Borrelia burgdorferi SensuStricto Is Clonal in Patients with Early Lyme Borreliosis

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Lyme borreliosis, the most commonly reported vector-borne disease in North America, is caused by the spirochete Borrelia burgdorferi. Given the extensive genetic polymorphism of B. burgdorferi, elucidation of the population genetic structure of the bacterium in clinical samples may be relevant for understanding disease pathogenesis and may have applicability for the development of diagnostic tests and vaccine preparations. In this investigation, the genetic polymorphism of the 16S-23S rRNA (rrs-rrlA) intergenic spacer and ospC was investigated at the sequence level in 127 clinical isolates obtained from patients with early Lyme borreliosis evaluated in suburban New York City. Sixteen distinct rrs-rrlA and 16 distinct ospC alleles were identified, representing virtually all of the genotypes previously found in questing Ixodes scapularis nymphs in this region. In addition, a new ospC group was identified in a single patient. The strong linkage observed between the chromosome-located rrs-rrlA and plasmid-borne ospC genes suggests a clonal structure of B. burgdorferi in these isolates, despite evidence of recombination at ospC.

Lyme borreliosis is the most commonly reported vector-borne disease in the United States, with approximately 20,000 new cases tabulated by the Centers for Disease Control and Prevention each year (4). In the United States, Lyme borreliosis appears to be caused exclusively by Borrelia burgdorferi sensu stricto (hereafter referred to as B. burgdorferi)(5). B. burgdorferi exhibits a high degree of genetic diversity (1, 3, 19, 29, 30, 46), and the genetic background of the bacterium appears to affect its pathogenic and ecological properties. For example, B. burgdorferi genotypes can infect a wide range of vertebrate species, and there is a significant fitness variation among genotypes that can result in different epidemiological behavior of this pathogen (13, 18). Furthermore, a growing number of clinical and animal studies have shown that the genotype of B. burgdorferi plays a critical role in spirochete dissemination and affects the severity of clinical manifestations in mice and humans (34, 43, 44, 47). Thus, knowledge of B. burgdorferi population structure in ticks, reservoir hosts, and patients is essential in assessing the risk of Lyme borreliosis to humans. This information may also be applicable for the development of diagnostic assays and candidate vaccine preparations.

Population genetic studies of B. burgdorferi frequently involve the rrs-rrlA rRNA intergenic spacer (IGS), located on the chromosome, and/or the plasmid-borne outer surface protein C gene (ospC) (2, 3, 12, 16, 30, 46). The noncoding rrs-rrl IGS has been shown to be a useful target for the study of bacterial phylogeny. In contrast to rRNA genes, which are well conserved throughout most bacterial species, rrs-rrl spacer regions exhibit sequence variation that allows intraspecies strain differentiation (9, 10, 17). Sequence analyses of B. burgdorferi at four genetic loci (ospA, ospC, p66, and rrs-rrlA) in a nymphal tick population in Connecticut confirmed the utility of rrs-rrlA as an important phylogenetic marker for B. burgdorferi and further separated the bacterial species into at least nine clonal IGS types (3). OspC, on the other hand, is an immunodominant, plasmid-encoded surface protein that exhibits significant immunological and genetic variation (39, 40, 46). To date, 16 ospC groups have been found in questing ticks from the northeastern United States (2, 30, 46). It has been suggested that the genomic diversity of B. burgdorferi in nature is maintained by balancing selection operating on OspC and that ospC groups represent evolutionarily stable clones (30, 31, 46).

To date, population genetic analyses of B. burgdorferi in human clinical specimens employing rrs-rrlA and/or ospC have been performed mainly by restriction fragment length polymorphism (RFLP) (16, 20) and single-stranded conformational polymorphism assays, respectively (34). These analytic methods, however, have limited discriminatory power when it comes to strain differentiation at the nucleotide level. Although several studies have used DNA sequencing of multiple loci, only a small number of clinical isolates was analyzed (1, 31).

In this study, the population structure of B. burgdorferi isolates from early Lyme borreliosis patients from suburban New York City and the phylogenetic relationship among these isolates were investigated at rrs-rrlA and ospC. This represents the largest survey of sequence diversity in human-derived strains of B. burgdorferi performed to date.

MATERIALS AND METHODS

Clinical isolates. B. burgdorferi was cultivated from erythema migrans lesions or blood of patients with early Lyme borreliosis associated with erythema migrans diagnosed at the Lyme Disease Practice of the Westchester Medical Center in Valhalla, NY, between 1991 and 2005, as described previously (33, 42, 47). One hundred twenty-seven isolates (35 blood and 92 skin isolates) from a col-
lection of more than 400 isolates that had been previously typed by both RFLP analysis of the \( \text{rrlA} \) IGS (20) and reverse line blot analyses of \( \text{ospC} \) (2, 30; G. P. Wormser et al., submitted for publication) were used in this study. Skin and blood isolates obtained from separate patients were chosen in order to cover the full diversity of \( B. burgdorferi \) genotypes found in the collection. The \( \text{rrlA} \) and \( \text{ospC} \) sequences of 11 of the isolates used in this study have been previously reported (44).

DNA extraction, PCR screening, and sequencing. DNA from low-passage (passages 1 to 5) \( B. burgdorferi \) cultivated in BSK-3 (42) was isolated with IsoQuick (Orca Research, Bothell, WA). A 941-bp fragment of \( \text{rrlA} \) was amplified by PCR with primers PB and P97 as described previously (19). The nearly complete \( \text{ospC} \) gene was amplified by PCR with primers \( \text{ospC}-\text{C} \) and \( \text{ospC}-\text{N} \) or \( \text{ospC}-\text{O} \) (+) and \( \text{ospC}-\text{O} \) (-) as described elsewhere (2, 45). PCR products were sequenced on both strands (Genewiz, Inc., South Plainfield, NJ) with PCR screening primers. Isolates that produced ambiguous sequence results were cloned by limit dilution, and sequence analyses of both loci were performed on two clones from each isolate.

Sequence alignments. Multiple sequence alignments were generated with the ClustalW algorithm and BioEdit software (11) by using default parameters, followed by manual inspection. For \( \text{ospC} \), alignments were made on the translated amino acid sequences and then back-translated to nucleotide sequences to ensure in-frame nucleotide alignment.

Phylogenetic analyses. Gene trees were inferred by PAUP*4.0b10 (37) under both maximum-parsimony and maximum-likelihood criteria. Insertion and deletion (indel) polymorphisms were excluded from all but parsimony analyses of the protein-encoding \( \text{ospC} \) region, where gaps were considered as a fifth state. Maximum-parsimony analysis used parsimony-informative sites only, 20 replicates of random taxon addition, and tree bisection-reconnection branch swapping. Support for internal nodes of the inferred phylogeny was estimated by the nonparametric bootstrap method, with 1,000 replications under a maximum-parsimony criterion and tree bisection-reconnection branch swapping. For the maximum-likelihood analysis, parameters including the proportion of invariable sites, nucleotide frequencies and substitution rates, and the gamma shape parameter were estimated by the Akaika information criterion implemented in Modeltest 3.06 (27). Rate heterogeneity among sites was examined by assuming a discrete gamma distribution with eight rate categories. The maximum-likelihood trees were generated by 20 replicates of random taxon addition and tree bisection-reconnection branch swapping. Starting trees for branch swapping were obtained by stepwise addition. Nonparametric bootstrapping with 100 replicates was performed by tree bisection-reconnection branch swapping. For both maximum-parsimony and maximum-likelihood analyses, all branches receiving 70% or higher bootstrap values were considered well supported. The topologies of two gene trees were compared by using the Shimodaira-Hasegawa test (35) implemented in PAUP*4.0b10. For a given gene, the Shimodaira-Hasegawa test compares the difference in log likelihoods of competing tree topologies. The null distribution of differences in log likelihoods was obtained with 1,000 replicates of nonparametric bootstrapping of re-estimated log likelihoods. To avoid potential bias toward higher levels of significance due to small numbers of topologies (41), 100 random topologies were added to each test. For both data sets, the two maximum-likelihood trees plus 100 random trees separately generated for each data set were compared by using the Shimodaira-Hasegawa test. Statistical significance was set at \( P = 0.05 \).

Recombination analyses. Putative recombination events were detected with RDP2.0808 (22). This program uses several methods to identify putative recombination events and breakpoints in DNA. The three methods chosen, Geneconv (32), MaxChi (23), and Chimaera (28), are based on patterns of nucleotide substitution and have been shown to be the most powerful recombination methods for divergent data sets and do not infer false-positive recombination events (28). The following settings were used for all of the methods: (i) sequences were linear, (ii) sequences in the alignment were screened in triplets, and (iii) statistical significance was set to \( P = 0.001 \) with Bonferroni correction for multiple comparisons. In Geneconv, the parameter GS CALE was set to 0. In MaxChi and Chimaera, a sliding window was used, the width was set to the number of polymorphic sites divided by 1.5 (28), the number of permutations was 1,000, and the permutation \( P \) value was 0.001. Only recombination events detected by at least two methods were considered further. The data were imported into RDP2.0808 in nexus file format.

The reference sequences used in this study were downloaded from GenBank. The accession numbers of the \( B. burgdorferi \) IGS types and subtypes used are AY275189 to AY275212 (3). The accession numbers of the \( B. burgdorferi \) \( \text{ospC} \) groups used are AF129860 to AF129865 for \( \text{ospC} \) A to F, AF129867 to AF129871 for \( \text{ospC} \) G to K, L42899 for \( \text{ospC} \) L, U01892 for \( \text{ospC} \) M, L42897 for \( \text{ospC} \) N, X84778 for \( \text{ospC} \) O, U91796 for \( \text{ospC} \) P, U91790 for \( \text{ospC} \) Q, U91791 for \( \text{ospC} \) R, U91793 for \( \text{ospC} \) S, AF065143 for \( \text{ospC} \) T, and AF065144 for \( \text{ospC} \) U (34, 46).

Nucleotide sequence accession numbers. The sequences for each ribosomal spacer (RSP) allele reported in this study have been deposited in GenBank with accession numbers EF649781 for RSP1, EF649783 for RSP3, EF649784 for RSP4, EF649786 for RSP6, EF649787 for RSP7, EF649789 for RSP9, EF649790 for RSP10, and EU477177 to EU477185 for RSP12 to RSP20. The accession numbers for the \( \text{ospC} \) alleles are EU482041 to EU482056.

### RESULTS

#### Sequence diversity of \( \text{rrlA} \).

Sequencing of 127 clinical isolates of \( B. burgdorferi \) revealed 16 distinct RSP alleles (for \( \text{rrlA} \), the term allele is used to indicate a unique sequence, although this locus does not encode a protein product). The 812-bp-long alignment started at nucleotide 47 and ended at nucleotide 859 of the \( \text{rrlA} \) IGS region of GenBank reference strain \( B. burgdorferi \) B31 (accession number U03396). The lengths of individual sequences varied from 805 to 812 nucleotides due to indel polymorphism. The mean nucleotide diversity per position (\( \pi \)) was 0.025. Out of 812 nucleotide positions, 63 (7.8%) were polymorphic and 47 (5.8%) were parsimony informative (Table 1). No nucleotide substitutions were detected in \( \text{ileT} \) (encoding tRNA\(^{\text{ileT}}\))(), which was included in the alignment. The sequence identity among RSP alleles ranged from 93.8 to 99.7%. The RSP alleles obtained in this study were compared to previously reported IGS types and subtypes found in a population of field-collected questing nymphs from Connecticut (3). A majority of the RSP types (11/16) identified in patients were identical to IGS sequences found in ticks. The remaining five RSP types (RSP6, -15, -16, -17, and -19) formed well-supported clusters with existing IGS sequences. Since there were no major topological differences between maximum-parsimony and maximum-likelihood trees, only the maximum-likelihood tree is shown (Fig. 1).

#### Sequence diversity of \( \text{ospC} \).

There were 16 \( \text{ospC} \) allele types found among the 127 sequences obtained from clinical isolates. The 522-bp-long alignment started at nucleotide 78 and ended at nucleotide 578 of the \( \text{ospC} \) coding region of GenBank reference strain \( B. burgdorferi \) B31 (accession number AE000792). The sequence alignment contained a total of 522 characters, of which 250 were constant, 67 were variable
but parsimony uninformative, and 205 were parsimony infor-
mative. The mean nucleotide diversity per position (\(\pi\)) was
0.193 (Table 1). The lengths of the nucleotide sequences varied
from 498 to 510, coding for 166 to 170 amino acids. Of the
variable sites, 162 were at first and/or second codon positions,
and 110 were at third codon positions. Pairwise nucleotide
sequence identity among \(ospC\) found in this study fell into 15 major
alleles. RFLP analyses of samples used in the present study
showed that the \(RST1\) genotype was linked with two \(rrs/rrlA\)
allele profiles, \(RST2\) with 4 and \(RST3\) with 10 \(ospC/rrs\)
allele profiles (Table 2). \(RST1\) and \(RST2\) each formed well-
supported clusters in the \(rrs/rrlA\) tree. In contrast, \(RST3\) types
were more diverse (Fig. 1). Each of the \(rrs/rrlA\) alleles corre-
sponded to at least one of nine previously identified IGS types
(3). The resolution of linkage among \(rrs/rrlA\) and \(ospC\) groups
observed in the present study, however, was not apparent in
the IGS typing framework. For example, \(ospC\) groups \(K\) and \(H\)
were exclusively associated with \(RSP\) alleles 3 and 4, respecti-
vely, in the present study, but both belonged to the same IGS
type (IGS2). Similarly, \(ospC\) groups \(U\) and \(T\) were exclusively
associated with \(RSP\) alleles 12 and 13, respectively, but both
belong to IGS type 8 (3) (Table 2).

Comparison of tree topologies. To determine whether the
linkage among genetic loci could be explained by a clonal
model, phylogenetic trees were constructed for each locus and
examined for congruence. The Shimodaira-Hasegawa test was
used to determine whether the same or different phylogenetic
information was obtained from the analysis of \(rrs/rrlA\) and
\(ospC\). The \(rrs/rrlA\) phylogenetic tree was incongruent with the
\(ospC\) tree (Shimodaira-Hasegawa test, \(P < 0.05\)) (Fig. 3). Some

![FIG. 1. Unrooted maximum-likelihood phylogenetic tree based on
\(rrs/rrlA\) data set and GenBank reference strains. Nonparametric boot-
strap values for nodes with \(>70\%\) support in both maximum-likelihood
and maximum-parsimony analyses are above and below the
branches, respectively. \(B. burgdorferi\) \(RSP\) alleles identified in this study
are in bold.](http://aem.asm.org/)

### Table 2. Comparison of \(rrs/rrlA\) and \(ospC\) alleles with different
typing systems

<table>
<thead>
<tr>
<th>(rrs/rrlA) IGS</th>
<th>(ospC) AT(\text{a})</th>
<th>(ospC) group(\text{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(RSP) (no. of isolates sharing the same allele)</td>
<td>IGS type(\text{a})</td>
<td>IGS subtype(\text{a})</td>
</tr>
<tr>
<td>1 (12)</td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>3 (25)</td>
<td>2</td>
<td>2A</td>
</tr>
<tr>
<td>4 (7)</td>
<td>2</td>
<td>2D</td>
</tr>
<tr>
<td>7 (11)</td>
<td>3</td>
<td>3A</td>
</tr>
<tr>
<td>6 (7)</td>
<td>4</td>
<td>NI(\text{b})</td>
</tr>
<tr>
<td>20 (15)</td>
<td>4</td>
<td>4A</td>
</tr>
<tr>
<td>14 (4)</td>
<td>5</td>
<td>NI</td>
</tr>
<tr>
<td>15 (3)</td>
<td>5</td>
<td>NI</td>
</tr>
<tr>
<td>16 (3)</td>
<td>5</td>
<td>NI</td>
</tr>
<tr>
<td>17 (1)</td>
<td>5</td>
<td>NI</td>
</tr>
<tr>
<td>9 (7)</td>
<td>6</td>
<td>6A</td>
</tr>
<tr>
<td>18 (6)</td>
<td>6</td>
<td>6B</td>
</tr>
<tr>
<td>10 (12)</td>
<td>7</td>
<td>7A</td>
</tr>
<tr>
<td>12 (6)</td>
<td>8</td>
<td>8A</td>
</tr>
<tr>
<td>13 (2)</td>
<td>8</td>
<td>8C</td>
</tr>
<tr>
<td>19 (6)</td>
<td>9</td>
<td>NI</td>
</tr>
</tbody>
</table>

\(\text{a}\) \(rrs/rrlA\) typing based on reference 3.

\(\text{b}\) \(rrs/rrlA\) typing based on reference 20.

\(\text{c}\) \(ospC\) groups according to references 34 and 46.

\(\text{d}\) NI, not identified.

\(\text{e}\) NT, new type.
of the incongruence involved nodes with strong bootstrap support in both trees. For example, ospC AT10/RSP16 and ospC AT8/RSP4 pairing was well supported in the ospC tree but both grouped with different ospC/RSP allele profiles with >70% bootstrap support on the rrs-rrlA tree.

Recombination. Three recombination methods (Geneconv, MaxChi, and Chimaera) were used to assess the presence of recombination at rrs-rrlA and ospC. None of the methods detected evidence of recombination at rrs-rrlA. The same analyses were performed on the ospC alignment. To reduce the risk that the clustering of polymorphic sites at ospC resulted from selection rather than recombination, an alignment that contained only third codon position nucleotides was used. Three putative recombination events were detected. All of them involved ospC group C as a putative recipient and ospC group B as one of the putative donors. In addition, ospC groups K, M, and H were detected as possible donors with a significance level of P < 0.001 (Fig. 4). These observations, in addition to the overlapping positions of recombination breakpoints, however, suggest that the detected recombination events most likely represent a single recombination involving three different possible combinations of parents. There were no traces of recombination identified in the newly described ospC group represented by ospC AT16.

DISCUSSION

The present study is the first large-scale sequence-based investigation of the genetic diversity of B. burgdorferi clinical isolates. rrs-rrlA and ospC have previously received much attention as genetic markers in epidemiological and population studies of B. burgdorferi, as well as loci associated with disease severity and dissemination in mice and humans (1, 3, 30, 34, 43, 44, 46, 47). In the present data set, 16 distinct rrs-rrlA and 16 distinct ospC alleles were identified. In addition, strong linkage

FIG. 2. Unrooted maximum-likelihood phylogenetic tree based on ospC data set and GenBank reference strains. Nonparametric bootstrap values for nodes with >70% support in both maximum-likelihood and maximum-parsimony analyses are above and below the branches, respectively. B. burgdorferi ospC alleles used in this study are in bold.

FIG. 3. Unrooted maximum-likelihood phylogenetic trees based on rrs-rrlA and ospC alleles. Nonparametric bootstrap values for nodes with >70% support in both maximum-likelihood and maximum-parsimony analyses are above and below the branches, respectively. The arrow indicates the differences between the maximum-likelihood and maximum-parsimony tree topologies supported by bootstrap values of >70% in either a maximum-likelihood or a maximum-parsimony analysis.
among these loci and the presence of recombination at *ospC*
were found.

Based on genetic variation, 21 *ospC* groups have been iden-
tified in *B. burgdorferi* worldwide, of which 16 have been found
in the northeastern United States (30, 34, 46). We identified 15
*ospC* alleles that were representative of 15 previously defined
*ospC* groups found in the northeastern United States. In addi-
tion, one unique *ospC* allele (*ospC* AT16) was found and,
based on the criterion of sequence similarity, can be consid-
ered a representative of a new *ospC* group (46). Recently, *B.
burgdorferi* was divided, based on *rrs*-mlA variation over a 250-bp
region, into at least nine major *rrs*-mlA (IGS) genotypes (3). Each of the 16 RSP alleles identified in the
present study corresponded to one of these nine major IGS
genotypes. However, three of the IGS types (2, 5, and 8)
represented more than one *ospC* group. In the present anal-
ysis, an 812-bp region of *rrs*-mlA was used. As a result of using
more extensive sequence information, it was possible to link
each *ospC* allele with a unique *rrs*-mlA allele. The association of
*ospC* with other genomic loci in *B. burgdorferi* has previously been reported (1, 3, 16, 30, 31, 46). It has been suggested,
based on the sequence analyses of one chromosomal locus and
multiple plasmid-borne polymorphic loci, that the *B. burgdor-
feri* population structure is dominated by balanced polymor-
phism at *ospC* and that the *ospC* groups could be viewed as
evolutionarily stable clonal complexes (31).

In the present data set, each *rrs*-mlA/*ospC* lineage was repre-
sented by a single *ospC* and a single *rrs*-mlA allele, despite the
fact that additional diversity of *rrs*-mlA and *ospC* alleles was
found in *Ixodes scapularis* ticks (3, 26, 46) and/or human-de-

tived samples (34). One could hypothesize that the clonal
lineages found in clinical samples of *B. burgdorferi* represent
more pathogenic genotypes and therefore underestimate the
genotypic diversity in the natural population and overestimate the
extent of clonality (36). Alternatively, the allelic com-
binations found in the present study could represent the predom-
inant clonal lineages that circulate in the ticks in the local area.
It is also possible that the observed clonal selection arose from
selective pressure during cultivation (20, 25).

The presence of additional *rrs*-mlA alleles may suggest that a
single *ospC* group can be associated not only with closely re-
lated *ospC* alleles (i.e., members of the same group have se-
quence difference of <2%) (46) but also with several different
*rrs*-mlA alleles. This phenomenon could be a result of diversi-
fication processes, such as point mutations and/or recombi-
nation, within each *ospC* clonal type that gives rise to *ospC* clonal
complexes (7, 31). It is also possible that *rrs*-mlA alleles repre-
sent different strains of *B. burgdorferi* that could share the same
*ospC* group. This would be consistent with the hypothesis that
balancing polymorphism may predate divergence among *B.
burgdorferi* species (31). Indeed, it has been shown previously
that *rrs*-mlA alleles match putative multilocus sequence types
(MLSTs) (1). However, this MLST was based on plasmid
genomes rather than housekeeping genes located on the chromo-
some. Furthermore, some of the loci included in the MLST
analysis are located on plasmids that may be absent in certain
*B. burgdorferi* strains (14, 38).

A number of studies have suggested extensive intra- and
interspecies recombination at *ospC* among *B. burgdorferi sensu
lato* species (6, 15, 21). Recombination at *ospC* was also re-
ported in *B. burgdorferi* strains found in the tick population in
the northeastern United States (3, 46). In the present study,
recombination at *ospC* in *B. burgdorferi* clinical isolates was
analyzed. The clonal model of bacterial evolution implies that
a genome forms a single linkage group and that all loci will
record the same evolutionary history (6, 8). The extent of
phylogenetic congruence among *rrs*-mlA and *ospC* phylogenetic
trees was investigated. The observed discrepancy between the
trees in statistically highly supported branches could be indica-
tive of a history of recombination. Therefore, the presence of
recombination at *ospC* was further investigated by analyzing
the distribution of polymorphic sites within a sequence align-
ment. The identification of clustered polymorphism from third
codon position in *ospC* group C provided the strongest evi-
dence of recombination at *ospC* within the local *B. burgdorferi*
population. Given the conservative nature of these recombi-
nation analyses, however, it is possible that the number of
putative recombination events has been underestimated.
The strong linkage between rrs-mtA and ospC appears to argue against recombination at ospC. Linkage disequilibrium is a well-acknowledged sign of clonality; however, it can be apparent even in recombining populations (7, 8). This might imply a low rate of recombination at ospC (6) that is not strong enough to disrupt the observed linkage. Since ospC recombinants are under strong positive selection (6), the recombination at ospC can be commonly detected, even if the frequency of recombination would be vanishingly small (8). Alternatively, ospC clones may have emerged and spread rapidly due to strong selective pressure but would be unstable over the long term (24). In addition, possible epistatic interactions among ospC and chromosomal loci, population bottlenecks accompanied by founder effects, and/or the presence of a strong balancing selection at ospC could result in the observed ospC-chromosome association (1, 30, 31, 46).

In the northeastern United States, I. scapularis nymphs are the main vectors involved in the transmission of B. burgdorferi to humans. The genetic diversity of B. burgdorferi in questing nymphs should reflect the entire spectrum of genotypes that humans might be exposed to in nature. The patients diagnosed at the Lyme Disease Practice of the Westchester Medical Center that were chosen for the present study were infected with B. burgdorferi genotypes that represent virtually all of the major IGS types and ospC groups found in the questing I. scapularis nymphs in this region (2, 3, 12, 30, 46). The exception was ospC group L, which was not identified in patients and was found only at a very low frequency in ticks, suggesting that the lack of this ospC group in clinical samples could simply be due to sampling error. In addition, a new ospC group that had not been previously described was isolated from one patient. The congruence of genotype diversity between clinical and tick-derived specimens suggests that any IGS or ospC genotype found in nature is capable of infecting humans.

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