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RESEARCH ARTICLE

Ovine and Caprine Toxoplasmosis: Experimental Study

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ABSTRACT

Eighteen mature ewes of the Mytilene breed and 18 mature Local- Damascus crossbred goats, seronegative for *Toxoplasma gondii (T. gondii)* by ELISA were used. All animals were mated after synchronization of estrus. On day 90 of pregnancy, animals were randomly assigned to 3 experimental groups; 6 ewes (S1) and 6 goats (G1) were orally inoculated by stomach tube with 1000 oocysts; 6 ewes (S2) and 6 goats (G2) were orally inoculated with a non-infected control inoculum. On day 140±2 of pregnancy, the remaining 6 ewes (S3) and 6 goats (G3) were inoculated by stomach tube with 3000 oocysts. Positive *T. godii* DNA was detected in 94% of fetal and maternal blood, 95% fetal tissue, 89% pre-colostral udder secretions and 12.5% milk samples using Polymerase Chain Reaction (PCR). Infected animals and their live newborns was seropositive (ELISA) until the end of the study. PCR was able to detect *T. gondii* DNA in maternal blood of infected animals 3-5 days before abortion occurred. This time period may be used to implement preventive and therapeutic measure to reduce abortion rate and associated economic losses. Since milk and milk products are important food sources in rural areas and in many cases it is used unpasteurized before consumption. The *T. gondii* DNA, detected by PCR in milk samples of infected animals, increases the possibility that the parasite is transmitted through consumption of unpasteurized milk which is a highly relevant result for public health considerations and providing valuable information for future research.

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INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by the intracellular protozoan *Toxoplasma gondii*, infecting worm blooded animals including humans world-wide (Hill and Dubey, 2013). Seroprevalence of *T. gondii* in sheep has been established in many countries and varied from 5.6 80% (5.6% South Africa, Samra *et al.*, 2007**;** 19.88% Southern Pakistan, Mushtaq *et al*., 2010; 54% Southern Europe, Petersen, 2007 and 80% Brazil, Rossi *et al.*, 2011). Toxoplasmosis causes abortions, reproductive problems and economic losses to sheep and goats industry all over the world (Buxton *et al*., 2007; Shabbir *et al*., 2013). In sheep toxoplasmosis seems to be controlled effectively using live vaccines applied 3 weeks before mating season (Buxton and Innes, 1995).

In humans, it is estimated that one third of the world population is infected by this parasite (Hill and Dubey, 2013). The infection can occur congenitally or postnatally. Congenitally, humans become at risk for the development of a number of fetal abnormalities, such as hydrocephalus and blindness. Postnatally, humans become infected by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with sporulated oocysts from the environment (Cenci *et al.*, 2011; Dubey and Hill, 2012). The ingestion of undercooked meat from infected sheep and goats was considered as a source of infection for pregnant women in Southern Europe (Tenter, 2009). A recent study revealed that *T. gondii* DNA has been detected in milk of naturally infected sheep (Camossi *et al.*, 2011).

The objectives of this experiment were to study blood and milk profiles of experimentally infected pregnant

sheep and goats by *T. gondii* during various stages of the gestation and lactation periods, and their fetuses using serological tests and PCR, to establish the time needed for the parasite to be detected in the blood of experimentally infected sheep and goats using PCR, its association with the occurrence of abortion and to study the time interval in which the parasite persists and execrated in milk.

MATERIALS AND METHODS

Animals and sampling: This study was conducted from October, 2010 to August, 2011 in a commercial private farm, Aristotle University of Thessaloniki, Greece and was authorized by the Ministry of Agriculture, Animal Research Ethics Committee (Protocol # 13/2011). Eighteen mature ewes of Mytilene breed (3-4 year-old) and 18 mature Local-Damascus crossbred goats (3-4 year-old), seronegative for *T. gondii* by an ELISA were used in this study. Animals were mated after synchronization of estrus.

Pregnancy diagnosis was done by ultrasound scanning at day 70 of gestation and revealed that all animals were pregnant. On day 90 (88-92) of pregnancy, all ewes and goats were randomly assigned to 3 experimental groups; 6 ewes (S1) and 6 goats (G1) were orally inoculated by stomach tube with 1000 oocysts; 6 ewes (S2) and 6 goats (G2) were orally inoculated with a non-infected control inoculum (negative control group). On day 140 (138-142) of pregnancy, the remaining 6 ewes (S3) and 6 goats (G3) were inoculated by stomach tube with 3000 oocysts. These experimental groups were housed separately in 6 different pens. Animals were closely monitored, physically examined and rectal temperature was recorded twice daily for each animal from day 2 before inoculation to day15 post-inoculation. Blood samples were collected prior to inoculation and at regular intervals post-inoculation (next day and every other day for the first 2 week following infection and then on weekly basis for 2 consecutive months). Vaginal swabs, placental and different fetal tissues such as kidney, liver, lung, and brain were collected from freshly aborted fetuses during 24 hours after abortion. Colostrum and milk samples were collected (S3 and G3) at regular intervals from each lactating animal (first milk sample was collected immediately after parturition and every other day for the first 2 weeks after parturition and then on weekly basis for 2 consecutive months). At birth, each lamb was ear tagged, weighted and pre-colostral blood sample was collected.

Serology: Blood samples were collected by jugular venipuncture from experimental and new born animals, separated on the same day and serum was stored at -20°C. To detect *T. gondii* specific IgG antibodies, ELISA was performed for serum samples as described previously (Haralabidis, 1984) using soluble *T. gondii* antigen (from *in vivo* cultured parasites) in a final concentration of 3 µg/mL.

DNA Extraction and molecular detection: The DNA was extracted from samples using DNA purification kit (Promega, USA) and was performed from 1g pooled sample of different fetal tissues (placenta, spleen, liver, lung and brain) and from 300 µL of fluids (blood and milk). Fluids were centrifuged at 6000xg for 5 min, and pellets were incubated at 55°C for 1.5 hrs in solution containing 200 µL of lysis buffer, and 40 µL of proteinase K, before starting the extraction according to the kit manufacturer's instructions. The concentration and purity of the DNA was determined spectrophotometrically, methods and procedure used were published previously elsewhere (Abu-Dalbouh *et al.*, 2012).

Statistical analysis: Statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, USA) Group mean values of all the parameters for experimental and control groups were analyzed by ANOVA (one-way) test followed by LSD testing, student's t-test or Fisher's exact test, as appropriate, $P \leq 0.05$ was considered significant.

RESULTS

Clinical signs, lamb and kid weight: On day 3 after inoculation, infected animals showed an increase in their temperature, reached a peak of 41.6°C on day 5 and 6 in sheep groups, and 41.3°C on day 7 and 8 in goats groups. The mean temperatures of the infected sheep and goat groups were significantly higher ($P<0.01$) than the control groups. No significant differences in temperature were detected among infected sheep and goats groups or the control groups (mean temperature for sheep was 39.2 ± 0.6°C and for goats 39.1 ± 0.5°C).

After infection, one ewe and one goat expelled their fetuses on day 8 and another ewe on day 11 and all had mucoid vaginal discharge before abortion (Table 1). In addition, one infected ewe of (S1) aborted 2 mummified lambs on day 135 of pregnancy. The control sheep and goat groups had a significantly ($P<0.05$) longer mean gestation period than the infected groups (S1 and G1) respectively (the mean gestation period in the uninfected groups was 152±5,152±4 days for sheep and goats, respectively) put not with S3 and G3 groups. The mean live lamb and kids body weight of infected groups was significantly lighter ($P<0.05$) than those born to ewes or goats of the control of groups, respectively (Table 1).

Serology: A total of Four hundred seventy two blood samples were collected during the study period from experimental and new born animals and tested by ELISA. All infected animals started to show an increase in the antibody titers on day 14 after infection and continued to rise staidly until day 60 of infection and then started to decline. Infected animals and their live newborns remained seropositive by ELISA until the end of the study.

Molecular detection (PCR): Positive *T. gondii* DNA was detected in 95% (42/44) tissue samples collected from aborted fetuses (tissue sample of one mummified fetus was PCR negative) (Fig. 1). Positive *T. gondii* DNA was detected in 94% (160/170) of blood samples that were collected from infected animals and live newborn lambs and kids (Fig. 2). *T. gondii* DNA was detected in blood on the 3th day after infection in all infected animals and continued for 21 day after infection. PCR detected *T. gondii* DNA in maternal blood of infected animals 3-5

Table 1: Experimental design, number of oocysts of *T. gondii* Inoculated, number of aborted, live born, mummified and stillborn fetuses

Animal groups	Oocyst dose	Number of aborted fetuses	Number of mummified fetuses	Number of live born fetuses	Mean±SD Kg BW Live fetuses**	Number of stillbirths
Sheep 1 (S1)	1000	4(2)	2(1)	5	2.9±0.2^{a}	2
Sheep 2 (S2)	-			11	3.5±0.4^{ab}	
Sheep 3 (S3)	3000	4(2)	-	7	2.8±0.3^{b}	2
Goat 1 (G1)	1000	3(1)	-	6	2.3±0.1^{cd}	2
Goat 2 (G2)	-	-	-	14	2.9±0.2^{c}	
Goat 3 (G3)	3000	3(1)		9	2.8±0.3^{d}	3
Total 36		14(6)	2(1)	52		9

In each group there were 6 animals. S3 and G3 were inoculated with *T. gondii* oocysts on day 90±2 and on day 140±2 of pregnancy, respectively. S2 and G2 were negative control groups; Values in parenthesis indicate number of animals aborted; **Mean±SD within same species in a column bearing difference alphabet differ significantly (P<0.05).

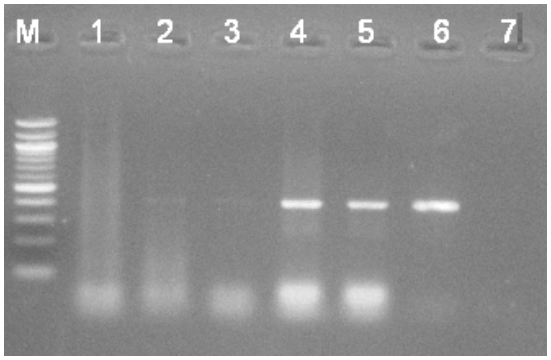

Fig. 1: Agarose gel electrophoresis of PCR-of Toxoplasmosis. The figure shows a single band, a 406-bp DNA fragment. Lanes: M, molecular 100 size ladder (in base pairs); 1-3: negative tissue samples; 4-5, Toxoplasmosis positive samples for fetal tissues; 6, positive control; 7, negative control.

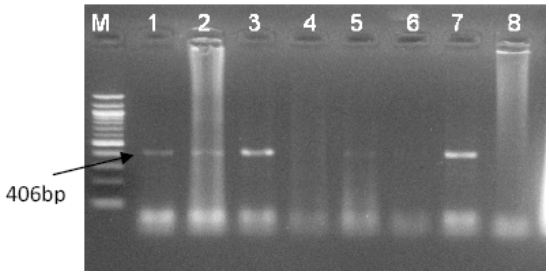

Fig. 2: Agarose gel electrophoresis of PCR-of Toxoplasmosis. The figure shows a single band, a 406-bp DNA fragment. Lanes: M, molecular 100 size ladder (in base pairs); 1-3: positive blood samples; 4-6, Toxoplasmosis negative samples for dams blood; 7, positive control; 8, negative control.

days before abortion occurred. Eighty eight percent (8/9) pre-colostral udder secretion and 12.5% (15/120) of colostral and milk samples collected during the 21 days period following the infection were PCR positive. No positive *T. gondii* DNA was detected in tissues, milk samples of the control groups or in milk samples that were obtained from infected animals after 28 days of infection.

DISCUSSION

Results revealed from this study showed that rectal temperature of all infected animals groups started to rise by day 3 and reached a mean maximum temperature of 41.5°C for sheep and 41.3°C for goats and retained to normal levels after day 11 after infection. These results were in agreement with results reported previously (AbouZeid *et al*., 2010; Gutierrez *et al*., 2010). Infection of sheep and goats on days 90±2 and 140±2 of gestation resulted in abortion of 6 animal expelled their fetuses on day 8-11 days after infection and had mucoid vaginal discharge before abortion. This result was concordant with Buxton *et al.* (1996).

The mean gestation period of the infected groups was shorter than the control and ewes and goats that completed their term of pregnancy produced viable and stillborn lambs and kids. Also, viable lambs and kids produced by infected ewes and goats were lighter than those born from uninfected ewes and goats. These results coincided with that reported by AbouZeid *et al*. (2010).

Infected ewes and goats and their live newborns carried detectable antibodies by ELISA until the end of the study. This result is in agreement with that reported previously for sheep (Edwards and Dubey, 2012) and for goats (Dubey, 2010) who reported that concentration of antibody may remain high until next breeding season.

Our results revealed that PCR gave some positive results in blood samples of infected animals as early as day 3 post infection and continued until 21 days after infection, and on 95% of the placenta and tissue samples of their aborted and mummified fetuses but gave negative results in control groups. These results agree with the report of AbouZeid *et al*. (2010). Other study showed that *T. gondii* DNA was not detected in the fetal and maternal blood of experimentally *T. gondii*-infected ewes (Gutierrez *et al*., 2010).

Toxoplasma gondii DNA was found in 23/129 pre-colostral, colostrum and milk samples obtained from the lactating infected animals. Related literature regarding the presence of the parasite or its DNA in experimentally infected ewes and goats is limited. However, recent study conducted by Camossi *et al.* (2011) revealed that *T. gondii* DNA was found in 7 milk samples from 5 seropositive naturally infected sheep. Also, Fusco *et al*. (2007) reported that 4 milk samples out of 117 examined by PCR were *T. gondii* DNA positive. Excretion of tachyzoites in milk of experimentally infected cats (Powell *et al*., 2001), and in naturally infected goats have been reported (Camossi *et al*., 2011). Also, tachyzoites have survived exposure to pepsin and trypsin (Dubey, 1998) and clinical toxoplasmosis has been linked to unpasteurized goat's milk (Skinner *et al*., 1990). A recent study revealed that consumption of unpasteurized goat's milk was considered as a positive risk factor for clinical toxoplasmosis in pregnant women in the USA (Jones *et al*., 2009) and in South-west of Iran (Fouladvand *et al*., 2010).

In general, positive PCR indicated the presence of *T. gondii* DNA in the milk but this is not enough to demonstrate the possible transmission of toxoplasmosis through milk ingestion, since the parasite may or may not

be alive. However, our obtained results provided important information for future research.

Conclusion: PCR was able to detect *T. gondii* DNA in the blood of experimentally infected sheep and goats 3-5 days before abortion occurred. This period of time could be used to implement preventive and therapeutic measure to reduce abortion rate and the associated economic losses. Since milk and milk products are important food sources in rural areas and in many cases it is used unpasteurized before consumption, the presence of *T. gondii* DNA, detected by the PCR in milk samples of infected animals, increases the possibility that the parasite is transmitted through consumption of unpasteurized milk which is a highly relevant result for public health considerations and providing valuable information for future research

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REFERENCES

Abu-Dalbouh MA, MM Ababneh, ND Giadinis and SQ Lafi, 2012. Ovine and caprine toxoplasmosis (*Toxoplasma gondii*) in aborted animals in Jordanian goat and sheep flocks. Trop Anim Health Prod, 44: 49-54.

AbouZeid NZ, Ha Amer, TM Barakat, AM Selim and FA Ei-Balkemey, 2010. Toxoplasmosis in naturally and experimentally infected goats. J Am Sci, 6: 122-129.

Buxton D and EA Innes, 1995. A commercial vaccine for ovine toxoplasmosis. Parasitology, 110: S11-S16.

Buxton D, SW Maley, SE Wright, S Rodger, P Bartley and EA Innes, 2007. *Toxoplasma gondii* and ovine toxoplasmosis: new aspects of an old story. Vet Parasitol, 149: 25-28.

Buxton D, S Wright, SW Maley, M Thomson, J Brebner and K Millard, 1996. Decoquinate and the control of experimental ovine toxoplasmosis. Vet Rec, 138: 434-436.

Cenci Goga BT, PV Rossitto, P Sechi, CME Mc Crindle and JS Cullor, 2011. Toxoplasma in animals, food and humans: an old parasite of new concern. Foodborne Pathog Dis, 8: 751-762.

Camossi LG, H Greca-Júnior, AP Corrêa, VB Richini-Pereira, RC Silva, AV Da Silva and H Langoni, 2011. Detection of *Toxoplasma gondii* DNA in the milk of naturally infected ewes. Vet Parasitol, 177: 256-261.

Dubey JP, 2010. Toxoplasmosis in Animals and Humans, 2nd Ed, CRC Press, Boca Raton, FL, pp: 1-313.

Dubey JR, 1998. Re-examination of resistance of *Toxoplasmosis gondii* tachyzpites and bradyzoits to pepsin and trysin digestion. Parasitology, 116: 43-50.

Dubey JP, DE Hill, DW Rozenboom , C Rajendran, S Choudhary, LR Ferreira, OC Kwok and S Su, 2012. High prevalence and genotypes of *Toxoplasma gondii* isolated from organic pigs in northern USA. Vet Parasitol, 188: 14-18.

Edwards JF and JP Dubey, 2012. *Toxoplasma gondii* abortion storm in sheep on a Texas farm and isolation of mouse virulent atypical genotype *T. gondii* from an aborted lamb from a chronically infected ewe. Vet Parasitol, 192: 129-136.

Fouladvand M, A Barazesh, B Naeimi, K Zandi and S Tajbakhsh, 2010. Seroprevalence of toxolasmosis in high school girls in Bushehr city, South-west of Iran, 2009. Afr J Microbiol Res, 4: 1117-1121.

Fusco G, L Rinaldi, A Guarino, YT Proroga, A Pesce, M de Giuseppina and G Cringoli, 2007. *Toxoplasma gondii* in sheep from the Campania region (Italy). Vet Parasitol, 149: 271-274.

Gutierrez J, J O'Donovan, E Williams, A Proctor, C Brady, PX Marques, S Worrall, JE Nally, M McElroy, H Basset, D Sammin, D Buxton, S Maley and BK Markey, 2010. Detection and quantification of *Toxoplasma gondii* in ovine maternal and foetal tissues from experimentally infected pregnant ewes using real-time PCR. Vet Parasitol, 172: 8-15.

Haralabidis S Th, 1984. The immunodiagnosis of the parasitic diseases and the immunoenzyme assay ELISA. Monography. Scientific Yearbook of the Veterinary Faculty of the Aristotle University of Thessaloniki, 22: 75-253.

Hill DE and JP Dubey, 2013. *Toxoplasma gondii* prevalence in farm animals in the United States. Int J Parasitol, 43: 107-113.

Jones JL, V Dargelas, J Roberts, C Press, JS Remington and JG Montoya, 2009. Risk factors for Toxoplasma gondii infection in the United States. Clin Infect Dis, 49: 878-884.

Mushtaq HL and T Zahid, 2010. Seroprevalence of toxoplasmosis in sheep in southern Punjab, Pakistan. Pak Vet J, 30: 91-94.

Petersen E, 2007. Toxoplasmosis. Semi Fetal Neonatal Med, 12: 214-223.

Powell CC, M Brewer and MR Lappin, 2001. Detection of *Toxoplasma gondii* in the milk of experimentally infected lactating cats. Vet Parasitol, 102: 29-33.

Rossi GF, DO Cabral, DP Ribeiro, AC Pajuaba, RR Corrêa, RQ Moreira, TW Mineo, JR Mineo and DA Silva, 2011. Evaluation of *Toxoplasma gondii* and *neospora caninum* infections in sheep from Uberlandia, minas gerais state, Brazil, by different serological methods. Vet Parasitol, 175: 252-259.

Samra NA, MC McCrindle, BL Penzhorn and B Cenci Goga, 2007. Seroprevalence of toxoplamosis in sheep in South Africa. J S Afr Vet Assoc, 78: 116-120.

Shabbir MZ, RK Khalid, DM Freitas, MT Javed, M Rabbani, T Yaqub, A Ahmad, MAB Shabbir and M Abbas, 2013. Serological evidence of selected abortifacients in a dairy herd with history of abortion. Pak Vet J, 33: 19-22.

Skinner LJ, AC Timperley, D Wightman, JMW (JM) Chatterton and DO Ho-Yen, 1990. Simultaneous diagnosis of toxoplasmosis in goats and goatowner's families. Scand J Infect Dis, 22: 359-361.

Tenter AM, 2009. *Toxoplasma gondii* in animals used for human consumption. Mem Inst Oswaldo Cruz, 104: 364-369.