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Seroprevalence of toxoplasmosis in quail birds (*Coturnix coturnix*) in Baghdad City, Iraq

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***Abstract*---**Toxoplasmosis is a common zoonotic parasitic disease in humans and animals worldwide, caused by the protozoan parasite *Toxoplasma gondii*. The study designed for detection the prevalence rate of Toxoplasmosis in quail birds (*Coturnix coturnix*) from different areas in Baghdad city, Iraq (Al- Ghazel souq**,** Al-Malhani**,** Al-Hurria, Al Shoula**'**a and quails farms in Abu-Ghraib Poultry Research Station) by indirect ELISA *IgG* test and determined the effects of some risk factors (sex, age, areas and months) on this rate during the period from the 1st January 2021, to 30th September 2021. One hundred and fifty sera of quails (75) hens and (75) cocks were examined by indirect *IgG* Enzyme Linked Immunosorbent Assay (ELISA). The results clarified the total infection rate was 19.33% (29/150) in quails 24% (18/75) hens and 14.66% (11/75) cocks. The higher infection rate 24% (24/100) was recorded in the age group ≤ Maturity period (35 day_s) ≤ Year (1-2y_s), while a lower infection rate 10% (5/50) was found in the age group < Maturity period (35 day_s). A higher infection rate 41.17% (7/17) was recorded in September, and lower infection rate 5.88% (1/17) was recorded in July. Al- Ghazel souq**,** Al-Malhani, Al-Hurria, Al-Shoula'a and quails farms in Abu-Ghraib Poultry Research Station areas were showed the infection rates 33.33% (10/30), 20.00% (6/30), 10.00% (3/30), 10.00% (3/30), and 23.33% (7/30) respectively, with significant differences (P≤0.05)**.** Hens recorded higher rate of infection (24%) than cocks (14.66%) increase with age without significant difference (P≤0.05). The results of this study revealed the presence of *T. gondii* infection in quails for the first time in Iraq.

Keywords*---Toxoplasma*, Quail birds, Seroprevalence, Indirect ELISA *IgG*.

Introduction

Toxoplasmosis is one of the most prevalent and important foodborne and waterborne zoonotic infectious diseases of medical and veterinary importance worldwide, caused by a highly successful obligate intracellular protozoan parasite *Toxoplasma gondii*, that can be life threatening when opportunistic or congenital in both humans and animals, so its diagnosis requires accurate and rapid techniques that rely mainly on serology and molecular method (Abbas *et al.*, 2020 and Robert *et al.*, 2021).

Toxoplasma gondii definitive host in which sexual reproduction occurs are strictly members of the felidae family only, but it is able to infect almost all warm blooded vertebrates, including humans and birds that harbour infective cysts in their tissues (Tenter *et al.*, 2000; Dubey, 2010a and Lorencova *et al.*, 2015).

Detection of *T. gondii* in ground feeding birds is considered as an excellent indicator for the heavy contamination of the environment with the oocyst excreted in cat feces (Dubey, 2020). Different birds such as chickens, ducks, turkey, pigeons and game have been reported to be infected with *T. gondii* and are regarded as potential sources for human infection if not handled and cooked properly, so it regarded as a significant public health problem worldwide (Hussein *et al* 2018 and Dubey, 2020).

Quail birds, one of the smallest members of the order Galliformes, are nutritious food consumed by humans and are also used as medicine in some parts of the world (Cong *et al.*, 2017). They are affected by common poultry diseases but are fairly disease resistant. Nowadays quail become important source of protein in Iraq 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 although it poses quality resistance to disease than those of chickens.

In birds, the infection is usually subclinical with formation of tissue cyst that may persist throughout the life (Dubey, 2002 and Atasever *et al.*, 2020). Moreover, previous study showed that *T. gondii* infection could cause death in quails with severe apathy, dyspnea and diarrhea (Casagrande *et al.*, 2015).

Considering the importance of birds and poultry in transmission of *T. gondii* to human and felids, since the prevalence of *T. gondii* in poultry is a bio-indicator for soil contamination with *T. gondii* oocysts (Al Khalid *et al.*, 2012 and Dubey, 2020) as well as study in Egypt highlights on the role of quail as a source of *Toxoplasma* infection for human (Hussein *et al.*, 2018) and according to previous knowledge, there is no information on *T.gondii* infection in quail birds intended for human consumption in Iraq is available. Therefore, we conducted the present serologic study for *T. gondii* infections in quail birds in Baghdad city.

Materials and Methods
Samples collection:

- Quail birds
  One hundred and fifty quail birds both sexes (75) cocks and (75) hens with age ranged from less than sexual maturity ($35day_s$) to more than one year purchased**,** from the local markets.
- Blood samples
  Three milliliter (ml) of blood was drawn from wing vein of each apparently healthy quail birds, nearly 17 blood samples every months, using a disposable syringe of 5 cc size**,** collected in gel tubes without anticoagulant for serum collection and left 30 minutes at room temperature to form clot**,** and subsequently centrifuged at 3000 round per minute (rpm) for 5 minutes**.** Sera aspirated by using micropipette**,** dispensed into another sterile fully labeled with date**,** serum and bird identification Eppendrof-tube kept in deep freeze at -20˚C until used for *Toxo-IgG*-ELISA test (Mikaeel and Al-Saeed, 2020) in parasitology laboratory of Kamal Al-Samarraee Hospital.

Avian–ELISA Kits-ID Screen® (ID Screen® -France) kit was used for detection of specific antibodies to *T. gondii* in quail birds by *IgG* ELISA technique (Mikaeel and AlSaeed, 2020). The optical densities of the reactions were determined according to the manufacturer's instructions by measuring the absorbance at 450 nm using a micro plate reader (Bio-Tek-USA). The results are expressed as (S/P%) percentage of the mean absorbance calculated according to the following formula:
S/P% = (OD sample-ODNC) / (ODPC-ODNC) ×100.
Depending on manufacturer's recommendation sera were regarded negative when S/P% < 50% and positive when S/P% ≥ 50%.

Statistical Analysis:

Statistical Analysis System-SAS (2012) used to detect the effects of difference factors such as age, sex, area and months in the infection rate and Chi-square test (x2) used to compare the significance (P ≤ 0.05 or 0.01) difference in this study (Al-Hadad and AlRubaie, 2021).

Results
Total infection rate:
The total infection rate of *T.gondii* by indirect ELISA *IgG* test in quail birds was 19.33% (29/150), as clarified in Table (1)

Table (1)
Total infection rate of *T. gondii* in quail birds by ELISA *IgG* test

Host	Examined No.	Positive No.	Percentage (%)
Quail birds	150	29	19.33

Infection rate of *T. gondi* in quail birds according to the sex by ELISA *IgG* test:

Hens quails recorded high rate of infection with *T. gondii* 24% (18/75) while cocks quails recorded 14.66% (11/75) without significant difference (NS) (P ≥ 0.05)**,** as clarified in Table (2).

Table (2)
Infection rate of *T. gondi* according to the sex of quail birds by indirect ELISA *IgG* test

Sex	Examined NO.	Positive NO.	Percentage (%)
Hens	75	18	24
Cocks	75	11	14.66
Total	150	29	19.33
Chi Square (x^2) P-value	---	---	1.689 NS 0.193
Non-Significant (NS).			
(P ≤ 0.05).			

Infection rate of *T. gondi* according to the age of quails birds by Indirect ELISA *IgG* test:

The result showed a significant difference between two age groups of quail birds that were infected with toxoplasmosis. Adult quail birds (≤ Maturity period (35 day_s) ≤ Year) reported a high infection rate 24% (24/100)**,** whilst the lower infection rate 10% (5/50) occurred in young quail birds **<** Maturity period with significant difference * (P≤0.05)**,** as clarified in Table (3).

Table (3)
Infection rate of *T. gondi* according to age groups of quail birds by indirect ELISA *IgG* test

Age groups		Examined No.	Positive No	Percentage (%)
Young	< Maturity period (35 day_s)	50	5	10
Adults	≤Maturity period ≤ Year	100	24	24
Total		150	29	19.33
Chi-Square (x^2)		4.92 *		
P value		0.0396		
* (P≤0.05).				

Infection rate of *T. gondii* in quail birds according to months by indirect ELISA *IgG* test:

Significant differences*(P≤0.05) were recorded between rate of infection according to months of study, a high infection rate 41.17% (7/17) was recorded in

September and the low infection rate 5.88% (1/17) found in July, as clarified in Table (4).

Table (4)
Infection rates of *T. gondii* in quail birds according to the months of the study by ELISA *IgG*:

Months/2021	Examined No.	Positive No.	Percentage (%)
January	14	3	21.42
February	17	5	29.41
March	17	2	11.76
April	17	2	11.76
May	17	4	23.52
June	17	3	17.64
July	17	1	5.88
August	17	2	11.76
September	17	7	41.17
Total	150	29	19.33
Chi-Square (x^2)	12.305 **		
P value	** (P≤0.01).		

Infection rate of *T. gondii* in quail birds according to the areas of study by indirect ELISA *IgG* test:

Higher significant difference ****(P≤0.01)** was recorded in the infection rate of *T. gondii* between areas of study in Quail birds**.** A high infection rate 33.33% (10/30) was recorded in AL-Ghazel Souq**,** while the low infection rate 10.00% (3/30) was found in ALHuria and Al-Shulla by ELISA *IgG* test**,** as clarified in Table (5).

Table (5)
Infection rate of *T. gondii* in quail birds according to the areas of study by ELISA *IgG* test

Areas	Examined No.	Positive No.	Percentage (%)
Al-Huria	30	3	10.00
Al- Ghazel Souq	30	10	33.33
Abu-Ghurib	30	7	23.33
Al-Malhani	30	6	20.00
Al-Shulla	30	3	10.00
Total	150	29	19.33
Chi-Square (x^2)	8.739 **		
P value	0.0052		
** (P≤0.01).			

Discussion

The choice of Indirect ELISA *IgG* as a diagnostic tool for the current study was made because of its high sensitivity and specificity compared to other serological tests (Shaapan *et al.*, 2008; Al Khalid *et al.*, 2012; Mikaeel and Al-Saeed, 2020).

The results of the current study showed that the total infection rate of toxoplasmosis in quail birds by indirect ELISA *IgG* test was 19.33% (29/150) that varied from that recorded in Giza, Egypt by Shaapan *et al.*, (2011) who recorded that The *T. gondii* antibodies prevalence was 29.8% (56/188) and 25.5% (48/188), by Modified Agglutination Test (MAT) and Latex Agglutination Test (LAT), respectively (lower prevalence 25.5% (25/98) and 22.4% (22/98) in bobwhite quails while higher prevalence 34.4% (31/90) and 28.8% (26/90) in brown quails). Also disagreed with Sadia *et al.*, (2012) who recorded an infection rate 4% (8/200) in quail birds among 200 captive birds in Lahore, Pakistan by LAT, and Cong *et al.*, (2017) who revealed that the presence of *T. gondii* infection 9.52% (59/620) in common quails in China by MAT, while agreed with Al-Dabbag *et al.*, (2021) who revealed by an ELISA test the infection percentage of *T. gondii* infection in chickens was17.5% (16/91) in Mosul city, and also agreed with Feng *et al.*, (2016) who found Antibodies to *T. gondii* were found in 18.86% (132/700) of the chickens by modified agglutination test in Henan Province of china. And with Mahmood *et al.*, (2014) who revealed by Indirect Hemagglutination Antibody (IHA) test the infection percentage of *T. gondii* infection in chickens was 18.86% (101/536).

The difference between the present study and the previously reported may be related to the many factors such as management practices and hygienic standards in breeding, cat densities and environmental condition are effect on the acquisition of *T. gondii* oocyst by animal, also these differences in the infection rates could be due to the difference in the sample numbers used and the kind of the samples**,** different serological techniques were used**,** MAT**,** LAT and ELISA**,** differing geographical locations of those countries**.** Also the difference in seroprevalence could be attributed to difference in breed and species of quail birds (Al-Khaled *et al.*, 2012; Gebremedhin *et al.*, 2015; Al abodi, 2017 and Yaowalark *et al.*, 2017). The increasing infection rates of *T. gondii* in poultry is believed to be due to the feeding pattern of the bird, where birds that feed from the ground directly are subjected to food contamination especially *T. gondii* oocyst, and can be regarded as important indicators for the environmental contamination and source for human infection (Dubey, 2010; Mohammed and Abdullah, 2013). In addition, it has been reported that cats could enhance the chickens' toxoplasmosis through their defecation on grass where chicken fed on (Mose *et al.*, 2016).

According to sex, the present study has also investigated the infection rate of *T. gondii* between both sexes. The data found that the infection rates 14.66% (11/75) were lower in cocks than that of hens 24.00% (18/75). These results are in line with some studies (Akhtar *et al.*, 2014 and Mahmood *et al.*, 2014) in Pakistan, and (Mikaeel and Al-Saeed, 2020; AlHadad and Al-Rubaie, 2021) in Iraq, who recorded an infection rate higher in hens than cocks, and agreed with Khan *et al.*, (2020) who recorded an infection rate higher in hens (16.7% *IgG*) than

cocks (11.7% *IgG*), while disagreed with Feng *et al.*, (2016) who mentioned the seropositivity rates varied with respect to sex, with antibodies being found in 33.59% of cocks and 14.48% of hens, and this due to *T. gondii* change the concentration of steroid hormones in the host and enhance the susceptibility of males or females to toxoplasmosis (Flegr *et al.*, 2008 and Franco *et al.*, 2014), and disagreed with Cong *et al.*, (2017) who detected *T. gondii* infection in cocks and hens common quails was 8.10% and 10.71%, respectively by MAT and also disagreed with Al-doury *et al.*, (2018) who detected *T. gondii* infection in cocks and hens was 27.3% and 12.7%, respectively by *Toxo*-Latex slide agglutination. It has been reported that the female hormones play an important role in the susceptibility of the animals to *T. gondii* through reducing their immune responses to the infection (Robert *et al.*, 1995 and Tilahun *et al.*, 2018). Estrogen has been enhanced antibodies production. The immunity can be broken down by various factors including nutrition, age, reproductive and environmental factors (Tasawar *et al.*, 2012).

The infection with *Toxoplasma* according to the months of the study showed variable rates with higher significant differences (P≤0.01). The highest rate of *Toxoplasma* infection was 41.17% (7/17) and seen in September, while the lowest was 5.88% (1/17) observed in July by ELISA. These results are in agreement with Andrade *et al.* (2013) in Brazil who found that infection rates increased in autumn months, also with Armand *et al.* (2016) recorded low rates in summer. The variation between months of study and the previously reported was attributed to fluctuation in environmental conditions and seasonal effects on *Toxoplasma* infection in quail and the resistance of oocysts for harsh weather conditions. AlKabi, (2016) who noted that existence of cats is principle important in epidemiology of toxoplasmosis based on environmental conditions which can remain oocysts for months and years and also *IgG* is exists for long time in animals and can be found at different months or seasons of year.

This study found higher differences in the infection rates between areas**,** where the highest rate 33.33% (10/30) was reported in Al-Ghazel Souq and the lowest rate 10.00% (3/30) was in Al-Huria & AlShulla. These results were in agreement with Mikaeel and Al-Saeed (2020) that showed a significant association between regions, while disagreed with AlHadad and Al-Rubaie (2021) who recorded no significant differences ($p \leq 0.05$) on the infection rate of *T. gondii* between areas of study in local chickens. These differences are assumed to be due to multi factorials such as number of quails, and feeding pattern as well as degree of contamination of food and water, or could be due high density of domestic cat feces that was existing in the areas (Hove *et al.*, 2005); cats may excrete millions of them after ingesting only one bradyzoite or tissue cyst, and many tissue cysts may be present in an infected animal (Dubey and Frenkel, 1972; Dubey, 2001 and Bawm *et al.*, 2020). Also cats and rodents which are distributed more in rural areas that lead to an increased chance of infection for the intermediate host and as result the final host (Bisson *et al.*, 2000), as well as this variation in the infection rates may be attributed to hosting parasite relationship and to the virulence of the parasite (Gamarra *et al.*, 2008); while the lowest prevalence in some areas may be due to supplied measurements of hygienic confinement prevention and keeping the shedding of oocysts away from the intermediate hosts

and feeding animals well cocked food with good hygienic controlling program (European Food Safety Authority, 2007).

Cong *et al.*, (2017) who recorded that common quails from northeast China (Changchun, Shenyang, and Dalian) have a lower infection rate to *T. gondii* than those from eastern China, and the great variations was found as a result of differences in climate changes which has great impact on the lifespan of oocysts, or differences in the used diagnostic techniques, the kind of the samples were depended and seasonality.

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Conflict of interest
The author declared that there is no conflict of interest.

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