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Molecular diagnosis of *Toxoplasma gondii* in Greylag goose (*Anser anser*)

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ABSTRACT

Thirty samples of Greylag goose (*Anser anser*) were collected from Al-Dalmage marshes in Wasit province –Iraq. The specimens of tissue was collected from brain and liver and grinded by grinder after treated with liquid nitrogen to prepare 'tissue powder', Genomic DNA purification kit (promega. USA) was used to extraction of sample's DNA according to company instructions and tested by Real time PCR using primers and probe targeting B1 gene of *Toxoplasma gondii* parasites. There are nine out of 30 (30%) of duck's brain samples was positive. Whereas 14 out of 30 (46.6%) samples of duck's liver was positive.

Key word : *Toxoplasma gondii, Greylag goose, Real Time PCR*

Introduction

The zoonotic disease, Toxoplasmosis caused by obligatory protozoan parasite *Toxoplasma gondii*, it is one of the most successful eukaryotic pathogen, it is worldwide prevalent in human, and most terrestrial animals (Dubey and Beattie, 1988; Dubey, 2010).

The wild birds are most common intermediate hosts of *T. gondii* (Dubey 2002). The sporulated oocysts are a common infective stage of parasites for herbivorous birds, which can be contaminates soil and water to which exposed these birds (Dubey, 2009). In epidemiology, the wild aquatic birds are very important in'toxoplasmosis' because they acts as reservoir hosts, and vectors of zoonotic pathogens such as *Toxoplasma gondii*. Some species of Geese such as "Ross's and Lesser Snow Geese"are routinely exposed to toxoplasmosis because they stay during winter and migrate to areas where parasites infective stages are likely to be environmentally present. Stacey (Stacey *et al.,* 2014) suggests that both populations of these samples from the central Canadian region were exposed to toxoplasmosis at several stages in their lives, supporting the hypothesis that aquatic birds"can be a source of *T. gondii* in the terrestrial Canadian region". The risk of transmission the parasites from geese to people and other wildlife animals"emphasizes" the need for trusted serological survey (McClintock *et al.,* 2010).

In the United State, *T. gondii* where is rarely documented as adeath causes in wild geese; there is only one report of fatal toxoplasma infection occurred in a Texas zoo as 16% Magpie Geese (*Anseranas semipalmata*) (Dubey *et al*., 2001).

Thierry (Thierry *et al*., 2016) identified two new genotypes that have not been previously reported in America or other regions based; these new genotypes of *T. gondii* detected in visceral organs of Hawaiian Nene Goose that died due to toxoplasmosis did not fit to those previously documented from mouflon sheep (Ovisammon) from Oahu (Verma *et al*., 2015) or Hawaiian Crows (Dubey *et al*., 2011), nor did they fit to the three major Types I, II, and III (Howe and Sibley, 1995).

The DNA of *Toxoplasma gondii* extracted from tachyzoites that isolates from infected Canadian

geese (*Brantacanadensis*) characterized using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), the results appear five different genotypes (Verma *et al*., 2016).

The most cases of prevalence of *T. gondii* in wild animals detected by serological tests, in which filter paper blood collection is a method that is used for detection of post-mortem antibody in these animals (Aston *et al*., 2014).

Seroprevalence, Genetic characterization and isolation of viable *Toxoplasma* parasite from a migratory population of Canadian geese was evaluated by (Verma, *et al*., 2016), by using modified agglutination technique the antibodies against parasites detected in 12 of 169 Canadiangeese.

The aim of this investigation was detection of *T. gondii* in greylag gooseducks rearing and consumption by human in Iraq.

Materials and Methods

Sample collection

Thirty samples of Greylag goose (Anseranser) were collected from Al-Dalmage marshes in Wasit province -Iraq. Collection of birds was done during January-April 2017. The weight of samples ranging from two to 3 kg. the samples of tissue collected from brain and liver and grinded by grinder after treated with liquid nitrogen to prepare 'tissue powder'.

DNA extraction

Genomic DNA extraction kit (promega. USA) was used to extraction of sample's DNA according to company instructions.

The primers and probe

Specific primers and probe was used to amplify a fragment (94 bp) of B1 gene of *Toxoplasma gondii* in several specimens. The primers and probes were design by (Mei-Huilin, 2000) and supplied by (Bioneercompany, Korea). Primers sequences was, TOXO-F"(5'- TCCCCTCTGCTGGCGAAAAGT-3') and TOXO-R (5'- AGCGTTCGTGGTCAACTAT CGATTG-3'); the TaqMan probe was specific for B1 gene in *Toxoplasma gondii*, the probe sequences was (5-6FAM-TCTGTGCAACTTTGGTGTATTCG CAG-TAMRA-3).

Real-Time PCR

This technique done according to (Mei-Huilin *et al*., 2000). The reaction was performed after initial activation of AmpliTaq Gold DNA polymerase at 95 °C for 10 min, 40 PCR cycles of 95 °C for 15 s and 60 °C for 1 min. The qPCR master mix was prepared by by (GoTaq®qPCR Master mix)."The master mix done according to company instructions"the reaction solution volume (25) mL containing"[DNA template 5 mL; Forward primer (10 pmol) 1 mL; Reverse primer (10pmol) 1mL; probe (20 pmol) 1 mL; qPCR master mix 12.5 mL; PCR water 4.5 mL]"put in Thermocycler (Bio Rad USA).

Results

The results of duck brain samples by real time PCR was elucidates in Fig. 1, there are 9 out of 30 (30%) of duck'sbrain specimens were positive. The maximum DNA levels of B1 gene fragment of *Toxoplasma gondii* that amplified of these specimens was (2.21) and the minimum was (0.895). Whereas 14 out of 30 (46.6%) specimens of duck'sliver was positive, the maximum DNA concentration of B1 gene fragment of *T.gondii* was (2.18), and the minimum was (0.721), Fig. 2.

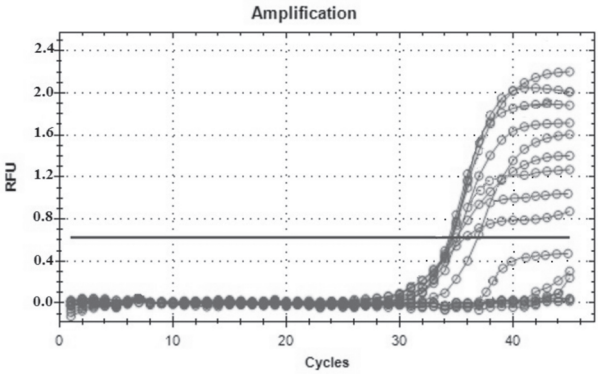

Fig. 1. Amplification plot of B1 gene of *Toxoplasma gondii* by Real-Time PCR in positive duck brain samples.

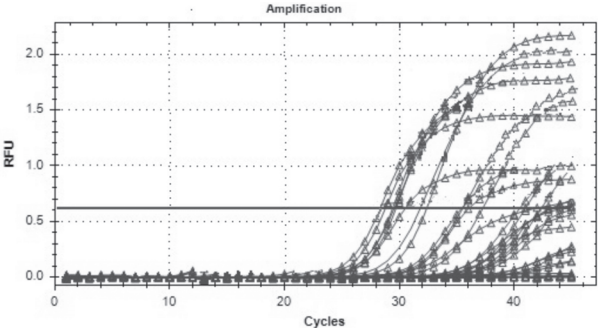

Fig. 2. Amplification plot of B1 gene of *Toxoplasma gondii* by Real-Time PCR in positive duck liver samples.

Discussion

Our investigation suggests that both liver and brain samples of Greylag goosewere infected with *T. Gondii,* this results agree with (Stacey *et al.,* 2014) suggestions that the populations of Ross's and Lesser Snow Geese collected from the central Canadian region were exposed to this parasite during 'migrating to the Karrak Lake area of Nunavut'and at some point in their lives. Although *T. gondii* infection was present in other species of wild goose (Murao *et al*., 2008; Sandstrom *et al*., 2013).

Our results also agree with (Lucie Skorpikovaa, *et al*., 2018) when recorded infection in brain and heart of'wild ducks and common pheasantsin the' Czech Republic by using Real-time PCR assay targeting B1 gene sequence, which remains conserved in almost of all strains of *T. gondii*. The authors also suggest overall prevalence of *T. gondii* was higher positivity in wild ducks with low in feathered game in comparison with common pheasants; this may be connectedto different natural environment of pheasants and wild duckssuch as their nutritional habits. The oocysts of *T. gondii* present in the upper parts of plants more than in below ground or in the ground, suggesting that diversity in feeding habits could explain the variations in parameter and serological prevalence data (Jonsson *et al*., 2013). In the Netherlands, Pink-footed Geese (Anserbrachyrhynchus) and adult Barnacle (*Branta leucopsis*) had a higher prevalence of Toxoplasmosis (Sandstrom *et al*., 2013). The antibody against *T. gondii* prevalence in nene (Hawaiian Geese, Brantasandvicensis) from the Hawaiian Islands was 21–48% (Thierry *et al*., 2016). Sandstrom (Sandstrom *et al*., 2013) diagnosis toxoplasmosis in Ross's and Lesser Snow Geese and other species of wild goose by detection antibodies using serological assay.

The species of Greylag goose (*Anseranser)* exposure to *T.gondii* may be occurs on winter season in the countries through migratory airways, where they feeding in farm fields at multi stopover stationsfrom Siberia, southeast, China, Kazakhstan, Iran and Iraq where they stay about four months in marshes and aquatic regions in middle and south of Iraq (Georg and Vielliard, 1970).

To our knowledge, this investigation is the first to document molecular survey to Greylag goose in Iraq.

References

Aston, E.J., Mayor, P., Bowman, D.D., Mohammed, H.O., Liotta, J.L. and Kwok, O. 2014. Use of filter papers to determine seroprevalence of *Toxoplasm agondii* among hunted ungulates in remote Peruvian Amazon. *Int. J. Parasitol. Parasites Wildl.* 3 (1) : 15–19.

Dubey, J.P. and Beattie, C.P. 1988. *Toxoplasmosis of Animals and Man*, CRC Press, Boca Raton. 220.

Dubey, J.P. 2010. *Toxoplasmosis of Animals and Humans*, 2nd edition, CRC Press, Boca Rotan. 117.

Dubey, J.P. 2002. A review of toxoplasmosis in wild birds. *Vet. Parasitol.*106 (2) : 121-153.

Dubey, J.P. 2009. *Toxoplasmosis of Animals and Humans.* CRC Press, Boca Raton. pp. 313.

Dubey, J.P., Velmurugan, G.V., Rajendran, C., Yabsley, M.J., Thomas, N.J. and Beckmen, K.B. 2011. Genetic characterisation of Toxoplasma gondii in wildlife from North America revealed widespread and high prevalence of the fourthclonal type. *Int J Parasitol*. 41: 1139–1147.

Dubey, J., Garner, M., Willette, M., Batey, K. and Gardiner, C. 2001. Disseminated to xoplasmosis in Magpie Geese (*Anseranas semipalmata*) with large numbers of tissuecysts. *J Parasitol.* 87 (1) : 219-223.

Georg, P.V. and Vielliard, J. 1970. Midwinter observations on birds of Central andSouth Iraq. *Bull. Iraq Nat. Hist. Mus.* IV (4) : 61-85.

Howe, D.K. and Sibley, L.D. 1995. *Toxoplasma gondii* comprises three clonal lineages, Correlation of parasite genotype with human disease. *J Infect Dis.* 172 : 1561–1566.

Jonsson, J.E., Ryder, J.P. and Alisauskas, R.T. 2013. Ross's Goose (*Chen rossii*). The Birds of North America Online. Cornell Lab of Ornithology. *Ithaca.*

Lucie Skorpikovaa, B., Nikol Reslovaa, B., Alena Lorencovaa, Radim Plhalc, Jakub Drimajc and Jiri Kamlerc, 2018. Molecular detection of *Toxoplasma gondii* in feathered game intended for human consumption in the Czech Republic. *Int. J. Food Microbiol.* 286 : 75–79.

Mei-Huilin, C. Tse-Ching, Tseng Tongkuo, T. Ching-Chung, and Ching-Ping, T. 2000. Real-Time PCR for Quantitative Detection of *Toxoplasma gondii*. *Journal of Clinical Microbiology.* 38 (11) : 4121–4125.

Murao, T., Omata, Y., Kano, R., Murata, S., Okada, T. and Konnai, S. 2008. Serological survey of *Toxoplasma gondii* in wild waterfowl in Chukotka, Kamchatka, Russia, and Hokkaido, Japan. *J. Parasitol.* 94 (4) : 830–833.

Stacey, A. Elmore, Kathryn, P. Huyvaert, Larissa, L. Bailey, Jared Milhous, Ray, T. Alisauskas and Alvin, A. Gajadhar, 2014. *Toxoplasma gondii* exposure in arctic-nesting geese: A multi-state occupancy framework and comparison of serological assays. *Int. J Parasitol: Parasites and Wildlife*. 3 (2) : 147-153.

McClintock, B.T., Nichols, J.D., Bailey, L.L ., MacKenzie, D.I., Kendall, W.L. and Franklin, A.B. 2010. Seeking a second opinion: uncertainty in disease ecology. *Ecol. Lett.* 13 : 659–674.

Montoya, J. G. and Liesenfeld, O. 2004. Toxoplasmosis. *The Lancet*. 363 (9425) : 1965-1976.

Sandstrom, C.A.M., Burma, A.G.J., Hoye, B.J., Prop, J., van der Jeugd, H. and Voslamber, B. 2013. Latitudinal variability in the seroprevalence of antibodies against *Toxoplasma gondii* in non-migrant and Arctic migratory geese. *Vet. Parasitol*. 194 (1) : 9–15.

Thierry, M., Work, Shiv K., Verma, Chunlei Su, John Medeiros, Thomas Kaiakapu, Oliverm C, Kwok, 2016. *Toxoplasma gondii* antibody prevalenceand two new genotype of the parasite in Endangered Hawaiian geese (Nene: *Branta sandvicensis*). *J. W. Dis*. 52 (2): 253–257.

Verma, S.K., Su, C. and Dubey, J.P. 2015. *Toxoplasma gondii* isolates from mouflon sheep (Ovisammon) from Hawaii, USA. *J Eukaryot Microbiol.* 62 : 141–143.

Verma, S.K., Calero-Bernal, R., Cerqueira-Cézar, C.K., Kwok, O.C., Dudley, M. and Jiang, T. 2016. Toxoplasmosis in geese and detection of two new atypical Toxoplasma gondii strains from naturally infected Canada geese (*Branta canadensis*). *Parasitol Res*. 115 (5) : 1767-1772.