Seroprevalence and Molecular Detection of *Toxoplasma* gondii Infection among chicken (Gallus domesticus) in Riyadh Region, Saudi Arabia.

Maha H Elamin[1*]

[1]Department of Zoology, Faculty of Science, King Saud University, Women's Students-Medical Studies & Sciences Section, Riyadh 11451, Saudi Arabia

*Corresponding author: Tel: 00966500731556, E-mail: mahaalamin@yahoo.com

Abstract

Toxoplasmosis is a widely prevalent zoonotic disease**.** The prevalence of *Toxoplasma gondii* (*T. gondii)*infection in chicken is a good indicator of soil contamination with *T.gondii*oocysts. Since chicken meat is widely consumed in many countries including Saudi Arabia, in the present report, the prevalence of *T.gondii* infection in chicken (*Gallus domesticus*) in Riyadh region, Saudi Arabia was investigated, moreover, the *T.gondii* recent infection was determined using PCR test. *T.gondii*Seroprevalence was studied in 200 chicken (*Gallus domesticus*) collected from different regions in Riyadh using ELISA IgG assay. The results revealed that 32% (64 out of 200) of the chicken investigated were seropositive. PCR test on the *T.gondii* B1 gene showed that 37.5% of the chicken had *T.gondii* DNA indicating recent infection. We concluded that, *T. gondii* infection may pose a potential threat to human and animal health in Riyadh region and attention should be given.

Key words: *Toxoplasma gondii*, Chicken (*Gallus domesticus*), Seroprevalence, PCR, Saudi Arabia.

1. Introduction

Toxoplasma gondii (T.gondii) is an obligate intracellular protozoan parasite that infects warm-blooded vertebrates. The infection is widely prevalent in human and animals worldwide [1,2]. Human become infected by consuming food or water contaminated with oocysts, ingesting tissue cysts from undercooked meat, or by accidentally ingesting oocysts from the environment. Felids are the most important hosts in the life cycle of *T. gondii*because they excrete environmentally resistant oocysts. Cats become infected with *T. gondii* by eating infected tissues from intermediate hosts. Meat from *T. gondii*-infected poultry (including chickens, ducks, geese, and pigeons) is consumed widely in many countries and is known to be the primary source of infection for humans [3,4]. Chickens are considered one of the most important hosts in the epidemiology of *T.gondii* infection because they are an efficient source of infection for cats that excrete the environmentally resistant oocysts and because humans may become infected with this parasite after eating undercooked infected chicken meat [5]. Detection of *T. gondii*oocysts in the environment is difficult, since domestic cats normally choose to bury their feces in soft and moist soil. However, free-range chicken have been used as 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 [5].

As there is lack of information in the role of chicken (*Gallus domesticus*) in environmental contamination and the transmission of *T.gondii* infection to human and animals in Riyadh region, Saudi Arabia, the investigation of this problem is undertaken in the present report.

2. Materials and methods

2.1 Study area

200 chicken (*Gallus domesticus*) were collected from different regions in Riyadh, Saudi Arabia, during the period February-July, 2013. All procedures for animal experimentation used were approved by the Institutional Animal Ethics Committee, King Saud University, Riyadh, Saudi Arabia.

2.2 Enzyme Linked Immunosorbent Assay (ELISA)

Toxoplasma IgG antibodies were detected using ELISA IgG test. Briefly, blood samples were collected from 200 chicken (Gallus domesticus).Sera were separated and diluted (1:50) in buffered7% NaCl and examined using ELISA IgG kit (BiocheckInc, USA). Diluted sera, positive and negative controls were added to the 96 well plates coated with toxoplasma antigens. Plates were incubated at 37C° for 30 minutes. After incubation, the plates were washed with diluted wash buffer (1X). Enzyme conjugate (100 µl) was added and plates were incubated at 37C° for 30 minutes.After washing, substrate was added and incubated for 10 minutes at 37C°. TMB reagent (100µl) was added followed by incubation at 37C° for 15 minutes. The reaction was stopped and the opticaldensity was measured using ELISA reader (Labsystemmultiskan EX), USA) at 450 wave length.

2.3 Polymerase Chain Reaction (PCR)

DNA was purified from blood using Puregene kit by following the instructions of the manufacturer (Gentra Systems, Minneapolis, USA).

PCR described by Burg *et al*.,[6] with some modificationswas performed using 10× PCR buffer (100 mMTris-HCl; 500 mMKCl; 15 mM $MgCl_2$; 0.01% gelatin) (Sigma Aldrich, USA), 1.5µM of each primer (Invitrogen- Life Technologies, Germany), 2U of Taq DNA polymerase (Invitrogen- Life Technologies, Germany) and 150-250 ng of DNA in a total reaction volume of 50µl. PCR reactions were conducted in a GeneAmp® PCR System 9700 (Applied Biosystems®).The first step of amplification was 10 min of denaturation at 95°C. This step was followed by 40 cycles, with 1 cycle consisting of 60 seconds at 95°C, 55 seconds at the annealing temperature for each pair of primers, and 60 seconds at 72°C. The final cycle was followed by an extension step of 10 min at 72°C. Primers used were B1F1 (5´GGAACTGCATCCGTTCATGAG3´), B1R1 (5´TCTTTAAAGCGTTCGTGGTC3´) (Invitrogen- Life Technologies, Germany). The primers correspond to the *T.gondii* B1 gene nucleotides 694-714 and 887-868, respectively. PCR was performed using PCR thermal cycler (Perkin Elmer, USA).

2.3.1 Nested PCR

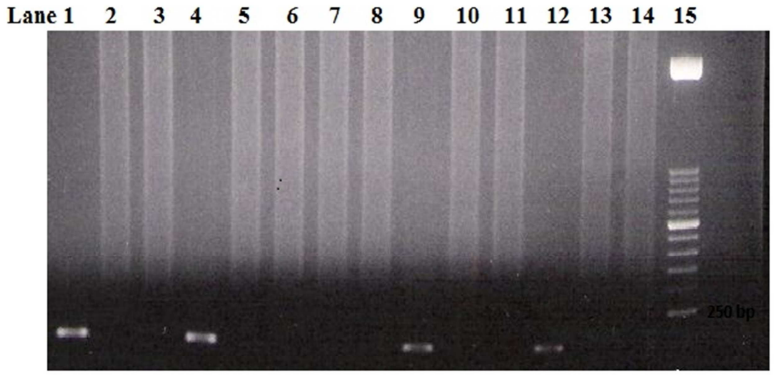
One µl of the 1:10 diluted PCR products of the first amplification were used as template for the second round the primers were replaced with the internal set B1F2 (5´TCTTTAAAGCGTTCGTGGTC3´), B1R2 (5´GGCGACCAATCTGCGAATACACC3´) (Invitrogen- Life Technologies, Germany), they correspond to the *T.gondii* B1gene nucleotides 757-776 and 853-831, respectively. The DNA products were amplified using the same PCR conditions, except for the annealing temperature which was 59C° for 2 min. Both control positive (*T.gondii* RH strain) and control negative (no DNA and ELISA negative samples) were used.PCR products were analyzed using 2% agarose gel electrophoresis

3. Results

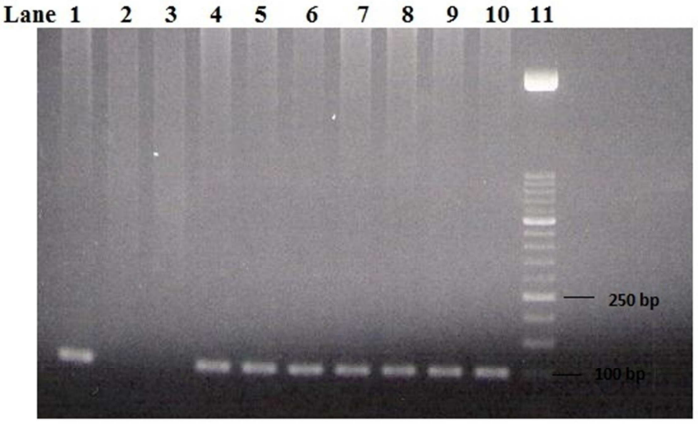
In the present study, ELISA IgG test revealed that, 64 (32%) out of the 200 chicken (*Gallus domesticus)*tested were positive.

T.gondii DNA was found in 75 (37.5%) of 200 chicken examined, and a fragment of 194 bp was obtained (Fig 1).Some of the IgG negative samples (11 samples) were positive by PCR test.

All PCR positive samples were confirmed by nested PCR which rendered the expected fragment of 97 bpincluding the reference RH strain (Fig2).



Amplification of *Toxoplasma gondii* B1 gene. Lane 1:Control positive. Lane 2,3: Control negative. Lane 4,9,12. Positive samples. Lane 15: 50bp DNA



Nested PCR on *Toxoplasma gondii* B1 gene. Lane 1. Control positive. Lane 2,3. Control negative. Lane 4-10 Positive samples. Lane 11. 50 bp DNA marker.

4. Discussion

Chicken are considered one of the most important hosts in the epidemiologyof *T.gondii* infection because they are an efficient source of infectionfor cats that excrete the environmentally resistant oocysts and because human may become infected with this parasite after eating undercooked infectedchicken meat [3].

In the present study, we found ELISA IgGseroprevalence of 32% in chicken (*Gallus domesticus*).To our knowledge, no studies were conducted for detection of *T. gondii*seroprevalence among

chicken(*Gallus domesticus*)in Saudi Arabia. Many *T.gondii*seroprevalencestudiesin Riyadh, Saudi Arabia,were conducted in animals. In a recent study in Riyadh, Saudi Arabia, among 412 healthy camels, antibodies to *T. gondii* were found in 27 (6.6%)[7]. During an outbreak of caprine abortion in a farm in Riyadh, anti-*T.gondii* antibodies were detected by ELISA in 63.2% of the goats [8]. The seroprevalence of *T.gondii* among clinically healthy horses used for sporting purposes in Riyadh was 31.6%[9]. In another report from Riyadh, a seroprevalence of 90% among stray cats was detected [10]. From the previous studies in Riyadh, it is clear that, there is a high prevalence of toxoplasmosis in some animals such as horses, goats and stray cats.

The seroprevalence in the present report(32%), was in agreement with others from Egypt performed among 600 chicken and reported that, 200 (33.3%) were positive [11].The seroprevalence obtained in the present study was higher than otherperformed in Egyptin which out of 85 chicken tested, 17 (20%) were found positiveusing the Dye test [12]. It was also found in Egypt that among 320chicken tested for the presence of *T.gondii* antibodies, 53.3% were infected, using the CFT test [11].

The discrepancy in results could be due to variation in the serological tests used.The results in the present report are in concordance with others obtained from Jordan in which *T. gondii*seroprevalence of 36% was detected [13]. In Iran, 23 (51%) out of 45 chicken were seropositive. The seropositivity percentage obtained in the present study was lower compared to Iran and this could be explained by thevariation in the number of chicken examined in each study[14]. Our results agree with other reports from India (Chennai, Madras) in which 39.5%*T.gondii*seroprevalence was obtained [15].The seroprevalence of *T. gondii* antibodies in 125 free-range chicken from Addis Ababa (Ethiopia) was 48 (38.4%) [16], which is also comparable to the prevalence obtained in the present study.

In USA, *T.gonii*seroprevalence of 16.9% [17] and 100% [4]were found in Ohio and Illinois, respectively. The discrepancy of the results of the present study from those conducted in USA could be explained by the variation in environmental conditions which affect oocyst survival [18,19]In Turkey and Mexico,*T.gondii*seroprevalenceamong free range chicken investigated,were 1.6%, [20] and 6.2%[21], respectively, The higher prevalence detected in Riyadh compared to those countries may be due to more hygienic practices in those countries which led to a decrease of *T.gondii*prevalence.Seroprevalence may also be affected by ecological and geographical factors, as well as chicken feeding conditions.
In the present report, using PCR test, 77 (38.5%) of chicken (*Gallus domesticus*) were found positive. There is clear evidence that PCR test showed higher positivity (38.5%) than ELISA test (32%).

PCR test on *T.gondii* B1 gene is highly sensitive and specific test in diagnosis of toxoplasmosis, because the B1 gene is a 35-fold repetitive gene sequence [6].Wastling*et al*. compared two genes sequences, one found in the *T.gondii* gene that codes protein P30 of the tachyzoite and the other in the *T.gondii* B1 gene. They concluded that use of the B1 sequence increased the sensitivity of PCRtest. In another study Burg *et al*. detected the presence of only one micro-organism using PCR test on the *T.gondii* B1 gene [6].

Conclusion

The results obtained in the present study indicated*T. gondii*infection in chicken in Riyadh, Saudi Arabia and suggested that the meat from the chicken might be an important source for human and animal infection by *T. gondii*parasite. Larger scale studiesare needed to investigate the seroepidemiology of *T.gondii*in Riyadh.Furthermore, molecular characterization of *T.gondii* in chicken *(Gallus domesticus)*in Riyadh, Saudi Arabiashould be investigated.

Acknowledgements

This work was supported by the National Plan for Science and Technology (NPST), funded by King Abdulaziz City for Science and Technology (KACST) through project number 10-ENV993-02.

References

1. Dubey JP. Toxoplasmosis of animals and humans.CRC Press, Boca Raton, Florida 2009.

2.TenterAM, Heckeroth AR and Weiss LM. *Toxoplasma gondii*: from animals to humans. Int J Parasitol 2000, 30:1217-1258.

3. Dubey JP. Toxoplasmosis of animals and humans.2nd edition. Boca Raton,Florida, New York: CRC Press Inc 2010a:1-313.

4. Dubey JP, Webb DM, Sundar N, Velmurugan GV, Bandini LA, Kwork OC and SuC: Endemic avian toxoplasmosis on a farm in Illinois: Clinical disease,diagnosis, biologic and genetic characteristics of *Toxoplasma gondii*isolates from chickens (*Gallus domesticus*), and a goose (Anseranser). VetParasitol 2007, 148: 207–212.

5. Dubey JP. *Toxoplasma gondii* Infections in Chickens (*Gallus domesticus*): Prevalence, Clinical Disease, Diagnosis and Public Health Significance. Zoonoses and Public Health 2010b, 57: 60-73.

6. Burg JL, Grover CM, Pouletty P and Boothroyd JC. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by Polymerase Chain Reaction. J ClinMicrobiol 1989, 27: 1787-1792.

7. Al-Anazi AD. Prevalence of Neosporacaninum and *Toxoplasma gondii* antibodies in sera from camels (Camelusdromedarius) in Riyadh Province, Saudi Arabia J Egypt SocParasitol 2011, 41: 245-250.

8. AL-Mufarrej SI, Hussein M, Aljumaah RS and Gar ElNabi AR. Toxoplasmosis in Goats in Riyadh, Saudi Arabia. J of Animal and Vet Advances 2011, 10: 2779-2782.

9. Alanazi AD, Alyousif MS. Prevalence of antibodies to *Toxoplasma gondii* in horses in Riyadh Province, Saudi Arabia. J Parasitol 2011a, 97: 943-945.

10. Al-Mohammed HI.Seroprevalence of *Toxoplasma gondii* Infection in Cats, Dogs and Ruminant Animals in Al-Ahsa Area in Saudi Arabia.Res J Med Sci, 2011: 190-192.

11. Hassanain MA, Derbala ZA, and. Kutkat MA. Serological diagnosis of *Toxoplasma gondii* (Apicomplexa: Toxoplasminae) infection in laying hens. Egypt JApplSci 1997, 12: 1-8.

12. Rifaat MA, Morsy TA, Sadek SM and Arafa MS, Azab ME, and Abdel Ghaffar FM. Isolation of Toxoplasma parasite from animals in Egypt (Review). J Egypt SocParasitol 1977, 7: 219-223.

13. Morsy TA, Michael SA, and El Refaii A: Toxoplasma antibodies in chickens in Aman, Jordan. J Egypt SocParasitol 1978, 8: 313-316.

14. Zia-Ali N, Fazaeli A, Khoramizadeh M, Ajzenberg D, Darde´M, and Keshavarz-Valian H. Isolation and molecular characterization of *Toxoplasma gondii* strains from different hosts in Iran.Parasitol Res 2007, 101: 111-115.

15. Devada K, Anandan R, and Dubey JP. Serologic prevalence of *Toxoplasma gondii* in chickens in Madras, India. J Parasitol 1998, 84: 621-622.

16. Tilahun G , Tiao N, Ferreira LR, Choudhary S, Oliveira S, Verma SK, Kwok OHC,Molla B, Saville WJA, Medhin G, Kassa T, Aleme H , Gebreyes WA , Su C, and Dubey JP. Prevalence of *Toxoplasma gondii* from Free-Range Chickens (Gallus domesticus) from Addis Ababa, Ethiopia. J Parasitol 2013, 99: 740-741.

17. Dubey JP, Graham DH, Dahl E, Sreekumar C, Lehmann T, Davis MF, and Morishita TY. *Toxoplasma gondii* isolates from free-ranging chickens from the United States. J Parasitol 2003, 89: 1060-1062.

18. Jones JL, Kruszon-Moran D and Wilson M. United States, 1999–2000. Emerging infectious diseases. 2003, 9: 1371.

19.Dubey JP, Graham DH, Blackston CR, Lehmann T, Gennari SM, Ragozo AMA , Nishi SM, Shen SK, Kwok OCH, Hill DE, and Thulliez P. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from Sa˜o Paulo, Brazil: unexpected findings. Int J Parasitol 2002, 32: 99-105.

20. Inci AC, Babu¨r, Dinc¸er S, and Erdal N. Detection of anti-*Toxoplasma gondii* antibodies using Sabin-Feldman dye test in domestic fowls in some provinces in Turkey. Tu¨rkiyeParazitolDerg 1998, 22: 420-423.

21.Dubey JP, Morales ES, and Lehmann T. Isolation and genotyping of *Toxoplasma gondii* from free-ranging chickens from Mexico. J Parasitol 2004b, 90: 411-413.

22. Wastling JM, Nicoll S, and Buxton D. Comparison of two gene amplification methods for the detection of *Toxoplasma gondii* in experimentally infected sheep. J Med Microbiol 1993, 38: 360-365.