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Molecular Characterization of *Toxoplasma gondii* from Bird Hosts

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Abstract

The determination of *Toxoplasma gondii* prevalence in a variety of domestic birds is thought as a good indicator of distribution of *T.gondii ooccyts* in the environment. To verify the potential role of domestic birds in circulating toxoplasmosis in Iran, the present study was conducted in Mazandaran Provinces, northern Iran. Latex agglutination test (LA) antibodies were found in 25 of 58 free-ranging chickens (*Gallus domesticus*) and ducks (*Anas* sp.). Also, viable *T. gondii* was isolated from 7 of 25 seropositive chickens and ducks by bioassay of their brains and hearts into mice. Most of the isolated strains were avirulant to mice. Genotyping of *T.gondii* isolates using Multiplex PCR for 5 microsatellite markers indicated that 7 isolates were type III. In this study type II and III isolates and mixed genotypes were not found. This study showed that domestic birds could have a potential role in transmitting toxoplasmosis to humans in Iran.

Keywords*:* Toxoplasma gondii*, Toxoplasmosis, Bird hosts,* Gallus domesticus*,* Anas *sp, Iran*

Introduction

Toxoplasma gondii causes toxoplasmosis in human and animals, a disease of cosmopolitan character (1). Infection in humans is probably established due to ingesting of tissue cysts found in raw or under cooked meat as. *T. gondii* has been recovered from a wide range of food animals including sheep, goats, pigs, rabbits, and domestic poultry (2-4). The determination of *T.gondii* prevalence in a variety of domestic birds is thought as a good indicator of distribution of *T.gondii ooccyts* in the environment (3). In domestic cycle of *T.gondii* (including cats, humans and meat producing animals, such as pigs, sheep and domestic fowls), there is a low diversity and *T.gondii* strains are classified into three genetic types (I, II, III) (5). The current study was to study the prevalence of *T. gondii* in different bird hosts used for food in Iran. In this study, genotypes of *T.gondii* strains from different domestic birds of Iran are reported for the first time.

Materials and Methods

Sample collection and parasites Forty-five samples of free-ranging chickens and 13 samples of ducks, purchased from different sources in Babol and Ghaem-Shahr cities in Mazandaran Province, northern part of Iran were bled and killed, then their hearts and brains were removed and transferred to laboratory under refrigeration conditions.

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Serologic evaluation *S*era were examined using the latex agglutination slide test (LA) (SGM Italia, Roma, Italy) as described earlier (6). The titer 1:8 or higher was considered as positive.

Bioassay in mice Isolation of *T. gondii* parasites from brains was carried out according to Beverley's method (7). Briefly, 20% suspension of samples from seropositive animals was inoculated intraperitoneally into mice. Brains and hearts of seropositive poultry samples were pooled. The samples were homogenized, and digested by acidic pepsin method as formerly illustrated (8). One ml of each digested sample was inoculated into 4-5 mice. Tissue imprints of dead mice were examined for *T. gondii* tachyzoites and/or tissue cysts. Survivors were bled on day 40 post-inoculation (PI), and 1:16 dilution of serum from each mouse was tested for *T. gondii* antibodies using immunofluorescence assay (IFAT). Mice were scarified 49 days PI and the brains examined for tissue cysts. The brain, spleen, liver and lung from serologically positive mice in which the parasite was not detected in direct smear, were subinoculated into another group of healthy mice.

Genotyping At first, *T.gondii* DNA was extracted from infected mouse tissues using the QIA amp DNA mini kit (QIA GENE, Courtaboeuf, France). Genotyping of domestic bird isolates was conducted with 5 microsatellite markers and the Multiplex PCR was used to determine the genetic type of *T.gondii* strains (9). Briefly, five pairs of primers were used for a multiplex assay. In each primer, one was 5-end labeled with fluorescent to allow sizing of PCR products with an automatic sequencer.

After amplification reaction, length polymorphism of microsatellite regions was assessed with an automatic sequencer and data analysis with Gene Scan software (Applied Biosystems).

Results

Antibodies to *Toxoplasma* in the serum of 25 out of 58 (43%) domestic birds of Mazandran Province were detected (Table 1). Viable *T. gondii* was isolated from 7 domestic fowls (Table 2). The latex agglutination titers of chickens are presented in Table 1.

Most of the strains isolated from birds were avirulent to mice (Table 2). *Toxoplasma* tissue cysts were not detected in direct microscopic examination of the samples prepared from the brains and hearts of birds examined. All of 7 isolates of *T.gondii* recovered from domestic fowls by mouse bioassay were type III (table 2). Type I, II or mixed genotypes were not found.

Table 1: Distribution of latex agglutination slide test antibodies to the *T. gondii* in chicken and ducks.

Animal	No. tested	Antibody titers						positive
		1:8	1:16	1:32	1:64	1:128	1:256	No (%)
Chicken	45	3	4	7	5	4	-	23 (51)
Ducks	13	1	-	1	-	-	-	2 (15.3)

Table 2: Isolation of *T. gondii* from tissues of chicken and ducks.

Animal No.	Antibody titer	Organ for Bioassay	No. mice Positive/ no. inoculated	No. dead	Day of death	Genotype by Microsatellite
Chicken						
103	1:128	Brain	2/4	0	Survived	III
140	1:32	Brain/heart	2/4	0	Survived	III
141	1:64	Brain/heart	1/5	1	3-4	III
157	1:64	Brain/heart	2/5	0	Survived	III
162	1:32	Brain/heart	1/4	0	Survived	III
164	1:8	Brain/heart	2/5	0	Survived	III
Duck						
38	1:32	Brain	1/4	0	Survived	III

Discussion

Toxoplasmosis encompasses a large variety of hosts including human, animals, birds and so on. One of the main sources of infecting humans, is bird meat, so besides another indicators to detect the distribution of *T.gondii* oocysts in the environment, the determination of *T.gondii* prevalence in domestic birds is of great importance (3). To determine and utilize this indicator in Iran, the present study was managed. Our findings demonstrated that anti-*Toxoplasma* antibodies were high in chickens in the studied area. It seems that chickens become infected mostly during feeding on the ground contaminated with oocysts. The humidity in northern Iran, allows the oocysts to live for a long period (10). The prevalence rate of anti-*Toxoplasma* antibody in chicken (43%) in present study was higher than that of Gharavi et al survey (30.3%), which was reported from chicken in this province (11). It is possible that the serological method, number of specimens and passing the time influenced this discrepancy. Our obtained data were in compliant with other studies undertaken in Egypt (3) and Brazil (12), where the rate of infection in free-ranging chickens was 40% and 39%, respectively. Of course, dissimilarity was seen with other studies conducted in America (13), India (14), Mexico (15).

Genetically, all the bird isolates in this study were type III, which is in compliant with animal isolates (5) and also to the results of free range chickens and ducks from Egypt (3). It seems that geographical and ecological conditions in these two countries were resourceful on this similarity.

In the present study, no type I isolates were recovered, which was disagreement with some other studies carried out elsewhere (12, 16, 17), where isolates were predominantly type II.

Various birds are one of the most important sources of meat in Iran, and the results of the present study indicate that these species are potentially involve as the important key animals in the transmission of the disease to humans.

It is worth mentioning that the present study is the first in consideration of *Toxoplasma* genotyping and also of isolation of *T.gondii* from ducks in Iran.

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