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ZOONOTIC TOXOPLASMOSIS IN CHICKEN

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Abstract

Modified agglutination test (MAT) was applied to 150 chicken sera (90 were farm-bred and 60 house-bred chickens). The prevalence of *Toxoplasma* antibodies was 18.7%. In house-bred chickens positive cases were 18 out of 60 (30.0%), while in farm-bred chickens positive cases were 10 out of 90 (11.1%). Histopathological sections of the seropositive chickens showed tissue lesions which were most likely to be induced by *T. gondii*. Tissue cysts of *T. gondii* were demonstrated in the liver, brain, heart and skeletal muscles of 22 (78.6%) out of 28 positive chickens. These tissue cysts were found mostly in the brain of seropositive chickens. Epidemiological implications of these data for public health significance were discussed.

Introduction

Toxoplasma gondii exists in three basic forms: trophozoite (proliferative form), tissue cyst and oocyst (Krick and Remington, 1978). Man may acquire *T. gondii* infection either transplacentally or by ingestion of tissue cysts in inadequately cooked or raw meat of infected animals (Stagno *et al.*, 1980) or ingestion of sporulated oocysts from cat faeces (Smith, 1993; Choi *et al.*, 1999). Many species of mammals and birds are intermediate host of *T. gondii* (Hubbard *et al.*, 1986; Dubey *et al.*, 1993; Lindsay *et al.*, 1994). Birds and rodents are important prey host of *T. gondii*, since they serve as a source of infection to cat, final

host (Dubey and Beattie, 1988). Felidae excrete the environmentally resistant *T. gondii* oocysts in their faeces after ingesting tissue cysts from infected animals (Bobic *et al.*, 1996). Flies, cockroaches, and earthworms were shown experimentally to be capable to carry oocysts (Dubey, 1998). Since soil is a continuous source of *T. gondii* infection for animals and humans, the prevalence of toxoplasmosis in feral chickens is also a good indicator of *T. gondii* in the environment according to the species eating habits (Jackson and Hutchison, 1989; El-Massry *et al.*, 2000). Despite their apparent resistance, infected birds can retain the parasite in their organs for several months after infection showing their possible role as reservoirs (Biancifiori *et al.*, 1986). Toxoplasmosis was reported to induce clinical symptoms among chicken (Goodwin *et al.*, 1994). Dubey (1994) proposed that *T. gondii* cysts can survive in tissues all over the life of the host. Tachyzoites are found during the acute phase whereas encysted bradyzoites (tissue cysts) are found predominately in the latent, chronic phase *T. gondii*. Isolation of *T. gondii* is the sure method of diagnosis, but due to difficulty of isolation, various techniques including serologic, histopathologic, immunohistochemical and molecular examinations can aid diagnosis. Modified agglutination test (MAT) was highly sensitive and specific for detecting antibodies to *T. gondii* infection in chickens (Dubey, 2002; Dubey *et al.*, 2002). Serological surveys of toxoplasmosis among chickens have been reported from various countries. The reported of *Toxoplasma* seropositive chickens were 27% in Jordan (Morsy *et al.*, 1978) 17% in Malaysia (Rajamanickam *et al.*, 1990); 5.1% in Czech Republic (Literak and Hejlíček, 1993); 39.5% in India (Devada *et al.*, 1998) and 0.4% in Croatia (Kuticic and Wikerhauser, 2000). In Egypt, there are scanty information on serological diagnosis of chicken toxoplasmosis. The reported of *Toxoplasma* seropositive chickens were 22% (Rifaat *et al.*, 1969), 33.3% (Hassanain *et al.*, 1997) and 47.2% (El-Massry *et al.*, 2000).

The present study aimed at evaluation of the anti-*Toxoplasma* antibodies in sera and to study *T. gondii* infected tissue(s) in chicken in the different bird shops in Assuit Governorate to clarify their epidemiological importance in infecting human.

Material and Methods

During the period from September, 2003 to August 2004, blood samples were obtained from 150 chickens (90 farm-bred and 60 house-bred chickens) slaughtered at bird shops in Assuit Governorate. Sera were stored at -20°C until used for serological diagnosis.

Preparation of antigen: The whole formalized tachyzoites antigen was prepared (Dubey and Desmonts, 1987). RH strain tachyzoites was maintained by intraperitoneal passage in laboratory mice in Department of Parasitology, Faculty of Medicine, Assuit University.

The sera were tested for *T. gondii* antibodies using formalized whole tachyzoites. Sera were screened at dilution 1:50 to determine the titer of anti-*Toxoplasma* antibodies by MAT to detect anti-*Toxoplasma* IgG (Dubey and Desmonts, 1987). A titer $\geq 1:50$ was considered positive. All data were subjected to statistical analysis using SPSS program (version 9).

The serologically examined chickens were necropsied after euthanasia. Portions of the heart, lung, brain, liver, and muscle tissues were collected. All tissues were kept in 10% neutral buffered formalin, paraffin embedded sections were cut at $50\mu\text{l}$ and stained with Haematoxylin and Eosin (Cormack, 1987). The stained sections were examined for *T. gondii* cysts and tachyzoites (Dubey, 1994).

Results

28 out of 150 chicken (18.7%) were positive for anti-*Toxoplasma* antibodies. The positive cases in the farm-bred chickens were 10 out of 90 (11.1%), while in house-bred ones, was 18 out of 60 (30.0%), with a significant P-value. Among positive cases, 18 out of 28 (64.3%) were house-bred chickens and 10 cases (35.7%) were farm-bred (Tab. 1).

Histopathological examination revealed the presence of *Toxoplasma* tissue cysts in the liver (Fig. 1-A), brain (Fig. 1-B), heart (Fig. 1-C), and skeletal muscles (Fig. 1-D) of 22 (78.6%) out of 28 positive chickens while no cysts were visualized in lung tissues. These tissue cysts were found mostly in the brain (10),

skeletal muscle (6), liver (4) and heart (2) of positive chickens. While mixed infection was in brain and liver (3), brain and skeletal muscles (2), brain and heart (1). Liver was grossly enlarged with areas of infarction. Heart (Fig. 1-C) and skeletal (1-D) muscles showed *T. gondii* cysts among muscles with marked degeneration in muscle fibers. The size of *T. gondii* cysts muscles (Fig. 1-D) was smaller than the size of those present in brain, liver, and heart.

Table 1: Prevalence of anti-*Toxoplasma* antibodies in chickens in Assiut Governorate.

	Farm-bred		House-bred		Total		P-value
	No.	%	No.	%	No.	%	
Positive	10	11.1	18	30.0	28	18.7	0.000
Negative	80	88.9	42	70.0	122	81.3	
Total	90	100.	60	100.	150	100.	

P-value is significant if < 0.05

Table 2: *T. gondii* tissue cysts in different organs of positive chickens.

Sero-positive chickens	Single infection				Double infection			All
	B.	L.	H.	S. m.	B+ L.	B.+ H.	B.+ S.m.	
22	10	4	2	6	3	1	2	0

B= Brain, L= Liver, H= Heart, S.m.= Skeletal muscle

Discussion

The seroprevalence of toxoplasmosis in chickens can give an idea about the extension of *T. gondii* infection. Chickens can become long-term carriers of *T. gondii* and responsible to be potentially reservoir host (Biancifiori *et al.*, 1986). In the present study, the prevalence of *Toxoplasma* antibodies among chickens was 18.7%. This result was lower than 27% reported by Morsy *et al.* (1978) in Jordan, 22% reported by Rifaat *et al.* (1969) in

Egypt, 33% reported by Ghorbani *et al.* (1990) in Iran, 39.5% reported by Devada *et al.* (1998) in India, 47.2% reported by El-Massry *et al.* (2000) in Egypt and 65.2% reported by da Silva *et al.* (2003) in Brazil. It was higher than 17% reported by Rajamanikam *et al.* (1990) in Malaysia 5.1% reported by Literak and Hejlícek (1993) in Czech Republic. So, it is not possible to make valid comparisons among data presented because of the differences in sensitivity and specificity of different serological tests, housing conditions, nature of soil, breeding of chickens near stray cats and improper sterilization of food used in rearing chickens, and difference in the level of contamination of soil with *T. gondii* oocysts. Food materials used as the main source in farms may be the responsible for *T. gondii* infection in farm bred-chickens. Cats which excrete *T. gondii* oocysts into the environment were commonly seen in the examined chicken-farms. The oocysts may be easily the source of chickens' infection by gathering their food.

The prevalence of *T. gondii* antibodies was high (30%) in house-bred chickens, while it was low (11.1) in farm-bred ones with significant P-value indicating that house-bred chickens were the main source of human infection than the farm-bred ones. Ruiz and Frenkel (1980) noticed that, the prevalence of *T. gondii* in the free-range chickens was a good indicator of *T. gondii* in the environment. So, the present study indicates that there was a high level contamination of soil with *T. gondii* infection in Assuit Governorate. These findings nearly agree with Dubey *et al.* (2003) using MAT and bioassay in mice, recorded that the seroprevalence of *T. gondii* antibodies was (40.5%) in free range chickens.

Of all intermediate hosts of *T. gondii*, chickens most actively peck the soil in search of grains, earthworms and other food heavily contaminated with soil. The prevalence of oocysts in soil could easily be assessed by means of chickens. Chickens develop cysts with bradyzoites between 8 and 10 days after the ingestion of oocysts (Ruiz and Frenkel, 1980).

The house-bred chickens may play an important role for humans infection with *T. gondii*. Tenter *et al.* (2000) stated that bradyzoites of *T. gondii* are more resistant to digestive enzymes, (i.e. pepsin and trypsin) than tachyzoites. Therefore, ingestion of

viable tissue cysts by a non-immune host usually results in *T. gondii* infection. Although tissue cysts are less resistant to environmental conditions than the oocysts, they are relatively resistant to changes in temperature and remain infective in refrigerated (1-4°C) carcasses or minced meat for up to 3 weeks, i.e. probably as long as the meat remains suitable for human consumption (Kaneto *et al.*, 1997).

In the present study, *Toxoplasma* tissue cysts demonstrated in 22 (78.6%) out of 28 seropositive chickens. *T. gondii* in tissues of chickens may represent one possible source of toxoplasmosis in man, particularly if hygiene control is lax when handling raw chicken tissues or when semi-raw or raw chicken meat is used for consumption. The contribution of raw meat (including poultry meat) in the prevalence of *Toxoplasma* antibodies in man was described (Konishi and Takahashi, 1987).

In conclusion, data obtained in the present study and those of others indicate that the longer a population is exposed to infection, the greater the proportion of infected individuals it will contain. To prevent infection of human beings by *T. gondii*, people should wash their hands thoroughly with soap and water after handling meat. Pregnant women should avoid contact with undercooked meat. Also parasites in poultry meat can be killed by exposure to extreme cold or heat. Tissue cysts are killed by heating the meat thoroughly to 67°C (Dubey *et al.*, 1990) or by cooling it to -13°C (Kotula *et al.*, 1991).

Since, *Toxoplasma* infection can be a possible cause for habitual and sporadic abortion and lead to miscarriage, still birth, or survival with growth problems (Kamel *et al.*, 1998; Baril *et al.*, 1999; Cook *et al.*, 2000), periodical examination of the indoor cats serologically and parasitologically. Cats should never be fed uncooked poultry meat and they must be nutritionally supplemented with only dry canned or boiled foods. Poultry meat for consumption should be cooked until the colour changes (Dubey, 2002).

Although poultry meat is generally cooked well before human consumption, handling of infected meat and poor hygiene could be a source of infection. Moreover, chickens are often slaughtered at home for local consumption. Viscera and meat scraps are left for scavengers. Ingestion of infected chicken viscera by cats

Egypt, 33% reported by Ghorbani *et al.* (1990) in Iran, 39.5% reported by Devada *et al.* (1998) in India, 47.2% reported by El-Massry *et al.* (2000) in Egypt and 65.2% reported by da Silva *et al.* (2003) in Brazil. It was higher than 17% reported by Rajamanikam *et al.* (1990) in Malaysia 5.1% reported by Literak and Hejlícek (1993) in Czech Republic. So, it is not possible to make valid comparisons among data presented because of the differences in sensitivity and specificity of different serological tests, housing conditions, nature of soil, breeding of chickens near stray cats and improper sterilization of food used in rearing chickens, and difference in the level of contamination of soil with *T. gondii* oocysts. Food materials used as the main source in farms may be the responsible for *T. gondii* infection in farm bred-chickens. Cats which excrete *T. gondii* oocysts into the environment were commonly seen in the examined chicken-farms. The oocysts may be easily the source of chickens' infection by gathering their food.

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Although poultry meat is generally cooked well before human consumption, handling of infected meat and poor hygiene could be a source of infection. Moreover, chickens are often slaughtered at home for local consumption. Viscera and meat scraps are left for scavengers. Ingestion of infected chicken viscera by cats

can lead to oocyst shedding and spread of *T. gondii* in the environment.

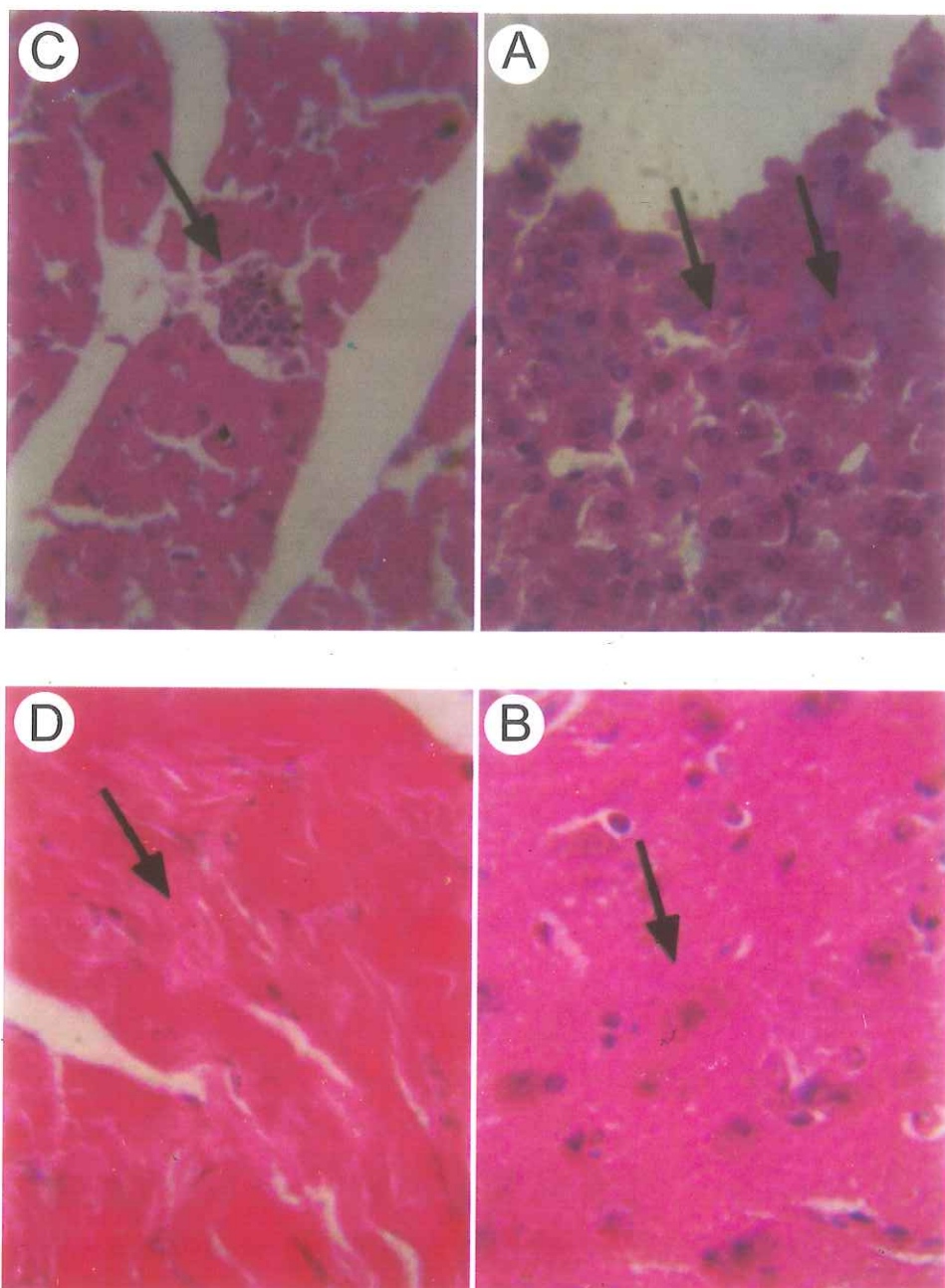
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- A. Liver tissue cyst of *T. gondii* (H&E, x700)
- B. Brain tissue cyst of *T. gondii* (H&E, x700)
- C. Tissue cyst of *T. gondii* in cardiac muscle (H&E, x700)
- D. Tissue cyst of *T. gondii* in skeletal muscle (H&E, x700)

الأهمية الصحية المشتركة لداء المصورات الليفانية (التكسوبلازموزس) فى الدجاج

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فى دراسة على الإصابة بطفيل تكسوبلازما جوندى فى الدجاج بمدينة أسيوط بجمهورية مصر العربية باستخدام اختبار التلازن المطور لتحديد مدى انتشار المرض وتقييم دور الدواجن فى نقل المرض للإنسان ، تم إجراء الاختبار السيرولوجى على مصل ١٥٠ دجاجة منهم ٩٠ دجاجة تم تربيتها فى مزارع لتسمين الدواجن و ٦٠ دجاجة تم تربيتها فى البيوت. وجد أن نسبة الإصابة الكلية بطفيل تكسوبلازما جوندى فى الدجاج ١٨,٧%. كانت الأجسام المضادة فى الدجاج الذى تم تربيتها فى المنازل ١٨ (٣٠%) أعلى منها فى الدجاج الذى تم تربيته فى المزارع ١٠ (١١,١%).

بالإضافة إلى الفحص الهستوباثولوجى الذى نتج عنه وجود حويصلات شبيهة بحويصلات التكسوبلازما النسيجية المتحوصلة فى أنسجة الكبد والمخ والقلب والعضلات فى ٢٢ (٧٨,٦%) من ٢٨ دجاجة موجبة للاختبار السيرولوجى. كم لوحظ أن وجود الطور النسيجى المتحوصل يكون غالباً فى المخ.

وقد نوقشت الأهمية الصحية المشتركة والوبائية للطفيل ومصادر التلوث المختلفة به بالإضافة إلى

مناقشة التوصيات للحد من انتشاره و الوقاية منه.

NOTICE TO CONTRIBUTORS

This is a regular journal published by the Egyptian Society of Parasitology and is concerned with the publication of research papers in the field of Parasitology and related subjects. The annual volume includes three numbers published in April, August and December. Two types of communications appear regularly; papers and correspondence. Letter to the editor is also published. Publication is not restricted to Fellows of the Society. Non-Fellows can publish on their own expense. Manuscripts should be signed by the author or authors concerned and sent to the Chief Editor. All the manuscripts are peer reviewed. However, the authors alone are responsible about all data and views they expressed.

Instructions for preparation of manuscripts: Paper should be sent in type-written, on one side of the paper only with double spacing and wide margins, on paper suitable for ink correction and on a floppy disk or CD. Papers should be as concise as possible and provided with a **brief abstract** (very short summary of your own results).

Illustrations (photographs, line drawings, graphs and tables) should be kept to the minimum. Details of results presented in this way should not be repeated in the text. Coloured plates can be included only at the author's expense. Line drawings, maps, graphs and tables should be approximately final size. Pronouns such as I, my, we, our, should not be used, thus my or our results should be in the present results. Discussion should be for your own results in relation to others and not in any way, a review of literature or aim of the work.

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