Survey of *Toxoplasma gondii* DNA in aborted Ovine and Caprine fetuses by nested PCR in Borno state, Nigeria

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Abstract

A study was designed to detect *T.gondii* DNA from tissues of aborted caprine and ovine fetuses by single tube nested PCR. A total of 327 tissues from 45 and 31 caprine and ovine aborted fetuses respectively were analyzed. Tissues analyzed were, 76 brains, 76 lungs, 70 livers, 65 hearts and 40 placentas. Approximately five grams of each tissue was finely chopped with a sterile scalpel blade and re-suspended in sterile distilled water. DNA extraction was conducted according to standard protocol. Two successive amplifications were performed in a single tube using 18S-5.8S rRNA ITS1 targeted primers in order to increase the sensitivity and specificity of the reaction and to minimize the chance of contamination. All samples analyzed were negative for *T.gondii* DNA. We conclude that the organism may not play important role in ovine and caprine abortions in the study area. Our next plan is to analyze tissues from the feline definitive host and rodents in the area in order to detect and sequence *T.gondii* DNA to enable us compare it to known clades.

Key Words: *Toxoplasma gondii,* Abortion, Ovine, Caprine, nPCR, Nigeria.

Introduction

Infectious organisms can cause significant production losses in farm ruminant as a result of abortion, embryonic damage and maternal infertility. Toxoplasma gondii is one of the principal agents causing abortion in sheep and goats (Dubey and Beatie 1988). Abortion in ruminants may constitutes a considerable public health risk as many of the pathogens that cause disease in ruminants may pose a significant danger to humans (Dubey and Beatie 1988). Several conditions due to *T. gondii* infection in many species of animals have been reviewed (Dubey and Beatie 1988, Tenter et.al., 2000). This includes embryonic death and resorption, fetal death and mummification, abortion, stillbirth and neonatal death in goats and sheep. When primary *T. gondii* infection occurs during pregnancy, it can lead to death of the fetus causing economical losses (Buxton, 1991).

Diagnosis of *T.gondii* abortion is usually based on clinical signs in the dam and gross or histological lesions, which are found mainly in the placenta and the brain of the fetus. However, the extension of these lesions is dependent on the timing of infection, and can sometimes be non-specific or difficult to diagnose due to autolysis (Buxton, 1991).

Serological tests to detect the presence of specific antibodies to *T. gondii* in fetal fluids by indirect fluorescent antibody test (IFAT) or latex agglutination test (LAT) can be employed but is not always detectable and isolation of *T. gondii* by bioassay is slow, hazardous and tedious (Buxton 1991, Salant et. al., 2007).

PCR amplification of different genes to detect *T. gondii* DNA in ovine fetal tissue and placental samples was considered a valuable tool for the diagnosis of congenital toxoplasmosis (Duncanson et.al, 2001, Hurtado et.al, 2001) and opens up a diagnostic approach to cases where aborted material is in an advanced stage of autolysis. PCR assays targeting different genes have been developed for the detection of *T. gondii*: the B1 repetitive sequence (35 copies) (Burg et.al., 1989); the P30 surface antigen (single copy) (Johnson et.al., 1990, Savva et.al., 1990); the ribosomal RNA (110 copies), both the small subunit rRNA gene (Tenter et.al., 1994) and the more variable internal transcribed spacer (ITS1) sequence (Payne and Ellis 1996). Some authors have used the single tube nested PCR developed for the detection of Neospora caninum DNA that minimizes the chances of contamination without losing detection power (Ellis et.al., 1999) for diagnosis of *T.gondii* in ovine aborted fetuses. In the present study, we also applied the single tube nested PCR for the diagnosis of *T. gondii* DNA in fetal tissues from naturally aborted lambs and kids in Borno State, Nigeria.

Material and methods

A preliminary questionnaire survey was conducted to determine the rate of abortion in small

ruminants in Borno state. A total of 24 farms located in three senatorial zones were visited and interviews conducted for farm owners.

Three hundred and twenty seven tissues from 45caprine and 31 Ovine aborted fetuses respectively were collected from August 2007- May 2009, from different farms in Borno State, Nigeria. Before sample collection, the fetuses were measured crown to rump to estimate the gestational stage at the time of abortion according to the method of Evans and Sack (1973). Tissues collected include 76 brains, 76 lungs, 65 hearts, 70 livers and 40 placentas. The condition of some of the fetuses was such that not all tissues could be collected.

The tissues were stored in absolute ethanol and transported to the Immunodiagnostic Laboratory, NVRI Vom, where they are stored at -20°C in sterile vials until used.

DNA extraction

Approximately 5 grams of tissues were finely chopped with a sterile scalpel blade and re-suspended in sterile distilled water. DNA extraction was conducted according to methods previously described (Payne and Ellis, 1999, Ellis et.al., 1999). For every 10 samples of DNA extraction, one control (400µl of TE without tissue sample) was included to detect any possible contamination occurring during the extraction process. Genomic DNA obtained from tachyzoites of three strains (GT1, PTG, CTG) of T. gondii, kindly provided by Prof C. Su (Dept of Microbiology, University of Tennessee, USA), were used as positive control in PCR amplifications.

Single tube nested PCR amplification

Two successive amplifications were performed in a single tube using 18S-5.8S rRNA ITS1 targeted primers in order to increase the sensitivity and specificity of the reaction as well as minimizing the chance of contamination as described by Hurtado et al., (2001). Two oligonucleotide primer pairs earlier designed by Hurtado et al., (2001) were commercially synthesized (Inqaba Biotechnical industries (Pty) Ltd) and employed for this work. The external primers were NN1 (5-CCTTTGAATCCCAAGCAAAACATGAG-3) and NN2 (5-GCGAGCCAAGACATCCATTGCTGA-3), that hybridize to a region of the ITS1 common to both *T. gondii* and *N. caninum.* The internal primers were Tg-NP1 (5-GTGATAGTATCGAAAGGTAT-3) and Tg-NP2 (5-ACTCTCTCTCAAATGTTCCT- 3), and amplify a region of 227 bp of the ITS1 of T. gondii.

The 25 ul reaction mixtures contained 250–500 ng of total genomic DNA, 10mM Tris– HCl (pH 8.3), 50mM KCl, 1.5mM MgCl2, 0.01% (w/v) gelatin, 0.2mM of each deoxynucleotide (Fermentas), 0.01 uM of each external primer, 0.04 uM of each internal primer and 1U of Thermus aquaticus (Taq) polymerase (Inqaba Biotechnical industries (Pty) Ltd).

The mixture was subjected to the following cycling conditions using Gene Amp® PCR System 9700 (Applied Biosystem): 94°C for 3 min, 15 cycles of 94°C for 30 s, 65°C for 45 s, 72°C for 1 min, 35 cycles of 94°C for 20 s, 53°C for 30 s, 72°C for 30 s and a final extension of 72°C for 5 min. Positive controls (purified *T. gondii* RH DNA) and negative controls (no template DNA) were included in each PCR run. The PCR products were analyzed by electrophoresis through 1.5% (w/v) agarose (Sigma) gels in TBE buffer containing 0.05% ethidium bromide and the bands were visualized under UV light on a Bioimaging Systems (Syngene).

Results

The questionnaire survey shows an average annual incidence of 8.9% and 10% abortion for caprine and ovine species respectively in the farms surveyed.

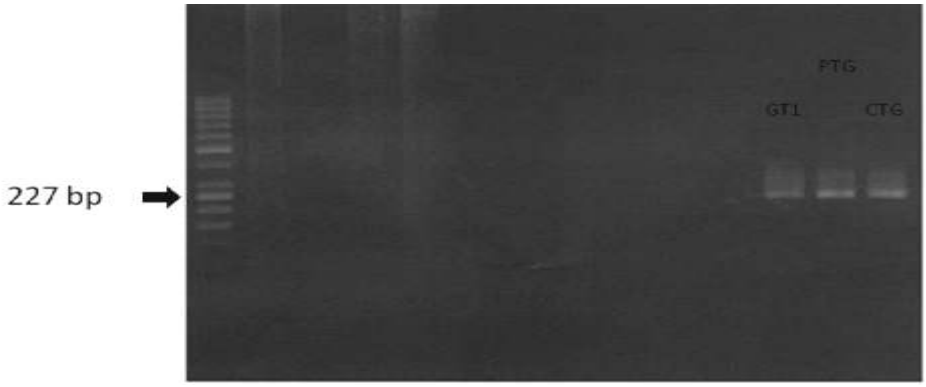

Figure 1.Gel electrophoresis of products amplified by single tube nested PCR from caprine and ovine fetal tissues. Lane1, 50kb molecular size marker. Lane 2-7 negative (2- brain tissue from caprine, 3- brain tissue fro ovines 4- placenta from caprine 5- placenta from ovine 6- heart from ovine ,7- heart from caprine). Lane 8, Negative control, Lane 9-11 positive control (9-GT1, 10- PTG , 11-CTG).

Of the 76 fetuses submitted, 20 (28.6%) were in first trimester, 47 (61.8%) in second trimester and 9 (11.8%) in third trimester of gestation. Some tissues like brain, heart, liver, lung and placenta tissues collected from aborted ovine and caprine fetuses were analyzed by nested PCR for the presence of *T.gondii* DNA. A total of 327 tissues being 194 from caprine and133 from ovine fetuses respectively were analyzed. All the tissues examined were negative for *T.gondii* DNA by single tube nested PCR (Fig.1) as none of the samples showed amplification.

Discussion

The aim of the present study was to determine the role of *T.gondii* in small ruminant abortion in Borno State, Nigeria. Using the single tube nested PCR targeting the multicopy ITS1 gene described by Hurtado et al., (2001); we were unable to detect *T.gondii* DNA by amplification at 227kb in any of the 194 caprine tissues from 45 fetuses and 133 ovine tissues from 31 fetuses (Fig. 1). Although the role of *T.gondii* in small ruminant abortion has been reported globally (Masala et. Al., 2003, Pereira-Bueno et al., 2004), our finding suggested that the organism does not play significant role in caprine and ovine abortion in the study area. A serological survey for anti *Toxoplasma gondii* antibodies in sheep and goats by ELISA in the study area by the author (Kamani et.al., 2010) shows a low seroprevalence of 6.7% and 4.6% respectively, compared to reports from other parts of the world (Pereira-Bueno et al., 2004, Bisson et al., 2000, Klun et al., 2006, Sharif et al., 2006) and the estimated global average of 31% (Fayer, 1981). The single tube nested format applied in this work minimizes the chances of PCR contamination and since it targets the multicopy ITS1 gene which is usually present in a high copy number (110 copies), the detection power of *T. gondii* DNA increases making it a highly sensitive method. This is particularly important when dealing with clinical or partially putrefied samples which is the case with most of the samples analyzed in this study. We were surprised by these finding considering the fact that the feline definitive host abound in the study area. One would expect a high seroprevalence as well as widespread *T.gondii* DNA in aborted tissues. The high ambient temperature experienced in the area most part of the year may be responsible for the low prevalence of the disease (Sharif et al.,2006) hence its minimal role in caprine and ovine abortion.

Our next plan is to isolate *T.gondii* DNA from cats and rodents in the study area, which will be sequenced and compared with known *T.gondii* clades.

Acknowledgements

We are grateful to the Executive Director, NVRI Vom for supporting this work. Wish to thank the following Dinchi T.A, Pauline D, Henry N. I and Dr Owoludu J for their technical assistance during the work. The positive controls were kindly provided by Prof C.Su, his effort in editing the draft manuscript is also highly appreciated.

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