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Zoonotic Chicken Toxoplasmosis in Some Egyptians Governorates

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Abstract: *Toxoplasmosis* is one of the most common diseases prevalent in the world, caused by a coccidian parasite *Toxoplasma gondii* which infects humans, animals and birds. Poultry consider reliable human source of food in addition it is considered an intermediate host in transmission of the disease to humans. Trails of isolation of local *T. gondii* chicken strain through bioassay of the suspected infected chicken tissues in mice was carried out and the isolated strain was confirmed as being *T. gondii* using Polymerase Chain Reaction (PCR). Seroprevalence of antibodies against *T. gondii* in chicken sera in six Egyptian governorates were conducted by enzyme linked immune-sorbent assay (ELISA) using the isolated chicken strain antigen. Moreover, comparison between the prevalence rates in different regions of the Egyptian governorates were been estimated. Isolation of local *T. gondii* chicken strain was accomplished from chicken tissues and confirmed by PCR technique. The total prevalence rate was 68.8% comprised of 59.5, 82.3, 67.1, 62.2, 75 and 50% in El Sharkia, El Gharbia, Kafr El sheikh, Cairo, Quena and Sohag governorates, respectively. The prevalence rates were higher among Free Range (FR) (69.5%) than commercial farm Chickens (C) (68.5%); while, the prevalence rate was less in Upper Egypt than Lower Egypt governorates and Cairo. This study is the first was used antigen from locally isolated *T. gondii* chicken strain for the diagnosis of chicken toxoplasmosis. The higher seroprevalence particularly in free range chickens (house-reared) refers to the public health importance of chickens as source of zoonotic toxoplasmosis to human.

Key words: *Toxoplasma gondii*, Chicken, ELISA, PCR, Egypt

INTRODUCTION

There is no doubt that the world suffers from the lack of nutrition especially those of animal origin, such problem is enlarged day after day especially in Egypt, poultry sector is one of the key elements in the economies of developed countries as it is one of the main sources for the provision of animal protein and meat consumed (Deyab and Hassanein, 2005). The improvement of poultry production is an important way to compensate the deficiency in protein requirements especially in Egypt, where Poultry production in Egypt and other middle-income countries totaled 37.5 million tons, compared with 29.3 million tons in high-income countries and only 3.6 million tons in low-income countries (Taha, 2003).

T. gondii is an obligate intracellular, the definitive hosts only are domestic and wild cats; in which the sexual phase of the life cycle occurs in the intestinal epithelium ended by the shedding of un-sporulated oocysts in feces and the sporulation occurs outside the host. In the intermediate hosts which are either animals, birds or man a transient acute phase caused by tachyzoites followed by the chronic phase which is characterized by formation of dormant tissue cysts contain bradyzoites (Dubey, 2010a). The *T. gondii* infection routes to human through ingesting undercooked meat containing the tissue cysts, consuming food or drink contaminated with oocysts and/or accidentally ingesting oocysts from the environment (Dubey *et al*., 2004).

Different serological tests were performed in many countries for serologic prevalence in free-range or backyard chickens worldwide. Prevalence varied from 2-100% depending on the source of the chickens. Prevalence of infection was 17.9% in India (Sreekumar *et al*., 2003), 38% in Brazil (Dubey *et al*., 2007a) and 85% in Nicaragua (Dubey *et al*., 2006) using Modified Agglutination Test (MAT). Recently, Bartova *et al*. (2009) reported, *T. gondii* antibodies in 1 of 293 chickens from the Czech Republic, Chumpolbanchorn *et al*. (2009) found *T. gondii* antibodies in 64% of 303 FR chickens from

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Thailand using Indirect Fluorescent Antibody Test(IFAT) and Yan *et al.* (2009) reported *T. gondii* antibodies in 11.4% of 361 FR chickens and in 4.1% of caged chickens in People's Republic of China using MAT. Few reports of chicken toxoplasmosis from Egypt, the infection rate were 47.2% (El-Massey *et al.*, 2000) and 40.4% (Dubey *et al.*, 2003) in free range chicken using MAT and in additionally Deyab and Hassanein, 2005, found MAT antibodies in 18.7% of 150 chickens from slaughterhouses in Egypt.

Chickens rarely have clinical toxoplasmosis (Dubey, 2010b). First reported of toxoplasmosis was confirmed in a flock of 40 White Leghorn hens from Norway by Erichsen and Harboe (1953) such an outbreak has not been reported in the literature in the last 50 years. Two reports of clinical toxoplasmosis were detected chickens from the United States; 1st from Georgia, peripheral neuritis in three chickens (Goodwin *et al.*, 1994) and 2nd in Illinois, 3 birds died suddenly out of a group of 14 backyard chickens with an unusual finding was the presence of numerous tissue cysts and tachyzoites in the lesions; the protozoa in the brain reacted positively with *T. gondii* antibodies and the remaining 11 chickens remained asymptomatic, though all contained viable *T. gondii* (Dubey *et al.*, 2007b).

Many serological tests were reported in the diagnosis of chicken toxoplasmosis; DT, like the CFT, does not detect antibodies to *T. gondii* in chicken sera or had only low titers even sera obtained from experimentally infected chickens with *T. gondii* (Dubey *et al.*, 1993). The IHAT is an insensitive test, in 13 experimentally infected chickens, its sensitivity was 46% and specificity of a negative test was 25% (Frenkel, 1981). There is limited information on the efficacy of IFAT test for the detection of *T. gondii* antibodies in chicken sera, viable *T. gondii* isolated from tissues of 9 of 15 (60%) chickens with IFAT titers of 1:16 or higher and from 2 of 13 (15.3%) sero-negative free-range chickens from Brazil (Brandao *et al*, 2006). ELISA, 1st studied in chickens fed oocysts, using the soluble fraction of tachyzoites, the chicken sera were diluted in buffered 7% NaCl (instead of 0.85%) aqueous solution (saline) because most chicken immunoglobulin precipitate when diluted with phosphate buffered saline (Biancifiori *et al.*, 1986). Although, the sensitivity and specificity of the MAT in naturally exposed chickens is under investigation in my laboratory using isolation of the parasite as a standard, MAT is efficient in detecting *T. gondii* antibodies in chickens (Dubey, 2010b).

T. gondii infected chickens has important role in the epidemiology of toxoplasmosis in humans. Therefore, the main objectives of this study are to isolate the isolate local *T. gondii* chicken strain through bioassay of the suspected infected chicken tissues in mice and confirm it by PCR. Also, to estimate the seroprevalence of *T. gondii* infection among chicken from free range and commercial farm in some Egyptian governorates using ELISA. Moreover, compare between the prevalence rates results in different regions of the Egyptian governorates.

MATERIALS AND METHODS

Experimental animals: This research study was conducted from 5-2012 to 12-2012. Swiss albino laboratory breed mice about 1 month-old were used for bioassay of the suspected infected chicken tissues and maintenance of isolated *T. gondii* local strain in the lab. All mice were kept in clean cages, fed on pelleted food and clean water supply with multivitamins.

Chicken tissue samples: Meat and tissue samples were obtained from slaughtered chicken at different regions, in Egypt. Pooled tissue sample, about 25-50 g, from heart, brain and pectoral muscles were prepared as described by Shaapan and Ghazy, 2007, the tissues and organs was cut into small cubes about 5×5×5 cm and stored at 4°C for few hours until used for bioassay in mice.

Isolation of local *T. gondii* chicken strain: Tissue samples were digested by pepsin according to method of Sharma and Dubey (1981), followed by microscopically examined at low and high powers for the presence of *T. gondii* tissue cysts containing the bradyzoites. Bioassay of the digested positive tissues in mice according method mentioned by Dubey (2010a), in order to obtain the tachyzoites stage which maintained in the laboratory through repeated intra peritoneal mouse inoculation in mice (Johnson *et al.*, 1979).

Chicken blood collection and sera preparation: Blood samples were collected in dry, sterile screw capped tubes of 15 cm from 439 apparently healthy chickens (free range and commercial farms) from six governorates include El Gharabia, Kafr El-shiekh, El Sharkia, Sohag Quena and Cairo. Samples were centrifuged at 3000 rpm for 15 min and stored at -20°C until used in serological investigation.

Serological survey for detection of *T. gondii* antibodies: The collected chicken sera were examined serologically for detection of *T. gondii* antibodies using the Enzyme Linked Immuno-sorbant Assay (ELISA), the whole soluble tachyzoites antigens were prepared as described by Waltman *et al.* (1984) and the procedures adopted according to Lind *et al.* (1997).

Polymerase chain reaction (PCR): In this study, PCR was adopted for confirmation that tachyzoites as previously isolated from chicken tissues and used in serological diagnosis (as local strain) belongs to *T. gondii* strain. Genomic DNA was extracted from tested *T. gondii* local

and RH (positive control) strains using a commercially available kit (Dneasy blood and Tissue kit, Qiagen Co., Cat. No. 69504) with modifications to the manufacturer's protocols (Howe *et al*., 1997). Primers used for PCR were targeting the repetitive 35-fold B1 gene. The amplified product was analyzed on 1% agarose gel and stained with Ethidium bromide. Every PCR run included controls (Burg *et al*., 1989).

RESULTS

Morphological studies of the isolated *T. gondii* developmental stages: Two developmental infective stages of *T. gondii* were isolated; trachyzoites and bradyzoites.

Tachyzoites: Tachyzoites were obtained from the peritoneal exudates of previously inoculated mice with infected digested chickens' tissues 6-8 post inoculation. Tachyzoites were found inside leucocytes (lymphocytes and macrophages) or free in the peritoneal exudates after rupture of leucocytes. The Giemsa stained isolated tachyzoites were often crescent in shape or banana shape pointed at one end and rounded or blunt at the other one, their size were 2×7 μm. It showed pale blue cytoplasm with reddish purple nucleus which centrally located or near the blunt end. The leucocytes appear as dark blue color (Fig. 1).

Tissue cysts containing bradyzoites: The tissue cysts of isolated *T. gondii* strain were noticed in the fresh smears after digestion of heart and brain tissues. The tissue cyst is the resting stage of parasite within the host, they were usually sub-spherical to spherical in shape and its cyst wall was thin elastic and will defined enclosing up several hundreds of crescent shaped Bradyzoites (Fig. 2).

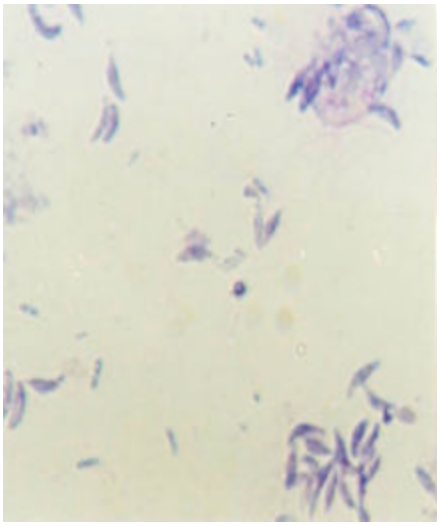

Fig. 1: Giemsa stained *T. gondii* tachyzoites (X400)

Confirmatory PCR results: As number of base pairs (94 pb) of the isolated *T. gondii* strain obtained from chicken tissues (lane, 3 and 4) was the same of compared positive control RH strain (lane, 2) with the presence of negative control sample (lane,1) and matched with standard marker (lane, M) (Fig. 3). So the PCR results confirm that tachyzoites which previously isolated is belong to *T. gondii*.

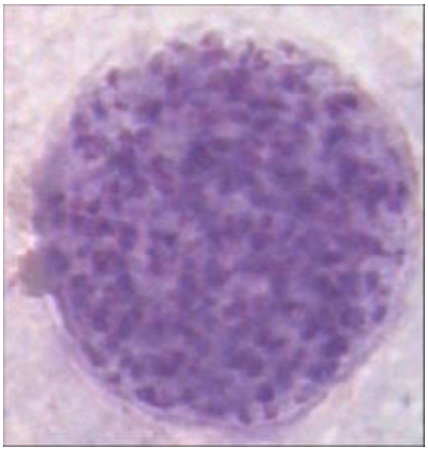

Fig. 2: Giemsa stained *T. gondii* tissue cysts (X1000)

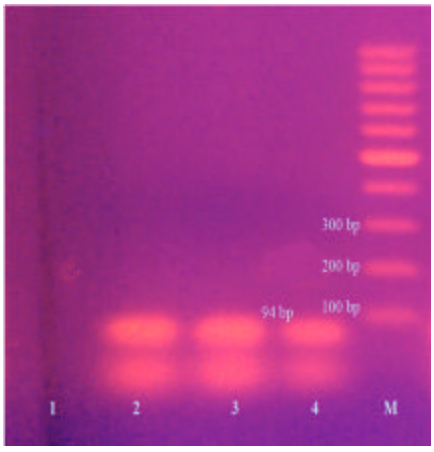

Fig. 3: Detection of *T. gondii* by PCR. Lane 1: Negative control, Lane 2: Positive control (RH stain), M: MW marker (100 bp ladder) and Lanes 3 and 4: Positive PCR samples of isolated chicken strain at 94 bp

Table 1: Seroprevalence of *T. gondii* in six governorates

Governorate	Source of chicken	No. of chickens	No. of positive	Infection (%)
El Sharkia	C	42	26	61.9
	FR	7	4	57.0
El Gharbia	C	133	106	79.6
	FR	20	17	85.0
Kafr El-Sheikh	C	-	-	-
	FR	76	51	67.1
Quena	C	90	56	62.2
	FR	-	-	-
Cairo	C	36	27	75.0
	FR	-	-	-
Sohag	C	30	12	40.0
	FR	5	3	60.0
Total	C	331	227	68.5
	FR	108	75	69.5
	Total	439	302	68.7

C: Commercial forms, FR: Free range

Serological investigation of *T. gondii* antibodies in chicken sera: Examination of the total 439 serum samples of chickens by ELISA revealed that 302 sample (68.8%) had antibodies against *T. gondii* which was considered the percentage of infection. The obtained results indicate that prevalence rate was slightly higher, (69.5%) in free range chicken (FR) than that (68.5%) in chicken from commercial farms (C) in the most of 6 examined governorate and also results refer to that the prevalence rates was less in Upper Egypt than governorates than that of Lower Egypt and Cairo (Table 1).

DISCUSSION

The high prevalence rate of chicken toxoplasmosis (68.8%) in the present study which comprised 6 examined Egyptian governorates; refer to the importance of chicken's meat as important source of infection among Egyptian consumers which agreed with that previously detected in Egypt by El-Massey *et al.* (1990), who reported that sheep, goat and chicken are important intermediate hosts for *T. gondii* and their meat are main source of infection to man if un-hygienically consumed.

However, present survey showed that seroprevalence rates was higher in Free-Range (FR) (69.5%) than commercial farm Chicken (C) (68.5%) indicated that the distribution of *T. gondii* in chicken might be related to the cats that disseminated the oocysts in the soil (Asgari *et al.*, 2006). On the other hand, cats could be infected from eating chicken meat, wild birds and rodents infected with *T. gondii* (Hassanain *et al.*, 2008). Therefore, *T. gondii* infection in chickens is epidemiologically significant and people have the habit of eating under-cooked chicken meat, should be aware of the risk of encountering *T. gondii* infection.

Little is known concerning the validity of the serologic tests for the detection of *T. gondii* antibodies in avian sera. Viable *T. gondii* was isolated from up to 100% of free-range chickens bio-assayed in mice (Dubey *et al.*, 2007b). The present study, ELISA test used for seroprevalence of 439 chicken blood samples collected from six Egyptian governorates. The ELISA test has the advantage that it specific and of great sensitivity, objective, quantitative, low coast and can be automated and is convenient for large-scale surveys of toxoplasmosis in human and animals but it need a refinement in the procedures and standardization (Shaapan *et al.*, 2008).

Many surveys detecting *T. gondii infection* in FR chickens have been conducted in a number of countries and the prevalence rates in most of these studies ranged between 17.9% and 85% (Deyab and Hassanein, 2005; Sreekumar *et al.*, 2003; Dubey *et al.*, 2007a; Dubey *et al.*, 2006; Yan *et al.*, 2009; El-Massey *et al.*, 2000; Dubey *et al.*, 2003). The seroprevalence of *T. gondii* in domestic birds varies within countries due to the testing methods, the number of examined animals, type and hygiene of animal breeding Dubey (2010a). The total seroprevalence *T. gondii* among chicken in our study (68.7%) was lower than some results other results (Sreekumar *et al.*, 2003; Dubey *et al.*, 2007a; Yan *et al.*, 2009; El-Massey *et al.*, 2000) similar to that (64%) in FR chicken in Thailand (Chumpolbanchorn *et al.*, 2009), While lower than 85% obtained in Nicaragua (Dubey *et al.*, 2006). Reasons for this variability could be many, including the age of the chicken, number examined, type of serological test used and the tissues bio-assayed. Overall, prevalence of viable *T. gondii* in chickens raised indoors was low (Dubey *et al.*, 2007a).

Concerning PCR results, the matched sequence of base pairs with the stander for *T. gondii* confirm that PCR assay is a specific, speedy, sensitive and cost-effective method for detecting *T. gondii* DNA in chickens. The choice of using the fragment of 94 bp from the B1 gene as target to PCR amplification was based on the observations made by other studies (Howe *et al.*, 1997; Dubey *et al.*, 2005).

CONCLUSION

In considering the above-mentioned findings, it could be concluded that *T. gondii* infection was spread widely in Egyptian poultry, so it give aware of the public health danger of poultry as an intermediate host which transmit the infection to man. The significantly higher infection rate than previous studies indicate that ELISA is more sensitive method for the diagnosis of toxoplasmosis. Moreover, this study is the first was used antigen from locally isolated *T. gondii* chicken strain for the diagnosis of chicken toxoplasmosis.

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