

Differential Survival of Lyme Borreliosis Spirochetes in Ticks That Feed on Birds

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The abilities of the most common European genospecies of *Borrelia burgdorferi* sensu lato to survive blood meals taken by ticks feeding on birds were analyzed. A pattern of differential survival of the spirochetes in feeding ticks was observed. The result is consistent with the concept of selective transmission of Lyme borreliosis spirochetes.

The genospecies of *Borrelia burgdorferi* sensu lato and, at times, their variants are maintained in nature by different sets of hosts (1, 3, 6–10, 15, 18, 20, 21, 23; K. Hanincová, S. M. Schäfer, S. Etti, H.-S. Sewell, V. Taragelová, D. Ziak, M. Labuda, and K. Kurtenbach, submitted for publication). At present, the key determinant of this host association is considered to be the interaction of *B. burgdorferi* sensu lato with the alternative pathway of the host's complement system (14, 16, 19, 22, 25). A recently proposed model of transmission predicts the selection of spirochetes by complement in the gut of feeding ticks (17, 18). In the present study, an avian model was used to test this prediction.

Two-week-old pheasants (*Phasianus colchicus*) were obtained from a breeder in Oxfordshire, United Kingdom. *Ixodes ricinus* larvae and nymphs were derived from a tick colony maintained at the NERC Centre for Ecology and Hydrology, Oxford, United Kingdom. Low-passage cultures of *B. burgdorferi* sensu stricto (ZS 7), *Borrelia afzelii* (ACA 1), *Borrelia garinii* (an isolate from Freiburg, Germany, with an *ospA* allele identical to that of the Rio2 strain [2]), and *Borrelia valaisiana* (strain UK) were expanded to a concentration of 10⁷ cells per ml of culture medium. The cultures were used to infect questing nymphs with spirochetes by glass capillary feeding. Of those ticks which took up more than 100 spirochetes, as calculated by the volume of culture imbibed, 20 were introduced to each bird by being confined within a neoprene cell glued with latex to the shaved throat of each bird (Fig. 1). Four groups composed of four birds each were challenged with ticks infected with one of the genospecies of *B. burgdorferi* sensu lato. Engorged nymphs were recovered, kept for 2 weeks, and then preserved in 70% ethanol.

For comparison, an additional four groups of 10 nymphs each (all of whom were preinfected with one of the four genospecies) were not allowed to feed on hosts but were starved for

several weeks and then preserved in ethanol. Four weeks after the challenge with infected nymphs, the pheasants were tested by xenodiagnosis for *Borrelia* through the introduction of >50 noninfected *I. ricinus* larvae per animal. These larvae were also contained in neoprene cells and glued to the back of the neck of each pheasant. Engorged larvae were allowed to molt to nymphs. After xenodiagnosis, two skin biopsy samples were taken from each bird, one from the feeding site of ticks and one from the eyelid. The 5S–23S intergenic spacer and the *ospA* locus of *B. burgdorferi* sensu lato were amplified by nested PCR from DNA extracted from *Borrelia* cultures, ticks, and biopsy samples (5, 15), and the PCR products were sequenced. In addition, wild-type strains from three natural sources were analyzed: (i) ticks engorged on wild pheasants captured during 1996 in a woodland near Wimborne St. Giles, Dorset, United Kingdom (15), (ii) questing *I. ricinus* ticks collected in the summer of 2001 from the same site, and (iii) questing ticks collected in the spring of 2000 in a forest near Ville d'Eu, Normandy, France.

Of the 80 experimentally infected nymphs introduced to each group of birds, between 27 (34%) and 56 (70%) were recovered in a fully engorged state. Approximately half of the preinfected nymphs tested positive for *B. burgdorferi* sensu stricto, *B. garinii*, and *B. valaisiana* 2 weeks post-repletion (Fisher's exact test, $P > 0.05$) (Table 1). *B. garinii* and *B. burgdorferi* sensu stricto were also detected in xenodiagnostic ticks. Xenodiagnosis performed on birds challenged with *B. valaisiana* was unsuccessful because the larvae from this group of birds, all kept in one desiccator, did not survive. *B. garinii* was significantly more prevalent than *B. burgdorferi* sensu stricto in xenodiagnostic ticks ($P < 0.05$). By contrast, *B. afzelii* was not detected 2 weeks after repletion in either preinfected nymphs or xenodiagnostic ticks. However, *B. afzelii* as well as the other genospecies were detected in all starved nymphs that had taken up >100 spirochetes. One out of four and three out of eight biopsy samples taken from birds challenged by nymphs infected with *B. garinii* and *B. valaisiana*, respectively, tested positive, but all the birds exposed to nymphs preinfected with *B. afzelii* were negative. The *ospA* sequences of the *B. garinii* and *B. valaisiana* cultures were compared to *ospA* sequences

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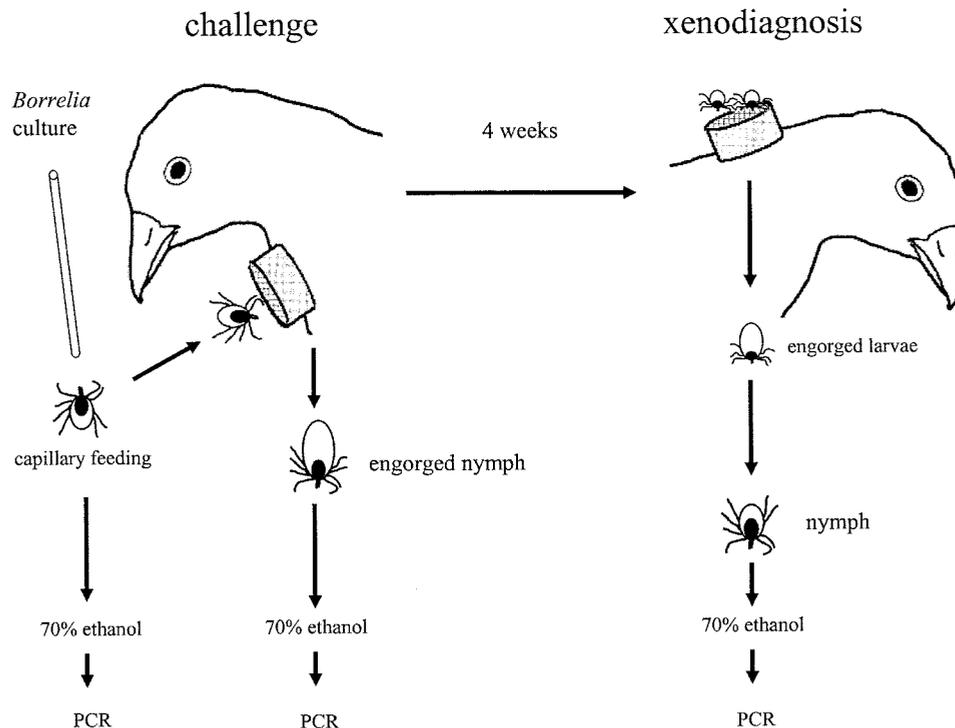


FIG. 1. Flow chart illustrating the experimental protocol to assess reservoir competence of pheasants for *B. burgdorferi* sensu lato. The life stages of ticks and the points where samples were taken and preserved are indicated.

detected in natural foci in the United Kingdom and France. In each pool from the field, the allele of the Rio2 strain of *B. garinii* (GenBank accession number AF227319; OspA serotype 3 [2]) and the allele of the UK strain of *B. valaisiana* (GenBank accession number AF09591) were found with considerable frequencies (Table 2).

The results show that *B. garinii*, *B. valaisiana*, and *B. burgdorferi* sensu lato survived the blood meal taken by ticks feeding on pheasants, whereas the *B. afzelii* strain was eliminated during the blood meal. The differential survival of spirochetes in the vector ticks was consistent with the xenodiagnostic data, confirming that pheasants are adequate reservoirs for *B. garinii* and *B. burgdorferi* sensu stricto (15). Although we did not assess the transmission of *B. valaisiana* from pheasants to ticks in this study, the survival of this genospecies in vector nymphs

and its detection in three out of eight biopsy samples suggest that pheasants are also adequate reservoirs for *B. valaisiana*. This is strongly supported by the presence of this genospecies in ticks that fed on wild pheasants (15) (Table 2).

It is very unlikely that the elimination of the ACA 1 strain of *B. afzelii* in ticks feeding on birds was due to the loss of infectivity of this particular isolate, because starved ticks remained infected with this strain. Rather, the results indicate that the uptake of avian blood triggered the elimination of *B. afzelii* in the tick. Furthermore, *B. afzelii* has been detected only occasionally in bird-derived ticks anywhere in the world. The experimental and field-derived data taken together strongly suggest that all strains of *B. afzelii* are killed in ticks feeding on birds.

The pattern of differential survival found in this study is consistent with in vitro findings on resistance or sensitivity of

TABLE 1. Infection chains of *B. burgdorferi* sensu lato genospecies with the pheasant as the avian host model, infected *I. ricinus* nymphs as vectors, and larvae as xenodiagnostic ticks^a

Genospecies	No. positive/total no. examined (%)		
	Vector ticks	Xenodiagnostic ticks ^b	Biopsy samples
<i>B. burgdorferi</i> sensu stricto	12/27 (44)	6/44 (13)	ND ^c
<i>B. afzelii</i>	0/56 (0)	0/33 (0)	0/8
<i>B. garinii</i>	14/31 (45)	31/72 (43)	1/4
<i>B. valaisiana</i>	23/38 (60)	NA ^d	3/8

^a Four birds were examined for each genospecies tested.

^b Tested for infection after molting to nymphs.

^c ND, not determined.

^d N/A, not available. The larvae from this group of birds did not survive.

TABLE 2. Frequency of *ospA* alleles of *B. garinii* and *B. valaisiana* in the field

Source ^a	No. of <i>B. garinii</i> sequences	No. of <i>B. garinii</i> alleles	Frequency of Rio2 strain allele ^b	No. of <i>B. valaisiana</i> sequences	No. of <i>B. valaisiana</i> alleles	Frequency of UK strain allele
A	16	6	3/16	6	2	4/6
B	35	12	3/35	9	1	9/9
C	27	14	2/27	12	3	10/12

^a A, engorged nymphs of *I. ricinus* fed on male pheasants captured in Dorset, United Kingdom, in 1996 (15); B, questing nymphal and adult *I. ricinus* ticks collected in Dorset, United Kingdom; C, questing nymphal and adult *I. ricinus* collected in Ville d'Eu, Normandy, France.

^b Rio2 is the *B. garinii* strain which represents OspA serotype 3 (2).

spirochetes to avian complement of studies using the same cultures of *B. burgdorferi* sensu lato (16). The data validate our recent model of selective transmission of *B. burgdorferi* sensu lato, which suggests that *B. afzelii* is lysed by avian complement in the gut of the feeding tick (17, 18). It remains to be determined whether *B. garinii* and *B. valaisiana* are eliminated in ticks feeding on rodents, as would be predicted by the high sensitivity to complement of *B. garinii* strains (other than OspA serotype 4 strains or NT 29 ribotypes that are associated with rodents [8, 21]) and *B. valaisiana* (16).

Resistance of spirochetes to complement is now known to be mediated by the expression of complement regulator-acquiring surface proteins (11–13). In fact, a general property of the proteins encoded by genes of the *erp* gene family appears to be their ability to bind host-derived complement control proteins in a species-specific pattern (24). Furthermore, the *erp* genes are expressed in infected, feeding *Ixodes scapularis* ticks (4). Altogether, the combined information points to the tick gut as a crucial site of selection during the life cycle of *B. burgdorferi* sensu lato. Thus, we suggest that *B. burgdorferi* sensu lato comprises distinct ecotypes that are determined by their *erp* gene repertoires.

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