Response to Dr. Randolph and Drs. Gern and Humair

To the Editor: We define reservoir competence of a host for a vector-borne pathogen in terms of three component questions: How susceptible is the putative reservoir host when the pathogen is delivered by the bite of an infected vector tick? How effectively does the pathogen proliferate and develop in this host? And how infective is the resulting infected host to vector ticks and for how long (1,2)? Drs. Gern and Humair insert the parenthesis (implied xenodiagnosis) into a citation of our text, thereby, equating reservoir competence with a simple xenodiagnostic test that partially addresses only the third component of this definition. At best, such a test records degree of infectivity to vector ticks at some arbitrary and often unknown point in time, a consideration that persuades us to limit our citations referring to reservoir competence. Conclusions derived from xenodiagnosis performed on field-derived animals differ from those that are obtained by an experimental study. With regard to acknowledging relevant research, we did cite the study on pheasants (3) in which these birds were infected in the laboratory by tick-borne spirochetes and subsequently infected only about a quarter of vector ticks. The cited study on blackbirds (4), on the other hand, used ticks solely to diagnose

infection in field-derived birds that had been infected in nature. Although a few of these animals proved to be infectious to xenodiagnostic ticks when tested 1-3 days after capture, this study failed to quantify susceptibility or to determine intensity and duration of infectiousness to vector ticks. Our rigorously standardized study (1) is the first to establish experimentally that birds are highly competent as reservoir hosts for Lyme disease spirochetes.

Drs. Gern and Humair disagree with our statement that "larval ticks seem not to feed on [pheasants], either in the laboratory or in nature." However, a field study on pheasants states that "no fully engorged larvae … were recovered from thirty adult male pheasants shot in a Dorset woodland" (5). The previously cited experimental study on these birds similarly demonstrated that larval infestations generally fail, and stated that "In fact, most of the introduced larvae died while attempting to feed on the pheasants" (3). Inflammatory responses directed against feeding larvae were advanced as a possible explanation for the observed failure to feed to repletion. The larval stage of the American vector similarly seems to feed poorly on chickens (6). Passerine birds, however, seem to serve effectively as hosts for the larval stages of this complex of ticks, and we find that larvae attach readily to American robins (1). Numerous larval *Ixodes ricinus* ticks feed on European blackbirds in nature, and larval ticks attach readily and repeatedly to such birds in the laboratory (7,8). Therefore, the limited attractiveness of gallinaceous birds to larval *Ixodes* ticks may render them less important than certain passerine birds as natural reservoir hosts for Lyme disease spirochetes.

The transmission cycle of the agent of Lyme disease tends to be more complex in Europe than in North America. The host range of the European vector tick, *I. ricinus*, is broader than that of its American cousin (*I. dammini*, frequently cited as *I. scapularis*), and the European pathogen in humans is more diverse, comprising several genospecies. Our study aimed to define the competence of the American robin, *Turdus migratorius*, as a reservoir host for rodent-infecting *Borrelia burgdorferi* sensu stricto—not *B. burgdorferi* sensu lato as stated in Drs. Gern and Humair's letter—and used American *Ixodes* ticks as vectors. The mode of perpetuation of the agents of human Lyme disease in Europe is peripheral to the subject of our article.

Dr. Randolph and Drs. Gern and Humair express commitment to the concept that different European spirochetal genospecies perpetuate simultaneously in distinct kinds of vertebrate reservoir hosts. Their concept requires that a larval *I. ricinus* tick that acquires a rodent-specific genospecies from a rodent host must, in its nymphal stage, again feed on a rodent. If this nymphal tick were to feed on a bird, the rodent-specific spirochete would not perpetuate because this nonpermissive host would function zooprophylactically. A suggested avian-specific spirochete would perpetuate reciprocally. According to the MacDonald concept of vectorial capacity (9), such a relationship would be unlikely if pathogens requiring different reservoir host populations were to be transmitted simultaneously by the same vector population. The studies cited in support of this concept rest on correlative evidence derived from field data. No confirmatory experimental proof demonstrates an especially intense association of *B. afzelii* with rodents and *B. garinii* or *B. valaisiana* with birds. Indeed, European larval ticks acquire *B. afzelii* as well as *B. garinii* infection from field-derived passerine birds (10). Various other observations also contradict the suggested close association between genospecies and particular kinds of hosts (11-13). One of the studies (14) cited as evidence for genospecies specificity was published even before the genospecies were differentiated; the other "consistent independent findings" derive from the laboratories of Drs. Randolph and Gern. Our findings that birds serve as competent hosts for an apparently mammal-perpetuated spirochetal genospecies would seem to contradict the concept of separate genospecies perpetuation. No rigorous evidence is yet in hand to support the theory that the same population of vector ticks perpetuates different European spirochetal genospecies differentially in particular kinds of reservoir hosts.

Dr. Randolph suggests that our experiments may have been confounded because Lyme disease spirochetes may have been inherited persistently within the laboratory colony of ticks used in our studies. Although an early observation points toward the possibility of such a mode of transovarial transmission

(15), subsequent experimental evidence suggests that vertical transmission rarely, if ever, occurs (16). Inherited infection in nature would be exceedingly infrequent because spirochetes infect less than 1% of naturally questing larvae, both in North America and in Europe (17, 18), and some of these larvae may have acquired infection by feeding partially on an infected host. We routinely seek evidence of spirochetal infection in each cohort of larval ticks used in our experiments but have never found spirochetes in a nonfed, laboratory-reared larva. Our reported frequency of experimental transmission of Lyme disease spirochetes from reservoir mice to vector ticks corresponds to that reported elsewhere (19-21).

Dr. Randolph's statistical analysis of our data confirms that the feeding success of nymphal ticks on robins exposed repeatedly to ticks varies nonsignificantly, supporting our conclusion that nymphal ticks readily feed repeatedly on tick-exposed robins. Although repeated nymphal infestations may protect inbred laboratory mice from tick-borne spirochetes (22), natural reservoir hosts, such as white-footed mice and American robins, remain susceptible to such spirochetes, regardless of prior exposure to ticks (1, 23).

Dania Richter,*† Andrew Spielman,†
Nicholas Komar,†‡ and
Franz-Rainer Matuschka*†

*Charité, Medizinische Fakultät der Humboldt-Universität zu Berlin, Berlin, Germany; †Harvard School of Public Health, Boston, Massachusetts, USA; ‡Current affiliation: Centers for Disease Control, Fort Collins, Colorado, USA.

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