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ORIGINAL ARTICLE

Prevalence and genotyping of *Toxoplasma gondii* among Saudi pregnant women in Saudi Arabia

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Abstract *Introduction:* Toxoplasma gondii (*T. gondii*) is an intracellular protozoan that can infect all mammals, who serve as intermediate host. It causes congenital, neurological, eyes complications and mild or asymptomatic infections in humans. *Purpose of this study:* To investigate not only the prevalence of *T. gondii*, but also to find out its genotyping using multiple sequential molecular methods to predict exactly the precise genotyping of *T. gondii* among Saudi pregnant women. *Methods:* A cross-sectional study was conducted using multi-stage methods. Initial stage involved enrolment of 250 Saudi pregnant women from multi-centre healthcare and community based settings in the capital of Saudi Arabia Riyadh. The second stage was embracement of the laboratory investigation that included Enzyme immunoassay (ELISA), DNA extraction, PCR, nested-PCR assay, and genotyping of the seropositive cases. *Results:* 203 women agreed to take part in our study with a response rate of 81.2% (203/250). Using ELISA, we found that the prevalence of Toxoplasma gondii IgG and IgM antibodies was 32.5% and 6.4%, respectively. We found that 29 samples (80.6%) were of genotype II; however 7 samples (19.4%) were of genotype III. *Conclusion:* Defining the population structure of *T. gondii* from Saudi Arabia has important implications for transmission, immunogenicity, pathogenesis, and in planning preventive strategies. Relationship between such variation in structure and disease manifestation in pregnant women is still difficult to assess due to the role of host immune status and genetic background on the control of infection, and of other parasitic features such as the infecting dose or parasite stage. Our finding

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of the genotyping of *T. gondii* might facilitate and inform future studies on comparative genomics and identification of genes that control important biological phenotypes including pathogenesis and transmission among Saudi women.

1. Introduction

Toxoplasma gondii (*T. gondii*) is a single-celled parasitic organism that can infect most animals and birds. Because it reproduces only in cats, wild and domestic felines are the parasite's ultimate host (Kravetz and Federman, 2005). *T. gondii* is the third greatest common reason of fatal food-borne infection in United States (Mead et al., 1999). It causes congenital, neurological, eyes complications and minor or asymptomatic infections in humans (Kong et al., 2012). During pregnancy, primary infection, which is transmitted trans-placentally, may cause congenital toxoplasmosis (Many and Koren, 2006). This includes a wide range of manifestations, extending from mild chorioretinitis, which can present many years after birth, to miscarriage, mental retardation, microcephaly, hydrocephalus and seizures (Kravetz and Federman, 2005). Hence, early and accurate diagnosis of toxoplasmosis can be crucial for the prevention and control of the disease, particularly in individual who are at risk, such as pregnant women.

Diagnosing *T. gondii* is usually achieved by identifying the parasite-specific antibodies in the serum with serological techniques such as Enzyme-linked Immunosorbent Assay (ELISA) and Immuno-fluorescence Antibody Assay (IFA) (Zhang et al., 2009; Lau et al., 2010). However, serology is very difficult to interpret in pregnant patients. Furthermore, Polymerase chain reaction (PCR) methods have substantial advancement for the detection of toxoplasmosis. Among these techniques, nested-PCR has been proved as the most sensitive diagnostic technique for the diagnosis of toxoplasmosis (Kong et al., 2012). There are different strains of *T. gondii* with variable genetic structure. Three genetically different types (strains) of Toxoplasma gondii are known. These are type-I, type-II and type-III. Due to their genetic differences, the three types also differ in the mode of infection and the severity of symptoms. Therefore, identifying the genetic type helps in better understanding the disease and probably finding the proper treatment (Fuentes et al., 2001). In this context, the identification of atypical strains is of clinical and epidemiological importance because atypical strains are usually associated with severe disease outcomes (Ajzenberg et al., 2010).

Approximately one-third of the global human population is infected with this pathogenic parasite including populations in Europe, South America, Africa and several Asian countries (Fallahi et al., 2014). The prevalence of positive serology for Toxoplasma varies in various regions and cultures. In Saudi Arabia, prevalence studies showed that 29.5–35.6% of pregnant women were found to have *T. gondii* during pregnancy (Ghazi et al., 2002; Al-Harthi et al., 2006). However, limited studies have been conducted to explore the trend and genotyping of such infection among Saudi pregnant women. Hence, the aim of this study was to investigate not only the prevalence of *T. gondii*, but also to find out its genotyping using multiple sequential molecular methods to predict exactly the precise genotyping of *T. gondii* among Saudi pregnant women.

2. Materials and methods

A cross-sectional study was conducted using multi-stage methods over a period of 9 months of the year 2011. Initial stage involved enrolment of 250 Saudi pregnant women from multi-centre healthcare and community based settings in the capital of Saudi Arabia Riyadh. These centres were selected from different regions involving rural and urban areas in order to maximize the variety of our sample. Participants were selected using convenient sampling. They were approached during waiting time for their antenatal care visits and explained the study aim and objectives, and a written consent was obtained accordingly. Inclusion criteria were pregnant women, Saudi, and aged 15–45 years.

The second stage was embracement of the laboratory investigation that included Enzyme immunoassay (ELISA), DNA extraction, PCR, nested-PCR assay, and genotyping of the seropositive cases.

2.1. Enzyme immunoassay

Blood samples were collected from the consented participants (6 mm). Each blood sample was divided into two tubes (3 ml each): one contained the EDTA (EDTA tube) for extracting the DNA and the second one was a plane tube kept to extract serum. The later samples were left for one hour at room temperature until clotting is established. Thereafter, the tubes were centrifuged for 10 min at a speed of 3000 cycles for 15 min. The resulted serum was transferred then into a clean dry tube (Eppendorf tube) and kept in under −20 °C for further analysis.

Specific IgG and IgM antibodies to *T. gondii* in the serum were measured by the ELISA test following the method of Engvall and his colleagues with the commercial ENZYWELL TOXOPLASMA IgG and IgM KIT, and using the BEP III system (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) according to the manufacturer's instructions (Engvall and Perlmann, 1971, 1972). The extinction value of calibrator identified the upper limit of the reference range of non-infected individual (Cut-off). Therewith, the findings were evaluated quantitatively by calculating a ratio of the extinction value of the control or patient samples over the extinction value of the calibrator. If the absorbance of the sample is higher than that of the Cut-off (>1.3 for IgG and >1.2 for IgM), the sample was considered positive for the presence of specific antibodies and negative if it was <0.7 of IgG and <0.8 for IgM. In case of doubt or borderline test results (0.7–1.3 for IgG and 0.8–1.2 for IgM), the test was repeated.