Serological and molecular detection of *Toxoplasma gondii* in *Columba livia* hunting pigeons of AL- Qadisiyah province

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Abstrsct

Toxoplasma gondii were detected and diagnosed in 80 *Columba livia* birds at the province of Al-Qadisiyah during the period started from January 2016 until June 2016 using latex test and rapid cassette test ,Moreover molecular diagnosis with Polymerase Chain Rraction technique for identification of B1 gene had been also used Results showed that antibodies were detected in 11 samples out of 80 (13.75%). of *Columba livia* pigeons in Al-Qadisiyah province, Since significant highest ($P \leq 0.05$) titration were indicated at 1/80 (36.37%),whereas the lowest titer indicated at 1/40 (9.09) and According to type of antibodies in *Columba livia* toxoplasmosis suspected samples using rapid test cassette, results were indicated that Six samples out of 80 (7.5%) were found positive. However IgG, IgM and IgG plus IgM were found in (33.34%),(16.66) and (50) respectively with no significance difference, furthermore results of PCR technique for detection of B1 gene revealed that 5% of samples from the *Columba livia* were only found positive ,Moreover the B1 gene were had a molecular weight of the private 399bp.It had been concluded that *Columba livia* birds were found infected with *T.gondii* with possible transmission to human being .

Keywords: *Columba livia*, serological tests, *Toxoplasma gondii*, PCR, Toxoplasmosis.

Introduction

Toxoplasmosis is one of the common diseases among animals ,However it considered as an important zoonotic one ,which caused by a parasite belong to Coccidia group, class Sporozoa known parasite *Toxoplasma gondii*, its an Intracellular obligate parasite and especially in nuclei of cells was also noted his presence in the nucleus (1).This parasite is characterized by the presence of three contagious phases and is developed oocyst that found at the external environment with the feces of infected cats, which contain inside spores and later developed to tachyzoite rapidly dividing inside both final and intermediate host cells and sometimes surrounded this phase objects cyst irregularly shaped thin wall, and bradyzoite phase, that breed slowly into the tissue cyst, which consists in the different organs of the host's body. This cyst different in size and shape depending on age and location of the infection, as it is circular or ovoid with a thick wall (2).The disease were transmitted to humans mainly by eating uncooked meat and the container on the bags of eggs in live tissue, food and water polluters bags eggs, in addition to blood transfusions are important in the transmission of the disease in the developed acute as well as the transfer of organs and tissues operations, which infected may be to healthy people who medications are given immunosuppressive drugs which plays an important role in the occurrence of disease (3). as well as the method of transmission from mother to offspring via placenta (2). The current studay came due to economic importance of the birds and the proximity and attach with a human and a private *Columba livia*, which operates as a carrier and medial to the parasite as well as cats, because of the high prevalence of toxoplasmosis in pregnant women in the mid-Euphrates region, which corroborated previous studies in the region in addition to the negative impact of the disease on the community because of the cost of treatment and checkups serological for pregnant women as well as for the loss in production rates and the infected children (4) current study aims to evaluated the incidence rates of *T. gondii* in *Columba livia* in the province of

Al-Qadisiyah to detect antibodies (Abs) Antibodies to the parasite in the birds sera using Latex Agglutination Test (LAT) and Rapid Test cassette (IVD) (IgG, IgM) as primary tests, and confirm the presence of the parasite *T. gonodii* using conventional Polymerase chain reaction technique (PCR) through the confirmation of the existence of the private B1 gene that the parasite in blood sample.

Material and Methods:

Collection of samples

Collection of bird specimens:

Eighty birds *C. livia* were collected during the period from different regions in Al-Qadisiyah province. The birds were brought up to the laboratory of the biology department in the college of science. Each birds was given a number and date of collection.

Collection of blood samples:

Blood samples were drained from a vein under the wing by vein puncture . samples were divided into two parts ,Plane part using for obtaining serum and EDTA added part using for PCR , samples were kept at -20c until used . (**5** and 6) .

Diagnostic procedures:

1- Latex agglutination test (Toxocell-latax , Spanish , Biokit) .

2- Rapid Test Cassette (Toxo-IgG / IgM) from(PLASMATEC) .

3--Polymerase Chain Reaction (PCR), this test was conducted and processed by BIONEER depending on primers used by (6) described in Table.1.:

Table(1): Single nucleotide sequence of the nitrogenous bases of the primers and the size of the output conventional PCR reaction

Primers	Sequence	Product size(bp)
Forward	5-GAACCACCAAAAATCGGAGA-3	399bp
Reverse	5-GATCCTTTTGCACGCACGGTTGTT-3	

This test occurs in three stages: extraction DNA oxygen deficient DNA from the samples and ensure the extraction of blood samples, amplification of DNA using a (Primers) own parasite *T. gondii* and determine the outcome of doubling the gel Alagaros (**7**).

Extraction DNA from blood samples:

DNA was extracted from blood samples by using AccuPrep ® Genomic DNA Extraction kit (Bioneer, Korea) and done according to protocol described by the manufacturer instructions.

Statistical Analysis

Data were analyzed using statistical program (SPSS version 10.5 software) and use at test X_{2}- Squre to determine the moral differences below the level of probability $P \leq 0.05$(8).

Results:

Results showed that antibodies were detected in 11 samples out of 80 (13.75%). of *Columba livia* pigeons in Al-Qadisiyah province, Since significant $P \leq 0.05$ highest titration were indicated at 1/80 (36.37%),whereas the lowest titer indicated at 1/40 (9.09).Table (2):

Table (2): Antibodies titer of of *T. gondii* in the sera of *Columba livia* using the latex test

samples		Total number	positive	Titration					
				1/20	1/40	1/80	1/160	1/320	1/640
				positive	positive	positive	positive	positive	positive
serum	NO.	80	11	2	1	4	2	2	0
	%		(13.75) a	(18.18) a	(9.09) a	(36.37) a	(18.18) a	(18.18) a	(0) a

According to the type of antibodies in *Columba livia toxoplasmosis* suspected samples using Rapid test cassette. Results were indicated that Six samples out of 80 (7.5%) were found positive, however IgG IgM and IgG plus IgM were found in (33.34%),(16.66) and (50) respectively, results indicated no significance difference .table (3):

Table. (3): Infection rate with *T.gondii* according to type of immunoglobuline using rapid test cassette.

samples		Total sample number	Positive Number (%)	Antibody Type		
				IgG	IgM	IgG + IgM
				Positive number (%)	Positive number (%)	Positive number (%)
Columba livia	NO.	80	6	2	1	3
	%		(7.5)	(33.34) a	(16.66) b	(50) c

Results of PCR technique for detection of B1 gene revealed that 5% of samples from the *Columba livia* were only found positive ,Moreover the B1 gene were had molecular weight of the private 399bp.Table (4) Fig (1).

Table. (4): PCR results for identification of B1 gene for *T. gondii* in the blood samples of *Columba livia*

Samples		Total sample number	Positive Number (%)	Negative Number (%)
EDTA blood	NO.	80	4	76
	%		(5 %)	(95 %)

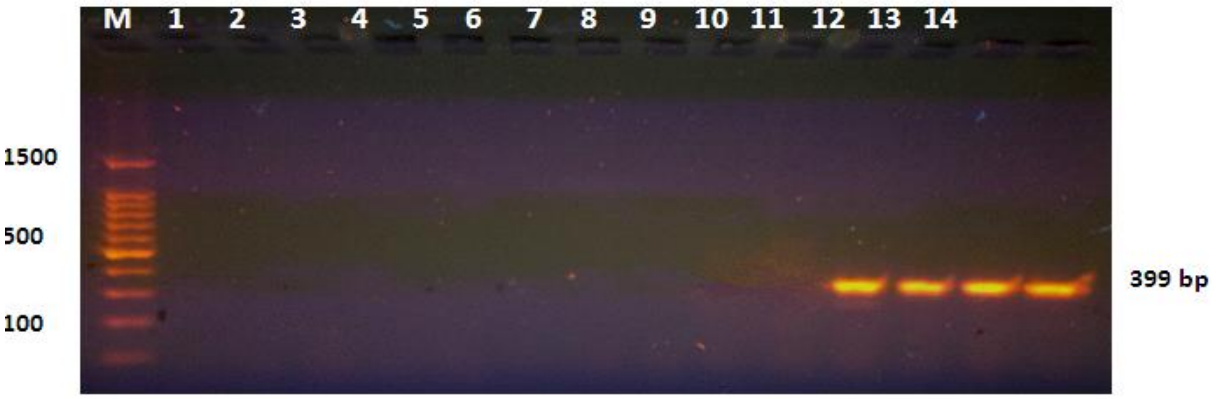

Fig.(1): (11-14) columns represent the blood of pigeons samples birds cationic polymerization reaction of the normal chain, where B1 with a molecular weight of the private 399bp parasite *Toxoplasma gondii* in four samples of the gene appears, while valuable columns represent (1-10) negative samples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

Discussion:

Birds are intermediate hosts that plays an important role in the epidemiology lot of parasites, especially *Toxoplasma gondii* parasite and transfer the infection to humans by eating raw or poorlycooked meat (9), and given the nature of feeding pigeons from contaminated soil bags eggs. *Toxoplasma gondii* that are resistance to various environmental conditions and survival for up to a year, leading to the injury of those birds (10), so The infection birds of *Toxoplasma gondii* parasite good indicator of soil pollution bags parasite eggs as a result of direct fed from the soil (11), birds and rodents infection may occure by eating food and water polluters bags eggs or contact with soil contaminated with feces of infected cats (12, 13). Serological and molecular diagnosis of *T.gondii* is too important simce the microscopic detect is difficult and might confused with other organisms especially *Sarcocystis spp* (14,15 and 16).The use of agglutination latex for diagnosis of *T. gondii* are common now a days, as it ease of use and lack of cost as well as lack of time and effort required for performing (17) and it appears the test result after 3-5 minutes and depends mainly on the efficiency and

accuracy of user skills in testing it also does not require expensive hardware, one of the tests used in epidemiological studies of toxoplasmosis in birds and in particular the local chickens, pigeons (18). Agglutination latex test and testing rapid cassette results is to check the birds samples birds explained the users that the proportion of injury parasite *Toxoplasma gondii* amounted to (11) 13.75% (11 appointed were positive), (6 were positive samples) 7.5% on the sequence, and this percentage is less than the percentage mentioned by (19) and higher than the percentage recorded using agglutination latex by both (20, 21 and 22) which amounted to 5.5%, 5%, 1.5%, respectively, as well as higher than the percentage registered by (22) using the test direct sparkle and which amounted to 0.83%. The incidence of infections recorded rates in the current study and previous studies is due to several factors, including the difference in the number of samples examined, differing geographical locations of those countries in addition to the difference in the ways of investigating the parasite *Toxoplasma gondii* sensitivity and specificity used for tests and this is in line with the sentiments (23), as most global studies rely on parasite diagnosis depending on *T. gondii* as a diagnostic preliminary to use clumping test for the advantage of doing this test in sensitivity and specificity in detecting toxoplasmosis in animals (24), in addition to the difference in the age of the birds that studies Previous and sex, as well as cats presence and the presence and vitality bags eggs in the study area and how the user stored in poultry feed may be an appropriate atmosphere for the growth of bacteria and fungi, which is one of the inhibitors immune and thus help to injury parasite *Toxoplasma gondii* (25, 26). while others attributed this difference researchers to environmental factors as the record (25) Higher infection rate incidence in the wet and warm areas compared with dry and cold areas that affect the vitality of the egg sacks in the environment, as well as the level of elevation above sea level and the size of breeding herds(27).The results using agglutination latex test, that the highest modular antibodies to the parasite *Toxoplasma gondii* in sera of birds when standard 1/80% (36.37) and the least at the standard 1/40 (9.09%).High concentrations of antibodies in the sera birds indicate to the presence of acute infection Acute infections of parasite *Toxoplasma gondii* demonstrates the pre-exposure to the parasite while concentrations of low-lying antibody indicate the presence of chronic injuries Chronic infections of parasite is consistent of with the sentiments (28), the reason for fluctuation happening in antibodies standards in sera birds may be due to the parasite *Toxoplasma gondii* which has the ability to stimulate the immune tribal Premonition in the bodies of hosts and by which the immune remain throughout the period of the parasite survival in their bodies until reaching the state of the immune balance, this is controlled by those hosts on the presence of the parasite and thus inhibit its effects pathological, but the event of any decline in the case of immune body for any reason it will lead to a revitalization of the bags textile inherent in the members of the host body and therefore torn and the emancipation the parasite which may react with pre-existing antibodies leading to a decrease in antibodies standards also lead to new stimulation of the immune system and thus the generation of antibodies and other increases antibodies standards (29).PCR technique was used to confirm the results of the serological tests, becaouse this technique high sensitivity and specificity replay when used to detect the parasite *Toxoplasma gondii* in various biological samples (30, 31).The results of using polymerase chain reaction to detect the private gene B1 (399bp private) parasite *T. gondii* in 80 blood samples from the *Columba livia* that the percentage of the presence of the gene B1 was 5% (4 samples were positive) and the proportion of the presence of the gene B1 recorded in the current study is less than the ratio mentioned by (32), amounting to 18.25% was attributed to the cause of the difference in the recorded rates in the current study and other studies, the difference in the number of samples tested and the laboratory conditions of uncontrolled is affecting on the polymerization chain reaction.

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