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ZOONOTIC IMPORTANCE OF TOXOPLASMA GONDII TISSUE CYSTS IN CHICKENS

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A B S T R A C T

Toxoplasmosis is a typical worldwide Zoonosis caused by *Toxoplasma gondii* (*T. gondii*), which is an obligate intracellular opportunistic coccidian protozoon of felids with an unusually wide range of intermediate hosts which are animals, birds or man. In the present study Brain tissues, hearts and breast muscles of 230 slaughtered domestic chickens (170 commercial farm and 60 free range or house reared) had been collected from three governorates, then digested , examined microscopically and were bio-assayed in mice for demonstration viable *T. gondii* bradyzoites. The results showed higher occurrence of tissue cysts in free-range chickens (FR) than commercial farm (CF) chickens. In addition to higher percentage of tissue cysts were in heart muscle than other organs. The percentage of tissue cysts in FR chicken was (11.6%) in brains, (31.6%) in hearts and (6.66%) in breast muscle while the percentage of tissue cysts in CF chicken was (2.90%) in brains, (5.29%) in hearts and (0.58%) in breast muscle. Moreover, isolation of local strain (isolates) from harvested tachyzoites was conducted after confirmation using PCR. Zoonotic importance was discussed and suggested recommendations were recorded to control such disease of great economic importance in veterinary field as well as its public health importance.

KEY WORDS: Chickens, *Toxoplasma gondii*, Zoonotic importance.

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1. I N T R O D U C T I O N

Nowadays, *T. gondii* found to be worldwide in distribution where nearly one-third of humanity has been exposed to this parasite, Moreover because of its broad host range, its high infection rates and its benign co-existence with the host. The definitive hosts only are domestic and wild cats in which the sexual phase of life cycle occurs in intestinal epithelium ended by shedding of unsporulated oocysts in feces and the sporulation occurs outside the host in the environment but in intermediate host which are either animals or human. A transient acute phase caused by tachyzoites followed by chronic phase which is characterized by formation of dormant tissue cysts contain bradyzoites [18]. Chickens are considered one of the most important hosts in the epidemiology of *T. gondii* infection because their infected tissues are an efficient source of infection for cats that excrete the environmentally resistant oocysts. Moreover, *T. gondii* infection in chickens are potential public health risks especially in free-range chickens (FR) as FR chickens are one of the best indicators for soil contamination with *T. gondii* oocysts [5] because they feed from the ground. However mostly *T. gondii* infection in poultry is asymptomatic. The clinical signs of toxoplasmosis in poultry include anorexia, emaciation, reduce eggs production, ataxia and even mortality rate may be as high 50% which is seldom observed [10].

Viable *toxoplasma* in edible tissues of food animals represent health hazard for human consumers; because if infected tissues of chickens ingested by cats, they may cause contamination of the environment with *T. gondii* oocysts [13]. Human may contract the infection horizontally by ingesting tissue cysts from undercooked meat or by consuming food or drink contaminated with oocysts accidentally [4] or vertically by trans-placental infection with tachyzoites passed from the mother to the offspring [16]. This high prevalence of infection in man and various modes of transmission prove the importance of toxoplasmosis as typical zoonotic disease for all warm-blooded animals including humans where the infection is usually either asymptomatic or mild flu-like symptoms. However, toxoplasmosis can be life threatening in Immuno-suppressed or immune-deficient individuals particularly AIDS patients in whom reactivation of tissue cysts occurs and suffered from fatal toxoplasmic encephalitis [15]. Moreover, if acquired the infection during pregnancy, toxoplasmosis can cause serious illness in pregnant women and female animals; it is a common cause of abortion or miscarriage. The disease becomes more dangerous if transmitted congenitally where congenital malformations affecting the brain, eyes or other organs of the fetus and causing blindness, megallocephally and mental retardation to children. Whereas, animal fetuses were mummified, macerated, and stillborn or may be borne weak and die within weeks after birth [19]. The present study was conducted to evaluate the distribution of *T. gondii* tissue cyst containing bradyzoites in different tissues of chickens (free range and commercial farm), isolation and confirmation of local strain using of PCR.

2. MATERIALS AND METHODS

2.1. *Microscopic demonstration of viable T. gondii tissue cyst in examined chicken tissues* .

In order to study distribution of viable *T. gondii* tissue cyst containing bradyzoites, collected chicken tissue samples were prepared after their digestion (using Acidic-pepsin-digestive solution that Composed of [2.6 g pepsin + 5 g Na Cl + 7 ml conc. Hcl + 500 ml water) according to Kotula *et al*., [11].

The number of chicken used and type of tissues for demonstration of viable *T. gondii* in tissues with its corresponding governorates and source of chickens summarized in table (1).

2.2. *Isolation of T.gondii local strain by Bioassay of chicken tissues in mice*.

The isolation of *T. gondii* local strain was carried out according to procedures described by Dubey and Beattie [6].

The samples of Brain tissues, hearts and breast muscles of 230 slaughtered domestic chickens (170 commercialfarm and 60 freerange or house reared) obtained from freshly slaughtered chickens which had been raised in small private farms and free range (FR); which reared in houses and fed commercial poultry feeds, supplemented with maize.

Table 1 Number of chicken used for demonstration of viable *T. gondii* in tissues with their corresponding governorates, age and source of chickens.

Governorate	Source	n	Age (days)	Brain	Heart	breast muscle
El Gharbia	CF	60	55	60	60	60
	FR	20	300	20	20	20
Kafr El-Shiekh	CF	35	42	35	35	35
	FR	25	350	25	25	25
Cairo	CF	75	45	75	75	75
	FR	15	150	15	15	15

(FR): free range chicken. (CF): commercial farm chicken.

Ten (10) grams were obtained from brain, heart and breast muscle of each slaughtered chicken and cut into small pieces then after their digestion. Each sample was homogenized in sterile sand and saline and intra-peritoneal injected into 64 Swiss albino laboratory breed mice about 1 month-old, their body weight; varying from 25 to 35 g and were kept in clean cages and fed on Toxoplasma free pelleted food and clean water supply with multivitamins in the National Research Center (NRC) with dose of inoculation was 1ml/mice where mice were used for bioassay of the suspected infected tissues. After 6 - 8 days, the peritoneal exudates of these injected mice were aspirated by sterile syringe and examined to demonstrate the tachyzoites of *T. gondii*, and then the recovered tachyzoites of *T. gondii* local strain (isolates) was maintained after serial passage. Six weeks later in the mice where toxoplasmosis was chronic, the mice were euthanatized and the fresh preparations of their brains microscopically examined for *T. gondii* tissue cysts.

2.3. *Maintenance of T. gondii local strain (isolates)*

T. gondii local strain was maintained similar to RH strain according to the procedures of Johnson *et al*. [9]. where the mice (64 mice during the study) about 1 month-old were injected with 2 x 106 tachyzoites of virulent local strain then was scarified by cervical dislocation 2–3 days after inoculation and fixed in dissecting tray. A small slit was carefully made in the mid-line of abdominal wall, muscles and peritoneum. Then the mouse peritoneal cavity was washed by about 5 ml of sterile normal saline using sterile Pasteur pipette. The peritoneal wash was examined microscopically for tachyzoites at high power (X 400), and then collected. The washing repeated until almost all parasites were recovered, then peritoneal wash containing tachyzoites were pooled and stored at 4 ºC, also to avoid bacterial contamination during aspiration of the peritoneal exudates, antibiotics (300 IU of penicillin and 100 mg of streptomycin per 5 ml of normal saline) were added. The last step included the peritoneal fluid was re-inoculated into 3 to 5 mice (0.2 ml / each) containing about 2X106tachyzoites, and further inoculation every 2 - 4 days

4. *Confirmatory Polymerase Chain Reaction (PCR)*

4.1. DNA extraction and PCR amplification:

Genomic DNA was extracted from *T. gondii* local strain (isolates) using a commercially available kit (Dneasy blood & Tissue kit, Qiagen Co., Cat. no. 69504) with the following modifications to the manufacturer's protocols by Burg *et al*. [2]: (i) Tachyzoites was first suspended in 300 µl of ATL buffer (included in the same kit). (ii) lysed tachyzoites suspensions were then incubated with 20µl proteinase K for 3 h at 56°C, followed by incubation with 300 µl ATL buffer at 70°C for 10 min with vortexing for 10 s every 3 min. DNA was purified through the columns according to the manufacturer's protocol and eluted in 50 µl of the supplied AE buffer and then stored at -20°C, then the steps of PCR amplification carried out after DNA extraction as discussed by [2]

3. RESULTS AND DISCUSSION

The two infective developmental stages of *T. gondii* were isolated and could be described as follows: The first developmental stage was Tachyzoites which obtained from the peritoneal exudates of previously inoculated mice 2-3 days earlier during maintenance of the *T. gondii* strain or obtained from mice inoculated with infected digested chickens' tissues after 6-8 days from inoculation. Tachyzoites found inside leucocytes (lymphocytes & macrophages) or free in the peritoneal exudates after rupture of leucocytes.The isolated tachyzoites in freshly obtained unstained peritoneal

exudates were often crescent in shape or banana shape, pointed at one end and rounded or blunt at the other one, their size ranged from (2 – 3) μm in width X (5 – 7) um in length (mean 2 X 7 μm). After the fixation and staining of peritoneal smear with Giemsa stain (Fig 1A), the tachyzoites showed pale blue cytoplasm with reddish purple nucleus, which centrally located or near the blunt end. The leucocytes appear as dark blue color and red blood cells if found appeared grey blue. While, the second stage was tissue cysts containing bradyzoites. The tissue cysts of *T. gondii* 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, 2B). Regarding, the distribution of *T. gondii* in tissue containing bradyzoites cyst in digested tissues during microscopic examination, it was differing from tissue to another and from Governorate to other Governorate. Generally, it's noticed that higher prevalence was in FR than commercial farm chickens and their number and percentage was as follow, (11.6%) 7 of 60 brains, (31.6%) 19 of 60 hearts, (6 .66%) 4 of 60 breast muscle pieces in FR chicken tissues. While, the percentage in CF chicken was as follows (2.9%) 5 of 170 brains, (5.29%) 9 of 170 hearts, (0 .58%) 1 of 170 breast muscle pieces (table 2). These results were lower than that reviewed by [4] in which collective study Of the 149 infected chickens whose individual tissues were bio-assayed, in which *T.gondii* could be detected in 89.5% (129 of 144) of hearts, 49.2% (67 of 136) of brains, 44.1% (15 of 34) of leg muscle and 18.6% (16 of 86) of breast muscle were found to be infected. Also, our results are significantly lower than that recorded by Deyab and Hassanein [3] in which the percentage of positive chickens in bioassay was 78.6%. Moreover these results were lower than that reported by [7] in Grenada where *T.gondii* viable tissue cysts could be detected in 68.5%, 94.2%, 5.7% for brain, heart and breast muscle pieces respectively out of 35 examined chickens. While, the percentage of positive chickens in bioassay was 66 %. Our results are higher than Kuticic and Wikerhauser [12] in which *T.gondii* was isolated in 0.4% of 716 Croatian chicken brain tissue using the Mouse bioassay.

Table 2 Number of chicken used for demonstration of viable *T. gondii* in tissues with their corresponding governorates, age and source of chickens.

Governorate	Source	n	Brain (%)	Heart (%)	Breast muscle (%)	Number of isolates (%)
El Gharbia	CF	60	1 (1.6)	3 (5.0)	0	4 (6.6)
	FR	20	3 (15.0)	7 (35.0)	1 (5.0)	11 (55.5)
Kafr El-Shiekh	CF	35	2 (5.7)	3 (8.5)	1 (2.8)	6 (14.0)
	FR	25	1 (4.0)	8 (32.0)	2 (8.0)	11 (44.0)
Cairo	CF	75	2 (2.6)	3 (4.0)	0	5 (6.6)
	FR	15	3 (20.0)	4 (26.6)	1 (6.6)	8 (53.3)
Total	CR	170	5 (2.9)	9 (5.29)	1 (0.58)	15 (8.8)
	FR	60	7 (11.6)	19 (31.6)	4 (6.6)	30 (50.0)
			12	28	5	45

(FR): free range chicken. (CF): commercial farm chicken.

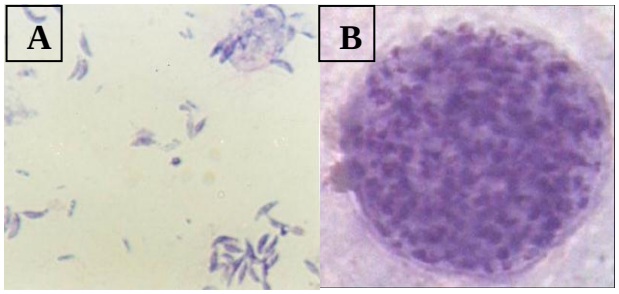

Fig 1 Giemsa stained *T. gondii* Tachyzoites (A; x400) and *T. gondii* tissue cysts (B; x1000)

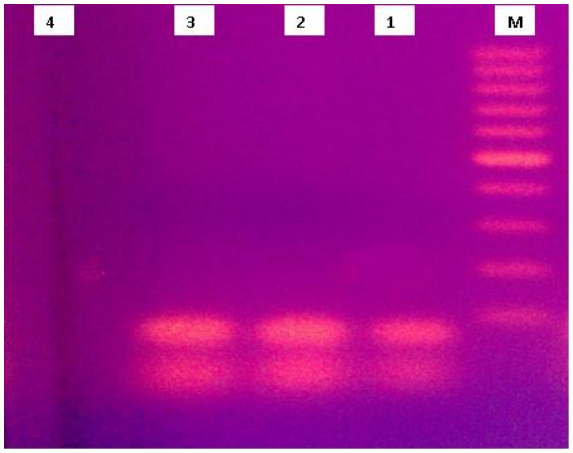

Fig 3 Detection of T. gondii in chicken tissue samples by PCR. M: Mol. wt. marker (100 bp ladder); Lane 1: negative control; lane 2: Positive control; lanes3&4: Positive PCR T. gondii samples at 94bp.

However, similar to that reported in Germany by Boch [1] and in the Czech Republic by Literak and Hejlíček [14]. Also, it's could be concluded from microscopic demonstration in the present study that the distribution of *T. gondii* bradyzoites in tissues and organs was more common in heart muscle than brain but it was very low in breast muscles. This was clear in the examined tissues and organs of FR chickens than that of CF chickens which may be resulted from open access of cats to house reared chickens (FR). As shown it was 31.6% of examined heart tissues in FR chickens while, the percentage was (5.29%) in CF chicken, following heart tissues in distribution of *T.gondii* tissue cyst, were brain tissues in which the percentage reached to 11.6% , 2.9% in FR ,CF chicken respectively. Moreover the lowest percentage of T.gondii bradyzoites could be detected in breast muscle samples in which the percentages were 6.6%, 0.58% in FR ,CF chicken respectively. It seems that the occurrence of toxoplasmosis in chickens depends on various factors, including feeding and management practices and, especially, the access of cats to poultry farms as stated by [12]. Reasons behind this variability in the present study from others could be many; including the age of the chicken, number examined, method of bioassay and the tissues bioassay but the results directed to higher distribution of the parasite in heart tissues than in brain tissues and muscle as stated by [4]. These findings are biologically interesting because *T. gondii* in chicken was considered a neurotropic parasite based on studies in rodents. However, there are now ample data from chickens and other animals' indicating that *T. gondii* encysts in heart more efficiently than in the brain as stated by Dubey and Beattie [6], which is in agreements with the present results. Considering the age, the present study showed that it was higher in older group (FR) than commercial farms (CF) group. The reasons behind this might be that the older animals had more opportunities to get infected than the younger ones; this results close to that discussed by [20]. Concerning PCR results, the main aim for using PCR in this study was confirmation from the isolate (as local strain) belong *T. gondii* and this shown in (Fig. 2), where the number of base pairs matched with stander for *T. gondii* and compared with negative control and, out of 9 isolates (local strain) that successively passage in mice, there is 5 isolates were positive by PCR and matched the stander number of base pairs concerning *T. gondii*. The matched sequence of base pairs with the stander for I in this study confirmed that PCR assay is a specific, speedy, sensitive and cost-effective method for detecting *T. gondii* DNA in chickens. It's noted that most of the previous serological studies in Egypt depend mainly on serological diagnosis while, in the present study confirmation carried out for the parasite by

DNA extraction for the tachyzoite of the local strain. This allows the recovery of parasite DNA at low concentration, from preserved organisms that have undergone special conditions of fast desiccation, low temperatures, high concentration of salt, and neutral pH, conditions which avoid its destruction by the action of the post-mortem autolytic phenomena as stated by [8]. The choice of using the fragment of 194 bp from the B1 gene as target to PCR amplification was based on the observations made by Paabo [17]. In studies with DNA showing a decrease in the efficiency of the amplification process when the DNA sequence target exceeds 200 bp. High specificity, repetitive nature, high level of sequence conservation between the different forms of the parasite and between several strains isolated from clinical samples [2], were factors which influenced the selection of B1 in this study. Thus, to minimize the changes that may have occurred at the genetic level during the parasite evolution history, the utilization of highly conserved sequences increase the chances to recover preserved DNA.It could be concluded from the obtained results that chicken meat should be considered as an important source of transmitting *T. gondii* to man particularly when consumed raw or insufficiently cooked, so improve the educational status of people and teaching them to be aware of the danger of *T. gondii* with reference to its life cycle and mode of transmission.

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الاهميه المشتركه للحويصلات النسيجيه لطفيل التوكسوبلازما جوندي في الدجاج

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الملخص العربي

يعتبر مرض التكسوبلازموزس { داء المقوسات القندية} من أكثر الامراض المشتركة انتشارا فى العالم،و يسبب المرض طفيل التوكسوبلازما جوندي وهو طفيل انتهازي اجباري التطفل وعائله النهائي القطط بجانب انه يصيب الانسان و الحيوان و الطيور كعائل وسيط. وتهدف هذه الدراسه الي تحديد نسب تواجد الحويصلات النسيجيه للطفيل وتوزيعها في انسجة الدجاج من خلال جمع عدد عينات 230 (170 من الدجاج المربي في المزارع و 60 من الدجاج المربي تربيه منزليه) وتشمل هذه العينات أنسجة دماغ، جزء من عضلة الصدرَ والقلبَ لهذا الدجاج المذبوح بنوعيه، ثم تم معاملة هذه الانسجه والاعضاء من خلال الهَضم ثمّ فَحصَها باستخدام الميكروسكوب وتسجيل اعداد ونسب الحويصلات النسيجيه الخاصه بالطفيل. تم ايضا الحقن في جرزان التجارب من اجل التمرير المتتالي للحصول علي العتره المحليه الخاصه بالطفيل بعد فحص اطوارها والتاكد منها، كما تضمنت الدراسه عْملَ مقارنةَ بين الدجاج المربي بالمنازل والدجاج المربي بالمزارع من حيث نسب تواجد الحويصلاتِ النسيجِيه وقد لوحظ أثناء الفحصِ المجهريِ للأنسجةِ ان إنتشارِ الحويصلات انسيجيه للطفيل أعلى في الدجاج المربي بالبيوت مِنْ دجاجِ المزرعةِ التجاريِ من حيث النسب والاعداد وايضا كانت النسب اعلي في العضلات القلبيه عن بقية الانسجه والاعضاء المفحوصه حيث كَانَت النسب الايجابيه في أنسجةِ الدجاجِ المربي بالبيوت كالتّالي، (11.6 %) في الدماغِ، (31.6 %) في القلبِ، (.6 66 %) في عضلةِ الصدرِ بينما كانت في الدجاج المربي بالمزارع (2.9 %) في الدماغِ، (5.29 %) في القلبِ، (.0 58 %) في عضلةِ الصدرِ. علاوة على ذلك أيضاً تم التوصل الي عزل العترِه المحليِّه مِنْ الطور السريع الحاد (Tachyzoites) بعد التأكدِ من مطابقتها لعدد ازواج القواعد باستخدام تفاعل البلمرة المتسلسل، كما تم ذكر التوصيات والمقترحات اللازم اتخاذها من اجل الوقايه من هذا المرض المشترك ذو الاهميه الاقتصاديه في الجانب البيطري بجانب تاثيره علي الصحه العامه.

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