MOLECULAR DETECTION AND CHARACTERIZATION OF *BORRELIA BURGDORFERI* SENSU LATO IN SMALL RODENTS

Jana Radzijevskaja[1], Algimantas Paulauskas[1], Olav Rosef[1,2]
[1]*Vytautas Magnus University, Department of Biology, Vileikos str. 8, Kaunas, LT-44404, Lithuania*
tel.: +37037327902; fax: +37037327916. E-mail: a.paulauskas@gmf.vdu.lt
[2]*ATP-Innovation AS, 3800 Bø i Telemark, Norway*

Summary. Lyme borreliosis (LB) caused by the spirochete *Borrelia burgdorferi* s.l. is the most frequently diagnosed tick-borne zoonozis in Europe, North America, and Asia. *B. burgdorferi* s.l. can infect humans and wild and domestic animals. Small rodents are the most important reservoir host of *B. burgdorferi* s.l. The aim of present study was to detect f *B. burgdorferi* s.l. in different tissue samples of small rodents and identify *Borrelia* genospecies and strains using different molecular markers and detection methods. Flagellin (*fla*) and outer surface protein A (*osp*A) encoding genes of *Borrelia* genome were used as targets for PCR amplification. We found that the *osp*A gene was a more sensitive marker for the detection of *B. burgdorferi* s.l. than *fla* gene. The presence of *B. burgdorferi* s.l. in urinary bladder, spleen and ear biopsy samples of 136 small rodents was compared. *B. burgdorferi* s.l. infection was detected with different rates in ear and bladder tissues, but was not found in spleen samples. Multiplex PCR assay based on *osp*A gene was used for identifying *B. burgdorferi s. s.*, *B. afzelii*, and *B. garinii* genospecies. *B. afzelii* was a single genospecies detected in small rodents from Norway and was predominant in rodents from Lithuania. *B. garinii* strains from rodents were identified by nucleotide sequencing of PCR products. Phylogenetic relationship between *B. garinii* strains and their correspondence to OspA serotype types were compared with the sequences registered in GenBank database. *B. garinii* strains detected in the present study showed similarities with sequences of OspA serotype 5 type and OspA serotype 6 type.

Keywords: Lyme borreliosis, rodents, *B. burgdorferi* s.l., detection, PCR, sequencing.

BORRELIA BURGDORFERI S. L. BAKTERIJŲ MOLEKULINIS NUSTATYMAS IR CHARAKTERIZAVIMAS SMULKIUOSE GRAUŽIKUOSE

Jana Radzijevskaja[1], Algimantas Paulauskas[1], Olav Rosef[1,2]
[1]*Biologijos katedra, Vytauto Didžiojo universitetas, Vileikos g. 8, Kaunas, LT-44404*
tel. +370 373 27 902; faks. +370 373 27 916; el. paštas: a.paulauskas@gmf.vdu.lt
[2]*ATP-Innovation AS, 3800 Bø i Telemark, Norvegija*

Santrauka. Laimo boreliozė, kurios sukėlėjai yra *Borrelia burgdorferi* sensu lato bakterijos, – multisisteminė erkių pernešama zoonozė, plačiai paplitusi Europoje, Šiaurės Amerikoje ir Azijoje. *B. burgdorferi* s. l. bakterijomis gali užsikrėsti žmonės, laukiniai ir naminiai gyvūnai. Tyrimo tikslas – nustatyti *B. burgdorferi* s. l. skirtinguose smulkiųjų graužikų audiniuose, identifikuoti *Borrelia* rūšis ir padermes, taikant skirtingus molekulinius žymenis ir metodus. Bakterijų DNR aptikti buvo naudojama PGR, kurios metu buvo dauginami flagelino (*fla*) ir išorinio paviršinio baltymo (OspA) genų žymenys. Efektyvesni ir jautresni aptinkant borelijas graužikų audiniuose buvo OspA geno žymenys. Palygintas *B. burgdorferi* s. l. paplitimas šlapimo pūslės, ausų ir blužnies audiniuose tarp 136 smulkiųjų graužikų. *B. burgdorferi* s. l. DNR buvo aptikta skirtingų dažnių ausų ir šlapimo pūslės mėginiuose, bet nerasta blužnies mėginiuose. *B. afzelii* ir *B. garinii* rūšys buvo identifikuotos su dauginės PGR pagalba, padauginus specifinius kiekvienai rūšiai OspA geno fragmentus. *B. afzelii* rūšis vyravo užsikrėtusiuose graužikuose iš Lietuvos ir buvo vienintelė rūšis, rasta graužikuose iš Norvegijos. *B. garinii* padermės buvo identifikuojamos atliekant sekvenavimą. Filogenetiniai ryšiai tarp *B. garinii* padermių ir jų atitikimas OspA serotipus buvo įvertinti palyginus su sekomis, registruotomis genų duomenų banke. Nustatyta, kad *B. garinii* padermės, identifikuotos šio tyrimo metu, buvo artimos OspA 5 ir OspA 6 serotipo sekoms.

Raktažodžiai: Laimo boreliozė, graužikai, *B. burgdorferi* s. l. nustatymas, PGR, sekvenavimas.

Introduction. Over the past two decades, tick- borne zoonoses have increased and now constitute a complex and diverse risk to humans and animals in the world. LB caused by the spirochete *Borrelia burgdorferi* s.l. is the most frequently diagnosed tick-borne zoonosis in Europe, North America, and Asia. To date, *B. burgdorferi* s.l. can be divided into at least 17 species (Margos et al., 2009; Vollmer et al., 2011). However, only *B. afzelii, B. garinii* and *B. burgdorferi* sensu stricto certainly are involved in clinical cases of LB in humans and mammals in Europe. The studies on different chromosomal and plasmid genetic loci discovered that each *Borrelia* species comprises a variety of strains (Wilske et al., 1993; Bunikis et al., 2004; Margos et al., 2009). It has been shown that specific associations exist between *Borrelia* species, reservoir hosts and different clinical manifestations of Lyme disease. *B. afzelii* predominates in the skin especially in cases of acrodermatitis chronica atrophicans (ACA), *B. burgdorferi* s.s is. associated with arthritis and *B. garinii* with neuroborreliosis in humans (van Dam et al., 1993; Ornstein et al., 2001;). *B. afzelii* is mainly associated with small rodents, while *B. garinii*

with birds. *B. burgdorferi* s.s. was found in both birds and rodents (Humair and Gern, 2000). However, *B. garinii* is very heterogeneous and some strains have also been identified in tissues from rodents (Ishiguro et al. 1996; Korenberg et al. 1997; Richter et al. 1999; Hanincova et al. 2003; Margos et al., 2009). The *ospA* gene of *B. garinii* is the most heterogenic. Based on genetic and immunological heterogeneity of the OspA protein from European strains, seven serotypes were isolated, and five of them 3, 4, 5, 6 and 7 constitute *B. garinii* (Wilske et al., 1993; 1996).

Tick-borne zoonoses occur in particular habitats with optimal conditions for the ticks and animals involved in the circulation of the pathogens. The sheep tick, *I. ricinus*, is the main vector for *B. burgdorferii* s.l. in Europe, and the taiga tick *I. persulcatus* in Eurasia. All developmental stages of these ticks are capable of acquiring and transmitting the spirochete between hosts. A variety of mammalian species (the majority of these are rodents) and birds are known to be reservoir hosts for *B. burgdorferi* s.l. (Gern et al., 1998). Wild cervids (roe deer and red deer) and other domesticated ungulates (sheep, cattle and goats) are known to be incompetent reservoirs for *B. burgdorferi* s.l., but they involved in the eco-epidemiology of borreliosis as hosts for the ticks (Kurtenbach et al., 2002; Rosef et al., 2009). Among domesticated animals, *B. burgdorferi* s.l. could cause recognizable signs of disease in dogs, horses and cattle (Blowey et al., 1994; Bushmick et al., 1994). The initial stage of LB in dogs, horses and cattle is associated with mild fever, slight depression, fatigue, muscle soreness and reduced appetite. In acute LB, lameness with pain in one or more joints is common. Horses show arthritis and an affection of the heart, more rarely infections of the eyes and encephalites (neuroborreliosis). Abortion, laminitis and chronic weight loss have been identified in cattle and horses. Neurologic sign and blindness have been reported in horses (Bushmick et al., 1994; Štefančíková et al., 2008; Durrani et al., 2011).

Diagnosis of LB depends on the recognition of clinical signs and identification of the spirochete in the affected animal. After injection of *B. burgdorferi* s.l. by the tick, the spirochete initially establishes a local infection in the skin at the site of the tick bite. Within day to weeks, the spirochete can disseminate via the bloodstream to many sites and establishes persistent infection in various tissues (skin, joints, heart, spleen and urinary bladder) (Rosa et al., 2005).

A variety of serologic tests are currently in use for the detection of LB, such as the enzyme-linked immunosorbant assay, indirect fluorescent antibody test, and the Western immunoblot. These methods only indirectly detect the host or patient's response to the pathogen. Serological evidence of *B. burgdorferi* s.l. infection in cattle has been reported in North America, Australia and Europe (Štefančíková et al., 2008; Durrani et al., 2011). Recent European studies showed that up to 30-40% of horses in an endemic area are seropositive for *B. burgdorferi* s. (Marie et al., 2005; Bhide et al., 2008). Antibodies against *B. burgdorferi* s. l. were found in sheep (Fridriksdottir et al., 1992). Spirochetes have been detected from the blood and urine of cows.

The polymerase chain reaction (PCR) assay has facilitated the diagnosis of LB. This method has been shown to be sensitive, rapid, and accurate for detection and genotyping of *Borrelia* spirochetes. A variety of genomic marker has been developed to amplify *B. burgdorferi* s.l. DNA by PCR from host and patient samples.

LB is the most common tick-borne infection in Lithuania, as in other European countries, and risk assessment prevention and control of disease, cannot be achieved without precise data on the occurrence of pathogens in ticks and rodent reservoirs. Since LB caused by several species and each *Borrelia* species comprises a variety of strains, the molecular identification and characterization of each strain is essential.

The aim of the present study was to detect f *B. burgdorferi* s.l. in different tissue samples of small rodents and identify *Borrelia* genospecies and strains using different molecular markers and detection methods.

Material and methods

Sample collections and preparation for molecular analysis

Habitat type and biogeographical region influence prevalence of *B. burgdorferi* s.l. in hosts (Humair and Gern, 2000), so the present study was performed in different habitat types in Lithuania and Norway. Small rodents were captured in deciduous and mixed forest, rocky, and ecotonal areas in Lithuania and Norway (permission to trap wild small mammals provided according to regulation No. 586 (2002-11-11) of Ministry of the Environment of the Republic of Lithuania) during the period from June to October 2005-2006. Rodents were live-trapped with own constructed wooden traps baited with sunflower oiled bread. Animals were identified to species level and their sex was recorded. A total of 135 small rodents belonging to 8 species of 2 families (Muridae and Cricetidae) were collected in Lithuania and Norway (Table 1). Ears, urinary bladder, and spleen samples were taken from each rodent and separately placed into 1.5 ml microcentrifuge tubes with 70% ethanol solution and stored at 4°C until processed. The scissors and tweezers used for sampling were dipped in 70% ethanol, flamed and quenched to avoid cross contamination.

DNA extraction

DNA from tissue samples (ears, urinary bladders and spleens) of 135 small rodents was extracted using Genomic DNA purification Kit (MBI Fermentas, Lithuania) according protocol that suggested by the manufacturer with some modifications. Rodent tissue samples were removed from the tubes with 70% ethanol and dried on sterile filter paper, and then they were either cut into small pieces using sterile scissors (spleens, urinary bladders) and disrupted using a homogenizer, or pulverized in liquid nitrogen (ears) with mortar and pestle. After these preparations samples were resuspended in 2 ml microcentrifuge tube with TE buffer, mixed with lysis solution and incubated at 65°C. Incubation time

ranges from 20 min (for spleen samples) to 60 and 90 min (for ear and urine bladder samples, respectively). Extracted DNA was dissolved in sterile deionised water. DNA concentration was measured spectrophotometrically. Samples were stored at -20°C until used as templates for PCR.

Detection of *B. burgdorferi* s.l.

Detection of *B. burgdorferi* s.l. infection was performed using direct PCR amplification of *B. burgdorferi* s.l. DNA from rodent tissue material. We used about 300 ng of extracted DNA in PCR amplification. As targets for amplification fragments of *fla* gene (localized on chromosome on the basis of data from GenBank AC X15660) and *ospA* gene (localized on lp-54 plasmid on the basis of data available in GenBank AC AE000790) in *B. burgdorferi* s.l. genome were used (Picken, 1992; Demaerschalck et al., 1995; Stańczak et al., 1999).

Amplification of fla gene with FL primers. A 276-bp fragment in conserved region of the chromosomal *fla* gene of *B. burgdorferi* (Picken, 1992) was amplified by using the following oligonucleotide primers: FL6 and FL7 (Roth, Germany) as described previously (Paulauskas et al., 2008).

Amplification of ospA gene with SL primers. PCR was performed using primers SL designed to amplify 307 bp fragment DNA of all genospecies of *B. burgdorferi* s. l. complex (Demaerschalck et al., 1995). Reaction volume of 25 µl contained 12.5 µl Multiplex PCR Master Mix with HotStart Taq Polymerase (Qiagen, USA), 10 pmol of each primer (SL-F and SL-R) (Roth, Germany), 8 µl double-distilled water. In each PCR run were used positive (DNA of positive ticks) and negative controls (double distilled water). PCR conditions were the following: 95° C for 15 min (for Taq Polymerase activation), followed by 40 cycles of 94°C for 20 sec; 63°C for 20 sec; and 72°C for 30 sec. Final extending was at 72°C for 2 min. The PCR products were separated by electrophoresis in 1.5% agarose gel with addition of ethidium bromide. 0.5 x Tris-Borate-EDTA (pH 8.2) was used as a running buffer. Gene Ruler^{TM} 50 bp marker (Fermentas, Lithuania) was applied for evaluation of the mass of the obtained product. The results of the PCR were visualized by UV transillumination (EASY Win32, Herolab, Germany).

Detection *of B. burgdorferi* s.l. genospecies

Based on the pronounced differences in the *ospA* sequences of *B. burgdorferi* s.s., *B. garinii*, and *B. afzelii* (Fukunaga et al., 1996) three species-specific primer sets (GI, GII, GIII) were designed by Demaerschalck et al. (1995) for identification of these species. We used these primer sets in Multiplex PCR for identification of *B. burgdorferi* s.l. genospecies (Demaerschalck et al. 1995; Paulauskas et al. 2008).

Sequencing of the *B. garinii ospA* gene

After purification of PCR product with a Nucleospin extract II purification Kit (Macherey-Nagel, GmbH & Co, Germany), the nucleotide sequences of 3 strains of *B. garinii* detected in small rodents and 4 strains previously detected in *I. ricinus* ticks from vegetation and small rodents were sequenced. Primers for sequencing were identical to initial PCR primers (GII-L/GII-R). BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) for PCR products sequencing reaction according to manufacturer recommendations was used. The sequencing product were cleaned by ethanol precipitation and applied to an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Nucleotide sequence access numbers

Partial sequences of the *ospA* gene were submitted to the GenBank under the following accession numbers: HM069329 (M.mus); HM119227 (K18); HM119228 (K38); HM119229 (Mi.arv); HM119230 (My.gla); HM119231 (J29), HM119232 (Tj 22-3). Strains of serotypes described for *B. garinii* (Wilske et al. 1993; Escudero et al. 2000) submitted in GenBank were included in phylogenetic analysis: WABSou (GenBank accession number X85441), PHei (X80251), N34 (Y10894), G25 (Z29086), T25 (X80254), and PBi (X80257).

The obtained sequences were edited using the BioEdit and Mega4 programs and aligned with *B. garinii ospA* gene sequences registered in GenBank database. A phylogenetic tree was constructed by the neighbour joining method.

Results and discussion

PCR detection

B. burgdorferi s.l. was detected with different prevalence in different species of small rodents and with higher rate in samples from Lithuania, than in Norway (Table 1). PCR analysis with *fla* gene target produced 31 positive DNA samples, and with *ospA* gene 32 positive samples. After a comparison of intensity of PCR bands amplified with *fla* and *ospA* genes we observed, that amplified fragments of *ospA* gene were more intensive, so have a higher amount of amplified PCR product than amplified fragments of *fla* gene.

Both plasmid and chromosomal targets used in this study have its advantages. The flagellin gene-targeted PCR analysis allows the detection of all borrelial infections irrespective of differences in the causative species because the *fla* gene is highly conserved among *Borrelia* species (Picken, 1992). As described in literature, targets carried on plasmids, such as *ospA* are present in multiple copies within each bacterium. Targets carried on plasmids, such as *ospA* are present in multiple copies within each bacterium. Our study found *ospA*-based amplification more suitable and confirmed the evidence, that *ospA* targets are more sensitive in detection of *Borrelia* DNA, than those single-copy chromosomal targets (Reed, 2002).

***B. burgdorferi* s.l. detection in different rodent tissue**

B. burgdorferi s.l. infection was detected in ears and bladders tissues, but not in spleen. From 135 paired comparisons of bladder and ear samples, 10 were positive for both ears and bladders samples, 22 were positive only for ears and 1 was positive only for bladder (Table 1).

Table 1. **A comparative analysis of ear and urinary bladder tissues for the detection of *B.burgdorferi* s.l in different species of rodents**

Collection area	Rodent species	Total number of tested	Detection of *B.burgdorferi* in rodent tissues*			
			Number ear+/ *bladder+*	Number ear+/ *bladder-*	Number ear-/ *bladder+*	Number of positive Combined data (prevalence)
Lithuania	*Myodes glareolus*	20	2	3	0	5 (25.0%)
	Microtus arvalis	14	5	3	0	8 (57.0%)
	Microtus agrestis	9	0	4	0	4 (44.0%)
		43	*7*	*10*	*0*	*17 (39.5%)*
	Apodemus flavicollis	21	1	8	0	8 (38.0%)
	Apodemus sylvaticus	1	0	0	0	0
	Apodemus agrarius	19	0	2	0	2 (10.5%)
	Rattus norvegicus	1	0	1	0	1
	Mus musculus	1	1	0	0	1
		43	*2*	*11*	*0*	*12 (27.9%)*
	Total	86	9	21	0	29 (33.7%)
Norway	*M. glareolus*	6	0	0	0	0
	M. agrestis	1	0	1	0	1
	A. sylvaticus	41	1	0	1	2 (4.8%)
	A. flavicollis	1	0	0	0	0
	Total	49	1	1	1	3 (6.1 %)

*Ear+/ *bladder+* indicate positive PCR results for ears and bladders. Ear+/ *bladder* indicates a positive ear but negative bladder, and ear-/ *bladder+* indicates a negative ear and positive bladder.

Bladder samples from rodents in Lithuania yielded significantly less positive results (9 bladder and 30 ear positive samples from 86 tested rodents; χ^2=9.31, df=1, p=0.0023) than ear samples. These results suggest difference in the densities pathogens in ear and bladder tissue and showed that ear samples proved more sensitive test for detecting *Borrelia* than urinary bladder samples. The comparison of voles with mice by double positive sample pairs and pairs showing different results indicates that in voles more often was detected both, localized (in ear) and disseminated (in bladder) *Borrelia* infection than in mice. In tissue samples taken from mice, *Borrelia* infection in ears was detected in higher prevalence, than in bladders.

Ears samples from rodents in Norway showed almost equal sensitivity to bladder samples. However, the overall prevalence of *B. burgdorferi* s.l. in rodents from Norway was much lower than they from Lithuania, but the number of comparisons was too low for conclusion about sensitivity of detection in different tissue samples.

Rodent ears are the zone were ticks mostly feeding. The peripheral margins and the bases of ears are the primary sites for feeding of *Ixodes* ticks (Hofmeister et al., 1992). Primary infection is localised near ticks bite site. In case of systematic infection, *Borrelia* pathogens have disseminated in different organs from the site of tick bite and can be detected in blood and different internal organs of host. The diagnostic tests for detection of *B. burgdorferi* s.l. in vertebrate tissues are still improving. The ear biopsy method is an efficient technique for obtaining tissue samples from naturally infected rodents for detecting *B. burgdorferi*, as this method could not require the removal of hosts from the natural population.

Our data confirmed that the estimated prevalence of *B. burgdorferi* in rodents can differ depending on the biopsy method used, rodent species and localities. Another study conducted in Central Europe (Petney et al., 1996) showed that ear biopsy samples led to a significantly lower estimate of infection than bladder biopsy samples. However, study conducted in United States showed that ear biopsy samples were almost equal by sensitivity to bladder biopsy samples for determining prevalence of *B. burgdorferi* in *Peromyscus leucopus* populations (Sinsky and Piesman 1989; Hofmeister et al., 1992).

Using of different biopsy methods helps in correct estimation of the prevalence of *Borrelia* infection in rodent population and determination of pathogen dissemination in individual rodent.

***B. burgdorferi* s.l. genospecies**

Genotyping of *B. burgdorferi* s.l. revealed that *B. afzelii* was the single genospecies detected in rodents from Norway. In Lithuania almost all rodents harboured only *B. afzelii,* but *B. garinii* was detected in three specimens: *M. musculus, M. glareolus* and *M. arvalis. M. arvalis* harboured both, *B. afzelii* and *B. garinii*. In Europe mostly only *B. afzelii* is associated with small rodents, but some studies conducted in Europe and Asia showed associations of wild rodents with *B. garinii* (Nakao et al., 1994; Korenberg et al. 1997; Richter et al. 1999; Masuzawa et al. 1997, 2001; Hanincova et al. 2003).

***B. garinii* strains**

Different *B. garinii OspA* strains have different pathogenicy and have been detected in different tissue of

humans and animals. Earlier studies have discovered that cerebrospinal fluid (CSF) isolates associated mostly with *B.* garinii (OspA 4-5 serotype). OspA type 7 (T25) and 6 (G25, N34) strains have previously been isolated from a ticks (Marconi et al., 1999; Wiske et al., 1993; Wiske et al., 2007). Since OspA type 6 strains are less frequent observed in patient, they seems to be a low pathogenic Some studies conducted in Europe have showed that *B. garinii* OspA serotype 4 strains are associated with rodents. These strains have rarely been cultivated from ticks, but frequently cultivated from CSF of patients with neuroborreliosis (Hu et al., 2001; Marconi et al., 1999; Wiske et al., 1993; Wiske et al., 2007). OspA serotype 5 strains (PHei, WABSou) have been detected in skin and CSF. According to Wilske et al. (1993), serotype 5 OspA resulted from genetic recombination of serotype 4 and 6 *ospA* genes.

In present study, the sequencing of the amplified 345 bp fragment of *ospA* gene confirmed the identification of *B. garinii*. To investigate the heterogeneity of *ospA* gene sequence of *B. garinii* we including in phylogenetic analysis the strains from rodents (n=3) and additionally strains (n=4) from *I. ricinus* ticks. The infected ticks were collected from vegetation in Lithuania from the same place where *B. garinii* infected rodents were captured (n=2), and in Norway from ticks feeding on rodents (n=2). To investigate the correspondence of strains to OspA serotype types, strains (n=6) of serotypes described for *B. garinii* submitted in GenBank were also included in phylogenetic analysis (Wilske et al. 1993; Escudero et al. 2000).

The alignment and phylogenetic analyses of sequences showed that *B. garinii* strains in *I. ricinus* ticks from Lithuania and Norway were identical (100% similarity). Strains from the rodents were heterogeneous and differed from strains detected in *I. ricinus* ticks (pairwise distances ranges from 0.9 to 3 %) (Fig. 1).

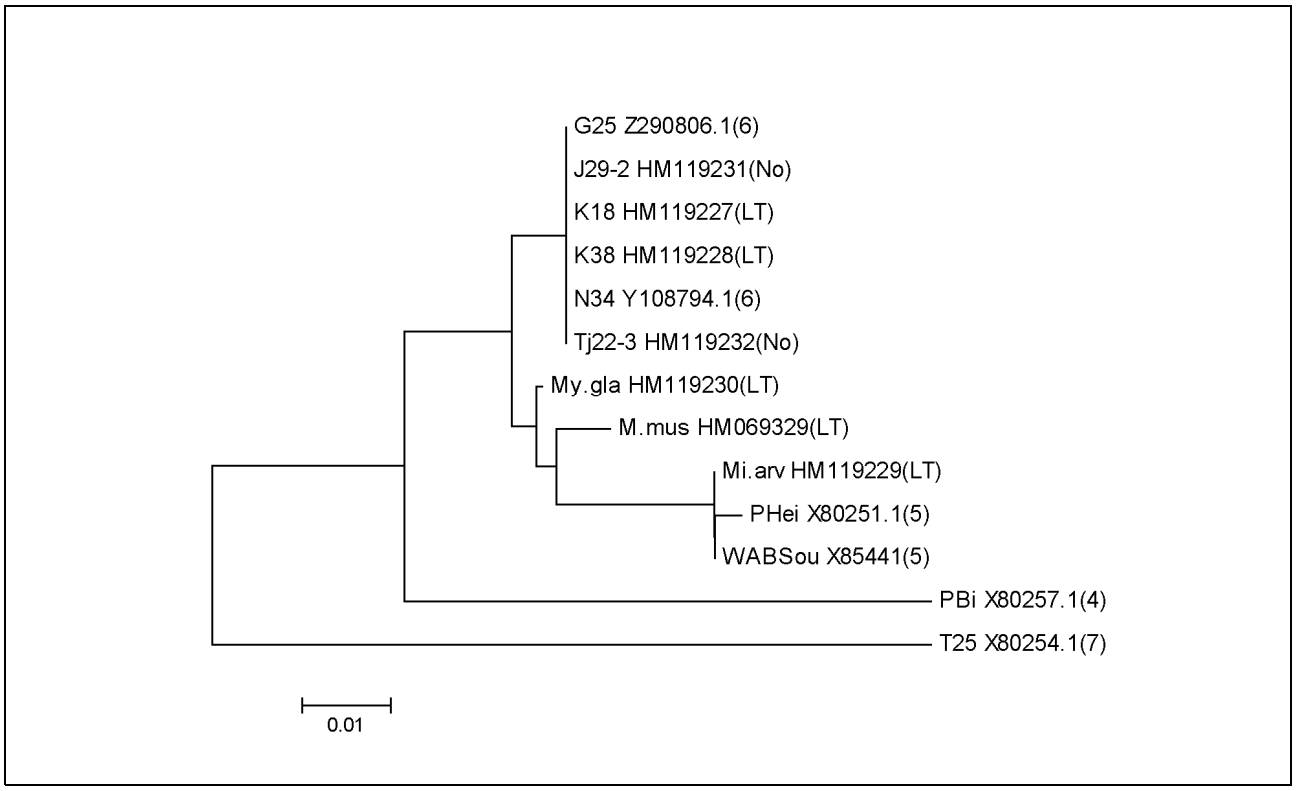

Figure 1. **Phylogenetic tree of *B. garinii* strains based on the sequence of the 345 bp fragments of ospA gene**

OspA serotypes of strain included in analysis for comparison from GenBank are given in parentheses. J29-2 – *B. garinii* detected in nymph from *A. flavicollis* (Jomfruland, Norway); Tj22-3 – in nymph from *M. agrestis* (Tjore, Norway); K-18, K-38 – in ticks from vegetation (Kaunas, Lithuania); M.mus – in *M. musculus*; Mi.arv - in *M. arvalis*; My.gla – in *M. glareolus* - all was collected in Kaunas, Lithuania.

OspA strains of *B. garinii* from *M. musculus*, *M. glareolus* and *M. arvalis* showed 97.3-100% similarity with OspA serotype 5 type strains (WABSou, PHei) and 97-99% similarity with OspA serotype 6 type strains (N34, G25). In phylogenetic tree these strains formed a separate cluster. *B. garinii* strain from *M. arvalis* showed 100% similarity with WABSou strain (Fig. 1). *B. garinii* strains from *I. ricinuis* ticks showed 100% similarity with OspA serotype 6 type strains (N34, G25) and formed other separate cluster (Fig 1).

Conclusions

1. The present study indicated that *ospA* encoding gene was more sensitive marker for the detection of *B. burgdorferi* s.l. in rodents than *fla* gene.

2. Our data confirmed that the estimated prevalence of *B. burgdorferi* in rodents differed depending on biopsy

method, rodent species and localities. *B. burgdorferi* s.l. infection was detected with different rates in ears and bladders tissues of small rodents, but was not found in spleen samples. Using of different biopsy methods could help in correct estimation of the prevalence of *Borrelia* infection in rodent population and determination of pathogen dissemination in individual rodent.

3. Both, *B. afzelii* and *B. garinii* were detected in small rodents from Lithuania. Rodents from Norway harboured only *B. afzelii* genospecies.

4. The alignment and phylogenetic analyses of sequences showed that *B. garinii* strains in *I. ricinus* ticks from Lithuania and Norway were identical (100% similarity), but strains from the rodents were heterogeneous and differed from strains detected in ticks. *B. garinii* strains from rodents showed similarity with sequences of OspA serotype 5 type and *B. garinii* strains from ticks with OspA serotype 6 type.

Acknowledgements

This work was supported by Lithuanian State Science and Studies Foundation and the Research Council of Lithuania (grant No. MIP-11174).

References

1. Bhide M., Zeki M., Esin G., Serhat T., Ivan M. Seroprevalence of anti-*Borrelia burgdorferi* antibodies in dogs and horses in Turkey. Ann. Agric. Environ. Med., 2008. Vol. 15. P. 85–90.

2. Blowey R.W., Carter S.D., White A.G., Barnes A. *Borrelia burgdorferi* infections in UK cattle: a possible association with digital dermatitis. Vet. Rec., 1994. Vol. 135. P. 577–578.

3. Bunikis J., Garpmo U., Tsao J., Berglund J., Fish D., Barbour A. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. Microbiology., 2004. Vol. 150. P. 1741–1755.

4. Bushmick S.L: Lyme Borreliosis in Domestic Animals. J Spirochetal & Tick-Borne Dis., 1994, Vol. 1. P. 24–30.

5. Demaerschalck I., Ben Messaoud A., De Kesel M., Hoyois B., Lobet Y., Hoet P., Bigaignon G., Bollen A., Godfroid E. Simultaneous presence of different Borrelia burgdorferi genospecies in biological fluids of Lyme disease patients. J Clin Microbiol., 1995. Vol. 33(3). P. 602–608.

6. Durrani A., Goyal S. Seroprevalence of borrelia burgdorferi in horses in Minnesota. J. Anim. Plant Sci., 2011. Vol. 21(1). P. 8–11.

7. Escudero R., Barral M., Perez A. et al. Molecular and pathogenic characterization of Borrelia burgdorferi sensu lato isolates from Spain. J Clin Microbiol., 2000. Vol. 38. P. 4026–4033.

8. Fridriksdottir V., Nesse L.L., Gudding R. Seroepidemiological studies of *Borrelia burgdosferi* infection in sheep in Norway. J. Clin. Microbiol., 1992. Vol. 30. P. 1271–1272.

9. Gern L., Estrada-Pena A., Frandsen F., Gray J., Jaenson T., Jongejan F., Kahl O., Korenberg E., Mehl R., Nuttall P. European reservoir hosts of *Borrelia burgdorferi* sensu lato. Zentbl. Bakteriol. Parasitenkd. Infektkrankh. Hyg. Abt. 1 Orig., 1998. Vol. 287. P. 196–204.

10. Hanincova K., Schafer S.M., Etti S., Sewell H.S., Taragelova V., Ziak D., Labuda M., Kurtenbach K. Association of *Borrelia afzelii* with rodents in Europe. Parasitology, 2003. Vol. 126. P. 11–20.

11. Hofmeister EK., Markham RB., Childs JE., Arthur RR. Comparison of polymerase chain reaction and culture for detection of *Borrelia burgdorferi* in naturally infected *Peromyscus leucopus* and experimentally infected C.B-17 *scid/scid* mice. J Clin Microbiol., 1992. Vol. 30. P. 2625–263.

12. Hu CM., Wilske B., Fingerle V., Lobet Y., Gern L. Transmission of *Borrelia garinii* OspA serotype 4 to BALB/c mice by *Ixodes ricinus* ticks collected in the field. J Clin Microbiol., 2001. Vol. 39. P. 1169–1171.

13. Huegli D., Hu CM., Humair PF., Wilske B., Gern L. *Apodemus* species mice are reservoir hosts of *Borrelia garinii* OspA serotype 4 in Switzerland. J Clin Microbiol., 2002. Vol. 40. P. 4735–4737.

14. Humair PF., Gern L. The wild hidden face of Lyme borreliosis in Europe. Microbes Infect., 2000. Vol. 2. P. 915–922.

15. Ishiguro F., Takada N., Nakata K., Yano Y., Suzuki H., Masuzawa T., Yanagihara Y. Reservoir competence of the vole, *Clethrionomys rufocanus* bedfordiae, for *Borrelia garinii* or *Borrelia afzelii*. Microbiol Immunol., 1996. Vol. 40. P. 67–69.

16. Korenberg, EI., Gorelova NB., Postic D., Kovalevskii IV., Baranton G., VoroÌeva NN. The reservoir hosts and vectors of *Borrelia*—the causative organisms of ixodid tick-borne borreliosis in Russia. Zh Mikrobiol Epidemiol Immunobiol., 1997. Vol. 6. P. 36–38.

17. Kurtenbach K., De Michelis S., Etti S., Schäfer SM., Sewell HS., Brade V., Kraiczy P. Host association of *Borrelia burgdorferi* sensu lato - the key role of host complement. Trends Microbiol., 2002. Vol. 10. P. 74–79.

18. Marconi RT.; Hohenberger S.; Jauris-Heipke S., et al. Genetic analysis of *Borrelia garinii* OspA serotype 4 strains associated with neuroborreliosis: Evidence for extensive genetic homogeneity J Clin Microbiol., 1999. Vol. 37: (12). P. 3965–3970.

19. Margos G., Vollmer SA., Cornet M., Garnier M., Fingerle V., Wilske B., Bormane A., Vitorino L., Pereira MC., Drancourt M., Kurtenbach K. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. Appl Environ

Microbiol., 2009. Vol. 75(16). P. 5410–5416.

20. Marie G. B., Hansen M., Line R., Thuesen M., Petersen R., Anders M.B. Seroprevalence of *Borrelia burgdorferi sensu lato* and *Anaplasma phagocytophilum* in Danish horses. Acta Vet. Scand., 2005. Vol. 52. P. 321–327.

21. Masuzawa T., Iwaki A., Sato Y., Miyamoto K., Korenberg E., Yanagihara Y. Genetic diversity of *Borrelia burgdorferi* sensu lato isolated in far eastern Russia. Microbiol Immunol., 1997. Vol. 41. P. 595–600.

22. Masuzawa T., Takada N., Kudeken M., Fukui T., Yano Y., Ishiguro F., Kawamura Y., Imai Y., Ezaki T. *Borrelia sinica* sp. nov., a Lyme disease-related *Borrelia* species isolated in China. Int J Syst Evol Microbiol., 2001. Vol. 51. P. 1817–1824.

23. Nakao M., Miyamoto K., Fukunaga M. Lyme disease spirochetes in Japan: enzootic transmission cycles in birds, rodents, and *Ixodes persulcatus* ticks. J Infect Dis., 1994. Vol. 170. P. 878–882.

24. Ornstein K., Berglund J., Nilsson I., Norrby R., Bergström S. Characterization of Lyme Borreliosis Isolates from Patients with Erythema Migrans and Neuroborreliosis in Southern Sweden. J Clin Microbiol. 2001. Vol. 39(4). P. 1294–1298.

25. Paulauskas A., Ambrasiene D., Radzijevskaja J., Rosef O., Turcinaviciene J. Diversity in prevalence and genospecies of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks and rodents in Lithuania and Norway. Int J Med Microbiol., 2008. Vol. 298 (S1). P. 180–187.

26. Petney TN., Hassler D., Brueckner M., Maiwald M. Comparison of urinary bladder and ear biopsy samples for determining prevalence of *Borrelia burgdorferi* in rodents in central Europe. J Clin Microbiol., 1996. Vol. 34. P. 1310–1312.

27. Picken R. Polymerase chain reaction primers and probes derived from flagellin gene sequences for specific detection of the agents of Lyme diseases and North American relapsing fever. J. Clin. Microbiol., 1992. Vol. 30. P. 99–114.

28. Reed K. Laboratory testing for Lyme disease: possibilities and practicalities. J Clin Microbiol., 2002. Vol. 40(2). P. 319–324.

29. Richter D., Endepols S., Ohlenbusch A., Eiffert H., Spielman A., Matuschka FR. Genospecies diversity of Lyme disease spirochetes in rodents reservoirs. Emerg Infect Dis., 1999. Vol. 5. P. 291–296.

30. Rosa P. A., Tilly K., Stewart P. E. The burgeoning molecular genetics of the Lyme disease spirochaete. Nat. Rev. Microbiol., 2005. Vol. 3. P. 129–143.

31. Rosef O., Paulauskas A., Radzijevskaja J. Prevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in questing *Ixodes ricinus* ticks in relation to the density of wild cervids. Acta Veterinaria Scandinavica. 2009. Vol. 51. P. 47.

32. Sinsky RJ., Piesman J. Ear punch biopsy method for detection and isolation of *Borrelia burgdorferi* from rodents. J Clin Microbiol., 1989. Vol. 27. P. 1723–1727.

33. Stańczak J., Racewicz B., Kubica-Biernat W., Kruminis-Lozowska J., Dabrowski A., Markowska M. Prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks (Acari, Ixodidae) in different Polish woodlands. Ann Agric Environ Med., 1999. Vol. 6. P. 127–132.

34. Štefančíková A., Adaszek Ł., Peťko B., Winiarczyk S., Dudiňák V. Serological and immunochemical evidence of *Borrelia burgdorferi* sensu lato in horses and cattle from Poland and diagnostic problems of Lyme borreliosis. Ann Agric Environ Med., 2008. Vol. 15. P. 37–43.

35. van Dam A., Kuiper H., Vos K., Widjojokusumo A., De Jongh B. M., Spanjard L., Ramselaar A. C. P., Kramer M. D., Dankert J. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. Clin. Infect. Dis., 1993. Vol. 17. P. 708–717.

36. Vollmer S., Bormane A., Dinnis R., Seelig F., D. M. Dobson A., Aanensen D., James M., Donaghy M., Randolph S., Feil E., Kurtenbach K., Margos G. Host migration impacts on the phylogeography of Lyme Borreliosis spirochaete species in Europe. Environmental Microbiology., 2011. Vol. 13(1). P. 184–192.

37. Wilske B, Busch U, Fingerle V, Jauris-Heipke S, Preac-Mursic V, Rossler D., Will G. 1996. Immunological and molecular variability of OspA and OspC. Implications for Borrelia vaccine development. Infection 24: 208–212.

38. Wilske B., Preac-Mursic V., Gobel UB., Graf B., Jauris S., Soutschek E., Schwab E., Zumstein G. An OspA serotyping system for Borrelia burgdorferi based on reactivity with monoclonal antibodies and OspA sequence analysis. J Clin Microbiol., 1993. Vol. 31. P. 340–350.

39. Wilske B., Fingerle V., Schulte-Spechtel U. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol., 2007. Vol. 49(1). P. 13–21.

Received 2 May 2011
Accepted 27 June 2011