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Research Article

Seroprevalence of toxoplasmosis in quail birds handlers and consumers at Baghdad city.

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Abstract

Toxoplasmosis is one of the most prevalent and important foodborne and waterborne zoonotic infectious parasitic diseases of medical and veterinary importance worldwide It is caused by an obligate apicomplexan intracellular protozoan parasite *Toxoplasma gondii (T. gondii)* that can be life threatening when opportunistic or congenital in both humans and animals. Therefore, its diagnosis requires accurate and rapid techniques that rely mostly on serology and molecular methods The infection can be acquired postnatally by consumption of inadequately cooked or raw meat of animals and birds involving infective tissue cysts, or from soil, foods or drinks contaminated with sporulation oocysts

Indirect ELISA targeting IgG was used to evaluated the prevalence of toxoplasmosis in(150) quail birds handlers and consumers and effect of some factors : gender, age, months and areas of study on prevalence also used minividas *Toxoplasma* –IgG avidity test to confirm the diagnosis. The total infection rate of toxoplasmosis in quail birds handlers and consumers by indirect ELISA, IgG was 44% (66/150), in which women and men recorded 49.33% (37/75) and 38.66% (29/75), respectively, without significant difference ($P \geq 0.05$). According to age, the highest infection rate 52 % (26/50) was found in ≥ 40 years age group, while the lowest 36% (18/50) was in ages of 20-29 years with significant difference ($P \leq 0.05$). A variation was found in the infection rates among the study areas, the highest 56.66% (17/30) was in Al-Ghazel market; and the lowest 33.33% (10/30) in Al-Shaula. According to the Months of the study the higher infection rate of toxoplasmosis 70.58% (12/17) was revealed in September, while the lowest 23.52% (4/17) recorded in June and July with highly significant** ($P \leq 0.01$) differences *Toxoplasma* specific IgG avidity (minividas) test showed a maximum value with high avidity (57/66) and only (7/66) with low avidity and 2/66 with intermediate avidity (borderline). The rates of high avidity (86.36%) were higher than the lower and intermediate (borderline) avidity (10.60%and 3.03%), respectively.

Aims of study: This study highlighted the seroprevalence of toxoplasmosis by ELISA IgG and avidity tests in quail birds handlers and consumers and effect of some factors as gender,age,months and areas of study in Baghdad city

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1. Introduction

Toxoplasma gondii definitive hosts where sexual reproduction occurs are strictly members of the felidae family only**,** but *T.gondii* is able to infect most warm-blooded vertebrates**,** including human and birds that harbor infective cysts in their tissues **(Dubey, J.P. 2010a and Lorencova, A.*, et al,* 2015)** In immunocompetent individuals, clinical symptoms of *Toxoplasma* infection present as mild and self-limiting, including lymphadenopathy, fever, muscle ache, headache, malaise,sore throat and fatigue. However, the infection is usually moresevere in immunosuppressed patients and first infected pregnant women, and may be accompanied by severe complications, such as encephalitis**,** retinochoroiditis**,** fetus abortion, splenomegaly**,**hydrocephalus(**Tenter, A. M, *et al,* 2000**) In birds**,** the infection is usually subclinical with formation of tissue cyst that may persist throughout the life **(Atasever, A., *et al,* 2020).**In addition to the cases of toxoplasmosis that occurred naturally in domestic birds**,** experimental studies have been conducted on a variety of species**,** including bobwhite quails**,** Japanese quails**,** pigeons**,** broilers**,** ducks**,** and pheasants (**Atasever, A., *et al,* 2017).**.**prevalence of *Toxoplasma* oocysts in cats was 27.3% ,and higher antibody titer 68% by latex test was observed in tested cats **(Osama J.Kallo, 2002).**

Rapid and accurate diagnosis of toxoplasmosis is essential for the treatment of human infections and it also a crucial research tool for understanding the role of animal reservoirs in the spread of illness**.** Indirect diagnosis by detection of antibodies (Ab) to *Toxoplasma* is feasible**,** but is less effective in determining the timing of infection**.** Immunoglobulin G (IgG) positivity often indicates *Toxoplasma* infection but does not provide a time frame **(Rahbar A,H. ,*et al,* 2012 and Tawfiq, S.K.,*et al,* 2019).** the development of an effective ELISA test to be used for detection *Toxoplasma* infection of slaughtered cattle would be exactly valuable since the important role that beef plays in epidemiology of *T.gondii* in particular the hazard of transmission to human and food safety**(Shaapan R.M., *et al.* 2021).** To discriminate between acute and chronic *Toxoplasma* infections, an *IgG* avidity test is often utilized **(Teimouri, A. *et al.*, 2020).**human consumption of quail birds meat and eggs as source of protein may increase the possibility of infection with toxoplasmosis ,

Materials and Methods

One hundred and fifty quails handlers and consumers of both genders (75) men and (75) women with ages ranging from 20 to more than 40 years**,**belonging to different socioeconomic classes**,** from different areas in Baghdad city (Al- Ghazel market, Al-Malhani, Al-Hurria, Al-Shaula and quails farms in Abu-Ghraib Poultry Research Station). Blood samples were collected during the period from the 1st January 2021 to 30th September 2021, and transferred by ice bags to the parasitology laboratory of Kamal Al-Samarraee Hospital, Baghdad, Iraq.

Blood samples

Five milliliters of blood were drawn from the vein of apparently healthy quail handlers and consumers, nearly 17 blood samples every month, using a sterile syringe 5 ml size and collected in gel tubes without anticoagulant. The samples were left 30 min at room temperature to form a clot, and subsequently centrifuged at 3000 rpm for 5 min for serum collection. Then, the serum was aspirated by using micropipette and dispensed into another sterile and labeled Eppendrof-tube. Each serum sample was divided into 2 labeled tubes, and kept in -- 20˚C until use **(Darweesh, N.H.,*et al.*, 2018).**

Indirect ELISA IgG for *T. gondii* detection in human sera

Toxoplasma IgG ELISA (Foresight**,** USA) kit was used for detection of IgG antibodies to *T. gondii* in human serum.The test was performed according to the manufacturer's instructions**.**According to the information included in the kit's insert**,** the catalog No. of the product was(I231-1091) and the immunoassay utilized had >99.9% sensitivity and 99.0% specificity **(Abdullhussein, H.S. 2017).**

avidity test

The IgG avidity test was performed as described previously by **(Montoya, J.G.,*et al*., 2004 and Murshid,M.K. and Abood, W.S., 2020)** using a commercial immune enzymatic (ELFA) kit (Vidas toxo IgG avidity kit; bioMérieux) according to the manufacturer's instructions. The test included two techniques, Vidas toxo 1gG –ELFA test and Vidas toxo IgG avidity–ELFA test **.**

Statistical Analysis

Statistical Analysis System **(SAS)** was used to detect the effect of different factors such as age, sex, area and months on the infection rate, and Chi-square test ($\chi 2$) was utilized to compare the significance ($P \leq 0.01$or 0.05) difference among groups of study(**Statistical Analysis System, 2012).**

Results

Indirect ELISA *IgG* :

Out of 150quails handlers and consumers sera examined using indirect ELISA IgG test, the overall prevalence of the positive samples was 44 % (66/150) in Baghdad city**.**

Women handlers and consumers recorded higher rate of 49.33% (37/75)than men who recorded 38.66% (29/75) without significant differences ($P \geq 0.05$) (**Table1)**

Table (1): Infection rate of toxoplasmosis in quail handlers and consumers (women and men) by ELISA IgG test.

Gender		Examined No.	Positive No.	Percentage (%)
Quail handlers	Women	75	37	49.33
	Men	75	29	38.66
Total		150	66	44
χ^2	0.969/ NS			
P value	0.325			

The infection rate of toxoplasmosis showed a significant relationship *($P \leq 0.05$)with the three age groups of quails handlers and consumers**.**The infection rate in the age group of ≥ 40years was 52% (26/50)**,** while the minimum infection rate 36% (18/50) was seen in the age group 20-29 years (Table**2).**

Table(2): Infection rates of toxoplasmosis in quail handlers and consumers according to age groups by indirect ELISA IgG test.

Age / years	Examined No.	Positive No.	Percentage (%)
20-29	50	18	36
30-39	50	22	44
≥ 40	50	26	52
Total	150	66	44
X	5.219 *		
P value	0.0218		
	*(P≤0.05)		

According to the Months of the study the higher infection rateof toxoplasmosis70.58% (12/17) was revealed at September**,** while the lowest percentage 23.52%(4/ was recorded at June & July**.** Statistical analysis of the data showed a highly significant****** (P≤0.01) differences in rate of infection between months**,** as clarified in **Table (3).**

Table (3): Infection rate of toxoplasmosis in quail handlers and consumers according to the months of the study by indirect ELISA *IgG* test:

Months/ 2021	Examined No.	Positive No.	Percentage(%)
January	17	8	47.05
February	17	10	58.82
March	14	7	50.00
April	17	10	58.82
May	17	6	35.29
June	17	4	23.52
July	17	4	23.52
August	17	5	29.41
September	17	12	70.58
Total	150	66	44
χ^2	14.376 **		
P- value	** (P≤0.01).		

The results showed significant **(P≤0.01) differences among the five areas of the study (Al-Huria, Al- Ghazelmarket, Abu-Ghraib, Al-Malhani and Al-Shaula).The highest infection rate was in Al- Ghazel market 56.66%(17/30) and the lowest infection rate was in Al-Shaula 33.33%(10/30) **(Table 4).**

Table(4):Infection rate of toxoplasmosis in quail handlers and consumers according to areas of study by indirect ELISA *IgG* Test.

Areas	Examined No.	Positive No.	Percentage(%)
Al-Huria	30	12	40
Al- Ghazel market	30	17	56.66
Abu-Ghraib	30	14	46.66
Al-Malhani	30	13	43.33
Al-Shaula	30	10	33.33
Chi-Square(χ^2)	--	--	9.622 **
P value	--	--	0.0061
** (P≤0.01).			

AvidityAssay

Avidity assay was done on 37 seropositive IgG women and 29 men quail handlers. and consumers.

1. VIDAS toxo 1gG –ELFA test

VIDAS toxo IgG II is a computerized quantitative test that is used to estimate the quantitation of anti-*Toxoplasma* IgG by ELFA.

All indirect ELISA IgG seropositive handlers and consumers samples were tested by VIDAS *toxo IgG* II apparatus. Quantitative measurement of anti-*Toxoplasma* IgG titer can be estimated and confirmed at ≥8 IU/ML by ELFA.

2. Minividas toxo-IgG avidity-ELFA test for seropositive Quail handlers and consumers

Cut off values or index recommended by the manufacturer for the *IgG* avidity (minividas) assay interpretation **(Table 5).**

Table(5): Cut off values or index recommended by the manufacturer for IgG avidity (Minividas) assay interpretation.

Toxo IgG avidity assay	Cut off value or index for the assay interpretation		
Vidas (bioMérieux)	Low avidity	Borderline (Gray zone)	High avidity
	<0.20	0.20 ≤ AI < 0.3	≥ 0.30

The interpretation of IgG avidity index (AI) results has been determined as follows: AI < 0.20 represents low avidity antibodies indicating an acute primary infection,0.20 ≤ AI < 0.3 represents borderline or intermediate avidity, andAI ≥ 0.30 represents high avidity antibodies indicating past infection. For this assay, a high avidity value allows exclusion of a primary infection within the previous four months **.**

On avidity test only 2/37 positive women had low antibody IgG avidity and 34/37 positive women had high antibody IgG avidity. While 1/37 positive women quail handlers and consumers had borderline. Only 5/29positive men had low antibody IgG avidity, 23/29 men quail handlers and consumers had high antibody IgG avidity, and 1/29 men quail handlers and consumers had borderline**.**

The total results of the IgG avidity test detected the maximum value with high avidity (57/66), 7/66 with low avidity, and minimum value (2/66) with borderline**.** The rates of high avidity (86.36%) were higher than low and borderline avidity (10.60**%** and 3.03%), respectively**.** The current study revealed that the rate of high avidity of toxo-IgG to various *Toxoplasma* antigens is higher than low and intermediate avidity **(Table 6).**

Table(6):Infection rate of toxoplasmosis in quail handlers and consumers (women and men) by mini-vidas toxo-IgG avidity-ELFA test

(Minividas) Anti- toxo- IgG avidity assay	ELFA (Index)	No. of examinedIgG positive sera samples			Percentage (%)
		Men	Women	Total	
Low avidity	<0.2	5/29 (17.24%)	2/37 (5.40%)	7	10.60
High avidity	0.3≥	23/29 (79.31%)	34/37 (91.89%)	57	86.36
Borderline	0.20 ≤ AI < 0.3	1/29 (3.44%)	1/37 (2.70%)	2	3.03
Total		29	37	66	100

* $P \leq 0.05$

Discussion :

Indirect ELISA *IgG* in quail handlers and consumers

The choice of indirect ELISA IgG as diagnostic tool for the current study was made because of its high sensitivity and specificity compared to other serological tests**((Mikaeel , F.B. and AL-Saeed , A.T., 2020).**

Out of 150quails handlers and consumers sera examined using indirect ELISA IgG test, the overall prevalence of the positive samples was 44 % (66/150) in Baghdad city**.**

This result was relatively in agreement with **(Al_Doori, A., 2010)** who recorded a rate of 49% rate in Tikrit**,** and **(Muhsin, S.S.,*et al*., 2019)** in Baghdad who recorded 42.5% in men by IgG ELISA**,** also agreed with **(Al-Kufaishi, Z.S.H., 2019)** in Babylon province who recorded 40% in rabbits handlers by ELISA IgG**.** On the other hand, the current study disagrees with **(Al-Dabbagh, S.M.K., 2019**) in Baghdad who recorded 51% in meat handlers by ELISA IgG**,** and with **(Mukhlif, M.M., *et al*., 2012)** recorded 38.4% in couples of husbands and wives in Al-Ramadi city**.** Likewise the results of this study are incompatible with(**Moses, A.E.,** *et al*.,**2021)** who demonstrated a prevalence of presumed ocular toxoplasmosis (POT) and ocular toxoplasmosis (OT) among livestock farmers and raw meat handlers in Uyo**,** Southern Nigeria (2.4% and 1.8%)**,** respectively**.**

Furthermore, **(Ekanem, U.S.,*et al*., 2018)** recorded 55.8% by IgG ELISA among abattoir workers in Uyo**,** Southern Nigeria. but higher than other studies, such as a rate of 11**.**9% in Diyala province **(Al-Griari, A.J.A., 2007)** 2.5%in Baghdad city **(Abdul-Aziz, N.S., 2009)** and 17.93%in Kut city(**AL-Khafaji, N.A., 2014).** the differences in the total infection rate with *Toxoplasma* in quail handlers and consumers by ELISA test attributed to several factors such as the number and size of samples collected**,** type of serological tests used, other factors related to the socioeconomic and cultural habits of community, the lifestyles of human in the living areas in Baghdad province, immune status of people hygienic practices and the favorable environmental conditions for oocysts infectivity**.** These factors are of epidemiological relevance and public health importance because of the recognized difference in the susceptibility of humans to *T. gondii* infections **(Zapata, M., *et al*., 2005 and Akhlaghi, L.,*et al*.,2013).**

Women handlers and consumers recorded higher rate of 49.33% (37/75)than men who recorded 38.66% (29/75) no significant difference was recorded between sexes by ELISA IgG because both sexes have the same chance of infection. The infection rate of the present study is more than that of **(AL-Bermani, S.M.K., 2012)** who recorded 36.08% in females and 32.07% in males and **(Mukhlif, M.M., *et al*., 2012)** in Al-Ramadi city recorded 30.7% (28/91)) in female and 13.1% (12/91) in male**.** However, the infection rate of the present study is lower than that of **(Acici, M.,*et al*., 2008)** in Turkey who recorded higher infection rate in females (65.24%). The infection rate of the present study is close to **(Jassam, H.M.S. and Salih, T.A., 2020)** who recorded 50% in women of Ramadi/Iraq, a study by **(Abdul-Hussain, S.Q., 2020)** showed that there was no significant difference recorded in the infection rate of *T. gondii* between males (40.4%) and females (40.67%). In addition, **(Al-Dabbagh, S.M.K., 2019)** showed that the infection with toxoplasmosis in meat handlers (butchers and women) was more prevalent in males (60%) than in females (47.14%). This relatively agrees with the current study result of women**.** Also the result disagrees with **(AL-Khafaji, N.A. ,2014)** who recorded higher infection in males (29.34%) than in females (6.52%)**.**

These variable results may be due to differences in the number of samples, types of serological tests used and other variable conditions in areas of study**.** One of the reasons for this high prevalence is related to women who handle raw meat and prepare food more frequently than men and they spend more time cooking at home and tasting foods during meal preparation**,** handling and chopping meat without wearing gloves, or may be due to lack of health education with poor hygienic conditions or because sex steroids, specifically androgens in males and estrogens in females, modulate several aspects of host immunity **(Kadir, M.A.,*et al*., 2011).** The infection rate of toxoplasmosis showed a significant relationship *(P≤0.05)with the three age groups of quail handlers and consumers**.** in age group of ≥ 40years was 52% (26/50)**,** whilst the minimum infection rate 36% (18/50) was in age group of 20-29 years. The result of the present study agrees with many previous studies, which stated that the prevalence increased with age but at different rates **(Khan, M.B., *et al*., 2020). Al-Dabbagh, S.M.K., (2019)** in Baghdad, showed that the maximum prevalence (66.66%) occurred in age group ≥ 40 years compared to lower rate 45.83% in the age group 20-30and 51.35% in the age group 30-40 years**,** without-significant difference in infection rate between age groups. Higher infection rate was recorded 22.2% in women of older age groups examined by ELISA test **(Alaa M.N., *et al*., 2016). AL-Taei, N.H.J., (2015)** in Thi-Qar province recorded a high prevalence in people aged ≥ 40 years. This result was similar to a higher rate of 39.68% seen in ages 20-45 years and a lower rate of 22.22% in ages < 20 years in the study carried out by **(AL-Bermani, S.M.K., 2012)** Whereas **(Alsaady, A.M., 2021)** in Maysan Province recorded high prevalence of infection 31.25% (5/16) in ages of 30 -39years compared to lower rate of 7.69% (2/26) in ages of ≥ 40 years. **Tammam, A.E., *et al*., (2013)** reported that the highest rate occurred in age <25 years and the lowest in > 40 years (42.1%**,** 1.1%), respectively**.**

Most studies worldwide showed that older ages were more likely to have *T. gondii* seropositive results than younger ages **(Daryani, A. M. *et al*., 2014; Negero, J.,*et al*., 2017and Khan, M.B., *et al*., 2020).** A significant relationship with age was noticed depending on the measurement of anti-*T.gondii* IgG antibodies**.** Thus**,** the most reasonable explanation for the higher involvement of older ages is the greater exposure of older ages to the parasite during their lives**,** because these antibodies persist for a long time**,** and the increment of the seroprevalence with age is associated with life-time exposure**(Turkey, S.A., 2019).** In Romania**, (Olariu, T.R., *et al*., 2008**) showed that *T. gondii* antibodies increased with age**.** In Germany**, (Wilking, H.,*et al*., 2016)** noted a significant interaction between age and gender since high seroprevalences were seen among more youthful men and older women**.** The variation in the infection rates between

the results of the present study and the previous studies attributed to several risk factors associated with infection, including differences in environmental conditions**,** behavioral patterns of life**,** immune status of people**,** and socio-cultural differences **(Akhlaghi, L. F.,*et al*., 2013).**

The highest percentage was shown in September(70.58%) while June and July recorded same lowest percentage (23.52%)witha highly significant****** (P≤0.01) differences in rate of infection between months**.** These results are in agreement with(**Al-Dabbagh, S.M.K., 2019)** in Baghdad, who showed that the maximum prevalence (71.42 %) in September and minimum infection rates(25%) in May and June without significant (P>0.05) difference, **A**utumn season recorded highest rate of infection with significant differences rather than other seasons**.** the effect of environmental conditions as temperature and humidity on life cycle of parasite. Epidemiology of toxoplasmosis is based on environmental conditions which can affect the longevity and viability of oocysts for months and years**;** Also**,***IgG* is existed for long time in hosts and can be found in different months or seasons of the year,other reasons for the variation between months of study was attributed to fluctuation in environmental conditions and seasonal effects on *Toxoplasma* infection in humans and the resistance of oocysts for harsh weather conditionsand the longer viability of *T.gondii* oocysts in moist or humid environments**(Kadir, M.A.,*et al*., 2011).**

Avidity Assay

Avidity assay was done on 37 seropositive IgG women and 29 men quail handlers and consumers. Positive IgG confirms the *Toxoplasma* infection**,** but gives no indication to when this event took place. Therefore, testing for IgG avidity was shown as an important diagnostic tool for discrimination between recent and past toxoplasmosis(**Li, S.,*et al*., 2000; Liesenfeld, O.,*et al*.,2001 and Jongert, E.,*et al*., 2007).**

VIDAS toxo IgG II is a computerized quantitative test that is used to estimate the quantitation of anti-*Toxoplasma* IgG by ELFA.

The results of the study showed significant ****(P≤0.01)** differences among the five areas of the study (Al-Huria**,** Al- Ghazelmarket**,** Abu-Ghraib**,** Al-Malhani and Al-Shaula).The highest infection rate was in Al- Ghazel market 56.66%(17/30) and the lowest infection rate was in Al-Shaula 33.33%(10/30) The results of the present study disagree with **(AL-Bermani, S.M.K. 2012)** who recorded the highest rate of infection in rural area (21.33%) and the lowest in urban (13.33%**).** All of the study areas are peri-urban**,** and a high infection rate may be due to lack of health education with poor hygienic conditions **(Kadir, M.A.,*et al*., 2011).** This variation in prevalence between areas within the same region may be attributed to differences in socioeconomic status**,** hygienic practices**,** health state**,** cat densities**,** pet animal contact, parasite strains and others **(Dubey, J.P., 2010).** Humans who ingested sporulated oocysts are thought to become infected for life**,** with detectable and persistent IgG antibody levels specific for *Toxoplasma* and that has aided the assessment of infection levels in many host species **(Dubey, J.P. and Jones, J.L., 2008).** the existence of cats is important with wide sources of infection, different human population based on differences in culture habits **(Al-Kabi, N.Ab.M., 2016),** other factors have been known to affect the prevalence of infection worldwide, such as cat population, differences in used diagnostic techniques used**,** and type of the samples involved **(Sakban F.M. and A,aiz N.N., 2020).**

All indirect ELISA IgG seropositive handler samples were tested by VIDAS *toxo IgG* II apparatus. Quantitative measurement of anti-*Toxoplasma* IgG titer can be estimated and confirmed at ≥8 IU/ML by ELFA, The results of the IgG avidity test may help to differentiate patients with acute infection from those with chronic infection better than do alternative assays**,** such as assays that measure IgM antibodies **(Lappalainen, M. and Hedman, K., 2004).**

The results were consistent with reports from other parts of the world, including Iraq

and some neighboring countries.These results are relatively in agreement with **(Saki, J.,*et al*., 2021)** in Khuzestan**,** Iran, in which the IgG avidity test in control groups showed that 35 (81.39%) and 2 (4.65%) of the samples had high and low avidity in this group**,** respectively**.** While the current study disagrees with the patient women groups with 60.16% of IgG-positive samples showed high avidity**.** While 27.64% showed low avidity**.** Another study in Iraq carried out by(**Turkey, S.A., *et al.*, 2014)** recorded that the rate of toxoplasmosis by *IgG* avidity test was 65.51% (76/116).

The difference between this study and others may be due to choosing a limited group, which included just quails handlers and consumers, low eco-social conditions and geographic area.Therefore, away from the result of *Toxoplasma* IgG, the avidity test can use as a diagnostic method to determine if the infection is past or current **(Teimouri, A.,*et al*., 2020)** .

Conclusions

For the first time in Baghdad/ Iraq, Toxoplasmosis was confirmed to be prevalent in quail birds handlers and consumers.

1. Infection rates of *T. gondii* were higher in females than males and increased with age in handlers and consumers during months and areas of the study.
2. Human minividas *IgG* avidity test detected maximum value with highest avidity and minimum value with borderline avidity and the rates of high avidity were higher than lower and borderline avidity**,** respectively**.**
3. Chronic infection rates of *T. gondii* by *IgG* avidity test were higher in quail handlers than the rates of *IgG* ELISA test

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