Electronic supplementary material

Relative frequencies of the major *ospC* group strains: In the present study, we report the relative frequencies rather than the absolute frequencies of the major *ospC* group strains for *B. afzelii* (Table 1) and *B. garinii* (Table 2). The relative frequency is the frequency of each major *ospC* group strain in the subset of nymphs infected with that particular *Borrelia* genospecies. For example, the relative frequency of *B. afzelii* major *ospC* group A10 is 0.647 (99 nymphs infected with strain A10/153 nymphs infected with *B. afzelii*; Table 1). In contrast, the absolute frequency refers to the frequency of each major *ospC* group strain in the population of *I. ricinus* nymphs, which includes both infected and uninfected individuals. The proportion of nymphs infected with *B. afzelii* was 0.118 (872 *B. afzelii*-infected nymphs/7400 total nymphs) and so the absolute frequency of *B. afzelii* major *ospC* group A10 is 0.118*0.647 = 0.076.

Native and exotic major *ospC* groups: The *Borrelia* genospecies for each nymph was determined using a reverse line blot (RLB) assay that targeted the 23S-5S spacer gene (1). We had previously used the terms ‘native’ and ‘exotic’ to indicate whether a given major *ospC* group corresponded to the *Borrelia* genospecies identification of the RLB assay or not (2). For example, the major *ospC* group A10 has been traditionally associated with *B. afzelii*. Thus major *ospC* group A10 is classified as native when it is recovered from a *B. afzelii*-infected nymph and as exotic when it is recovered from a *B. garinii*-infected nymph. In the manuscript, we removed the exotic major *ospC* groups from the statistical analysis.

Of the 13 major *ospC* groups found in this study, 6 have been traditionally associated with *B. afzelii* (A1, A2, A7, A9, A10, A14), and 7 with *B. garinii* (G2, G4, G6, G7, G13, G14, G15). Of the 68631 reads from the *B. afzelii*-infected nymphs, 68518 native reads (99.84%) and 113 exotic reads (0.16%) clustered with the major *ospC* groups of *B. afzelii* and *B. garinii*,

respectively. Conversely, of the 24014 reads from the *B. garinii*-infected nymphs, 22178 native reads (92.35%) and 1836 exotic reads (7.65%) clustered with the *ospC* major groups of *B. garinii* and *B. afzelii*, respectively.

REFERENCES

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