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COMPARATIVE EXPERIMENTAL INFECTION OF *IXODES RICINUS* AND *DERMACENTOR RETICULATUS* (ACARI: IXODIDAE) WITH *BORRELIA BURGDORFERI SENSU LATO*

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Borrelia burgdorferi s. l., the causative agent of Lyme disease, is transmitted principally by ticks of the *Ixodes ricinus* L. complex. However, borreliae have occasionally been found in some species of the genus *Dermacentor*, viz. *D. variabilis* (Say), *D. albipictus* Packard and *D. occidentalis* Marx in North America (Magnarelli L. A., Anderson J. F., Apperson C. S., Fish D., Johnson R. C., Chappell W. 1986: J. Wildl. Dis. 22: 178–188; Lane R. S., Lavoie P. E. 1988: Ann. N. Y. Acad. Sci. 539: 192–203; Piesman J., Sinsky R. J. 1988: J. Med. Entomol. 25: 336–339), and *D. reticulatus* Koch in Europe (Kahl O., Janetzki C., Gray J. S., Stein J., Bauch R. J. 1992: Med. Vet. Entomol. 6: 363–366; Doby J. M., Bigaignon G., Degeilh B., Guifuen C. 1994: Revue Méd. Vét. 10: 743–748). However, other investigators did not observe spirochetes in *D. reticulatus* (Aeschlimann A., Chamot E., Gigot F., Jeanneret J. P., Kesseler D., Walther C. 1986: Zbl. Bakt. A 263: 450–458; Kmety E., Řeháček J., Výrosteková V., Kocianová E., Guryčová D. 1990: Bratisl. Lek. Listy 91: 251–266; Doby J. M., Bigaignon G., Auberti M., Imbert G. 1991: Bull. Soc. Fr. Parasitol. 9: 279–288; Hubálek Z., Juřicová Z., Halouzka J. 1991: In: F. Dusbábek and V. Bukva, (Eds.), Modern Acarology, Vol. 2, Academia, Prague, pp. 49–53). The aim of our study was to test experimentally the differences between *I. ricinus* and *D. reticulatus* in the survival of *B. burgdorferi* s. l. in the ticks.

Ticks were collected by flagging at Valtice (Czech Republic) in spring 1994. The mean infection rate of local adult ixodid ticks with *Borrelia burgdorferi* s. l. is 0 % for *D. reticulatus* and 18 % for *I. ricinus*. However, less than 1 % of female *I. ricinus* contain > 100 borreliae in the spring (Hubálek Z., Halouzka J., Juřicová Z., Svobodová Š. 1994: Zbl. Bakt. 280: 423–431). To standardise the procedure, the capillary method of artificial tick feeding and infection was used (Burgdorfer W. 1957: J. Inf. Dis. 100: 212–214). Female ticks were attached on microscope slides by adhesive tape and the glass capillary tubes filled with culture of *Borrelia garinii* isolate BR-14 (2nd passage in BSK II medium; the genospecies has been identified by Dr. J. F. Anderson, The Connecticut Agricultural Experiment Station, U.S.A.) were pushed over the hypostome and chelicerae. The density of the culture was 3.10^3 spirochetes μl^{-1}. The ticks were fed in an incubator at 23°C and relative humidity > 90 % for 5 days. The volume of ingested culture was measured in each tick by weighing. After feeding, the ticks were kept starving under the same conditions. The numbers of borreliae were determined by darkfield microscopy of systematically searched slides at specific intervals up to 21 days.

Sixty percent of 80 *I. ricinus* and 63 % of 100 *D. reticulatus* ingested the medium with borreliae. These ticks were then used in the experiment. The mean volume of ingested culture

Table 1. The survival of borreliae in female *Ixodes ricinus* and *Dermacentor reticulatus* in relation to time of starvation.

Days after feeding	Number[a] of borreliae	Average	Median	Number[a] of borreliae	Average	Median
	Dermacentor reticulatus			*Ixodes ricinus*		
0	4, 38, 53, 11, 262	73.6	38	429, 42, 27, 84	145.5	63.0
2	5, 29, 77, 11, 19	28.2	19	0, 720, 43, 13	194	28.0
4	17, 21, 5, 16, 0	11.8	16	36, 19, 52, 4	27.75	27.5
6	11, 6, 4, 9, 0	6.0	6	nt	nt	nt
9	4, 0, 0, 0, 7	2.2	0	25, 42, 0, 0	16.75	12.5
12	0, 2, 0, 1, 1	0.8	1	38, 15, 0, 0	13.25	7.5
15	0, 3, 0, 0, 1	0.8	0	56, 13, 0, 0	17.25	6.5
18	0, 0, 0, 2, 0	0.4	0	164, 0, 11, 5	45.00	8.0
21	2, 0, 0, 0, 0	0.4	0	7, 33, 11	17.00	11.0

[a] – per individual ticks
nt – not tested

used in the experiment. The mean volume of ingested culture was 1.37 µl in *I. ricinus* and 1.86 µl in *D. reticulatus*. The number of ingested spirochetes varied greatly and did not depend on the volume of engorged culture. *I. ricinus* ingested about twice as much spirochetes (from 27 to 429) as *D. reticulatus* (from 4 to 262) – see Table 1. During the first week after feeding, there was an apparent decrease of borrelial numbers in both tick species, but different trends were found thereafter. In *D. reticulatus*, the spirochetes disappeared from the ticks after nine days, or it was only possible to find 1–3 non-motile borreliae. In *I. ricinus*, the numbers of spirochetes declined to one-fifth of its beginning value within the first week, and they remained stable thereafter. The differences in spirochete counts between *I. ricinus* and *D. reticulatus* from the day 9 till 21 post-infection have been significant ($P < 0.05$) when evaluated by the *t*-test ($t = 2.41$; unequal variance) and Mann-Whitney test. Moreover, only non-motile spirochetes have been observed in *D. reticulatus* (contrary to *I. ricinus*) since the 12th day post-inoculation.

The ability of borreliae to survive in *I. ricinus* and their transstadial and transovarial transmission by this tick species were proved experimentally (Gern L., Zhu Z., Aeschlimann A. 1990: Ann. Parasitol. Hum. Comp. 65: 89–93; Monin R., Gern L., Aeschlimann A. 1989: in G. Stanek (Ed.), Lyme Borreliosis II, Zbl. Bakt. Suppl. 18, pp. 14–20). A lower infection rate in *D. variabilis* (19 %) than in *Ixodes scapularis* Say (76 %) was detected after their experimental feeding on infected animals (Piesman J., Sinsky R. J. 1988: J. Med. Entomol. 25: 336–339). Our observations have shown a gradual decline and the loss of spirochetes in *D. reticulatus* during short time after infection and indicate the disability of this ixodid species to serve as a competent vector of *B. burgdorferi*. Similar results were noted in *D. variabilis*, in that individuals collected in nature contained borreliae, but the vector competence was not confirmed in laboratory experiments. When *I. scapularis, D. variabilis* and *Amblyomma americanum* (L.) were inoculated by feeding on experimentally infected mice, *D. variabilis* and *A. americanum* lost borreliae after the transstadial molt in contrast to *I. scapularis* (Mather T. N., Mather M. E. 1990: J. Med. Entomol. 27: 646–650). The physiological mechanisms of vector competence in various tick species have remained unexplained and require further studies.

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