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# Investigation of haematophagous arthropods for borreliae – summarized data, 1988-1996*)

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**Abstract.** Blood-sucking arthropods, collected in South Moravia, Czech Republic, were examined by darkfield microscopy for borreliae from 1988 to 1996. Among host-seeking ixodid ticks (8481 *Ixodes ricinus* (L.), 372 *Dermacentor reticulatus* (Fabr.), 167 *Haemaphysalis concinna* Koch), borreliae were only observed in adult (23.2%), nymphal (17.2%) and larval (6.3%) *I. ricinus*. The prevalence of borreliae in *I. ricinus* did not vary considerably among habitats except for lower values in agroecosystems, xerothermic oak woods and grasslands. The frequency of intensity of spirochaetal infection ($\log_{10}$ counts of borreliae per tick) in *I. ricinus* approximated the negative binomial distribution. The proportions of host-seeking female and nymphal ticks containing >100 borreliae were 5.0% and 1.7%, respectively. Among preimaginal ticks (749 *I. ricinus*, 222 *D. reticulatus*, 82 *H. concinna*) parasitizing free-living forest birds and small mammals, borreliae were detected in 6.1% of larval and 10.3% of nymphal *I. ricinus*, and in one larval *H. concinna*; 3.2% of the birds and 19.4% of the mammals carried infected ticks. Among 3464 female mosquitoes (Culicidae) of 6 species, 4.1% contained spirochaetes: 1.4% of *Aedes vexans* Meig., 1.3% of *A. cantans* (Meig.), 2.2% of *A. sticticus* (Meig.), 2.2% of *Culex pipiens pipiens* L. and 5.9% of *C. p. molestus* Forskal. Borreliae were also detected in 8.4% of 142 fleas (Siphonaptera, largely *Ctenophthalmus agyrtes* Heller and *Hystrichopsylla talpae* Curtis) collected from small mammals. Twelve isolates of *B. burgdorferi sensu lato* have been identified to genospecies: 6 strains from *I. ricinus* (4 *Borrelia garinii* Baranton et al., 1 *B. afzelii* Canica et al. and 1 *B. lusitaniae* Le Fleche et al.), 1 strain from *A. vexans* (*B. afzelii*), 2 strains from *C. agyrtes* (*B. afzelii*), and 3 strains from host rodents (*B. afzelii*).

The ecology of the Lyme disease agent, *Borrelia burgdorferi sensu lato*, has been investigated mainly in ixodid ticks (see Gern et al. 1993), much less often in other blood-sucking athropods. We have been studying borreliae in various haematophagous arthropods in South Moravia, Czech Republic, since 1988. This contribution summarizes our results over the whole period, and involves published (Hubálek et al. 1990, 1991a,b, 1993, 1994, 1996a,b, Kryuchechnikov et al. 1990, Halouzka 1993, Halouzka et al. 1995, 1997, Mátlová et al. 1996) as well as unpublished data.

## MATERIALS AND METHODS

Arthropods were collected in various localities and habitats of South Moravia by dragging white flannel flags over low vegetation (host-seeking ixodid ticks: March to November), from 123 live-captured small mammals of 7 species (ixodid ticks and fleas: June to July) and 411 netted birds of 33 species (ixodid ticks: April to October) or by aspirator on human bait (mosquitoes, including hibernating ones: all the year round). They were stored in humidified tubes at 5°C until examination (i.e., 1-10 days), and only living arthropods were examined. Abdomen organs (mainly midgut) of arthropods were homogenized in a drop of saline and examined individually by darkfield microscopy; borreliae were counted. Isolation attempts in BSK II or BSK-H (Sigma) medium were only carried out from preparations containing high numbers of spirochaetes. Inoculated test tubes were incubated at 33°C for up to 6 weeks. Characterization and identification of borrelial isolates were done with monoclonal antibodies and with probes directed against flagellin and OspA genes (Wallich et al. 1992) or ribosomal genes (Postic et al. 1994). Statistical analyses were performed with the SOLO package (BMDP), and the fitting of the negative binomial distribution curve was done according to Ludwig and Reynolds (1988).

## RESULTS AND DISCUSSION

### Borreliae in host-seeking ixodid ticks

Of the unfed ixodid ticks (8481 *Ixodes ricinus*, 372 *Dermacentor reticulatus*, 167 *Haemaphysalis concinna*), examined between 1988 and 1996 (Table 1), *Borrelia burgdorferi sensu lato* has only been observed in adult (23.2%), nymphal (17.2%) and larval (6.3%) *I. ricinus* (Hubálek et al. 1990, 1991a, 1994, Halouzka et al. 1995). The presence of borreliae in about 6% of

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**Table 1.** Borreliae in host-seeking ixodid ticks, 1988-1996 (darkfield microscopy): no. infected/no. examined.

| | *Ixodes ricinus* | *Dermacentor reticulatus* | *Haemaphysalis concinna* |
|---|---|---|---|
| Females | 539/2380 (22.6%) | 0/223 - | 0/19 - |
| Males | 573/2413 (23.7%) | 0/149 - | 0/17 - |
| Nymphs | 611/3546 (17.2%) | - | 0/99 - |
| Larvae | 9/142 (6.3%) | - | 0/32 - |
| Total | 1732/8481 (20.4%) | 0/372 - | 0/167 - |

**Table 2.** Borreliae in ixodid ticks parasitizing birds and small mammals, 1993-95 (darkfield microscopy): no. infected/no. examined.

| | *Ixodes ricinus* | *Dermacentor reticulatus* | *Haemaphysalis concinna* |
|---|---|---|---|
| Birds* | | | |
| larval ticks | 8/156 (5.1%) | - | 0/8 - |
| nymphal ticks | 11/94 (11.7%) | - | 0/5 - |
| Rodents** | | | |
| larval ticks | 28/437 (6.4%) | 0/125 - | 1/65 (1.5%) |
| nymphal ticks | 5/62 (8.1%) | 0/97 - | 0/4 - |

*Avian hosts with infected ticks: *Erithacus rubecula* 5/59, *Turdus merula* 3/8, *Sylvia atricapilla* 2/42, *Phylloscopus collybita* 1/69, *Parus major* 1/15, *Garrulus glandarius* 1/2

**Rodent hosts with infected ticks: *Apodemus flavicollis* 16/64, *Clethrionomys glareolus* 7/49, *Mus musculus* 1/1.

**Table 3.** Numbers ($\log_{10}$) of borreliae in host-seeking *Ixodes ricinus*, 1988-1996 (F - females; M - males; N - nymphs; I - adults).

| | F | M | N | I |
|---|---|---|---|---|
| No.infected ticks | 435 | 476 | 573 | 911 |
| No. borreliae per tick: | | | | |
| Minimum⁺) | 1 | 1 | 1 | 1 |
| Maximum⁺) | 7075 | 4340 | 11000 | 7075 |
| Median⁺) | 33 | 25 | 18 | 28 |
| Mean⁺) | 37.3 | 25.4 | 20.4 | 30.5 |
| Standard deviation | 0.693 | 0.639 | 0.614 | 0.67 |
| Skewness | 0.446 | 0.551 | 0.864 | 0.514 |
| Kurtosis⁺) | 3.24 | 6.54 | 67.78 | 4.42 |
| Normality test $K^2$ | 17.3*** | 29.7*** | 82.4*** | 46.3*** |

⁺) antilog values

*** different from normal distribution ($P < 0.001$)

unfed larval *I. ricinus* has demonstrated a relatively frequent transovarial transmission of *B. burgdorferi sensu lato* in enzootic foci of Lyme borreliosis. Analogous data have been published by Zhioua et al. (1994) in Switzerland (3.1% of 652 larvae infected), Rijpkema et al. (1994) and Rijpkema and Bruinink (1996) in the Netherlands (10.8% of 204 larvae and 5.3% of 57 larvae, respectively, infected with *B. afzelii*, *B. garinii* and *B. valaisiana*), and modelled by Randolph and Craine (1995). Moreover, the transovarial transmission of *B. burgdorferi* in *I. ricinus* was already demonstrated by Burgdorfer et al. (1983). This means that *I. ricinus* is not only the vector, but also a long-term reservoir of Lyme borreliosis.

A few spirochaetes were observed in a larval *H. concinna*, but they were thicker than *B. burgdorferi* and had pointed tips; therefore they were not included in Table 1. No borreliae have been detected in host-seeking *H. concinna* either in South Moravia or elsewhere: Slovakia (Kmety et al. 1990; n = 119), eastern Germany (Kahl et al. 1992; n = 96) or the Far East (Korenberg et al. 1989). Doby et al. (1994) reported 2.6% of *H. concinna* (n = 38) with spirochaetes in France, but those ticks were collected from mammalian hosts.

We have not found any spirochaetes in 594 unfed *D. reticulatus* collected in an enzootic area of Lyme borreliosis in South Moravia, and similar findings were reported from Switzerland (Aeschlimann et al. 1986, Péter et al. 1995) and Slovakia (Kmety et al. 1990; n = 1491). On the other hand, Kahl et al. (1992) observed borreliae in 9.5% of host-seeking adult *D. reticulatus* (n = 116) in eastern Germany. Doby et al. (1994) found spirochaetes in 2.3% of *D. reticulatus* (n = 257) in western France, although these ticks were collected on mammals. Our experiment has demonstrated that *D. reticulatus*, in contrast to *I. ricinus*, does not seem to be a competent vector for *B. garinii* (see below).

We have also studied seasonal variability and longitudinal prevalence of *B. burgdorferi s.l.* in the *I. ricinus* population in a mixed oak forest near Valtice. The results have shown a very complex pattern of spirochaetal distribution (see Hubálek et al. 1994, 1996b).

## Borreliae in ixodid ticks parasitizing vertebrates

A significant proportion of the birds (28.7%) and mammals (88.6%), captured alive in a mixed oak forest at Valtice between 1993 and 1995, were infested by preimaginal ticks *I. ricinus*, *H. concinna* and/or *D. reticulatus* (the latter species was not found on birds: see Hubálek et al. 1996a). Thirteen birds (3.2%) and 24 mammals (19.4%) were parasitized with infected ticks (Table 2). Interestingly, these usually partially engorged ticks revealed equal or even lesser prevalence rates as compared with the host-seeking ticks. While *D. reticulatus* did not contain any spirochaetes, 40 morphologically typical borreliae were seen in a larval *H. concinna* taken from a *Clethrionomys glareolus* (Schreber).

**Table 4.** Percentage of host-seeking *Ixodes ricinus* with high numbers of borreliae, 1988-1996.

| | Females | Males | Nymphs |
|---|---|---|---|
| No. of ticks examined | 2380 | 2413 | 3546 |
| No. of ticks with borreliae | 539 | 573 | 611 |
| Percentage of all ticks | 22.6% | 23.7% | 17.2% |
| No. of ticks with >100 borreliae | 119 | 73 | 61 |
| Percentage of infected ticks | 22.1% | 12.7% | 10.0% |
| Percentage of all ticks | 5.0% | 3.0% | 1.7% |
| No. of ticks with >1000 borreliae | 15 | 12 | 11 |
| Percentage of infected ticks | 2.8% | 2.1% | 1.8% |
| Percentage of all ticks | 0.6% | 0.5% | 0.3% |

**Table 5.** Prevalence of borreliae in host-seeking *Ixodes ricinus* according to main habitat groups in South Moravia, 1988-1996: no. infected/no. examined.

| | Adults | Nymphs |
|---|---|---|
| Xerothermic oak woods and grasslands (Quercetalia pubescentis), Mikulov area | 80/622 (12.9%) | 27/237 (11.4%) |
| Xerophilic heather (*Calluna*) stands, National Park Podyjí | 61/201 (30.3%) | 119/458 (26.0%) |
| Xerophilic oak forest with pines on loess (Potentillo-Quercentum), Valtice area | 641/2648 (24.2%) | 355/2124 (16.7%) |
| Mesophilic oak forest on slopes (Querceto-Carpinetum), NP Podyjí | 81/445 (18.2%) | 52/330 (15.8%) |
| 'Hardwood' flood-plain forests (Ulmeto/Querceto-Fraxinetum), Břeclav area | 106/391 (27.1%) | 31/234 (13.2%) |
| 'Softwood' flood-plain forests (Saliceto-Alnetum), Břeclav area | 58/146 (39.7%) | 15/52 (28.8%) |
| Dry balks between arable fields (shrub and grass communities), NP Podyjí | 10/75 (13.3%) | 5/51 (9.8%) |
| Urban parks (Brno, Valtice) | 74/244 (30.3%) | 7/60 (11.7%) |

**Table 6.** Prevalence of spirochaetes in female mosquitoes (Culicidae), 1993-1995 (darkfield microscopy).

| Species | Inf./exam. | Prevalence |
|---|---|---|
| *Aedes vexans* | 6/425 | 1.4% |
| *Aedes cantans* | 5/398 | 1.3% |
| *Aedes sticticus* | 5/225 | 2.2% |
| *Aedes geniculatus* | 0/48 | - |
| *Culex pipiens pipiens* | 8/357 | 2.2% |
| *Culex pipiens molestus* | | |
| spring to autumn collections | 2/58 | 3.4% |
| winter collections (hibernating) | 114/1901 | 6.0% |
| *Culiseta annulata* | | |
| spring to autumn collectins | 0/30 | - |
| winter collections (hibernating) | 0/22 | - |
| Total | 142/3464 | 4.1% |

**Table 7.** Genospecies of *Borrelia burgdorferi s. l.* isolated in South Moravia, 1989-1995.

| Strain | Source | Year | Genospecies |
|---|---|---|---|
| BR-41 | *Ixodes ricinus* (M) | 1989 | *B. lusitaniae* |
| BR-5 | *Ixodes ricinus* (F) | 1991 | *B. garinii* |
| BR-14 | *Ixodes ricinus* (N) | 1991 | *B. garinii* |
| BR-33 | *Ixodes ricinus* (F) | 1993 | *B. afzelii* |
| BR-34 | *Ixodes ricinus* (N) (on a *Turdus merula*) | 1993 | *B. garinii* |
| BR-53 | *Aedes vexans* (F) | 1994 | *B. afzelii* |
| BR-64 | *Ixodes ricinus* (N) | 1995 | *B. garinii* |
| BR-72 | *Ctenophthalmus agyrtes* (on a *Clethrionomys glareolus*) | 1995 | *B. afzelii* |
| BR-75 | *Ctenophthalmus agyrtes* (on another *C. glareolus*) | 1995 | *B. afzelii* |
| BRM-6 | *Clethrionomys glareolus* | 1995 | *B. afzelii* |
| BRM-11 | *Apodemus flavicollis* | 1995 | *B. afzelii* |
| BRM-40 | *Apodemus flavicollis* | 1995 | *B. afzelii* |

## Experimental inoculation of *Dermacentor reticulatus*

During our studies, we have never observed any spirochaetes in a total of 125 larval, 97 nymphal and 372 adult *D. reticulatus*. When about 60 females of each *D. reticulatus* and *I. ricinus* were inoculated by capillary feeding of *Borrelia garinii* strain BR-14 grown in BSK medium, the borreliae virtually disappeared from *D. reticulatus* within 1-2 weeks (for details, see Mátlová et al. 1996), whereas they survived in *I. ricinus* for at least 3 weeks post-feeding (Fig. 1).

## Distribution of borrelial counts in *Ixodes ricinus* population

Host-seeking adult and nymphal *I. ricinus* infected with borreliae were analyzed for their individual spirochaetal burden. The borrelial counts have been expressed as common logarithms, and the results are shown in Figs. 2 and 3, and summarized in Table 3. The frequency curves of the intensity of infection for nymphal and adult *I. ricinus* deviate significantly from the normal (Gauss) and Poisson distributions, but approximate the negative binomial distribution when evaluated by the chi-square goodness-of-fit test. The histograms of Fig. 4 demonstrate the distribution pattern of intensity of infection put into four classes only (1-9; 10-99; 100-999; 1000+). Ticks within the latter two classes (>100 spirochaetes per tick) can play an important role as vectors in the epidemiology of Lyme borreliosis and might be used for the transmission risk assessment of enzootic foci (cf. Hubálek et al. 1996b). The overall proportion of these ticks was about 2% in nymphs and 5% in females; 0.3% of nymphs and 0.6% of females harboured even more than 1000 borreliae (Table 4).

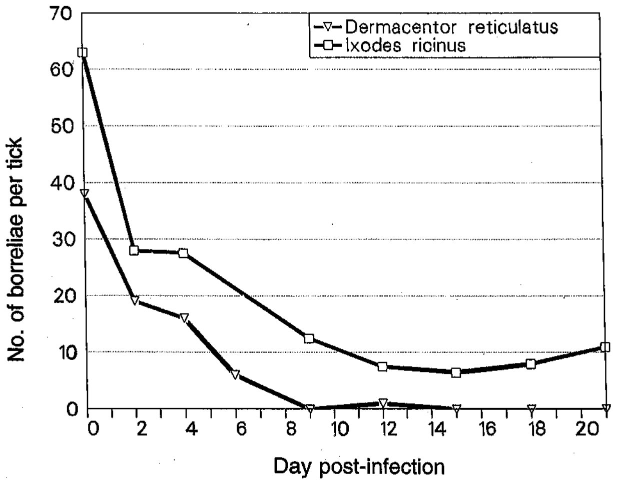


**Fig. 1.** Survival of *Borrelia garinii* in experimentally inoculated female *Dermacentor reticulatus* and *Ixodes ricinus* (based on data of Mátlová et al. 1996): median values, 4-5 ticks per interval.

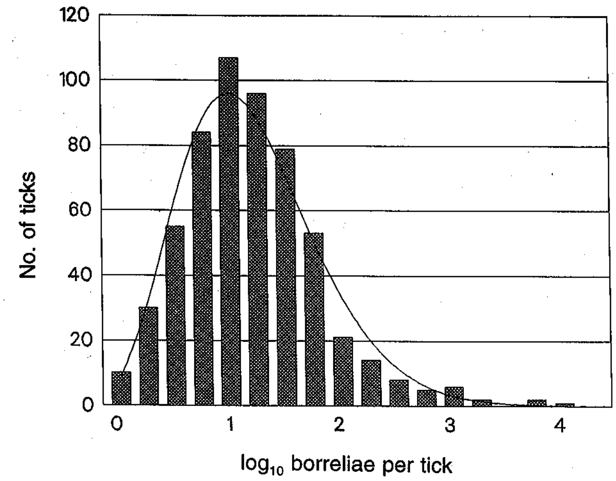


**Fig. 2.** Distribution of borrelial numbers in unfed nymphal *Ixodes ricinus*, 1988-1996. The curve approximates the negative binomial distribution of the data.

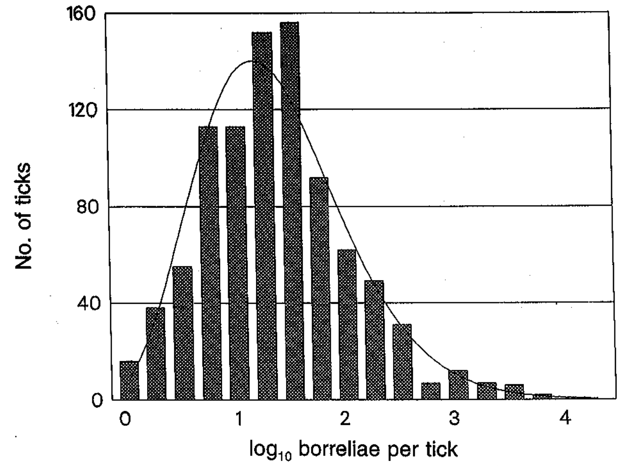


**Fig. 3.** Distribution of borrelial numbers in unfed adult *Ixodes ricinus*, 1988-1996. The curve approximates the negative binomial distribution of the data.

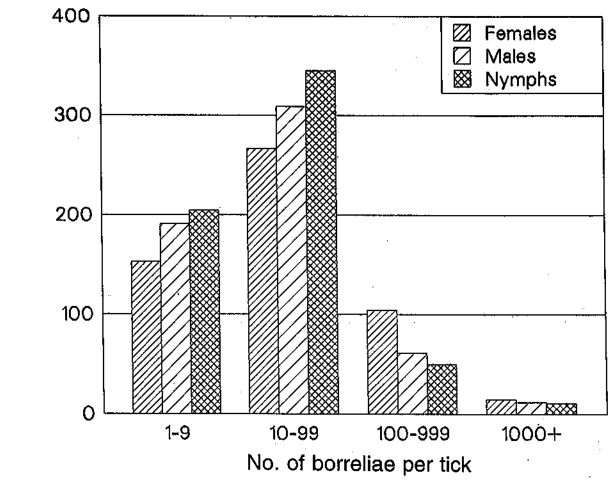


**Fig. 4.** Distribution of borrelial frequency classes in unfed *Ixodes ricinus*, 1988-1996.

### Prevalence of infected *Ixodes ricinus* according to habitats

We have not found a very heterogeneous prevalence of borreliae in host-seeking *I. ricinus* collected from different habitat groups. However, the proportion of infected ticks has been low in agroecosystems (dry balks between arable fields), and xerothermic oak woods and grasslands (Table 5). A high prevalence of infected ticks has been detected, interestingly, in floodplain forests and in urban parks (Hubálek et al. 1993).

### Spirochaetes in mosquitoes

Between 1993 and 1995, 3464 female mosquitoes (Culicidae) were examined by darkfield microscopy (Halouzka 1993, Halouzka et al. 1997). In 4.1% of them, spirochaetes morphologically similar to *B. burgdorferi s.l.* were found (Table 6). Of the infected mosquitoes, 32.6% contained 1 to 9 spirochaetes, 49.6% between 10 and 99 spirochaetes, and 17.7% >100 spirochaetes. Motile spirochaetes have also been detected in 6% of hibernating female *Culex pipiens molestus* Forskål.

Spirochaetes were previously detected in 7-8% of *Aedes* spp. mosquitoes in Connecticut, U.S.A. (Magnarelli et al. 1986), 3.8% of mosquitoes in South Moravia (Halouzka 1993), 1.1% of *Aedes* spp. and 0.3% of *Culex* spp. in northeastern Poland (J. Stanczak, pers. comm.). In one experiment, a few *Aedes* mosquitoes remained infected for 2 weeks post-inoculation (Magnarelli et al. 1987). At least one human case of *erythema migrans* has been described after mosquito bites in Sweden (Hard 1966). However, no *B. burgdorferi s.l.* have been isolated from mosquitoes until the work of Halouzka et al. (1997). It is probable that mosquitoes may play a role of secondary vectors of Lyme borreliosis, at least as mechanical carriers of *B. afzelii*. However, it remains to be proved whether or not mosquitoes can transmit borreliae to a host. The mosquito species found to carry spirochaetes in our study, *Aedes vexans* Meig., *A. cantans* (Meig.), *A. sticticus* (Meig.), *Culex p. molestus* and *Culiseta annulata* regularly attack man, whereas the other species (*A. geniculatus*, *C. p. pipiens* L.) bite humans sporadically.

**Borreliae in fleas**

Fleas (Siphonaptera) of a few species, largely *Ctenophthalmus agyrtes* Heller and *Hystrichopsylla talpae* Curtis, were collected from 64.2% of the captured small mammals. Darkfield microscopy revealed borreliae morphologically similar to *B. burgdorferi s.l.* in 8.4% of 142 examined specimens of fleas (*C. agyrtes*). The hosts carrying infected fleas were *C. glareolus*, *Microtus subterraneus* de Sélys-Longchamps, *Apodemus flavicollis* (Melchior) and *Sorex araneus* L. In the 12 infected fleas, the spirochaetal counts were >100 in 9 cases (four fleas collected from two *C. glareolus*, *M. subterraneus* and *A. flavicollis*, harboured >1000 spirochaetes) while only three fleas contained <100 spirochaetes.

Borreliae have occasionally been observed in fleas parasitizing small mammals, e.g. in France (8.1% of 197 *Ctenophthalmus baeticus*, 4.8% of 63 *Megabothris turbidus*, 1 of 6 *Spilopsyllus cuniculi* – Doby et al. 1990, 1991), E. Slovakia (6.2% of *Ctenophthalmus solutus* and *M. turbidus*: B. Peťko, pers. comm.). One strain of *B. burgdorferi sensu stricto* was isolated from the cat flea, *Ctenocephalides felis* in Texas (Teltow et al. 1991). Fleas might therefore be significant vectors of *B. burgdorferi s.l.* in the enzootic cycles among rodents; the cat flea, moreover, occasionally attacks humans.

**Genospecies of *B. burgdorferi s.l.* isolated**

Table 7 lists our spirochaetal isolates which have been identified to genospecies in laboratories abroad: strains BR-14 and BR-34 at the Connecticut Agricultural Experiment Station in New Haven (Dr. John F. Anderson), and the rest at the Pasteur Institute in Paris (Dr. Danielle Postic). Interesting strains are BR-53 (the first isolate of *B. afzelii* from mosquitoes – Halouzka et al. 1997), BR-41 (*Borrelia lusitaniae*, i.e. group PoTiB2 – the second isolate in Europe after the Portugal prototype), BR-72 and BR-75 (first isolations of *B. afzelii* from fleas), and BR-34 (*B. garinii* from nymphal *I. ricinus* infesting a young blackbird, *Turdus merula* L. – Hubálek et al. 1996a). The list also includes three strains of *B. afzelii* isolated from the urinary bladder of forest rodents – tick hosts.

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**REFERENCES**

AESCHLIMANN A., CHAMOT E., GIGON F., JEANNERET J.P., KESSELER D., WALTHER C. 1986: *Borrelia burgdorferi* in Switzerland. Zbl. Bakt. A 263: 450-458.

BURGDORFER W., BARBOUR A.G., HAYES S.F., PÉTER O., AESCHLIMANN A. 1983: *Erythema chronicum migrans* – a tick-borne spirochetosis? Acta Trop. 40: 79-83.

DOBY J.M., BIGAIGNON G., AUBERTI M., IMBERT G. 1991: Ectoparasites du renard et borreliose de Lyme. Recherche de *Borrelia burgdorferi* chez les tiques Ixodidae et insectes Siphonaptera. Bull. Soc. Fr. Parasitol. 9: 279-288.

DOBY J.M., BIGAIGNON G., DEGEILH B., GUIGUEN C. 1994: Ectoparasites des grands mammifères sauvages, cervidés et suidés, et borreliose de Lyme. Rev. Méd. Vét. 145: 743-748.

DOBY J.M., BIGAIGNON G., LAUNAY H., COSTIL C., LORVELLEC O. 1990: Présence de *Borrelia burgdorferi*, agent de spirochétoses à tiques, chez *Ixodes* (*Exopalpiger*) *trianguliceps* Birula, 1895 et *Ixodes* (*Ixodes*) *acuminatus* Neumann, 1901 (Acariens Ixodidae) et chez *Ctenophthalmus baeticus arvernus* Jordan, 1931 et *Megabothris turbidus* (Rothschild, 1909) (Insectes Siphonaptera), ectoparasites de micromammifères des fôrets dans l'Ouest de la France. Bull. Soc. Fr. Parasitol. 8: 311-322.

GERN L., BURGDORFER W., AESCHLIMANN A., KRAMPITZ H.E. 1993: The ecology of Lyme borreliosis in Europe. In: K. Weber and W. Burgdorfer (Eds.), Aspects of Lyme Borreliosis. Springer, Berlin, pp. 59-69.

HALOUZKA J. 1993: Borreliae in *Aedes vexans* and hibernating *Culex pipiens molestus* mosquitoes. Biológia 48: 123-124.

HALOUZKA J., JUŘICOVÁ Z., MÁTLOVÁ L., HUBÁLEK Z., 1995: Borreliae in larval *Ixodes ricinus* ticks. Med. Vet. Entomol. 9: 205-206.

HALOUZKA J., POSTIC D., HUBÁLEK Z. 1997: Isolation of *Borrelia afzelii* from mosquitoes. Med. Vet. Entomol., submitted. 12:101-103.

HARD S. 1966: *Erythema chronicum migrans* (Afzelii) associated with mosquito bite. Acta Derm. Venerol. 46: 473-476.

HUBÁLEK Z., ANDERSON J.F., HALOUZKA J., HÁJEK V. 1996a: Borreliae in immature *Ixodes ricinus* (Acari: Ixodidae) ticks parasitizing birds in the Czech Republic. J. Med. Entomol. 33: 766-771.

HUBÁLEK Z., HALOUZKA J., JUŘICOVÁ Z. 1991a: A comparison of the occurrence of borreliae in nymphal and adult *Ixodes ricinus* ticks. Zbl. Bakt. 275: 133-137.

HUBÁLEK Z., HALOUZKA J., JUŘICOVÁ Z. 1993: Prevalence of borreliae in *Ixodes ricinus* ticks from urban parks. Folia Parasitol. 40: 236.

HUBÁLEK Z., HALOUZKA J., JUŘICOVÁ Z. 1996b: A simple method of transmission risk assessment in enzootic foci of Lyme borreliosis. Eur. J. Epid. 12: 331-333.

HUBÁLEK Z., HALOUZKA J., JUŘICOVÁ Z., SVOBODOVÁ Š. 1994: Seasonal distribution of borreliae in *Ixodes ricinus* ticks. Zbl. Bakt. 280: 423-431.

HUBÁLEK Z., JUŘICOVÁ Z., HALOUZKA J. 1991b: Tick-borne viruses and bacteria isolated in Czechoslovakia. In: F. Dusbábek and V. Bukva (Eds.), Modern Acarology. Vol. 2. Academia Prague - SPB Academic, The Hague, pp. 49-53.

HUBÁLEK Z., KORENBERG E.I., JUŘICOVÁ Z., KOVALEVSKI Yu.V., HALOUZKA J., SHCHERBAKOV S.V. 1990: Prevalence of borreliae in *Ixodes ricinus* ticks from southern Moravia, Czechoslovakia. Folia Parasitol. 37: 359-362.

KAHL O., JANETZKI C., GRAY J.S., STEIN J., BAUCH R.J. 1992: Tick infection rates with *Borrelia*: *Ixodes ricinus* versus *Haemaphysalis concinna* and *Dermacentor reticulatus* in two locations in eastern Germany. Med. Vet. Entomol. 6: 363-366.

KMETY E., ŘEHÁČEK J., VÝROSTEKOVÁ V., KOCIANOVÁ E., GURYČOVÁ D. 1990: Prevalence of *Borrelia burgdorferi* and *Francisella tularensis* in ixodid ticks in Slovakia. Bratisl. Lek. listy 91: 251-266. (In Slovak.)

KORENBERG E.I., SHCHERBAKOV S.V., ZAKHARYCHEVA T.A., LEVIN M.L., KALININ M.I., KRYUCHECHNIKOV V.N. 1989: Lyme disease in Khabarovsk territory. Med. Parazitol. 5: 74-78. (In Russian.)

KRYUCHECHNIKOV V.N., KORENBERG E.I., SHCHERBAKOV S.V., GORELOVA N.B., JUŘICOVÁ Z., HALOUZKA J., HUBÁLEK Z., KOVALEVSKI Yu.V., LEVIN M.L., BUNIKIS I.A. 1990: Identification of *Borrelia* isolated in the USSR and Czechoslovakia from *Ixodes ricinus* (L.). Zh. Mikrobiol. Epid. Immunobiol. No. 6: 10-13. (In Russian.)

LUDWIG J.A., REYNOLDS J.F. 1988: Statistical Ecology. A Primer on Methods and Computing. J. Wiley, New York, 337 pp.

MAGNARELLI L.A., ANDERSON J.F., BARBOUR A.G. 1986: The etiologic agent of Lyme disease in deer flies, horse flies, and mosquitoes. J. Inf. Dis. 154: 355-358.

MAGNARELLI L.A., FREIER J.E., ANDERSON J.F. 1987: Experimental infections of mosquitoes with *Borrelia burgdorferi*, the etiologic agent of Lyme disease. J. Inf. Dis. 156: 694-695.

MÁTLOVÁ L., HALOUZKA J., JUŘICOVÁ Z., HUBÁLEK Z. 1996. Comparative experimental infection of *Ixodes ricinus* and *Dermacentor reticulatus* (Acari: Ixodidae) with *Borrelia burgdorferi sensu lato*. Folia Parasitol. 43: 159-160.

PÉTER O., BRETZ A.-G., BEE D. 1995: Occurrence of different genospecies of *Borrelia burgdorferi sensu lato* in ixodid ticks of Valais, Switzerland. Eur. J. Epid. 11: 463-467.

POSTIC D.M., ASSOUS V., GRIMONT P.A.D., BARANTON G. 1994: Diversity of *Borrelia burgdorferi sensu lato* evidenced by restriction fragment length polymorphism of *rrf* (5S) *rrl* (23S) intergenic spacer amplicons. Int. J. Syst. Bacteriol. 44: 743-752.

RANDOLPH S.E., CRAINE N.G. 1995: General framework for comparative quantitative studies on transmission of tick-borne diseases using Lyme borreliosis in Europe as an example. J. Med. Entomol. 32: 765-777.

RIJPKEMA S., BRUININK H. 1996: Detection of *Borrelia burgdorferi sensu lato* by PCR in questing *Ixodes ricinus* larvae from the Dutch North Sea island of Ameland. Exp. Appl. Acarol. 20: 381-385.

RIJPKEMA S., NIEUWENHUIJS J., FRANSSEN F.F.J., JONGEJAN F. 1994: Infection rates of *Borrelia burgdorferi* in different instars of *Ixodes ricinus* ticks from the Dutch North Sea island of Ameland. Exp. Appl. Acarol. 18: 531-542.

TELTOW G.J., FOURNIER P.V., RAWLINGS J.A. 1991: Isolation of *Borrelia burgdorferi* from arthropods collected in Texas. Am. J. Trop. Med. Hyg. 44: 469-474.

WALLICH R., HOLMES, C., SCHAIBLE U.E., LEBET Y., MOTER S.E., KRAMER M.D., SIMON M.M. 1992: Evaluation of genetic divergence among *Borrelia burgdorferi* isolates bu use of *ospA*, *fla*, HSP60, and HSP70 gene products. Infect. Immun. 60: 4856-4866.

ZHIOUA E., AESCHLIMANN A., GERN L. 1994: Infection of field-collected *Ixodes ricinus* (Acari: Ixodidae) larvae with *Borrelia burgdorferi* in Switzerland. J. Med. Entomol. 31: 763-766.

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