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Detection of tick-borne pathogens by molecular methods

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The use of molecular methods such as species-specific PCR, Multiplex-PCR, RT-PCR, reverse line blot hybridisation in investigations of tick-borne pathogens allowed to detect and identify the causative agents of Lyme borreliosis, anaplasmosis, and babesiosis in ticks and rodents in Lithuania and Norway. The overall prevalence of *Borrelia burgdorferi* s. l. infection detected in Lithuanian and Norwegian ticks was found to be 13.3% (223/1679) and 5.4% (75 /1383), respectively. A total 68 of 398 (17.1%) rodent ear extractions screened by PCR were found positive for *B. burgdorferi* s. l. infection. *B. burgdorferi* s. l. was detected in 53 of 428 (12.4%) immature *Ixodes ricinus* ticks collected on rodents in Lithuania and in 30 of 782 (3.8%) collected on rodents in Norway. In 24 of 173 (13.8%) ticks feeding on passerine migrating birds collected in Norway, *B. burgdorferi* s. l. pathogens were detected. Three clinically important species (*B. afzelii*, *B. garinii* and *B. burgdorferi s. s.*) were identified in ticks and rodents. *Anaplasma* sp. was detected in 5% of questing ticks and 19.6% of ticks collected from birds in Lithuania. *A. phagocytophilum* pathogens were detected in 7.1% of ticks from birds and in 93 of 1634 (5.7%) *I. ricinus* ticks collected from vegetation in Norway. To *Babesia divergens*, positive were 2% and 0.9% of questing ticks collected in Lithuania and Norway respectively.

Key words: tick-borne pathogens, *Ixodes ricinus*, PCR, molecular detection methods

INTRODUCTION

Over the past two decades, tick-borne diseases have increased and now constitute a major health problem in many parts of Europe and North America, accounting for tens of thousands of new cases yearly in both regions. The most common of these diseases are tick-borne encephalitis, Lyme disease, anaplasmosis and babesiosis. According to the data of World Health Organization, in Lithuania the incidence of tick-borne encephalitis and Lyme boreliosis has markedly increased since 1990 and is the highest in Northern Europe [1].

The study and identification of tick-transmitted pathogens is complicated as require investigation of all levels of the pathogen–host system – the pathogen, its competent vector and the reservoir host. Several methods have been developed for a direct detection of pathogens in infected vectors, host tissue, and clinical specimens from a patient. These include microscope-based assays, antigen detection assays, *in vitro* cultivation, and nucleic acid-based detection. However, detection methods such as culture isolation, dark-field microscopy and indirect antibody and antigen detection assays, are either time-consuming or prone to contamination, or have limited specificity and sensitivity [2]. Using only phenotypic methods, it is impossible to resolve genetic differences among the different species of pathogens. In the recent years, molecular detection methods based on PCR amplification of the DNA of the pathogen have been shown to be effective for the diagnosis of tick-borne diseases and for elucidation of their epidemiology. Various polymerase chain reaction-based molecular methods, such as nested PCR, RFLP/PCR, Multiplex PCR, Real-time PCR, Real-time multiplex PCR, DNA–DNA hybridization analysis, nucleic acid sequence analysis have shown an increasing significance in the detection and typing of bacterial pathogens and parasitic protozoa [3]. These above-mentioned detailed researches have been started recently and have successfully extended in different countries of the world. In Lithuania, scientific research studies dedicated to molecular detection of tick-borne pathogens in invertebrate vectors and vertebrate hosts start to develop. During the last five years, molecular detection and identification of causative agents of tick-borne encephalitis, Lyme boreliosis, anaplasmosis and babesiosis in ticks and their hosts was carried out [4–8].

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MATERIALS AND METHODS

During 2004–2007, detection of tick-borne pathogens was carried out in Lithuania and Norway.

Sample collection

A total of 3848 specimens of *Ixodes ricinus* ticks were collected from different locations; 2805 feeding ticks were removed from 398 live-trapped small rodents, and 816 of *I. ricinus* ticks were collected from 194 migrating passerine birds.

DNA extraction from ticks and rodent tissue samples

To prepare ticks and rodent tissue samples for PCR analysis, it is first necessary to extract nucleic acids. Extraction of DNA from ticks which contain little blood was carried out by lyses of ticks in ammonium hydroxide (NH_4OH) as previously described by

Ambrasiene et al. [5]. DNA from engorged ticks and from rodent tissue samples was extracted using the Genomic DNA Purification Kit (MBI Fermentas, Lithuania) according to the protocol suggested by the manufacturer.

Taxonomical identification of *I. ricinus* ticks by molecular methods

For an accurate taxonomic identification, because of difficulties caused by identification of immature stages or damaged mouthparts and the adjacent structures of ticks during removing them from hosts, molecular methods were used. In addition, in western Lithuania, the transpalearctic species *I. persulcatus* is overlapped by a typically European distribution of *I. ricinus*, and differences in the morphology of these two species are very small [9]. *I. persulcatus* was found only once in the northern part of Lithuania in 1972. Oligonucleotide primers Ixri-F: 5. GGA AAT CCC GTC GCA CG 3 and Ixri-R: 5. CAA ACG CGC CAA CGA AC 3 designed by A. Jenkins (A/S Telelab, Skien, Norway) on the basis of data on available genomes in GenBank (Accession N D88863.; [10]) were used in the PCR reaction. These primers amplify a 150 bp segment of the 5.8 s rRNA gene, which is specific of *I. ricinus* [10] (Fig. 1).

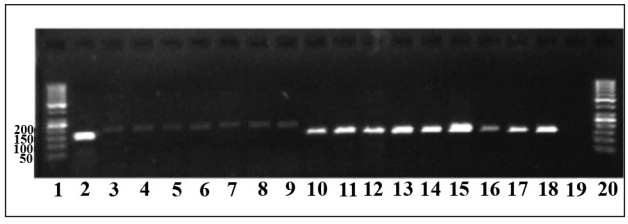

Fig. 1. Molecular taxonomical identification of *Ixodes ricinus* by PCR assay.
Lanes 1 and 20 – 50 bp marker; ane 19 – negative control; lanes 3–9 – negative results for larval ticks morphologically identified as *I. trianguliceps*: amplified other (200 bp) fragments; lanes 10–18 – positive results: amplified 150 bp specific fragment for *I. ricinus*; lane 2 – positive control of *I. ricinus* (150 bp)

Detection of *Borrelia burgdorferi* s. l. by species-specific PCR

Detection of *B. burgdorferi* s. l. infection was performed using direct PCR amplification of *B. burgdorferi* s. l. DNA from tick and rodent tissue material. As targets for amplification, fragments of *fla* gene (localized on chromosome; on the basis of data on available genomes in GenBank (AC X15660; [11]) and *OspA* gene (localized on lp-54 plasmid; on the basis of data on available genomes in GenBank (AC AE000790; [12]) in *B. burgdorferi* s. l. genome were used [2].

Amplification of *fla* gene fragment with FL primers

PCR was performed according to Stańczak et al. [13]. A 276-bp fragment in the conserved region of the chromosomal *fla* gene of *B. burgdorferi* was amplified using the following oligonucleotide primers: FL6 and FL7 (Roth, Germany). Plasmid with a specific 276 bp fragment of *B. burgdorferi* s. l. (Fermentas, Lithuania) was used as the positive control, and double-distilled water was used as the negative control in each PCR run. The PCR products were separated by electrophoresis on 1.5% agarose gel and visualized by UV transillumination. The obtained specific fragment of 276 base pairs was considered as a positive result (Fig. 2).

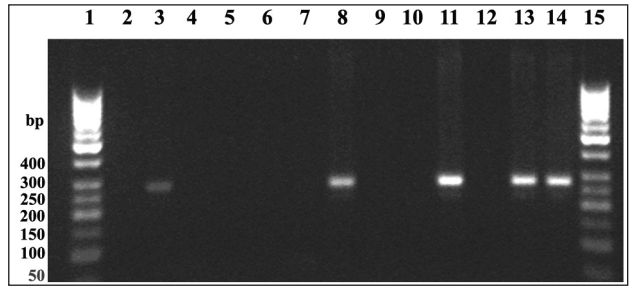

Fig. 2. PCR amplification of the *fla* gene of *Borrelia burgdorferi* s. l. from *Ixodes ricinus* lysates.
Lanes 1 and 15: 50-bp marker; lane 2: negative control; lanes 3, 8, 11, 13: positive tick samples (276-bp fragment); lanes 4–7, 9, 10, 12: negative tick samples; lane 14: positive control of *B. burgdorferi* s. l.

Amplification of *ospA* gene fragment with SL primers

PCR with SL-F and SL-R primers designed to amplify the plasmid-encoded *Borrelia ospA* gene was performed according to Demaerschalck et al. [14]. PCR amplification products were separated on 2% agarose gel and visualized by UV transillumination. The obtained specific products of 307 base pairs were considered as a positive result.

Multiplex PCR using for identification of three clinical important *Borrelia* species

For detection of *B. burgdorferi* s. s, *B. garinii* and *B. afzelii* species we used multiplex PCR with genospecies-specific primers GI-L/GI-R (*B. burgdorferi* s. s), GII-L/GII-R (*B. garinii*) and GIII-L / GIII-R (*B. afzelii*) designed by Demaerschalck et al. [14] based on differences existing in the *ospA* sequences of these three *Borrelia* species [15]. PCR amplification products were resolved onto 2.0% agarose gel electrophoresis and visualized under UV light. The specific products of 544 bp (*B. burgdorferi* s. s), 345 bp (*B. garinii*) and 189 bp (*B. afzelii*), were considered to represent positive results (Fig. 3).

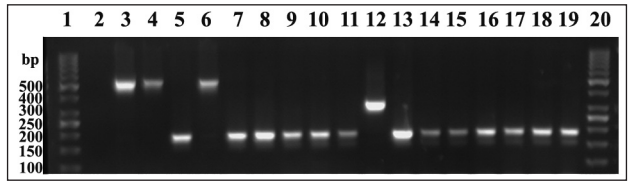

Fig. 3. Identification of *Borrelia burgdorferi* s.s., *B. garinii*, and *B. afzelii* in 2% agarose gel after PCR amplification of the *ospA* gene of *B. burgdorferi* s. l.
Lanes 1 and 20: 50-bp marker; lane 2: negative control; lane 3: positive control (544 bp); lanes 4 and 6: the presence of DNA bands indicates samples infected with *B. burgdorferi* s. s. (544 bp); lanes 5 and 7–11, 13–18: infected with *B. afzelii* (189 bp); lane 12: sample infected with *B. garinii* (345 bp); lane 19: positive control for *B. afzelii* (189 bp).

Detection of *Anaplasma* sp.

PCR screening

The presence *Anaplasma* group pathogens was determined by PCR with *Anaplasma*-specific primers EHR 521/747 of *16s rRNR* gene as described before [16, 17]. The specific PCR products of 247 bp obtained by agarose gel electrophoresis were considered as a positive result of *Anaplasma* sp. (Fig. 4).

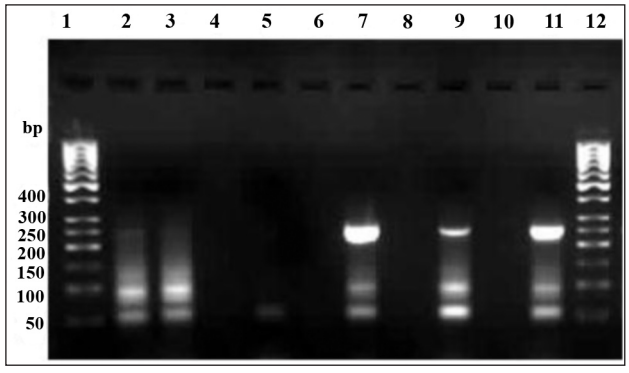

Fig. 4. PCR amplification of *16s rRNR* gene fragment of *Anaplasma* sp. with Ehr 521/747 primers from *I. ricinus* lysates.
Lanes 1 and 12: 50-bp marker; lane 4: negative control; lanes 9, 11: positive tick samples (247 bp fragment); lanes 2, 3, 5, 6, 8, 10: negative tick samples; lane 7: positive control of *Anaplasma* sp.

Reverse line blot hybridization

All positive *Anaplasma* sp. samples were re-amplified and labelled by PCR according to Schouls et al. [17] for *Anaplasma* species identification. These PCR products were used in a reverse line blot hybridization assay in which oligonucleotide probes are covalently linked to a membrane in parallel lines. In order to identify the species, the biotinylated *Anaplasma* PCR product was hybridized with seven different oligonucleotide probes in the reverse line blot assay. Hybridization of the samples with oligonucleotide probes on this membrane enabled a simultaneous detection and identification of Anaplasma species. The reverse line blot technique is a method for a simultaneous detection and identification of microorganisms such as ticks in field samples [17].

Detection of *A. phagocytophilum* by Taq Man based Real time PCR

The *A. phagocytophilum msp2* gene encodes a unique to *Anaplasma* species 44-kDa immunodominant outer membrane protein which was chosen as amplification targets in the analysis [17]. The species-specific primers ApMSP2f, ApMSP2r and the TaqMan probe ApMSP2p-FAM were used to amplify a 77-bp fragment of the *msp2* gene [18]. PCR was performed by using TaqMan® Universal PCR Master Mix (Applied Biosystems, USA) in a Bio-Rad quantitative thermal cycler. In all runs, both negative and positive controls were included. The results of analyses were evaluated according to the PCR Baseline Subtracted Curve (Fig. 5).

Use of Taq Man-based real time PCR using for detection of *Babesia divergens*

For detection the primers Bdi F, BdiR and TaqMan probe BdiT were used to amplify the 62 bp fragment in *18srRNA* gene of *B. divergens* [5, 19]. PCR was performed in a reaction volume of 30 µl using TaqMan Master Mix. The PCR conditions were: initial denaturation at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of a 15 s denaturation at 95 °C, followed by a 1 min annealing-extension step at 60 °C. The finial extension was at 72 °C for 2 min. The negative and positive controls were included in all runs.

RESULTS AND DISCUSSION

All ticks collected from vegetation were identified as *I. ricinus* by both morphological and molecular methods. Several larvae and nymphs collected from rodents by the molecular identification method, proved not to be *I. ricinus* (Fig. 1). They were identified as *I. trianguliceps* by the morphological keys.

***B. burgdorferi* s. l. in ticks and rodents**

The flagellin gene-targeted PCR analysis allows the detection of all borrelial infections irrespective of differences in the causative species because the flagellin gene is highly conserved among *Borrelia* species [2]. In this study, we also used amplification with *ospA* gene fragments. It was done because PCR targets carried on plasmids, such as *ospA*, *ospC*, are present in multiple copies within each bacterium, and it was expected that analysis with these targets could be more sensitive than that employing single-copy chromosomal targets [20]. In our study, we found a 95% similarity of results by using these two genes (*fla, ospA*) as targets in PCR amplifications of *B. burgdorferi* s. l. DNR. More sensitive were *ospA* gene-based primers (5% of *B. burgdorferi* s. l. infection was possible to detect only by using *ospA* gene primers).

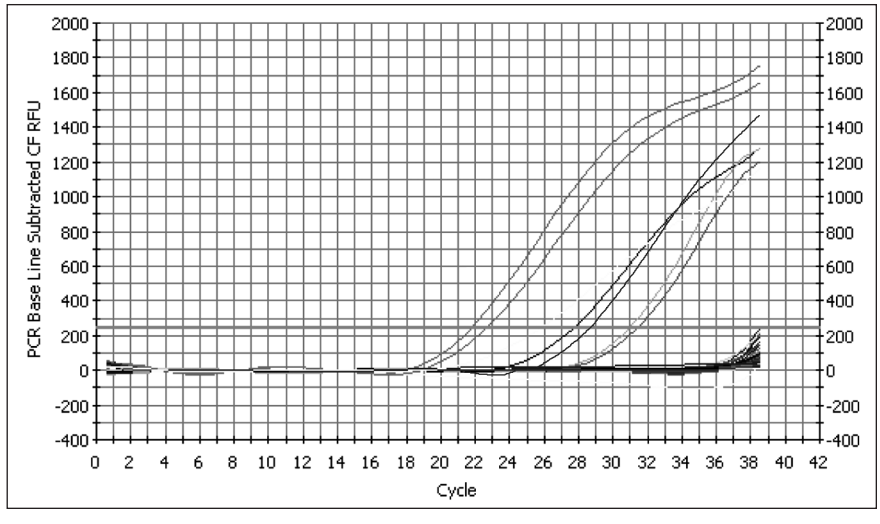

Fig. 5. PCR Baseline Subtracted Curve from TaqMan based RT-PCR (Cycle Graph for FAM-490) with ApMSP2f and ApMSP2r primers demonstrated positive (above baseline) and negative samples (below baseline) for *A. phagocytophilum*

Table. **The prevalence of tick-borne pathogens in *I. ricinus* ticks and rodents in Lithuania and Norway (N = number of tested ticks; n = number of infected ticks)**

	Borrelia burgdorferi s. l.			*B. afzelii*			*B. garinii*			*B. burgdorferi* s. s.			*Anaplasma* sp.			*A. phagocythophylum*			*B. divergens*		
	N	Prevalence		N	Prevalence		N	Prevalence		N	Prevalence		N	Prevalence		N	Prevalence		N	Prevalence	
		n	%		n	%		n	%		n	%		n	%		n	%		n	%
LITHUANIA																					
Questing *I. ricinus*	1679	223	13.3	190	143	76	190	19	10	190	14	7	240	12	5	240	6	2.5	383	8	2
Rodents	248	58	23.4	58	57	98.3	58	3	5.2	58	0	0	–	–	–	58	0	0	–	–	–
I. ricinus ticks from rodents	428	53	12.4	53	53	100	53	0	0	53	0	0	–	–	–	53	0	0	–	–	–
I. ricinus ticks from birds	56	0	0	56	0	0	56	0	0	56	0	0	56	11	19.6	56	0	0	–	–	–
NORWAY																					
Questing *I. ricinus*	1383	75	5.4	28	19	68	28	6	21	28	3	11	–	–	–	1634	93	5.7	224	2	0.9
Rodents	150	10	6.7	10	10	100	10	0	0	10	1	10	–	–	–	10	0	0	–	–	–
I. ricinus ticks from rodents	782	30	3.8	30	27	90	30	1	3.3	30	4	13.3	–	–	–	30	0	0	–	–	–
I. ricinus ticks from birds	173	24	13.8	24	0	0	24	19	76	24	1	4.2	668	185	27.7	668	48	7.1	–	–	–

A total of 3062 (1679 from Lithuania and 1383 from Norway) ticks collected from vegetation were investigated by PCR for the presence of *B. burgdoferi* s. l. The prevalence of *B. burgdorferi* s. l. in ticks varied from 0% to 29.21% in different locations. The overall prevalence of *B. burgdorferi* s. l. infection detected in Lithuanian ticks was found to be 13.3% (223/1679) and in Norwegian ticks 5.4% (75/1383) (Table).

A total 68 of 398 (17.1%) rodent ear extractions screened by PCR were found positive for *B. burgdorferi* s. l. infection. *B. burgdorferi* s. l. was detected in 53 of 428 (12.4%) immature *I. ricinus* ticks collected on rodents in Lithuania and in 30 of 782 (3.8 %) collected on rodents in Norway (Table).

In 24 of 173 (13.8%) ticks feeding on passerine migrating birds collected in Norway, *B. burgdorferi* s. l. pathogens were detected (Table).

For the detection of *B. burgdorferi* s. l. in ticks it is possible to use the hemolymph test [21]. As bacteria may invade and propagate in all organs and fluids of ticks, their detection in the hemolymph or salivary glands is relatively simple. *Borrelia* also may be seen by dark field microscopy, and they stain well with various aniline dyes. Immunodetection methods are also available for the detection of *Borrelia*, *Anaplasma*, and *Francisella tularensis* [22]. But all of these methods seem to be time-consuming or prone to contamination, or have a limited specificity and sensitivity.

Studies of *Borrelia* detection in Lithuanian ticks had been performed since 1987 by dark-field microscopy. Since 2001, the prevalence of *B. burgdorferi* s. l. infection in *I. ricinus* has been determined by molecular methods [5]. Using the dark-field microscopy method, sometimes the microorganisms seen in dark-field-positive ticks may represent species other than *B. burgdorferi* s. l. [23]. Furthermore, the PCR have proven to be a specific and sensitive method of detecting the species-specific agent of Lyme borreliosis in infected ticks.

Two methods are often used for detecting bacterial tick-borne pathogens in a vertebrate host: bacterium isolation by BSK cultivation of tissue samples and pathogen DNA detection in host tissue after PCR amplification. The isolation has the disadvantage of being a fastidious and not so sensitive technique. In contrast, PCR amplification has the advantage of being very sensitive, allowing detection of 1–10 spirochetes.

B. burgdorferi s. l. genospecies identified in *I. ricinus* ticks and rodents

Data from a number of molecular and phenotypic studies resulted in the subdivision of the *B. burgdorferi* s. l. complex into different taxonomic entities named genospecies or genotypes [24, 25]. According to the latest data, the *B. burgdorferi* s. l. complex is subdivided into 12 species. Three genospecies, commonly associated with human infection, include *B. burgdorferi* s. s. (distributed mostly in North America), *B. afzelii* (distributed in Western Europe, Central Europe and Russia), and *B. garinii* (distributed in Europe, Russia and northern Asia).

B. afzelii has been found as the most dominant 76% (143) genospecies in questing *I. ricinus* ticks collected from various localities in Lithuania. *B. garinii* has been identified in 10% (19) and *B. burgdorferi* s. s. in 7% (14) of the tick lysates examined (190) (Table). Double infections have been observed in 1% of the

infected ticks. The other 12 samples (6%) of *Borrelia* infections haven't been identified to the genotype level by this method.

The genotyping of *B. burgdorferi* s. l. in ticks which had fed on rodents collected in Lithuania showed that 100% of the infected ticks harboured only *B. afzelii*. In the majority of ticks which had fed on rodents collected in Norway we also detected *B. afzelii* species. Double infections (*B. afzelli* with *B. garinii* or *B. burgdorferi* s. s.) have been found in 12% of the infected ticks.

B. garinii was found to be dominant genospecies in *I. ricinus* ticks collected from birds in Norway. *B. garinii* was been identified in 76% (19/24) of ticks, and double infections (*B. garinii* and *B. burgdorferi* s. s.) were observed in 4% (1/24) of the infected ticks (Table). Three samples (12.5%) of *Borrelia* infections haven't been identified to the species level by this method.

As the analysis has shown, for identification of *B. burgdorferi* s. l. species, which were not identified in our study, other methods are needed. Sequencing of PCR product is used for genotyping of *Borrelia* species [26] and for estimating the true rate of mixed infections [27]. Schouls et al. [17] detected *Borrelia* genospecies in Dutch *I. ricinus* using the reverse line blot hybridization method.

***Anaplasma* sp. in ticks**

Ehrlichioses and anaplasmoses have been known for a long time in veterinary medicine. However, in recent years, three bacteria have been recognized as emerging tick-borne pathogens in humans: human monocytic ehrlichiosis due to *Ehrlichia chaffeensis*, *Ehrlichia ewingii* granulocytic ehrlichiosis, and human granulocytic anaplasmosis (formerly human granulocytic ehrlichiosis) due to *A. phagocytophilum* (formerly named the HGE agent, *E. phagocytophila* and *E. equi*) [28, 29]. According to the new systematic classification based on genetic analysis of 16S rRNA, *groESL* and surface proteins, the former *Ehrlichia phagocytophila*, *Ehrlichia equi* and the human granulocytic ehrlichiosis agent are currently assigned to the same species, *A. phagocytophilum*, because of their genetic similarity [28].

Numerous PCR-based assays and primer sets for the detection of *A. phagocytophilum* have been reported [30]. For the detection of *Anaplasma* group pathogens in ticks, were used PCR with primers Ehr521/Ehr747 which are very sensitive. Although PCR assays have been shown to be sensitive and specific, they are time-consuming and labour-intensive, particularly when testing for pathogens in a large number of samples. An innovation in PCR technology has been the development of equipment and techniques that allow the monitoring of PCR in real time. Monitoring in real time removes the need for electrophoretic detection of amplified products and simplifies quantitative PCR by allowing the identification of the cycle at which amplification starts. The development of fluorescence-based reporter probe systems has improved the sensitivity and specificity of real-time PCR. Real-time PCR have been used for the detection of tick-borne pathogens like *Borrelia, Anaplasma, and Babesia* [18, 31]. The TaqMan based RT-PCR assay for the detection of *A. phagocytophilum* used in the present study eliminates the need for electrophoretic analysis of amplified products and the second-round of amplification. The specific primers amplify the conserved amino-terminal coding region of the *msp2* gene and amplify the multiple copies of the gene that are in the *A. phagocytophilum* genome for increasing the sensitivity [18]. So, the characteristics of this assay made it suitable for population studies and a large-scale testing.

In the present study, screening of tick lysates by PCR with primers specific to *Anaplasma* sp. Ehr521/Ehr747 demonstrated positive results in 12 (5%) samples of Lithuanian questing ticks (Table). *Anaplasma* sp. – *A. phagocytophila*, HGE variant and *E. schotti* – has been determined by using the reverse line blot assay. According to PCR with Ehr521/Ehr747 primers, a total of 19.6% (11/56) of ticks collected from birds in Lithuania and 27.7% (185/668) in Norway were found to be infected with *Anaplasma* sp. (Table). However, using TaqMan based RT-PCR and specific APMSP2f/r primers, *A. phagocytophilum* pathogens were detected only in 7.1% of ticks from birds collected in Norway, but not in ticks from Lithuania. In addition, by using the same TaqMan-based RT-PCR method, in 93 of 1634 (5.7%) questing *I. ricinus* ticks collected from vegetation in Norway, *A. phagocytophilum* pathogens were detected (Table).

***B. divergens* in ticks**

The real-time PCR method with primers and probe specific of *B. divergens* showed positive results in 8 (2%) of 383 Lithuanian ticks and in 2 (0.9%) of 224 Norwegian ticks used for the analysis (Table).

CONCLUSIONS

To understand the epidemiology of tick-transmitted diseases, the interaction among the pathogen, vectors and vertebrate hosts should be investigated. Various analytical methods must be validated for each host species, for each tissue type and for each genospecies of pathogens.

The use of molecular methods such as species-specific PCR, Multiplex-PCR, RT-PCR, reverse line blot hybridisation and sequencing in investigations of tick-borne pathogens allowed to detect and identify the causative agents of Lyme borreliosis, anaplasmosis, and babesiosis in ticks and rodents in Lithuania and Norway. Using molecular detection methods, the role of small rodents as zoonotic reservoirs of *B. burgdorferi* s. l. and the role of birds in the dissemination of *B. burgdorferi* s. l. and *A. phagocytophilum* pathogens in different ecological and biogeographical zones of Lithuania and Norway have been determined. Such investigations of the prevalence of tick-borne pathogens in vectors and reservoir hosts assessed the risk of infection in the human population and explained the circulation of these pathogens in nature.

Although in Lithuania HGA and babesiosis have so far not been diagnosed in humans, findings that ticks in Lithuania are infected with *A. phagocytophilum* and *B. divergens* confirm data that *I. ricinus* might be also involved in the circulation of this parasite in Europe [32] and the possible risk of transmitting these infections to humans.

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ERKIŲ PERNEŠAMŲ PATOGENŲ NUSTATYMAS MOLEKULINIAIS METODAIS

Santrauka

Skirtingais molekuliniais tyrimo metodais (PGR, Multiplex-PGR, RT-PGR, sekvenavimas) buvo nustatyti įvairūs erkių pernešami patogenai – Laimo boreliozės, anaplazmozės ir babeziozės sukėlėjai erkėse ir graužikuose iš Lietuvos ir Norvegijos. Lietuvoje bendras erkių, surinktų nuo augalų, užkrėstumas *B. burgdorferi* s. l. buvo 13,3% (223/1679), Norvegijoje – 5,4% (75 /383). *B. burgdorferi* s. l. nustatyta 53 iš 428 (12,4%) *I. ricinus* erkių, rastų ant graužikų Lietuvoje, ir 30 iš 782 (3,8%) Norvegijoje. Nustatyta, kad migruojantys paukščiai perneša *B. burgdorferi* s. l. užsikrėtusias erkes (24 iš 173 (13,8%)). Atlikus graužikų ausų ekstraktų PGR analizę, teigiama *B. burgdorferi* s. l. infekcija nustatyta 68 iš 398 (17,1%) graužikų. Rastos trys žmogui patogeniškos borelijų rūšys (*B. afzelii*, *B. garinii* ir *B. burgdorferi s.s.*) erkėse ir graužikuose. Tag Man RT-PGR metodu nustatyta, kad Lietuvoje 2,5 % erkių, rastų ant augalų, užsikrėtusios *Anaplasma phagocytophylum*, o Norvegijoje – 5,7% (93 iš 1634) erkių ant augalų bei 7,1% erkių ant paukščių.