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Original article

Serological and molecular studies of ovine and human toxoplasmosis with a trial of treatment of infected ewes

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ABSTRACT

The aims of the present study were to diagnose toxoplasmosis in pregnant ewes and women serologically and molecularly, treat naturally infected ewes, and diagnose congenital toxoplasmosis. Blood samples were taken from 30 and 60 pregnant ewes and women, respectively, and used for diagnosis of toxoplasmosis through Latex agglutination test (LAT). Seropositive samples were confirmed by PCR for detection of acute infection. Ten infected pregnant ewes were classified into two groups. The first group was treated with sulfadimidine 33.3%, 200 mg (0.6 ml) / kg.b.wt, and the other group was treated with normal saline. At titers ≥ 1:64, serological diagnosis indicated that 16 (53.33%) ewes and 29 (48.3%) women were seropositive and the seroprevalence increased in older ewe and younger women. Positive LAT samples were used for amplification of DNA and showed bands at 193 bp, analogues to that of the RH strain in 12 (40%) and 15 (25%) blood samples of ewes and women, respectively. All treated ewes with sulfadimidine 33.3% delivered healthy lambs with normal gestation period, whereas untreated ewes delivered 4 abortuses and 3 stillbirths. The tissue cysts were demonstrated microscopically in stained smears from tissues of dead fetuses. Local strain of *T. gondii* was isolated through intra peritoneal injection in mice from tissues of abortuses and stillbirths and maintained in the lab. The DNA of both RH (a virulent

strain) and the local strains expressed diagnostic amplified DNA bands at 193 bp, indicating a zoonotic importance of *T. gondii* and the role of sheep a as source of human infection.

1. Introduction

Toxoplasmosis is an important zoonotic disease caused by the intracellular protozoan parasite, *Toxoplasma gondii* (Family: Sarcocystidae). Felidae species are final hosts, whereas an array of warm blooded animals, including humans and cats serve as intermediate hosts. *T. gondii* infection is established by rapid multiplication of the tachyzoite stage of the parasite. During the chronic phase of toxoplasmosis, intermediate hosts may harbor tissue cysts in the brain and other tissues causing chronic infection that persists for the lifetime of the host (Dubey, 2010). A number of reports focused on outbreaks of food borne toxoplasmosis as a result of ingestion of hamburger, sausage, and grilled meat, containing infective tissue cysts (Kijlstra and Jongert, 2008; CDC, 2011).

The risk factors associated with infection of pregnant women were soil contact and eating raw or undercooked meat (Nimri et al., 2004). Sheep are important in the epidemiology of *T. gondii* infection worldwide (Buxton et al, 2007). Seropositive sheep can be assumed to harbor large numbers of tissue cysts in their meat (Dubey and Jones, 2008). Transmission of *T. gondii* tachyzoites in unpasteurized sheep or goat milk also may occur (Tenter et al., 2001). Under laboratory conditions, cats can shed as many as 500 million oocysts after ingesting one *T. gondii*-infected mouse (Dubey and Frenkel, 1972). Millions of oocysts were shed by cats fed even a few bradyzoites (Dubey, 2001). Intermediate hosts get also infected through accidental ingestion of oocysts, shed by cats, from the environment. Transplacental infection occurs in case of sheep, goat, and humans (Edward and Dubey 2013; Ramadan et al., 2007; Hill and Dubey, 2002).

It is estimated that one third of people around the world have been exposed to *T. gondii* during some point of their lives (Dubey, 2010). When women infected during the first trimester, the result is abortion, stillbirth or severe disease of fetus (Lin et al., 2000). The severity of disease decrease if the infection occurs in the second or third trimester, but the risk for transmission from mother to fetus increases (Romand et al., 2001). It can cause blindness, mental retardation in congenitally infected children (Hill and Dubey, 2002). Toxoplasmosis is a leading cause of death following a severe systemic infection, especially in AIDS patients and those receiving an immunosuppressive therapy (Haberkorn, 1996). The cost of illness in the US caused by *Toxoplasma* has been estimated to be nearly US $3 billion and an annual loss of 11,000 quality-adjusted life year (QALY) (Batz et al., 2012). Recent publications have linked suicide and schizophrenia to *Toxoplasma* infection (Torrey et al., 2012; Webster et al., 2013).

Infection of sheep poses a risk to public health, as well as economic losses due to reproductive failure (Edward and Dubey, 2013). Immunosuppressed sheep may also develop a nervous form of the disease (Mobini et al., 2002) as it is well known that *T. gondii*- tissue cysts are reactivated when the host immune system is weaken (Dubey, 2010). Toxoplasmosis is generally detected by serological examinations and also by direct microscopic examination, mouse inoculation, and PCR (Mazumder et al., 1988; Dubey, 2009, 2010; Ramadan et al., 2007). The agglutination of latex particles detects small quantities of an antibody or antigen in a fluid test sample. Some advantages of this assay are that the procedures are simple, widely applicable, speedy, and nonhazardous (Molina-Boli´Var and Galisteo-Gonza´ Lez, 2005). Molecular assays such as PCR make it possible to detect small quantities of target DNA and potentially provide an alternative sensitive diagnostic tool (Habibi et al., 2012; Moazeni Jula et al., 2013; Tavassoli et al., 2013). B1 gene is the most sensitive and widely used target for detecting both tachyzoite and cysts in all (21) strains of *T. gondii* (Bastein, 2002).

Early diagnosis of infection is of great consequence for reducing the severity of the disease and the risk of congenital toxoplasmosis (Behbehani and Al-Karmi, 1980; Edward and Dubey, 2013). Administration of suitable treatment is crucial to stop abortion and to present normal birth with satisfactory clinical and laboratory response. Although ovine toxoplasmosis was reported worldwide (Dubey, 2009), few studies have been conducted about its prevalence in Egypt (El Ridi et al., 1990; Shaapan et al., 2008; Shaapan and Fathia Khalil, 2008), treatment of pregnant ewes, and the role of *T. gondii* in ovine abortion; therefore, the aims of the present study were to

diagnose toxoplasmosis in pregnant ewes and women serologically and molecularly, treat naturally infected ewes, and diagnose congenital toxoplasmosis.

2. Materials and methods

2.1. Animals

A sheep flock (100 heads) in a private farm in Toukh, Qalyubia Governorate, Egypt, had a history of abortion according to the owner, of which pregnant ewes (1-3 years old, 8-17 weeks of gestation) were used for diagnosis of toxoplasmosis. For chemotherapy, ten ewes (1-3 years old and 8-10 weeks of gestation) were used and kept indoors in two separate holdings throughout the studying period, received a balanced ration and clean source of water, and protected from cats and pests. The animal rooms were cleaned periodically with appropriate detergents and disinfectants. Bedding was removed and replaced with fresh materials as often as necessary to keep the animals clean and dry. The animals were fed palatable, non-contaminated, and nutritionally adequate food. Food and water were available *ad labitum.*

2.1.1. Blood samplings

Two types of blood samples were collected from jugular veins of 30 pregnant ewes and collected from brachial veins of 60 pregnant women (21-41 years old, 8-32 weeks of gestation and admitted to the Gynecological and Obstetric Department at Toukh and Benha Hospitals, Qalyubia Governorate, Egypt). The first type of blood samples were collected in tubes and left few minutes to obtain serum for serologic evidence of *Toxoplasma* antibodies. The other type of blood samples were collected in sterile tubes with EDTA for amplification by PCR. The collected sera and blood samples were coded and preserved at -20 °C till used.

2.2. Latex agglutination test

Sheep sera were tested for *T. gondii* antibodies using a Latex agglutination test (LAT), (Sigma Scientific Services Co., Cairo, Egypt) as described by Zaki (1995). The results for LAT were interpreted as agglutination 1:16 was indicative of no immunity to toxoplasmosis; whereas a titer of ≥ 1:64 was considered indicative of *T. gondii* seropositivity to acquired toxoplasmosis or evolving immunity (i.e. 1: 64 is the cut- off titer).

2.3. Polymerase chain reaction (PCR)

Positive LAT blood samples were confirmed by PCR. Each 5 ml of blood samples with EDTA was suspended in 10µL of Tris-EDTA buffer (PH 7.5; 10mM Tris- HCL, 1mM EDTA) and stored at -20 °C until PCR protocol was performed.

2.3.1. *Toxoplasma gondii* RH strain

RH strain is a virulent RH strain of *T. gondii*, maintained at the Department of Zoonoses, National Research Center, Egypt, was used as a positive control DNA for PCR.

2.3.2. DNA extraction

Genomic DNA was extracted from blood samples of the positive reactors against *T. gondii* antigen and from *T. gondii s*train RH which used for bioassay standardization and as positive control of PCR assays (Dubey et al., 1999), using a commercially available kit, QIAamp DNA, blood and tissue kit (Qiagen, Hilden, Germany). DNA extraction and purification protocol was recommended by the manufacturer.

2.3.3. Primer selection

PCR was performed to amplify *T. gondii* by using primers 1 and 4 described by Burg *et al.* (1989), but modified by one nucleotide to avoid primer dimer amplification and false results, according to Van de Ven et al. (1991). The primers are located on the B1 gene. This gene is about 35-fold repetitive. The primers were manufactured by Sigma, Co., Egypt. The primer 1 (5`- TTG CAT AGG TTG CAG TCA CT-`3) (position 694 to 714) and primer 4 (5`-TCT TTA AAG CGT TCG TGG TC-`3) (positions 868 to 888).

2.3.4. PCR technique

PCR amplification was carried out in 25 µL containing 2µLRH strain DNA sample (positive control) or 5µL of blood DNA sample,1µLof each primer (100 pmol), and 12.5µL PCR Master Mix (Jena Bioscience Co. Jena, Germany). Thermal profiles were performed in thermal cycler (T-Biometra). Thermocycler condition included one min of denaturation at 95 °C, followed by 39 cycles of two seconds of denaturation at 94 °C, 5 seconds of annealing at 48 °C and 25 seconds of primer extension at 72 °C and final extension step of 72 °C for 10 min. A 10 µL aliquot of the amplified product was analyzed on 1.5 % agarose gel and stained with ethidium bromide and visulized under a transilluminator with a 100 bp DNA ladder (Fermentas, Canada). Every PCR run included positive and negative controls. The product length of the positive result was indicative at 193 bp.

2.4. Chemotherapy

Once diagnosis of toxoplasmosis with both LAT and PCR was confirmed, ten infected pregnant ewes were classified into two groups (5 animals each). The first group was treated with sulfadimidine 33.3%, according to manufacture (ADWIA Co., 10th of Ramadan City, Egypt). Animals were injected, slowly intravenous, with 200 mg (0.6 ml)/ kg.b.wt. at the first day as an initial dose and subsequent maintenance dose with 100 mg/ kg.b.wt. for 4 successive days. The second group was considered as a control group and injected with normal saline. All abortuses and stillbirths were submitted for testing for toxoplasmosis.

2.5. Direct microscopic examination

Impression smears from the brains of the abortuses and stillbirths (3-4 smears for each sampled brain) were prepared according to the method described by Dubey (1996). A cerebral portion (enough to fill an area under 22 mm cover slip) was crashed between a glass slide and a cover slip and examined unstained under light microscope for tissue cysts at 1000× magnification. In addition, one half of the brain of each animal was homogenized with a manual glass homogenizer in 0.5 mL of normal saline. A small portion of the previously mentioned homogenate was spread on three slides and allowed to air dried. Samples were stained with Giemsa and examined for *T. gondii* tissue cysts at a magnification of 1000×, according to Giadinis et al. (2011).

2.6. Mice inoculation

The isolation of *T. gondii* local strain from suspected infected aborted lambs' tissues was carried out according to the procedure described by Dubey and Beattie (1988) by intra peritoneal injection of bioassay of tissues in albino mice. After one week of inoculation, the peritoneal exudates of the injected mice were examined microscopically for identification and isolation of tachyzoites (local strain).

The RH strain and the locally isolated strain of *T. gondii*, from abortuses and stillbirths, were maintained separately in the laboratory by serial passages in mice, according to the procedure cited by Johnson et al. (1979). Both strains, isolated from the peritoneal fluids of infected mice, were stored at -20 °C till used for the PCR technique as previously described.

3. Results

Serological diagnosis of toxoplasmosis through LAT indicated that 16 (53.33%) out of 30 pregnant ewes were seropositive at titers ≥ 1:64. The seroprevalence of *T. gondii* in pregnant ewes, in relation to age groups increased from 5 (38.5%) in in ewes younger than two years to 11 (64.7%) in ewes older than two years. The seroprevalence of *T. gondii* antibodies in pregnant women was 29 (48.3%) at titer ≥ 1:64. The seroprevalence of *T. gondii* antibodies decreased as the age increased form 19 (57.6%) to 10 (37%) (Table 1).

Blood samples of positive LAT were used for amplification of DNA and showed bands at 193 bp, analogues to that of the RH strain (Fig. 1) in 12 (40%) and 15 (25%) blood samples of ewes and women, respectively (Table 1).

All treated ewes with sulfadimidine 33.3% delivered healthy lambs with normal gestation periods, whereas the untreated ewes were febrile > 41 °C, in appetent, and delivered 4 abortuses and 3 stillbirths.

The tissue cysts were demonstrated microscopically in stained smears from tissues of abortuses and stillbirths. The local strain of *T. gondii* was isolated through intra peritoneal injection in mice from tissues of

abortuses and stillbirths and maintained in the laboratory. The DNA of both local and RH strains expressed diagnostic amplified DNA bands at 193 bp (Fig. 1).

4. Discussion

Infection with *T. gondii* during pregnancy may lead to severe complications, if the infection is not fatal to the fetus (Remington *et al.,* 1995). Therefore, emphasis is placed on preventive measures and early diagnosis for prevention of severe complications of infection.

Toxoplasmosis is wide spread in Egypt, 10.75% in cattle (Ibrahim et al., 2009); 15.7% in ducks (Dubey et al., 2003); 16.7% in camels and 30% in buffaloes (Khalifa et al., 2005); 35.42% in goats (Ramadan et at. 2007; 40.4% in chicken (Dubey et al., 2003); 52% in horses (Shaapan et al., 2012); and 65.6% in donkeys (El-Ghaysh, 1998). Serology is widely used in epidemiological surveys, such tests include: LAT, indirect haemagglutination test (IHAT), indirect immunofluorescence assay test (IFAT), and enzyme-linked immunosorbent assay (ELISA) (Dubey, 2009).

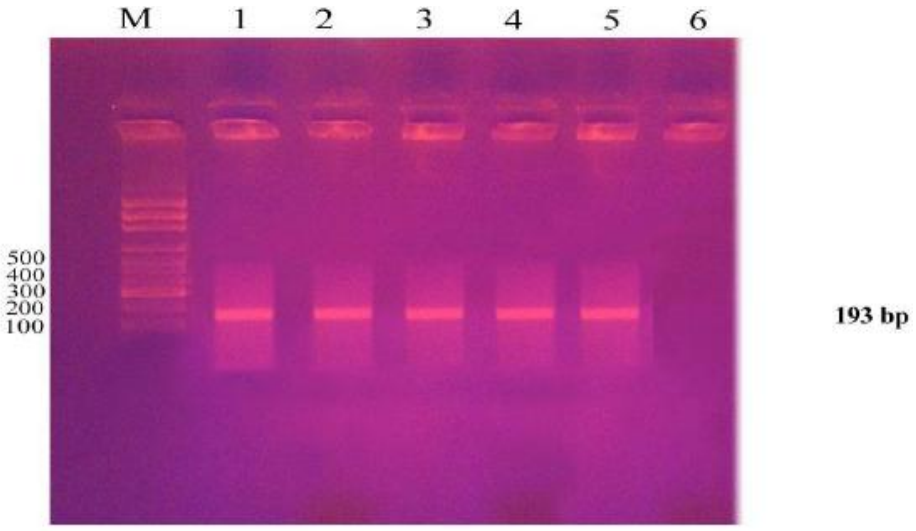

Fig. 1. PCR amplification for detection of *Toxoplasma gondii*. Lane M: a 100 bp molecular size marker. Lane 1: 193 bp product of DNA extracted from the RH strain of *T. gondii* (the positive control), Lane 2: blood sample of pregnant sheep, Lane 3: blood sample of a pregnant woman, Lane 4: blood sample of an aborted sheep, Lane 5: a local strain isolate from an aborted fetus, and Lane 6: the negative control.

Table 1
Prevalence of *Toxoplasma gondii* in the examined pregnant ewes and women in relation to age groups.

Age/ year	LAT							PCR	
	Negative			Titers					
	Total No.	No.	%	1:16	%	≥1:64	%	No.	%
Ewes									
1-2	13	4	30.8	4	30.8	5	38.5	3	23.1
2-3	17	4	23.5	2	11.8	11	64.7	9	52.9
Total	30	6	20.0	8	26.7	16	53.3	12	40.0
Women									
21-30	33	10.0	30.3	4	12.1	19	57.6	11	33.3
31-41	27	15.0	55.6	2	7.4	10	37.0	4	14.8
Total	60	25.0	41.7	6	10.0	29	48.3	15	25.0

LAT provides a rapid, specific, inexpensive and efficacious method for routine serological screening for antibodies to *T. gondii* (Mazumder et al., 1988). The agglutination reaction involves in vitro aggregation of

microscopic carrier particles (usually of polymeric nature, referred to as latex). This aggregation is mediated by the specific reaction between antibodies and antigens, one of which is immobilized on the surface of the latex particles to enhance the sensitivity and extend the point of equivalence (Molina-Boli´Var and Galisteo-Gonza´ Lez, 2005).

The present study indicated that out of 30 sera samples of ewes, 16 (53.3%) samples showed agglutination at dilutions ≥ 1:64 indicating acquired toxoplasmosis or evolving immunity against *T. gondii*. This seropositivity is in agreement with those reported in Egypt, 43.3%, 43.7%, and 50% (Khalifa et al., 2005; Shaapan et al., 2008; El-Ghaysh and Mansour 1994, respectively); Bulgaria, 48.2% (Prelezov et al., 2008); Turkey, 49.5% (Acici et al., 2008); Italy, 49.9% (Vesco et al., 2007); Jordan, 54% (Nimri et al., 2004); Scotland, 56.6% (Katzer et al., 2011); and Sudan, 57.5% (Khalil and Elrayah, 2011).

Our finding is higher than those reported elsewhere for ovine toxoplasmosis, 3 % in Pakistan (Zaki, 1995); 6.7% in Nigeria (Kamani et al., 2010); 20.6% in Jordan (Harps, 1993); 22.1% in Brazil (Andrade et al., 2013); 24.8% in Iran (Moazeni Jula et al., 2013); 27.6% in Morocco (Sawadogo et al., 2005); 29% and 39% in Egypt (El Ridi et al., 1990; Shaapan and Fathia Khalil, 2008, respectively); and 31% in Turkey (Oncel and Vural, 2006). Our result is lower than those recorded in different countries, 60% in Italy (Vitale et al., 2008); 95.7% in Turkey (Mor and Arslan, 2007); 92% in France (Cabannes et al., 1997); and 94.8% in USA (Edward and Dubey, 2013).

It has been reported here that older sheep had a higher prevalence of toxoplasmosis compared to younger sheep. Analogues finding was reported (Vesco et al., 2007; Katzer et al., 2011; Moazeni Jula et al., 2013). The age susceptibility suggested that most animals acquire infections post-natally (Dubey, 2009) due to prolonged exposure of sheep, reared outdoors, to infection due to heavy environmental contamination with oocysts shed form the observed stray cats in the farm. Up to 13 million *T. gondii* oocysts were present per gram of cat feces (Schares et al., 2008) and sporulated oocysts can survive in moist soil or sand for 12–18 months (Frenkel et al., 1975). Because the oocysts have an impermeable outer shell, they are resistant to many disinfectants (Wainwright et al., 2007). Moreover, human consumption of *T. gondii*- tissue cysts harboring undercooked or raw meat, meat derived products, or offals as well as contact with unprocessed meat are important routes of infection with human toxoplasmosis (El-Tras et al., 2011).

Serological studies show a considerable variation in the prevalence of human toxoplasmosis from 7.5 to 95% in different parts of the world, and indeed between different population groups within the same country (Asthana et al., 2006). Our results indicated that the seroprevalence of *T. gondii* among pregnant women was 48.3%, at dilutions ≥ 1:64. Similar seroprevalences were reported; 44.8% in Iran (Abdi et al., 2008); 51.49% in Dakahlia governorate, Egypt (Ibrahim et al., 2009); 54% in Jordan (Nimri et al., 2004); and 57.6% and 58.1% in Qualubia governorate, Egypt (Hussein et al., 2001, El-Gozamy et al., 2009, respectively). On the other hand, our finding is higher than those reported in Pakistan, 11.18% (Samad et al., 1997); in Egypt, 27.3% (Azab et al., 1993); in Iraq, 30.7% (Mohammad et al., 2012); and in Egypt, 39% (Aly et al., 2004). In addition, our result is much lower than those reported in Egypt, 80% (Khalifa et al., 2005) and in Kuwait, > 75 % (Behbehani and Al-Karmi, 1980).

Our data indicated that positive serum samples of women decreases with aging. The highest positive reaction detected at age group (21-30) years and decline after that. Similar observation had been recorded (Nimri et al., 2004; Khalifa et al., 2005). This age preference could be explained as most Egyptian women (age 20-31) seek treatment from toxoplasmosis before or after being subjected to abortion, and they are hygienically educated about the mode of transmission, which reduces infections after the age of 31- years old. In contrast to our results, positive serum samples rise with ageing (El Gozamy et al., 2009).

The differences of the prevalences of toxoplasmosis may be attributed to the breed susceptibility (Williams et al., 2005); age (Kamani et al., 2010; Andrade et al., 2013); sex, diagnostic techniques, and cut-off values used to determine seropositivity (Kamani et al., 2010; Moazeni Jula et al., 2013); breeding condition (Kamani et al., 2010); demographics of the populations (Kamani et al., 2010; Katzer et al., 2011; Andrade et al., 2013); using surface water as drinking water (Vesco et al., 2007); Andrade et al., 2013), farm size (Vesco et al., 2007 and management (Abu Samra et al., 2007); methods used for disposal of aborted fetuses (Abu-Dalbouh et al., 2012); geographic structure of *T. gondii* in relation to genetic variation (Lehmann et al., 2006), immune status, timing of infection, and genetic composition of the host and the organism (Suzuki, 2002); or distribution and behavior of cats (Abu-Dalbouh et al., 2012; Andrade et al., 2013).

PCR provides a simple and safe method for accurate and early diagnosis of toxoplasmosis (Tavassoli et al., 2009; Moazeni Jula et al., 2013). Seropositivity of *T. gondii*, in the present study, was confirmed by PCR technique which had been carried on samples of ewes, women, and the locally isolated strain and their DNA expressed

diagnostic amplified bands at 193bp when compared to that of the RH strain (positive control) indicating a zoonotic relationship.

Previous studies confirmed that PCR could actually detect *T. gondii* in blood specimens of women before or during pregnancy (Hussein et al., 2002).The clearance time for *Toxoplasma* DNA from the blood of patients with acute toxoplasmic lymphadenopathy was estimated to be 5.5−13 weeks (Guy and Joynson, 1995). Based on this, the presence of *Toxoplasma* DNA in the maternal blood most probably indicates a recent infection or an indicator of apparent parasitemia, which is likely to be clinically significant (Nimri et al., 2004; Iqbal and Khalid, 2007) for both ewes (40%) and women (25%), in the present study.

Burg et al. (1989) detected *T. gondii* DNA from a single tachyzoite using the B1 gene by PCR method for the first time. Several subsequent PCR tests have been developed using different gene targets. As a general rule, this technique has been recognized as a valuable method in diagnosis of clinical toxoplasmosis (Dubey, 2008). The B1 gene referred to as B1 repeat, is a 2214 base pair (bp) sequence that is repeated 35 times in the genome of *T. gondii* (Burg et al., 1989). Recently, B1-PCR has been shown as the most sensitive protocol for detection of infection with *T. gondii* (Tavassoli et al., 2013).

Although the B1 gene had a 35-fold repetitive sequence; this may be insufficient when compared with the targets used for the other microorganisms. To solve this problem, we used repeated copy numbers to avoid primer dimers, which may lead to insufficient sensitivity of the primer, according to Van de Ven et al. (1991).

Although some previous studies have reported the higher sensitivity of PCR targeting AF146527 over that of B1 gene which is frequently used for diagnosis of toxoplasmosis, a number of recent studies suggests that the AF146527 element was absent in 4.8% of human *T. gondii*- positive samples, this explains why the B1 PCR technique is the test of choice (Wahab et al., 2010; Menotti et al., 2010).

Since the discovery of *T. gondii* as an abortfacient in sheep in New Zealand by Hartley et al., (1954), toxoplasmosis associated abortions have been reported in many countries (Dubey and Beattie, 1988; Dubey, 2009). Reproductive failure due to toxoplasmosis, in the present study, resulted in infectious placentitis and subsequent abortion and stillbirth due to placental insufficiency (Owen et al., 1998). Accurate knowledge of acute *Toxoplasma* infections during pregnancy is needed for risk assessment of vertical transmission of infections as basis for counseling, prevention, and treatment (Vimercati et al., 2000).

Microscopic examination, mice inoculation, and PCR, in the present study, indicated that *T. gondii* infection is the major causative agents for abortion in ewes. Congenital toxoplasmosis had also been confirmed. Similar finding has been reported (Habibi et al., 2012; Edward and Dubey, 2013; Moazeni Jula et al., 2013).

Even though direct microscopic examination of aborted tissues is very helpful for preliminary diagnosis of congenital toxoplasmosis; accurate diagnosis needs more confirmatory tests. The isolation of the parasite in mice is considered the "gold standard," but the technique is cumbersome and time-consuming. Success of such technique is impacted by variable susceptibility of the mice, virulence of the infecting parasite, the route and dose of the inoculum as well as the content of potentially viable parasite (Derouin et al., 1989). An assay based on amplification of the BI target would be faster than xenodiagnosis and offer the potential for enhanced sensitivity by detection of nonviable as well as viable parasites (Burg et al., 1989).

The severity of infection is associated with the stage of pregnancy at which ewe becomes infected. Infection during the early stage of gestation can result in fetal death, resorption, and abortion, while infection in the latter stage of gestation (fetal immunity is relatively well developed), may have no clinical effect and lambs are usually born normal but infected and immune (Dubey and Beattie, 1988; Buxton et al., 2007; Edward and Dubey, 2013).

Although it is well known that toxoplasmosis can cause abortion only once in infected animals and sheep can be saved for future breeding, recently published papers have reported that repeated transplacental transmission of *T. gondii* in sheep may be more common than previously believed (Williams et al., 2005; Buxton et al, 2007; Hide et al., 2009) In endemic areas, the annual abortion rate is low, i.e. up to 2%. In animals that were previously non-infected, abortion rate from toxoplasmosis can be high, as it has been shown in experimental infections with *T. gondii* in seronegative flocks (Buxton et al., 1993, 1996). Abortion storms also could occur in sheep flocks (Giadinis et al., 2009; Edward and Dubey, 2013).

Toxoplasmosis causes considerable economic losses to sheep industry worldwide (Dubey and Beattie, 1988; Buxton et al., 2007) due to both the large numbers of the aborted fetuses as well as loss of milk production, as abortions took place at 110-130 days of pregnancy, when the udder was not yet developed for the following lactation period (Smokovitis, 1990). Toxoplasmosis could be controlled effectively with the use of live vaccines, applied before the mating period, although their use may have some disadvantages (Buxton and Rodger, 2007).

Vaccines cannot be used in flocks with pregnant sheep that abort in consequence of toxoplasmosis; in such cases, treatment of pregnant ewes is necessary to stop the occurrence of abortions.

From the already existing treatments, sulfadimidine is an easily available substance in the Egyptian market. Treatment of pregnant ewes with 33.3% sulfadimidine, in the present study, reduced the abortion rate and subsequently helped sheep to have a normal length of gestation and subsequent normal production. Analogous treatment with sulfadimidine had been recorded (Giadinis et al., 2011).

Several drugs were used to treat toxoplasmosis with good results such as decoquinate (Buxton et al., 1996; Kul et al., 2013), combination of pyrimethamine and sulfadimidine, vaquilopruim and sulfadimidine or trimethoprim and sulfadimidine (Buxton et al., 1993), toltrazuril (kul et al., 2013); and ponazuril (Mitchell et al., 2004). Studies have been conducted to control experimental infections with *T. gondii* in non-dairy sheep using monensin (Buxton et al., 1988); combination of sulfadimidine and pyrimethamine (Buxton et al., 1993); and combination of sulfadimidine and baquiloprim (Buxton and Rodger, 2007). Furthermore, clindamycin, spiramycin, atavaquone, arithromycin, clarithromycin, and dapsone have been used with various results in non-ruminant species and humans (Khalifa et al., 2005; Giadinis et al., 2009).

Sulfademidine 33.3%, in our previous work, successfully treated infections in pregnant does and prevent abortion (Ramadan et al., 2007). Botanically, the essential oil of *Thymus broussonetii* Boiss (Arabic names are za'itra,za'atar el-hamir and ra'atar essouiri, whereas the English name is broussonet thyme) was administered to mice at 20 µg/ animal orally at the time of infection with *T. gondii* and for several days thereafter. This resulted in total absence of intracerebral cysts in treated mice (Dahbi et al., 2010).

4. Conclusion

In conclusion, toxoplasmosis continues to be significant public health problem in Egypt. Accurate knowledge of acute toxoplasmosis during pregnancy is crucial for risk assessment of vertical transmission of infections as basis for counseling, prevention, and treatment. Therefore, this study highlights the need for a confirmatory test to detect primary acute toxoplasmosis in pregnant ewes and women. A single PCR- positive sample in conjugation with a LAT- positive test confirms a recent infection. Also, the high prevalence of *Toxoplasma* infection in sheep indicates that the risk of infection from sheep to human is high as consumption of grilled mutton is popular among Egyptians. Appropriate treatment in pregnant ewes with sulfadimidine is crucial to stop reproductive failure especially in unvaccinated sheep flocks. Further studies are needed to explore whether some sheep breeds have a particular genetic susceptibility to *T. gondii* and to detect predominant genotypes of the zoonotic parasite.

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