Invasive potential of *Borrelia burgdorferi* sensu stricto ospC type L strains increases the possible disease risk to humans in the regions of their distribution

Maryna Golovchenko1,2, Radek Sima1, Ondrej Hajdusek1, Libor Grubhoffer1,3, James H Oliver Jr2 and Nataliia Rudenko1,2*

**Abstract**

**Background:** Analysis of *Borrelia burgdorferi* ospC types from the southeastern U.S.A. supported the common belief that various ospC types are geographically restricted and host specific. Being widely distributed in the region, the southeastern population of *B. burgdorferi* is represented by a surprisingly small number of ospC types. Types B, G and H are dominant or common and are invasive, while scarce type L, restricted mostly to the southeastern U.S.A., is believed to rarely if ever cause human Lyme disease. OspC type B and L strains are represented in the region at the same rate, however their distribution among tick vectors and vertebrate hosts is unequal.

**Findings:** Direct diagnostics was used to analyze the ability of *B. burgdorferi* ospC type L strains to disseminate into host tissues. Mice were infected by subcutaneous injections of *B. burgdorferi* strains of various ospC types with different invasive capability. Spirochete levels were examined in ear, heart, bladder and joint tissues. Noninfected *I. ricinus* larvae were fed on infected mice until repletion. Infection rates were determined in molted nymphs. Infected nymphs were then fed on naive mice, and spirochete transmission from infected nymphs to mice was confirmed.

**Conclusions:** *B. burgdorferi* ospC type L strains from the southeastern U.S.A. have comparable potential to disseminate into host tissues as ospC types strains commonly associated with human Lyme disease in endemic European and North American regions. We found no difference in the invasive ability of ospC type B and L strains originated either from tick vectors or vertebrate hosts.

**Keywords:** *B. burgdorferi* ospC type, Invasive potential, Lyme disease, Southeastern U.S.A., Tick vector, Vertebrate host

**Findings**

**Background**

It is known that each *Borrelia burgdorferi* sensu lato (s.l.) species is characterized by its tick vectors, host spectrum, geographical distribution and, for the pathogenic species, its organotropism [1]. The relative invasiveness of various *B. burgdorferi* strains, classified by ospC type, reveals the ratio between that type’s frequency in vector ticks compared to human patients [2].

Published data on prevalence of Lyme disease (LD) spirochetes in vector ticks and vertebrate hosts in the southeastern U.S.A. qualifies this region as *B. burgdorferi* s.l. endemic area, despite prevailing dogma concerning LD in the United States [3]. Briefly, current dogma states that *Ixodes scapularis* (formerly, *I. dammini*) is the only vector of the spirochetes in the eastern U.S.; *B. burgdorferi* is antigenically and genetically uniform in North America in contrast to the situation in Europe; *B. burgdorferi* does not occur in wildlife in the southern U.S.A. and thus, humans in the Southeast could not acquire LD [3,4]. It is now known that the often referenced *I. scapularis*-Peromyscus leucopus enzootic cycle in the northeastern U.S.A. is mirrored by the *I. scapularis*-P. gossypinus transmission cycle.

* Correspondence: natasha@paru.cas.cz
1Biology Centre AS CR, Institute of Parasitology, Ceske Budejovice 37005, Czech Republic
2Georgia Southern University, James H. Oliver, Jr. Institute of Coastal Plain Sciences, Statesboro, GA 30460-8056, USA
Full list of author information is available at the end of the article

© 2014 Golovchenko et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
in the Southeast which is further enhanced by *Ixodes affinis* and *Ixodes minor* as vital maintenance vectors of *Borrelia* in areas where they occur, and *Sigmodon hispidus* and *Neotoma floridana* as additional major reservoir hosts [3,5]. As the tick vectors and reservoir hosts differ significantly between the northeastern and southeastern regions, it is highly possible that *B. burgdorferi* strains that cause LD in both areas will differ as well. Our previous research showed that of the 4 *ospC* types, B, G, H and N that have been detected in LD patients in the northeastern and midwestern U.S.A., 3 types, B, G, and H, at a lower rate, are widely distributed in the southeastern United States [5]. While *ospC* type B is associated with severe LD around the world [6,7], *ospC* types H and G are commonly detected in tissues at disseminated sites of LD patients from the northeastern and midwestern U.S.A. [2,8]. *B. burgdorferi* *ospC* type L strains were not considered to have any impact in LD in North America in general and, in the southeastern U.S.A. particularly, because: i) *ospC* type L was considered to be restricted to Europe only; ii) *ospC* type L is recognized as very rare worldwide; and iii) *ospC* type L strains were previously detected in ticks only [2,6-9].

Globally rare *B. burgdorferi* *ospC* type L strains are associated with the non-human biting tick *I. affinis* that feeds on the same vertebrate hosts as human-biting *I. scapularis* in the southeastern U.S.A. [9]. However, due to the common reservoir hosts in the feeding cycle, *Borrelia* strains from *I. affinis* might be transmitted to humans [3]. *B. burgdorferi* *ospC* types B and L strains are the most prevalent in the region. They are represented at the same rate overall, but their distribution among the tick vectors and the rodent hosts is unequal. While 75% of highly invasive *ospC* type B strains were isolated from rodents and 25% from ticks, in the case of *ospC* type L, host- and vector-originated strains represented 37.5% and 62.5%, respectively. For a long time it was believed that *ospC* type L strains are incapable of causing human LD [2]. Nevertheless, a *B. burgdorferi* *ospC* type L strain (SLV-1) was cultured from a skin biopsy from a Slovenian patient with acrodermatitis chronica atrophicans (ACA), commonly associated with *B. afzelii* infection [10]. Additional strains were isolated from *I. ricinus* nymphs collected in Switzerland (NE5222 and NE5266) and Slovakia (SKT-2) [5], European LD endemic regions. Another *ospC* type L strain (SCW-9) was isolated from secondary sites of infection of cotton rat (*Sigmodon hispidus*) trapped in South Carolina, U.S.A. [5,9]. The question about Lyme disease in the southeastern U.S.A. is still controversial and confounded by multiple facts and fallacies. Because *ospC* type L strains are one of the two most prevalent in this region [9], the goal of this study was to analyze the capability of *B. burgdorferi* *ospC* type L strains to disseminate into vertebrate hosts and to compare their invasive potential with infective strains.

**Methods**

Low passage *Borrelia* strains were grown in BSK-H medium (Sigma-Aldrich, U.S.A.) according to the protocol described previously [11,12]. Strains used for infection of laboratory mice were characterized in our previous study [5] and were selected on the basis of their *ospC* types defined earlier [5]: human originated *ospC* type B strain SLV-2 (collection site 46°3′17″N,14°30′21″E), cotton mouse (*Peromyscus gossypinus*) originated *ospC* type L strain SCCH-30 (32°47′00″N, 79°56′00″W), *I. ricinus* nymphs originated *ospC* type L strain NE5222, *ospC* type B strain NE5264 and *ospC* type V strain NE5248 (46°59′34.72″N, 6°55′54.96″E) and *I. affinis* female originated *ospC* type B strain SCW-53 (33°10′17″N, 79°23′57″W). Six weeks female C3H/HeN mice (Jackson Laboratory, Sulzfeld, Germany) were infected by subcutaneous injections of 10⁵ spirochetes in 100 μl of BSK-H medium per mouse (2 mice per strain). Presence of spirochetes in ear biopsies was determined at weekly intervals by PCR. Total DNA was extracted using a NucleoSpin Tissue Kit (Macherey-Nagel, Germany) according to the manufacturer’s protocol. Detection of spirochetes was performed by amplification of a 154 bp fragment of flagellin gene using primers FlaF1 (AAGCAATTTAGGTGCTTTCCAA) and FlaR1 (GCA ATCATGCCCATTGCAGA) [12]. At the 3rd week after infection the presence of spirochetes was confirmed in all ear biopsy samples regardless of the *ospC* type of strain used for infection. *I. ricinus* larvae were obtained from the pathogen-free tick colony of the Institute of Parasitology (Czech Republic). At the 4th week after inoculation, non-infected larvae were fed on infected mice until repletion (100 larvae per mouse) and left to molt. Infection rates were determined in pools of ten molted nymphs using PCR described above. Nymphs were considered to be infected if >80% of them were PCR positive. Ten infected nymphs were then fed on naïve mice (5 mice per strain); spirochete transmission from infected nymph to mice was determined as described above. At week 6th, spirochete load was determined in positive biopsies from mouse ear, heart, bladder and joint by quantitative PCR using primers described above, the TaqMan FlaProbe1 (TGCTACAA CTCATCTGTCACTTAGCATCTTTATTG) and a LightCycler 480 (Roche) [13]. Spirochete burden in tissues was normalized to mouse actin using previously described methods [14]. Amplification and sequencing of spirochete *ospC* genes from final mouse samples confirmed their identity to the *ospC* genes of *B. burgdorferi* strains used for initial infection [5]. All laboratory animals were treated in accordance with the Animal Protection Law of the Czech Republic No. 246/1992 Sb, ethics approval No. 137/2008.

**Results and conclusions**

PCR was used to confirm infection of laboratory mice with invasive *B. burgdorferi* *ospC* type B strains and *ospC*
type L strains, previously found only in ticks (Table 1). An ospC type V strain was included in the analysis for comparison, as it was found in ticks and in sites of local infection in humans. The same pattern of transmission of ospC type B and type L strains of the LD spirochete from infected host to tick vector and then from infected tick vector to uninfected host was revealed using the designed protocol. It is interesting to note that the results of dissemination of host originated ospC type B (human, SLV-2) and L (rodent, SCCH-30) strains were comparable in each tissue analyzed (Table 1). Dissemination ability of ospC type B (NE5264) and L (NE5222) strains originated from human biting I. ricinus was almost equal in each tissue (Table 1). However, the load of spirochetes of ospC type B (SCW-53) originated from non-human biting I. affinis was more than 10 times higher in mouse joints than was the spirochete load of human-originated ospC type B (SLV-2) or human-biting I. ricinus originated (NE5264) strains. Our results confirm that tick originated strains NE5264 and NE5222 of ospC type B and L and host originated ospC type B strain SLV-2, show the same pattern of dissemination in all analyzed host tissues, with no preferential site of infection. Identical pattern of dissemination was revealed for non-human biting tick originated ospC type B strain SCW-53, and rodent originated ospC type L strain SCCH-30 (strains from South Carolina, U.S.A.), with joints as the preferable site of infection. Identical results show the same pattern of dissemination in all analyzed host tissues, with no preferential site of infection. In contrast to type B and L, this ospC type preferentially disseminates into bladder, not joints (Table 1).

*B. burgdorferi* ospC type L strains originated from human, rodent or hard tick were able to disseminate into laboratory mice as well as invasive ospC type B strains that are responsible for systemic disease in humans. Therefore, further study on the pathogenicity of *B. burgdorferi* ospC type L strains is warranted. The qPCR results in this study defined heart as a tissue with the lowest load of spirochetes, while the joints showed the highest load of borrelia in mice infected either with ospC type B or L strains (Table 1). Our results support the association of 

B. burgdorferi with Lyme arthritis [15,16]. The confirmed ability of ospC type L strains to disseminate into vertebrate host tissues in the same manner as invasive ospC type B strains, known to be responsible for severe disease in humans worldwide, increases the possible disease risk to humans in the southeastern U.S.A., the region, where studied strains are widely distributed [5].

### Table 1 Invasive potential of *B. burgdorferi* sensu stricto strains with different ospC types

| Mice B. burgdorferi | Ear punch biopsy (week) | qPCR results (#) |
|---------------------|------------------------|------------------|
| Qty | Strain | Species | Source | ospC | 1 | 2 | 3 | 4 | 5 | 6* | Ear | Bladder | Joint | Heart |
| 2 | C3H/HeN Europe Bb ss. SLV-2 human | type B | - | + | + | + | 2/2 | 12.0 ± 3.0 | 9.5 ± 2.5 | 15.0 ± 9.0 | 3.0 ± 1.0 |
| 5 | C3H/HeN U.S.A. Bb ss. SCCH-30 P.gossypinus | type L | - | + | + | + | 4/5 | 7.0 ± 2.4 | 7.0 ± 1.2 | 33.0 ± 18.5 | 5.0 ± 1.0 |
| 2 | C3H/HeN Europe Bb ss. NE5264 l. ricinus (n) | type B | - | + | + | + | 2/2 | 18.5 ± 5.3 | 18.7 ± 9.5 | 14.6 ± 1.8 | 2.9 ± 0.4 |
| 5 | C3H/HeN Europe Bb ss. NE5222 l. ricinus (n) | type L | - | + | + | + | 3/5 | 14.6 ± 2.7 | 20.7 ± 6.2 | 14.0 ± 3.4 | 2.5 ± 0.3 |
| 2 | C3H/HeN U.S.A. Bb ss. SCW-53 l. affinis (f) | type B | - | + | + | + | 2/2 | 6.5 ± 0.5 | 12.0 ± 1.0 | 17.4 ± 70.5 | 3.0 ± 2.0 |
| 2 | C3H/HeN Europe Bb ss. NE5248 l. ricinus (n) | type V | - | + | + | + | 1/2 | 8 | 20 | 6 | 4 |

Qty - quantity; # of mice/experiment; (n) - nymph; (f) - female; 6* - total number of infected mice at 6 weeks time point; qPCR results are expressed as means ± SEM, (#) - number of spirochetes/10^6 marine genomes.
4. Lane RS, Piesman J, Burgdorfer W. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annu Rev Entomol 1991, 36:587–609.

5. Rudenko N, Golovchenko M, Höning V, Mallistová N, Kršková L, Mikulášek P, Fedorova N, Belfiore NM, Grubhoffer L, Lane RS, Oliver HJ Jr. Detection of Borrelia burgdorferi sensu stricto ospC alleles associated with human Lyme borreliosis worldwide in non-human-biting tick Ixodes affinis and rodent hosts in southeastern United States. Appl Environ Microbiol 2013, 79:1444–1453.

6. Seinost G, Dykhuizen DE, Dattwyler RJ, Golde WT, Dunn JJ, Wang IN, Wormser GP, Schriefer ME, Luft BJ. Four clones of Borrelia burgdorferi sensu stricto cause invasive infection in humans. Infect Immun 1999, 67:3518–3524.

7. Qiu WG, Bruno JF, McCaig WD, Xu Y, Livey I, Schriefer ME, Luft BJ. Wide distribution of a high-virulence Borrelia burgdorferi clone in Europe and North America. Emerg Infect Dis 2008, 14:1097–1104.

8. Brisson D, Baxamusa N, Schwartz I, Wormser GP. Biodiversity of Borrelia burgdorferi strains in tissues of Lyme disease patients. PLoS One 2011, 6:e22926.

9. Rudenko N, Golovchenko M, Grubhoffer L, Oliver HJ Jr. The rare ospC allele L of Borrelia burgdorferi sensu stricto, commonly found among samples collected in a coastal plain area of the southeastern United States, is associated with Ixodes affinis ticks and local rodent hosts Peromyscus gossypinus and Sigmodon hispidus. Appl Environ Microbiol 2013, 79:1403–1406.

10. Ruzić-Sabljić E, Zore A, Stire M. Characterization of Borrelia burgdorferi sensu lato isolates by pulsed-field gel electrophoresis after MluI restriction of genomic DNA. Res Microbiol 2008, 159:441–448.

11. Rudenko N, Golovchenko M, Grubhoffer L, Oliver HJ Jr. Borrelia carolinensis sp. nov., a new (14th) member of the Borrelia burgdorferi sensu lato complex from the southeastern region of the United States. J Clin Microbiol 2009, 47:134–141.

12. Rudenko N, Golovchenko M, Lin T, Gao L, Grubhoffer L, Oliver HJ Jr. Delineation of a new species of the Borrelia burgdorferi sensu lato complex, Borrelia americana sp. nov. J Clin Microbiol 2009, 47:3875–3880.

13. Schweiger M, Péter O, Cassinotti P. Routine diagnosis of Borrelia burgdorferi (sensu lato) infections using a real-time PCR assay. Clin Microbiol Infect 2001, 7:461–469.

14. Dai J, Wang P, Adusumilli S, Booth C, Naraśman S, Anguita J, Fikrig E. Antibodies against a tick protein, Salp15, protect mice from the Lyme disease agent. Cell Host Microbe 2009, 6:482–492.

15. Steere A. Lyme disease. N Engl J Med 1998, 328:586–596.

16. Ochsannrn P, Dormdorff W, Hering C, Schäfer C, Wellensiek HJ, Pfaffhauk KW. Stages and syndromes of neuroborreliosis. J Neurol 1998, 245:362–372.

Cite this article as: Golovchenko et al.: Invasive potential of Borrelia burgdorferi sensu stricto ospC type L strains increases the possible disease risk to humans in the regions of their distribution. Parasites & Vectors 2014, 7:538.

doi:10.1186/s13071-014-0538-y

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit