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Review

Genetic diversity of *Toxoplasma gondii* isolates from ruminants: A systematic review

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ABSTRACT

Toxoplasma gondii is a protozoan capable of infecting all warm-blooded animals. This parasite has been classified into three major lineages. Our aim was to assess and compare the identified Types and genotypes in ruminants. From November 2014 to April 2015, four English language databases and four Persian databases that reported data on the *T. gondii* genotyping in ruminants were searched. Overall, typing results of the 250/307 *T. gondii* isolates in all animals showed that Type II was a predominant Type (81.4%). In addition, genotyping data from the 82/215 *T. gondii* isolates or strains indicated that atypical genotypes were predominant (38.13%). This systematic review has demonstrated a large degree of genetic diversity in some countries. However, in the new nomenclature of genotyping, there are atypical or exotic genotypes, such as Chinese 1, Types Br (I, II, III and IV), and Type 12. Further genotyping studies are required to corroborate the current results.

1. Introduction

Toxoplasma gondii (*T. gondii*) is a ubiquitous protozoan parasite capable of infecting virtually all warm-blooded animals, including humans, livestock, and marine mammals (Dubey, 2010a). Toxoplasmosis is a challenging zoonosis, particularly in vulnerable groups such as pregnant women and immunocompromised patients (Weiss and Dubey, 2009). Human infection occurs via three main modes: (I) transplacental transmission of the tachyzoite from non-immune mother during primary infection; (II) ingestion of food or water contaminated with sporulated oocysts from infected cat feces; (III) consumption of raw or undercooked meat containing tissue cysts (Tenter et al., 2000). The European Food Safety Authority has recognized toxoplasmosis as a food-borne disease that has the highest human incidence (Olivier et al., 2007). Risk-factor analysis indicates that 30% to 63% of human infections can be attributed to the consumption of raw or undercooked meat (Cook et al., 2000). However, human infections depend on prevalence of *T. gondii* in animals and on eating habits (Alvarez et al., 2015; Opsteegh et al., 2010).

Recently, diverse options have been discussed to assess the infection-risk for the human population (Kijlstra and Jongert, 2008). The inoculum dose, infecting stage, and the genetic diversity of the infectious strain may be related to variation in disease outcome (Olivier et al., 2007). Recent studies have been focused on the genetic variability among *T. gondii* isolates from different hosts (Grigg and Sundar, 2009). Studies performed in Europe and North America have shown that approximately 90% of *T. gondii* isolates from humans and animals have been classified into three clonal lineage Types (I, II and III) with low genetic diversity (Sibley et al., 2002). There are limited reports in Asia that identified two genotypes (Dubey et al., 2007). In contrast, recent reports have revealed a large degree of genetic diversity from Central and South America, Africa, and Germany (Ajzenberg et al., 2004; Al-Kappany et al., 2010; Herrmann et al., 2010; Pena et al., 2008). However, since data related to non-clonal strains, as the fourth clonal lineage (atypical and recombinant) was presented, it revealed that the population structure is more diverse than previously thought (Khan et al., 2011).

In this review, we aimed to collect information related to the global diversity of *T. gondii* in ruminants, because data on the population structure of *T. gondii* is a significant subject to realize its disease variable manifestations and use them for developing new strategies for treatment, vaccination, and diagnosis of toxoplasmosis.

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2. Materials and methods

This review followed the preferred reporting items for systematic reviews (PRISMA) guidelines.

2.1. Search strategy

We have systematically searched four English language databases (PubMed, Science Direct, Scopus and Google Scholar) and four Persian databases (Magiran, Scientific Information Database, IranMedex, and IranDoc) from November 2014 to April 2015.

Language restriction was applied to English and Persian language articles. The abstracts of all chosen articles were interpreted to identify the potentially eligible articles.

The present investigation was carried out using terms such as: "Toxoplasmosis", "*Toxoplasma gondii*", "Genotype", "Genotyping", "Molecular characterization", "Livestock", "Sheep", "Ovine", "Cattle", "Goat", "Camel", "Alpaca", "Llama", "Aborted fetus", "Meat-producing animals", "Human consumption meat", "Domestic animals" and "Slaughtered animals" alone or combined (in both languages).

2.2. Gray literature search

The gray literatures such as national proceedings of conferences and graduate student dissertations were searched manually.

2.3. Study selection

Abstracts were reviewed independently by four authors (MS, AA, AM, and SS) and selected for further use if they met one of the following inclusions criteria: 1) genotyping studies based on genetic markers 2) genotyping articles based on molecular markers and 3) *Toxoplasma* genotyping papers for various hosts such as sheep, goats, cattle, camels, and aborted fetuses. Discrepancies were resolved by discussion and consensus. In addition, the collected publications were screened carefully and duplicate articles, which did not meet our inclusion criteria, were excluded from study.

2.4. Data extraction

Data extraction protocol was evaluated by the research group. Full-texts of included abstracts were retrieved and reviewed independently by four authors, using data extracted sheets (AA, AM, SAH, and SS). Any disagreements were resolved with AD and MA. All information including year of publication, first author, tissue location, host, total sample size, serological examination technique, number of subjects with positive tests, bioassay, number of isolates/strains, molecular identification assay, Type and genotype analysis, and ToxoDB PCR-RFLP genotypes were collected.

3. Results

3.1. Search results

We identified that among 5067 studies of the literature search, 39 records were potentially appropriate for inclusion in this systematic review. Fig. 1 shows the process of search in this systematic review article.

3.2. Descriptive analysis

In total, 1429 goats, 2894 sheep, 2670 cattle, 70 camels, 843 aborted fetuses, and 164 meat products were analyzed in the 39 studies included in this review. Applied techniques and genetic characterizations of these animals were assessed according to Types (n = 4946) and genotypes (n = 3124). Characteristics of the selected studies are summarized in Tables 1 and 2.

The typing results by kind of animal and country are summarized in Table 3. PCR-RFLP based on monolocus typing and microsatellite markers in each animal revealed that Type II was predominant. Isolates obtained from some studies performed in Spain, Romania, Eastern Slovakia, Netherlands, France, UK, and Iran showed only Type II (100%). Overall, typing of the 307 *T. gondii* isolates in all animals also showed that Type II was a predominant Type (81.4%) (Table 3).

Genotyping results by kind of animal and country are listed in Table 4. Genetic characterization among *T. gondii* isolates or strains, using PCR-RFLP based on multilocus typing displayed that atypical genotypes were predominant (43.08% in goats and 50% in sheep); whereas in other animals, Type II lineage had the highest frequency and its prevalence in the aborted fetuses was 100%. Overall, genotyping of the 215 *T. gondii* isolates or strains confirmed that atypical genotypes were predominant (38.13%) (Table 4).

3.3. Genetic diversity

Comparison of identified genotypes among these animals revealed that genotype #1 was the most common genotype in these animals. Also, genotypes Br #1 and Br #10 (n = 4) in goats and genotype #1 (n = 17) and genotype #4 (n = 10) in sheep were most common (Table 2).

Moreover, this study showed that genotypes #10, #118, #143, #154, #156, #167, Br #4, Br #5, Br #10, and Br #11 were seen only in goats and genotypes #7, #11, #17, #19, #32, #54, #72, #73, #74, #110, #111, #131, #230, #249, #TgShUs35, #Unique, Br #2, Br #3, Br #6, Br #7, Br #8, and Br #9 were also observed just in sheep. Further information about genotypes identified, including ToxoDB PCR-RFLP are presented in Table 5.

4. Discussion

This systematic review has attempted to provide a survey on existing genotypes of *T. gondii* obtained from animals (sheep, goats, cattle, camels, and aborted fetuses) around the world. *Toxoplasma* infections are broadly prevalent in livestock in many countries (Dubey, 2010b). Some studies showed that the seroprevalence in sheep and goats was as high as that in humans, for a particular region (Cook et al., 2000). These animals are economically important in many countries because they are required for the production of meat and milk, and breeding. Most animals acquire this infection after birth (Halos et al., 2010). Ruminants are mainly infected because of a contamination of their environment (food and water) with infective oocysts (Tenter, 2009). Therefore, handling or consumption of raw or uncooked meat, and unprocessed milk, especially among immunocompromised persons and pregnant women, could be a potential risk factor for acquiring the infection (Sharif et al., 2010; Tenter, 2000). The recent findings in Europe showed that imported meat is important source for transmission of this parasite. In France, severe or fatal outcomes caused by atypical strains probably attained by ingestion of raw horse meat imported from South America (Elbez-Rubinstein et al., 2009; Pomares et al., 2011).

In the past two decades, the diagnostic techniques for *T. gondii* infection were based on direct detection of parasite nucleic acids in biological and clinical samples of humans and animals (Bessieres et al., 2009). The different molecular methods used to detect *T. gondii* deoxyribonucleic acid (DNA) can be divided into two classes. In the first class, methods that are applied for specific detection of *T. gondii* DNA in suspected samples include the conventional PCR, nested PCR (n- PCR), and quantitative real-time PCR (qPCR). In the second class, these methods are applied to study the genetic make-up and analysis of *T. gondii* DNA, including multilocus PCR-RFLP, microsatellite and multilocus sequence typing (MLST) of single copy DNA sequences (Su et al., 2010). Genetic typing methods are being progressively perfected to test human and animal samples (Dardé, 2004). However, there are some