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B1 gene based semi nested PCR for detection of toxoplasmosis from poultry hearts

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Toxoplasma gondii is an obligate intracellular coccidian parasite with utmost zoonotic relevance. Consumption of undercooked meat of secondary hosts mainly poultry, pig and sheep is a major route of transmission of the disease to humans (Tenter *et al*. 2000). The condition also causes severe economic losses in terms of abortions and death of young stock and newborns (Dubey and Beattie 1998) alongside its life-threatening consequences in immunecompromised human patients. Detection of the toxoplasmosis in the intermediate hosts through parasitological techniques is difficult as none of the biological stages are demonstrable in the blood and/or natural excretions or secretions. The PCR based diagnosis remains the only viable tool for detection of toxoplasmosis in slaughtered food animals where other tools like serology fails to detect the infection. The present paper describes the use of universal *B1* gene of *T. gondii* to detect toxoplasmosis in meat samples of poultry reared under semi arid conditions.

Poultry hearts (40) were procured from local meat shops of Mathura. Small piece from apex of heart was cut and was finely chopped. Thereafter the chopped tissue was put in three times the volume in TE lysis solution consisting of 10 mM tris acid and 1mM EDTA. Then 20 µl of 25 mg/ml of Proteinase K and 100 µl of 10% SDS were added to it, and the sample was kept at 65°C overnight. Thereafter, the DNA was extracted as per standard phenol chloroform protocol. The concentration of DNA in each sample was determined using a nanodrop (Eppendroff). A final concentration of approximately 250 ng in each sample was adjusted using nuclease free water. The nucleotide sequences of the primers used for the amplification of *B1*gene (Sreekumar *et al.* 2004) included forward primer, B1F 5'/-GGAACTGCATCCGTTCATGAG -3' and reverse primer, B1R 5 '-TCTTTAAAGCGTTCGTGGTC -3'. The PCR amplification was carried out in a 20 µl PCR reaction mixture consisting of 2 µl of sample DNA (500 ng), and 10 µl Green Taq PCR Master Mix (Fermentas), and 1.5 µl (15 picomoles) each of forward and backward primers. The final volume was made 20 µl by addition of nuclease free water. The PCR conditions for the semi nested PCR consisted of initial denaturation of DNA strands at 94°C for 5 min followed by 10 cycles of denaturation at 94°C for 45 sec, primer annealing at 60°C for 60 sec and strand elongation at 72°C for 60 sec. This was followed by another 30 cycles of denaturation at 94°C for 45 sec, primer annealing at 55°C for 60 sec and strand elongation at 72°C for 60 sec. A final extension of the strands was made at 72°C for 10 min. The amplification was confirmed by visualization of the PCR products on 1.5% agarose gel incorporated with ethidium bromide following electrophoresis.

Out of 40 heart samples processed, 2 were found to be positive for *T. gondii* DNA as they yielded 198 bp amplicon specific for *B1*gene. A wide range of gene targets like *B1* gene (Sreekumar *et al.* 2004), Tox4 and Tox5 genes (Jacquet *et al.* 2001), SAG 3 (Sudan *et al.* 2016) have already been used for the PCR based diagnosis of toxoplasmosis. All these gene targets are having their own advantages and limitations. Most of the times, these are either present in only one of the infective stages of the parasite and thereby, their presence is affected by the course of the infection. The repeated *B1* gene has got immense value for being used as the polymorphism marker for direct genotyping of *T. gondii* from clinical specimens without the use of any isolation or cultures (Costa *et al*. 2013).

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 (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

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detect viable parasites (Wastling *et al.* 1993). Moreover, the size of the sample analyzed is very important for detection as in bioassays it is quite possible that the parasitic stage in the sample to be tested is either low or sparse and show 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 and even a very few tachyzoites are sufficient in accurate diagnosis no matter if they are living or dead. Alongside there are a few reports of toxoplasmosis based on serology from India (Sudan *et al.* 2015, Singh *et al.* 2014) but these studies can be done on field and cannot be possible for meat samples available in market in Indian conditions.

Many reports of outbreaks of human toxoplasmosis have been reported in these areas stressing the threat the human population is facing in India (Balasundaram *et al.* 2010). Hence, well planed surveys need to be taken at larger areas to give the accurate picture of prevalence of this parasite in all the susceptible food animals so as to develop suitable control strategies in the near future.

SUMMARY

The WTO guidelines on control strategies regarding food-borne diseases insist on mandatory serological investigations of the causative agent(s) at the farm level and in slaughtered animals. But in countries like India, it is very difficult to go for serological based diagnosis on slaughtered meat found in local markets. Hence, in these cases, PCR remains the sole source of detection of infectious agents. The aim of the study was to find out the presence of toxoplasmosis in meat samples of poultry reared under semi arid conditions of Mathura. The samples were subjected to semi nested PCR, using primers directed against the multicopy of universal *B1* gene. Out of 40 heart samples screened, 2 were found to be positive for *Toxoplasma gondii* DNA.

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