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EPIDEMIOLOGICAL STUDY ON THE ROLE OF QUAILS IN TRANSMITTING OF *TOXOPLASMA GONDII* TO MAN

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ABSTRACT

This study aimed to detect *Toxoplasma gondii* in quail samples collected from different houses in Sohag Governorate by histopathological examination and determine the seroprevalence of IgM and IgG in aborted women using ELISA test. The results revealed that 10% of quail samples were positive for *Toxoplasma gondii*, 48.9 % of aborted women were seropositive for IgM (5.4%), IgG (38%) and both IgM and IgG (5.4%). Higher infection rate was reported in age group ranging from18 to 20 years, in women with first time of abortion and in women live in rural areas. This study highlights on the role of quail as a source of *Toxoplasma* infection for human.

Key words: Quails, *Toxoplasma gondii*

INTRODUCTION

Toxoplasma gondii is an obligatory intracellular protozoan parasite which appears to have broad host specificity. Cats and wild felines are the only definitive host while all other worm-blooded animals including humans are intermediate hosts (Remington *et al.,* 2001). In birds, the disease is usually subclinical with formation of tissue cyst that may persist throughout the life (Dubey, 2002). Nowadays quail become important source of protein in Egypt because it is palatable and cheap in price. Also, it was considered a main dish beside meat and chicken in many houses and hotels, so it is important to chick the presence of *T.gondii* in it.

Beside the vertical transmission of *T.gondii* in human, consumption of under cooked meat with tissue cysts of *T. gondii* can result in the horizontal transmission (Dubey, 2004).

Congenital infection can lead to a wide range of manifestations in the fetus including spontaneous miscarriage or still-birth. Complications in a living infant with congenital toxoplasmosis include microcephalus or hydrocephalus, retinochorioditis and cerebral calcifications, failure to thrive or an apparently normal infant who develops symptoms of central nervous system later in life (Remington *et al.,* 2006).

Toxoplasmosis infection in many individuals especially those with efficient immunity may be a symptomatic or with non specific symptoms, therefore diagnosis relies on serological tests mainly for pregnant women. Serological tests determine whether the infection was acquired recently or in the distant past which is important for treatment (Gieta and Majid, 2012).

MATERIALS AND METHODS

1- Collection and preparation of samples

A- Quail samples

A total of 30 quail samples were collected randomly (3 samples were taken from each quail including liver, brain and lung) from different quail houses in Sohag Governorate. The three organs of each bird were kept in sterilized labeled bottle filled with 10% neutral formalin until histopathological examination.

B- Human samples

A total of 92 aborted women blood samples were collected randomly from Governmental hospital and private clinics in Sohag Governorate. Blood samples were collected by vein puncture and allowed to clott at room temperature then centrifuged for 10 minutes

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at 1000rpm. Serum samples were labeled and kept in deep freeze at -20ºC until examined.

2- Histopathological examination of quail samples

This work carried out in Pathology and clinical pathology department in Assiut University. Specimens (liver, brain and lung) were fixed in 10% neutral formalin then dehydrated in a graded alcohol series, cleared with methyl benzoate and embedded in paraffin wax. Sections of 5µm were cut and stained with Haematoxylin and Eosin for light microscopic examination. Stained sections were examined under light microscope (Olympus CX31, Japan) and photographed using digital camera (Olympus, Camedia C-5060, and Japan).

3- Serological examination of human samples

ELISA technique used for examination of human serum samples using *Toxoplasam* IgG ENZYME IMMUNOASSAY TEST KIT (Cat. No. BC-1085. BioCheck, Inc) for IgG and *Toxoplasam* IgM ENZYMEIMMUNOASSAY TEST KIT (Cat. No. BC-1087, BioCheck, Inc) for IgM.

4- Statistical analysis

The obtained results were analyzed using Statistical Analysis Software (SAS) and the significances of the results were evaluated by Chi-Square (χ^2).

RESULTS

From a total of 30 quail birds three (10%) shown to be infected with *T. gondii* using histopathological examination. Histopathological examination of quail organs revealed that two (6.7%) out of 30 livers, one (3.3%) out of 30 brain, two (6.7%) out of 30 lung show histoplthological lesions for *T. gondii* as shown in figures (1,2,3,4,5,6).

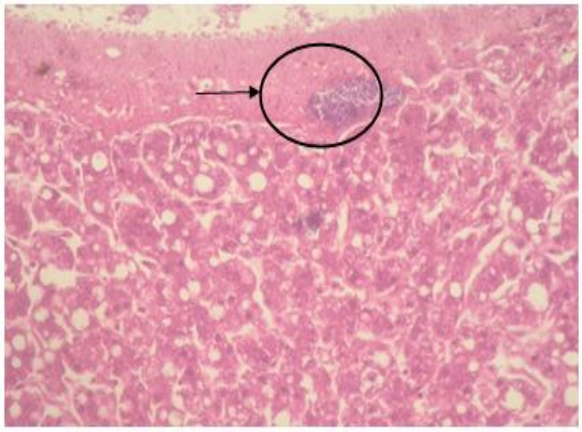

Fig.1: Photomicrograph of liver section showing *T. gondii* cyst in hepatic parenchyma (arrow) associated with hydropic degeneration of the hepatocystes. H&E.×10.

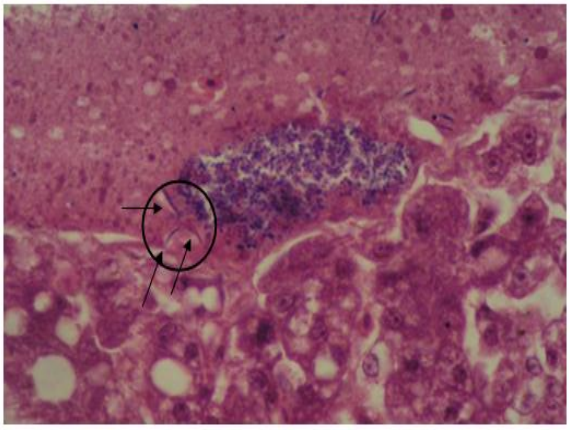

Fig 2: Higher magnification of Fig. 1 showing appearance of *T. gondii* trophozoite (arrow) beside the cyst. H&E.×40.

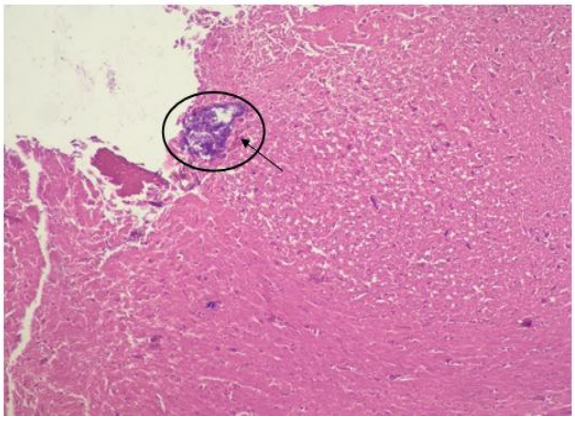

Fig 3: Photomicrograph of brain section showing *T. gondii* cyst in the cereberal tissue (arrow). H&E. ×10.

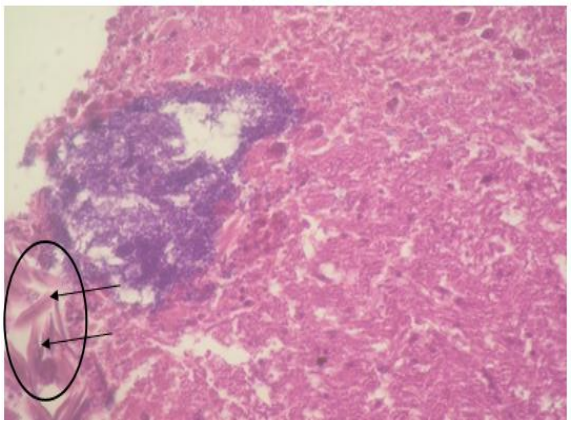

Fig. 4: Higher magnification of Fig. 3 showing trophozoite beside the *T. gondii* cyst (arrow). H&E .×100

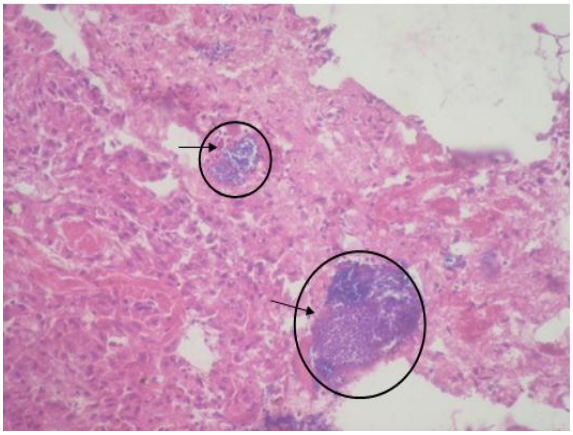

Fig 5: Photomicrograph of lung section showing appearance of *T. gondii* cysts (arrow) in lung tissue associated with inter stitial pneumonia. H&E ×40.

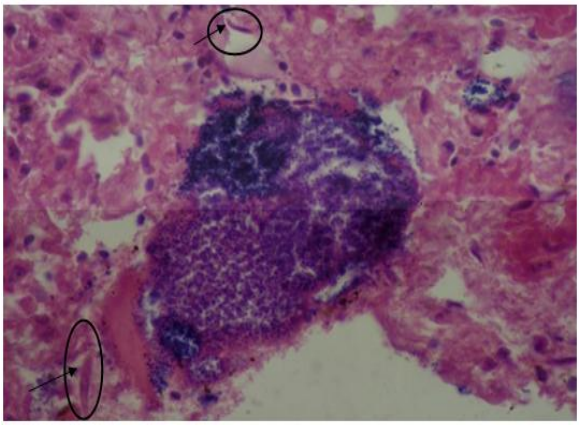

Fig. 6: Higher magnification of Fig. 5 showing appearance of trophozoite beside the *T. gondii* cyst (arrow). H&E ×100.

Table 1: Occurrence of *T.gondii* in aborted women using ELISA test.

No. of tested samples	Seropositive cases		ELISA					
			IgG		IgM		IgG&IgM	
	No.	%	No.	%	No.	%	No.	%
92	45	48.9	35	38	5	5.4	5	5.4

Table 2: Occurrence of *T.gondii* in aborted women according to age.

Age group (years)	No. of examined cases	Seropositive cases			*Toxoplasma* antibodies								
					IgG			IgM			IgG&IgM		
	No.	No.	%	χ^2	No.	%	χ^2	No.	%	χ^2	No.	%	χ^2
18 to 20	7	4	57.1	50.86 P <.0001	3	42.9	21.57 P <.0001	_ _	_ _	55.07 P <.0001	1	14.3	62.53 P <.0001
21 to 29	67	31	46.3		24	35.8		4	5.9		3	4.5	
≥ 30	18	10	55.6		8	44.4		1	5.6		1	5.6	
Total	92	45	48.9		35	38		5	5.4		5	5.4	

Table 3: Occurrence of *T.gondii* in aborted women according to number of abortion.

No. of abortion	No. of examined cases	Seropositive cases			*Toxoplasma* antibodies								
					IgG			IgM			IgG&IgM		
	No.	No.	%	χ^2	No.	%	χ^2	No.	%	χ^2	No.	%	χ^2
1time	37	19	51.4	95.04 P<.0001	15	40.5	14.4 P<.0001	1	2.7	47.4 P<.0001	3	8.1	52.85 P<.0001
2 time	31	16	51.6		13	41.9		2	6.5		1	3.2	
3 time	11	5	45.6		2	18.2		2	18.2		1	9.1	
4 time	10	4	40		4	40		--	--		--	--	
5 time	3	1	33.3		1	33.3		--	--		--	--	
Total	92	45	48.9		35	38		5	5.4		5	5.4	

Table 4: Occurrence of *T.gondii* in aborted women according to residency.

Residency	No. of examined cases	Seropositive cases			*Toxoplasma* antibodies					
					IgG		IgM		IgG&IgM	
	No.	No.	%	χ^2	No.	%	No.	%	No.	%
Rural	58	29	50	44.33 P <.0001	22	37.9	3	5.2	4	6.9
Urban	34	16	47.1		13	38.2	2	5.9	1	2.9
Total	92	45	48.9		35	38	5	5.4	5	5.4

DISCUSSION

Histopathological examination of 30 quail birds revealed that three (10%) were positive for *Toxoplasma* parasite. Raising of quail in Sohag governorate usually occurs in small number at houses which may have access cats near birds food and water, so contaminate them by *Toxoplasma* oocysts.

As shown in figures1,2,3,4,5 and 6; histopathological examination of quail organs revealed that two (6.7%) out of 30 livers, one (3.3%) out of 30 brains and two (6.7%) out of 30 lungs show histopathological lesions for *T.gondii.* This result was lower than that obtained by Dubey *et al.* (1994) who found *T.gondii* in 6/13 (34.6%) of livers, 7/13 (53.8%) of lungs and 7/14 (50%) of brains in infected Japanese quail. The parasite distribution in bird tissue is random; so, the negative result should be interpreted carefully because it is possible that the parasite could be present in unexamined parts of the target tissue (Asgari *et al.,* 2008).

The occurrence of *T.gondii* in aborted women illustrated in Table (1) revealed that out of 92 examined aborted women serum samples, 45(48.9%) were positive for *T.gondii* using ELISA test. The obtained result was higher than that reported by Moalaee *et al.* (1999) and consistent with Abdi *et al.* (2008). The prevalence rate varies with age, cultural habits and environmental factors (Dubey *et al.*, 1998).

The rate of *T.gondii* IgG positive result among aborted women with spontaneous abortion was 35 (38%) indicating chronic infection.

By comparing the obtained result with that reported by some researchers; it was found that our seropositivity was lower than that reported by Laila *et al.* (2004), Harma *et al.* (2004), Jamshaid and Nabila (2007), Nahed *et al.* (2009), Parviz *et al.* (2014) and Samira *et al.* (2016), while Ebadi *et al.* (2011) found a result near to ours. On the other hand our seropositivity rate was high when compared to Al –Qurashi *et al.* (2001), Tabbara and Saleh (2005), Pietro *et al.* (2011) and Zakieh *et al.* (2016). In addition; five (5.4%) out of 92 aborted women were positive for IgM antibodies indicating acute infection.

Generally, detection of anti-toxoplasma specific IgM antibodies is a sensitive indicator of recent infection. A similar result was reported by AL-Qurashi *et al.* (2001) and higher than the prevalence estimated in previous studies of Laila *et al.* (2004), Harma *et al.* (2004), Parviz *et al.* (2014) and Zakieh *et al.* (2016). Remarkably, a much higher prevalence rate was reported by Jamshaid and Nabila (2007), Nahed *et al.* (2009), Adnan and Abdel Monem (2009) and Samira *et al.* (2016).

Occurrence of both *T.gondii* IgG and IgM in aborted women serum was five (5.4%) suggesting subacute infection, this result was higher than Saeed *et al.* (2006) and lower than Samira *et al.* (2016). In pregnancy, it is mandatory to perform additional conclusive tests that must include IgG avidity test, PCR, IgA and IgE on individuals with both positive IgG and IgM (Pereira *et al.*, 2010), because specific *Toxoplasma* IgM antibodies may be persist for 18 months after acute acquired infection (El-sheikha *et al.,* 2009).

Results illustrated in Table (2) represented the occurrence of *T.gondii* in aborted women sera according to age. *T.gondii* infection was reported among age groups ranging from18 to 20 years 4/7(57.1%), 21 to 29 years 31/67 (46.3%), and ≥ 30 years 10/18 (55.6%), the highest prevalence was at age of18 to20 years and ≥30 years and the lowest prevalence was at age of 21to 29 years. This result revealed a significant correlation between the seroprevalence of *T.gondii* infection and different age groups.

High incidence of infection at the age of 18 to 20 (the marriage age in women especially in rural areas) suggested that infection may occurred during childhood due to the playing habits of children but disease doesn't discovered until marriage and pregnancy because of low information about *Toxoplasam* and its health hazards (Al –Qurashi, 2004).

Higher prevalence among the group elevated age indicating a higher risk of exposure as age increases (Rai *et al.,* 1999). When women increased in age they are more likely to be involved in house work, agricultural activities, rearing of animals (especially in rural areas) and taking care of children, and therefore have a higher chance to being exposed to *T. gondii* infection. This completely agree with Hung *et al.* (2007) who mentioned that older age group of ≥ 35 years old had a significantly higher seroprevalence than that of the younger age group of 15 to 25 Years.

Our result revealed that the prevalence of IgG at age of 18 to 20 years, 21 to 29 years and ≥ 30 years was 42.9%, 35.8% and 44.4% respectively. This result was within the range of Nahed *et al.* (2009) and higher than the result reported by Adnan and Abdel Monem (2009). On the other hand, a rate of IgM in our investigation at 21 to 29 years and ≥ 30 years were 5.9% and 5.6% in agreement with Pietro *et al.* (2011). From this result it is clear that seropositivity rate increased with age; this may be explained by the fact that older women have been exposed to infection for a longer period of time and may retain a steady level of anti-*Toxoplasma* IgG in serum for years (Babaie *et al.,* 2013).

A significant correlation between seropositive cases and number of abortion was illustrated in Table 3 which clarify that the seroprevalence for1 time (51.4%), 2 times (51.6%),3 times (45.6%),4 times (40%) and 5 times (33.3%). This revealed that higher abortion time was in first and second times and slightly decreased in third, fourth and fifth times. High frequency of repeated abortion indicated a re-exposure or reactivation of *Toxoplasma* infection during pregnancy. Such percentage is considered higher than that previously reported by Adnan and Abdel Monem (2009), while Laila *et al.* (2004) detect a higher result 143 (96.6%) for 1-2 time of abortion and a lower result 5(3.4) for ≥ 3 abortion time. The same conclusion was recorded by Muna and Nadham (1996) who indicated a clear association between *Toxoplasma* infection and habitual abortion.

With regarding to location, significant difference was observed between rural and urban residency. Our seropositivity rates indicated that women from rural areas were found to be more risky to *Toxoplasma* infection (50%) than that live in urban areas (47.1%) (Table 4). The possible reason for this difference is that women from villages were found more exposed to *Toxoplasma* infection than who reside in city because they are in frequent contact with animals, soil, fresh infected meats, drinking of untreated water, eating of raw vegetables, drinking unboiled milk and poor sanitary facilities. This result was disagree with Laila *et al.* (2004) who detected 25.7% rural and 74.3% urban women at high risk.

It was obviously that in our study the prevalence in urban areas was not low. This may be due to high income of urban areas and their different habits in eating poultry and junk food from restaurants which have been found to be a major source for *T.gondii* transmission (Sroka, 2010). Consumption of under cooked meat was a significant risk factor, this may be attributed to the habit of eating some Egyptian food containing under cooked meat as kabbab, shawerma, hawawshy and luncheon (Amany *et al.,* 2015). In addition, with the continuous development of society in Sohag; more people are starting to keep pets as cats and dogs, with inadequate veterinary inspection enhance the risk to pet owners of toxoplasmosis.

In the present study it was observed that titre of IgG antibodies were high in all groups of women as compared to titre of IgM antibodies. This may be due to that infection with *T.gondii* at acute stage usually asymptomatic or have flu like symptoms that resolve spontaneously, so disease usually not discovered in early stage (containing IgM antibodies), but detected at chronic stage after formation of IgG antibodies. This is supported by the fact that IgM antibody titre rises from 5 days to weeks followed acute infection, reaching a maximum after 1 to 2 months and decline more rapidly than IgG (Stray, 1993). But IgG antibodies are detected for years after acquired infection and are usually present throughout the life (Liesenfeld *et al.,* 1997).

Toxoplasmosis will remain a problem, mainly in risk groups such as pregnant women and immunocompromised patients. So both IgG and IgM tests are recommended as routine tests among pregnant women at Sohag Governorate. Improvement can be attained by increasing prevention and reducing the risk factors, so a health program should provide the public with information about the disease, its risk factors, and the negative influence on the fetus. On the other hand a program should be established to allow all pregnant women to be screened in their first trimester.

CONCLUSION

Good hygienic practice should be followed during quail breeding to reduce their infection with *T.gondi* and continuous surveillance by agriculture and veterinary authorities is required to reduce toxoplasmosis transmission. Early detection of *toxoplasma* antibodies in pregnant women is important to detect acute and chronic infection which play a role in treatment and prevent congenital transmission.

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دراسة وبائية عن الدور الذي تلعبه طيور السمان في نقل مرض التكسوبلازما للإنسان

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تهدف هذه الدراسة إلي تشخيص التكسوبلازما جوندي بالفحص الخلوي في عينات طيور السمان والمجمعة من مزارع متفرقة بمحافظة سوهاج. وبإختبار القياسة المناعية الإنزيمية (ELISA) لكل من الأجسام المضادة IgM و IgG والمجمعة من سيرم الدم من السيدات المجهضات. وقد أسفرت النتائج عن وجود طفيل التكسوبلازما في طيور السمان بنسبة ١٠% و بنسبة ٤٨,٩% في سيرم السيدات المجهضات بواقع ٥,٤% للأجسام المضادة IgM و ٣٨% للأجسام المضادة IgG و كلاهما معا ٥,٤%. كما سجلت أعلي نسبة للإصابة في الفئة العمرية من ١٧ – ٢٠ عاما (٥٧,١%) وفي السيدات المجهضات لأول مرة (٥١,٤%) وكذلك اللواتي يقيمن في الريف (٥٠%). وهذه الدراسة تسلط الضوء علي الدور الذي تلعبه طيور السمان كمصدر للعدوي بمرض التكسوبلازما في الإنسان.