An investigation of Toxoplasmosis in Free Range chickens, Industrial chickens and Duck in mid Euphrates area of

Iraq

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Abstract

The current study determined the prevalence of Toxoplasmosis among the free Range Chicken, Industrial Chicken and Duck by using LAT and ELISA. A total of 200 FR Chicken purchased (70 Al-qadisyia province, 65Al-najaf province, and 65 Babylon province), 200 Industrial Chicken purchased from ten Industrial farms in geographical different region of Al-Diwania city and 50 Duck purchased(rural area and bird sale places of Al-Diwania city) were involved in this study, one hundred thirty four (67%) of FR Chicken, Sixty two (31%) of Industrial chicken and twenty eight (56%) of Duck, were diagnosed primarily as Toxoplasmosis by LAT.Out of 90 (45 FR Chicken and 45 Industrial Chicken) of LAT positive cases, only 36% were positive by ELISA which considered as confirmed Toxoplasmosis cases .Results revealed ,that 51.11% and 28.88% of FR chicken and Industrial chicken positive by ELISA respectively. The percentage of T. gondii antibodies in FR chickenby LAT were 71.42%,69.23% and 55.38% in Al-Qadissiya, Babylon and in Al-najaf provinces respectively whereas the percentage by ELISA were 66.66%, 46.66% and 33.33% in Al-Qadisiya, Babylon and in Al-najaf respectively. Although the difference observed in the percentage of T. gondii antibodies among different provinces, there was no significant differences P > 0.05 detected by LAT whereas in the ELISA there was significant differences P>0.05.The highest titer in LAT were 1/128 (34.32%) in FR chicken, 1/8 (29.3%) in the Industrial chicken and 1/32(35.7 %) in the Duck, The lowest titer were 1/2 (5.22%) in FR chicken, 1/128 (0%) in the Industrial chicken and 1/4(0%) in the Duck.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan that infects humans and a wide range of mammalian and bird (1).Its high infection rates and its benign co-existence with the host, T.gondii is regarded as one of the most successful parasites on earth . is a global parasites with no known geographical boundaries (2).Serological surveys done in various parts of the world show that in some countries more than a third of the human population have antibodies against T.gondii .This high prevalence of infection in human proves the importance of toxoplasmosis as azoonotic disease(3,4).T.gondii infection in freechickens range (FR) is considered important as FR chickens are one of the best indicators for soil contamination with T.gondii oocysts because they feed from the ground, and tissues of infected chickens are considered a good source of infection for cats. Additionally, ingestion of infected chicken meat can be a source of infection for *T.gondii* infection in humans and other animals. Rarely, toxoplasmosis can cause clinical disease in chickens (5).Soil contaminated with oocysts can be taken up by pastoral animals, such as sheep and goats, during grazing. Poultry having also outdoor access will take up considerable amounts of soil and can thus become infected with Toxoplasma. Therefore, free-ranging chickens are now used as sentinel animals to isolate and characterise **Toxoplasma** strains throughout the world (6).

The aim of the study:

Investigate the utility of enzyme linked immunosorbent assay (ELISA) for detection infections with T. gondii in chickens and whether it can be used as a confirmatory assay to determine the positive infections with Latex Agglutination Test(LAT) results.

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Materials and Methods

- Samples Collection:

- Free range chicken samples

A total 200 chicken samples were cluster screening randomly purchased from chickens of rural geographical properties of AL-Qadisiya, AL-Najaf and Babylon provinces in 2010. Since female gender ranged 2-5 years old was destined for meat and egg production, they were dominated more than 90% in this study.

- Industrial chicken samples:

Totally 200 chicken samples were purchased from ten industrial farms. in geographically different regions of AL-Qadisiya province.

- Duck

A total of 50 Duck purchased from rural area and AL- Diwaniyah bird sale places in geographically different regions of of AL- Qadisiya province .

- Blood samples

Five milliliter (ml) of blood was drawn from each bird heart by disposable syringe. blood were collected in sterile plain tubes and left 30 minutes at room temperature to clot, , then centrifuged at 3000 rpm for 5 minutes for serum collection which was aspirated by using micropipette and dispensed into another sterile tubes, each serum was divided into 2 tubes, and kept in deep freeze at -20°C.

- The Diagnostic Methods:

- Latex Agglutination Test, [PLASMATEC company / United Kingdom]

Two hundred sample of free Ranging chicken, Two hundred sample of industrial chicken and fifty Ducks sera were involved in this test.

- Enzyme Linked Immunosorbant Assay (ELISA).

This is kit product of the European Veterinary Laboratories (EVL), Holland origin, and was performed according to the manufacturer's instructions, this kit used the whole parasite protein of cell membrane as antigen and determined the antibodies in the sera of animals, and stored at $\pm 4^{\circ}$ C until used. figure (1).Ninety sera (45 free Ranging chicken, 45 industrial chicken) sero-positive sera by LAT test.



Fig. (1)Toxoplasma (ELISA kit)

- Statistical Analysis

Data were analyzed by digital interactive Chi-square test program, P

value ≤ 0.05 was considered statistically significant (7)

Result and Discussion

Sero -Incidence:

Latex Agglutination Test (LAT):Incidence of Toxoplasmosis Cases

According to breed of chicken.

(FR chicken and Industrial chicken)

According to LAT, the high rate of Toxoplasmosis cases (67%) in FR chicken and the lowest rate of Toxoplasmosis cases among industrial chicken (31%) Table(1)

Type of bird	Total	Positive No.	Percentage %
FR chicken	200	134	67
Industrial chicken	200	62	31
Duck	50	28	56

Table(1) No. of sero-positive cases of toxoplasmosis in FR chicken, Industrial chicken and Duck by LAT.

 $X^2 = 47.8$

The LAT was used for the general detection of toxoplasmosis because it is relatively a simple, cheap and provided an efficacious method for routine serological screening for antibodies to T. gondii (8). Then the LAT results were further tested with ELISA test to increase the accuracy of diagnosis because of the high specificity and sensitivity of this test (9). This present study showed the high percentage of antibody titer against T. positive by LAT among FR gondii chicken. This result indicate high distribution of toxoplasmosis among chicken in the studied area.Our finding demonstrated that anti T. gondii antibodies were high in FR chickens in the studied area .it seems that become infected mostly during feeding on the ground contaminated with oocysts. (10). The prevalence rate of Toxoplasma antibody in FR anti chicken(67%) in present study was higher than that of ELmassry etal survey(47.2%) from Giza province in Egypt (11). The role of these FR chicken as intermediate host and disseminator of oocysts (12). The prevalence of infection in industrial chickens was 31% that is close to that of Ghorbani etal survey 30.3% which was reported in Iran (13). The 31% prevalence of T. gondii antibodies in chickens was used for food in Iraq was higher than 9.5% prevalence in chicken reported from India (14).Since industrial chicken that reared in saloons rose for meat production in short duration and less exposed to cat feces,

these chicken had the lowest prevalence compared to FR chicken .T. gondii antibodies were reported to be present in 53.3% of chickens in Egypt by sabinfeldman dye test .indirect hemagglutination assay (IHA) ,or the complement fixation test (15.16).However, IHA and the dye test were found to be insensitive for detecting T. gondii antibodies in experimentally infected chicken The prevalence of T. gondii in chickens ,as determined by the modified agglutination test (MAT)(17) varies within countries, ranging from 10% to 47% (11,14,18,19,20). The many factors such as management and hygienic standards in breeding, density of cat and environmental condition are effect on the acquisition of T. gondii oocyst by animal.

Ducks

Results revealed that the LAT detected 28seropositive cases from total selected 50 case in percentage 56%. The prevalence of infection in Duck 56% was nearly similar to those recorded in Duck of Egypt 50% (11). The 56% seroprevalence of *T. gondii* in Duck in the present study is markedly higher than the 1.7% prevalence reported by (21). In the table (2) the result recorded the highest percentage of the presence of T. gondii antibodies detected by LAT was in AL-Qadisiyia 71.42%, whereas the lowest percentage was in the AL-Najaf 55.38%, results showed no significant difference these among provinces.

P<0.05

Table (2) sero-positive cases and *T.gondii* antibodies percentage in different provinces by LAT in FR chicken.

Province	Total	Serpositive No.	Percentage %
AL-Qadisiyia	70	50	71.42
AL-Najaf	65	36	55.38
Babylon	65	45	69.23
Total	200	134	67

 $X^2 = 6.5$

P<0.05

Result showed that no significant differences was present among different provinces by LAT test, which may be due to all these provinces included in this study located in mid Euphrates area of Iraq which was similar in the nature of climatic condition.

Antibodies titration

The highest titers were 1/128 (34.32%) in FR chicken , 1/8 (29.3%) in

the Industrial chicken and 1/32(35.7% %)in the Duck respectively, as illustrated in Table (3).The percentage of antibody against *T. gondii* in FR chicken at a titer 1/128 was 34.32% this is lower than that reported in FR chicken of Iran by(22).The present study result is not compatible with the result recorded by Lindsay etal. Who reported 45.45% that was at atiter 1/160 in broiler chicken (23).

Table (3)Percentages of antibody titers in (FR chicken, Industrial chicken and Duck) by LAT.

Titer Avian	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
FR Chicken	5.22%	8.2%	7.46%	9.7%	10.44%	12.68%	34.32%	11.94%
Industrial Chicken	6.45%	9.67%	29.3%	16.12%	12.9%	6.45%	0%	4.83%
Duck	7.14%	0%	10.71%	3.57%	35.7%	13.71%	17.85%	14.28%

L.S.D= 31.3

P<0.05

The high prevalent of antibodies titer in FR Chicken at titer 1/128 and in Duck at titer 1/32 in this study may be attributed to the nature of infection in which most of FR Chicken at age (2-5 years) which may be previously suffered from chronic infection while the high prevalent of antibodies titer infected Industrial Chicken at a titer 1/8 may be due to the modern infection in those Industrial chicken which less stimulated to Immunity.

ELISA

-Incidence of Toxoplasmosis Cases According to breed of chicken.

out off (90) sera samples which were seropositive in LAT tested a total of 36 were positive. The highest percentage of T. *gondii* antibodies was in FR chicken 51.11% but the lowest percentage was in the industrial chicken 28.88% as shown in table (4).

Type of Chicken	No. of examined	Positive No.	Percentage %
FR Chicken	45	23	51.11
Industrial Chicken	45	13	28.88
total	90	36	40

X²= 6 P<0.05

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ELISA was used for confirming the results of latex agglutination test (LAT), because of the ELISA is of a great sensitivity, objective, quantitative and may be automatically adopted, although it needs a refinement in the procedures.(24).A significant difference P≤0.05 was detected in the percentage of T. gondii antibodies among FR Chicken and Industrial chicken by ELISA, Bird and rodents are two of the most important intermediate hosts. they become infected easily through ingestion of oocysts (25). Dubey et al. (1993) studied the serologic response of four-week-old chickens to T. gondii following oral oocyst inoculation. In his study, both the modified agglutination test and ELISA test detected antibodies within two weeks of inoculation and to the termination of the experiment at 68 days post-inoculation. Other workers have used an ELISA test to demonstrate that chickens and pigeons inoculated with T. gondii oocysts seroconvert within 2 and 3 weeks, respectively(26). Soil is the most important infection source of for intermediate hosts and, owing to the feeding behavior of terrestrial species ,e. g. chickens and partridges ,the prevalence of T. gondii in these hosts is a good indicator of environmental contamination with parasite oocysts (14). which indicates that FR Chicken are more likely to get infection than those which are Industrial chicken.The prevalence rate of toxoplasmosis in FR chicken by ELISA

was nearly similar to those of Chinese infected FR Chicken (34.7%) while The prevalence rate of toxoplasmosis in Industrial chicken by ELISA was much than that reported in Chinese higher infected caged chicken (2.8%) (27). The lower prevalence rate in the Industrial chicken in the present study could be due to various reasons. It possible that the samples that were analyzed here in originated from younger Chicken. this theory is supported by the fact that the highest titer obtained in this study was only 1/8, which was much less than that obtained elsewhere (28). Thus it is entirely possible that a younger age of industrial chicken were sampled leading to lower prevalence. A significant difference P≤0.05 was detected among different province of in the percentage of *T. gondii* antibodies in FR chicken by ELISA, and the highest percentage of T.gondii antibodies was seen in AL-Qadisiyia 66.66%, while the lowest was detected in AL-Najaf 40%, as shown below.The significant table (5) in differences that was observed in the percentage of T. gondii antibodies among different provinces, which could be attributed to increasing opportunities of exposure several sources of to Toxoplasmosis infection and this were evident that ELISA were more accurate, and could be detected different parts of antibodies .

Type of chicken	province	total	Positive No.	Percentage %
FR	AL-Qadisiyia	15	10	66.66
FR	Babylon	15	7	46.66
FR	AL-Najaf	15	6	40
industrial	AL-Qadisiyia	45	13	28.88
	total		36	40

Table (5) No. of sero-positive	cases of toxoplasmosis in o	different chicken and	provinces by
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X²= 5.9 P<0.05

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التحري عن داء المقوسات في الدجاج حر المعيشة والدجاج التجاري والبط في وسط العراق

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صممت الدراسة الحالية لتحديد مدى انتشار داء المقوسات بين الدجاج حر المعيشة والدجاج التجاري والبط باستخدام اختبارين هما اختبار تلازن حبيبات اللاتكس واختبار التألق المناعي المرتبط بالأنزيم(الأليزا) .جمعت 200 عينة من الدجاج حر المعيشة بواقع (70 دجاجة من محافظة القادسية و65 دجاجة من محافظة النجف و65 دجاجة من محافظة بابل) وكذلك تم جمع200عينة من الدجاج التجاري من عشرة حقول تجارية في مناطق مختلفة من محافظة القادسية وكذلك تم جمع 50 عينة من البط من مناطق ريفية وأماكن بيع الدواجن في محافظة القادسية. خضعت جميع هذه العينات للفحص السيرولوجي باستخدام اختبار تلازن جزيئات اللاتكس وأظهرت نتائج هذا الفحص ان 134(67%) من الدجاج حر المعيشة و62(31%) من الدجاج التجاري و28(56%) من البط كانت مصابة بداء المقوسات كتشخيص أولى.تم تأكيد التشخيص باستعمال اختبار التألق المناعى المرتبط بالأنزيم وذلك بفحص 90 عينة مصل (45 من الدجاج حر المعيشة و45 من الدجاج التجاري) من عينات المصل الموجبة لاختبار تلازن اللاتكس ،حيث ظهر ان النسبة الكلية للدجاج المصاب 36(40%) حالة مصابة إصابة حقيقية، وتبين ان النسبة المئوية الأعلى للإصابة كانت في الدجاج حر المعيشة حيث بلغت 51.11% بينما كانت النسبة الأدنى في الدجاج التجاري حيث بلغت 28.88% .أظهرت النتائج ان النسبة المئوية للدجاج حر المعيشة الحامل لأضداد مقوسات كوندي والمفحوصة باختبار تلازن حبيبات اللاتكس كانت71.42. % ، 69.23% و 55.38 % في المحافظات القادسية ، بابل والنجف على التوالي بينما بلغت النسب66.66% ، 46.66% و 40% في القادسية ، بابل و النجف على التوالي في اختبار ارتباط الخميرة للأدمصاص المناعي.على الرغم من الفروقات آلتي تبينت من النسب المئوية لأضداد المقوسات الكوندية بين المحافظات المختلفة ألاان لم يكن هنالك فرقاً معنوياً(P>0.05) باختبار تلازن حبيبات اللاتكس بينما كان هنالك فرقاً معنوياً معنوياً (P<0.05) بأختبار ارتباط الخميرة للأدمصاص المناعى. كان المعيار الأعلى في اختبار تلازن حبيبات اللاتكس 1/128 (34.32 %) في الدجاج حر المعيشة ،1/8 (29.3%) في الدجاج التجاري و 1/32 (35.7%) في البط بينما كان المعيار الأدنى 1/2 (5.22 %) في الدجاج حر المعيشة ، 1/2(0%) في الدجاج التجاري و 1/4 (0%) في البط.