Incidence from coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus

S. E. RANDOLPH[1]*, D. MIKLISOVÁ[2], J. LYSY[2], D. J. ROGERS[1] *and* M. LABUDA[2]

[1] *Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*
[2] *Institute of Zoology, Slovak Academy of Sciences, 842 06 Bratislava, Slovakia*

(*Received 20 June 1998; revised 26 July 1998; accepted 26 July 1998*)

SUMMARY

Tick-borne encephalitis (TBE) virus has a highly focal distribution through Eurasia. Endemic cycles appear to depend on the transmission of non-systemic infections between ticks co-feeding on the same rodent hosts. The particular features of seasonal dynamics and infestation patterns of larval and nymphal *Ixodes ricinus*, but not *Dermacentor reticulatus*, from 4 regions within TBE foci in Slovakia, are such as to promote TBE virus transmission. The distributions of larvae and nymphs on their principal rodent hosts are highly aggregated and, rather than being independent, the distributions of each stage are coincident so that the same *ca*. 20% of hosts feed about three-quarters of both larvae and nymphs. This results in twice the number of infectible larvae feeding alongside potentially infected nymphs compared with the null hypothesis of independent distributions. Overall, co-feeding transmission under these circumstances brings the reproductive number (R_0) for TBE virus to a level that accounts quantitatively for maintained endemic cycles. Essential for coincident aggregated distributions of larvae and nymphs is their synchronous seasonal activity. Preliminary comparisons support the prediction of a greater degree of coincident seasonality within recorded TBE foci than outside. This identifies the particular climatic factors that permit such patterns of tick seasonal dynamics as the primary predictors for the focal distribution of TBE.

Key words: tick-borne encephalitis, *Ixodes ricinus*, disease foci, seasonal dynamics.

INTRODUCTION

The two most important tick-borne infections of humans in Central Europe, tick-borne encephalitis (TBE) virus and Lyme borreliosis caused by the spirochaete *Borrelia burgdorferi* s.l., differ markedly in their epidemiology. Within Europe, both are transmitted by the tick *Ixodes ricinus* L which has a very wide host range, including mammals and birds of all sizes (Milne, 1949; Humair *et al*. 1993*a*, *b*; Craine, Randolph & Nuttall, 1995) and even lizards (Matuschka *et al*. 1991). While ungulates such as deer, sheep and goats feed large numbers of all tick stages but do not support TBE virus transmission to ticks (Milne, 1949; Steele & Randolph, 1985; Labuda, Kozuch & Lysy, 1997*a*), the ubiquitous rodent species (*Clethrionomys glareolus* Schreber and *Apodemus* spp. L.) feed the immature stages and play a central rôle in the enzootic transmission cycles of both TBE virus and *B. burgdorferi* s.l. (Kozuch *et al*. 1990; Kurtenbach *et al*. 1995). Whereas Lyme borreliosis occurs extensively throughout Europe wherever the vector ticks occur (O'Connell *et al*. 1998), TBE is far more focal in its distribution (Korenberg, 1994; IMMUNO AG, 1997), suggesting that the conditions for its maintenance are more narrowly defined. Furthermore, the infection prevalence in *I. ricinus* is much higher for Lyme spirochaetes, typically 2–20% in questing nymphs (Gray *et al*. 1998), than for TBE virus, typically 0·1–5% (Kunz, 1992).

One of the major differences between these two infections is the very much shorter duration of infectivity within the vertebrate host, only 2–3 days for TBE virus (Kozuch *et al*. 1981), but many months for the Lyme spirochaete (Gern *et al*. 1994). This largely accounts for an estimated 60-fold difference in the relative value of the reproductive number, R_0, of the two infections (Randolph, Gern & Nuttall, 1996) (where R_0 is defined as the number of new infections that will arise from one current infection given an entirely susceptible host populations – Anderson & May, 1991). Indeed, it appears from the best available data on transmission parameters that TBE virus would barely be maintained in Nature were it not for 2 recently discovered features of the transmission process (Randolph *et al*. 1996). First, TBE virus is transmitted most efficiently between infected and uninfected ticks co-feeding on the same host in the absence of a systemic

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* Corresponding author: Department of Zoology, South Parks Road, Oxford OX1 3PS, UK. Tel: +01865 271241. Fax: +01865 271240. E-mail: sarah.randolph@zoology.ox.ac.uk

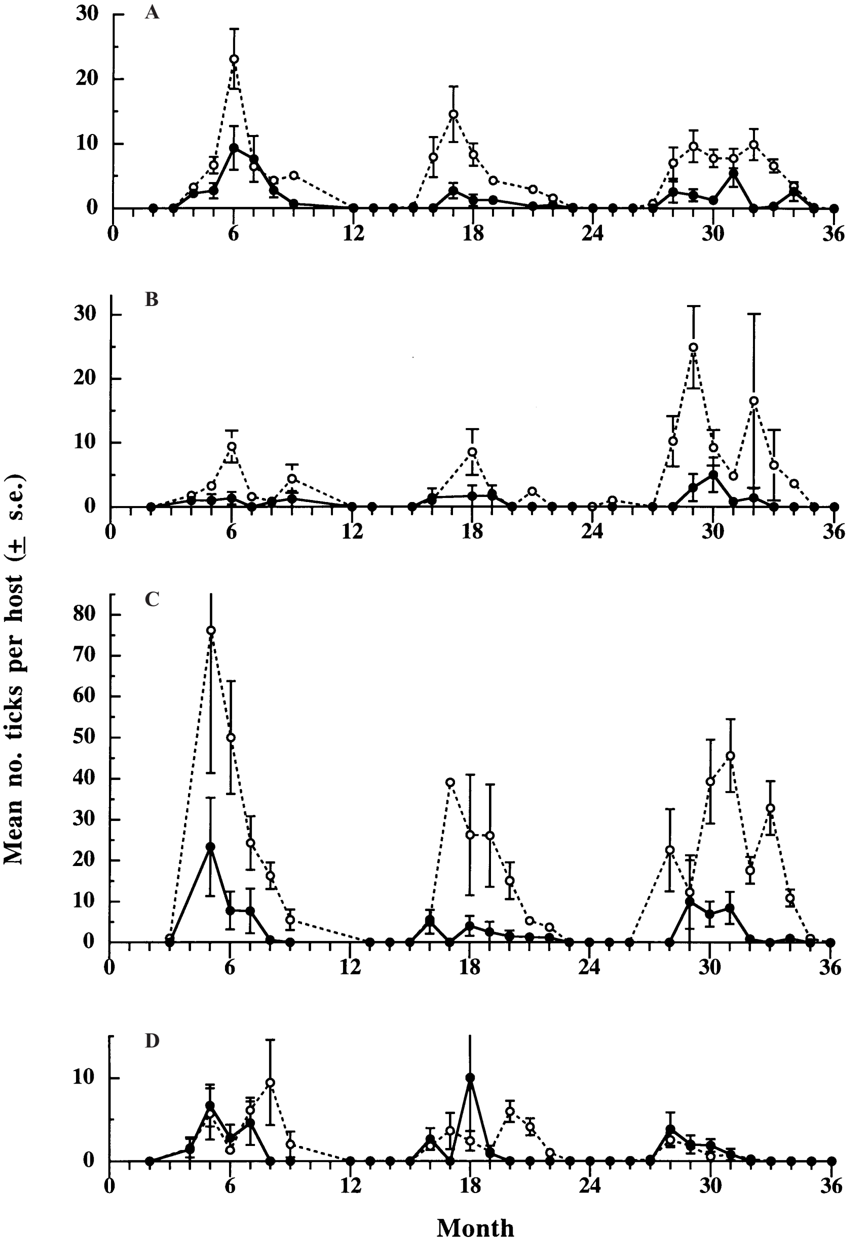

Fig. 1. The seasonal patterns of mean numbers (±1 s.e.) of larval (○) and nymphal (●) *Ixodes ricinus* ticks on rodents (*Clethrionomys glareolus* and *Apodemus flavicollis*) in 4 regions of Slovakia, (A) Borská nízina, (B) Small Carpathians, (C) Danube steppe, and (D) Danube lowland. Month 1 is January 1987. For clarity, the numbers of nymphs are shown multiplied by 10.

viraemia (Labuda *et al.* 1993) and, secondly, this transmission route is possible even on TBE virus-immune hosts (Labuda *et al.* 1997*b*). The suggestion from these laboratory observations is that in certain parts of the vector's range, natural patterns of tick infestations on rodents are such that they facilitate this transmission route. To test this suggestion, tick infestation patterns on rodents trapped within known foci of TBE in Slovakia were examined for the incidence of co-feeding larval and nymphal ticks. These were compared with patterns from elsewhere in Europe where TBE does not occur.

In this paper, we present the first quantitative explanation for the maintenance of natural cycles of TBE virus and, consequently, identify the primary predictors for the focal distribution of TBE.

MATERIALS AND METHODS

Study sites

Within western Slovakia (17° 0′–17° 30′ E, 47° 53′–48° 41′ N), 10 sites were selected from 3 distinct geographical areas: (1) lowland along the Danube River southeast of Bratislava; most sites were within the inundated plain where the vegetation was mixed poplar-willow (*Populus* and *Salix* spp.), but 1 site, Kopác, was a forest-steppe habitat; (2) foothills of the Small Carpathian Mountains running northeast

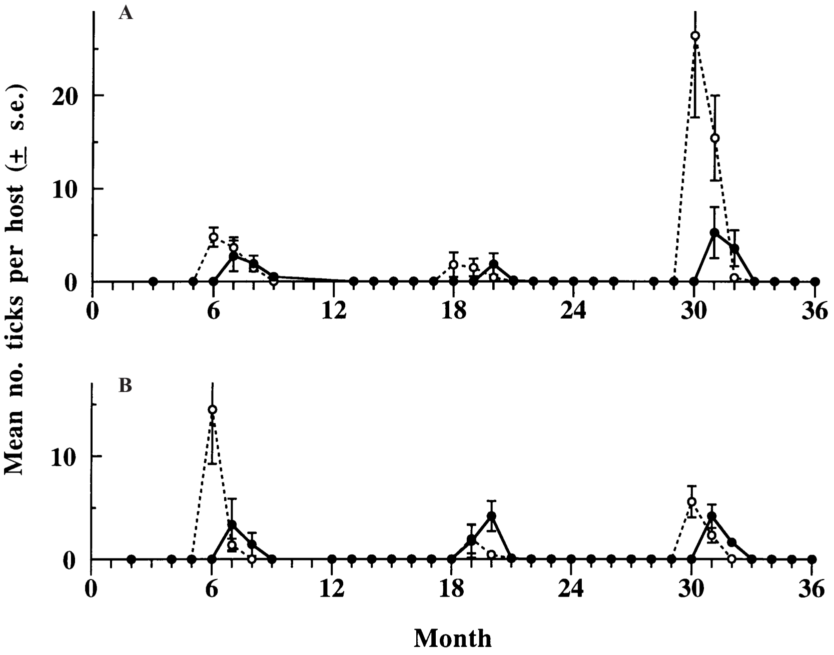

Fig. 2. The seasonal patterns of mean numbers (±1 S.E.) of larval (○) and nymphal (●) *Dermacentor reticulatus* ticks on rodents (*Clethrionomys glareolus* and *Apodemus flavicollis*) in 2 regions of Slovakia, (A) Danube steppe and (B) Danube lowland. Month 1 is January 1987.

from Bratislava, dominated by beech (*Fagus sylvaticus*); (3) the Borská nízina lowland *ca*. 60 km due north of Bratislava, with mixed pine forests (*Pinus nigra*), alder stands (*Alnus glutinosa*), shrubs and untended fruit trees.

Mammal trapping and tick collection

Small mammals were live-trapped in Swedish bridge metal traps baited with oat flakes. A line of 25 traps, 6–8 m apart, was set in each collecting site once a month for 2 consecutive nights. Six sites were worked from February 1987 to December 1989, to which were added 1 more site in the Carpathians from January 1988 to December 1989, and 3 extra sites in the Danube lowlands from January to December 1989. Trapped animals were euthanized, placed individually in cotton bags and transported to the laboratory for examination. All ectoparasites were collected from each animal individually and stored in 70 % ethanol. Ticks were selected from the material, identified and counted.

RESULTS

Overall patterns of abundance of ticks and hosts

In Central Europe, seasonal tick activity is limited to the period April–October, when all but 39 of the total 16004 ticks were collected and 1964 (74·2 %) of the small mammals were trapped, of which 1452 (74 %) were infested with at least 1 tick. The intensity of infestation was very high, with an overall mean of 11 ticks per infested host. The highest observed infestation was 242 ticks on 1 male *A. flavicollis* and each of the 25 most infested rodents carried more than 100 ticks. Ticks habitually attached in groups, preferentially on ears. *I. ricinus* was the most abundant tick feeding on these hosts, whose larvae (12032) and nymphs (400) comprised 77·7 % of the total ticks counted, followed by *Dermacentor reticulatus* (1991 larvae and 872 nymphs, 17·9 %). The least common tick, *Haemaphysalis conncina* (687 larvae and 22 nymphs) made up only 4·4 % of the tick sample and is excluded from the following analysis.

Of the total 2646 small mammals of 11 species that were trapped, the 5 most abundant species formed 98·5 %. Within the season of tick activity, April–October, the bank vole *Clethrionomys glareolus* comprised 60·4 %, followed by the yellow-necked mouse *Apodemus flavicollis* (20·8 %), the woodmouse *A. sylvaticus* (7·0 %), common shrew *Sorex araneus* (6·4 %) and common vole *Microtus arvalis* (4·1 %). The latter 3 species were only numerous in the Danube lowland region, predominantly in 1989. Therefore, detailed analysis of tick infestation patterns will be presented for the 2 most abundant rodent species which are represented in almost all monthly samples from all sites. Infestations on the other species differed only in that shrews carried average numbers of larval ticks, but vanishingly few nymphs, a total of only eight amongst 123 hosts trapped between April and October.

Seasonal patterns of tick infestations

Although the distribution of ticks amongst their hosts was highly aggregated (see below), the seasonal

Table 1. Mean tick infestations on *Apodemus flavicollis* and *Clethrionomys glareolus* for *Ixodes ricinus*, *Dermacentor reticulatus* and both tick species together

(Also shown are the *k* exponent of, and goodness of fit to, the negative binomial distribution.)

	Larvae			Nymphs				Mean no. larvae per nymph-feeding host		
	Mean no. LL per host	*k*	χ^2† (D.F.)‡	Prevalence NN¶ (%)	Mean no. NN per host	*k*	χ^2 (D.F.)	Observed	Null prediction§	Relative increase
Ixodes ricinus (April–July)										
Borská nízina	8·7	0·790	$28{\cdot}8_{(27)}$	73/366 (20)	0·475	0·153	$13{\cdot}6_{(5)}$*	19·8	8·7	× 2·3
Small Carpathians	6·7	0·525	$20{\cdot}9_{(14)}$	14/123 (11)	0·146	0·350	$2{\cdot}2_{(2)}$	13·4	6·7	× 2·0
Danube steppe	36·4	0·885	$14{\cdot}6_{(20)}$	27/86 (31)	0·791	0·278	$1{\cdot}9_{(5)}$	64·9	36·5	× 1·8
Danube lowland	1·65	0·339	$13{\cdot}9_{(11)}$	38/308 (12)	0·214	0·161	$0{\cdot}5_{(4)}$	4·5	1·7	× 2·6
Dermacentor reticulatus (July–August)										
Danube steppe	5·1	0·278	$3{\cdot}1_{(10)}$	26/77 (34)	3·1	0·130	$3{\cdot}4_{(6)}$	11·2	5·1	× 2·2
Danube lowland	1·2	0·112	$3{\cdot}6_{(9)}$	104/200 (52)	2·7	0·284	$25{\cdot}6_{(11)}$**	1·9	1·2	× 1·7
Ixodes ricinus + *Dermacentor reticulatus* (April–August)										
Danube steppe	36·7	1·008	$22{\cdot}3_{(28)}$	49/125 (39)	2·45	0·183	$7{\cdot}0_{(8)}$	51·0	36·5	× 1·4
Danube lowland	3·9	0·321	$26{\cdot}1_{(18)}$	143/399 (36)	1·6	0·187	$29{\cdot}5_{(12)}$**	4·4	3·9	× 1·1

† Chi-square for goodness of fit to the negative binomial model; * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

‡ Degrees of freedom, based on grouping the frequency distributions so that no class has an expected frequency of less than 3.

¶ Nymphal infestation prevalence: number of hosts feeding nymphs/number of hosts examined (and percentage).

§ The null hypothesis assumes independent aggregated distributions of larval and nymphal ticks.

Table 2. The median numbers (with 10 and 90 percentiles) of *Ixodes ricinus* (April–July) and *Dermacentor reticulatus* (July–August) larvae and nymphs on voles (*Clethrionomys glareolus*) and mice (*Apodemus flavicollis*) in the Danube region of Slovakia

	Danube steppe				Danube lowland			
	I. ricinus		*D. reticulatus*		*I. ricinus*		*D. reticulatus*	
	C. g.	*A. f.*	*C. g.*	*A. f.*	*C. g.*	*A. f.*	*C. g.*	*A. f.*
No. of hosts	21	65	22	55	269	39	178	22
Larvae								
Median no. of larvae	11	33	3	1	0	1	0	0
90–10 %	1–34	5–96	0–24	0–6	0–4	0–10	0–3	0–0
Z statistic†	3·16**		2·34*		3·15**		1·43 N.S.	
Nymphs								
Median no. of nymphs	0	0	7·5	0	0	0	1	0
90–10 %	0–0	0–3	0–23	0–1	0–1	0–1	0–8	0–0
Z statistic†	1·65 N.S.		4·82***		0·61 N.S.		4·16***	

† Results of the Mann–Whitney test comparing the infestation levels on voles and mice.
N.S., Non-significant; * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

Table 3. Comparison of estimates of absolute R_0 values for TBE virus in 4 regions of Slovakia depending on the route of transmission (systemic or co-feeding non-systemic) and the pattern of tick infestations (independent or coincident aggregated distributions of larvae and nymphs)

	Systemic infection; *independent distributions of larvae and nymphs (null hypothesis) R_0 index‡ = $0{\cdot}98\ \mu N/H$¶ $\mu = 0{\cdot}1$§		Non-systemic infection; †coincident distributions of larvae and nymphs (observed) R_0 index = $1{\cdot}65\ \mu N/H$ $\mu = 0{\cdot}1$	
	N/H	R_0	N/H	R_0
Danube lowland	1·7	0·17	4·5	0·74
Small Carpathians	6·7	0·66	13·4	2·21
Borská nízina	8·7	0·85	19·8	3·27
Danube steppe	36·5	3·58	64·9	10·71

* Transmission of systemic infections.
† Transmission of non-systemic infections via co-feeding ticks.
‡ As defined and estimated in Randolph *et al.* (1996).
¶ Mean vector–host ratio, where N is the total number of larval ticks feeding on H hosts.
§ Equivalent to an approximately 10 % interstadial survival from larvae to nymphs.

patterns are presented as means (Figs 1 and 2), because the median numbers of nymphs rarely differed from zero even at the peak of their feeding season. Whether based on mean or median infestation levels, the salient point is the synchroneity of larval and nymphal feeding periods of *I. ricinus*, whose larvae and nymphs both appeared on rodents in April each year, reached peak numbers in May to June, and ceased feeding by the end of October at the latest (Fig. 1). The larval period tended to persist a month or two later in the year than the nymphal period. As a result, *I. ricinus* nymphs never fed at a time when *I. ricinus* larvae were not also feeding.

D. reticulatus was only present in significant numbers in the Danube region, where larvae started to feed a month or two after *I. ricinus*, with a peak in June (Fig. 2). This tick has a very rapid development rate, engorged larvae moulting and giving rise to

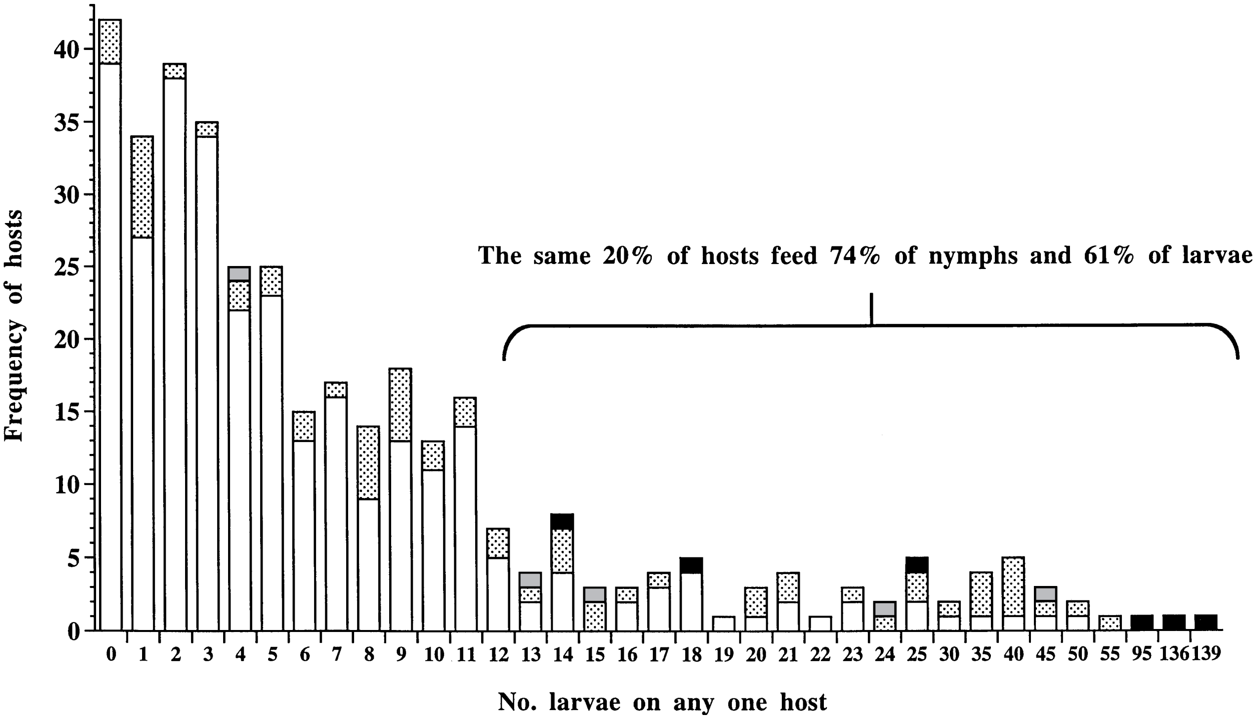

Fig. 3. The coincident aggregated frequency distributions of larval and nymphal *Ixodes ricinus* ticks on rodents (*Clethrionomys glareolus* and *Apodemus flavicollis*) in the Borská nízina lowland, Slovakia. At each intensity of larval infestation (x-axis), the numbers of hosts coincidentally feeding zero (□), 1–2 (░), 3–4 (▒) or 5–23 (■) nymphs are shown.

feeding nymphs within a month, and the whole generation being completed within a few months. The brief period of nymphal feeding therefore followed the larval period by 1 month, with only partial overlap between stages.

As the critical factor in virus transmission is the number of larvae that feed alongside infected nymphs, further analysis will be confined to the periods when the nymphs of each species fed, April to July when 86% of *I. ricinus* nymphs fed, and July to August when all but 1 of *D. reticulatus* nymphs in the Danube region fed.

Patterns of tick infestations on individual hosts

Both larvae and nymphs were highly over-dispersed on their host population, showing distributions that can usually (13 cases out of 16) be adequately described by the negative binomial model (Table 1). The null hypothesis is that these aggregated distributions for each tick stage are independent, in which case the number of larvae co-feeding with at least 1 nymph can be predicted from the negative binomial distributions of each stage. This is calculated as the sum of the products of the predicted frequency of each number of larvae feeding per host and the predicted probability that any host feeds 1 or more nymph. In fact, the observed mean number of larvae co-feeding with nymphs was consistently about twice as high as that predicted by the null hypothesis (Table 1), because the aggregated distributions were coincident rather than independent. Such coincident aggregated distributions of nymphs and larvae amongst their hosts (Fig. 3 for an example from Borská nízina) result in a consistent pattern for both tick species: in each region, the same 17·5–26% most heavily infested of hosts feed both 61–75% of the nymphs and 65–86% (in one case 100%) of the larvae. Overall, only 3·1% (10/324) *I. ricinus* nymphs were recorded on hosts that were not also carrying at least 1 larva, compared with 28% (219/782) of *D. reticulatus* nymphs ($\chi^2 = 86{\cdot}6$, $P < 0{\cdot}001$).

As both tick species are competent vectors of TBE virus in the laboratory (Kozuch & Nosek, 1971, 1977, 1985), the virus could potentially be exchanged between ticks of each species where they co-exist; for example, uninfected *D. reticulatus* larvae might acquire virus by co-feeding with infected *I. ricinus* nymphs. These tick species, however, make differential use of voles and mice as hosts; more *D. reticulatus* were recorded on *C. glareolus* than on *A. flavicollis*, while *I. ricinus* showed the reverse host association (Table 2). The aggregated distributions of both tick species combined from April to August are, therefore, not coincident, resulting in an observed mean number of larvae co-feeding with nymphs little greater than predicted by independent aggregated distributions of each stage (Table 1).

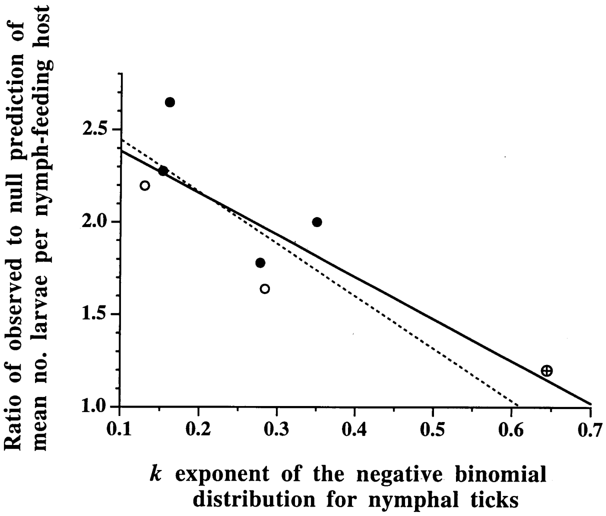

Fig. 4. The relationship between the degree of aggregation of nymphs on their host population (*k* exponent of the fitted negative binomial model) and the relative increase in the mean number of tick larvae per nymph-feeding host as observed or as predicted by the null hypothesis of independent aggregated distributions of larvae and nymphs. The relationship for *Ixodes ricinus* (●) and *Dermacentor reticulatus* (○) on voles and mice from the 4 study sites in Slovakia is not statistically significant ($Y = -2{\cdot}821X + 2{\cdot}727$, $r = 0{\cdot}694$, $n = 6$, ------) without additional data for *Ixodes ricinus* on squirrels in Thetford Forest, UK (⊕) ($Y = -2{\cdot}275X + 2{\cdot}612$, $r = 0{\cdot}856$, $n = 7$, $P < 0{\cdot}05$, ——).

DISCUSSION

Although the infection prevalence in unfed *I. ricinus* nymphs is low ($< 0{\cdot}2\%$, Labuda *et al.* 1997*a*), this tick appears to be the only significant vector in this region of Slovakia. Even though *D. reticulatus* is a competent vector of TBE virus in the laboratory, infection has not been recorded in ticks of this species in the field (Labuda *et al.* 1997*a*). This almost certainly arises from the particular pattern of this tick's life-cycle. Nymphs only start to feed in July and these are derived from larvae that fed in June on hosts that cannot, therefore, have been infected by *D. reticulatus* nymphs. This means that the larvae that feed in July will also not become infected via hosts fed on by conspecific nymphs, and so on. The potential for *D. reticulatus* larvae to pick up infections that are transmitted by *I. ricinus* nymphs is reduced by 2 factors. First, the peak of larval *D. reticulatus* does not coincide with the peak of nymphal *I. ricinus*, and secondly the 2 tick species feed principally on different host species. Furthermore, transmission of TBE virus via *C. glareolus*, the major host for *D. reticulatus*, is much less efficient than via *A. flavicollis* (Labuda *et al.* 1993). *I. ricinus* nymphs, on the other hand, are derived from larvae that had been exposed to a certain chance of infection by co-feeding the previous year with conspecific nymphs, principally on *A. flavicollis*. The probability of viral transmission is increased by the markedly coincident aggregated distributions of larval and nymphal *I. ricinus*. The differential potential of these 2 tick species to act as vectors for TBE virus in the field is therefore due to their different ecology (seasonality and tick-host relations), rather than any known biological (virus-tick interactions) constraint on their transmission competence.

Causes of coincident aggregated distributions

One factor that contributes to the aggregated distribution of ticks on their hosts is the monthly variation in mean tick infestation levels within the periods when most nymphs of *I. ricinus* feed (April–July). However, the distributions of larvae and nymphs also approximate to the negative binomial in the majority of individual monthly samples, where these are large enough for such analysis. Clearly, at any one time there are factors that cause some hosts to feed more ticks than others: for example, the more wide-ranging sexually mature male mice and voles pick up more ticks than do juveniles or females (Randolph, 1975, 1977), while such voles with high testosterone levels, known to be immunosuppressive (Grossman, 1985), show reduced acquired immunity to ticks and so permit more of the attached ticks to feed successfully (Hughes, 1998). The observed positive correlation in numbers of larvae and nymphs on the same individual hosts will arise if these factors are common to both immature tick stages, as they are likely to be.

Added to this, the closely synchronous marked monthly variation in numbers of larvae and nymphs exacerbates their coincident aggregated distributions across host populations over the whole 4-month nymphal feeding season.

Consequences of coincident aggregated distributions

Rodents are the principal amplifying hosts for TBE virus (Labuda *et al.* 1993, 1997*a*), despite the fact that they typically feed very few nymphal *I. ricinus*. In the samples from Slovakia, nymphs on average made up just $4{\cdot}1\%$ of the total number of *I. ricinus* counted on *C. glareolus* and *A. flavicollis* from April to July, and these nymphs were very highly clumped on a fraction (on average 17%) of the rodent population (the *k* exponent of the negative binomial model $\leqslant 0{\cdot}350$). These factors could mitigate against the maintenance of TBE virus: if the distributions of nymphs and larvae were independent, the more aggregated are these few nymphal ticks on their hosts, the lower the chance of their feeding alongside, and transmitting TBE virus to, larvae. Coincident aggregated distributions of larvae and nymphs, however, result in about twice as many larvae co-feeding alongside nymphs as would occur if the distributions were independent. As expected, therefore, the greater the degree of aggregation of the nymphs, the greater the amplifying effect of the observed coincident distributions of larvae and nymphs, although this correlation is not statistically significant for the six single-species observations available from Slovakia. By comparison the numbers of larvae and nymphs feeding on any one squirrel in Thetford Forest, UK, were also positively correlated (Craine *et al.* 1995), but between April and July 90% of squirrels carried nymphs, the monthly variation in median infestation levels was less pronounced and the resultant degree of aggregation ($k = 0{\cdot}644$) was less than on the small mammals in Slovakia. As a result, the numbers of larvae feeding alongside nymphs was little different from the null prediction of independent distributions.

There is an accessory route of amplification of TBE virus prevalence in unfed questing nymphs. Any transovarially infected larvae, however few (typically $< 1\%$ of any infected egg batch – Danielová & Holubová, 1991), will co-feed with much larger numbers of uninfected larvae. The resulting infected nymphs can only pass the infection on as they feed on rodents, making the coincident aggregated distributions of both larvae and nymphs a significant element in TBE virus maintenance whichever the precise route of co-feeding transmission.

The exact timing of tick feeding is also conducive to TBE virus transmission. Peak numbers of potentially infected nymphs co-feed with larvae in May and June, just as new-born susceptible hosts are being recruited to the rodent populations (data on monthly rodent body weight distributions not shown). Although co-feeding transmission of TBE virus can occur *via* immune hosts, on *A. flavicollis* it is 3 times more efficient (72 *vs.* 24%) and on *C. glareolus* it is one and a half times more efficient (42 *vs.* 29%) on non-immune hosts than on hosts immunized by the bite of an infected tick (Labuda *et al.* 1997*b*). If the seasonal onset of feeding by nymphs preceded that of larvae to any extent, even if the activity seasons overlapped later in the summer/autumn, hosts would have been immunized before co-feeding transmission could occur.

The quantitative effect of the infestation patterns of ticks on small rodents is highly significant for TBE virus transmission, as it effectively doubles the vector–host ratio for that fraction of the host population most likely to be infective. Starting from a single infected tick bite on a susceptible host, and taking the best available estimates of the transmission coefficients, the latent period and duration of infection in rodents, the relative index of the R_0 value was calculated as 0·98 via the classically supposed systemic pathway compared with 1·65 via the non-systemic co-feeding pathway (Randolph *et al.* 1996). The latter gives $> 60\%$ greater degree of amplification of TBE virus. In each case, this index value must be multiplied by the mean vector–host ratio and the proportional interstadial (larval–nymphal) survival to give absolute estimates of R_0. There is no evidence that tick survival is affected by the mode of virus transmission, and a mean value of 10% would allow tick population persistence (Randolph & Craine, 1995). We can now also insert the vector–host ratios (mean number of larvae per nymph-feeding host) from each site of this study. Those predicted from the null hypothesis of independent larval and nymphal distributions would be appropriate for the systemic route, giving estimates of absolute R_0 values from 0·2 to 3·6; the observed coincident distributions are appropriate for the non-systemic route, giving values of 0·7 to 10·7, an approximately 3-fold greater chance of TBE virus survival.

For the first time, we have crude estimates of R_0 values for TBE virus, and we show that it is the particular patterns of tick infestations on the transmission-competent rodent hosts that provide a quantitative explanation for the maintenance of TBE virus in certain parts of Europe. It is not possible to relate regional differences in R_0 estimates to observed prevalences of TBE infection in either rodents or ticks. Over the years 1981–1986, the mean prevalence of TBE-specific neutralizing antibody in small mammals was very similar in all regions (13·3% in Borská nízina, 15·1% in the Danube lowland, 16·3% in the small Carpathians) (Kozuch *et al.* 1990). Only 91 isolates of TBE virus were obtained from 44437 nymphal and adult *I. ricinus* ticks (0·2% mean prevalence) collected between 1964 and 1996 from 33 natural foci within Slovakia, including the regions of the present study. With such low prevalences, and with considerable temporal variation recorded in any one place (Labuda *et al.* 1997*a*), no clear regional patterns are evident.

Predictors for the focal distribution of TBE

The *sine qua non* of coincident aggregated distributions is the coincident seasonal activity of nymphs and larvae, which is not true of *I. ricinus* throughout its geographical range. If this really is a critical factor for the maintenance of TBE, there should be a greater degree of coincident seasonality within recorded TBE foci than outside them. In the UK and most of France, for example, where TBE is absent, larval ticks usually start to quest and reach peak numbers about 2 months after nymphs (Gilot *et al.* 1975; Steele & Randolph, 1985; Craine *et al.* 1995; L'Hostis *et al.* 1995). In the weak but stable, most westerly focus of TBE (Perez-Eid, Hannoun & Rodhain, 1992) in the Alsace region of France, however, larval and nymphal *I. ricinus* both start their seasonal activity in March, peak together in May and continue until October (Perez-Eid, 1989, 1990). Here, nymphs show a mean infection prevalence with TBE virus of $\leqslant 0{\cdot}12$ (Perez-Eid *et al.* 1992) and make up only 1 % of the *I. ricinus* feeding on rodents (Perez-Eid, 1990), but a tick infestation pattern similar to that seen in Slovakia might yield over 7 larvae per nymph-feeding host (double the recorded overall mean number of larvae per host from May to October over 4 years; Perez-Eid (1990) recorded 9056 ticks on 2511 rodents, of which 99 % were larvae $= 8965/2511 = 3{\cdot}6$ larvae per rodent). This would give a crude R_0 value of about 1·2, theoretically just sufficient to maintain the focus.

Interestingly, in parts of the British Isles where louping-ill is or was prevalent, larval and nymphal *I. ricinus* show coincident seasonality (Lees & Milne, 1951; Walton & O'Donnell, 1969; Ogden, Nuttall & Randolph, 1997). It is generally thought that the louping-ill virus is a new variant of TBE virus, having replaced the ancestral form in Ireland and northern Britain when large scale sheep farming was introduced into upland ecosystems where rough pastures are inhabited by ticks (Reid & McQuire, 1997). Sheep are highly transmission-competent for louping-ill virus, but not for European strains of TBE virus, while small rodents are very scarce in these sheep-grazed habitats (Ogden *et al.* 1997). Co-feeding transmission of louping-ill virus *via* hares has been demonstrated and suggested as an essential element in virus maintenance where sheep are removed from the cycle by vaccination (Jones *et al.* 1997). Possibly, the particular features of tick seasonality account for the occurrence of louping-ill virus only in these parts of the British Isles.

In conclusion, having identified coincident feeding by larvae and nymphs as the critical factor in the maintenance of TBE virus transmission, we can focus on the particular climatic factors that permit such tick phenology as the primary predictors for the focal distribution of TBE. This is a specific example of the considerable qualitative impact of the seasonal dynamics of ticks on their potential to transmit parasites, additional to the more general quantitative effects (Randolph, 1998).

The study was supported by Slovak Grant Agency VEGA grants no. 95/5305/358 and 1/5160/98. SER is supported by a Wellcome Trust Senior Research Fellowship in Basic Biomedical Science.

REFERENCES

ANDERSON, R. M. & MAY, R. M. (1991). *Infectious Diseases of Humans: Dynamics and Control*. Oxford University Press, Oxford.

CRAINE, N. G., RANDOLPH, S. E. & NUTTALL, P. A. (1995). Seasonal variation in the rôle of grey squirrels as hosts of *Ixodes ricinus*, the tick vector of the Lyme disease spirochaete, in a British woodland. *Folia Parasitologica* **42**, 73–80.

DANIELOVÁ, V. & HOLUBOVÁ, J. (1991). Transovarial transmission rate of tick-borne encephalitis virus in *Ixodes ricinus* ticks. In *Modern Acarology*, vol. 2 (ed. Dusbabek, F. & Bukva, V.), pp. 7–10. Academia, Prague and SPB Academic Publishing. The Hague.

GERN, L., SIEGENTHALER, M., HU, C. M., LEUBA-GARCIA, S., HUMAIR, P. F. & MORET, J. (1994). *Borrelia burgdorferi* in rodents (*Apodemus flavicollis* and *A. sylvaticus*) – duration and enhancement of infectivity for *Ixodes ricinus* ticks. *European Journal of Epidemiology* **10**, 75–80.

GILOT, B., PAUTOU, G., MONCADA, E. & AIN, G. (1975). Première contribution à l'étude écologique d'*Ixodes ricinus* (Linné, 1758) (Acarina, Ixodoidea) dans le Sud-Est de la France. *Acta Tropica* **32**, 355–381.

GRAY, J., KAHL, O., ROBERTSON, J. N., DANIEL, M., ESTRADA-PENA, A., GETTINBY, G., JAENSON, T. G. T., JENSEN, P., JONGEJAN, F., KORENBERG, E., KURTENBACH, K. & ZEMAN, P. (1998). Lyme borreliosis habitat assessment. *Zentralblatt für Bakteriologie* **287**, 211–228.

GROSSMAN, C. J. (1985). Interactions between the gonadal steroids and the immune system. *Science* **227**, 257–261.

HOODLESS, A. N., KURTENBACH, K., NUTTALL, P. A. & RANDOLPH, S. E. (1998). Ticks reduce pheasant fitness, with consequences for the transmission of Lyme disease spirochaetes. *Proceedings of the Royal Society, B* (under revision).

HUGHES, V. (1998). The effects of testosterone on parasitic infections in rodents, with respect to *Babesia microti* transmission by ticks. D.Phil. thesis, University of Oxford.

HUMAIR, P.-F., TURRIAN, N., AESCHLIMANN, A. & GERN, L. (1993*a*). *Borrelia burgdorferi* in a focus of Lyme borreliosis: epizootiologic contribution of small mammals. *Folia Parasitologica* **40**, 65–70.

HUMAIR, P.-F., TURRIAN, N., ASESCHLIMANN, A. & GERN, L. (1993*b*). *Ixodes ricinus* immatures on birds in a focus of Lyme borreliosis. *Folia Parasitologica* **40**, 237–242.

IMMUNO AG (1997). Tick-borne encephalitis (TBE) and its immunoprophylaxis. Vienna, Austria.

JONES, L. D., GAUNT, M., HAILS, R. S., LAURENSON, K., HUDSON, P. J., REID, H., HENBLEST, P. & GOULD, E. A. (1997). Transmission of louping ill virus between infected and uninfected ticks co-feeding on mountain hares. *Medical and Veterinary Entomology* **11**, 172–176.

KORENBERG, E. I. (1994). Comparative ecology and epidemiology of Lyme disease and tick-borne encephalitis in the former Soviet Union. *Parasitology Today* **10**, 157–160.

KOZUCH, O., CHUNIKHIN, S. P., GRESIKOVA, M., NOSEK, J., KURENKOV, V. B. & LYSY, J. (1981). Experimental characteristics of viraemia caused by two strains of tick-borne encephalitis virus in small rodents. *Acta Virologica* **25**, 219–224.

KOZUCH, O., LABUDA, M., LYSY, J., WIESMANN, P. & KRIPPEL, E. (1990). Longitudinal study of natural foci of Central European encephalitis virus in West Slovakia. *Acta Virologica* **34**, 537–544.

KOZUCH, O. & NOSEK, J. (1971). Transmission of tick-borne encephalitis (TBE) virus by *Dermacentor marginatus* and *D. reticulatus*. *Acta Virologica* **15**, 334.

KOZUCH, O. & NOSEK, J. (1977). Survival of TBE virus during the life of *Dermacentor reticulatus* nymphs. *Folia Parasitologica* **24**, 186.

KOZUCH, O. & NOSEK, J. (1985). Replication of tick-borne encephalitis (TBE) virus in *Ixodes ricinus* ticks. *Folia Parasitologica* **32**, 373–375.

KUNZ, C. (1992). Tick-borne encephalitis in Europe. *Acta Leidensia* **66**, 1–14.

KURTENBACH, K., KAMPEN, H., DIZIJ, A., ARNDT, S., SEITZ, H. M., SCHAIBLE, U. E. & SIMON, M. (1995). Infestation of rodents with larval *Ixodes ricinus* (Acari: Ixodidae) is an important factor in the transmission cycle of *Borrelia burgdorferi* s.l. in German woodlands. *Journal of Medical Entomology* **32**, 807–817.

LABUDA, M., NUTTALL, P. A., KOZUCH, O., ELECKOVÁ, E., WILLIAMS, T., ZUFFOVA, E. & SABÓ, A. (1993). Non-viraemic transmission of tick-borne encephalitis virus: a mechanism for arbovirus survival in nature. *Experientia* **49**, 802–805.

LABUDA, M., KOZUCH, O. & LYSY, J. (1997*a*). Tick-borne encephalitis virus natural foci in Slovakia: ticks, rodents, and…goats. In *4th International Potsdam Symposium on Tick-borne Disease: Tick-borne Encephalitis and Lyme Borreliosis, February 1997* (ed. Suss, J. & Kahl, O.), pp. 34–46. Pabst Science Publishers, Lengerich.

LABUDA, M., KOZUCH, O., ZUFFOVA, E., ELECKOVÁ, E., HAILS, R. S. & NUTTALL, P. A. (1997*b*). Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. *Virology* **235**, 138–143.

LEES, A. D. & MILNE, A. (1951). The seasonal and diurnal activities of individual sheep ticks (*Ixodes ricinus* L.). *Parasitology* **41**, 189–208.

L'HOSTIS, M., DUMON, H., DORCHIES, B., BOISDRON, F. & GORENFLOT, A. (1995). Seasonal incidence and ecology of the tick *Ixodes ricinus* (Acari: Ixodidae) on pastures in western France. *Experimental and Applied Acarology* **19**, 211–220.

MATUSCHKA, F.-R., FISCHER, P., MUSGRAVE, K., RICHTER, D. & SPIELMAN, A. (1991). Hosts on which *Ixodes ricinus* nymphs most abundantly feed. *American Journal of Tropical Medicine and Hygiene* **44**, 100–107.

MILNE, A. (1949). The ecology of the sheep tick *Ixodes ricinus* L. Host relationships of the tick. Part 2. Observations on hill and moorland grazings in Northern England. *Parasitology* **39**, 173–194.

O'CONNELL, S., GRANSTRÖM, M., GRAY, J. S. & STANEK, G. (1998). Epidemiology of European Lyme borreliosis. *Zentralblatt für Bakteriologie* **287**, 229–240.

OGDEN, N. H., NUTTALL, P. A. & RANDOLPH, S. E. (1997). Natural Lyme disease cycles maintained *via* sheep by co-feeding ticks. *Parasitology* **115**, 591–599.

PEREZ-EID, C. (1989). Dynamique saisonnière des nymphes et adultes d'*Ixodes ricinus* en phase libre sur la végétation, dans le foyer Alsacien d'encéphalite à tiques. *Acarologia* **30**, 355–360.

PEREZ-EID, C. (1990). Les relations tiques – petits mammifères dans le foyer Alsacien d'encéphalite à tiques. *Acarologia* **31**, 131–141.

PEREZ-EID, C., HANNOUN, C. & RODHAIN, F. (1992). The Alsatian tick-borne encephalitis focus: presence of the virus among ticks and small mammals. *European Journal of Epidemiology* **8**, 178–186.

RANDOLPH, S. E. (1975). Patterns of distribution of the tick *Ixodes trianguliceps* Birula on its host. *Journal of Animal Ecology* **44**, 451–474.

RANDOLPH, S. E. (1977). Changing spatial relationships in a population of *Apodemus sylvaticus* with the onset of breeding. *Journal of Animal Ecology* **46**, 653–676.

RANDOLPH, S. E. (1998). Ticks are not insects: consequences of contrasting vector biology for transmission potential. *Parasitology Today* **14**, 186–192.

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. *Journal of Medical Entomology* **32**, 765–777.

RANDOLPH, S. E., GERN, L. & NUTTALL, P. A. (1996). Co-feeding ticks: epidemiological significance for tick-borne pathogen transmission. *Parasitology Today* **12**, 472–479.

REID, H. W. & MCQUIRE, K. (1997). Ecoepidemiology of Louping-ill. In *4th International Potsdam Symposium on Tick-borne Disease: Tick-borne Encephalitis and Lyme Borreliosis, February 1997* (ed. Suss, J. & Kahl, O.), pp. 128–134. Pabst Science Publishers, Lengerich.

STEELE, G. M. & RANDOLPH, S. E. (1985). An experimental evaluation of conventional control measures against the sheep tick *Ixodes ricinus* (L) (Acari: Ixodidae). I. A unimodal seasonal activity pattern. *Bulletin of Entomological Research* **75**, 489–499.

WALTON, G. A. & O'DONNELL, T. G. (1969). Observations on *Ixodes ricinus* and louping ill virus in Ireland. In *Proceedings of the 2nd International Congress of Acarology, Prague, Czechoslovakia, September 1967*, pp. 601–608.