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

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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 toxoplasmosis[17], and there is an ideal correlation between acute virulence in *Toxoplasma*-infected mice and type I strains[4].

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

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of *T. gondii* from chickens was conducted by Ruiz and Frenkel[21]. 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 mice[9]. However, isolates from Brazil and Colombia were more pathogenic in mice in the absence of the clonal type II lineage[9].

Free-range chickens are a good indicator of the *T. gondii* oocyst prevalence in the environment[21]. 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 km^2) 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 saline[6]. 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 previously[13]. 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 *Nci*I (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		

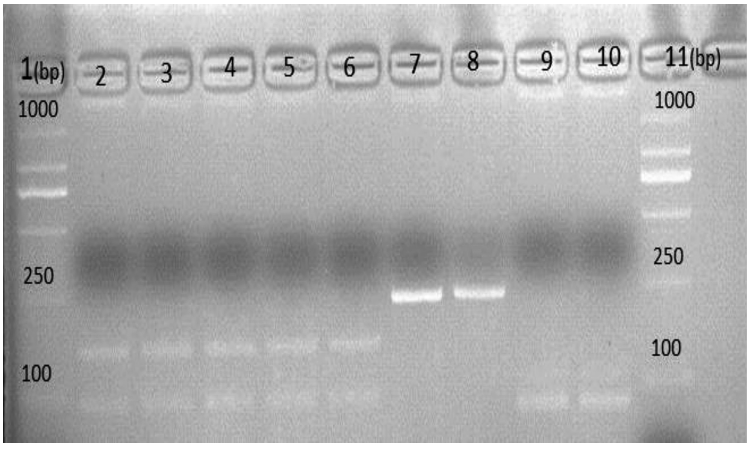

Fig. 1. (Al Nasr I.)

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 Egypt[8] and Iran[19], 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 expected[22]. Other researchers showed that *T. gondii* strains could be categorized into three types, namely types I, II, and III[18]. 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 Portugal[23] and Spain[16]. 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 animals[1], 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 genotype[1]. Meanwhile, type II (80.6%) and III (19.4%) were recorded in pregnant women in Riyadh, Saudi Arabia[2], and type II (59.1%) and III (31.8%) were also found in *Rattus rattus* in this area[14]. In the United Arab Emirates (UAE) and Qatar, type II was found in sand cats, in addition to atypical strains[12]. Worldwide, *T. gondii* type II has been reported in many animal foods[21]. 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 Iran[27], while only type I was identified in meat-producing animals in Iran[19]. The absence of the type I strain in the Eastern Mediterranean Region in general has been reported[8]. None of the samples in the present study harbored *T. gondii* type I; however, it has been detected in Madinah[1].

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.

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