test results were show 58.3% (56/96) of IgG seropositive as chronic infection and 39.9% (38/96) IgM seropositive as reactivated chronic infection as show in figure (2). The PCR technique results shown detection of *Toxoplasma gondii* in positive at (24%) and (23/96) respectively. The PCR technique based repetitive DNA sequence for detection of *Toxoplasma gondii* were show good PCR amplification in extracted DNA from fecal samples as shown in figure (3).

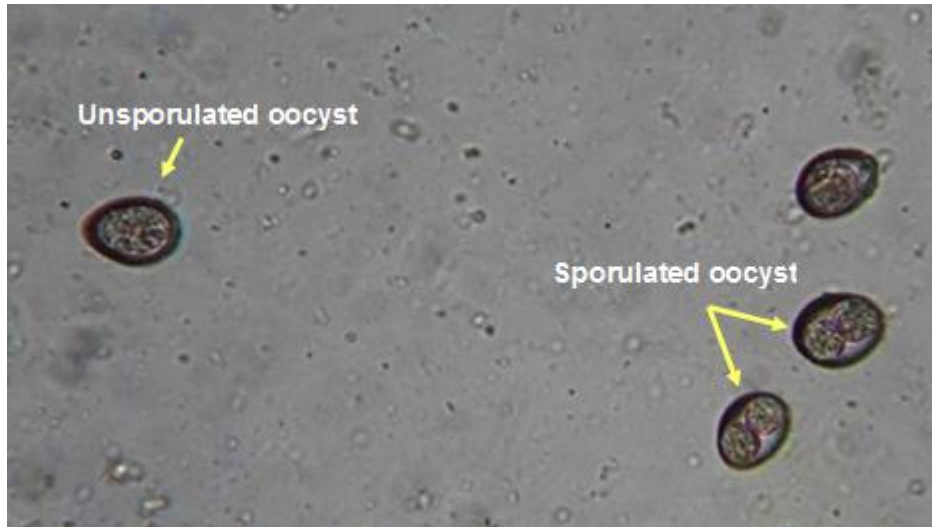


Figure (1): The flotation methods image using light microscope (400X power) from fresh cat fecal sample that show the *Toxoplasma gondii* sporulated oocyst and unsporulated oocyst.

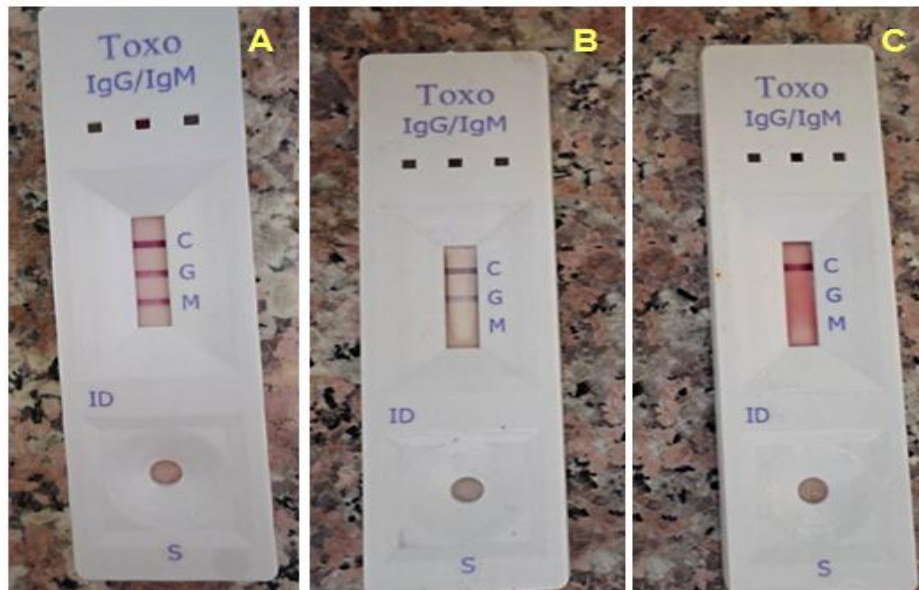


Figure (2): The *Toxoplasma* IgG/IgM Rapid Test results from fresh serum blood sample that show A: Toxo IgG and IgM positive reaction, B: Toxo IgG positive reaction, and C: negative reaction.

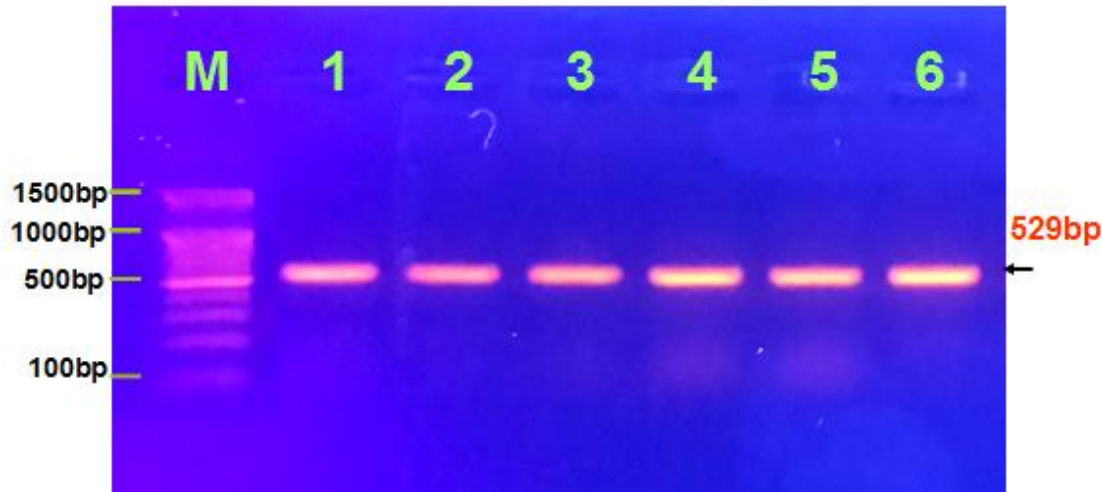


Figure (3): Agarose gel electrophoresis image that show the PCR product of repetitive DNA sequence of that using in detection *Toxoplasma gondii* in cat fecal samples. Where M: Marker (2000-100bp), lane (1-6) some positive samples at 529bp PCR product size.

The low prevalence of *Toxoplasma gondii* in cats were recorded by many studies by (12,13) who recording prevalence at 35.3% and 46.67% respectively. Other study were recorded high prevalence of *Toxoplasma gondii* in cats by (14,15) who recording prevalence at 66% and 86% respectively. The difference in *Toxoplasma gondii* infection prevalence by serological tests may be due to variation in environmental, geographical factors and living conditions for cats in general as well as the type of serological tests that used in detection *Toxoplasma gondii* antibodies. So the eggs of the *Toxoplasma gondii* it more likely to survive in hot and humid environments (16). The hot and humid climate in Iraq is not suitable for transmitted of *Toxoplasma* parasite, However, the transmission of parasite may be due to the food system of cats which includes hunting rodents and wild birds and dead animals (17). In epidemiological outbreak it is advisable to conduct serological studies for detection of antibodies against the *Toxoplasma gondii* parasite and estimation of the immune status for cats (18). The negative result of serum which refers that the cats have not yet exposed to the parasite *Toxoplasma gondii* remains susceptible in the future. According to the positive result of serum that cats already infected *Toxoplasma* parasite (IgG) or has been activated this infection at the present time (IgM) (19). The polymerase chain reaction test (PCR) is more sensitive than traditional detection tests (20) and the difference in the result of serological test and molecular test (PCR) that serum test indicates the presence of antibodies formed by cats as a result of parasite infection in the previous time or the current time. As molecular testing (PCR) Fidel on the presence of the parasite in the host himself (cats). This parasite is one of very important zoonotic pathogens between human and animal and the cats play an important role in spreading infection through contamination of food and water by feces containing the parasite eggs (21). In conclusion, our study was determine the prevalence of *Toxoplasma gondii* chronic, acute or reactivated infection in domestic cats using Immunoassay Rapid IgM /IgG test and polymerase chain reaction (PCR).

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