MOLECULAR DETECTION OF *Toxoplasma gondii* IN CHICKEN LICE (*Menacanthus stramineus*)

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Keywords: ectoparasites, *Toxoplasma gondii*, chickens.

ABSTRACT

The current study was carried out to demonstrate the ability of chicken lice *Menacanthus stramineus* to transmit *Toxoplasma gondii* by using PCR technique. The blood was collected from the jugular vein of 85 chickens infested with ectoparasites to investigate the existence of *Toxoplasma gondii* by latex agglutination test. The results showed that 42 (49.41%) of chicken were infected with lice, lice samples of *Menacanthus stramineus* were collected from chickens infected with *T.gondii* for PCR investigation to confirm the presence of *T.gondii* in lice tissues and the results were revealed the presence of *T. gondii B1* genein 18(42.85 %) samples.

INTRODUCTION

Poultry is a domesticated birds kept for the production of meat and eggs. This involves birds like chicken, goose, turkey, duck, pheasant, quail, guinea fowl and peafowl**.** Chickens are the most important poultry worldwide irrespective of civilization and religion because chicken products have very high nutritive values. Chickens are an economic and efficient supply of animal protein within the shortest possible time, playing a vital role in narrowing down the animal protein source gap (1,2). Like all other animals, backyard poultry too suffer from a wide range of diseases. In semi-intensive system, chickens are found to be infested with several species of ectoparasites including different species of lice, mites etc (3,4). Lice consider one of the biggest entirely parasitic insect order and their all members are obligate ectoparasites of mammals and birds. According to the last researches approximately 4500 which described louse species. Moreover, the majority of these

species (more than 85%) are infests birds. Based on classification, they have its place to the Exopterygota cluster of insects and are classified into 4 sub orders which including: Amblycera, Ischnocera, Rhynchophthirina, and Anoplura. The first divisions as Mallophaga (chewing lice) and Anoplura (sucking lice) have been no longer to be uses anymore such as Mallophaga turned out to be paraphyletic (5). The first hugest problems for lice is the mode of transition to a new host individual, as they are wingless, the spreading usually includes direct physical contact between host individuals (6). Toxoplasmosis is a zoonotic disease broadly distributed through and all over the globe. It is caused by the intracellular blood parasite called *Toxoplasma gondii*. On the other hand, the domestic cats and the entire cat family are consider the main host of the parasite, where in the parasite reproduces sexually. *T. gondii* can also infect a varied types of intermediate hosts, the most affecting are the warm-blooded animals (7). *T. gondii* is a parasite that is often distribution through the world, with a higher incidence in tropical regions and a decrease when the latitude upsurges (8). The felids play significant role in the spreading of *T. gondii* to humans and other animals, through excretion of environmentally resistant oocysts in their fece (9). Moreover, the characterization and identification of *T. gondii* has been done in several animal species including wild, companion and production animals (10). The first protocol for molecular detection of *T.gondii*, for standard PCR targeting *B1* sequence, was developed in 1989 and has since been changed and optimized in several laboratories (11, 12). The *B1* sequence, though of unknown perform, is wide exploited in a variety of diagnostic and epidemiologic studies due to its specificity and sensitivity (13) .The present study was conduct to prove the role of chewing lice in transmission of *Toxoplasma gondi* to chicken by polymerase chain reaction (PCR).

MATERIALS AND METHODS

A total of 85 Chickens infested with ectoparasites were collected from different areas in Basrah Governorate. Blood sample was withdrawn from the jugular vein (5 ml) of the infected chicken and placed in a vacuum tube (not contain anticoagulant). Then, the tubes was centrifuged at 3,000 rpm for 5 min to collect serum to investigate the presence of *T.gondii* using latex agglutination test.

Latex agglutination assay: Detection of *Toxoplasma gondii* in serum of the infected chicken was done using latex agglutination kit (Netherland, Saluce). Briefly, Forty microliter of the serum were distributed on the single circle of the test slide, this step was repeated for the positive and negative controls. Serum was then distributed over the entire test circle. The toxo latex reagent was shaken well and the reagent suck up with the needle of the kit. After that, one fall drop of the latex reagent was distributed on each test circle that containing sample. Finally, the mixture was mixed well for 4 min. The agglutination was then examined under 4x force of dissecting microscope.

Polymerase Chain Reaction (PCR) assay: Detection B1 gene of *Toxoplasma gondii* in lice tissue was done using PCR technique. The lice sample was prepared for DNA extraction by crushed twenty five milligrams of lice (*Menacanthus stramineus)* by using the mortar and pestle. The sample was then transferred in the eppendroff tubes (1.5 ml). The DNA extracted steps from the lice tissue was done according to the procedure of the AccuPrep ® Genomic DNA Extraction kit (Bioneer, Korea).

The PCR technique used to detect the *T. gondii B1* gene using specific foreword primer (5-GAACCACCAAAAATCGGAGA-3) and reverse primer (5-GATCCTTTTGCACGGTTGTT-3), which amplify (399bp) verified as positive for *B1*gene (14).The reaction of PCR was consisted of Master Mix(3 µl), forward primer (0.5µl), reverse primer (0.5µl), and DNA templates (10µl). The final volumes of the reaction were 20 µl. by adding nuclease-free water (6 µl) The PCR amplification method was accomplished by employing a Thermocycler (Esco, Singapore), with athletics conditions consisting of initial denaturation at 94°C for five min, followed by thirty cycles of amplification (denaturation at 94°C for thirty sec, tempering at 53°C for thirty seconds, extension at 72°C for forty five sec, and final elongation at 72°C for five min). The PCR products were analyzed using 2% of agarose gel electrophoresis. Molecular weight marker a 100 bp of DNA ladder (Bioneer, Korea) was used. The gel was stained with ethidium bromide and visualized under ultraviolet (UV) illumination (E-graph – ATTO/Japan).

RESULTS

A total of 85 sera of chickens infested with ectoparasites were tested by latex agglutination test for detection of *T.gondii.* The current study showed that 42 samples were positive and the rate of infection was 49.41% .DNA from 42 samples isolates from lice tissue were conducted for PCR assay to detect the presence of *B1* gene and the results of PCR amplification of *B1*gene of *T.gondii* from lice tissue revealed that 18 (42.85 %) isolates had clear band of approximately 399bp which corresponded to identification of *T.gondii* (Figure 1).

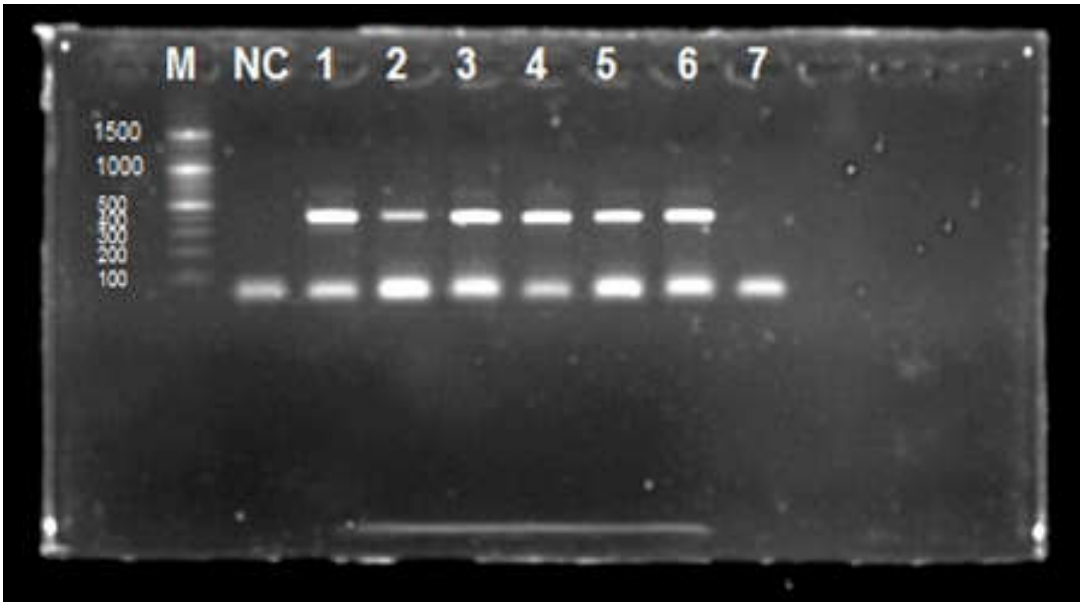

Figure 1 :PCR amplification results for *B1* gene of *T. gondii.* M: 100 bp of DNA Ladder. Lane 2:negative control. Lanes 3-6: positive PCR result for *B1* gene (399bp), lane 7 negative PCR result.

DISCUSSION

Ectoparasites are frequently found in all birds, where they nourish on their blood, feathers, skin, and scales. This is may cause a series of a symptoms, which including discomfort, irritation, loss of feathers, growth was stunted and the eggs production and hatchability were reduced egg, anemia, increased feed costs, elevated mortality, and susceptibility to different infections (15).

The result of latex Agglutination test for *T.gondii* was 49.41 % (42/85). This result was closed to result that obtained by (16) in Austria and (17) in Iran as they

reported seroprevalence of 40% and 43% respectively in chicken. On the other hand, (18) found that the high seroprevalence of *T. gondii* in chicken is 9%.While, (19) reported 81% in Nineveh in Broiler chicken. The management and hygienic standards in breeding, density of felines and different environmental conditions are effective factors on the gaining of *T .gondii* oocysts by animals (20). The rate of infection in free-ranging chicken is a crucial indicator of environmental contamination as a result of food habits (21, 22).

PCR technique was applied in the present study to detect the pathogenic protozoan *T.gondii* in tissue of lice (*Menacanthus stramineus*) and to confirm the result of latex agglutination test. Result of PCR technique for detection of *B1* gene (399bp) in 42 samples revealed only 18 samples were positive with a rate 42.85 %. This may confirms the ability of lice to transfer *T.gondii* from infested chicken to healthy chicken as a result of transmission between chicken and feed on their blood. It has been found that *Menacanthus stramineus* have the ability to transmit *T.gondii* (23). In addition, the ability of lice to transport the *T.gondii* among chicken leads to an increase in cases of infection with *T.gondii* (24,18). Previously, detection of *T.gondii* in lice tissue has been detected using *B1* gene with percentage 62.8 % in al-AL-Diwaniyah Governorate, Iraq (25).

B1 gene has a high specificity in *T. gondii* and has been repeated 35 times in its genome, so it is used as a target for amplification in polymerase chain reaction to detect parasites in clinical materials such as the blood and tissues (11). Additionally, the number and concentration of such organisms in a such sample play critical point in concentration of DNA that extract with the influence of the types and kit procedure used for purified the *T.gondii* from the ectoparasites. All these factors might be the reason for low percentage of infection in the present study.

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