



HOKKAIDO UNIVERSITY

Title	Seroprevalence and Genotyping of <i>Toxoplasma gondii</i> from Free-range Chickens in Qassim, Saudi Arabia
Author(s)	Nasr, Ibrahim Al; EL-Ashram, Saeed; Huang, Shujian
Citation	Japanese Journal of Veterinary Research, 66(2), 113-117
Issue Date	2018-05
DOI	https://doi.org/10.14943/jjvr.66.2.113
Doc URL	https://hdl.handle.net/2115/70497
Type	departmental bulletin paper
File Information	113-117 Ibrahim_Al_Nasr.pdf



Seroprevalence and Genotyping of *Toxoplasma gondii* from Free-range Chickens in Qassim, Saudi Arabia

Ibrahim Al Nasr^{1, 2, *}, Saeed El-Ashram^{3, 4)} and Shujian Huang³⁾

¹⁾ College of Science and Arts in Unaizah, Qassim University, Unaizah, Saudi Arabia

²⁾ College of Applied Health Sciences in Ar Rass, Qassim University, Ar Rass 51921, Saudi Arabia

³⁾ College of life science and Engineering, Foshan university, 18 Jiangwan street, Foshan 528231, Guangdong province, China

⁴⁾ Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh, Egypt

Received for publication, July 19, 2017; accepted, December 25, 2017

Abstract

Toxoplasma gondii is an intracellular apicomplexan parasite that infects a wide range of warm-blooded vertebrate hosts, including birds. The present study was undertaken to isolate and genotype *T. gondii* from free-range chickens (*Gallus domesticus*) in Qassim, Saudi Arabia. A total of 244 chickens were examined for *T. gondii* infection. Antibodies to *T. gondii* were detected in 29 (11.9%) chickens using the direct agglutination test (DAT). The brains of 29 chickens were bioassayed in mice. Four (13.8%) bioassayed mouse samples were positive for *T. gondii* DNA. Genotyping using the surface membrane antigen 3 (SAG3) locus indicated that all four isolates were type III. This is the first report on the genetic characterization of *T. gondii* isolated from chickens in Saudi Arabia.

Key Words: *T. gondii*, Genotyping, Saudi Arabia

Toxoplasma gondii, a zoonotic protozoan parasite, infects all warm-blooded animals. One-third of the global human population is estimated to be chronically infected by this parasite^{3,15,23)}. An important opportunistic pathogen, *T. gondii* results in extreme neurological complications in immunocompromised individuals, disseminated congenital infections in the developing fetus, and ocular symptoms in otherwise healthy individuals^{10,20,25)}. *T. gondii* isolates have been classified into three genetic types (I, II, and III) based on restriction fragment length polymorphism (RFLP)¹⁷⁾. Furthermore, they have

been phenotypically categorized as mouse virulent or avirulent. Type I strains are considered mouse virulent, whereas type II and III strains were avirulent or mildly virulent for mice. Although type II strains have a higher prevalence in human toxoplasmosis, type I strains are excessively represented in congenital toxoplasmosis¹⁷⁾, and there is an ideal correlation between acute virulence in *Toxoplasma*-infected mice and type I strains⁴⁾.

There have been many studies concerning the *T. gondii* genotypes circulating in chickens worldwide. The first report regarding the isolation

*Corresponding author: Ibrahim Al Nasr, College of Applied Health Sciences in Ar Rass, Qassim University, Ar Rass 51921, Saudi Arabia
Fax: +966-16-333-4564. E-mail: Insar@qu.edu.sa
doi: 10.14943/jjvr.66.2.113

of *T. gondii* from chickens was conducted by Ruiz and Frenkel²¹. Dubey *et al.* and Lehmann *et al.* recently studied the biological and genetic characteristics of *T. gondii* isolates from free-range chickens from many countries, and they found that *T. gondii* isolates from chickens in Brazil, Colombia, and South America were biologically and genetically distinct from isolates collected from the rest of the world^{7,11,13,18}. Most isolates from the United States were clonal type II strains, but they were not pathogenic for mice⁹. However, isolates from Brazil and Colombia were more pathogenic in mice in the absence of the clonal type II lineage⁹.

Free-range chickens are a good indicator of the *T. gondii* oocyst prevalence in the environment²¹. A greater access to hidden feline feces is facilitated by chickens' ground-scratching and feeding habits. Moreover, chickens serve as a substantial source of meat in Saudi Arabia, and they may be an important source of human infection. The purpose of this study was to determine the prevalence of *T. gondii* infection in free-range chickens in the Qassim region and characterize *T. gondii* isolates using the surface membrane antigen 3 (SAG3) locus.

All the animals used in the experiments were handled using procedures approved in the ethical committee guidelines at Qassim University. Blood and brain samples were collected from 244 free-range chickens from different locations in the Qassim region (located near the center of the Arabian Peninsula, with an area of 58,046 km²) between March and September 2013, and transported to the laboratory at the College of Applied Health Sciences, Ar Rass, Qassim University, Saudi Arabia. The blood was centrifuged, and the serum was separated. The sera and brains were then stored at 4°C.

The sera of free-range chickens were checked for *T. gondii* antibodies using the direct agglutination test (DAT; Toxo-Screen DA, bioMérieux®, France) following the manufacturer's instructions. Sera were screened at dilutions of 1:10, 1:20, 1:40, 1:80, 1:160, and 1:320.

Titers equal to or greater than 1:10 were considered positive.

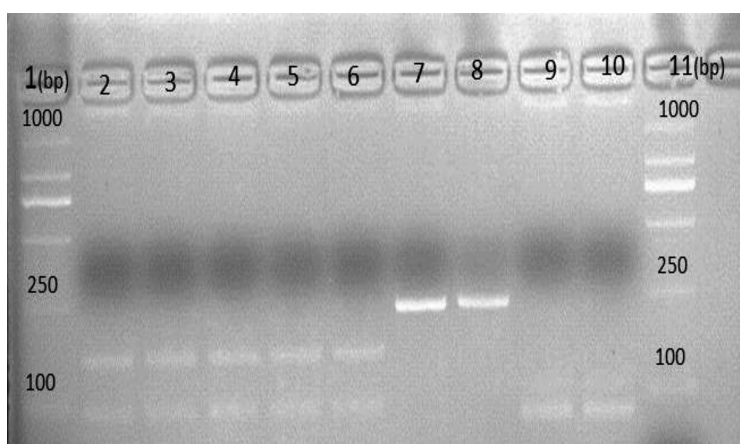
Brains from 29 seropositive chickens were bioassayed for *T. gondii* in Swiss Webster albino female mice. Each brain was chopped and gently blended in a laboratory mixer without any fluid. NaCl saline was then poured into the mixer and homogenized for 30 s at the maximum speed. This homogenate was incubated with an acid pepsin solution for 1 h at 37°C, centrifuged, neutralized, and suspended in antibiotic saline⁶. The homogenate was inoculated intraperitoneally into four mice (1 ml/mouse). The tissue imprints of the mice were examined for *T. gondii* tachyzoites or tissue cysts. After 45 days of inoculation, the surviving mice were euthanized via cerebral dislocation. The brains of all the mice were examined for tissue cysts, as described previously¹³. The brains were removed, homogenized in 1 ml of phosphate-buffered saline (PBS), and then examined for tissue cysts.

DNA was isolated from the brains of the infected mice using a commercial DNA extraction kit (Qiagen Pudong, Shanghai, China) following the manufacturer's instructions. The polymorphic region of the SAG3 gene was used for *T. gondii* genotyping following Su *et al.*²⁴, and the polymerase chain reaction (PCR) was performed using Phusion® High-Fidelity DNA Polymerase (New England BioLabs, Ipswich, MA, USA) following the manufacturer's instructions. After checking the amplified DNA fragment of interest by agarose gel electrophoresis, RFLP analysis was performed using digestion of the SAG3 amplified products with *NciI* (New England BioLabs, Ipswich, MA, USA) following the manufacturer's instructions. Five microliters of PCR products were examined by electrophoresis in 3% agarose gel containing 15 µl of GoldView™ nucleic acid stains and visualized under ultraviolet (UV) light.

Antibodies to *T. gondii* (1:10 and above) were found in 29 (11.9%) of 244 chickens. The titers were 1:10 in 10 chickens, 1:20 in 7 chickens, 1:40 in 3 chickens, 1:80 in 3 chickens,

Table 1.

DAT titers	No. of infected chickens	No. of infected chickens successfully bioassayed in mice	Clonal type
1 : 10	10	2	III
1 : 20	7		
1 : 40	3	2	III
1 : 80	3		
1 : 160	5		
1 : 320	1		

**Fig. 1. (Al Nasr I.)**

1 : 160 in 5 chickens, and 1 : 320 in 1 chicken. In addition, *T. gondii* DNA was detected in tissues from four mice using PCR (13.8%). *T. gondii* was detected from the tissues of chickens with DAT titers of 1 : 10 (2; 6.8%) and 1 : 40 (2; 6.8%). Tissue cysts were found in the brains of mice inoculated with these tissue samples (Table 1). None of these isolates were pathogenic to mice, and all four isolates were identified as type III lineage (Fig. 1).

In the present study, a prevalence rate of 11.9% was recorded using the DAT. However, seroprevalence of *T. gondii* in chickens was 4% and 8% in Egypt⁸⁾ and Iran¹⁹⁾, respectively. These discrepancies may be related to differences in the climatic conditions, study design, chicken age, number of samples, and cat densities. To determine the clonal type lineages of *T. gondii* in Saudi Arabia, molecular genotyping analysis and mouse bioassay were conducted for detecting the

pathogenicity of *T. gondii* strains. Our results showed that all chickens were infected with *T. gondii* type III.

Earliest studies on the molecular identification of *T. gondii* strains indicated that they have a lower genetic complexity than expected²²⁾. Other researchers showed that *T. gondii* strains could be categorized into three types, namely types I, II, and III¹⁸⁾. Although these types exhibit small differences in their sequences, their virulence is highly diversified in the hosts. The distribution of these types is related to the region in which the host is reared. Studies on SAG2 or dense granule antigen (GRA6) showed that type II is prevalent in North America and Europe^{4,26)}, while types I and III are prevalent in Portugal²³⁾ and Spain¹⁶⁾. Until now, no genotyping investigations have been performed in chickens in Saudi Arabia. Nevertheless, genotypes I and III were reported in 20.9% and

21.9% of cases in humans and farm animals¹⁾, respectively, and the type II isolate was the prevalent one (45.1%) in Madinah, Saudi Arabia. These researchers also showed that 12.1% of the samples were of unknown genotype¹⁾. Meanwhile, type II (80.6%) and III (19.4%) were recorded in pregnant women in Riyadh, Saudi Arabia²⁾, and type II (59.1%) and III (31.8%) were also found in *Rattus rattus* in this area¹⁴⁾. In the United Arab Emirates (UAE) and Qatar, type II was found in sand cats, in addition to atypical strains¹²⁾. Worldwide, *T. gondii* type II has been reported in many animal foods²¹⁾. Moreover, types II and III were reported in chickens in Egypt, with a predominance of type III^{8,26)}. Similarly, these two types were reported in Iran²⁷⁾, while only type I was identified in meat-producing animals in Iran¹⁹⁾. The absence of the type I strain in the Eastern Mediterranean Region in general has been reported⁸⁾. None of the samples in the present study harbored *T. gondii* type I; however, it has been detected in Madinah¹⁾.

The present study represents the first report on *T. gondii* genotyping in chickens in Saudi Arabia. In addition, the results provide preliminary data for further approaches for the epidemiology and genotyping of *T. gondii* in Saudi Arabia and the Eastern Mediterranean Region.

Acknowledgement

This work was supported by a Scientific Research Deanship, Qassim University, Kingdom of Saudi Arabia (project number 734).

References

- 1) Abd El-Aal A, Habib A, Sheikh B, Harak M, Shalaby A. *Toxoplasma* genotyping among infected human and animal hosts using PCR-restriction fragment length polymorphism: study in Al-Madinah, Saudi Arabia. *Int J Health Sci*, III, 309–311, 2010.
- 2) Algamdi J, Elamin M, Alhabib S. Prevalence and genotyping of *Toxoplasma gondii* among Saudi pregnant women in Saudi Arabia. *SPJ*, 24, 645–651, 2016.
- 3) Al Nasr I, Ahmed F, Pullishery F, El-Ashram S, Venkata R. Toxoplasmosis and anti-*Toxoplasma* effects of medicinal plant extracts-A mini-review. *Asian Pac J Trop Med*, 9, 730–734, 2016.
- 4) Barragan A, Sibley L. Transepithelial migration of *Toxoplasma gondii* is linked to parasite motility and virulence. *J Exp Med*, 17, 195, 1625–33, 2002.
- 5) Dardé M.L, Bouteille B, Pestre-Alexandre M. Isoenzyme analysis of 35 *Toxoplasma gondii* isolates and the biological and epidemiological implications. *J Parasitol*, 78, 786–794, 1992.
- 6) Dubey J. Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. *Vet Parasitol*, Jan 15, 74: 75–7, 1998.
- 7) Dubey J, Applewhaite L, Sundar N, Velmurugan G, Bandini L, Kwok O, Hill R, Su C. Molecular and biological characterization of *Toxoplasma gondii* isolates from free-range chickens from Guyana, South America identified several unique and common parasite genotypes. *Parasitology*, 134, 1–7, 2007.
- 8) Dubey J, Graham D, Dahl E, Hilali M, El-Ghaysh A, Sreekumar C, Kwok O, Shen S, Lehmann T. Isolation and molecular characterization of *Toxoplasma gondii* from chickens and ducks from Egypt. *Vet Parasitol*, 30, 89–95, 2003.
- 9) Dubey J, Graham D, Dahl E, Sreekumar C, Lehmann T, Davis M, Morishita T. *Toxoplasma gondii* isolates from free-ranging chickens from the United States. *J Parasitol*, 89, 1060–1062, 2003.
- 10) Dubey J, Ruff M, Camargo M, Shen S, Wilkins G, Kwok O, Thulliez P. Serologic and parasitologic responses of domestic chickens after oral inoculation with *Toxoplasma gondii* oocysts. *Am J Vet Res*, 54, 1668–72, 1993.
- 11) Dubey J, Sundar N, Gennari S, Minervino A, Farias N, Ruas J, dos Santos T, Cavalcante G, Kwok O, Su C. Biologic and genetic comparison of *Toxoplasma gondii* isolates in free-range chickens from the northern Para state and the southern state Rio Grande do sul, Brazil revealed highly diverse and distinct parasite populations. *Vet Parasitol*, 143, 182–188, 2007.
- 12) Dubey J, Pas A, Rajendran C, Kwok O, Ferreira L, Martins J, Hebel C, Hammer S, Su C. Toxoplasmosis in sand cats (*Felis*

- margarita*) and other animals in the breeding centre for endangered Arabian wildlife in the United Arab Emirates and Al Wabra wildlife preservation, the State of Qatar. *Vet Parasitol*, 20, 195–203, 2010.
- 13) Dubey J, Venturini M, Venturini L, Piscopo M, Graham D, Dahl E, Sreekumar C, Vianna M, Lehmann T. Isolation and genotyping of *Toxoplasma gondii* from free-ranging chickens from Argentina. *J Parasitol*, 89, 1063–4, 2003.
 - 14) Elamin M. Genotyping of *Toxoplasma gondii* from Rats (*Rattus rattus*) in Riyadh, Saudi Arabia. *Korean J Parasitol*, 52, 257–261, 2014.
 - 15) El-Ashram S, Yin Q, Barta JR, Khan J, Liu X, Suo X. Immunoproteomic technology offers an extraordinary diagnostic approach for *Toxoplasma gondii* infection. *J Microbiol Methods*, 119, 18–30, 2015.
 - 16) Fuentes I, Rubio J, Ramirez, Alvar J. Genotypic characterization of *Toxoplasma gondii* strains associated with human toxoplasmosis in Spain: direct analysis from clinical samples. *J Clin Microbiol*, 39, 1566–1570, 2001.
 - 17) Howe D, Sibley L. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *J Infect Dis*, 172, 1561–6, 1995.
 - 18) Lehmann T, Marcet P, Graham D, Dahl E, Dubey J. Globalization and the population structure of *Toxoplasma gondii*. *Proc Natl Acad Sci*, 103, 11423–11428, 2006.
 - 19) Mahami-Oskoue M, Moradi M, Fallah E, Hamidi F, Akbari N. Molecular Detection and Genotyping of *Toxoplasma gondii* in Chicken, Beef, and Lamb Meat Consumed in Northwestern, Iran. *Iran J Parasitol*, 12, 38–45, 2017.
 - 20) Mondragon R, Howe D, Dubey J, Sibley L. Genotypic analysis of *Toxoplasma gondii* isolates in pigs. *J Parasitol*, 84, 639–641, 1998.
 - 21) Ruiz A, Frenkel J. Intermediate and transport hosts of *Toxoplasma gondii* in Costa Rica. *Am J Trop Med Hyg*, 29, 1161–6, 1980.
 - 22) Sibley L, Boothroyd J. Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. *Nature*, 359, 82–85, 1992.
 - 23) de Sousa S, Ajzenberg D, Canada N, Freire L, de Costa J, Dardé M.L, Thulliez P, Dubey J.P. Biologic and molecular characterization of *Toxoplasma gondii* isolates from pigs from Portugal. *Veterinary Parasitology*, 135, 133–136, 2006.
 - 24) Su C, Zhang X, Dubey J. Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. *Int J Parasitol*, 36, 841–848, 2006.
 - 25) Tenter A, Heckeroth A, Weiss L. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol*, 30, 1217–58, 2000.
 - 26) Velmurugan GV, Dubey JP, Su C. Genotyping studies of *Toxoplasma gondii* isolates from Africa revealed that the archetypal clonal lineages predominate as in North America and Europe. *Vet Parasitol*, 17, 314–8, 2008.
 - 27) Zia-Ali N, Fazaeli A, Khoramizadeh M, Ajzenberg D, Darde M, Keshavarz-Valian H. Isolation and molecular characterization of *Toxoplasma gondii* strains from different hosts in Iran. *Parasitol Res*, 101, 111–115, 2007.