ISSN: 2577 - 8455

Research Article

Archives of Infectious Diseases & Therapy

Molecular Studies on Toxoplasma Gondii in Sheep and Goats in Ismailia Province, Egypt

El-Gawady HM*, Abdel-Aal AA, Sallam NH and Youssif EM

Suez Canal University, Egypt

***Corresponding author**
El-Gawady HM, Suez Canal University, Egypt, E-mail: gawady555@hotmail.com

Submitted: 10 Sep 2018; **Accepted:** 17 Sep 2018; **Published:** 05 Oct 2018

Abstract

Diagnosis of Toxoplasma gondii in 20 brain samples (10 from sheep and 10 from goats) using nested PCR techniques by amplification of the T. gondii B1 gene revealed that, six sheep's brain samples were positive (60%) and six goat's brain samples were positive (60%). Bioassay was done by inoculation of T. gondii positive PCR samples into healthy mice. Histopathological examination of the experimental mice brains, declared pathological lesions with demonstration of pseudocysts containing bradizoites within the host cell cytoplasm in the mice killed 30th day and 35th day post inoculation.

Introduction

Toxoplasmosis is an important parasitic disease caused by the protozoan species; *Toxoplasma gondii*. The parasite firstly discovered by ***Nicolle and Manceaux***, in the gundi (*Ctenodactylusgundi*), a North African rodent [1]. The name *Toxoplasma gondii* ("*toxon*"= arc; "*plasma*"= form in Greek) is derived from its crescent shape and the animal firstly isolated. The entire life cycle of *T. gondii* was not fully described until 1970 [2]. Several studies have shown the sensitivity of the PCR for the detection of *Toxoplasma* DNA in various biological samples [3]. In addition, studies have compared the traditional methods of *Toxoplasma* diagnosis, which include the detection of specific antibodies, mouse inoculation and immunohistochemistry, with the PCR and have shown a good correlation between the different techniques in the diagnosis of toxoplasmosis [4].

Although the PCR was extremely sensitive, in many situations a more reliable diagnosis will be gained if it is used in diagnosis of *T. gondii* in combination with other diagnostic data (serology and mouse inoculation) [5]. An increasingly popular detection method involving amplification of the *T. gondii* B1 gene by PCR that was able to detect 0.05pg of DNA, which corresponded approximately to the DNA content of one tachyzoite [6]. This work aimed to, diagnosis of Toxoplasma gondii in brain samples from sheep and goats by nested PCR technique and bioassay through inoculation of *T. gondii* positive PCR samples into mice.

Materials and methods

Polymerase chain reaction (PCR) according to Esteban-Redondo et al. [7]:

DNA extraction: This method consists of extracting DNA by using the conventional phenol chloroform method [8].

Specimens from brains of 10 sheep and 10 goats were used for homogenization and DNA extraction.

PCR amplification and sequence analysis: [9] T. gondii DNA was determined via nested-PCR protocol. ATP Binding Cassette B1 gene region was targeted for the generation of specific primer sets for T. gondii

Table 1: Primers and nucleotide positions according to the RH strain (Genbank Accession number: AF 179871)

Name	sequence (5'-3')	Position
Toxo-1 First round forward	ggaactgcatccgttcatgag	(694-714)
Toxo-2 First round reverse	tctttaaagcgttcgtggtc	(868-887)
Toxo-3 Second round forward	tgcataggttgccagtcactg	(757-776)
Toxo-4 Second round reverse	ggcgaccaatctgcgaatacacc	(831-853)

Protocol of PCR:

The PCR mixture contained:

- 0.5 µM of each primer,10 mM of Tris-HCl,1.5 mM of MgCl2,50 mM of KCl (pH 8.3), 0.2 mM of each deoxynucleoside triphosphate (Fermentas®, Lithuania),1.25 U of *Taq* DNA polymerase (Fermentas®, Lithuania).
- The reaction volume was 50µl containing 10µl of DNA extracts.

Amplification was carried out within a PTC-200 MJ Research thermal cycler. The first PCR (an initial step at 94°C for 3 min, 45 cycles of PCR amplification 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, final extension at 72°C for 8 min) was performed with Toxo-1 and Toxo-2 primers. Three microliters of the reaction product were used for nested-PCR (under the same conditions), which was performed with the primer pairs, Toxo-3 and Toxo- 4, respectively, resulting in amplification of a 96bp fragment. DNA from the RH strain of *T. gondii* was used as positive control. Distilled water was used as negative controls. Then Separation of PCR amplicons by Gel Electrophoresis.

Determination of Toxoplasma Infection by bioassay in mice

A 50 grams specimen of each brain samples of some selected PCR positive *T. gondii* sheep (5) were homogenized digested in pepsin according to ***Sharma and Dubey*** [10], the prepared inoculums inoculated in 10 swiss albino mice where 2 ml of suspension were inoculated I/p in each mouse for each sample. Other 10 mice were control group which inoculated only with saline solution mixed with digestion solution and last 10 mice left without any treatment. One mouse of each group was sacrificed at 3rd, 5th, 7th, 14th, 21st, 28th, 30th, 35th, 40th and 54th day after inoculation. Their peritoneal exudates were examined microscopically for T. gondii tachyzoites.

Impression smears of lung, brain, liver, heart of mice were fixed in methanol, stained with Giemsa stain and examined microscopically for T. gondii. For histopathological examination, specimens from lung, brain, liver, heart, spleen and kidney of each mouse were fixed in 10% formaline saline, embedded in paraffin and cut 5u thick, stained with Haematoxy line & Eosin stain [11].

Results

Table 2: showed that, in sheep, out of ten examined cases, six were positive and four cases were negative for *T. gondii* using PCR techniques. In goats, out of ten examined cases, six were positive and four cases were negative for *T. gondii* using PCR techniques.

	PCR			
Species	**+ve**	**%**	**-ve**	**%**
Sheep	6	60	4	40
Goats	6	60	4	40
Total	12	60	8	40

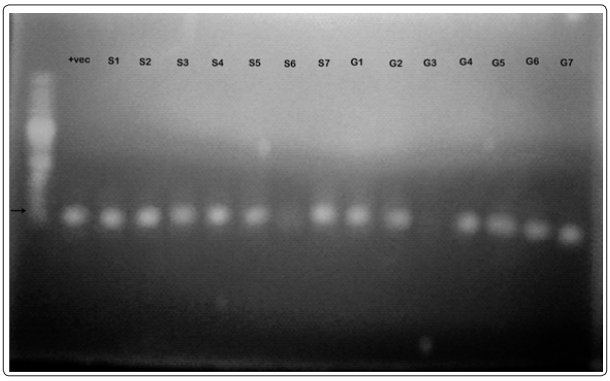

Figure 1: Detection of *Toxoplasma gondii* RH strain by nested PCR in brains of examined sheep and goats.

1st lane: +ve control, 2nd, 3rd, 4th, 5th, 7th and 8th lanes: +ve sheep samples of *T. gondii* with PCR (96 bp), 6th lane: -ve sheep sample of *T. gondii* with PCR.

9th, 10th, 12th, 13th, 14th and15th lanes: +ve goats samples of *T. gondii* with PCR (96 bp) 11th lane: -ve goat sample of *T. gondii* with PCR.

Histopathological findings for tissues of mice inoculated with brain homogenate of some positive PCR cases:

Brain: Moderate to severe congestion of meningeal blood vessels with mononuclear leukocytic cell infiltrations mainly lymphocytes and macrophages were observed (Figure 2).

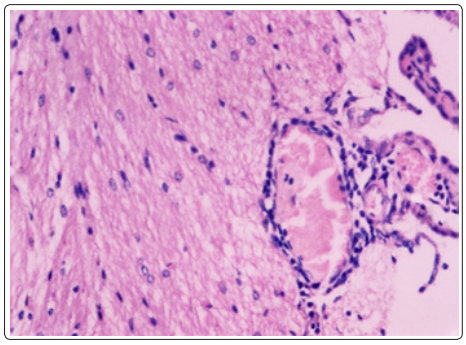

Figure 2

Large typical pseudocysts containing bradyzoites were also demonstrated. Tissue cyst was spheroidal or ovoid and measured about 30 - 40 µm. The cyst wall was composed of host cells and enclosed lancet shape bradyzoites. The tissue cyst develops within the host cell cytoplasm. (Figure 3 & 4).

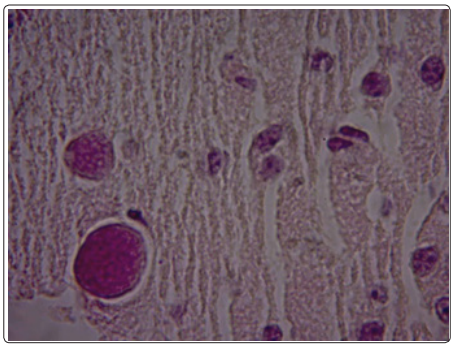

Figure 3

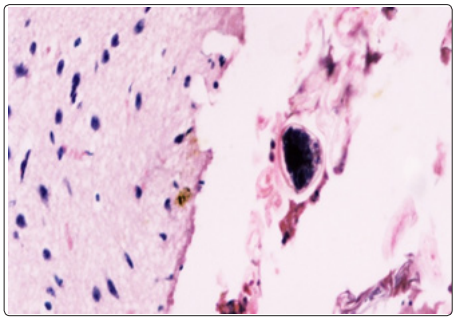

Figure 4

Mild necrosis in cerebrum and cerebellum also observed, in addition to perivascular cuffing and gliosis (Figure 5).

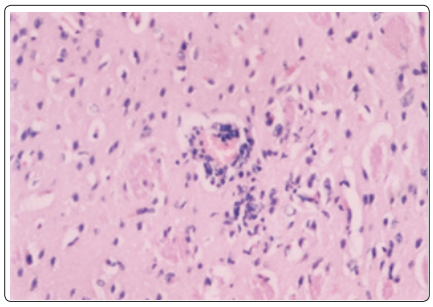

Figure 5

Liver: grossly: grayish whitish nodules about 1mm. in diameter were scattered all over the hepatic parenchyma. Liver was pale and friable in consistency.

Histopathology: multiple multifocal necrotic foci with mononuclear leucocytic infiltration mainly lymphocytes and few neutrophils were observed in hepatic parenchyma. Mild congestion of central veins and sinusoids in addition to vacuolar degeneration and focal coagulative necrosis were present (Figure 6).

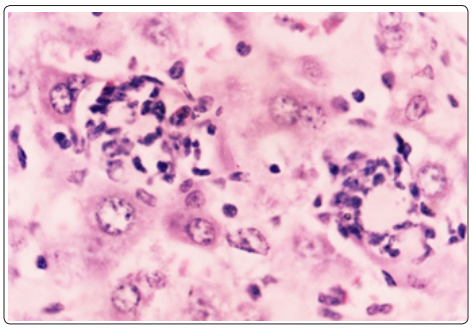

Figure 6

Kidneys: grossly: the examined kidneys of the mice were pale in color, enlarged in size with congested cut surface.

Histopathology: marked congestion of intertubular capillaries and glomerular tufts. Interstitial focal lymphocytic aggregation was seen. Most renal epithelial cells were swollen (Figure 7).

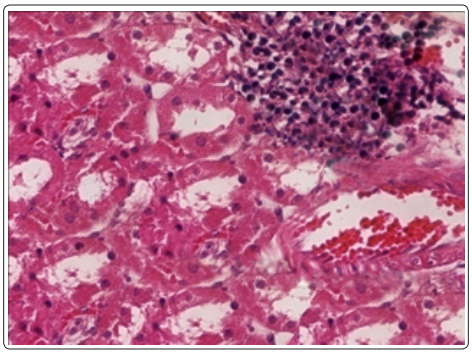

Figure 7

Lung: Moderate congestion of interalveolar capillaries with focal interstitial thickening of alveolar septa and mononuclear inflammatory cell infiltration mainly lymphocytes and few macrophages (Figure 8).

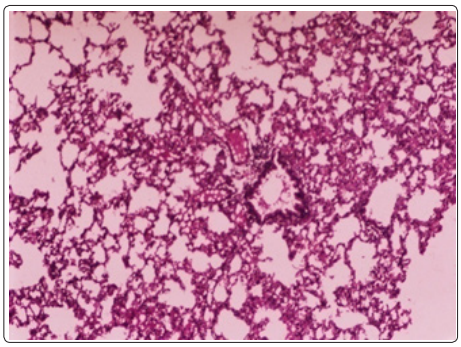

Figure 8

Spleen: the examined spleens of the infected mice showed depletion of lymphocytes of the white pulp and slight infiltration of neutrophils (Figure 9).

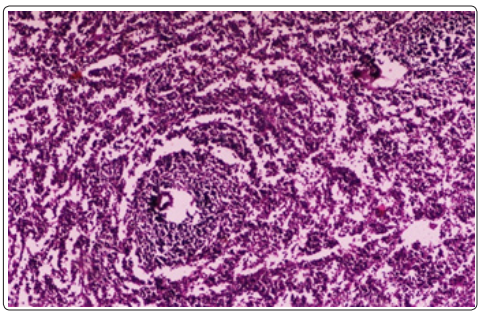

Figure 9

Impression smears:tachyzoites were detected from impression smear of the liver stained by Giemsa stain, from the mouse sacrifized in the 3rd day of inoculation. The tachyzoite is often crescent shaped, about 1.5 x 6 µm, with a pointed anterior (conoidal) end and a rounded posterior one (Figure 10).

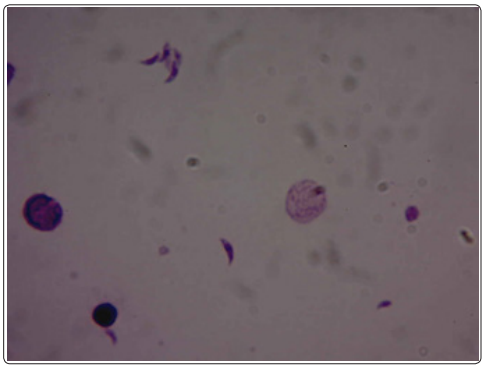

Figure 10

Discussion

The present results showed that from 20 examined brain samples (10 from sheep and 10 from goats) by nested PCR, six samples were positive in sheep (60%) and six from goats (60%). The obtained results agreed with those of ***Esteban-Redondo et al***., who reported that, by using PCR, the presence of *T* [7]. *gondii* in sheep that was more frequently and consistently detected in particular within brain and heart tissues. Also agreed with ***Hurtado et al***. who reported that PCR is a good diagnostic tool for the detection of T [12]. *gondii* in ovine; although *T. gondii* DNA was amplified from different types of tissues, brain was the tissue with the highest detection rate.

In this study, B1 gene region was targeted for the generation of specific primer sets for *T. gondii*. The B1 gene has theoretical advantages because each *T. gondii* contains approximately 35 copies of this gene [13]. TheB1 specific gene probe used for the detection of *T. gondii* does not cross-react with other tissue-resident microorganisms such as *Sarcocystis, Neospora*, and *Trichinella* [14]. Studies have reported that the B1 geneis able to detect a single tachyzoite in ocular fluids [13, 15].

In the present results, histopathological examination of mice inoculated with brain homogenates of Toxoplasma positive PCR samples declared pathological lesions in the brain represented by congestion, perivascular cuffing, degenerated neurons and gliosis

with demonstration of pseudocysts containing bradyzoites in 2 mice killed 30th and 35th day after inoculation. These results agreed with those of ***Dubey and Beattie*** who mentioned that although tissue cysts should be sought 6 weeks after inoculation, even though tissue cysts may appear earlier because early tissue cysts are small and may be missed [16]. On the other hand, the results obtained by ***Ito et al***., ***and Michael*** revealed that the main findings were congested and mild degenerative changes in the brain of the experimental animals, but the parasites were not seen in the histopathological section [17, 18].

In the liver, the pathological lesions were multiple multifocal coagulative necrotic foci with mononuclear leucocytic infiltration mainly lymphocytes and few neutrophils and congestion of central veins and sinusoids. The kidney showed congestion of blood vessels with focal areas of mononuclear leucocytic infiltration. The spleen showed depletion of lymphocytes of the white pulp and slight infiltration of neutrophils. The present results were in agreement with the results obtained by ***Remington et al., Rifaat et al. and Youssef*** [19-21].

On the other hand, microscopic granuloma in the liver of mice was not recognized in this study demonstrated by ***Ito et al. and Michael*** [17, 18]. In our opinion, this is may be attributed to the fact that the general resistance of individual animals differs to some extent. In animals with a low general resistance no granuloma could be expected to appear in the infected liver. Furthermore, granuloma appeared in long standing chronic cases, which didn't occur within this study as the last day of sacrificing was 54th post inoculation.

In the present study, the lung showed congestion of peribronchial blood vessels and thickening of alveolar septa and mononuclear leucocytic infiltration. This was in agreement with the results obtained by Ito et al. and El- Manyawy [17, 22].

Tachyzoits were detected from impression smear of the liver stained by Giemsa stain, from the mouse sacrificed in the 3rd day of inoculation. The tachyzoite is crescent shaped, about 2 x 6 µm, with a pointed anterior (conoidal) end and a rounded posterior one. These results agreed with those of Dubey, [23].

In the present study, the mice inoculation with brain homogenates of Toxoplasma positive PCR samples give positive histopathological results, with the presence of tachyzoites in the stained impression smear of liver, and cyst in the brain. The present results were in agreements with those of Wastling et al. who mentioned that PCR technique was known to be a sensitive method for the diagnosis of Toxoplasma infection, and added that mouse inoculation detected viable parasites, whereas PCR detected specific DNA [24].

Also the present results were in agreements with those of Steuber et al. and Buxton et al. who mentioned that, the detection of live T [4, 25]. Gondii and parasite DNA by mouse inoculation and the polymerase chain reaction respectively, gave similar results Also ***Owen et al***. proved that PCR was as sensitive as mouse inoculation in the diagnosis of *Toxoplasma* abortion in sheep and goats when tissues from the aborted feti and placentas from the live lambs were examined. However, ***Mahittikorn et al***. declared that, the advantages of the n-PCR assay over mouse inoculation, histology, or other assays are its rapidity, specificity and sensitivity [26, 27].

References

1. Nicolle C and Manceaux L (1908) Surune infection a corps de Leishman (ouorganismesvoisins) du gondi. C. R. Seances Acad. Sci 147: 763-766.
2. Frenkel JK (1970) Pursuing Toxoplasma. J. Infect. Dis 122: 553-559.
3. Ho-Yen DO, Joss AWL, Balfour AH, Smyth ETM, Baird D, et al. (1992) Use of the polymerase chain reaction to detect Toxoplasma gondii in human blood samples. J. Clin. Pathol 45: 910-913.
4. Steuber S, Niu A, Bauer C, Reetz J, Roth A, et al. (1995) Detection of Toxoplasma gondii in tissues of ovine abortion using the polymerase chain reaction. Deutsch TierarztlicheWochenschrift 102: 91-93.
5. Ausubel F, Brent R and Kingstone RE (1993) Current protocols in molecular biology. Wiley/ Interscience 1: 134-137.
6. Savva D, Morris JC, Johnson JD and Holliman R E (1990) Polymerase chain reaction for detection of Toxoplasma gondii. J. Med. Microbiol 32: 25-31.
7. Esteban-Redondo I, Maley SW, Thomson K, Nicoll S, Wright S, et al. (1999) Detection of Toxoplasma gondii in tissues of sheep and cattle following oral infection. Vet. Parasitol 86: 155-171.
8. Sambrook J, Russell DW and Sambrook J (2001) Molecular cloning: a laboratory manual, 3rd ed. NY: Cold Spring Harbor Lab. Press.
9. Ergin S, Ciftcioglu G, Midilli K, Issa G and Gargili A (2009) Detection of Toxoplasma gondii from meat and meat products by the Nested- PCR method and its relationship with seroprevalence in slaughtered animals. Bull. Vet. Inst. Puawy 53: 657-661.
10. Sharma SP and Dubey JP (1981) Quantitative survival of Toxoplasma gondiitachyzoites and bradyzoites in pepsin and in trypsin solutions. Am. J. Vet. Res 42: 128-130.
11. Drug RA, Wallington E.A and Cancerson R (1967) Carleton's Histopathological Techniques. 4th Ed. Oxford Uni. Press, London, New York, Toronto.
12. Hurtado A, Aduriz G, Moreno B, Barandika J and Garcia-Perez AL (2001) Single tube nested PCR for the detection of Toxoplasma gondii in fetal tissues from naturally aborted ewes. Vet. Parasitol 102: 17-27.
13. Burg JL, Grover CM, Pouletty P and Boothroyd JC (1989) Direct and sensitive detection of a pathogenic protozoan, Toxoplasma gondii, by polymerase chain reaction. J. Clin. Microbiol 27: 1787-1792.
14. Silva AV and Langoni H (2001) The detection of Toxoplasma gondiiby comparing cytology, histopathology, bioassay in mice, and the polymerase chain reaction(PCR). Vet. Parasitol 97: 191-198.
15. Bretagne S, Costa J.M, Vidaud J, Tran Van Nhieu J and Feith JF (1993) Detection of Toxoplasma gondii by competitive DNA amplification of bronchoalveolar lavage sample. J. Infect. Dis 168: 1585-1588.
16. Dubey JP and Beattie CP (1988) Toxoplasmosis of animals and man. CRC Press, Boca Raton, FL 01-220.
17. Ito S, Tsunoda K and Suzuki K (1967) Distribution of Toxoplasma gondii, RH strain in infected mice as determined by the Fluorescent antibody technique and the histopathology of toxoplasmosis. Nat. Inst. Health Quart 7: 208-220.
18. Michael SA (1966) Rats and mice as experaminal animal for Toxoplasma infection. Inougural Dissertation, Dr. Med. Vet. Bonn University.

19. Remington JS, Jacobs L and Kaufman HE (1967) Toxoplasmosis in the adult. New Engl. J. Med 262: 180-186.
20. Rifaat MA, Mahdi AH, Arafa MS, Nasr NT and Sadek MSM (1971) Isolation of Toxoplasma for Ratusnorveqicus in Egypt. Trns. Roy. Soc. Trop. Med. Hyg 65: 788-789.
21. Youssef MLGE (1993) Clinico-pathological studies on Toxoplasmosis inAlbino rat. M. V. SC. Thesis Faculty of Veterinary Medicine, Zagazig University.
22. El-Manyawy SMA (1984) some biological studies for the detection of Toxoplasma gondii antibodies during the different stages of infection among rat. M.V. SC. Thesis Faculty of Veterinary Medicine, Cairo University.
23. Dubey JP (1997) Validation of the specificity of the modified agglutination test for toxoplasmosis in pigs. Vet. Parasitol 71: 307-310.
24. Wastling JM, Nicoll S and Buxton D (1993) Comparison of two gene amplification methods for the detection of Toxoplasma gondii in experimentally infected sheep. J. Medical Microbiol 38: 360-365.
25. Buxton D, Thomson KM, Maley S, Wastling JM, Innes EA, et al. (1994) Primary and secondary responses of the ovine lymph node to Toxoplasma gondii: Cell output in efferent lymph and parasite detection. J. Comp. Pathol 11: 231-241.
26. Owen MR, Clarkson MJ and Trees AJ (1998) Diagnosis of Toxoplasma abortion in ewes by polymerase chain reaction. Vet. Rec 25: 445-448.
27. Mahittikorn A, Wickert H and Sukthana Y (2005) Comparison of five DNA extraction methods and optimization of A B1 gene nested PCR (nPCR) for detection of T. gondii tissue cyst in mouth brain. Southeast Asian J. Trop. Med. Public Health 6: 1377-1382.