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Abortion Due to Toxoplasmosis in Small Ruminants

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Abstract: The present study was carried out on a flock of sheep and goats suffered from late abortion with incidences of 35.6 and 43.7%, respectively. Toxoplasmosis was a prime suspect. Blood samples were taken from infected dams for serological examination. Tissue samples were collected from internal organs of aborted feti for bacteriological examination, PCR and histopathological study. Serological examinations of all aborted ewes and does revealed positive reaction for *Toxoplasma gondii* infection. A nested PCR assay yielded a 94 bp amplification product consistent with *T. gondii*. Myocarditis, non suppurative encephalitis, hepatitis and diffuse interstitial pneumonia are the most predominant histopathological changes in aborted feti. There were numerous clusters of dark purple banana-shape of *T. gondii* within the cardiac tissue in most of examined cases. It could be concluded that demonstration of the parasite associated with the characteristic histopathological changes in aborted fetal organs and using of nested PCR on paraffin embedded tissues are of great importance in diagnosis of ovine and caprine toxoplasmic abortion.

Key words: *Toxoplasma gondii* · Abortion · Sheep · Goats · Nested PCR · Histopathology

INTRODUCTION

Toxoplasmosis is one of the main causes of infectious reproductive failure in small ruminants in the world. It causes fetal resorption, abortion, stillbirth and neonatal mortalities resulting in great economic losses [1,2]. In Egypt, serological surveys indicated that the incidence of infection averaged 9.4and 6.9% (Direct agglutination Test) in sheep and goats, respectively [3] and 31.08% (indirect ELISA) in sheep [4].

The main histopathological changes associated with *T. gondii* infection were recorded in the placenta, brain and heart of fetus [5-9].

Currently, diagnosis of abortion due to *T.gondii* is based on detection of specific antibodies in fetal fluids by indirect fluorescent antibody technique (IFAT) or latex agglutination test (LAT) [10,11] and demonstration of parasite in tissues by immunohistochemistry techniques [6]. Also, the identification of characteristic histopathological picture in the placenta and the brain of fetus are indicative for toxoplasmosis [5]. Recently, methods of molecular biology have been increasingly used in diagnosis. Several PCR-based assays targeting genes have been developed for detection of *T.gondii*: the B1 repetitive sequence (30copies) [12]; the P30 surface antigen (single copy) [13]; the ribosomal RNA (110copies), both the small subunit rRNA gene [14] and the 529bp DNA fragment [15]. At the present, PCR has been shown to be useful and reliable confirmative technique for diagnosis of *T.gondii* infection in ovine and caprine placenta and aborted fetuses[15-18] this investigation was done to confirm wether toxoplasmosis is the etiological cause of abortion in sheep and goat flock by using serological tests, PCR and pathological examination.

MATERIAL AND METHODS

The present study was carried out on small flock of Baladi sheep and goats. Animals were reared in private farm located in Abou-Rawash, Giza governorate, Egypt. The incidence of late abortion was 35.6 and 43.7 % for sheep and goats, respectively, during the breeding season. Animals had previous case history of reproductive disorders including pyometra, abortions, stillbirths and births of weak lambs and kids. Several cats were observed moving in and out of the farm.

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Samples Collection:

- Blood samples were collected from ewes and does just after abortion. Sera were separated and stored-20°C until used for serological investigations.
- Tissue samples were taken randomly from 12 aborted feti (8 sheep and 4 goats). These samples were used for bacteriological and pathological studies as well as PCR assay.

Bacteriological Examination: Tissue specimens from internal organs of individual aborted feti were randomly collected after abortion under aseptic conditions and submitted to the laboratory. Swabs from the tissue specimens were inoculated onto blood agar, nutrient agar, brain heart infusion agar, MacConkey agar, Brucella agar, SS agar, Skirrow media [20] and Sabouroud dextrose agar media at 37°C for 24-48 hours. Isolation and identification of growing colonies were carried out according to [21,22].

Serological Examination: Sera of aborted ewes and does were examined for presence of specific antibodies against abortifacient pathogens: *Brucella* spp by using Rose Bengal Plate Agglutination test and Rivanol test [23]; *Salmonella* by using commercial SAS febrile antigen [24]; *Chlamydia psittaci* by using Complement Fixation test [25] and *Toxoplasma gondii* by using SAS *toxoplasma* latex kit [26].

Polymerase Chain Reaction (PCR) Assay

A-Detection of DNA *T.gondii:* Paraffin embedded tissue specimens from internal organs of aborted feti were used for nested PCR assay. Samples were treated with xylene followed by ethanol and washed with PBS. DNA was extracted by using commercial kits (Dneasy Tissue Kite from QIAGEN, Basel, Switzerland). Detection of *T.gondii* DNA was done according to [12]. Two external primers were initially used.

B1 5′-GGAACTGCATCCGTTCATGAG-3′
B2 5′-CTTTAAAGCGTTCGTGGTC-3′

The first amplified PCR product was followed by a second round of amplification using a set of internal primers.

B3 5′-TGCATAGGTTGCAGTCACTG-3′
B4 5′-GGCGACCAATCTGCGAATACACC-3′

The PCR amplified products were visualized by electrophoresis on agarose gel 1.5% stained with ethidium bromide. Distilled water was used as negative control and *T.gondii* was used as positive control.

B-Detection of DNA *Neospora caninum.*

For possible role of *Neospora caninum* as an etiological agent of abortion in sheep and goat, paraffin embedded tissue specimens from internal organs of aborted feti were also subjected to PCR analysis. Detection of DNA of *N. caninum* (amplified product is 270 bp) was done by using a set of external primers according to [27].

NS2f 5′-CATGTGATTTTGCA-3′
NR1r 5′-AAATAACGGGTGGGAAAA-3′

The PCR amplified products were visualized by electrophoresis on agarose gel 1.5% stained with ethidium bromide.

Histopathological Study: Tissue samples were taken from internal organs of aborted feti and were fixed in 10% neutral buffered formalin. The fixed specimens were washed, dehydrated and embedded in paraffin wax. The tissues were sectioned at 4-5 thickness and stained with haematoxylin and eosin (H and E) as routine work for histopathological examinations according to [28]. Some tissue sections were stained with special stains as: Periodic acid Schiff (PAS), Giemsa stain, Modified Ziehl Nelson stain, Von Kossa stain [29] and Gimenez stain as special stain for *Chlamydia psittaci* according to[28].

RESULTS

Clinical Symptoms: Animals in this flock showed abortion in the late stage of gestation during the breeding season with incidence of 35.6 and 43.7 % for sheep and goats, respectively. The aborted ewes showed dyspnea and mild nervous symptoms manifested by unsteady gaits. Meanwhile aborted does expressed signs of anorexia and diarrhea just before abortion. Few cases were died after abortion due to secondary bacterial infection.

Bacteriological Isolation: The isolated microorganisms from the internal organs of aborted feti were *Staphylococcus aureus (*in five cases), *Salmonella* spp. (five cases), *Corynebacterium pyogen*es (four cases),

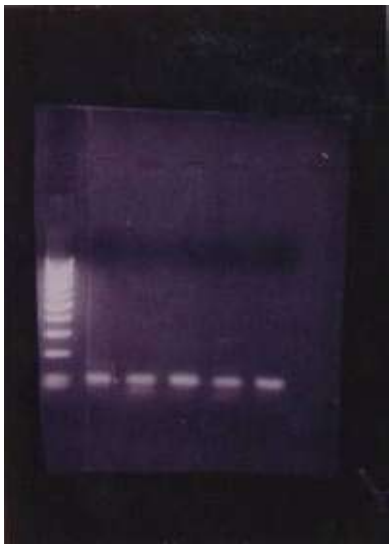

Fig. 1: Showing electrophoretic pattern of second PCR product (94bp) on 1.5% agarose gel stained with ethidium bromide. Lane 1: DNA marker (100 base-pair), Lane2: positive control (*T. gondii* DNA), Lanes 3, 4, 5 and 6: amplified *T. gondii* DNA (94 bp) in aborted fetal tissue samples. Lane7: negative control.

*Streptococcus pyogene*s(four cases) and *anthracoids* contaminants (five cases). More than one organism was isolated from each case.

Serological Examination: All serum samples of aborted ewes and does revealed positive reaction to *T. gondii* antibodies titre. Other performed serology tests for *Brucella, Salmonella and Chlamydia* proved negative results.

PCR Assay: The PCR assay using the external primers yielded an amplified product of 193 bp whereas the second PCR with the nested primers produced a product of 94 bp (Fig.1). At the same time, no detectable amplified products were seen using primers for *Neospora caninum*.

Pathological Findings: Postmortem examination of late gestation stage aborted feti, revealed generalized subcutaneous edema associated with accumulation of moderate quantities of blood-tinged fluids in the thoracic and abdominal cavities. Most of aborted feti revealed enlarged, congested and friable liver with rounded borders. Moreover, the lungs of aborted cases were congested and showed pneumonia with hemorrhages. Microscopically, liver showed irregular multiple necrotic foci scattered throughout the hepatic lobules associated with hepatic cords dissociation. Van Kupffer cells showed hyperactivity. Severe congestion of hepatic sinusoids was also seen. The portal areas were greatly infiltrated with lymphocytes. Hyperplasia of biliary epithelium was evident. The most prominent renal lesions are focal interstitial aggregations of mononuclear cells mainly lymphocytes. The renal tissue revealed severe focal necrosis and desquamation of the epithelial lining of the most renal tubules. There were dilatation and congestion of renal blood vessels and glomerular tufts. Mononuclear inflammatory cells were infiltrated in the interstitial tissues in the peritubular and periglomerular areas. Lungs showed congestion of pulmonary blood vessels associated with severe sub pleural hemorrhage. The lung in many cases showed diffuse interstitial pneumonia. Concerning, the alveolar wall, there were hyperplasia and metaplasia of the epithelial lining of the alveoli with the accumulation of large mononuclear cells and some leukocytes in the

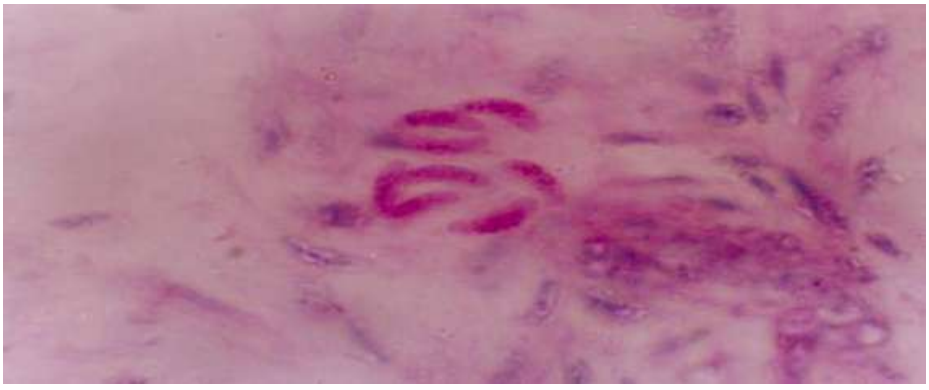

Fig. 2: Heart of aborted caprine fetus showing presence of dark purple tachyzoites in between the muscle fibers. [PAS stain, X400]

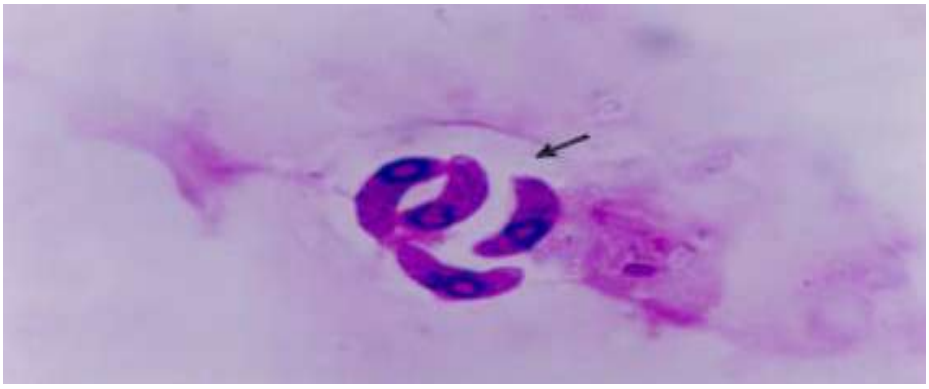

Fig. 3: Heart of aborted caprine fetus showing clusters of dark purple banana-shaped tachyzoites (arrow) with apical nucleus and granulated cytoplasm. [PAS stain, X1000].

alveolar lumen. The heart showed edema of the interstitial tissue with intermuscular focal areas of mononuclear cells infiltrations mainly lymphocytes. Sections of the heart of aborted feti stained with PAS showed several numbers of tachyzoites, which were dark purple banana-shaped with apical nucleus and granular cytoplasm in between cardiac muscles (Fig. 2 and 3). Serous pericarditis was also seen in few cases. There is lymphocytic encephalitis, specially submeningealy. Accumulation of lymphocytes in Virchow-Robin spaces was also a common finding besides to focal gliosis, edema with severe dilatation and congestion of both meningeal and cerebral blood vessels.

DISCUSSION

The present reported rate of abortion (35.6 and 43.7% in goats and sheep, respectively) due to *T. gondii* infection represents a serious impact on the fertility and overall production of the involved animals. It was reported abortion percentage due to toxoplasmosis may vary from 5 to 100% due to ewes immunity, time of exposure and dose of oocysts [30]. Fetal death is likely caused by the multiplication of *T. gondii* in the placenta rather than invasion of the fetus by the parasite[7]. When infection is initiated in the placentomes, parasite multiplication causes foci of placental necrosis and inflammatory reactions which expand throughout the remainder of gestation until abortion or birth [5] The continued multiplication of the parasite in the placenta and fetus is probably due local suppression of immune mechanisms in the maternal placenta and to immaturity of the fetal immune system [5,31]. It was reported that, the ovine fetus become capable of mounting an immune response at around 60 days of gestation but the immunocompetence is not sufficient to confer protection until the last month before birth [5] Thus, infection prior to 40 days of gestation causes embryonic death and fetal resorption, while infection between 40 and 120 results in fetal maceration, mummification or abortion and infection after 120 days produces stillbirth or birth of weak or healthy lambs [32]. Moreover, it has been found that the inflammatory reactions induced in the placenta by *T. gondii* are capable of stimulating synthesis and release of prostaglandin $F_{2\acute{a}}$ which has a luteolytic action leading to decreased progesterone level and subsequent abortion [33,34].

Myocarditis, interstitial nephritis, non suppurative encephalitis, hepatitis and diffuse interstitial pneumonia were the most predominant histopathological changes of aborted feti. Similar findings were reported in experimentally induced and naturally occurring toxoplasmosis in fetal lambs and kids by [7,8,31,36,37]. Occurrence of such changes and identification of the parasite in fetal heart indicate transplacental infection. Placenta, brain and heart are the most commonly affected organs in *Toxoplama* induced abortion in sheep and goat [7,38]. It was reported that necrotic foci followed by mononuclear inflammatory reactions are the most prominent lesions of toxoplasmosis in organs heavily infected with tachyzoites and this appears to be directly related to the rapid intracellular replication of the parasite, with no evidence that *T.gondii* produce toxins [39]. The ability of *T. gondii* to survive in the intracellular environment is apparently due to failure of fusion of lysosomal membrane with membrane of parasitophorous vacuole [39] and that could be due absence of host protein markers in parasitophorous vacuole resulted from its unique non fusigenic properties[40].

In the present study nested PCR assay was done on paraffin embedded aborted fetal tissues in order to increase the sensitivity and specificity of the reaction minimizing the chance of contamination. All the examined samples gave the expected amplification products specific for *T. gondii* (94 bp) and were consistent with *T.gondii* associated histopathological changes and serological results. Similar results were recorded by [16-19]. Nested PCR was used with two pairs of primers to amplify B1 gene which is highly conserved within a species and has high copy number (35) in genome of *T. gondii*, since the sensitivity of PCR depends on the copy number of the gene amplified [12,41]. By using PCR; it can easily detect the parasite in poorly stored tissues or in contaminated samples which are unsuitable for mouse inoculation [16]. Also, it was found that PCR has the advantage over serology in its ability to diagnose *T.gondii* infection at early stages of gestation when the fetus is not yet immunocompetent or, in lambs that have taken colostrum [17].

It could be concluded that demonstration of the parasite associated with the characteristic histopathological changes in aborted fetal organs and using of nested PCR on paraffin embedded tissues are of great importance in diagnosis of ovine and caprine toxoplasmic abortion.

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