Detection of Prevalence of *Toxoplasma gondii* from Iranian Native Cattle

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**Abstract**—Infections by the protozoan parasite *Toxoplasma gondii* are widely prevalent worldwide in animals and humans. *T. gondii* is the causative agent of toxoplasmosis, one of the most prevalent parasitic infections to humans and domestic animals. The disease occurs throughout the world and also in Iran. The present study aimed to determine of *T. gondii* isolates from native cattle in south west Iran by molecular methods. In this study, 155 blood samples were collected and genomic DNA was extracted using DNA extraction Kit (Cinna Gen, Iran) according to the manufacturer protocol and PCR was performed using specific primers (ITS-F and ITS-R). Sixteen (6.95%) cattle were positive to *T. gondii* infection. The positive control samples showed the excepted amplification product specific for *T. gondii* (171 bp). The results present showed low prevalence of *T. gondii* infection in Chaharmahal va Bakhtiari native cattle. In our opinion control and eradication programs for prevent of prevalence this infection factor and also economic losses are necessary.

**Keywords**—Non-pasteurised, tachyzoites, *Toxoplasma gondii*, toxoplasmosis

I. INTRODUCTION

*Toxoplasma gondii* (*T. gondii*) is a protozoan parasite mandatory which infects all warm-blooded animals such as marine, mammals, humans, animals and birds [1]. *T. gondii* infection causes mental retardation loss of vision, and other congenital health problems in human newborns [2]. Toxoplasmosis is an infection that created by *T. gondii* and also infected with this parasite found in one-third of the world population [3]-[4]. Belonging to the branch apicomplexa, which is including main pathogens of humans and domestic animals such as *Plasmodium* (agent malaria), *Cryptosporidium* (diarrhea), *Eimeria* (coccidiosis in poultry) and *Theileria* (East Coast Fever in cattle) [5]. The infection asymptomatic there are in persons that have a healthy immune system hosts, but can be fatal in immunocompromised people. Infection during pregnancy possible result feeble offspring, abortion and stillbirth in birth defects. Moreover, ocular diseases have been reported that sometimes disease is severe in healthy adults resulting in loss of vision, cognitive function and motor even fatal encephalitis [6]-[7]. Two main routes of transmission exist in nature (sexual and asexual) and three various growth form sporozoites, bradyzoites and tachyzoitessexu [6]. But the sexual period of the parasite just occur in the intestine of the definitive host, the domestic and wild cat and result in the excretion of oocystes in the stool [8]. Generally in undercooked meat, pigs, wild meat, lamb, and soil contaminated and in cat feces, on raw fruits and greengrocery are the main sources of the parasite from transmission to humans [9]. Three important clonal lineages of *T. gondii*, specified as Type I, II and III, were classified according to their genetic multiform and also, there are atypical and recombinant species. It has been shown that type I and some of the recombinant or atypical species are virulent in mice, whilst genotype II and III are non-virulent [10]. Type II strains are relatively virulence in mice, however they easily show chronic infections characterized by tissue cysts that are very infectious by the oral way [11]. In around the world prevalence of this disease in human is about 10–30% [12]. During over the past decade, use Polymerase Chain Reaction (PCR) assay have significant advances for detection of toxoplasmosis both congenital diseases in the time before birth and in cases of acute toxoplasmosis in immunocompromised patients. Between these assays, nested-PCR followed by hybridization has been reported as the most sensitive detection technique for the detection of toxoplasmosis [13].

Internal transcribed spacer (*ITS*) is unusual in a fragment of non-functional RNA situated among structural ribosomal RNAs (rRNA) on a common pioneer transcript [14]. Read from 5' to 3', this polycistronic rRNA forerunner transcript has the 5' external transcribed sequence (5'ETS), 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA and at the end ewhich show higher rates of divergence [15]. ITS-1 is the spacer region between the 18s and 5.8s genes [16]. The purpose of this study was prevalence of *Toxoplasma gondii* from Iranian native cattle by molecular assay.

II. MATERIALS AND METHODS

**Samples collection**

One hundred fifty five (155) native cattle blood samples were collected from slaughterhouse in Chaharmahal va Bakhtiari province and transfer into a sterile tube and stored in 10% 0.5 M EDTA-coated. Sterile tubes (BD Vacutainer Systems, Plymouth, UK). DNA extraction Genomic DNA was

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isolated from specimens using DNA Extraction Kit (CinnaGen, Iran) according to the manufacturer’s protocol. The extracted DNA was quantified by spectrophotometric measurement at a wavelength of 260 nm. The extracted DNA of each specimen was kept frozen at −20°C until used.

Gene amplification

Specific primers for ITS-1 of *T. gondii* were used for gene amplification. Forward primer begins at base 97 with the following sequence Toxo-F: 5'-CATTGGAGAGATTTGCATTC -3' and reverse primer begins at base 240 with the sequence Toxo-R: 5'-ATCAGTATCCCAACAGAGACAC -3', therefore it is intended to amplify a fragment of 171 base pairs. PCR test was performed in final volume of 25 μl PCR reactions containing 2 μl of DNA template, concentration of 1.5 mM MgCl2, 200 μM dNTPS, 2 mM of each primers and a unit of Taq DNA polymerase. Thermal PCR conditions consisted of 5 min at 95°C and then 32 cycles initial temperature of 94°C, temperature of 56 and 72°C connector at each end for 1 min, and last extension was for 5 min at 72°C.

The amplification products were analyzed in 1.5% agarose gel electrophoresis. Electrode buffer was TBE (Tris-base 10.8 g, 89 mM, Boric acid 5.5 g, 2 mM EDTA (pH 8.0) 4 ml of 0.5 MEDTA (pH 8.0), with all components joined in enough H2O and stirred to dissolve). Gels were stained with ethidium bromide, aliquots of 10 μl of PCR products were applied to the gel. Constant voltage of 80 V for 30 min was used for products separation. After electrophoresis, generated bands were screened and digitally photographed under UV light (UK).

**Statistical Analysis**

All data for were analyzed by the chi-square test using the SPSS 17 (SPSS Inc. Chicago, IL, USA) software. P values <0.05 were considered significant.

**III. RESULTS**

Agarose gel electrophoresis of positive samples revealed a 171 bp fragment. An example of PCR amplification of blood samples is shown on Fig. 1. In this study from 155 samples collected from Slaughterhouse in Chaharmahal Va Bakhtiari province *T. gondii* DNA was found in 16 of 155 (6.95%) cattle blood samples. The results showed a low frequency of *T. gondii* isolate in native cattle in the Chaharmahal Va Bakhtiari province.

**IV. DISCUSSION**

Toxoplasmosis is a zoonotic disease cause by *T. gondii* and has been known in many countries since 1908 reported that the occurrence of toxoplasmosis varies between countries, according to traditions, customs and the life styles of the population. *T. gondii* infection in cattle is distributed worldwide. Hence not possible comparison prevalence data of studies which used different serological tests with variable sensitivity and specificity therefore we selective bio assay for(isolate *T. gondii* from cattle [17]. There are multitude reports on comparison of different techniques on diagnosis of *Toxoplasma* infection [18]. PCR has been used to detect *T. gondii* is very efficient in rapid diagnosis of toxoplasmosis [36]. PCR where a part of DNA genome of *T. gondii* is detectable, because of proportionate sensitivity and specificity, is preferred to other techniques and reception immediate results are also another benefit [19]. Blood samples are the most available sample required to do PCR in diagnosis of animal, birds and human cases [36], but in other perusal, the positivity value which was observed were more or less than values of our finding including 7.8% in France [20], 15.7% in Spain [21], Serbia with 76.3% [22], 5% in America [23] and 10.5% in Vietnam [24]. In another study from Brazil, the number of *T. gondii* positive animals from farms that had a history of abortion or stillbirth was much higher than in animals from farms without abortion history (P=0.019) [25]. Prevalence of toxoplasmosis has been found around zero in some areas, Mexico, Australia, Indonesia, Canada and Egypt to 100% [26]. Another report states that age is an important factor in sheep toxoplasmosis. In this research, infection rate in younger animals was higher than in other age groups [27]. In another study, the prevalence rates were reported to be between 1.6-15.9% in North-West of Iran, and zero in North of Iran [27]-[28]-[29]-[30]. In Iran did not detect *T. gondii* in cattle using Latex Agglutination (LAT), Indirect Hemagglutination Tests (IHAT), direct microscopy and bioassay in mice as a result we have used the PCR method [31]. In another assay performed a study on dairy cows and showed the prevalence rate of 21.6% on the coastal region of Caspian Sea (North Iran) and 32%, in Khuzestan province (South West Iran) [32] In addition, it is in accordance with the with 13.8% rate in Ahvaz city (South west Iran) using Latex test, and with a 15.9% rate in Tabriz city (North West Iran) using IF method [33]-[34]. They showed that cattle under the age of one year were significantly (P>0.05) more infected the older cattle [30].

In conclusion, the results presented low presence of *T. gondi* infection in cattle samples and suggested that control and eradication programs for prevent and reduce of economic loses of this infection it seems to be necessary. Furthermore, the results of the present study suggest that PCR was highly sensitive and specific for identification and differentiation of *T. gondi* and that it could be a suitable tool for diagnosis of *T. gondi*. Thus, it is essential to screen in all region regularly to prevent the spread of the disease and laboratory support is an important tool in the diagnosis of the disease. Seemingly, PCR is one of the best ways to detect and characterize *T. gondi* fast, less hazardous and sensitive method.

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Fig. 1. Gel electrophoresis for detection of T. gondii infection in blood samples. Lane M is 100 bp DNA ladder (Fermentas, Germany); lane 1 is a negative sample, lane 2 and 3 are positive samples, lane 4 is negative control.