

emphasize determining the reservoirs, the modes of virus transmission to people, and the possible distinct clinical forms of hantavirus infections.

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## Israeli Spotted Fever *Rickettsia* in Sicilian *Rhipicephalus sanguineus* Ticks

**To the Editor:** Mediterranean spotted fever (MSF) is endemic in Italy, where it is a reportable disease. From 1992 to 1998, the Italian Ministry of Health was notified of approximately 8,500 cases of human rickettsioses presumed to be MSF. MSF occurs more commonly in some central (Lazio) and southern (Sardinia, Sicily, and Calabria) regions (1,2); in 1998, an average of 8.8 cases occurred for every 100,000 persons in Sicily, compared with the national average of 1.6 cases per 100,000 persons. *Rickettsia conorii* has been thought to be the only pathogenic *Rickettsia* of the spotted fever group in Sicily (3,4) or the western Mediterranean area. Recently, three different spotted fever group rickettsiae, including *R. helvetica*, were detected in *Ixodes ricinus* ticks from central and northern Italy. This finding suggests that bacteria other than *R. conorii* are involved in rickettsial diseases in Italy (5).

To investigate whether unusual tick-transmitted rickettsiae are also present in Sicily, we used molecular-sequence-based identification techniques to study two strains isolated from the hemolymph of *Rhipicephalus sanguineus* ticks collected in 1990 in western Sicily. These isolates had been previously identified by serologic tests as belonging to the spotted fever group rickettsiae. We obtained bacterial DNA and performed polymerase chain reaction (PCR) for *ompA* gene and restriction analysis under conditions previously described by Roux et al. (6). Our observation of a peculiar *PstI* profile allowed a presumptive identification of one of the two tick isolates as belonging to the Israeli spotted fever rickettsiae, while the other showed a restriction profile corresponding to that of *R. conorii* strain Seven. To confirm the identification of the Israeli spotted fever *Rickettsia* isolate, we sequenced the PCR-amplified fragment of *ompA* gene (MWG-Biotech AG, Ebersberg, Germany) and aligned sequence data with homologous sequences of reference strains of the spotted fever group rickettsiae retrieved from the GenBank database. Sequence analysis showed

100% similarity with the homologous sequence of Israeli spotted fever *Rickettsia* reference strain ISTT CDC1 (GenBank accession no. U43797). The Israeli spotted fever *Rickettsia* belongs to the *R. conorii* complex (7,8) and was first isolated in 1974 from ticks and humans. Initially, Israeli spotted fever rickettsiae distribution appeared to be restricted to Israel (9), but more recently the organism has also been isolated from patients with MSF in Portugal (10). Our finding of Israeli spotted fever *Rickettsia* infection in a *R. sanguineus* tick, the main vector for MSF in Sicily, also suggests that the geographic distribution of Israeli spotted fever might be wider than previously thought, including not only Israel and the Iberian Peninsula but also Italy.

Molecular analysis of spotted fever group *Rickettsia* isolates from Sicilian MSF patients is under way to verify this hypothesis. Because initial signs and symptoms of Israeli spotted fever are particularly uncharacteristic, awareness of the presence of Israeli spotted fever *Rickettsia* in our geographic area may hasten provision of the appropriate treatment. The Sicilian *ompA* gene sequence described in this study has been

deposited in the GenBank database (accession no. AY197565).

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## Co-feeding Transmission and Its Contribution to the Perpetuation of the Lyme Disease Spirochete *Borrelia afzelii*

**In Reply:** Richter et al. (1) have asked an important question: To what extent does the transmission of non-systemic infections of the Lyme borreliosis spirochete (*Borrelia afzelii*) between co-feeding nymphal and larval *Ixodes ricinus* ticks apply to natural tick infestations on wild rodents? The authors conclude that the transmission of infections 3 days after inoculation by tick bite is >100 times less efficient than the transmission of infections that have lasted at least 14 days. That answer depends on a critical calculation based on experimental results combined with field observations. Unfortunately, this calculation is incorrect by a factor of approximately 20.

When hairless laboratory mice were restrained within wire mesh tubes and larvae were allowed to

attach at random over their bodies, 13.6% of these larvae became infected with *B. afzelii* if they fed 3 days after the attachment of a single infected nymph (i.e., transmission probability of 0.136, as used below). By contrast, 85.4% of larvae that fed 14 days after the nymph became infected (1). At three sites in Germany and France, over the period April–October in each of the years from 1993 through 1995, 17.6% of mice (*Apodemus flavicollis* and *A. sylvaticus*) and voles (*Clethrionomys glareolus*) fed larval and nymphal ticks together, while 1.5% fed nymphs alone. Of these nymphs, 26.4% were infected with *B. burgdorferi* s.l. before attachment. The probability of a larva's acquiring an infection equals the product of 1) the probability of transmission from host to larva and 2) the probability of the host's being infected while the larva feeds via an infected nymphal tick bite. For a short-lived (3-day) infection, the probability is  $0.136 \times 0.176 \times 0.264 = 0.0063$ ; for longer-lived (14-day) infections, the probability is  $0.854 \times (0.176 + 0.015) \times 0.264 = 0.0431$ . The ratio is therefore 1:6.8. Richter et al. erroneously con-

cluded that the ratio was 1:116 because they did not take into account the probability of wild rodents' acquiring a long-lived, "systemic" infection; the authors assumed the probability was 1. A greater proportion of garden dormice (*Eliomys quercinus*) carried ticks and so would yield much higher transmission probabilities but in almost the same ratio, 1:6.4.

In fact, how much of the increase from 13.6% transmission at day 3 to 85.4% at day 14 was due to the development of systemic infections (i.e., disseminated to parts of the hosts' bodies >2 cm from the infected tick bite) is not clear because the feeding sites of the larvae attached ad libitum on the hairless mice were not reported. In the original discovery of co-feeding transmission of *B. burgdorferi* s.l. (2), the infection prevalence in larvae feeding close to infected nymphs increased from 33% on day 2 to 96% on day 11 and 100% on day 14 (3; see Figure 2 therein) in the demonstrated absence of a systemic infection. Mice skin and ticks feeding at distant sites remained uninfected. Only after day 14 had a systemic