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Serodiagnosis of *Toxoplasma gondii* in ducks from Behera Governorate, Egypt

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ABSTRACT

Toxoplasma gondii is an important zoonotic parasite. The diagnosis of infection in animals is an important tool to prevent human infection. In the present study, *Toxoplasma gondii* was diagnosed by using the modified agglutination test (MAT) in ducks from Behera Governorate, Egypt. The prevalence of *Toxoplasma gondii* was 13.9%. The prevalence of *Toxoplasma gondii* was the highest in the Native breed (17.65%) and in 6-8-months age group (19.4%). End-point titer of 1:25, 1:50, and 1:100 were recorded in 61.9%, 19.05%, and 19.05% of the positive samples, respectively. Duck meat is one source of the *Toxoplasma gondii* infection for human in Egypt.

Keywords: *Toxoplamsa gondii*; Modified agglutination test (MAT); Ducks; Egypt.

INTRODUCTION

Ducks are strong animals and good scavengers. They are easier and cheaper to keep than chickens. This makes duck keeping for the production of eggs and meat an attractive enterprise. About 700 million ducks are kept worldwide. The majority, more than 500 million, are in Asia. Despite this uneven distribution, it is possible to raise ducks in other parts of the world, including Africa and Latin America. Ducks can be reared for eggs and meat for own use or for sale. Other products from ducks which can also be sold include down, feathers, fattened livers (van der Meulen and den Dikken, 2004).

Ducks are of 2 types, Muscovy ducks (*Cairina moschata*) and the mallard ducks (*Anas platyrhynchus*) the ancestor of the domestic ducks in its many different breeds (Harrison and Greensmith, 1993). There are several breeds of ducks in Egypt. The old breeds include Native, Sudanese, and White Peckin. The new breeds include Muscovy and Campbell.

Toxoplasma gondii is an intracellular parasite. The cat is the final host. *T. gondii* affects most species of warm-blooded animals, including birds (Dubey, 2002). Infection occurs primarily by ingesting tissue cysts from undercooked meat or from food or drink contaminated with oocysts shed in cat feces. However, only a small percentage of exposed adult humans or animals develop clinical signs (Dubey *et al*., 2003). Infection has resulted in abortion in human and sheep. Fatal toxoplasmosis has been recorded in ducks from Argentina (Boehringer *et al*., 1962), and infection has been reported in ducks worldwide (Zardi *et al*., 1967; Literák and Hejlícek, 1993; El-Massry *et al*., 2000; Dubey *et al*., 2003; Yan *et al*., 2009). Therefore, the diagnosis of toxoplasmosis is necessary for the protection of human from acquiring the disease.

Diagnosis of *T. gondii* infection can be conducted by using serological, histopathological, immunohistochemical, and molecular examinations (Dubey and Beattie, 1988; Dubey *et al*., 1993a, b; Dubey and Odening, 2001). Serological tests are widely used for the diagnosis of *T. gondii* infection in birds. The Sabin-Feldman dye test (DT) (Feldman and Sabin, 1949) has been used for the diagnosis of infection in ducks (Havlik and Huebner, 1960; Zástéra *et al*., 1963; Grzywinski, 1967; Zardi *et al*., 1967a, b; Literák and

Hejlícek, 1993). The indirect hemagglutination test (IHT) has also been used for the diagnosis of infection (Yu *et al*., 1985; Zhang, 1989; Chen *et al*., 1986; Zhai *et al*., 1987; Lv, 1993). Among different serological tests available, the modified agglutination test (MAT) (Dubey and Desmonts, 1987) is the most useful one because it is sensitive and specific, does not require special equipment, and works well with all species of birds tested (Dubey, 2002). MAT was efficient for the diagnosis of the *T. gondii* infection in ducks (El-Massry *et al*., 2000; Dubey *et al*., 2003). Little is known about the prevalence of *T. gondii* infection in ducks from Egypt. The aim of this study was to study the prevalence of antibodies to *T. gondii* and their relationship with the age and breed of ducks by using the MAT.

MATERIALS AND METHODS

Birds

Serological examinations were conducted on 151 duck serum samples of different breeds (Native, Sudanese, White Peckin, Campbell, and Muscovy). They were submitted for slaughtering at the avian market in Damanhur City, the capital of Behera Governorate, Egypt, in 2001-2003. Their ages were varied from 3 months to > 12 months, and their weights from 1-5 kg.

Collection of blood samples

Five ml of blood was obtained separately from each of the 151 ducks in clean, sterile, centrifuge tubes without an anticoagulant. The blood was left to coagulate at room temperature for 2 hours. The clot was separated from the wall of each tube using a fine clean needle. The blood was centrifuged at 3,000 rpm for 15 minutes. The produced sera were transferred to other clean and dry labeled tubes and stored at -20^{o}C until used.

Modified agglutination test (MAT)

The MAT was performed as previously described (Dubey and Desmonts, 1987). The sera were tested for detection of *T. gondii* antibodies using the formalized whole tachyzoites as an antigen that was prepared from the RH strain of *T. gondii* cultivated along with mouse TG 180 sarcoma cells in the peritoneal cavities of mice (formalized whole tachyzoites). A diluting Tris buffer (0.5 M and pH 8.5) was prepared. Four hundred µl of the antigen was mixed with 3.5 ml Tris buffer, 45 µl 2-mercaptoethanol, and 70 µl of (1:10) Evan's blue solution. The serum samples were examined at predetermined dilution (1:25, 1:50, 1:100, and 1:200) after titration using positive and negative control sera with a Tris buffer. The test was performed in a micro titration plate (U-shaped bottom, 96 well). Positive and negative control sera were included in each plate. The plates were covered with sealing tape and incubated at 37^{o}C overnight. A blue button at the bottom of the well indicates a negative result, while a clear bottom indicates a positive one.

RESULTS

T. gondii infection was recognized in ducks through serodiagnosis. A total of 151 duck serum samples were examined by the MAT for the detection of antibodies to *T. gondii*. MAT detected antibodies against *T. gondii* in 13.9% (21/151) of the total samples tested. Thirteen samples (61.9%) from these positives samples showed an end-point titer of ≥ 1:25. Four samples (19.05%) had an end-point titer of ≥ 1:50 and 4 samples (19.05%) had an end-point titer of ≥ 1:100. No seropositive samples were reported at the titer of 1:200.

The relationship between the serologically positive ducks to their breed was studied. The prevalence of infection with *T. gondii* was 7.87% in Sudanese, 11.11% in Campbell and Muscovy, 16.67% in White Peckin, and 17.65% in Native (Table 1). The relationship between *T. gondii* infection and the age of ducks was also examined. The infection rate was 10.42% (5/48) in the 3-5-month age group, 19.4% (12/67) in the 6-8-month age group, and 8.33% (3/36) in the 9-12-month age group (Table 2).

Table 1. *Toxoplasma gondii*-seropositive ducks in relation to their breed

Breed	Number examined	Positive number	Percentage of positive
White Peckin	48	8	16.67
Muscovy	9	1	11.11
Sudanese	38	3	7.89
Campbell	27	3	11.11
Native	34	6	17.65
Total	151	21	13.9

Table 2. *Toxoplasma gondii*-seropositive ducks in relation to their age

Age	Number examined	Positive number	Positive percent
3-5 M	48	5	10.42
6-8 M	67	13	19.40
9-12 M	36	3	8.33
Total	151	21	13.9

M= Month

DISCUSSION

The prevalence of *T. gondii* infection in ducks was evaluated through the detection of antibodies in the sera of ducks using the MAT. The positive cases were 13.9% (21/151). Among the positive ducks 13 (61.9%) had an end-point titer of 1:25, 4 (19.05%) had an end-point titer of 1:50, and 4 (19.05%) had end point titer of 1:100, while no cases were positive at a titer of 1:200. Prevalence of *T. gondii* infections of 50% at a titer of 1:25 (El-Massry *et al*., 2000) and 15.7% at a titer of 1:80 (Dubey *et al*., 2003) has been reported in ducks from Egypt; prevalence of 16% at a titer of 1:5 has been reported in ducks from China. The prevalence of *T. gondii* detected by the DT in the duck specimens from Czechoslovakia was 0% (Havlik and Huebner, 1960), 12.5% (Zástéra *et al*., 1963), and 1.70% (Literák and Hejlícek, 1993). Grzywiński (1967) did not detect any antibodies in the 100 ducks examined by the dye test in Poland; on the other hand, a prevalence of 56% was detected by the dye test from ducks in Italy (Zardi *et al*., 1967b). The prevalence of *T. gondii* infection by the IHT was 6% in ducks from Florida (Burridge *et al*., 1979); on the other hand, prevalence was 0% (Yu *et al*., 1985), 20% (Chen *et al*., 1986), 32.19% (Zhai *et al*., 1987), 23.33% (Zhang, 1989), and 3.93% (Lv, 1993) in the ducks from China.

A positive reaction at 1:25 dilution was considered indicative of old exposure to *T. gondii* that revealed a low antibody titer. A positive reaction at 1:50 dilution was considered indicative of a slightly old infection. A positive reaction at 1:100 dilution was considered indicative of a moderate period of exposure to the infection. The prevalence of *T. gondii* infection in the young age group may be due to their ill-developed immune system. The high prevalence that was encountered in 6-8-month age group may be due

to the fact that this was the most commonly exposed group to infection. The prevalence was the lowest in ducks in the 9-12-month age group, as they had well-developed immune systems.

Concerning the relationship between the prevalence of *T. gondii* infection and the breed of ducks, four positive Native in the 5-11-month old had an antibody titer of 1:25, which indicates an old infection that reveals a low antibody titer; 2 others were positive, with 1:25 and 1:50. The Campbell had 2 positives in the 4-month age group gave an antibody titer of 1:50; one other in the 5-month age group gave titers of 1:25, 1:50, and 1:100, which may indicate a recent infection yielding a high antibody titer. In the White Peckin, 3 samples in the 5-6-month group gave an antibody titer of 1:25; 2 samples in the 7-month group gave antibody titer of 1:25 and 1:50; 3 positive samples in the 6-8-month group gave titers of 1:25, 1:50, and 1:100, which may be due to the current large-scale rearing of this breed in Egypt, which yields these days which yield high exposure to the infection. One Muscovy sample gave an antibody titer of 1:25, and 3 Sudanese ducks gave a 1:25 antibody titer; this might indicate one of the following: that this was an old infection, that the Muscovy and Sudanese were similar in body immune response against infection, or that they may have been infected for a long time and, hence, be immune to new infections. Ducks may become infected by pecking the sporulated oocysts that contaminate the soil and feed. This occurs commonly among ducks reared outdoors, as they are in the villages of the Behera Governorate, and suggests that the meat from the ducks could be an important source for human infection by *T. gondii*.

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