The genospecies *B. burgdorferi* s.l., isolated from ticks and from neurological patients with suspected Lyme borreliosis

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This paper is dedicated to the 70th birthday of Prof. Traubner

Abstract

OBJECTIVE: Lyme borreliosis (LB) is the most disseminated tick-borne disease in the Northern hemisphere, and infestation with ticks is one of the essential factors influencing transmission of the disease to humans. This work intends to compare the occurrence of borrelia circulating in indigenous ticks and in patients suffering from neurological diseases.

MATERIALS & METHODS: The total of 660 nymphs and 567 adult ticks from the Bratislava and Košice areas was examined over the years 2001–2004, and the cerebrospinal fluid (CSF) of 82 neurological patients suffering from suspected Lyme borreliosis infection was investigated in the 2007–2009 period, using the polymerase chain reaction method (PCR).

RESULTS: PCR investigation proved presence of borrelia in 23.3% of the total 1227 ticks; of these, co-infection was found in 2.7% of all ticks. *Borrelia garinii* (9.9%) and *B. valaisaina* (9.2%) were the prevalent types. PCR investigation of the CSF samples of 32 patients with clinically diagnosed Lyme borreliosis showed the presence of *B. burgdorferi* s.l. in 17 cases. Positive results were found also in patients with unclear or different diagnoses. In cases where the genospecies could be identified, *B. garinii* was most frequently found (8x), followed with *B. burgdorferi* s.s. (4×) and *B. afzelii* (3×).

CONCLUSIONS: The high infestation level of ticks with borrelia, mainly with *B. garinii* which is the most-often documented borrelia species identified in neurological patients, is indicative of a high risk of this contamination in Slovakia. *B. garinii* were found also in our neuroborreliosis patients, whereas their proof in the CSF of patients with suspected neuroborreliosis or with a different clinical diagnosis pointed upon their persistence after an infectious experience. However,

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knowledge of not only the genospecies but also of the genotypes capable of eliciting an invasive disorder would be necessary for better clarification of the relationship between borrelia and their peccant capacity. Identification of the invasive borrelia types circulating in nature, and clarification of the vector vs. human infection incidence relationship is of importance from the aspect of detailed knowledge of the epidemiology of this disease.

INTRODUCTION

Lyme borreliosis (LB) is the most disseminated tick-borne disease in the Northern hemisphere. In nature, *Borrelia burgdorferi* s.l. (sensu lato) circulates among various hosts (wild animals) and ticks, whereby the tick *Ixodes ricinus* is known as the main European vector. Of the 18 known genospecies (Stanek and Reiter, 2011), *B. burgdorferi* s.s.(sensu stricto), *B. garinii* and *B. afzelii* represent the etiological agents of localized, disseminated and chronic Lyme borreliosis in Europe, while in the North American population affected with the disease only *B. burgdorferi* s.s. has been appearing. Other European types, viz. *B. spielmanii,* were described in connection with localized early disorders, along with *B. bissettii* and *B. valaisiana* linked to very sporadically occurring diseases and *B. luisitaniae,* the significance of which in human afflictions is not known. The extensive variability of borrelia and their different geographic distribution in relatively small areas were pointed out in several studies (Bunikins *et al.* 2004, Derdáková and Lenčáková, 2005, Lenčáková *et al.* 2006). Tick infestation is one of the essential factors influencing transmission of the disease also to humans. Repeated tick studies in Slovakia confirmed the high level of infestation of this country, oscillating between 2.1% and 41.7% in dependence on the region and the method of detection (Derdáková and Lenčáková, 2005, Smatanová *et al.* 2007). Different genospecies are linked to specific clinical diseases; *B garinii,* found in Europe, mostly connects to neurological diseases, *B. afzelii* to dermal disorders, as e.g. erythema migrans and acrodermatitis chronica (Lebech 2002, Strle *et al.* 2006). The objective of our work was to compare the occurrence of borrelia circulating in ticks originating in Slovakia, and in patients suffering from neurological diseases.

MATERIALS AND METHODS

During years 2001–2004 questing ticks were flagged in suburban forests around two largest cities in Slovakia (Bratislava and Košice). Total of 567 adult and 660 nymphal *I. ricinus* were analyzed. Extracted DNA was subjected to nested or single PCR targeting the 5S – 23S intergenic spacer (IGS) (Rjipkema *et al.* 1995; Derdáková *et al.* 2003). Spirochetes were assigned to a species by RFLP (Derdáková *et al.* 2003) or reverse-lineblotassay (Rjipkema *et al.* 1995).

Cerebrospinal fluid (CSF) from 82 neurological patients suffering from suspected Lyme borreliosis was examined in the 2007 – 2009 period, using the polymerase chain reaction (PCR) – commercial Amlisens test, detecting 16S rRNA *Borrelia burdorferi* s.l and, in parts of the patients, also by DNA isolation with the easy tissue Qiagen kit and diagnostics rrf(5S) – rrl nested PCR method.

Clinically, the disease was diagnosed on the basis of recommendations for Lyme borreliosis and neuroborreliosis diagnoses (Blanc *et al.* 2007; Stanek *et al.* 2011).

Tab. 1. Prevalence of *Borrelia burgdorferi* s.l. in *Ixodes ricinus* ticks from two areas in Slovakia.

locality		No. of ticks examined	No. of ticks Positive	% of positive ticks
Košice area	nymphs	240	36	15
	adults	279	71	25.5
	Total	519	107	20.6
	co-infections	519	18	3.5
Bratislava area	nymphs	420	88	21
	adults	288	91	31.6
	total	708	179	25.3
	co-infections	708	15	2.1
Total	nymphs	660	124	18.8
	adults	567	162	28.6
	total	1227	286	23.3
	co-infections	1227	33	2.7

RESULTS

In the PCR examination of 1227 ticks, borrelias were found in 23.3% of all cases, with co-infection appearing in 2.7% (Tables 1 and 2). Findings of *Borrelia garinii* (9.9%) and of *B. valaisaina* (9.2%) prevailed, with infestation appearing in adult ticks more profoundly than in nymphs. *B. valaisiana* was most frequently found in West Slovakia. Least incidence of the main pathogen types was found in *B. burgdorferi* s.s., relatively more often found in ticks captured in the Košice area, as opposed to ticks around Bratislava.

Of all 32 patients clinically diagnosed with Lyme borreliosis, the presence of *B. burgdorferi* s.l. was proven in 17 cases by PCR in the CSF (Table 3). In addition, 11 out of 23 patients were also found positive for the organism, although their diagnoses were not unequivocal and failed to fully meet the criteria of neuroborelliosis, mainly in cases of protracted duration of the disease; nevertheless, the clinical course of the disorder and overall examination had been pointing toward this

Tab. 2. Distribution of *Borrelia burgdorferi* s.l. genospecies in *I. ricinus* ticks from two areas in Slovakia including co-infections, in Bratislava district 17 borrelial infections could not be typed to the species.

Locality	stage	No of ticks Examined	Distribution of *Borrelia* genospecies in *I. ricinus**			
			B.garinii	*B. valaisiana*	*B. afzelii*	*B. burgdorferi* s.s.
Košice area	Nymphs	240	18	9	7	8
	Adults	279	29	21	16	17
	Total (%)	519	47 (9.1)	30 (5.8)	23 (4.5)	25 (4.8)
Bratislava area	Nymphs	420	39	32	31	1
	Adults	288	35	51	9	3
	Total (%)	708	74 (10.5)	83 (11.7)	40 (5.6)	4 (0.6)
Total (%)		**1227**	**121 (9.9)**	**113 (9.2)**	**63 (5.1)**	**29 (2.4)**

aetiology, and these patients were conclusively characterized as suspected neuroborelliosis. Furthermore, 5 patients suffering from other basic diseases were also found positive.

In patients where the genospecies could be identified, *B. garinii* was most frequently found (8× – Table 4); of these, neuroborelliosis was clinically confirmed in five cases, and suspected in one case. The diagnoses of 2 patients were different (demyelinating disease); the anamnesis of one of these showed past LB experience. *B. burgdorferi* s.s. was found in 4 patients, two of whom were clinically confirmed as neuroborreliosis, and 2 clinically concluded as suspected neuroborreliosis. *B. afzelii* was found in 3 patients in the form of co-infections with *B. garinii* (1× – susp. neuroborreliosis, 2× – other diagnoses).

Tab. 3. Detection of *B. burgdorferi* s.l. by polymerase chain reaction in CSF in patients with suspected Lyme borreliosis.

Clinical diagnosis	number of patients	PCR	
		posit.	negat.
neuroborreliosis	32	17	15
Lyme arthritis	8	–	8
susp. neuroborreliosis	19	11	8
	59	**28**	
Other diseases:			
demyelinating disease	4	4	–
M. Parkinson	2	1	1
tumor	2	–	2
lumboischiating sy	3	–	3
cervical myelopathy	3	–	3
cephalea	8	–	8
neurolues	1	–	1
Total	**82**	**33**	**49**

Tab. 4. Distribution of genospecies *B. burgdorferi* in CSF in patients with suspected lyme borreliosis

Genospecies	neuroborreliosis	clinical diagnosis susp. neuroborreliosis	other
B. burgdorferi s.s.	2	2	–
B. garinii	5	1	2
B. ajzelii	–	1	2

DISCUSSION

The high infestation of ticks with borrelia, mainly *B. garinii* is indicative of a high risk to contract neuroborreliosis. We wished to clarify this relationship by examining the cerebrospinal fluid (CSF) of suspected Lyme borreliosis patients, using the polymerase chain reaction method (PCR). The importance of this examination in the diagnostics of neuroborreliosis is not quite clear. The tests are not standardized, and the results are affected by a multitude of factors including procedural methodology, sample provenience, and different aspects applied in the individual studies (Cerar *et al.* 2008; Moravcová *et al.* 2009). Most often, the declared test sensitivity range is 12–46% (Honegr *et al.* 2001; Pícha *et al.* 2008; Cerar *et al.* 2008; Stanek *et al.* 2011), or somewhat higher at early-stage neurological patients (Moravcová *et al.* 2009); the findings in our study were comparable. The low sensitivity level is caused mainly by the low primary concentration of borrelia in the organism, their genetic heterogeneity, and technical factors relating to sample processing and to the selection of patient groups. Our patients diagnosed with neuroborreliosis most manifested *B. garinii,* which is the most frequent type of borrelia confirmed in patients with neurological disorders (Strle *et al.* 2006, Cerar *et al.* 2008). We have found *B. garinii* also in patients suffering from other basic diseases. Prolonged persistence of borrelia after an infectious experience is well documented – presence of the organism was found

in the central nervous system also after an asymptomatic infection (Pícha *et al.* 2008; Cerar *et al.* 2008). The anamnesis of one of our patients with *B. garinii* DNA found in the CSF and diagnosed with demyelinating disease showed LB experience (Ďurovská *et al.* 2011), while others, positively tested by PCR, might have been subclinically affected, or the infection remained unnoticed with slight and non-specific clinical symptoms. References report that major parts (minimally 20–30%) of *B. burgdorferi* infections are asymptomatic, or show nonspecific symptoms (Honegr *et al.* 2001; Pícha *et al.* 2008).

Several genotypes are known within the genospecies *B. garinii,* based on analyses of OspC and Osp A genes; of these, OspC is linked to tick saliva and host invasiveness (Fingerle *et al.* 2002; 2007). The genes are differently distributed within geographic regions, hosts and vectors, and their capability to induce invasive diseases is different (Jones *et al.* 2006). From this viewpoint, determination of genotypes in the identification of *B. garinii* would be of interest in our patients and in ticks.

In our examination, 2.7% of all ticks were co-infected with two or three genospecies. Recent studies pointed out high incidence of compound tick infection (Lenčáková *et al.* 2006; Smatanova *et al.* 2007; Crowder *et al.* 2010). In North America as much as 39% of all examined adult *Ixodes scapularis* ticks were infected with more than one *B. burgdorferi* s.s. genotype. The examination of tick genotypes in Slovak regions would obviously show even more co-infections, which are the potential sources of growing dissemination risks of the disease in humans.

Borrelia are highly variable, and their multilocular sequential analysis and typification point upon their higher diversity than originally reported (Bunikis *et al.* 2004). Recently, 53 genotypes were identified within the genospecies *B. burgdorferi* s.s. alone, of these 44 were found in Northern America. Only 6.6% American genotypes were found in Europe as well, while 27% of European genotypes were observed in America (Crowder *et al.* 2010). The capability of individual genotypes to induce a local or invasive disease is not known. Further studies may be necessary to clarify whether a specific combination of genotypes is involved in the tick co-infection, or relates to affliction in humans.

CONCLUSION

Different clone complexes of *B. burgdorferi* prefer different host types, and their pathogenity to humans varies, some of them being more frequently linked to invasive disorders. Identification of the invasive types of borrelia circulating in the natural environment, and their relation to the disease contracted by humans is important from aspects of improved knowledge of its epidemiology. From this viewpoint close cooperation is necessary between workers in the field of environmental and microbial ecology on one hand, and health care researchers on the other hand. The capability to rapidly identify, from clinical materials, the individual types of borrelia and mainly the genotypes linked to invasive disorders would be very important from viewpoints of treatment of these patients.

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