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

Hamidreza Kabiri[1*], Abbas Doosti[2], Esmaeil Mahmoudi[2], Payam Ghasemi Dehkordi[2]

***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 aprotozoan 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 oocytes in the stool [8]. Generally in undercooked meat, pigs, wild game 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

[1]Young Researchers and Elite Club, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran. Corresponding Author: **Hamidreza Kabiri** (Address: Islamic Azad University, Shahrekord Branch, Shahrekord, Iran, Postal Box: 166. Tel: +98-381-3361001, Fax: +98-381-3361001. Mobile Number: +09138872574, Email: biologyshki@yahoo.com)

[2]Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

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 stired 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 world-wide. Hence not possible comparison prevalence data of studies which used different serological tests with variable sensitivity and specificity therefore we selective bio assay for isolitate *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*as fast, less hazardous and sensitive method.

Acknowledgment

The authors would like to express their deepsense of gratitude and sincere thanks to thestaff of the Biotechnology Research Center ofIslamic Azad University of Shahrekord branch in southwest Iran.

REFERENCES

[1] L. Holec-Gąsior, D. Drapała, B. Dominiak-Górski, J. ózefkur, "Epidemiological study of Toxoplasma gondii infection among cattle in Northern Poland," *Ann Agric Environ Med*., vol. 20, no. 4, pp. 653-656, 2013.

[2] J. P. Dubey, C. Rajendran, L. R. Ferreira, J. Martins, O. C. Kwok, D. E. Hill, I. Villena, H. Zhou, C. Su, J. L. Jones, "High prevalence and genotypes of Toxoplasma gondii isolated from goats, from a retail meat store, destined for human consumption in the USA. Int J Parasitol,", vol. 41, no. 8, pp. 827-833, 2011.
http://dx.doi.org/10.1016/j.ijpara.2011.03.006

[3] T. Okada, D. Marmansari, Z. M. Li, A. Adilbish, S. Canko, A. Ueno, H. Shono, H. Furuoka, M. Igarashi, " A novel dense granule protein, GRA22, is involved in regulating parasite egress in Toxoplasma gondii," *Mol Biochem Parasitol*., , vol. 189, no. 1-2, pp. 5-13, 2013.
http://dx.doi.org/10.1016/j.molbiopara.2013.04.005

[4] K.M. Choi, J. Gang, J. Yun, "Anti-Toxoplasma gondii RH strain activity of herbal extracts used in traditional medicine," *Int J Antimicrob Agents*., vol. 32, no. 4, pp. 360-362, 2008.
http://dx.doi.org/10.1016/j.ijantimicag.2008.04.012

[5] S. Pratt, N. K. Wansadhipathi-Kannangara, C. R. Bruce, J. G. Mina, H. Shams-Eldin, J. Casas, K. Hanada, R. T. Schwarz, S. Sonda, P. W. Denny, "Sphingolipid synthesis and scavenging in the intracellular apicomplexan parasite, Toxoplasma gondii," *Mol Biochem Parasitol*, vol. 187, no.1, pp. 43-51, 2013.
http://dx.doi.org/10.1016/j.molbiopara.2012.11.007

[6] M. E. Grigg, N. Sundar, "Sexual recombination punctuated by outbreaks and clonal expansions predicts Toxoplasma gondii population genetics," *Int J Parasitol*., vol. 39, no. 8, pp. 925-933, 2009.
http://dx.doi.org/10.1016/j.ijpara.2009.02.005

[7] T. Masatani, T. Matsuo, T. Tanaka, M. A. Terkawi, E. G. Lee, Y. K. Goo, G. O. Aboge, J. Yamagishi, K. Hayashi, K. Kameyama, S. Cao, Y. Nishikawa, X. Xuan, "TgGRA23, a novel Toxoplasma gondii dense granule protein associated with the parasitophorous vacuole membrane and intravacuolar network," *Parasitol Int*, vol. 62, no. 4, pp.372-379, 2013
http://dx.doi.org/10.1016/j.parint.2013.04.003

[8] C. M. Miller, N. R. Boulter, R. J. Ikin, N. C. Smith, "The immunobiology of the innate response to Toxoplasma gondii," *Int J Parasitol*., vol. 39, no. 1, pp. 23-39, 2009.
http://dx.doi.org/10.1016/j.ijpara.2008.08.002

[9] D Qu, J. Han, A. Du, "Enhancement of protective immune response to recombinant Toxoplasma gondii ROP18 antigen by ginsenoside Re," *Exp Parasitol*., vol. 135, no. 2, pp. 234-239, 2013.
http://dx.doi.org/10.1016/j.exppara.2013.07.013

[10] M. Döşkaya, A. Caner, D. Ajzenberg, A. Değirmenci, M. L. Dardé, H. Can, D. D. Erdoğan, M. Korkmaz, A. Uner, C. Güngör, K. Altıntaş, Y. Gürüz, "Isolation of Toxoplasma gondii strains similar to Africa 1 genotype in Turkey," *Parasitol Int*, vol. 62, no. 5, pp. 471-474, 2013.
http://dx.doi.org/10.1016/j.parint.2013.06.008

[11] B. A. Fox, A. Falla, L. M Rommereim, T. Tomita, J. P. Gigley, C. Mercier, M. F. Cesbron-Delauw, L. M. Weiss, D. J. Bzik, "Type II Toxoplasma gondii KU80 knockout strains enable functional analysis of genes required for cyst development and latent infection," *Eukaryot Cell*., vol. 10, no. 9, pp. 1193-1206, 2011.
http://dx.doi.org/10.1128/EC.00297-10

[12] W. D. Lopes, J. D. Rodriguez, F. A. Souza, T. R. dos Santos, R. S. dos Santos, W. M. Rosanese, W. R. Lopes, C. A. Sakamoto, A. J. da Costa, "Sexual transmission of Toxoplasma gondii in sheep," *Vet Parasitol*, vol. 195, no. 1-2, pp. 47-56, 2013.
http://dx.doi.org/10.1016/j.vetpar.2012.12.056

[13] S. H. Fallahi, B. Kazemi, S. J. Seyyed Tabaei, M. Bandehpour, Z. Lasjerdi, N. Taghipour, N. Zebardast, B. Nikmanesh, V. F. Omrani, F. Ebrahimzadeh, "Comparison of the RE and B1 gene for detection of Toxoplasma gondii infection in children with cancer," *Parasitol Int*., , vol. 63, no. 1, pp. 37-41, 2014.
http://dx.doi.org/10.1016/j.parint.2013.08.005

[14] S. Rajaratnam , T. Thiagarajan, "Molecular characterization of wild mushroom Euro," *J. Exp. Bio.*,vol. 2, no. 2, pp. 369-373, 2012.

[15] T. Kent, Y. R. Lapik, D. G. Pestov, "The 5' external transcribed spacer in mouse ribosomal RNA contains two cleavage sites," *RNA*., , vol. 15, no. 1, pp. 14-20, 2009.
http://dx.doi.org/10.1261/rna.1384709

[16] C. E. Ritland, K. Ritland, N. A. Straus, "Variation in the ribosomal internal transcribed spacers (ITS1 and ITS2) among eight taxa of the Mimulus guttatus species complex," *Mol Biol Evol*., , vol. 10, no. 6, pp. 1273-1288, 1993

[17] T. Oncel, G. Vural, "Occurrence of *Toxoplasma gondii* antibodies in sheep in Istanbul, Turkey," *VET ARHIV*, vol. 76, no 6, pp. 547-553, 2006

[18] F. Sevinsc, "The seroprevalence of Toxoplasma gondii in goats detected by indirect hemagglutination (IHA) and indirect florescent antibody (IFA) tests in the region of Konya," *Acta Parasitologica Turnica*, vol. 24, no. 1, pp. 57-80, 2000

[19] S. J. GREG, S. VITALI G, J. D. DAVID, L. G. GWENDOLYN, " Comparison of cell culture, mouse inoculation and PCR for detection of Toxoplasma gondii: Effects of storage conditions on sensitivity," *J CLIN MICROBIOL*., vol. 34, pp. 1572-1575, 1996.

[20] E. Gliot-Fromont, D. Aubert, S. Belkilani, P. Hermitte, O. Gibout, R. Geers, I. Villena, "Landscape, herd management and within herd seroprevalence of *Toxoplasma gondii* in beef cattle herds from champagne-Ardenne," France. *Vet. Parasitol*. Vol. 161, pp. 36-40, 2009.
http://dx.doi.org/10.1016/j.vetpar.2008.12.004

[21] M. González-Warleta, J. A. Castro-Hermida, C. Carro-Corral, J. Cortizo-Mella, M. Mezo, "Epidemiology of neosporosis in dairy cattle in Galicia (NW Spain)," *Parasitol Res*., vol. 102, no. 2, pp. 243-249, 2008.
http://dx.doi.org/10.1007/s00436-007-0753-y

[22] I. Klun, O. Djurković-Djaković, S. Katić-Radivojević, A. Nikolić, "Cross-sectional survey on Toxoplasma gondii infection in cattle, sheep and pigs in Serbia: seroprevalence and risk factors," *Vet Parasitol*., vol. 135, no. 2, pp. 121-131, 2006.
http://dx.doi.org/10.1016/j.vetpar.2005.08.010

[23] J. P. Dubey, "Isolation of Toxoplasma gondii from a naturally infected beef cow," *J Parasitol*., vol. 78, no. 1, pp. 151-153, 1992.
http://dx.doi.org/10.2307/3283705

[24] L. T. Huong, B. L. Ljungström, A. Uggla, C. Björkman, "Prevalence of antibodies to Neospora caninum and Toxoplasma gondii in cattle and water buffaloes in southern Vietnam," *Vet Parasitol*., vol. 75, no. 1, pp. 53-57, 1998.
http://dx.doi.org/10.1016/S0304-4017(97)00178-7

[25] H. V. Fajardo, S. D'ávila, R. Rocha Bastos, C. Dutra Cyrino, M. de Lima Detoni, J, Luis Garcia, L. Batista das Neves, J. Leonardo Nicolau, M. Regina Reis Amendoeira, "Seroprevalence and risk factors of toxoplasmosis in cattle from extensive and semi-intensive rearing systems at Zona da Mata, Minas Gerais state, Southern Brazil," *Parasites & Vectors*., vol. 6, pp.191, 2013.
http://dx.doi.org/10.1186/1756-3305-6-191

[26] J. P. Dubey, "A review of toxoplasmosis in cattle," *Vet Parasitol*., vol. 22, no. 3-4, pp. 177-202, 1986.
http://dx.doi.org/10.1016/0304-4017(86)90106-8

[27] C. Ghazaei, "Serological survey of antibodies to Toxoplasma gondii African," *J. Health*., vol. 12, no. 3-4, pp. 114-117, 2005.

[28] M. Sharif, S. Gholami, H. Ziaei, A. Daryani, B. Laktarashi, S. P. Ziapour, "Seroprevalence of Toxoplasma gondii in cattle, sheep and goats slaughtered for food in Mazandaran province, Iran during ," *Vet. J*., vol. 174, no. 2, pp. 422-424, 2005.
http://dx.doi.org/10.1016/j.tvjl.2006.07.004

[29] A. Nematollahi, G.Moghadam, "Survey on seroprevalence of anti-Toxoplasma gondii antibodies in cattle in Tabriz, Iran by IFAT," *American. J. Anim. Vet*., vol. 3, no. 1, pp. 40-42, 2008.
http://dx.doi.org/10.3844/ajavsp.2008.40.42

[30] S. Raeghi, A. Akbarei, S. Sadeghi, "Seroprevalence of Toxoplasma gondii in sheep, cattle and horses in Urmia North-West of Iran," *Iranian. J. Parasitol*., vol. 6, no. 4, pp. 90-94, 2011.

[31] R. Hashemi-Fesharaki, "Seroprevalence of Toxoplasma gondii in cattle, sheep and goats in Iran," *Vet. Parasitol*., Vol. 61, no. 1-2, pp. 1-3, 1996.
http://dx.doi.org/10.1016/0304-4017(95)00818-7

[32] M. Gorbani, A. Hafizi, M. T. Sbegerfcar, M. Rezaiean, A. *Nadim*, M. Anvar , A. Afshar, "Animal Toxoplasmosis in Iran," *J. Trop. Med. Hyg.*, vol. 86, no. 2,73-6. 1983.

[33] N, Hoghooghi-Rad, M. Afraa, "Prevalence of toxoplasmosis in humans and domestic animals in Ahwaz, capital of Khoozestan Province, south-west Iran," *J Trop Med Hyg*., vol. 96, no. 3, pp. 163-8, 1993.

[34] A. Nematollahi, G. Moghaddam, "Survey on Seroprevalence of Anti-Toxoplasma Gondii Antibodies in Cattle in Tabriz (Iran) by IFAT," *Am. J. Anim. Vet*., Vol. 3, no. 1, pp. 40-42, 2008.
http://dx.doi.org/10.3844/ajavsp.2008.40.42

[35] J. P. Dubey, "Toxoplasmosis in sheep--the last 20 years. Vet Parasitol," *SCI*., vol. 163, no. 1-2, pp. 1-14, 2009.

[36] M. Tavassoli , M. Ghorbanzadehghan , B. Esmaeilnejad, "A survey on infection of animals with Toxoplasma gondii using PCR and genetic differences using RFLP in Urmia, Iran," *Pazhoohesh va Sazandegi*., vol. 58, pp. 61-66, 2009.

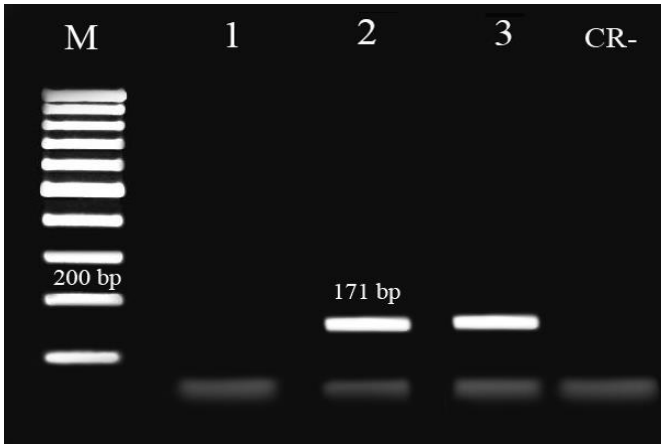

Fig. 1. Gel electrophoresis for detection of T.gondii infection in blood samples. Lane M is 100 bp DNA ladder (Fermentas, Germany); lane 1 is a negative sample, lane 2 and 3 are positive samples, lane 4 is negative control.