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Cryptosporidiosis and *Toxoplasmosis* in Native Quails of Egypt

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ABSTRACT

Cryptosporidium spp. and *Toxoplasma gondii* are public health important protozoan parasites. The presence of *Cryptosporidium* spp. oocysts and anti-*Toxoplasma gondii* antibodies in fecal and serum samples from native quails in different farms in Giza, Egypt has been carried out, respectively for the first time in Egypt., through the parasitological and serological examination. The extent of *Cryptosporidium* oocysts invasion was found to be 31.9% (lower extent 30.8% in bobwhite and higher 33.3% in brown quails). Three *Cryptosporidium* spp. oocysts were detected varied from small (*Cryptosporidium meleagridis*), medium (*Cryptosporidium baileyi*) and large size (*Cryptosporidium galli*). The *T. gondii* antibodies prevalence was 29.8 and 25.5%, using Modified Agglutination Test (MAT) and Latex Agglutination Test (LAT), respectively (lower prevalence 25.5 and 22.4% in bobwhite while higher prevalence 34.4 and 28.8% in brown quails). The obtained results indicated that Egyptian quails are reservoirs for both zoonotic *Cryptosporidium* spp. and *Toxoplasma gondii*. So that, it is imperative for human to avoid consuming insufficiently cooked quail meat. Also fecal contamination from quail should be controlled.

Key words: *Cryptosporidium* spp., *Toxoplasma gondii*, fecal examination, modified agglutination test, latex agglutination test

INTRODUCTION

Cryptosporidia and *Toxoplasma* are emerging coccidian parasite zoonoses that transmitted to humans either by ingesting environmentally robust transmissible resistant stage oocysts through contact with contaminated faeces or ingestion of contaminated water and food in case of both *Cryptosporidia* and *Toxoplasma* or by eating raw or undercooked meat containing infective tissue stages in case of *Toxoplasma* (Nissapatorn, 2007; Dorny *et al.*, 2009; Smith and Nichols, 2010).

Cryptosporidiosis is one of the most prevalent parasitic infections in domesticated, caged and wild birds (Ryan, 2010). *Cryptosporidium* species have been reported in more than 30 avian species worldwide (Sreter and Varga, 2000; Ng *et al.*, 2006; Seva Ada *et al.*, 2011). The infection caused by several genotypically diverse *Cryptosporidium* species, has been dynamically changing over the past decade from that of a rare, largely asymptomatic infection to an acute enteric disease of animals and humans (Ramirez *et al.*, 2004; Moghaddam, 2007; Fayer and Xiao, 2008).

There have been extensive studies conducted on avian cryptosporidiosis; however, the prevalence data for the determination of *Cryptosporidium* infection in quails in the different

localities of the world is much less known. In USA, examined intestinal content from different quail species for *Cryptosporidium* oocysts, only 18 (14.9%) of 121 birds had *Cryptosporidium* oocysts in their feces at time of collection (Duszynski and Gutierrez, 1981). In Azerbaijan, the invasion extensities of *Cryptosporidium* oocysts invasion in chickens, pheasants, peacocks and quails were 50.6% (135 out of 269), 7.9% (16 out of 203), 12.9% (8 out of 62) and 21.7% (26 out of 120), respectively (Musaev *et al.*, 1998).

However, many avian species are intermediate hosts of *Toxoplasma gondii*, the prevalence of *T. gondii* infection in quail species is little known, experimental toxoplasmosis in bobwhite and Japanese quails through oral inoculation with ME 49 strain *T. gondii* oocysts had been done by Dubey *et al.* (1993) and Dubey *et al.* (1994), respectively, the infective stages were isolated from the brains, hearts and skeletal muscles of all quail by bioassays in mice and quails that survived develop severe protozoal pneumonia, myocarditis or meningo-encephalitis. The *T. gondii* antibodies were found in quail sera 63 Days After Infection (DAI) using MAT and LAT. Also, Albuquerque *et al.* (2001), observed *T. gondii* tachyzoites from 7th DAI, in the liver, lung and spleen and the *T. gondii* tissue cysts observed in 70th DAI in brain and heart of Japanese quails experimentally inoculated with tachyzoites of 'P' strain while no parasites were observed in any animals after inoculation with tachyzoites of SERO-47 strain of *T. gondii*.

Since largely unknown concerning *Cryptosporidium* and *T. gondii* infection prevalence in Egyptian native quails, so the objectives of the study reported here were to investigate the native quails parasitological and serological to *Cryptosporidium* and *T. gondii* and refer to their zoonotic importance in transmission of infection to human.

MATERIALS AND METHODS

Fecal and blood samples: This research project was conducted from 1-2011 to 4-2011. A total of 210 quail fecal samples (120 from bobwhite and 90 from brown quails) and 188 quail blood samples (98 from bobwhite and 90 from brown quails) were collected from different quail farms in Giza, Egypt. Sera were separated, labeled and kept at -20°C until use.

Fecal examination: Fine feces smears fixated with methanol spirit and stained with Modified Ziehl-Neelsen Stain (MZN) for detection of *Cryptosporidian* oocysts according to the procedure described by Henriksen and Pohlenz (1981). The preparations were observed and the oocysts were measured with help of stage micrometer conjugated with the light microscope at the eyepiece 10x and the objective 100x. The all measurements are in micrometers (μm) for about 20-50 oocysts, with the range in parenthesis following the mean (Yatswako *et al.*, 2007; Fayer and Xiao, 2008).

Serological assay

Modified Agglutination Test (MAT): Formalized-fixed whole tachyzoites antigen was prepared using RH strain of *T. gondii* secured in Department of Zoonotic Diseases, National Research Center, Egypt as described by Desmonts and Remington (1980) and the test procedures was carried out according to the method of Dubey and Desmonts (1987), considering 1:25 serum dilution as the cut-off point.

Latex Agglutination Test (LAT): The collected quail sera were testing against *Toxoplasma* infection using latex agglutination test kit (Toxocheck-MT; Eiken Chemical, Tokyo, Japan)

Table 1: Prevalence of *Cryptosporidium* infection among quail

Quail spp.	Examined No.	Positive No.	Positive (%)
Bobwhite quails	120	37	30.8
Brown quails	90	30	33.3
Total quail spp.	210	67	31.9

Table 2: The dimensions of *Cryptosporidium* oocysts detected in quails

Quail spp.	Dimensions of the oocysts (µm)		Shape index
	Length×Width	Mean	(SI) L/W
<i>Cryptosporidium meleagridis</i>	(4.5-6.0)×(4.2-4.6)	5.2×4.4	1.0-1.2 (1.1)
<i>Cryptosporidium baileyi</i>	(5.5-7.4)×(4.4-5.3)	6.4×4.8	1.1-1.4 (1.3)
<i>Cryptosporidium galli</i>	(8.0-8.5)×(6.2-6.4)	8.2×6.3	1.0-1.3 (1.3)

Table 3: Prevalence of *T. gondii* antibodies among quail sera using different serological tests

Quail sp.	No. of examined	Prevalence			
		MAT		LAT	
		Positive No.	(%)	Positive No.	(%)
Bobwhite quails	98	25	25.5	22	22.4
Brown quails	90	31	34.4	26	28.8
Total quail spp.	188	56	29.8	48	25.5

MAT: Modified agglutination test, LAT: Latex agglutination test

following the manufacturer's instructions. Sample was considered positive when agglutination observed at dilutions of 1:64 and greater.

RESULTS

Detection of *Cryptosporidium* oocysts in quails by fecal examination: Quail Fecal samples have been examined concerning the presence of *Cryptosporidium* spp. oocysts. The extent of invasion was 31.9% (67 out of 210) of quail fecal samples were found to be infected with *Cryptosporidium* spp. oocysts. The bobwhite quails revealed lower prevalence 30.8% (37 out of 120) while the brown quails showed higher *Cryptosporidium* infection 33.3% (30 out of 90) (Table 1).

The dimensions of *Cryptosporidium* oocysts detected in quail: The measurements of detected *Cryptosporidium* oocysts in the examined quail feces were revealed three *Cryptosporidium* species of round or slight ovoid shaped oocysts. They were varied from small sized (*Cryptosporidium meleagridis*) oocysts of 5.2×4.4 µm dimensions and 1.1 µm in shape index; medium sized (*Cryptosporidium baileyi*) oocysts of 6.4×4.8 µm dimensions and shape index 1.3 µm and large sized (*Cryptosporidium galli*) oocysts 8.2×6.3 µm in dimensions and 1.3 µm in shape index (Table 2, Fig. 1a-c).

Serological examination for the detection of *T. gondii* antibodies: Examination of the 188 serum samples from slaughtered quails through MAT and LAT revealed that 56 (29.8%) and 48 (25.5%), respectively had antibodies against *T. gondii*. The bobwhite quails revealed lower prevalence 25.5 and 22.4% (25 and 22 out of 98) while the brown quails showed higher *T. gondii* infection 34.4 and 28.8% (31 and 26 out of 90), respectively by MAT and LAT (Table 3).

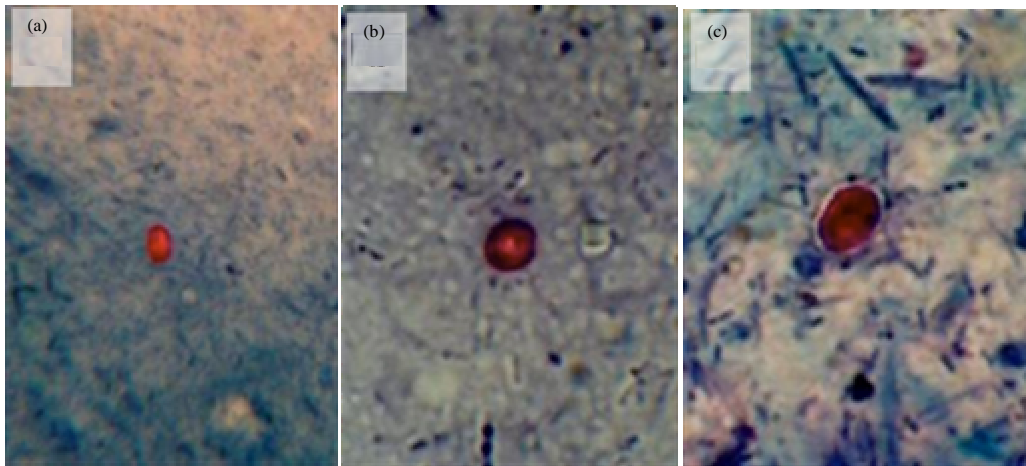


Fig. 1 (a-c): *Cryptosporidium* oocysts in quail fecal smears stained with Modified Ziehl-Neelsen (MZN) (x100). (a) *Cryptosporidium meleagridis*, (b) *Cryptosporidium baileyi* and (c) *Cryptosporidium galli*

DISCUSSION

The extent of *Cryptosporidium* oocysts invasion in quails in this study was found to be 31.9% (lower extent 30.8% in bobwhite and higher 33.3% in brown quails). These finding was higher than (14.9 and 21.7%) detected by Duszynski and Gutierrez (1981) in different quail species from USA and Musaev *et al.* (1998) in quails from different localities in Azerbaijan.

The variation in the invasion extensivities of *Cryptosporidium* oocysts in quails examined during the present study and those previously surveyed are probably attributed to the number of birds examined, time of feces collection and examination and the hygienic conditions of the quail farms and its surrounding environment. On the same approach, O'Donoghue (1995), Fayer *et al.* (2000) and Ramirez *et al.* (2004), also proved that the variation in *Coccidian* oocysts incidence in birds was associated with the age and sex of the bird examined, the methods used to make the diagnosis, climatic conditions, stresses exposed by birds and differences in management methods.

The three *Cryptosporidium* species (*Cryptosporidium meleagridis*, *Cryptosporidium baileyi* and *Cryptosporidium galli*) detected in quail fecal smears in this study are morphologically similar with the same species detected in quails and birds in many previous studies (Zha and Jiang, 1994; Fujino, 1996; Ryan *et al.*, 2003). The *Cryptosporidium* spp. identification was depending upon the conventional criteria, such as oocyst morphology and measurements, this opinion was agreed with Fayer *et al.* (2000) and Morgan-Ryan *et al.* (2002), who cited that morphometric measurement of oocysts represents the cornerstone of *Cryptosporidium* taxonomy and is one of the requirements for establishing a new species.

A coccidian parasite of humans and animals was first believed to be an opportunistic organism but now is recognized as a primary pathogen, several studies have implicated animals as a source of human infection (Levine *et al.*, 1988; Caver *et al.*, 1996). Because strains of *Cryptosporidium* detected in this study are cross-transmissible especially *Cryptosporidium meleagridis*, awareness of cryptosporidiosis as a potential zoonotic infection has emerged as a significant public health concern (Hoerr *et al.*, 1986).

Quail in the present study responded serologically to *T. gondii* through MAT and LAT; high levels of *Toxoplasma gondii* antibody titers were higher with the MAT (29.8%) than with the LAT (25.5%). These results are nearly agreed with that proved by Dubey *et al.* (1993) and Dubey *et al.* (1994) who they found that antibody titers to *T. gondii* in sera of experimentally infected quails by the MAT using whole tachyzoites were higher than in LAT and Indirect Hemagglutination Tests (IHT) that used soluble antigens and antibodies were not detected in quail sera examined by the Sabin-Feldman dye test.

However, *T. gondii* prevalence in quails with the MAT (29.8%) and the LAT (25.5%) in this study are slightly lower than that results obtained with Asgari *et al.* (2008) among free-ranging chickens (27.1%) in Southern Iran while it is largely lower than that obtained with Dubey *et al.* (2003) in free range chickens (40.4%) from a rural area surrounding Giza, Egypt using MAT and with Butty (2009) among turkeys (76.63%) from ten regions in Ninevah governorate, Iraq using LAT and this finding may due to chickens and turkeys become infected mostly by feeding from the ground contaminated with oocysts and they considered as a good indicator of prevalence of *T. gondii* in their environment (Ruiz and Frenkel, 1980).

The Modified Agglutination Test (MAT) is considered the more sensitive and recommended test for diagnose the *T. gondii* infection in several animals and man (Dubey, 1997; Garcia *et al.*, 1999). Moreover the highest sensitivity and specificity among all serological tests of MAT was confirmed by the results obtained by Shaapan *et al.* (2008) and Shaapan *et al.* (2010) who demonstrated the benefits of using more sensitive and specific MAT for the detection of *T. gondii* antibodies in sheep, goat and buffaloes sera which is cheaper, easier than other tests and does not need special sophisticated equipment.

CONCLUSION

It is of interest to point out that, this study is the first report in Egypt for the detection of *Cryptosporidium* oocysts and *Toxoplasma gondii* antibodies in native quails. The high prevalence rates obtained by this study indicate the potential risk of its transmission to humans through contact with feces or meat consumption of quails. Also biological and molecular characteristics of the detected *Cryptosporidium* spp. will necessary to be investigated to confirm their diagnosis.

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