INVESTIGATION OF TICK-DERIVED LYME DISEASE BORRELIA STRAINS ISOLATED IN STYRIA, AUSTRIA

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ABSTRACT

In Styria, Austria, several areas are known to be endemic for Lyme disease. The *Borrelia* spirochetes which are the causative agent for this illness are transmitted by the tick vector *Ixodes ricinus*. The present study was undertaken to survey the tick population of Styria for the presence of *Borrelia* and to characterize the strains isolated. Ticks were collected in biotopes known to be natural foci of tick borne encephalitis, and *Borrelia* were cultivated from tick extracts. Each isolate was characterized by species and, from several strains, the sequence of the *ospC* gene was determined. Sixteen Lyme disease *Borrelia* strains were isolated, ten of which were further characterized. These included three *Borrelia burgdorferi* sensu stricto, three *Borrelia afzelii* and four *Borrelia garinii* isolates. Sequence analysis of two of the *Borrelia afzelii* isolates and three of the *Borrelia garinii* isolates indicated that two of the strains have *ospC* genes identical to alleles previously described (RFLP types 20 and 34), and three of the genes were novel variants. In conclusion, it was demonstrated for the first time that the three major Lyme disease *Borrelia* species are present in the tick population of Styria. The *ospC* genes of the analyzed strains showed a high degree of variability.

KEY WORDS

Lyme disease, borrelia strains, ospC gene, RFLP types, tick isolates, Styria

INTRODUCTION

Lyme disease (LD) is a vector-transmitted infection caused by spirochetes of the genus *Borrelia* (1,2). In the Austrian province of Styria, the primary vector transmitting the infective agent is the tick *Ixodes ricinus* (3,4). In humans, LD is a multi-systemic illness with a wide range of symptoms (dermatological, neurological, articular or cardiological) (5-8). Correlations between clinical manifestations of LD and *Borrelia* species have been reported (9,10). The major species responsible for LD are *Borrelia burgdorferi* (*Bb*) sensu stricto, *B. afzelii* and *B. garinii*. LD *Borrelia* are extremely heterogeneous (11-13); one of the most variable proteins expressed by *Borrelia* is the outer surface protein OspC (14-16).

Pior to this study, no data was available regarding LD *Borrelia* strains present in the tick population of

Table I. Collection areas, number of collected ticks and number of isolates.

COLLECTION AREAS (n = 5)	NUMBER OF TICKS (n = 1004)	NUMBER OF ISOLATES (n = 16)	ISOLATE/(POOL)
Tobelbad	284	5	Z1 (5 nymphs) Z2 (5 nymphs) Z12 (3 nymphs) Z13 (2 nymphs) Z14 (2 females)
Fernitz	500	6	Z3 (2 females) Z4 (5 nymphs) Z5 (5 nymphs) Z6 (5 nymphs) Z7 (5 nymphs) Z16 (2 nymphs)
Grambach	71	1	Z15 (2 nymphs)
St. Veit / Vogau	53	1	Z8 (2 females)
Kleinsemmering	98	3	Z9 (5 nymphs) Z10 (2 males) Z11 (2 males)

Styria. The aim of the present study was to survey ticks from this region for the presence of *Borrelia* and to perform a preliminary characterization of the spirochete strains isolated. Ticks were therefore collected from five different areas. *Borrelia* were isolated from the ticks, and the species of each spirochete isolate identified. Additionally, for several isolates, the nucleotide sequence of the *ospC* gene was determined.

MATERIALS AND METHODS

Five biotopes were selected for tick collection (Tab. I and II). These areas are endemic for human manifestations of LD and natural foci of tick borne encephalitis. A total of 1004 ticks, including adults, nymphs and larvae, were collected by the flag dragging method; all were identified as *Ixodes ricinus*. Ticks were pooled (2-5 per pool) and cultivated in BSK-II- medium (Sigma Aldrich Chemie, Deisenhofen, Germany) with 12 mg/ml SXT at 33°C. After three to five passages, cultures were harvested in the logarithmic growing period. The protein profile was examined by SDS-PAGE using the PHAST-Gel-Gradient 8-25 in a Phast System Separation and Development Unit (Pharmacia, Uppsala, Sweden). The protein patterns were visualized by silver staining (data not shown).

Species determination was performed for isolates (n = 10) with less than six passages in culture. Species-specific primers were used in the polymerase chain reaction (PCR) to amplify 16S rRNA sequences from the strains as described (17).

The *ospC* gene was amplified from five of the strains and the nucleotide sequence of the PCR product determined as recently described (16). Sequences were analyzed using GNASIS and PROSIS software.

RESULTS AND DISCUSSION

Cultivation of pools of the ticks resulted in the isolation of sixteen *Borrelia* isolates (Tab. I). Species-specific identification of those isolates with less than six passages in culture (n = 10) revealed that three isolates were *Bb* sensu stricto, three isolates *B. afzelii*, and four isolates *B. garinii*. More than one

Table II. Species-specific identified isolates of B. burgdorferi s.l. in 5 Styrian regions endemic for borrelial infections.

COLLECTION AREAS (n = 5)	ISOLATE (n = 10)	SPECIES
Tobelbad	Z12	*B. garinii*
	Z13	*B. garinii*
Fernitz	Z5	*B. afzelii*
	Z6	*B. burgdorferi s.s.*
	Z16	*B. afzelii*
Grambach	Z15	*B. afzelii*
St. Veit/Vogau	Z8	*B. garinii*
Kleinsemmering	Z9	*B. burgdorferi s.s.*
	Z10	*B. garinii*
	Z11	*B. burgdorferi s.s.*

species was found in two of the five collection areas (Tab. II). The *ospC* gene was sequenced from five of the isolates. Each strain examined had a different *ospC* allele; one of the *B. garinii* strains had RFLP type 20, another had RFLP type 34 and the remaining three *ospC* genes from the isolates Z5, Z13 and Z16 were novel alleles.

In the present study it is shown for the first time that the three major LD *Borrelia* species *Bb* sensu stricto, *B. afzelii* and *B. garinii* are present in the tick population of Styria. In two of the five collection areas, more than one *Borrelia* species could be isolated, indicating co-existence of multiple species. Among the Styrian isolates, the *ospC* gene is highly variable. Three of the five determined *ospC* RFLP types have not been described until now. The other two RFLP types were described in *Borrelia* strains originating from varying geographical locations. The *ospC* gene of isolate Z8 showed RFLP type 20, which has been found in the Czech Republic, France and Switzerland. Isolate Z10 possessed an *ospC* gene with RFLP type 34, also found in France and Finland. It is concluded that the *Borrelia* strains infecting *Ixodes ricinus* ticks in the Austrian province of Styria are genetically highly diverse.

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