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SEROPREVALENCE OF ANTI-TOXOPLASMA GONDII ANTIBODIES IN THE MOST CONSUMED LIVESTOCK AND POULTRY IN GAZA – PALESTINE

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Abstract:

It is commonly established that warm blooded animals are extremely vulnerable to the infectious coccidian parasite, Toxoplasma gondii. T. gondii can be transmitted to poultry and livestock through the ingestion of oocyst (fecal-oral), and placenta (congenital toxoplasmosis) in livestock. Cattle, sheep, and rabbits are some of the most consumed livestock in Palestine. As for poultry, backyard chicken, caged chicken, and caged turkeys are some of the most consumed birds. The aim of the present study is to detect the seroprevalence of Anti-T. gondii antibodies (IgG/IgM) among the frequently consumed livestock and poultry, which is collected from farms and slaughterhouses in Gaza, Palestine. For this specific study, blood samples from 213 cattle, 100 sheep, and 99 rabbits were collected. On the other hand, 50 blood samples from each type of poultry mentioned above were also collected. The criteria of samples collection based on a quick survey was performed among animal breeders and slaughterhouses owners in the study area, under the guidance of the colleagues in the Palestinian Ministry of Agriculture (veterinary department), as well as with the support of documented references as the targeted animal groups are the most suitable for meat production regardless of age and gender. The collected samples were analyzed through the use of the ELISA technique and Rapid Cassette Test. The results of the present study showed a high seroprevalence of Anti-T. gondii antibodies among sheep, which was 64.00 % and 13.14% among cattle. On the other hand, the results among backyard chicken and caged chicken were 26.00% and 2.00%, respectively. However, no antibodies were detected in the serum of rabbits and caged turkeys.

Keywords:

Toxoplasma gondii - Seroprevalence - ELISA - Rapid Cassette - Livestock - Poultry - Gaza Palestine.

1. Introduction:

First discovered in 1908 in a desert rodent in Tunisia, T. gondii is an intracellular parasite that can inhabit many tissues in the host, such as muscles and intestinal. This parasite has three infectious stages which are: the tachyzoite stage, which is also known as trophozoite; bradyzoite which gathers in the tissue cyst; and lastly the sporozoite which is found in the oocyst, that is also known as the environmental stage (Hill *et al*., 2005; Roberts *et al*., 2009). While felids including cats (both, stray and domestic)are the definitive hostjust asall other warm-blooded animals are the intermediate hosts, the parasite can only complete its sexual reproduction in felids only. While oocysts are the environmental stage that can resist all harsh environmental conditions, they can be shed with the feces of the infected felids only. This parasite causes toxoplasmosis, which is one of the most common parasitic diseases as it is estimated to infect at least 30% of the human population, and it is transmitted through several ways (Frenkel *et al*., 1970; Tenter *et al*., 2001; Dubey, 2010). More specifically, those modes of transmission include the consumption of raw or undercooked infected meat, infected raw fruits and vegetables, and/or the ingestion of oocysts that cats have passed on their faces (CDC, 2019). Moreover, food is often contaminated with the oocysts upon its contact with flies and cockroaches or other mechanical vectors, given that these species hold a primary role in carrying-out the infective stages from cats to food consumers (Bogitsh *et al*., 2018). Heinemann and Chandler, in addition to Desmonts were the first researchers who documented that carnivorism and fecal-oral route are the modes of transmission of *T. gondii* among definitive and intermediate hosts (Gunn & Pitt 2012; Bogitsh *et al*., 2018). It is highly possible for livestock and poultry to be infected through the ingestion of food and/or water, which was infected by sporulated oocysts, whereas new-born livestock could get infected by the already infected mother through the placenta (Dubey, 2008; OTHMAN and ALZUHEIR, 2014). The seroprevalence of *T. gondii* infection among pregnant women in the Arab world ranges between (8-67.5%). Egypt holds the highest level of infection rate followed by Lebanon, Kuwait, and Saudi Arabia (Alsammani, 2016). Taking this statistic into consideration, it is only logical to consider toxoplasmosis as a major public health concern in our region; hence it must be addressed with more precedence. Even though the infection among adults is almost asymptomatic, congenital infection can lead to abortion, stillbirth, or

neonatal diseases with encephalitis, chorioretinitis and hepatosplenomegaly, fever, jaundice, and inter cranial calcification(Assafa et al., 2006). Through their research study, Nijem & AL Amleh observed that eating undercooked meat and raw vegetables, keeping direct contact with cats as well as with soil, and drinking untreated rainwater are potential causes of *T. gondii* infections among pregnant women in Hebron, Palestine (Al Amleh, S. and Nijem., 2009). Furthermore, a number of research studies have documented the impact of the seroprevalence *Toxoplasma* among pregnant women in Gaza, Palestine. For instance, Al-Jarousha *et al*. recorded a high seropositivity of 30.90% among 255 pregnant women surveyed in Gaza. On top of this, another study was conducted by Al Hindi *et al*., which reported a high prevalence of *T. gondii* among pregnant women in Gaza with the following findings: IgG was reported with an average of 33.2% while IgM had an average of 21.00 % (Al-Jarousha, 2012; Al-Hindi *et al*., 2017). Although there are many studies documenting the occurrence of *T. gondii* in human serum, the studies discussing the prevalence of *T. gondii* in livestock and poultry are limited. Meat sources from cattle, sheep and rabbits are considered the most consumed livestock in Palestine. Approximately 38 to 42 thousand of lambs as well as 33 to 38 thousands of cattle were consumed in 2016 and 2017, respectively. In addition, more than seven million poultry have been consumed during 2016, thus making caged chicken, backyard chicken, and caged turkeys the most consumed poultry in Palestine (Municipalities, 2016; Municipalities., 2017). A rare study was pursued by Othman *et al.*, which was carried out in order to investigate the prevalence of toxoplasmosis in sheep herds in Palestine. The study tested the presence of *T. gondii* antibodies among sheep in three different locations throughout the Northern part of the West Bank as well as two cities along the Jordanian Valley in the Palestinian territories. This study demonstrated that almost 20.5% of 2479 sheep were infected (Othman *et al*., 2017). Sheep and other small ruminants are more susceptible to *T. gondii* infection all over the world. Beside its clinical effect on animal health, toxoplasmosis plays a major role in the reproductive loss through causing abortion to the infected sheep. Moreover, it is a zoonosis disease so it can be transmitted to humans through infected ruminants as more than 200 studies have reported the high prevalence of *T. gondii* antibodies in sheep and other small ruminants (Dubey, 2009;

Opsteegh *et al*., 2016). Furthermore, the significance of toxoplasmosis is higher in sheep followed by rabbits, and poultry, than in cattle. This is due to the less common presence of the parasite in their tissues; however, it is still considered a real risk factor of human infection since consuming under-cooked beef is still common, particularly in Europe. There, among 26 documented outbreaks of human toxoplasmosis, four of them had undercooked beef meat as the risk factor from 1965 to 2000 (Dubey, 1986; Cook *et al*., 2000; FSSA, 2005). While many other studies have reported the occurrence of *T. gondii* in poultry, regarding the way of ingestion of their food from the ground, which make them vulnerable to be infected by the accidental ingestion of oocyst from soil or water, poultry rarely develop any clinical signs as the result of this infection (Dubey, 2010). At the same time, studies like this have not been performed to investigate the infection status among other livestock or even poultry. Moreover, the main purpose of the current study is to evaluate the seroprevalence of Anti- *T. gondii* antibodies in the most consumed livestock and poultry in Gaza - Palestine.

2- Materials and Methods

Ethical Approval

An ethical approval was obtained from the Ethical Research Committee at the Islamic University of Gaza on 5 Aug 2019. While the approval ID serial number is 5 Aug 2019.

Sample Collection

The samples collection process was executed in different areas within Gaza city during the time period from March to July 2019. The criteria of samples collection was performed based on the most used animals for meat production, in addition to the consultant of the veterinarians at the Ministry of Agriculture. Animals were selected upon the best criteria for meat production regardless of the gender and age, based on that all the livestock and turkeys were males while other poultry were females, as shown in Table (1), below. For the investigation of the seroprevalence of Anti-*T. gondii* antibodies in cattle, sheep, and rabbits; 213, 100 and 99 blood samples were collected from each livestock type, respectively. The blood samples of cattle and

sheep were taken through a puncture in the jugular vein, while the blood samples of rabbits were obtained from the slaughterhouses Similarly, 50 blood samples from each poultry of the following; caged chicken, backyard chicken and turkey were collected from slaughterhouses as well. The blood samples were centrifuged to obtain serum, upon which they were transported to the Central Veterinary Laboratory of the Ministry of Agriculture. Collected serum samples were stored at a 4 °C temperature to be tested within a week.

Table (1): ***The livestock and poultry which were collected for the present study.***

Livestock/Poultry	No of Samples	Gender	Market age
Cattle	213	M	2-3 Years
Sheep	100	M	5-8 Months
Rabbits	99	M	8-10 Weeks
Broiler or (Caged) chicken	50	F	6-8 Weeks
Fryer or Caged turkeys	50	M	50-55 Weeks
Backyard chicken	50	F	16-24 Weeks

Enzyme linked Immunosorbent Assay (ELISA)

Serum samples of livestock were examined for the presence of anti-T. gondii antibodies using the Vet Line Toxoplasma ELISA kit (The Nova Tec Vet Line Toxoplasma ELISA, Germany). Per the instructions displayed on the manual, the kit is designed to determine antibodies against *T. gondii* in mammalian serum. Due to the fact that this kit is designed to detect Anti *T. gondii* antibodies generally, consequently the result ascertains the occurrence of these antibodies, however, does not determine which one of them is present, whether it is IgG or IgM. The results were pronounced as optical density (OD). While the absorbance was read at 450 nm and the wells of each microplate were coated with *T. gondii* antigens, and subsequently, the

peroxidase (HRP) conjugate was added. This HRP substance addition plays a major role in the formation of the antigen–antibody complex. In order to draw the validation purpose from each test, positive and negative controls supplied by the kit manufacturer were utilized. Samples with values

greater than 35 IU/ml were considered to be positive and vice versa for negative samples (VetLine *Toxoplasma*, 2021).

Rapid Cassette Test

The Rapid Cassette Test (Toxo-IgG /IgM) by Plasmatic was employed to determine the occurrence of anti-*T. gondii* antibodies in the poultry serum. Each substance used in this test was remained still to reach room temperature between 15 to 30 °C. Following this, two drops of serum were vertically dropped in each specimen cassette as well as two drops of buffer, consecutively. After that, a timer was set to 15 minutes in order to read the results (Hussein *et al*., 2019).

Statistical Analysis

Through the utilization of the SPSS (USA, II, Chicago, SPSS Inc) software package v.15.0, and the data collected by the subject study, a statistical analysis was implemented. A Chi- square test was performed for the comparison between the seroprevalence of anti-*T. gondii* antibodies in (livestock and poultry) and animal husbandry methods. P value less than 0.05 was considered significant.

3- Results

Among the 213 cattle serum samples collected, anti-*T. gondii* antibodies IgG/IgM antibodies were detected in 28 (13.14%) samples. Yet, out of the 100 sheep sampled, 64 sheep were positive, hence returning a 64.00% positive rate. On the other hand, all rabbit samples were negative for anti-*T. gondii* antibodies. These results are shown below in table (2).

Pertaining the seroprevalence of Anti-*T. gondii* antibodies among poultry, the results showed that antibodies were detected in 13 out of 50 samples (26.00%) of backyard chicken serum, while just one sample (2.00%) of caged chicken turned out positive. All serum samples of turkeys were negative for Anti-*T. gondii*, as shown below in Table (3). Moreover, IgM was found in 10 out of13 backyard chicken serum samples (76.92%), while IgG was only found in 2 samples (15.38%). Meanwhile, a sample of both caged and backyard chicken found to be seropositive for both

antibodies (IgG +IgM),as shown below in table (4). The findings of the present study revolving around the prevalence of Anti-T. gondii antibodies among livestock and poultry is summarized by the bar-chart diagram displayed in Figure (1) below.

Table (2): The prevalence of anti-T. gondii antibodies in cattle, sheep and rabbits in Gaza – Palestine.

Livestock	No of Samples	Antibodies (+) IgG/IgM	Antibodies (-) IgG/IgM	% Positive
Cattle	213	28	185	13.14
Sheep	100	64	36	64.00
Rabbits	99	0	99	0.00

Pertaining the seroprevalence of Anti-*T. gondii* antibodies among poultry, the results showed that antibodies were detected in 13 out of 50 samples

(26.00%) of backyard chicken serum, while just one sample (2.00%) chicken turned out positive. All serum samples of turkeys were negative for Anti-*T. gondii*, as shown below in Table (3).

Table (3): *The prevalence of Anti-T. gondii antibodies in backyard chicken, caged chicken and turkey.*

Poultry	*No of Samples*	*Antibodies (+) IgG / IgM*	*% Positive*
Backyard chicken	*50*	*13*	*26.00*
Caged chicken	*50*	*1*	*2.00*
Turkey	*50*	*0*	*0.00*

Moreover, IgM was found in 10 out of13 backyard chicken serum samples (76.92%), while IgG was only found in 2 samples (15.38%). Meanwhile, a sample of both caged

and backyard chicken found to be seropositive for both antibodies (IgG +IgM),as shown below in table (4).

Table (4): The detected (IgG, IgM), Anti-T. gondii antibodies in poultry.

Poultry	*No of (+) Samples*	*IgG (+)%*		*IgM (+)%*		*(IgG +IgM) (+)%*	
Backyard chicken	*13*	*10*	*76.92*	*2*	*15.38*	*1*	*7.70*
Caged chicken	*1*	*0*	*0*	*0*	*0.00*	*1*	*100.00*
Turkey	*0*	*0*	*0*	*0*	*0.00*	*0*	*0.00*

The findings of the present study revolving around the prevalence of Anti-T. gondii antibodies among livestock and poultry is summarized by the bar-chart diagram displayed in Figure (1) below.

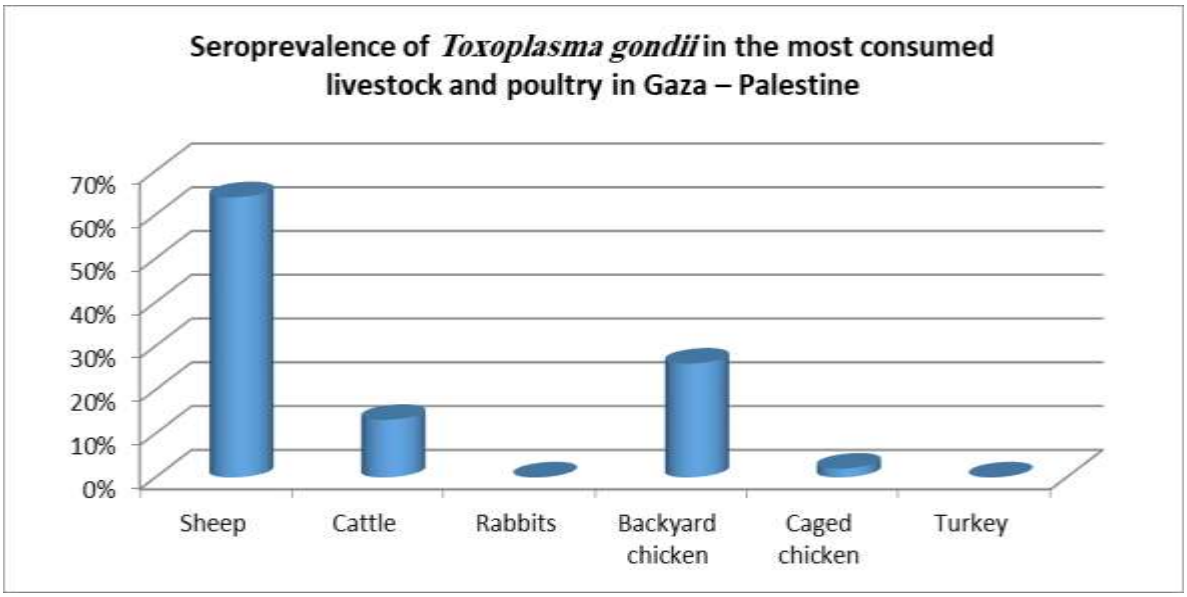

Figure (1): The prevalence of Anti-T. gondii antibodies among the most consumed livestock and poultry in Gaza-Palestine.

Along with Fig (1), and based on animals' husbandry methods, the results of the present study at hand clearly indicated that the prevalence of Anti-*T. gondii* antibodies among animals raised in closed barns and that have

mostly concentrated feed as part of their diet were less than those dependent on grazing on open pastures as shown in table (5). Given the statistical analyses implemented on the collected data, it was found that there was a statistically significant relationship between the prevalence of Anti-*T. gondii* antibodies in both livestock and poultry as well as the animal husbandry methods. This statistical significance of $\chi 2 = 131.117$ and P 0.000< 0.05is shown below in table (5).

Table(5): ***The seroprevalence of Anti-T. gondii antibodies among livestock and poultry, based on the husbandry methods, in Gaza- Palestine.***

Husbandry method	Animals	No Samples	(+) Samples	% Positive
Concentrated feed only.	Caged chicken and turkey	100	1	1.00
Less (Food), that the farmers bring + More concentrated feed	Cattle and rabbits	312	28	8.97
More grazing in open area + Less concentrated feed	Sheep and backyard chicken	150	77	51.33
Total samples		562	106	18.86
$\chi 2 = 131.117$	P 0.000		P < 0.05.	
	Statistically significant			

4- Discussion

Although there has been extensive data documenting the existence of the parasite among humans and in particular pregnant women, data documenting the prevalence of *T. gondii* in livestock and poultry, until now, has been quite limited in Palestine (Al-Jarousha, 2012; Al-Hindi *et al*., 2017). It is very important when consumers buy meat searching for eating quality, which is related to the animal age as increased age results in decreased quality (Pethick *et al*., 2005; Warner *et al*., 2010), this is due to the increase of collagen concentration in mature animals which affects muscle tenderness Thompson *et al*., 2005). So, in this study the ages of animals were the one of main criteria to select the suitable animals for producing meat. While the gender for the selected animals was selected according to the recommendations of Palestinian Ministry of agriculture, in addition to the meetings with some animals breeders, based on the most appropriate for meat production. Nonetheless, in the present study, many other resources supported the suitable ages for slaughtering poultry in order to obtain the food quality, which were taken in consideration, such as, the European Food Safety authority and National Chicken Council in the USA, in addition to the criteria which was suggested by Sams, regarding the ages and weights of selected poultry (Sams, 2001; EFSA, 2010; National Chicken Council, 2020). As for lambs, those of new season aged from 5 – 8 months were preferred because of the increase of their eating quality (Pethick et al., 2005). For this reason, in addition to the reasons mentioned above in the samples' collections section, sheep aged 5-8 months were selected. The results of the present study showed that 28 out of 213 samples (13.14%) were positive for Anti-*T. gondii* antibodies IgG/IgM in cattle. It is worth noting that this result is lower than the 23.60% reported in Egypt, the seropositive range of 2.00% up to 92.00% in Europe, and the 15.90 % figure reported in Iran(Tenter *et al*., 200; Nematollahi A, and Moghddam , 2008; Fereig *et al*., 2016). No previous studies were carried out about the prevalence of Anti-*T. gondii* antibodies in cattle in the study area, but few studies have been carried out in neighboring countries, In Egypt, around 20.00% of the tested cows were infected (Hassanain *et al*., 2012), while in Europe, the seropositivity of Anti-*T. gondii* antibodies in cattle is more than 45.00 % in Switzerland (Berger-Schoch *et al*., 2011). High seroprevalences of up to 92.00% in sheep has been described in Europe, which is considered to be a result of free-range husbandry and constant exposure of small ruminants with oocysts in the environment (Hill and Dubey, 2002).

Eating raw or undercooked mutton/lamb has not only been identified in several European case-control studies as an important risk factor for acquiring toxoplasmosis during pregnancy ((Hill and Dubey, 2002; Robert-Gangneux and Dardé, 2012; EFSA, 2013), but was also the source of infection during an outbreak in France (Ginsbourge *et al*., 2012). On the contrary, the figure reported was higher than the 12.00% findings reported in Tunisia and 2.30 % in China (Yu *et al*.,

2007; Lahmar *et al*., 2015). Likewise, this result is quite consistent with similar results documented in Sudan (Elfahal *et al*., 2013). On the other hand, the results of this study showed a high seroprevalence of Anti- *T. gondii* in sheep (64%), which was higher than that reported in Tunisia, 34.50% (Lahmar *et al*., 2015), and in Egypt, 38.70% (Fereig *et al*., 2016). In addition, the results were higher than those reported in Morocco, where Anti- *T. gondii* antibodies were detected in 9 out of 23 aborted sheep herds (Benkirane *et al*., 1990). As revealed by this study, all tested rabbits for Anti- *T. gondii* were found to be seronegative. In contrast, results reported in Korea showed a *T. gondii* seropositive of 10.00% (Shin *et al*., 2013). The current study reported that backyard chicken had the highest level of Anti- *T. gondii*, whereas the seropositive antibodies were found to be 26.00%, followed by caged chicken with 2.00%. seropositive. All Turkeys that were tested were found to be seronegative for Anti- *T. gondii*. The findings of this study were higher than those reported in Portugal, where the prevalence of Anti- *T. gondii* was 5.6.00% in free-range and 0.00% in broiler chickens (Rodrigues *et al*., 2019). On the contrary, the reported results were lower than those reported in Egypt among turkeys, chickens, and ducks, which were 56.50%, 47.20%, and 50.00%, respectively (El-Massry, 2000). The differences between seroprevalence of Anti- *T. gondii* among livestock and poultry throughout some of the countries mentioned could perhaps be influenced by the collection period, sample sizes, and analysis techniques employed in the experiment. Moreover, it could possibly depend on the husbandry methods and the susceptibility of animals infected with *T. gondii.* Sheep and backyard chicken in Gaza feed on grazing partially of pastures and often in potentially contaminated environments given that stray cats are abundant and usually defecate in house-yards and on roofs. First, by taking a closer look and assessing the vicinity in which the animals used in this study were raised, we can clearly detect those animals that are vulnerable to infections. Given the fact that some cattle, rabbits, caged chicken, and turkeys are raised within closed barns and are mostly fed industrial feed, this environment and diet lower their risk of getting infected. Though, it is more common for sheep and backyard chicken to feed on a diet that is likely to be contaminated with oocysts. Also, it was observed that some of the sampled sheep in the present study were grazing on pastures near sewage lagoons and trash dumping stations. Undoubtedly, this occurrence drastically raises the risk factor of feeding on contaminated food. Traditionally, Gaza relies on importing cattle and broiler poultry more than sheep and backyard chicken. It is not far-fetched that some of those imported animals are already infected from the exporter. Another possibility of animals catching infections is through feeding on some of the wild-plants sold by farmers to animal herders. Moreover, milk can be easily contaminated with tachyzoites of *Toxoplasma*, hence raising an additional risk factor (Dehkordi *et al*., 2013). Although the (The Novatec VetLine *Toxoplasma* ELISA, Germany) technique utilized in this study is capable

of detecting the Anti-*T. gondii* antibodies of both IgG/IgM, it cannot distinguish between the specific types of antibodies detected. This leads to ambiguity in knowing the certain date in which the infection was caught, whether recent or old. In other words, it is restrained to only giving us the general condition of seroprevalence *Toxoplasma* in livestock. Given the findings recorded by a study carried out in Iraq, which focused on detecting Anti-*T. gondii* antibodies in (*Columba livia*) hunted pigeons, the Rapid Test used to detect the prevalence of Anti-*T. gondii* antibodies in poultry seemed to be more sensitive than latex Agglutination. The study carried out in Iraq revealed that the infected pigeons using latex agglutination were 11, while 6 pigeons were found infected through Rapid Cassette and only 4 pigeons were detected using the PCR technique (Al-abodi, 2017).

5- Conclusion

The present study revealed that there is a high seroprevalence of Anti- *T. gondii* among sheep at 64%, backyard chicken at 26%, cattle at 13.14%, and caged chicken at 2%, while the tested rabbits and turkey were found to be free of Anti- *T. gondii* antibodies. Lastly, this study enforces the fact that cattle, chicken, and sheep are potential sources of infection with *T. gondii* in the surrounding research areas carried within this study.

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