



PCR based detection of toxoplasmosis in tissue samples: A step towards detection of toxoplasmosis in meat and post mortem samples

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ABSTRACT

The aim of the study was to find out a suitable marker gene for diagnosis of toxoplasmosis in tissue and meat samples during meat inspection and/or during post mortem studies. PCR was used to diagnose toxoplasmosis in ten inbred Swiss albino mice after experimental inoculation of 100 tachyzoites of laboratory maintained human RH strain of the parasite. Blood, peritoneal lavage and tissue from lung, liver spleen, brain, heart and kidney were taken from experimental murine models in duplicate. The samples were subjected to PCR, using primers directed to the multicopy of Surface Antigen 3 (SAG 3 gene). Blood, kidney and heart tissue were found negative while peritoneal lavage along with lung, liver spleen and brain tissue yielded desired positive amplicons. The significance of the studied molecule vis-à-vis future projections in regard to diagnosis of toxoplasmosis during meat sample examination and/or during routine post mortem is being described.

Key words: PCR based detection, SAG 3 amplicon, Tissue samples, Toxoplasmosis

Toxoplasma gondii is an obligate intra-cellular coccidian parasite with utmost zoonotic relevance. Consumption of undercooked meat of secondary hosts mainly pig and sheep is a major route of transmission of the disease to human population (Tenter *et al.* 2000). The condition causes severe economic losses in terms of abortions and death of young stock and newborn animals (Dubey and Beattie 1998) alongwith its life-threatening consequences both in immuno-compromised human patients suffering from acquired immune deficiency syndrome (AIDS) and those with organ transplants. The PCR based diagnosis remains the only viable tool for detection of toxoplasmosis in food like animal meat samples and/or in post mortem examinations where because of death of animal, other tools like serology fails to detect the infection. The present paper describes the use of SAG 3 gene of *T. gondii* to detect toxoplasmosis in tissue samples of experimentally infected murine models.

MATERIALS AND METHODS

Ten inbred Swiss albino mice (either sex, age group 6–8 weeks weighing approximately 22–25 g) were used in the present study and subsequently infected intraperitoneally with 100 live virulent human RH strain

of *T. gondii*. The strain was being maintained through regular passaging in murine model for over a decade in a cryopreserved stock line in Divisional Protozoology Laboratory, IVRI. The experiments on the laboratory animal were done as per the approval of University Ethical Committee. The infected mice were daily monitored for the development of signs infection. Such mice were euthanized on day 7 PI and the peritoneal fluid was aspirated after inoculation of 5 ml of sterile phosphate buffered saline (PBS, pH 7.2) in the peritoneal cavity with due care to avoid injury to visceral organs. The process was repeated until the peritoneal contents were clear. Similarly, blood was also aspirated directly from the heart. Small pieces (matchstick head size) of lungs, liver, brain, spleen, kidney and heart were excised and placed in about 150 µl of distilled water and grounded thoroughly with a pestle and mortar. Duplicate samples, from these grounded samples along with peritoneal washings and blood, were placed in the tubes and were heated in boiling water for 15 minutes. From these boiled crude samples, 15 µl of the supernatant was taken and served as the DNA template for PCR amplification. The nucleotide sequences of the primers for the amplification of SAG 3 gene (Sudan *et al.* 2012) were as follows: forward primer, TS3F 5-ATGCAGCTGTGGCGGCGCAG-3 and reverse, TS3R 5-TTAGGCAGCCACATGCACAAG-3. The PCR amplification was carried out in a 25 µl PCR reaction mixture consisting of 15 µl of sample, 2.5 µl of 10X PCR buffer, 2.5 µl of 2.5 mM dNTP mix, 0.5 µl (1.5 U) of Taq polymerase and 1 µl each of forward and backward primers. The final volume was made 25 µl by addition of adequate

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nuclease free water. The PCR conditions consisted of initial denaturation of DNA strands at 95°C for 5 min followed by 32 cycles of denaturation at 95°C for 50 sec, primer annealing at 62°C for 75 sec and strand elongation at 7°C for 50 sec. Thereafter, one cycle of final extension of the strands was given at 72°C for 12 min. The PCR amplification was confirmed by visualization of the PCR products with appropriate volume of 6X loading dye on 1.5% agarose gel stained with ethidium bromide following electrophoresis.

RESULTS AND DISCUSSION

All the infected mice started showing characteristic signs of the disease from day 5 post infection (Sudan *et al.* 2014a, 2014b). The clinical signs included raised and rough hair coat, pendulous abdomen, severe ascites, dullness and tachypnoea marked by resting with fore legs on walls of the cages, the nozzle of water bottle or on other resting mice. Direct amplification of the samples was observed in peritoneal washings, liver, lungs, spleen and brain. A characteristic specific band suggesting amplification was seen at 1158 bp distance that confirmed the presence of *T. gondii* DNA in these samples. No amplification was observed in blood, kidneys and heart (Fig 1). Known positive and negative samples were also run alongside.

It is known that multiplication of tachyzoites occurs freely in the lymph nodes 9 and the lymphoid tissues particularly during acute phase of infection and 100 virulent tachyzoites of human RH strain are sufficient to cause acute toxoplasmosis in mice (Sudan *et al.* 2014a, 2014b). The macrophages in the peritoneal fluid account for presence of tachyzoites and hence amplification was seen in peritoneal lavage. Similarly, lymphoid tissue drained to liver and spleen account for amplification in these tissues. Brain and lungs are found to be centers of tissue reactions caused due to acute toxoplasmosis (Sreekumar *et al.* 2004, Sudan *et al.* 2014a, 2014b). The absence of amplification in the blood might be attributed to two reasons viz. firstly, there might be absence of tachyzoites in the blood and the animals

were euthanized much before the paracetemia reached the blood and secondly that the paracetemia was present but the boiling of the blood samples might have caused a change in the constituents like haeme and proteinases, which are potent PCR inhibitors. Thus, the continued presence of the inhibitory substances could have prevented the amplification of desired amplicon. The kidney and heart samples did not show any amplification and it could be attributed to absence of tachyzoites in these tissues owing to acute phase of infection and tachyzoites did not get enough time to get converted to bradyzoites and actually get settled in these tissues as pseudocysts.

PCR is a highly sensitive, specific and rapid technique for detecting *T. gondii* (Hurtado *et al.* 2001). No doubt, murine bioassays are more sensitive than PCR with respect to the isolation of *T. gondii* (Hurtado *et al.* 2001) but indeed the ultimate significance of PCR based detection of toxoplasmosis is that PCR can detect the DNA of parasites even when the tissues available for testing are in state of decomposition; bioassays, in contrast, can only detect viable parasites (Wastling *et al.* 1993). Moreover, the size of the sample analyzed is very important in the detection even by bioassays and it is quite possible that the parasitic stage in the sample to be tested is either low or sparse and shows focal distribution in the tissues or it may be all together dead. Bioassay will miss detection in either of these cases. But PCR will give amplification even if the parasitic stage is dead (Yai *et al.* 2003) and/or very less in number. PCR can even detect 0.1 pg of DNA (MacPherson and Gajadhar 1993) and even a very few tachyzoites are sufficient in accurate diagnosis no matter if they are living or dead.

A wide range of gene targets like B1 gene (Sreekumar *et al.* 2004), Tox4 and Tox5 genes (Jacquet *et al.* 2001) are being used for the PCR based diagnosis of toxoplasmosis from time over and again. All these gene targets are having their own advantages and limitations. Most of the times, these are either present in either one of the infective stage of the parasite and thereby their presence is affected by the course of the infection. Surface antigen 3 (SAG3) is a glycosylphosphatidylinositol (GPI) anchored membrane bound protein commonly found on both the developmental stages of parasite viz. tachyzoites and bradyzoites (Burg *et al.* 1999). Hence, this molecule was selected so that it can detect tachyzoites in acute infections as well as tissue cysts in form of bradyzoites in chronic cases.

Considering toxoplasmosis a significant emerging zoonotic problem, the findings reported herein have undoubtedly opened newer frontier of research. It would be interesting to precisely investigate through well planned experimental studies to elucidate effective well planned research aiming at SAG 3 molecule to be used as a potent candidate for diagnosis of toxoplasmosis from meat tissues and/or dead animals in referral labs. Further detailed research in all these aspects is thereby warranted.

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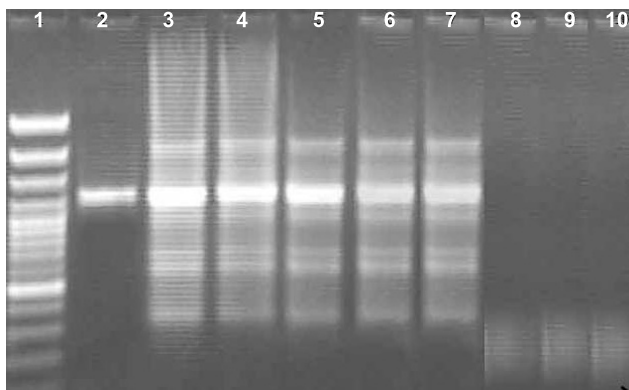


Fig. 1. Gel showing the desired 1158 bp amplicon of SAG 3. Lane 1, 100 bp plus ladder; lane 2, known positive; lane 3, peritoneal lavage; lane 4, liver tissue; lane 5, lung tissue; lane 6, spleen tissue; lane 7, brain tissue; lane 8, kidney tissue; lane 9, heart tissue; lane 10, known negative.

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