Molecular detection of *Toxoplasma gondii* infection in aborted fetuses in sheep in Khorasan Razavi province, Iran

Danehchin, L.[1], Razmi, Gh.[2*], Naghibi, A.[3]

[1]*Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*

[2]*Center of Excellence in Ruminant Abortion and Neonatal Mortality, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*

[3]*Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*

Key words:
abortion, brain, nested-PCR, sheep, *Toxoplasma gondii*

Correspondence
Razmi, Gh.
Center of Excellence in Ruminant Abortion and Neonatal Mortality, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
Tel: +98(51) 38788944
Fax: +98(51) 38763853
Email: razmi@um.ac.ir

Received: 26 October 2016
Accepted: 14 January 2017

Abstract:

BACKGROUND: *Toxoplasma gondii* is a significant cause of abortion in sheep and goats in the world. Toxoplasmosis causes reproduction disorders such as fetal resorption, early embryonic death, mummification, abortion, stillbirth, neonatal and fetal death in sheep. **OBJECTIVES:** The aim of this study was to detect *T. gondii* infection in ovine aborted fetuses in Khorasan Razavi province. Methods: From June 2009 to July 2013, 112 brain samples of aborted ovine fetuses were collected and examined to detect *T.gondii* DNA by nested- PCR. The association of the frequency of *T.gondii* infection with age and geographical location of aborted fetuses was also studied. **RESULTS:** The results showed that 18 (16.07%) brain samples of aborted fetuses were Toxoplasm positive in PCR reaction. The frequency of *T.gondii* in the age group ≥120 days was more than other age groups of infected aborted fetuses ($p<0.05$). All the infected fetuses belonged to the sheep flocks in the northern part of the province. **CONCLUSIONS:** The results showed the moderate *T.gondii* infection among ovine aborted fetuses in the northern part of Khorasan Razavi province.

Introduction

Toxoplasma gondii was recognized for the first time as a significant cause of abortion in sheep in New Zealand and later in many countries (Buxton et al. 2007; Dubey, 2009). *T.gondii* infection during pregnancy can result in embryo/early fetal loss, fetal death and abortion/mummification and birth of weak lambs. (Buxton 1991; Scott 2007). This parasite is transmitted to sheep via ingesting food and water contaminated with oocysts which are excreted by cats or by other transmission rout from mother to fetus (Buxton et al. 2007). Furthermore, recent molecular studies showed that repeated transmission of *T. gondii* from dam to fetuses may be more common than previously observed in sheep (Duncanson et al. 200l; Morley et al. 2007; Hide et al. 2009).

The diagnosis of *T. gondii* infection is usually based on serological assay, histopathological examination and mouse bioassay. Many epidemiological studies have been carried out on ovine toxoplasmosis in different regions of Iran. The se-

roprevalence of ovine toxoplasmosis was reported between 13.8% -35% in different areas of Iran (Ghorbani et al. 1983; Rahbari&Razmi1992; Hashemi-Fesharki et al. 1996; Sharif et al. 2007). *T.gondii* infection was also detected in ovine fetuses using PCR and bioassay methods from Iran (Rassouli et al. 2011; Razmi et al. 2010; Zia-ali et al. 2007; Habibi et al. 2012). The aim of this study was to determine the frequency of Toxoplasma infection in aborted ovine fetuses in the province using molecular method.

Materials and Methods

The field of study: Khorasan Razavi province is located in northeastern Iran between 33°30′-37°41′ N latitude and 56°19′-61°18′ E longitude, with an area of more than 127,000 km2. The northern part of the province has a mountainous climate and suitable conditions for agricultural activity and animal husbandry. The southern part is mostly semi-desert and desert, with poor vegetation cover.

Sampling: From June 2009 to July 2013, one hundred-twelve aborted ovine fetuses were examined. The fetuses and fetal membranes were grossly examined for any macroscopic lesions and the age of the aborted fetuses was also determined by crown-rump length (Evans & Sack 1973).

The fetuses were necropsied and the brain samples were collected and stored at −20 °C for PCR analysis. The frozen fetal brain tissues were homogenized by mortar and pestle in Tris-HCl (pH 8•0) followed by treatment with proteinase K (Fermentas®, Lithuania) at a final concentration of 200 µgml. The phenol-chloroform and ethanol precipitation method was used for DNA extraction procedures. The purified DNA samples were resuspended in 20 µl of TE (10mM Tris and 1mM EDTA, pH8•0) and stored at −20 °C. DNA extracted from 10^6 particles of the RH strain of *T. gondii* was used as a source of positive control sample. DNA samples were tested by nested-PCR based on the amplification of B1 gene (35 copies per parasite) using 2 sets of oligonucleotide primers as was described by Burg et al. (1989). Amplification was performed in 20 µl reaction volumes (Accupower PCR premix kit,Bioneer®, South Korea) with a final concentration of each dNTP of 250 µM in 10mM Tris-HCl pH 9•0, 30mM KCl and 2mM MgCl2, 1U Taq DNA polymerase and 10 pmol of each PCR primer(Denazist. Iran). Then 1 µl of DNA template (250-500 ng) was added to each reaction and the remaining 20 µl reaction volume was filled with sterile distilled water. The PCR conditions were slightly modified. The following cycling conditions using a Bio-Rad thermocycler were applied: The first step of amplification was 3min of denaturation at 94 °C, this step was followed by 38 cycles, with 1 cycle consisting of 1min at 94 °C, 1min of annealing at 50°Cfor each pair of primers, and1min at72 °C, an extension step of 7min at 72 °C was planned after the eventual cycle. The PCR primer pair was derived from the B1 gene sequence (Burgetal.1989) , B1F0:5′GGAACTGCATCCGTTCATGAG3′ and BIR0:5′TCTTTAAAGCGTTCGTGGTC-3′ (position 694-714 and 887-868 nucleotide respectively).The PCR products were electrophoresed through a 1.8% agarose gel to assess the presence of a 193 bp band (if any) in the first round. All PCR products were included in the second round of PCR. The nested-PCR reaction condition was similar to the first round, except for the annealing temperature which was 52 °C,

the number of cycles was 30. The resulted amplification products were diluted to 1:10 in water and then a second amplification was done with the internal primers (Burg et al 1989), B1F1:5′-TGCATAGGTTG-CAGTCACTG-3andB1R1:5′-GGCGAC-CAATCTGCGAATACACC-3′ (position 757-776 and 853-831 nucleotide , respectively), using 1µl of the diluted product as the template nested PCR. The amplified products were detected in 1.8% gel stained by electrophoresis and viewed under ultraviolet light.

The presence of specific bands of 193 bp in primary PCR and 96 bp in nested -PCR on agarose gel was considered as positive sample.

Statistical analysis: The Chi-square test was used to analyze the association between the *T.gondii* infection with fetal age and geographic region.

All risk factors were analyzed in a multivariable logistic regression model and Odds-ratios (OR) with 95% confidence intervals are calculated. Statistical analyses were performed with the statistics package SPSS, version 16.0 for Windows (IBM, New York, USA).

Results

T.gondii DNA was detected in 18 out of 112 (16.07%) brain samples of ovine fetuses by nested- PCR (Table.1) Three positive samples demonstrated a detectable reaction in both rounds of PCR (Fig.2) and 15 positive samples were detected in second round (Fig. 3). Based on the geographical location, all Toxoplasma infection was detected in the aborted fetuses of sheep in the north areas ($p<0.01$, Table 2). In this study, significant differences were found between Toxoplasma infection in different age groups of fetuses ($p<0.05$, Table 2). No significant difference was shown between the frequency of Toxoplasma infection in different time periods ($p>0.05$, Table2).

Table 1. Frequency of *T.gondii* infection in aborted fetuses of sheep in different areas of Khorasan province.

Location	Number of positive	Total
Mashhad	10	47
Chenaran	1	17
Kalat	2	14
Fariman	0	7
Ghochan	0	2
Dargaz	2	4
Serakhas	0	6
Toos	1	2
Bojnord	1	2
Shirvan	1	3
Esfaryein	0	1
Torbat heidareih	0	2
Neishabour	0	1
Gonabad	0	1
Torbat Jaam	0	1
Khaf	0	2
Total	18	112

Table 2. Frequency of *T.gondii* infection in aborted fetuses of sheep from the Khorasan Razavi Province, Iran, in relation to possible risk factors.

Variable	PCR results		Total
	Negative No	Positive No (%)	
Age			
<120 day	15	0 (0)	15
≥120 day	69	18 (20.6%)	87
unknown	10	0 (0)	10
Region			
The North	72	18(20%)	90
The South	22	0 (0)	22
Year			
2009-2010	28	4(12.5%)	32
2010-2011	10	3(23%)	13
2011-2012	19	1(5%)	20
2012-2013	37	10 (21.28)	47

Discussion

Several PCR methods have been devel-

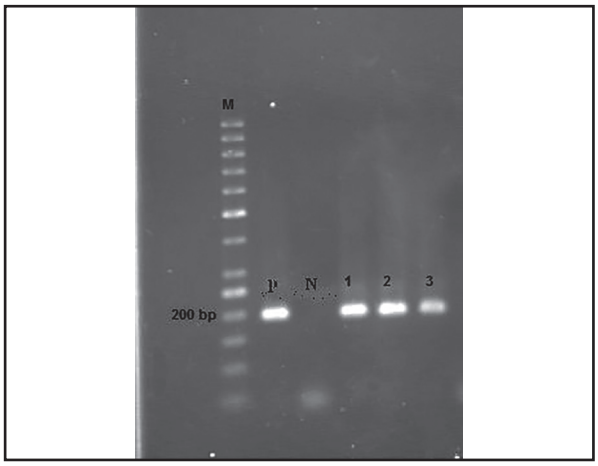

Figure 1. Electrophoretic analysis of PCR-amplified products stained with ethidium bromide in 1.8 % agarose gel. (M) Lane M, 50 bp DNA ladder marker; lane 1, negative control ; Lane2, positive control DNA sample of *T.gondii* (193 bp) ; lane 1-3 samples : DNA samples of blood.

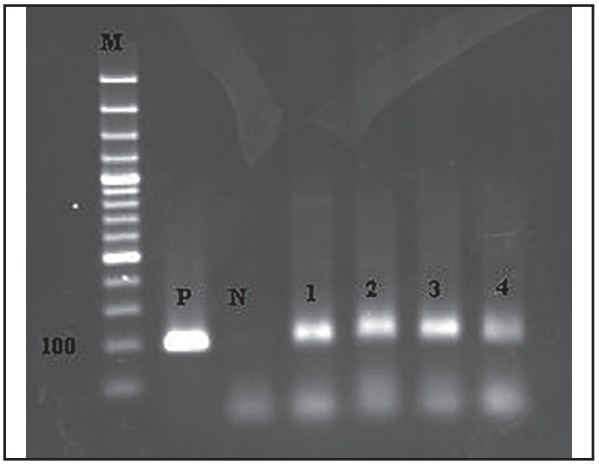

Figure 2. Electrophoretic analysis of PCR-amplified products stained with ethidium bromide in 1.8 % agarose gel . (M) Lane M, 50 bp DNA ladder marker; (P) lane P, positive control ; LaneN, negative control DNA sample of *T.gondii* (96 bp) ; lane 1-5 samples : DNA samples of blood.

oped for *T. gondii* detection in ovine abortion cases, (Hurtado and Aduriz 2007). Among these methods, the B1 gene is widely used as a target in many Toxoplasma PCR detection methods, mainly as a nested protocol based on that described by Burg et al (1989). In the present study, *T. gondii* infection was detected in 16.07% of aborted ovine fetuses by nested PCR.

Among the regions studied in Rio Grande do Norte, seroprevalence varied from 17.8% in the Central Potiguar region to 26.3% in the Leste Potiguar region (24).

In Iran, *T.gondii* DNA has been detected in aborted fetuses to range between 13.5% to 69% in Khorasan, Qazvin and East Azarbeijan provinces by different PCR assays (Rassouli et al. 2009; Habibi et al. 2011; Mooazeni Jula et al. 2013). These results show that the moutainous climate of Qazvin and East Azarbeijan provinces are more favorable conditions to the development and maintenance of *T. gondii* oocysts in the environment. Oocysts can survive in the environment for months, depending on moisture and temperature. Thus, low humidity and high temperatures of the Khorasan Razavi province is deleterious to the viability of Toxoplasma oocysts in the pasture. In other countries, *T. gondii* PCR positivity was detected to range between 3.5 and11.1% in Italy (Masala et al. 2003; Pereira-Bueno et al. 2004; Chessa et al. 2014)., 5.4 to16.9% in Spain (Hurtado et al. 2001, Moreno et al. 2012), 14% in Ireland (Gutierrez et a l. 2012), 14.3% in Brazil (de Moraes, et al. 2011) and 29.8% in Jordan (Abu-Dalbouh et al. 2012). The differences of PCR results would be related to many factors such as different PCR methods, climatic condition in each country.

All infected fetuses in this study belonged to the areas that are located in the northern part of the province. The northern part of the province has mountainous climate and mean annual rainfall is more than the southern areas with semi desert climate. The mountainous climate confers favorable conditions for Toxoplasma oocysts survival, because the viability of *T. gondii* oocysts is dependent on the moisture and temperature of the soil (Lélu et al. 2012; Du et al. 2012 ;). The higher prevalence of ovine toxoplas-

mosis has been reported in the humid and temperate areas compared to dry areas (Kamani et al. 2010; Alvarado-Esquivel et al. 2013; Gebremedhin et al. 2013; Andrade et al. 2013; Hamidinejat et al. 2008).

In the present study, most of the infected fetuses had more than 120 days of age. The clinical signs of ovine toxoplasmosis in pregnant ewes depends on the age and immune status of fetus. Fetal death is more likely to occur from infection during the first trimester of pregnancy when the fetal immune system is relatively immature. Infection at mid-gestation can result in birth of a stillborn or weak lamb which may have an accompanying small mummified fetus, whereas infection in later gestation may result in birth of a live, clinically normal, but infected lamb (Buxton 1991; Scott 2007; Innes et al. 2009). Other studies have shown that the abortion associated ovine toxoplasmosis generally occurs during the mid pregnancy (Pereira-Bueno et al. 2004) when the *T. gondii* aborted fetuses have 110-130 days of age (Giadinis et al. 2011).

Based on our results, the moderate frequency of *T.gondii* infection was detected among ovine aborted fetuses in this area. However, a definite diagnosis of Toxoplasma infection in causing abortion in sheep needs to combine pathological and molecular examination

Conflict of interest: The authors declare that they have no conflict of interest

Acknowledgments

This study was supported by a grant VPRTFM-3.27748 from the Vice President of Research and Technology of Ferdowsi University of Mashhad, Iran. We would like to thank Dr Zahra Naseri for help sampling and Dr Fazaeli kindly provided the control positive PCR from the Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran.

References

Abu-Dalbouh, M.A., Ababneh, M.M., Giadinis, N.D., Lafi, S.Q. (2012) Ovine and caprine toxoplasmosis (*Toxoplasma gondii*) in aborted animals in Jordanian goat and sheep flocks. Trop Anim Health Prod. 44: 49-54.

Alvarado-Esquivel, C., Estrada-Malacón, M.A., Reyes-Hernández, S.O., Pérez-Ramírez, J.A., Trujillo-López, J.I.,Villena, I., Dubey, J.P. (2013) Seroprevalence of *Toxoplasma gondii* in domestic sheep in Oaxaca State, Mexico. J Parasitol. 99: 151-2 .

Andrade, M.M., Carneiro, M., Medeiros, A.D., Andrade Neto, V., Vitor, R.W. (2013) Seroprevalence and risk factors associated with ovine toxoplasmosis in Northeast Brazil. Parasite. doi: 10.1051/parasite/2013019.

Burg, J.L., Grover, C.M., Pouletty, P., Boothroyd, J.C. (1989) Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J Clin Microbiol. 27:1787-1792.

Buxton, D. (1991) Toxoplasmosis. In: Diseases of Sheep. Martin, W. B., Aitken, I.D. (eds.). (2nd ed.). CAB Wallingford, UK. p. 49-58.

Buxton, D., Maley, S.W., Wright, S. E., Rodger, S., Bartley, P., Innes, E. A. (2007) *Toxoplasma gondii* and ovine toxoplasmosis: new aspects of an old story. Vet Parasitol. 149: 25-28 .

Chessa, G., Chisu,V., Porcu, R., Masala, G. (2014) Molecular characterization of *Toxoplasma gondii* Type II in sheep abortion in sardinia, Italy. Parasite. Doi: 10.1051/parasite/2014007.

de Moraes, E.P., da Costa, M.M., Dantas, A.F., da Silva, J.C., Mota, R.A. (2011) *Toxoplasma*

gondii diagnosis in ovine aborted fetuses and stillborns in the State of Pernambuco, Brazil. Vet Parasitol. 183: 152-5.

Dubey, J. P. (2009) Toxoplasmosis in sheep-The last 20 years.Vet Parasitol. 163: 1-14.

Duncanson, P., Terry, R.S., Smith, J.E., Hide, G. (2001) High levels of congenital transmission of *Toxoplasma gondii* in a commercial sheep flock. Int J Parasitol. 31: 1699-1703.

Du, F., Feng, H.L., Nie, H., Tu, P., Zhang, Q.L., Hu, M., Zhou, Y.Q., Zhao, J.L. (2012) Survey on the contamination of *Toxoplasma gondii* oocysts in the soil of public parks of Wuhan, China. Vet Parasitol. 184: 141-6.

Evans, H.E., Sack, W.O. (1973) Prenatal development of domesticand laboratory mammals: growth curves, external features and selected refrences. Anat Histol Embryol. 2: 11-45.

Gebremedhin, E.Z., Agonafir, A., Tessema, T.S., Tilahun, G., Medhin, G., Vitale, M., Di Marco, V. (2013) Some risk factors for reproductive failures and contribution of *Toxoplasma gondii* infection in sheep and goats of Central Ethiopia: a cross-sectional study. Res Vet Sci. 95: 894-900 .

Ghorbani, M., Hafizi, A., Shegerfcar, M.T., Rezaian, M., Nadim, A., Anwar, M., Afshar, A.(1983) Animal toxoplasmosis in Iran.J Trop Med Hyg. 86: 73-6.

Giadinis, N.D., Terpsidis, K., Diakou, A., Siarkou, V., Loukopolos, P., Osman, R., Karatzias, H., Papazahariadou, M. (2011) Massive Toxoplasma abortions in a dairy sheep flock and therapeutic approach with diff erent doses of sulfadimidine. Turk Vet Anim Sci. 35: 207-2011.

Gutierrez, J., O'Donovan, J., Williams, E., Proctor, A., Brady, C., Marques, P. X., Worrall, S., Nally, J. E., McElroy, M., Bassett, H., Sammin, D., Buxton, D., Maley, S., Markey, B.K. (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.

Hamidinejat, H., Goraninejad, S., Ghorbanpoor, M., Nabavi, L.,Akbarnejad, F. (2008) Role of *Toxoplasma gondii* in abortion of ewesin Ahvaz (South-West Iran). Bull Vet Inst Pulawy. 52: 369-371.

Hartley, W.J., Jebson, J.L., McFarlane, D. (1954) New Zealand type II abortion in ewes. Aust Vet J. 30: 216-218.

Habibi, G.R., Imani, A.R., Gholami, A.R., Hablolvarid, A.R., Behroozikhah, A.M., Lotfi, M., Kamalzade, M., Najjar, E., Esmaeil-Nia, K., Bozorgi, S. (2012) Detection and Identification of *Toxoplasma gondii* Type One Infection in Sheep Aborted Fetuses in Qazvin Province of Iran. Iran J Parasitol. 7: 64-72.

Hashemi-Fesharki, R. (1996) Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats in Iran. Vet Parasitol. 61: 1-3.

Hide, G., Morley, E.K., Hughes, J.M., Gerwash, O.M., Elmahaishi, S., Elmahaishi, K.H., Thomasson, D., Wright, E.A.,Williams, R.H., Murphy, R.G., Smith, J.E. (2009) Evidence for high levels of vertical transmission in *Toxoplasma gondii*. Parasitol. 136: 877-1885.

Hurtado, A., Aduriz, G., Moreno, B., Barandika, J., García-Pérez, A.L. (2001) Single tube nested PCR for the detection of *Toxoplasma gondii* in fetal tissues from naturally aborted ewes. Vet Parasitol. 102: 17-27 .

Hurtado, A., Aduriz, G. (2007) Polymerase Chain reaction. In: Protozoal Abortion in Farm Animal. Ortega, L.M., Gottstein, B., Conraths, F.J., Buxton, D. (eds.). Protozoal abortion in Farm animal. CAB International, Wallingford, UK. p. 136-141.

Innes, E.A., Barley, P.M., Buxton, D., Katzer, F. (2009) Ovine toxoplasmosis. Parasitol. 136:

1887-1894.

Kamani, J., Mani, A.U., Egwu, G.O. (2010) Seroprevalence of *Toxoplasma gondii* infection in domestic sheep and goats in Borno state, Nigeria. Trop Anim Health Prod. 42: 793-7.

Lélu, M., Villena, I., Dardé, M.L., Aubert, D., Geers, R., Dupuis, E., Marnef, F., Poulle, M.L., Gotteland, C., Dumètre, A., Gilot-Fromont, E. (2012) Quantitative estimation of the viability of *Toxoplasma gondii* oocysts in soil. Appl Environ Microbiol. 78: 5127-32.

Masala, G., Porch, R., Madau, L., Tanda, A., Ibba, B., Satta, G., Tola, S. (2003) Survey of ovine and caprine toxoplasmosis by IFAT and PCR assays in Sardinia, Italy. Vet Parasitol. 117: 15-21.

Morley, E.K., Williams, R.H., Hughes, J.M., Thomasson, D., Terry, R.S., Duncanson, P., Smith, J.E., Hide, G. (2007) Evidence that primary infection of Charollais sheep with *Toxoplasma gondii* may not prevent fetal infection and abortion in subsequent lambings. Parasitology. 135: 169-173.

Moreno, B., Collantes-Fernández, E., Villa, A., Navarro, A., Regidor-Cerrillo, J., Ortega-Mora, L.M. (2012) Occurrence of *Neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. Vet Parasitol. 187: 312-318.

Moazeni Jula, F., Moazeni Jula1, G., Nowzari, N., Kavari1, H., Hashemzadeh Farhang, H. (2013) A Serological and Molecular study on *Toxoplasma gondii* infection in sheep and goat in Tabriz. Arch Razi Inst. 68: 29-35.

Pereira-Bueno, J., Quintanilla-Gozalo, A., Pérez-Pérez, V., Alvarez-García, G., Collantes-Fernández, E., Ortega-Mora, L.M. (2004) Evaluation of ovine abortion associated with *Toxoplasma gondii* in Spain by different diagnostic techniques. Vet Parasitol. 121: 33-43.

Rahbari, S., Razmi, G.R., Nurozian, E. (1991) A seroepidemiology survey for toxoplasmosis in sheep of Mazandran province.J Fac Vet Med Univ Tehran. 50: 39-49.

Rassouli, M., Razmi, G.R., Bassami, M., Movassaghi, A.R., Azizzadeh, M. (2011) Study on ovine abortion associated with *Toxoplasma gondii* in affected herds of Khorasan Razavi Province, Iran based on PCR detection of fetal brains and maternal serology. Parasitol. 138: 691-697.

Razmi, G.R., Ghezi, K., Mahooti, A., Naseri, Z. (2010) A Serological Study and Subsequent Isolation of *Toxoplasma gondii* From Aborted ovine Fetuses in Mashhad Area, Iran. J Parasitol. 96: 812-814.

Scott, P.R., Sargison, N.D., Wilson, D.J. (2007) The potential for improving welfare standards and productivity in U.K. sheep flocks using veterinary flock health plans. Vet J. 173: 522-531.

Sharifi, M., Gholami, Sh., Ziaei, H., Daryani, A., Laktarashi, B., Ziapour, S.P., Rafiei, A., Vahedi, M. (2007) Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats slaughtered for food in Mazandaran province, Iran, during 2005. Vet J. 174: 422-4.

Zia-Ali, N., Fazaeli, A., Khoramizadeh, M., Ajzenberg, D., Darde, M., Keshavarz-Valian, H. (2007) Isolation and molecular characterization of *Toxoplasma gondii* strains from different hosts in Iran. Parasitol Res. 101: 111-5.

مجله طب دامی ایران، ۱۳۹۶، دوره ۱۱، شماره ۲، ۱۵۳-۱۴۷

تعیین ملکولی آلودگی تو کسو پلاسما گوندیی در جنین‌های سقطی گوسفندان استان خراسان رضوی

لیلا دانه چین[۱] غلامرضا رزمی[۲]* ابوالقاسم نقیبی[۳]

۱) دانش آموخته انگل شناسی، دانشکده دامپزشکی دانشگاه فردوسی مشهد، مشهد، ایران
۲) قطب سقط چنین و بیماری نوزادان نشخوارکننده ، دانشکده دامپزشکی دانشگاه فردوسی مشهد، مشهد، ایران
۳) گروه پاتوبیولوژی، دانشکده دامپزشکی دانشگاه فردوسی مشهد، مشهد، ایران

(دریافت مقاله: ۵ آبان ماه ۱۳۹۵، پذیرش نهایی: ۲۵ دی ماه ۱۳۹۵)

چکیده

زمینه مطالعه: تو کسوپلاســما گوندی یک عامل مهم ســقط جنین در گوسفندان و بزان در دنیا می‌باشد. توکسوپلاسموز سبب اختلالات تولید مثل در جنین از جمله جذب جنین، مرگ زودرس جنین، مومیایی شدن، سقط جنین، مرده‌زایی و مرگ نوزادان در گوسفندان می‌شود. **هدف:** هدف این مطالعه شناسایی آلودگی تو کسوپلاسما گوندیی در جنین‌های سقطی گوسفندان استان خراسان رضوی می‌باشد. **روش کار:** ازخرداد ۱۳۸۹ تا تیر ماه ۱۳۹۲ تعداد ۱۱۲ نمونه مغز جنین‌های سقط شده گوسفندان جمع‌آوری شده و مورد آزمایش nested- PCR جهت تعیین آلودگی توکسوپلاسما قرار گرفتند. ارتباط فراوانی آلودگی توکسو پلاسما گوندی با فاکتور سن و منطقه جغرافیایی مورد بررسی قرار گرفت. **نتایج:** نتایج آزمایش PCR نشان دهنده آلودگی ۱۸ نمونه مغزی (۱۶/۸٪) به توکسوپلاسما بودند. در این مطالعه فراوانی توکسوپلاسما در جنین‌های سقط شده بالای ۱۲۰ روز در مقایسه با سایر سنین بالاتر بود ($p<0/05$). تمام جنین‌های سقط شده آلوده به توکسو پلاسما متعلق به مناطق شمالی استان خراسان رضوی بودند. **نتیجه‌گیری نهایی:** نتایج بدست آمده نشان دهنده آلودگی متوسط تو کسوپلاسما گوندی در جنین‌های سقطی مناطق شمالی استان خراسان رضوی می‌باشد.

واژه های کلیدی: سقط جنین، مغز، Nested-PCR، گوسفند، تو کسوپلاسما گوندیی

---

*) نویسنده مسؤول: تلفن: ۳۸۷۸۸۹۴۴ (۵۱)۹۸+ نمابر: ۳۸۷۶۳۸۵۳ (۵۱)۹۸+ Email: razmi@um.ac.ir