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Dairy goats – indicators of some zoonotic pathogens in the environment

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Summary

970 samples of Ixodes ricinus were collected in various areas within the investigated terrain in order to detect the causes of Tick Borne Encephalitis (TBE) in humans. At the same time the prevalence of antibodies to Toxoplasma gondii and TBE virus were detected in goat population within this area. Blood sera were tested by the Waldeland modified micro titration Dye Test (DT) for Toxoplasma gondii, and ELISA and HAI tests were also used to investigate seroprevalence to the TBE virus. It was found that 4 out of the 5 tested goats' herds had Toxoplasma gondii and TBE virus antibodies. 52.63 % of small ruminants had antibodies to T. gondii and 11.8 % to TBE virus in the infected herds of the tested goats. Significant differences were also observed between age groups (P<0.05).

Keywords: goats' milk, Toxoplasma gondii, virus of Tick Borne Encephalitis

Toxoplasmosis is a disease causing a big economical damage for sheep and goats. Ooysts, the parasite stage responsible for initiating infection, are produced following primary infection in cats. Infected with *Toxoplasma gondii* goats can establish a placental and fetal death and resorbtion, abortion or stillbirth (3, 6, 22).

Goats' toxoplasmosis also has a very big social significance. People become infected with *T. gondii* mainly by ingesting tissue cysts in undercooked goat meat/meat products, or oocysts from an environmental contaminated with infected feline feces (7). Although most postnatally acquired infection in human are asymptomatic, *Toxoplasma* may result fetal death abortion and can cause mental retardation and loss of vision in congenitally infected children and fatal encephalitis in patients with Immunodeficience Syndrome (AIDS). According to the statistical data 0.03% of humans (from all infectious diseases detected in this area) had been registered toxoplasmosis and 3.57% – TBE in Kaunas region, Lithuania.

During an acute infection in goats toxoplasms may be excreted in the goat milk and be a possible source of human infection if drunk unpastereused. Infection in humans has been documented from ingestion of raw goat milk (19, 21). This way of infection was confirmed during experimental study (4). It was determined that tachyzoites can survive up to 3 days at +4°C, while samples of goat milk spiked with large numbers of tachyzoites were positive for 7 days at 4°C, but were killed after freezing of up to –20°C after 1 h, but did survive in Hanks Balanced Salt Solution without calcium and magnesium (HBSS) for 1 h at this temperature (32).

Preliminary clinical diagnosis of goats is detected according to epidemiological investigation, and clinical signs. Cotyledons on the accompanying placenta also show small necrotic foci visible to the naked eye. A large numbers of IFA, ELISA and others serological tests have also been described and marketed by commercial firms (7, 28), although it is generally that the majority of these are not as specific or as sensitive as the Dye Test (DT), suggested in 1948 by A.B. Sabin and H.A Feldman (20).

Tick Borne Encephalitis (TBE) – disease of central nervous system, which causes by virus. Ticks are the main carries of TBE virus in the nature. TBE virus is a member of the genus *Flavivirus* within the family *Flaviviridae* and is prevalent over a wide area of the Eurasian continent (many European countries, Russia, far-east Asia and Japan). TBE viruses cause severe encephalitis in humans, with serious squeal, and have a significant impact on public health in these endemic regions (11). Based on geographical origin and antigenic characteristics, TBE viruses were originally subdivided into two subtypes, European and far-eastern.

Ticks of these 5 species: *Ixodes (I.) persulcatus*, *Ixodes (I.) ricinus*, *Dermacentor (D.) silvarum*,

Haemophysalis (H.) concinna, *Haemophysalis (H.) japonica* are the main vectors of TBE disease transmission. Before mentioned 2 species of ticks are very common in Lithuania, and have a very important epidemiological role on transmission of TBE virus (1).

For TBE infection it is very characteristic some sources in nature there virus circulates. Ticks act as both the vector and reservoir for TBE. The main hosts are small rodents, with humans being accidental hosts. Animals are feeding hosts for the ticks, but do not play a role in maintenance of the virus. All kinds of animals, rodents, birds and human become infected through tick bites or by drinking unpasteurized raw goats or bovine milk (12). Dairy goats are housed near or in the farm house; infection in goats is a good indicator of the contamination of the environment by *T. gondii* oocysts. Because ingestion of oocysts from cat feces is the only mode of primary infection in goats, results indicate a heavy contamination of the environment by *T. gondii* oocysts (9). The seroprevalence to TBE virus in goats shows also a contamination of environmental with infected by TBE virus ticks (2).

TBE had been reported when more than 600 people had been infected after drinking unpasteurized raw goats or bovine milk. In Slovakia during the last 10 years this way of infection had been determined in 27% TBE cases, and drinking of raw goat milk was the main source of TBE infection in Sankt-Petersburg region of Russia (2, 10).

There are some reports on surviving of TBE virus in the milk or milk products during their keeping. It had been determined that during 2 weeks keeping of butter, virus titer was the same during the 2 weeks keeping in freezer, and virus survived after 2 months keeping in butter and sour cream.

The aim of this study was to test the level the seroprevalence to *Toxoplasma gondii* and TBE virus in goats' herds.

Material and methods

The ticks had been collected in April and October, 2004 on the special flags by the methodology confirmed by Lithuanian Ministry of Health at the stationers, forests and parks of Kaunas city and Kaunas regions areas. It was gathered 970 units of ticks and sortered according to their sex. In the Kaunas Center of Public Health all collected ticks were divided into separate specimens. Before the investigations all the ticks were stored at –70°C until analyzed.

The number of cats had been observed in the goats housing region and owners of cats were asked for keeping of cats in their farms.

Paralelly 76 blood samples were collected from 5 goats herds in the same area. For serological investigation all samples was taken from *v. yugularis* of goats. The samples were collected into sterile tubes and stored at the room temperature for 8 h and centrifuged at 15 000 × g for 10-15 min. The serum was taken out, and 1 : 10.000 Merthiolate was added and frozen at –20°C until tested.

Seroprevalence to *Toxoplasma gondii* was analyzed at the Department of Sheep and Goat Research, Norwegian College of Veterinary Medicine by the Dye Test (DT), described by Sabin and Feldman (20) and modified by Waldeland (30) using of RH strain *Toxoplasma gondii* tachyzoites were taken from peritoneal cavity of infected mice. All samples before analyzing were inactivated by heating +60°C at 1 h. Positive and negative controls were included. Sera were diluted into 1 : 16, 1 : 32, 1 : 64, and 1 : 128, 1 : 256, 1 : 512 solutions. Sera that gave less than 1 : 16 titer (dilution) were considered seronegative. The titres recorded corresponded to the terminal serum dilution in which 50% f 100 counted parasites were unstained or modified as revealed by phase contrast microscope.

The seroprevalence to TBE was detected by Haemoglutination Inhibition Test (HAI) in Finland National Health Institute, and by Enzyme-Linked Immunosorbent Assay (ELISA) in the Lithuanian National Veterinary Laboratory. For HAI test were used antibodies of EEV Kumlige and Sofjin strains (Test line, BRNO EIA TBE IG, Slovakia). Nonspecific sera inhibitors were absorbed by kaolin and geese erythrocytes. Analyzing goat sera in dilutions 1 : 10 and 1 : 640 were added into microtitration plates. Later 3 units of the haemagglutination antigen were added. The 2% of geese erythrocytes solution was used during incubation. To control nonspecifity of test the Semlich – Forest antigens of viruses and *Chlamydia spp*. antigens were used also. Titer of specific antigens was equating to the last dilution in which inhibition of haemagglutination was noticed. The test was evaluated by 4+ systems.

The same blood sera had been tested paralelly by ELISA. The TEST-LINE diagnostic kits (BRNO EIA TBE IG, Slovakia) was used in this study. A microtitration plates were coated by TBE virus antigen. The serum samples were incubated with antibodies against mice anti TBE virus. Conjugate (pigs anti mice antibodies, labeled by peroxydasis) and connected with hard matter, amount was directly in proportion to amount of antibodies of mice anti TBE virus. This had been detected by the color reaction with substrate (TMB, peroxide). For the test control, antibody positive and antibody negative controls were used. Absorbance of each dilution was measured by automated Micro ELISA reader equipment with 450 nm filter. For the determination of ELISA the optical density (OD) of the test blank (antigen, conjugate substrate) was subtracted from each serum OD. The OD of each serum was divided by the reference serum. The results of test were counted according to this formula:

$$X = OT_1/OT_2$$

there: OT_1 – optical density of tested sample;
OT_2 – optical density of negative control;
Note: test positive, when $X < 2$;
test disputed, when $2 < X < 3$;
test positive,when $X > 3$.

The positive reaction was kept, when optical density was twice and more times less than absorption of control serum.

For statistical data analysis SPSS (Statistical Package for Social Sciences) was used. Arithmetical mean (M) and standard deviation (SD) was calculated. Comparison of the means was performed using Fisher's t-tests with a significance level of 0.05.

All investigations were performed in the compliance of the „Principles of laboratory animal care" (NIH publication

No 86-23, revised 1985) and with the laws of Lithuania for „Animal Protection, Welfare and Use" („State News" Vol. 108, 28/11/1997), with the laws of Lithuanian State Food and Veterinary Service „The Veterinary Requirements for Breeding and Keeping of Laboratory Animals, their Welfare and Transportation" (31/12/1998, No 4-361) and „The Use of Laboratory Animals in the Scientific Investigation" (19/01/1999).

Results and discussion

The analysis of done parasitological investigation had shown, that the activity of Ixodes ricinus ticks are in relationships with warmer climatic conditions in Kaunas region, there the shorter winter had been noticed during the 2004 year season. During this time period the activity the first ticks had been observed in the first part of April. In the ticks stationers the biggest amount of ticks in 1 km way had been found in second half of May. During the time of investigation 970 units of ticks were collected. Some different numbers of ticks had been found in the tested areas: Kaunas city – 260, Kaunas region – 710 units.

During the rainy and cool season in 2004 year the increasing activity of ticks had been observed in the second half of August; in average 15.6 units of ticks had been gathered at 1 km of investigated area; in Vandziogala area – 14.7, and in Kaunas region stationers – 16.2 of ticks/nymphs had been found on the flags during the time of investigation.

During the investigation domestic cats had seen near the all goats farms. From the tested 5 goats herds 4 owners of goats had cats in their farms.

The serological investigation in Kaunas region goats' herds had shown that the prevalence of antibodies to *Toxoplasma gondii* was different in the all of tested areas. The biggest number (83.3%) had been observed in III herd (tab. 1). From the data of investigated sera, it had been found that more than a half of goats (52.63%) had been found the antibodies to this parasite.

In the blood sera of goats, kept in IV herd, antitoxoplasma antibodies had not been found; although in other herds (I, II, III, V) 62.38% of tested animals had the positive seroprevalence. It had been noticed that in infected by *Toxoplasma herds* more than 18.1% (in average. 69.45% and 56.87% respectively) of positive seroprevalence had been noticed in Kaunas city herds (I, III) than in the district herds (II, V). Not any one goat seroprevalence to *Toxoplasma gondii* found in IV herd, where goats had kept indoor, blood sera.

The serological investigation of goats herds for sero-prevalence to TBE virus had shown that the biggest seroprevalence (tab. 2) was found in Kaunas city herds (I, III), that involved 22.23% and 33.3% of tested goats respectively. It has been noticed 12.1% of seroprevalence in V herd.

Tab. 1. The prevalence of antibodies to *Toxoplasma gondii*

Number of		Number of positive (%)
herds	tested goats	
I	9	5 (55.60)
II	12	6 (50.00)
III	6	5 (83.30)
IV	16	0
V	33	20 (60.60)
Total	76	36 (52.63)

Tab. 2. The prevalence of antibodies to TBE virus

Number of		Number of positive (%)	
herds	tested goats	HAI	ELISA
I	9	0 (0)	2 (22.2)
II	12	0 (0)	1 (8.3)
III	6	0 (0)	2 (33.3)
IV	16	0 (0)	0 (0)
V	33	9 (27.3)	4 (12.1)
Total	76	9 (11.8)	9 (11.8)

Seropositivity in Kaunas distric (II herd) was 8.3% of tested goats. In the IV herd positive animals were found neither by HAI, nor ELISA tests.

In resume, when the all goats had been tested by HAI and ELISA, 11.8% of investigated animals had the antibodies to TBE virus. The HASR test had been shown that haemaglutination inhibition had been detected in sera solutions from 1 : 10 up to 1 : 256.

The distribution of antibody titres in the seropositive to *Toxoplasma gondii* goats sera presented in tab. 3. The antibodies have been detected in sera solution diluted from 1 : 16 to 1 : 256. In V herd have been detected 36.3% of all seropositive small ruminants, which titers were 1 : 64-1 : 256. From the all positive tested sera, antibodies to *T. gondii* had been detected in 1 : 256 dilution 1.3%, 1 : 128 – 3.9%, 1 : 64 – 15.8%, 1 : 32 – 13.2%, 1 : 16 – 13.2% of all cases. The highest titers of antibodies had been detected in herds of sheep, kept in Kaunas district than in Kaunas city.

It have been noticed that the biggest seroprevalence antibodies to *T. gondii* had been found in the goats, aged more than 3 years old (fig. 1). In older than 3 years old goats sera the seroprevalence to *Toxoplasma gondii* was 2 time (50%) higher than in other two groups aged between 1-2, or 2-3 years respectively ($P < 0.05$).

The same tendency was noticed in the same goats' sera, tested for TBE virus by HASAR and ELISA.

According to the results of this study seroprevalence to TBE virus had been increased regarding the goats age. In I group (1-2 year old) the seroprevalence was 22.2%, although in the II group (2-3 year old) this data was 1.5 times higher, and in III group (more than

Tab. 3. Distribution of DT endpoint titres to *Toxoplasma gondii* in seropositive goats

Number of		Number of DT positive sera	Titres of antibodies				
herds	tested sera		1 : 16	1 : 32	1 : 64	1 : 128	1 : 256
I	9	5	2	3	–	–	–
II	12	6	1	1	3	1	–
III	6	5	3	2	–	–	–
V	33	20	4	4	9	2	1
Total	60	36	10	10	12	3	1

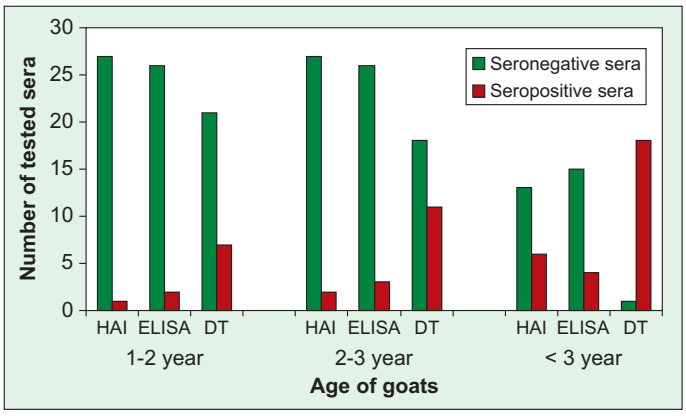

Fig. 1. Antibodies to *T. gondii* and TBE virus dependence on age of goats

3 years old) the seroprevalence was 2 times higher than in I group respectively ($P < 0.05$).

In the nature the reservoir of TBE virus are *Ixodes spp*. ticks. In the ticks saliva had been detected viruses, bacteria, mycoplasma spp., fungi, *Toxoplasma gondii*, *Chlamydia spp*. etc. (1, 26). Tick female usually sucks the blood of animals or birds 6-8 days, and male-only 1.5 h. After sucking and fecundating tick female falls out from the animal or bird to the grass and puts 350-5500 eggs. The environmental humidity and temperature have a high influence on the development of larvae. Low temperature – lowering down the metamorphosis of ticks, high – accelerates. In the favorable conditions the life cycle of lasts 3 months. But usually this process spreads from 2 till 3 years. In extremely unfavorable conditions this process can last up to 7 years. Tick eggs and larvae at minus 7-8°C usually dead. The development of ticks from egg to larvae and imago can be expressed in this proportion: 240 : 40 : 1 (2, 12). The analysis of parasitological investigation had shown that the ticks in Kaunas region are very active in average 8 months during all year. According to data, taken from 10 last years, it had been noticed the tendency of increasing number of ticks population (in average 9.3 times; from 1.8 till 17.8 ticks on the flag in the 1 km of way (2) in Lithuania.

In Kaunas region like as in all Lithuania the activity of ticks depends on the climatic conditions. The biggest number of ticks (16.2) on 1 km of the way had been detected on the second part of the May. According to the data of present study based on the results of HAI ELISA tests, the highest seroprevalence to TBE virus (22.2 and 33.3%) have been detected in that territories, where the increasing of ticks number and their activity had been noticed.

Although the clinical signs of toxoplasmosis and TBE are not noticed frequently in the goats' population. However the main way to detect these infections is the serological tests. Usually *Toxoplasma gondii* detects by direct noticing in the tissue samples by the microscope, or by the indirect methods – detection of specific antibodies by serological tests. Sabin-Feldman Dye test – is one of the oldest, high specific and sensitive test still using as an etalon of other serological tests for *Toxoplasma gondii* detection. DT is a definitive test. However, it is not used routinely because it is semiquantitative, requires an accessory factor, is as subjective, and is potentially hazardous for laboratory workers, because a live *T. gondii* tachyzoites are using there. Recently the Enzyme – Linked Immunosorbent Assay (ELISA) has been adapted for the detection of *Toxoplasma gondii* antibodies in goats' blood sera. It is relatively simple assay that is highly sensitive and specific. The assay may be fully or semiautomated and is nonhazardous to laboratory workers.

According to the investigation of researchers, goats, like sheep, had very high seroprevalence to *Toxoplasma gondii* (5, 13-15).

The seroprevalence of animals depends on a lot of factors, from which the main is their contact with cats. The importance of close contact between young cats and goats is consistent with the biology of disease, as young cats primarily responsible for the shedding of oocysts (7, 24). Cats, are, hovewer, likely to be found in almost all areas where goats are kept, and the probability that young cat may shed oocysts on a farm will always be present as oocysts survival in soil for up to two years (25, 29). There are some data that *Ixodes spp*. ticks may be a vector of transmission of *Toxoplasma gondii* in humans (26). So it is possible that grazing goats had a contact with the ticks, which could transmit the disease during these goats, and they had a higher seroprevalence. To confirm this hypothesis more and detail investigations should be done. In the IV herd, where goats were kept in stable the prevalence of antibodies to *T. gondii* and TBE virus were not found because the goats had not a direct contact with the cats and ticks. The owners of this farm had not any cat there. During the time of investigation it had been noticed that the highest seroprevalence (60.6% had been detected in the V herd, there goats had grazed near by the forest and big bushes, ant the owners of this herd has one old female cats with 2 young 60 days old teen age kittens. Also a lot of cats had been noticed in the city area, there goats grazed. The antibodies to *T. gondii* had been noticed in the 80% of tested goats blood sera in dilutions 1 : 32 and higher. This data shows the permanent infection and active monogenetic process in the herds. In the present study seroprevalence was 50% higher in older goats than in young age goats respectively. This age related difference in acquisition of *T. gondii* infection is expected because older goats are exposed to *T. gondii* oocysts for longer periods than are younger goats (5, 7, 13, 15, 16).

It is known that there are a good correlation between antibody of DT titers and presence of vialable cysts in the meat (31). Although in the present study the 4 goats had an antibody titer more than 1 : 128 but not always results of quantities analysis indicates about

the prolonging of infection. The presence of high antibody titers to *T. gondii* in the serum is not necessarily diagnostic of recent infection, because titers may remain high into the next breeding season (6). After the analysis of this study data and results from other researches it may be that the infection in goats goes permanently, when the transmissions of toxoplasmosis not over, because sources of infection (*T. gondii* oocysts) remains in the environmental.

Although the importance of cats and meat in spreading chain of *Toxoplasma gondii* in animals and humans is well established but the other ways of this infection it is not good known (7).

Toxoplasma gondii has been found in milk of experimentally infection of goats, cows, mice and cats (7, 18). Very often the unpasteurized goat milk is used instead of pasteurized cows milk, because it is an opinion exist that pasteurization decreases the biological value of milk, and it become less digestible. It is known that unpasteurised goats milk have some of anticancer factors and stimulates the development of useful bacteria's in the digestible tract of children. Toxoplasmosis has been reported in human beings after drinking raw goats' milk. It has been reported (9, 23, 27).

Toxoplasma tachyzoites are resistant to the some conditions. Direct microscopical examination of tachyzoites in acid pepsin solution revealed that tachyzoites were damaged immediately; they were more granular, less retractile, and became ghost-like within 15-30 min. So it is still exists opinion that tachyzoites cannot survive in human gastric juice and proteolytic enzymes destroy them (7). Alternatively, infection by tachyzoites might occur by penetration of oropharyngeal mucosa (17, 20). Although the new the investigation had shown that tachyzoites can survive for 2 h in acid pepsin solution. After feeding higher doses (> 1000) extracellular tachyzoites they were also infectious orally for mice and cats (8). Therefore, these data might explain the some cases of toxoplasmosis in humans after drinking unpasteurised goats milk. According to this data the milk of infected with *T. gondii* may be seriously dangerous for pregnant women and for immunosupressed patients.

In resume it has been detection the increasing in 9.3% ($P < 0.05$) of *Ixodes ricinus* tics in Kaunas region due to changing of climatic conditions. According to the serological investigation 80% of tested herds are seroprevalent to *Toxoplasma gondii*. The antibodies to *T. gondii* had been detected in 52.63% of tested by DT goats sera. The prevalence to *T. gondii* were 50% higher in the older goats group than younger once ($P < 0.05$). The seroprevalence to TBE virus had been detected in 11.8% of tested goats' sera.

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