Animal welfare, etológia és tartástechnológia

Animal welfare, ethology and housing systems

Volume 9 Issue 3

Különszám/Special Issue

Gödöllő
2013

DIAGNOSTICS OF *TOXOPLASMA GONDII* IN MILK SAMPLES OF SHEEP FROM EASTERN SLOVAKIA

Lenka LUPTÁKOVÁ[1], Eva PETROVOVÁ [2], Ľubica ŠŤAVOVÁ[1]*

[1]University of Veterinary Medicine and Pharmacy, Department of Biology and Genetics, Komenského 73, 041 81 Kosice, Slovakia
[2]University of Veterinary Medicine and Pharmacy, Department of Anatomy, Histology and Physiology, Komenského 73, 041 81 Kosice, Slovakia
* MVDr. Lenka Luptáková, PhD, luptakova.l@gmail.com

Abstract
The paper presents the results of examination of 62 sheep from flocks of eastern Slovakia for the presence of *Toxoplasma gondii* antibodies by complement fixation test. Thirteen samples were found to be positive at the titre of 1:64, 10 were positive at 1:128 and two at 1:256 dilution. Subsequently, DNA was isolated from blood samples of these animals. PCR analysis with specific primers was then used in search for *T. gondii* DNA. In PCR analysis, 5 of 25 (20%) samples tested positive.

Key words: milk, real time PCR, sheep, *Toxoplasma gondii*

Introduction

Toxoplasmosis is one of the most common parasitic zoonoses of mammals and birds transmissible to humans. The agent of the disease is *Toxoplasma gondii (T. gondii)*, the definitive hosts of which are the representatives of the family F e l i d a e, infected with oocysts from the environment and with tachyzoites and bradyzoites from intermediate hosts. Intermediate hosts are all vertebrates including man (Frenkel, 2000). It is a panthropic cosmopolite, a facultative heteroxenic coccidium. Sheep were in fact the first mammals in which congenital toxoplasmosis were proven with abortions, dead-born fetuses and frequent manifestations of infection including infertility. The first case of manifest toxoplasmosis in sheep with symptoms of encephalomyelitis and tachycardia was described by Olafson and Monlux. Sheep play a significant role in the economy of many countries since they are important source of meat and milk products. Toxoplasmosis is the major parasitic disease affecting sheep. It is important for veterinary medicine, animal science and public health since it causes reproductive and economic losses in the herd, as well as damaging human health due to consumption of contaminated meat and milk, which can facilitate zoonotic transmission (Camossi et al., 2011).

Material and methods

Samples: A total of 50 milk samples of adult sheep were examined by real time PCR. Sheep originated from farm located 30 km of Kosice in eastern Slovakia. In the farm proper sanitary conditions were respected and sheep were regularly pastured on grassland where they could contamined with *T. gondii*.
Molecular diagnostics: Examination was carried out on samples of sheep milk.
Isolation of DNA: DNA was isolated from 50 milk samples by commercially available kit (DNA-sorb-B, Amplisens, Russia). DNA was isolated according to the manufacturer´s protocol and stored at -20 °C for futher examination.
Detection of DNA by real time PCR: Amplification of the isolated DNA was carried out by the real time PCR with SYBR green as a detection system from the *T. gondii* gene region TGR1E, repeated in the genome 30-35 times, using the specific primers TGR1E-1 and TGR1E-2 (Cristina et al., 1991). Cloned *T. gondii* TGR gene (GenExpress, Germany) diluted to 10^4-10^9 was used for the calibration curve. In each reaction, a melting analysis (comparison of the melting temperature (Tm) of PCR products) was determined to differentiate specific and non-specific PCR products. The reaction volume was 25 µl, which contained commercial FastStart Universal SYBR Green Master (Roche,

Germany) and 0.2 μM primers (TGR1-1 and TGR1E-2). Real-time PCR was completed using a thermocycler Line GeneK with the software Line GeneK Fluorescent Quantitative Detection system (BIOER Technology, China). After incubation at 50°C for 2 minutes and initial denaturation at 95°C for 10 minutes, 40 amplification cycles were performed (95°C for 15 s, 60°C for 1 minute). Melting analysis was carried out at temperatures ranging from 60°C to 95°C, in which the temperature was gradually increased by 0.5°C and the period of measurement at individual steps was 15 s. Every PCR run included a control without DNA (containing the reaction mix alone and nuclease-free water).

Results

Using quantitative real-time PCR the presence of *T. gondii* DNA was detected and the number of copies quantified in the 50 milk samples of sheep. Using real-time PCR, *T. gondii* DNA was detected and quantified in 5 samples of milk. Standards with the known dilution of *Toxoplasma* DNA were used to determine the detection limit of a modified real-time PCR and to create a calibration curve that ranged from 10^9-10^4 copies of *Toxoplasma* DNA. The correlation coefficient of the calibration curve was 0.998. As SYBR Green a fluorescent dye, was used as a detection system, a melting analysis was a part of the real-time PCR to distinguish between specific and non-specific products. During the melting analysis, the melting temperature (Tm) of a positive control and positive samples was 84°C. In quantifying the examined samples within a 40-cycle protocol for the real-time PCR, the number of copies detected in the positive samples ranged from 1.1 x10^2 to 1.30 x10^5 (Table 1).

Table 1

The number of DNA copies in positive samples

sample	DNA copies	C_t value
Sample1	**1.1 x 10^2**	**19,50**
Sample 5	**1.3 x 10^5**	**18,72**
Sample 23	**5.7 x 10^4**	**19,37**

Discussion

Consumption of raw food, especially among immunocompromised persons and pregnant women, poses a potential threat to public health. The milk of sheep and goats represent risk factors as they are consumed raw (Spisak at al., 2010).

Consumption of unpasteurized goat, sheep or cow milk or its products represents a risk because tachyzoites, the stage most likely present in milk are thought to be immediately destroyed by the gastric juice (Dubey, 1998). However, evidence indicates that the part of ingested tachyzoites is not destroyed by gastric juice and it can cause infection (Cook et al., 2000). Toxoplasmosis transmission by unpasteurized or inadequately processed milk or fresh cheese, important food sources in rural areas, can be a significant means of contamination by this agent (Hiramoto et al., 2001)

In the period of 1999 – 2002, studies conducted in Sardinia analyzed 9639 samples of sera and 815 samples from abortions (670 aborted fetus and 145 placentas) obtained from 964 sheep and goat farms. The sampled sera were examined for presence of IgG and IgM specific antibodies against *Toxoplasma gondii* by means of indirect immunofluorescence method while fetus and placenta were examined by PCR. Specific antibodies against IgG were diagnosed in 652 (9%) sheep. of the total number of 2471 samples of sheep fetuses including muscles, liver, abomasums, spleen, brain and placenta, PCR proved positivity in 271 (11.1%) cases. on the one hand, these

results indicate relatively high seroprevalence and, on the other hand point to the important role of PCR diagnostics related to sheep abortions (Masala et al., 2003).

In southern Italy, region Campania, 117 sheep flocks kept on pasture were examined. Samples of blood and milk were taken from 10 adult sheep (> 18 months old) from each flock. Thus altogether 1170 sheep were subjected to examination. Blood sera were examined for the presence of IgG antibodies by indirect immunofluorescence. Of the examined samples collected from 1170 sheep 333 samples were positive (28.5%) and PCR proved presence of *Toxoplasma gondii* in 4 samples of milk, which represents 3.4% of the total analyzed samples, demonstrating the transmission potential of ovine milk and dairy products (Fusco et al., 2007).

The tachyzoites presented in sheep or goat milk are generally not considered as an important source of oral transmission of *T. gondii* because they are rapidly killed outside the host and because they are considered sensitive to proteolytic enzymes (Powell et al., 2001). But the contamination of milk should not be underestimated since it can represent a critical point in food safety. Some local home-made cheeses deriving from mass-milk production and destined to be consumed fresh can represent a risk factor for public health if they are produced in small family-based farms without previous milk pasteurization.

Acknowledgement

The paper is a result of research work done within the frames of grant projects VEGA of the Ministry of Education of the Slovak Republic No. 1/0063/13.

References

Frenkel J.K. 2000. Biology of *Toxoplasma gondii*. In: Ambroise-Thomas P, Peterse E. (ed): Congenital toxoplasmosis: scientific background, clinical management and control. Springer-Verlag, Paris, pp. 9-25.

Olafson, P., Monlux, W.S. 1942. *Toxoplasma* infections in animals. Cornell Veterinarian, 32, 16-190.

Camossi, L.G., Greca-Júnior, H., Correa, A.P.F.L., Richini-Pereira, V.B., Silva, R.C., Da Silva, A.V., Langoni, H. 2011. Detection of *Toxoplasma gondii* DNA in the milk of naturally infected ewes. Veterinary Parasitology, 177, 256-261.

Cristina, N., Liaud, M.F., Santoro, F., Oury, B., Ambroise-Thomas, P. 1991. A family of repeated DNA sequences in *Toxoplasma gondii*: cloning, sequence analysis, and use in strain characterization. Experimental Parasitology, 1991, 73, 73-81

Spisak, F., Turcekova, L., Reiterova, K., Spilovska, S., Dubinsky P. 2010. Prevalence estimation and genotypization of *Toxoplasma gondii* in goats. Biologia, 65, 670-674.

Dubey, J.P. 1998. Re-examination of resistance of Toxoplasma gondii tachyzoites and bradyzoites to pepsin and trypsin digestion. Journal of Parasitology, 116, 43-50.

Cook, A.J.C., Gilbert, R.E., Buffolano, W., Zufferey, J., Peterse, E., Jenum, P.A., Foulon, W., Semprini, A.E., Dunn, D.T. 2000. Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. BMJ, 321, 142-147.

Hiramoto, R.M., Mayrbaurl-Borges, M., Galisteo, A.J., Meireles, L.R., Macre, M.S., Andrade, Jr., H.F. 2001. Infectivity of cystis of the ME-49 *Toxoplasma gondii* strain in bovine milk and homemade cheese. Revista de Saúde Pública, 35, 113-118.

Masala G, Porcu 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. Veterinary Parasitology 117, 15-21.

Fusco G, Rinaldi L, Guarino A, Proroga YTR, Pesce A, Giuseppina DM, Cringoli G. 2007. *Toxoplasma gondii* in sheep from the Campania region (Italy). *Periodical* Veterinary parasitology 149, 1-4.

Powell, C.C., Brewer, M., Lappin, M.R. 2001. Detection of *Toxoplasma gondii* in the milk of experimentally infecting lacting cats. Veterinary Parasitology, 102, 29-33.