Investigate the *Toxoplasma gondii* infection in the consumed beef in Al-Diwaniyah province

F. M. Sakban *Noaman N. A'aiz

Dept. of Microbiology and Parasitology, College of Veterinary Medicine, Univ. of Al-Qadisiyah
*Corresponding author: noaman.a'aiz@qu.edu.iq

Abstract

Toxoplasmosis is very important zoonotic disease in the world cause by an obligate intracellular protozoan parasite called *Toxoplasma gondii* can infect human and all warm-blood animals, beef consider from most important source for infection with *T.gondii* and there is no really data and study about the rate of the infection in beef in Diwaniyah, the aim of this work was to determine the prevalence of *T.gondii* in local and imported beef by using molecular methods with study the effect of certain factors on its prevalence in Diwaniyah province.

A total of 300 samples which collected from heart, tongue, muscles, of 100 slaughtered beef of local and imported cattle, throughout the period from September 2017 to May 2018 ,initially examined microscopically for searching on *T.gondii* bradyzoits then all suspected samples was subjected to conventional PCR technique through *B1*gene amplification to confirm the infection with *T.gondii*, in addition to analyzed the recorded data for each sample to determine the effect of some factors on prevalence of infection like organ, season and animal age.

Out of 300 tested samples only 53 were confirm positive *T.gondii* DNA. The infection in local beef was higher than in imported beef 33/150 (22%) and 20/150 (13.5%) respectively and there is no difference ($p < 0.05$) in infection among different examined organs. Regarding to effect of some factors, the autumn season recorded highest rate of infection with significant differences ($p < 0.05$) rather than others seasons in both local and imported beef, while the age appears with no effect on infection ($p < 0.05$).

The local cattle meat are more risky than the imported due to the higher rate of infection with *T.gondii*, and the animal age cannot effected on the infection rate, whereas the season play role in this rate.

Key words : Toxoplasma gondii, beef , Iraq, Diwaniyah, PCR.

التحري عن الاصابة بمقوسة كوندي في لحوم الابقار المحلية و المستوردة في محافظة الديوانية

فرح محمد صكبان نعمان ناجي عايز

فرع الاحياء المجهرية والطفيليات/ كلية الطب البيطري/ جامعة القادسية

الخلاصة

داء المقوسات هو من اهم الامراض المشتركة حول العالم ويسببه طفيلي مقوسة كوندي , وهو من الاوالي مجبرة التطفل داخل الخلايا والتي تصيب الانسان ومعظم حيوانات الدم الحار, وتعتبر اللحوم احد اهم المصادر لنقل الاصابة و لا يوجد هناك دراسات او بيانات اولية حول نسبة اصابة لحوم الابقار المحلية والمستوردة في محافظة الديوانية باستخدام طريقة الفحص الجزيئي. أن الهدف من الدراسة هو لتحديد نسبة انتشار طفيلي مقوسة كوندي في لحوم الابقار المحلية والمستوردة بأستخدام الطرق الجزيئية مع دراسة تأثير بعض العوامل على نسبة انتشاره في محافظة الديوانية.

تم اجراء الفحص المجهري لـ 300 عينة مأخوذة من القلب واللسان والعضلات لـ 100 ذبيحة لأبقار من سلالات محلية ومستوردة وذلك للكشف عن الطور الساكن ثم تم اجراء تقنية تفاعل سلسلة البلمرة لبعض العينات من خلال مضاعفة جين*B1*، بالإضافة إلى تحليل البيانات المسجلة لكل عينة لتحديد تأثير بعض العوامل على انتشار العدوى مثل العضو و الموسم وعمر الحيوان.

تم تأكيد الاصابة بطفيلي مقوسة كوندي لـ53 نموذج فقط من اصل 300 وكانت نسبة الاصابة في اللحوم المحلية اعلى منها في اللحوم المستوردة حيث كانت (150/33) 22% ,(150/20) 13.5 % على التوالي.

تعتبر لحوم الماشية المحلية أكثر خطورة من المستوردة بسبب ارتفاع معدل الاصابة فيها بطفيلي مقوسة كوندي، ولا يمكن لمعدل الإصابة أن يتأثر بعمر الحيوانات او العضو، في حين يلعب الموسم دور في هذا المعدل.

الكلمات الافتتاحية: مقوسة كوندي, , اللحوم، الديوانية، العراق، تفاعل سلسلة البلمرة.

Introduction

Toxoplasma gondii is an protozoan obligate intracellular parasite and its cause Toxoplasmosis, zoonosis disease worldwide distribution can infected all warm –blood animals which act as intermediate host and felids like domestic cats known as definitive hosts (1)

The importance of this disease occurring during pregnancy period especially in women, where this parasite cause many congenital defects in fetus in addition its severe life-threating infections, also immunocompromised individuals consider the target to this parasite, and can cause illness in many features, in both the weakness of immune system play important role in occurrence of the infection (2)

The main ways of disease transmission are ingestion of oocyst that contain sporozoites by food, water, or any way lead to contact with cat feces , transmission via placenta , and consumption of raw or under cooked meat until dealing with it, so about 28% pregnant women get infection by this way (3).

The role of beef in transmission of *T.gondii* infection to human is unclear yet because of the infection by it is reported as the one of the risk factors that cause acute infection, on the other hand the theory believed that cattle poor persistence of parasite and the tissue cyst are not very persistent in cattle ,thus it is necessary make large scale screening of cattle for the present of *T.gondii* to clarify the role of cattle as a source of infection (4).

According to the importance of toxoplasmosis as a zoonotic disease and it's close relationship to the health of community, role of meat in transporting of *T.gondii* infection to human, and there is no previous studies in this field in AL- Diwaniyah province the aim of study was designed.

Material and Method

Three hundred samples were used in this study which collected from heart, tongue, muscles, of 100 slaughted animal of local and imported cattle.

- Isolation of *T.gondii* bradyzoits

meat samples were subjected to a peptic digestion method for isolate suspected toxoplasma bradyzoites from the examined tissue to prepare it for DNA extraction, briefly 10 g of meat from each sample was cut by scissors for a very small pieces and they were transferred into a sterile test tube, 10 ml from digestive solution (pepsin2.6 g, NaCl 5g, HCL7ml, Distilled water 485 ml this amounts for 500 ml from digestive solution) were added to the tube and incubated at 40°C for 30 minutes, then the digestate was poured through two layers of gauze into a flask and then the filtrate was centrifuged (5000 rpm for 10 min), the supernatant was discarded and 5ml of sodium bicarbonate solution(NaHCO3. 6 g+ 500 ml distilled water) was added to precipitate then mixed well and re-centrifuged (5000 rpm for 10 min), after that supernatant was discarded again and the precipitate was examined microscopically. The positive sample's precipitates were suspended in 600 µl normal saline and mixed well, then used for DNA extraction.

- Genomic DNA Extraction

Genomic DNA were extracted from each prepared samples of meat by used the gSYAN DNA extraction Kit (Geneaid, USA), depending on the company's instructions.

-PCR Amplification

Conventional PCR was performed for detection *T.gondii* based on amplify the *B1* gene, this procedure was applied according to technique explained by (5) this gene was target to produce the specific primer ,as forward primer (5'AAAAATGTGGGAATGAAAGAG 3'), and revers primer (5'ACGAATCAACGGAACTGTAAT 3'), and the conditions of PCR thermo cycler (Amplification steps) included initial denaturation at 95°C for 5min.,30 cycle for denaturation at 95 °C for 30sec., annealing at 56 °C for 30sec., extension at 72 °C for 30sec., 1 cycle for final extension at 72 °C for 5 min., the PCR products of *B1* gene was analyzed by 1% agarose gel electrophoresis to detect an expect 469 bp product.

Results

The result of molecular examination showed that 22% (33/150) and 13.5% (20/150) of local and imported cattle's meat respectively were infected with *T.gondii* protozoan, (fig. 1).

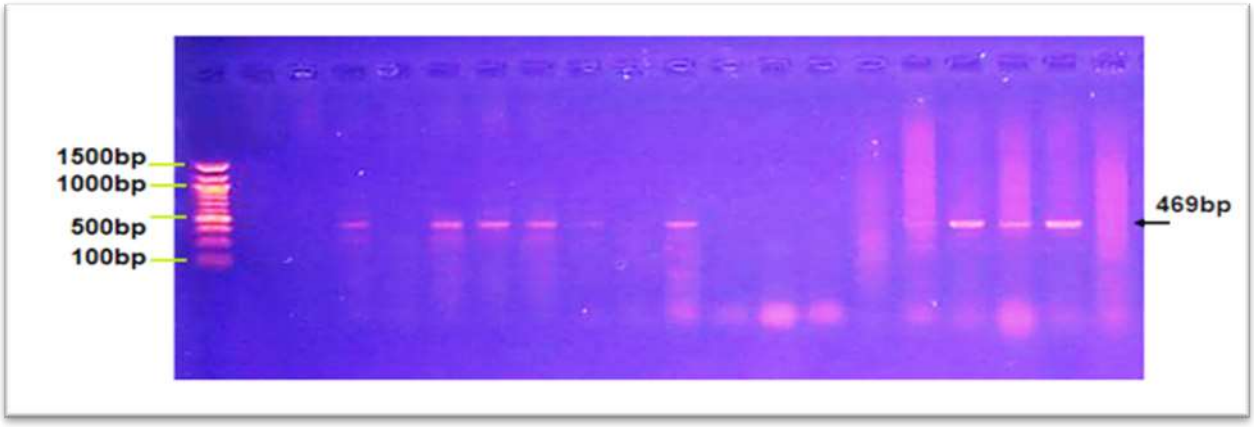

Fig.(1):Molecular-Positive result of *T.gondii* infection according to *B1* gene (469 bp) analysis.

Regarding to the distribution of infection among examined organs the results showed there were 31.5%(15/48), 21%(10/47) and 15.9%(8/55) of the muscles, tongues, and hearts respectively from the local and 18% (9/50), 12.9% (7/54) and 8.6% (4/46) for muscles, tongues and hearts respectively from imported meat were infected but with no significant difference ($p< 0.05$), (table1).

The rate of infection according to seasonal variation appeared highest in Autumn (September to November) in both local and imported meat and recordded14.7% , 5.4% respectively when comparing with other studied seasons, (table 2). According to the effect of age on the rate of infection the result revealed that all studied ages can infect with *T. gondii* but there are no significant difference ($p < 0.05$) among them although the ages' categories of 1-3 years and more than 3 years appeared with highly infection in local and imported meat respectively in comparing with other, (table 3).

Table(1): distribution of *T.gondii* infection in local and imported meat according to the organs.

Organs		**Examined No.**	**Positive No.**	**Percentage (%)**		**Examined No.**	**Positive No.**	**Percentage (%)**
Muscle	**Local meat**	48	15	31.5	**Imported meat**	50	9	18
Tongue		47	10	21		54	7	12.9
Heart		55	8	15.90		46	4	8.6
Total		150	33	22		150	20	13.5
X2		4.189				1.805		
P value		0.123				0.406		

Table (2): infection of *T.gondii* in local and imported meat according to the seasons.

months	**Local meat**	**Examined No.**	**Positive No.**	**Percentage (%)**	**Imported meat**	**Examined No.**	**Positive No**	**Percentage (%)**
Autumn (Sept.Nov.)		70	21	30		45	12	26.6
Winter (Dec.- Fab.)		40	4	10		66	0	0
Spring (Mar.-May)		40	8	20		39	8	20.5
X2		6.061*				18.817*		
P value		0.048				0		

X2 : chi-square * significant difference at $p < 0.05$

Table (3): infection of *T.gondii* in local and imported meat according to the age.

Age	**Local meat**	**Examined No.**	**Positive No.**	**Percentage (%)**	**Imported meat**	**Examined No.**	**Positive No**	**Percentage (%)**
>1 year		30	4	13		15	1	0.15
1-3 year		57	17	30		48	8	16.6
< 3 year		64	12	19		87	11	12.6
X2		3.757				1.074		
P value		0.153				0.584		

Discussion

Meat is an important source of human food, and among different kinds of meat, beef represent one of the most common consumption type (6). *T.gondii* infects beef as whereas other meat and lead to many health problems like congenital defects and abortion in both humans and livestock (7).

This is the first study that deals with the *T.gondii* infection in local and imported beef in in AL-Diwaniyah / Iraq, in which molecular technique was used to detect the infection through amplification of *B1*gene, serological study associated with beef was done by Zakaria (8) in Iraq (Mosul) to detect the *T.gondii* infection in different meat juices by LAT test, while another many Iraqi studies were deals with the prevalence of infection in sheep ,aborted women, chicken, stray cats (9,10,11, 12, 13) and there are many researches on pregnant and aborted women in Iraq.

Out of 300 beef samples 33(22%) and 22(13.3%) for local and imported beef respectively were achieved a positive results. This results were compatible to the results of Ehsan in Iraq (Mosul) who mentioned in serological study that 17% of examined sample revealed *T. gondii* infection also in molecular study was done by Amdouni *et al*.,(14) from Tunisia showed that among 150 cattle meat samples that 19.3% (29/150) gave positive result. So the agreement between the results of the previous studies and the current study revealed the risky in consumption of infected beef with *T.gondii* .

The results of the present work was less than that values found in Northern Portugal (50%) by Lopes *et al.,(*15), and the study in Colombia included 180 samples from chicken, swine, and beef ,targeted *B1* gene which recorded 37% by Franco-Hernandez *et al.,* (16). In another side the prevalence of *T.gondii* in present study was higher than those recorded in Brazil and Switzerland (2% and 3.8%, respectively) (17; 18), also the study that where done in Iran by (19) which recorded 4% rate of infection.

In comparing study by Navari *et.al.,* (20) between local and imported meat in Iran, showed that the imported meat recorded higher infection rate 26% than local meat 6%, this results are conflict with present study which found the infection in local animals more than infection in imported, thus at the end all these differences among different studies may depend upon many factors like climate conditions, numbers of tested samples, rearing methods, false negative, and different sample size.

Concerning to the infection of *T.gondii* in different examined organs no significant difference ($p< 0.05$) was recorded in the current study. This recording is in agreement with other previous study made by Burrells *et al.,* (21) on calves when studied the probable variations of different organs infection and showed that there is no clear predilection site within bovine tissues.

According to the effect of seasons on infection with *T.gondii*, Autumn was recorded the highest rate of infection with significant difference ($p < 0.05$), the present study consistent with the study (22) in China where they revealed that *T.gondii* was found in 6 (3.33%) samples collected in Autumn and 1 (0.56%) collected in winter, this indicated that seasons might have important impacts on the presence of *T. gondii* , , likewise (23) recorded rising in infection rate during winter and spring than Autumn and summer and referred to that seasonal variation of

T.gondii infection in human are remarkably different than animals, also seasonal variation study on pregnant women in France appeared that the maximal risk of infection between end of summer to end of Autumn (24). this may be related to the availability of favorable conditions for oocysts maturation and sporulation to become infective (25).

Regarding to the animal's age the current study mention that the highest prevalence of infection was recorded in age 1- 3years (30%) (16.6)for local and imported respectively, while lowest prevalence rate observed in ages less than 1year (1.2%). This results near to many another like the study did by (20) and he referred to that infection in cattle and sheep in Iran were increase during ageing in rate 3.7% for animals less than 2 years while the rate of infection was 9.09% in animals with ages range from 2-4 years, another molecular study was done by (14), referred to that younger animals less infection than the older animals, serological study in Sudan conducted by Elfahal *et.al.* (26) for detection of *T.gondii* in cattle, mentioned that the prevalence of *T.gondii* infection was significantly ($P < 0.05$) higher (36.4%) in animals less than one year old than those above two years (12.8%).

In fact many factors impact on *T.gondii* infection incidence such as the type of livestock management and production, the hygiene standards of abattoirs, food processing, the density of cats in the environment, and the habits of human consumers, also the geographical location with respect to altitude, and the prevailing climatic conditions and the important factor is present of the final host (27).

Conclusion

The infection of consumed meat with *T.gondii* can't be negligible due to the valuable rate of infection severity of the problem on community healthy, in addition to that the local cattle meat are more risky than the imported due to the higher rate of infection with *T.gondii*,The parasite can infect all examined organs (Heart, Muscles, Tongue) in the same rates Approximately, and the infection rate can be effected by season but not by age of animals.

Acknowledgment

I extend my thanks and appreciation to all Veterinarians who cooperated with me for getting samples in Al-Diwaniyah abattoirs.

REFERANCE

1- Dubey JP. Sources of Toxoplasma gondii infection in pregnancy. Until rates of congenital toxoplasmosis fall, control measures are essential. BMJ (Clinical research ed.).2000; 321(7254), 127-8.

2-Halonen SK and Weiss LM. Toxoplasmosis. Handb Clin Neurol. 2013;114: 125-45.

3-Kravetz JD and Federman DG. Toxoplasmosis in pregnancy. The American journal of medicine. 2005; 118 (3): 212-6.

4- Jones JL, Dargelas V, Roberts J, Press C, Remington JS, Montoya JG. Risk factors for Toxoplasma gondii infection in the United States. Clin Infect Dis. 2009; 49 (6):878-84.

5- Ortega-Pacheco A, Acosta Viana KY, Guzmán-Marín E, Segura-Correa JC, Alvarez-Fleites M, and Jiménez-Coello M. Prevalence and risk factors of Toxoplasma gondii in fattening pigs farm from Yucatan, Mexico. BioMed research international. 2013; 231497.

6- Dubey JP. A review of toxoplasmosis in cattle. Vet. Parasitol. 1986; 22:177–202.

7- Saadatnia G, and Golkar M. A review on human toxoplasmosis. Scand. J. Infect. Dis. 2012; 44:805–814.

8-Zakaria EG. Detection of Toxoplasma gondii Antibodies in Different Meat Juices. Raf. J. Sci. 2011;Vol. 22, (4):17-25.

9- Alkhaled MJA, Yakoob AY, AL-hamadani AHU. An investigation of Toxoplasmosis in Free Range chickens, Industrial chickens and Duck in mid Euphrates area of Iraq AL-Qadisiya Journal of Vet.Med.Sci. 2012; 11(2).

10-Switzer AD, McMillan-Cole AC, Kasten RW, Stuckey MJ, Kass PH, and Chomel BB. Bartonella and Toxoplasma infections in stray cats from Iraq. The American journal of tropical medicine and hygiene. 2013; 89 (6), 1219-24

11- Mohammed AA and Abdullah Sh H. Diagnostic Study of Toxoplasmosis in Domestic Chickens in Sulaimani Province. AL-Qadisiya Journal of Vet.Med.Sci. 2013; 12 (2).

12-Mohammed NS, Al-A'ssie AH and Al-saqur IM. Genotyping of Toxoplasma gondii Isolated from Aborted Iraqi Women. Diyala Journal of Medicine. 2015; Vol. 9, Issue 1.

13- A'aiz NN. Determination of Toxoplasma gondii lineages of sheep in Wasit, Iraq. Iraqi Journal of Veterinary Sciences. 2016; Vol. 30, No. 2, 2016 (23-26).

14-Amdouni Y, Rjeibi MR, Rouatbi M, Amairia S, Awadi S and Gharbi M. Molecular detection of Toxoplasma gondii infection in slaughtered ruminants (sheep, goats and cattle) in Northwest Tunisia. Meat Science. 2017; 133, 180–184.

15- Lopes AP, Vilares A, Neto F, Rodrigues A, Martins T, Ferreira I, Gargaté MJ, Rodrigues M, Cardoso L. Genotyping Characterization of Toxoplasma gondii in Cattle, Sheep, Goats and Swine from the North of Portugal. Iran J Parasitol. .2015; 10: 465– 472.

16- Franco-Hernandez EN, Acosta A, Cortés-Vecino J and Gómez-Marín JE. Survey for Toxoplasma gondii by PCR detection in meat for human consumption in Colombia. Parasitology Research. 2015;115(2): 691–695.

17- Santos SL, Costa KDS and Gondim L. Q.Investigation of Neospora caninum, Hammondia sp. and Toxoplasma gondii in tissues from slaughtered beef cattle in BahiaBrazil. Parasitology Research. 2010;106(2), 457–461.

18- Berger-Schoch AE, Herrmann DC, Schares G, Müller N, Bernet D, Gottstein B, and Frey C F. Prevalence and genotypes of Toxoplasma gondii in feline faeces (oocysts) and meat from sheep, cattle and pigs in Switzerland. Veterinary Parasitology.2011; 177(3-4):290–297.

19- Rahdar M, Samarbaf-Zadeh A, Arab L. Evaluating the Prevalence of Toxoplasma gondii in Meat and Meat Products in Ahvaz by PCR Method, Jundishapur J Microbiol. 2012; 5(4):570-573.

20-Navari D, Saadati D, Nabavi R and AL-ipour Eskandani M. Epidemiology and Molecular Prevalence of Toxoplasma gondii in Cattle Slaughtered in Zahedan and Zabol Districts, South East of Iran Iran. J Parasitol. 2017; Vol. 13, No. 1, pp.114-119

21- Burrells A, Taroda A, Opsteegh M, Schares G, Benavides J, Dam-Deisz C, Bartley PM, Chianini F, Villena I, van der Giessen J, Innes E A, … Katzer F. Detection and dissemination of Toxoplasma gondii in experimentally infected calves, a single test does not tell the whole story. Parasites & vectors. 2018; 11(1), 45.

22- Liu X, He Y, Han D, Zhang Z, Li K, Wang S, Xu L, Yan R and Li X. Detection of Toxoplasma gondii in chicken and soil of chicken farms in Nanjing region, China Infectious Diseases of Poverty. 2017; 6:62.

23- Logar J, Soba B, Premru-Srsen T, Novak-Antolic Z. Seasonal variations in acute toxoplasmosis in pregnant women in Slovenia.Clin Microbiol Infect. 2005; 11(10):852-5.

24- Morin L, Lobry JR, Peyron F and Wallon M. Seasonal variations in acute toxoplasmosis in pregnant women in the Rhoˆne-Alpes region (France) Clin Microbiol Infect. 2012; 18: E401–E403.

25- Yan C, Liang LJ, Zheng KY and Zhu XQ. Impact of environmental factors on the emergence, transmission and distribution of Toxoplasma gondii. Parasites and vectors. 2016; 9, 137.

26- Elfahal AM, Elhassan AM, Hussien MO, Enan KA, Musa AB, and ElHussein AM. Seroprevalence of Toxoplasma gondii in Dairy Cattle with Reproductive Problems in Sudan. ISRN Veterinary Science. 2013; Article ID 895165, 4 pages.

27- Hall S, Ryan KA and Buxton D. The epidemiology of toxoplasma infection. In Toxoplasmosis: a comprehensive clinical guide (eds Joynson D. H., Wreghitt T. J., editors.). 2001; pp. 58–124 Cambridge, UK: Cambridge University Press.