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Survival Strategy of Tick-borne Encephalitis Virus: Cellular Basis and Environmental Determinants

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Summary

Although TBE virus can be transmitted in the laboratory by a wide variety of ixodid tick species to a wide variety of vertebrate host species, nevertheless in nature endemic cycles of TBE virus depend principally on just two tick species, *Ixodes ricinus* in the western and *I. persulcatus* in the eastern Palaearctic. A complete transmission cycle, from tick to tick *via* vertebrates, occurs most efficiently between co-feeding ticks in the absence of a systemic viraemia. This non-systemic route depends on TBE virus replication within particular immunocompetent cells in the skin, and only certain vertebrate species, notably *Apodemus* mice, are susceptible to this. Amongst the potential tick vectors in Europe, only *I. ricinus* has the correct host relationships and appropriate natural life cycle to support such non-systemic transmission cycles. Within the wide European distribution of this tick-host relationship, only in certain places do larval and nymphal ticks feed together on the same hosts with sufficient coincidence to ensure TBE virus survival. The environmental factors that determine this seasonal coincidence are being identified with the help of remotely-sensed meteorological satellite imagery to create predictive risk maps of TBE foci.

Key words: Tick-borne encephalitis virus, non-systemic transmission, *Ixodes ricinus*, vector-virus-vertebrate interactions, climate

Introduction

Tick-borne encephalitis (TBE) virus, medically the most important arbovirus in Europe, occurs endemically over widespread but disjunct regions of Europe and northern Asia, occupying much less then the full geographical range of the main ixodid tick vectors, *Ixodes ricinus* and *I. persulcatus*, (10). As an arbovi-

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rus, TBE virus relies on two different types of hosts for its survival: ticks act as both virus vectors and reservoir hosts, and vertebrates amplify the virus infection by acting as a source of infection for feeding ticks. Specific reciprocal interactions between TBE virus and tick vector, TBE virus and vertebrate host, and also between tick vector and vertebrate host, together create unique conditions for virus survival only within certain geographical areas (12, 29, 34). Here we highlight and review some of the interactions we consider as the most important for the perpetuation of TBE virus transmission cycles in nature.

The vector: one amongst many

Ixodid ticks are the only group of arthropods shown to be able to replicate and transmit TBE virus or play any role in TBE virus circulation. At least 18 species have been implicated as vectors (17). In addition to *I. ricinus* in Europe and *I. persulcatus* in northeastern Europe and east of the Ural Mountains, several other Eurasian tick species (e. g. *I. hexagonus, I. arboricola, Haemaphysalis concinna, H. punctata, H. inermis, Dermacentor reticulatus, D. marginatus)* and even a non-Eurasian species *(Rhipicephalus appendiculatus)* have been shown to transmit TBE virus between vertebrates in the laboratory (28). Yet virus has been isolated repeatedly only from field-collected specimens of *I. ricinus* and *I. persulcatus*, which appear to be the only vectors and reservoirs responsible for long-term TBE virus survival in nature (28). In other words, although many ixodid tick species can support the replication of TBE virus and can transmit it experimentally, they do not support natural TBE virus transmission cycles and contribute very little, if anything, to virus persistence within any particular focus. Hence, TBE virus survival depends not on the specific virus susceptibilities of certain vector species (as is the case for many other arboviruses), but rather on the intimate ecological association of *I. ricinus* or *I. persulcatus* with transmission-competent vertebrate hosts (see below).

With respect to their role as vectors of TBE virus, the critical ecological features of *I. ricinus* ticks which distinguish them from other sympatric tick species are: i) their prolonged life cycle lasting several (two to six) years (5) and involving long survival of infected larvae and nymphs; ii) the overlapping seasonal periods of feeding activity by larvae and nymphs (34); and iii) the particular range of small mammal species upon which the immature stages feed (28).

The vertebrate host: viraemia, too much of a good thing

Many vertebrates have been implicated as maintenance hosts of TBE virus in central Europe, although the special significance of rodents has long been recognised (3) and ungulates (e. g. goats and deer) contribute only indirectly by

feeding adult ticks and therefore maintaining the vector population (23, 26, 27). Studies based on detection of host infection and seroconversion showed that many different taxa of mammals, some species of birds and even lizards were susceptible to TBE virus infection. Any species which showed a sufficiently high level of viraemia was assumed to act as a maintenance and amplifying host of TBE virus. Hosts can only maintain virus transmission cycles, however, if they succeed in passing virus on to ticks feeding on them, and recent work has challenged the concept of a viraemia as a condition either necessary for, or even correlated with, the ability to infect feeding ticks. First experiments compared the ability of *R. appendiculatus* ticks to transmit Thogoto virus (family Orthomyxoviridae) *via* either non-viraemic guinea pigs or highly viraemic hamsters, when uninfected ticks fed simultaneously alongside infected ticks as they do naturally. A much higher percentage of these uninfected ticks acquired infection *via* guinea pigs than *via* hamsters, yet the Thogoto virus viraemia in guinea pigs was undetectable and well below the expected threshold to infect ticks (14). Similarly efficient non-viraemic transmission *via* guinea pigs occurred when TBE virus-infected and uninfected ticks fed together. Transmission was equally efficient using either *I. ricinus* or *D. reticulatus* or even non-Eurasian species, and *R. appendiculatus* (1, 2, 21), indicating again that no one tick species possesses certain intrinsic biological (as opposed to ecological, see below) features that make it a more efficient TBE virus vector than any other ixodid species.

To determine whether non-viraemic transmission also occurs *via* natural host species, mammals and birds captured from natural TBE-free sites were infested in the laboratory with infected and uninfected *I. ricinus* (25). Hedgehogs *(Erinaceus europaeus)*, goats, pheasants *(Phasianus colchicus)* and blackbirds *(Turdus merula)* were comparatively resistant to infection. Virus recovered from their blood was evidence of successful virus delivery to these hosts, but they did not support virus transmission: no nymphal tick fed on pheasants or goats, and only 5–10 % of those fed on hedgehogs or blackbirds, acquired infection. In contrast, pine voles *(Pitymys subterraneus)* were highly susceptible to infection. They had high levels of virus in their blood and internal organs, and three out of six animals died before the ticks had completed engorgement. Therefore, although most of the engorged nymphs from pine voles were infected (71 %), the overall transmission efficiency was low (10 %) because so few of the exposed ticks fed successfully. In striking contrast, field mice *(Apodemus flavicollis, A. agrarius)* showed comparatively low, even undetectable, levels of virus infection but infected a high proportion of ticks that fed on them (68%). They fed ticks very well, and so produced the greatest yield of infected ticks (46 % of those exposed). Viraemia in bank voles *(Clethrionomys glareolus)* was higher, but transmission was less efficient (28% of engorged ticks) (24, 25). Furthermore, voles show strong immune responses to feeding ticks (8, 32), so that only 13 % of ticks exposed to voles became infected. These experiments demonstrated great heterogeneity among vertebrate species in their ability to support TBE virus transmission,

clearly identifying rodents, and particularly *Apodemus* field mice, as the most important amplifying hosts (among those tested). The preference of *A. agrarius* for open landscape and cultivated fields reduces its natural contact with ticks and thus diminishes the role of this species in the ecology of TBE virus (37).

The above results allow us to reconsider some of the published data. Although in the laboratory *I. hexagonus* can transmit TBE virus to its principal host species, hedgehogs (38, 39), TBE virus has been isolated from field-collected *I. hexagonus* only once (18). The low efficiency of the transmission from tick to tick *via* hedgehogs (see above) explains the apparently limited role of this tick species in nature.

For the long-term survival of any tick-borne parasite, one infected tick must on average give rise to at least one new infection in another tick, having passed *via* a host. Then the basic reproduction number, R_0, equals one, the minimum value necessary to perpetuate the transmission cycle. According to the traditional paradigm (transmission *via* systemic viraemia), the best available parameter estimates indicate that this value is barely achievable for TBE virus (33). The additional non-systemic pathway, however, changes this. With rodents as amplifying hosts, a quantitative comparison indicated that the non-viraemic pathway (relative R_0, index = 1.65) affords a 50 % greater degree of amplification of TBE virus than does the viraemic pathway (relative R_0-index = 0.98) (33). This suggests that non-viraemic transmission between co-feeding ticks rather than classical viraemic transmission is the main mechanism by which TBE virus survives in its natural ecosystem. Because the difference arises principally from the high mortality associated with high viraemia in feral rodents, it is likely to be the hosts which develop the weakest viraemia that contribute most to TBE maintenance.

The effective reproduction number may be increased still further by the recent discovery that vertebrate hosts with specific immunity to TBE virus can still participate in transmission. When *A. flavicollis* and *C. glareolus* were immunised against TBE virus and produced neutralizing antibodies, 89 % of immune animals that did not produce any detectable viraemia nevertheless supported virus transmission between co-feeding ticks, at about half the level seen on non-immune control animals (24). Previously, such immune hosts, which seasonally constitute at least 25 % of the population, were considered to be dead-end hosts, but these results show that they do not prevent virus transmission between co-feeding ticks.

The partnership: coincident optima for virus and vector

The cellular basis for non-viraemic transmission during co-feeding by ticks is now known to depend on the very mechanisms with which the vertebrate tries to defend itself against feeding ticks. The virus exploits this antagonistic partnership to the full, but, again, only in certain vertebrate species. Infected ticks

deliver virus in their saliva into the feeding site in the host skin. It is there that TBE virus first replicates, promoted by one or more proteins synthesized in the tick salivary glands and secreted in saliva (15, 22). In fact, the virus actually invades the neutrophils, monocytes/macrophages and Langerhans cells that are attracted to the feeding site (20). These cells of the host's immune system migrate to the lymph nodes that drain the skin site, where further virus replication takes place and, importantly, they are also attracted to the feeding sites of uninfected co-feeding ticks. Ticks therefore take an active part in the whole process. A feeding lesion is produced, and it becomes a specific site of inflammation, immunomodulated by pharmacologically active substances in tick saliva (41).

This cellular basis of non-viraemic transmission explains results from experiments designed to investigate virus dissemination in hosts. Ticks were fed in two separate chambers: into one chamber attached to the host's back were placed 2 infected females accompanied by 2 uninfected males and 20 uninfected nymphs; into a second chamber, a few cm from the first, were placed only 20 uninfected nymphs. As a rule, fewer ticks became infected in chamber 2 than in chamber 1, suggesting that certain extra conditions are necessary for dissemination of the virus. This was best demonstrated on pine voles, on which, even in the presence of high viraemia, only 20 % of the nymphs that fed in chamber 2 became infected, in contrast to 92 % in chamber 1 (25). On *A. flavicollis*, 51 % of nymphs that fed in chamber 2 became infected compared with 80 % in chamber 1, and for *C. glareolus* the figures were 25 % and 33 %, respectiviely. Transmission on immune animals was even more localized within chamber 1. Disseminated transmission to chamber 2 was evidently reduced by the presence of TBE-specific antibodies, and it was reduced more on *C. glareolus* than on *A. flavicollis* (24). Dissemination is important for virus transmission because not all ticks feed at exactly the same site, nor do they all feed synchronously. Experiments with the same design as above showed that *A. flavicollis*, but not *C. glareolus*, could support disseminated transmission to more distant skin sites when feeding there by uninfected ticks was delayed by 24 hours relative to the infected tick. This highlights once more the particular significance of *A. flavicollis* as a more efficient amplifying host than *C. glareolus*.

Even without a full knowledge of all the underlying mechanisms, these observed phenomena lead to a conclusion of considerable epizootiological consequence. Only those host species susceptible to TBE virus replication within particular immunocompetent cells in the skin can support transmission, and this is a much more restricted range of species than was once thought on the basis of observed viraemias.

In addition to supporting virus transmission, hosts must also come into contact with ticks and feed them in sufficient quantity to ensure both the acquisition and transmission of virus. Vanishingly few larvae are infected transovarially with TBE virus, so the normal cycle depends on transmission by infected nymphs and acquisition by co-feeding larvae. These larvae then

develop in turn to infected nymphs. Clearly, the sequential feeding of tick stages on rodents occurs as part of the normal course of the tick's life cycle, and therefore both species-specific tick-feeding habits and the timing of the tick's life cycle are critical factors. As will be seen, environmental conditions play a significant role in determining these factors, and this establishes a connection between geography and natural endemic cycles of TBE virus. *I. ricinus* ticks have a very wide host range, including many species of mammals, birds and even lizards. In spite of such potential variety, the majority of ticks feed on only a few mammalian species; tick infestation is normally concentrated on only a fraction of the host population, both inter- and intraspecifically. For example, in central Europe, *A. flavicollis* and *C. glareolus* are typically the most abundant rodent species and also the principal rodent hosts for *I. ricinus* immatures (23). Within these species, certain classes of individuals (typically adult males) feed many more ticks than do other age and sex classes, resulting in markedly overdispersed distributions of ticks amongst their hosts; most hosts carry very few ticks, but a significant proportion of hosts carry large numbers of ticks feeding together.

Overdispersion arises from the non-random distribution of questing ticks (especially larvae that arise from egg-batches) in the habitat, and from genetic, behavioural and immunological heterogeneities in the host population that determine the differential probability of ticks being picked up and fed successfully. These latter factors apply to both immature stages feeding on rodents, so that the aggregated distributions of *I. ricinus* larvae and nymphs are coincident, rather than independent, on their hosts (34); i. e. the same individual hosts feed large numbers of both larvae and nymphs. For example, within the mouse and vole populations from each of four sites in western Slovakia, the same 20% of hosts feed about three quarters of both larvae and nymphs. The consistent effect of this is to double the number of infectible larvae feeding alongside any one, potentially infected, nymph compared with the number if the distribution of these two tick stages was independent. Inserting these empirically observed numbers of infectible larvae per host into the R_0-equation, we now have estimates of absolute R_0, values ranging from 0.74 to 10.7, high enough to account for the persistence of TBE virus in nature (34).

Because of the different life cycle characteristics of the sympatric tick species in this region, this observed pattern of co-feeding larvae and nymphs does not apply to the other major tick species, *D. reticulatus*, to nearly the same extent as to *I. ricinus*. Development times of *D. reticulatus* are short, allowing the life cycle to be completed within a single year, with most larvae feeding one month before the nymphs. Thus, whereas only 3% of *I. ricinus* nymphs were recorded on hosts that were not carrying at least one larva, as many as 28% of *D. reticulatus* nymphs were not co-feeding with larvae of the same species. As both species transmitted TBE virus in the laboratory, the virus could potentially be exchanged between ticks of each species where they coexist. 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 (34), so they do not co-feed to a significant extent. The principal host of immature *I. ricinus*, mice, are also the more efficient amplifiers of TBE virus (see above).

It now appears that the biological versatility of TBE virus transmission, the potential to use several tick species to achieve passage *via* several vertebrate host species, is severely restricted by ecological factors. We have shown how R_0-values, and thus the chances of TBE virus survival, may be increased by several distinct phenomena involving each interaction between tick, virus and host, but coming together in a concerted partnership: the non-systemic route of virus transmission, transmission *via* immune animals, more efficient transmission *via Apodemus* species, the dominance of *A. flavicollis* as hosts for immature *I. ricinus*, and the coincident aggregated pattern of these ticks. For the first time, we have crude estimates of R_0-values for TBE virus which provide a quantitative explanation for the maintenance of TBE virus. It remains, however, to explain the focal nature of TBE virus distribution. Given the ubiquity of *I. ricinus*, of woodmice and of larger hosts for adult ticks (and indeed of *Borrelia burgdorferi* s. l., whose cycles also depend on these biotic components) across wide regions of Europe, which environmental factors determine TBE virus circulation only in certain parts of Europe?

The place: environmental gate-keepers

The endemic distribution of TBE virus over a huge territory of Europe has been well documented (19), with no evidence that the size and location of endemic areas of TBE virus have changed significantly during the last decades (12). The situation in Sweden provides a good example. TBE virus is limited to the south-eastern part of the country along the coastline and to the islands in the Baltic Sea, not spreading to other areas despite the prevalence of *I. ricinus* all over southern Sweden. The areas with TBE virus-infected tick populations have been fairly constant, with only minor local changes throughout the years. A detailed study of 25 human cases demonstrated that the areas where people became infected in 1989 (11) were already natural endemic foci as early as 1956 (42). Similarly, in Finland TBE virus remains almost exclusively limited to the islands of the Åland archipelago in the south-western part of the country (4, 16, 40).

We now have a testable hypothesis for the reasons for such limited, well-defined foci of TBE virus. We can go beyond the obvious assumption that outside the foci at least some of the requirements for virus survival are not accomplished, and identify exactly what those limiting critical factors are. Factors such as large and relatively stable populations of rodents (particularly mice) and larger wildlife to support tick populations are clearly necessary, but are not sufficient on their own. Similarly, certain climatic and habitat conditions appear to be necessary, but not sufficient. In Slovakia, for example, the distribution of 33 distinct TBE virus natural foci has been associated with the 8 °C

annual isotherm, with a mean annual rainfall of at least 800 mm and with plant communities of thermophilic growth within mixed oak and blacklocust forests (23). Not all areas fulfilling these criteria, however, are TBE virus foci. In the relatively small area of the Tribec mountains of western Slovakia, nine foci were identified, all situated within ecotones of forests and open cultivated landscape. More detailed investigations revealed that within the natural foci there was an aggregated distribution of distinct "microfoci", with TBE virus-infected ticks associated mostly with animal feeding and resting places (3). These foci clearly depend on the stability of the micro-structure of habitat, repeatedly bringing ticks and hosts together.

To these observations derived from intensive studies on a small-scale, we can now add quantitative explanations, also derived from intensive studies, that should allow us to extrapolate to extensive patterns and so produce a predictive risk map of TBE throughout Europe. To fulfill the quantitative conditions for virus persistence, ticks and hosts must come together according to specific requirements. First, *I. ricinus* nymphs must feed on rodents in sufficient numbers to deliver infections, and secondly, larvae must co-feed with these nymphs. The first condition may vary on smaller scales than the second, creating microfoci with broader foci. Experimental manipulation has revealed that under increasingly dry microclimatic conditions, nymphs descend the vegetation to the lower moister layers, and there they contact and attach to small rodents in greater numbers than under wetter microclimatic conditions (36). The numbers of nymphs counted feeding on voles were directly correlated with the maximum saturation deficit (dryness) of the atmosphere. Such microclimatic variation could be introduced, for example, by different soil or vegetation types, a factor likely to differ between sites on a smaller scale than weather patterns (6). Any local factor that increases the contact between nymphs and rodents will increase the probability of TBE virus transmission to rodents.

Nevertheless, endemic TBE virus cycles can be maintained even where nymphs make up only a tiny proportion of the immature ticks feeding on rodents. In the Alsace region of France, for example, vanishingly few nymphal *I. ricinus* (on average 0.04 per host) feed on rodents alongside 100 times as many larvae (30), but this is evidently sufficient to maintain weak but persistent TBE virus cycles (31). This appears to be due to the very large numbers of larvae co-feeding with each nymph, crucial in allowing sufficient amplification of virus prevalence in the tick population. The essential factor, the highly coincident aggregated distribution of nymphs and larvae, can only occur where the seasonal activity of nymphs and larvae is coincident, which is not true of *I. ricinus* throughout its geographical range. Only under certain climatic circumstances will larvae become active and feed early in the year at the same time as the nymphs derived from the previous generation of larvae. We predict that this climatically determined, specific pattern of tick seasonal dynamics will largely coincide with the focal distribution of TBE. In other areas where unusually large numbers of nymphs feed on rodents, nymph-to-nymph co-feeding transmission may occur, resulting in significant amplification of infection as nymphs feed and

therefore in a high infection prevalence in adult ticks. These adult ticks usually feed on ungulates and therefore do not contribute to endemic transmission cycles (23), nevertheless they may pose a significant risk of infection to humans.

In Oxford, we are using two complementary approaches to identify these critical climatic conditions, and thereby to map the risk of TBE. At the same time, because climatic data are not available on a sufficiently fine temporal or spatial resolution for epidemiological mapping, remotely sensed meteorological satellite data are being harnessed as surrogates for climate. First, the critical environmental factors are being identified by matching satellite images visually and statistically to the recorded TBE distribution and patterns of tick seasonality across Eurasia, using a geographical information system (GIS). This is similar in principle, although based on different statistical methods, to the matching of recorded cases of TBE infection with vegetation types as detected by Landsat-TM satellite imagery over an area of 4900 km^2 in western central Bohemia in the Czech Republic (7). In that case, however, the chosen satellite imagery gave high spatial resolution (30 m) at the expense of temporal resolution (at most a few images per year). Effectively, *Daniel* et al. (7) were investigating the vegetational correlates of micro-foci. In our case, we prefer to use advanced very high resolution radiometer (AVHRR) data provided by the National Oceanic and Atmospheric Administration's (NOAA) meteorological satellites because, although the spatial resolution is only down to 1 km, the much finer temporal resolution (one image every ten days) allows us to interrogate the seasonal climatic and micro-climatic patterns. These, we think, are the main determinants of wide-scale patterns of TBE foci.

To date, satellite images that have been processed to display the seasonal signatures of temperature and vegetational indices, reveal encouraging correspondence between TBE foci and regions where, for example, high peak temperatures occur relatively late in the summer (*R. M. Green* and *S. E. Randolph*, unpublished). It is apparent from such images that climatic conditions in France are very different from those immediately to the East where TBE is endemic. This is consistent with recent phylogenetic analyses of flaviviruses (9) showing a geographical cline from western type TBE virus to Spanish sheep encephalitis (SSE) virus to Louping ill (LI) viruses in Ireland and UK. Both SSE and LI viruses occupy similar niches on the sheep-rearing hillsides in the Basque region and north and west UK respectively. The suggestion is of an environmental barrier to the westward spread of WTBE virus into France, driving the cline southwards instead, presumably via a corridor of permissive climatic conditions, ultimately reaching Ireland and then UK where larval and nymphal *I. ricinus* do indeed show coincident seasonal peaks in regions where LI viruses occur (34). This hypothesis could be tested by examining the transmission paths of SSE virus and patterns of tick seasonal dynamics in Spain. This would not be the first time that satellite imagery has revealed abiotic factors responsible for directing evolutionary events (13).

Secondly, and more powerfully, a population model for *I. ricinus*, driven by climatic factors and their satellite surrogates, will allow dynamic predictions

of tick distribution, abundance and seasonal patterns under both present, and any future changed, conditions. At three field sites in UK, where *I. ricinus* shows nicely contrasting seasonal patterns of abundance, we are relating tick population parameters to recorded microclimatic factors and concurrent satellite imagery. From these data we are establishing the precise abiotic determinants of the tick's demographic (development and mortality) rates, and the correct relationships between meteorological satellite data and these critical climatic factors. These relationships will be used in a generic population model similar in structure to the one already developed for the African tick *Rhipicephalus appendiculatus*, which captures the geographically variable patterns of seasonal population dynamics throughout the tick's extensive range (35). Such a model for *I. ricinus* will allow us to capture and map the proposed essential feature for TBE virus foci, the seasonal coincidence of nymphs and larvae. The climatic circumstances that generate such coincident seasonality, and its timing each year, are unlikely to be uniform over such a heterogeneous, extensive continental region as Europe. For the first time, we have tools that are sufficiently versatile and broad-scale to capture this spatial heterogeneity, and which can also up-date the resulting risk maps in real time as conditions change.

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References

1. *Alekseev, A. N.* and *S. P. Chunikhin:* Exchange of tick-borne encephalitis virus between Ixodidae simultaneously feeding on animals with subthreshold levels of viraemia. Med. Parazitol. Parazit. Bolezni 2 (1990) 48–50
2. *Alekseev, A. N., S. P. Chunikhin, M. Y. Rukhkyan,* and *L. F. Stefutkina:* Possible role of Ixodidae salivary gland substrate as an adjuvant enhancing arbovirus transmission. Med. Parazitol. Parazit. Bolezni 1 (1991) 28–31
3. *Blaskovic, D.* and *J. Nosek:* The ecological approach to the study of tick-borne encephalitis. Prog. med. Virol. 14 (1972) 275–320
4. *Brummer-Korvenkontio, M., M. Saikku, P. Korhonen,* and *N. Oker-Blom:* Arboviruses in Finland. 1. Isolation of tick-borne encephalitis (TBE) virus from arthropods, vertebrates and patients. Am. J. Trop. Med. Hyg. 22 (1973) 382–389
5. *Cerny, V.:* The knowledge on the developmental cycle of *Ixodes ricinus* ticks in the relation to the veterinary praxis. Veterinarstvi (1957) 136–137
6. *Daniel, M.* and *F. Dusbábek:* Micrometeorological and microhabitat factors affecting maintenance and dissemination of tick-borne diseases in the environment. In: Ecological Dynamics of Tick-borne Zoonoses (*D. E. Sonenshine* and *T. N. Mather,* eds.). Oxford University Press, New York, Oxford (1994) 91–138
7. *Daniel, M., J. Kolar, P. Zeman, K. Pavelka,* and *J. Sádlo:* Predictive map of *Ixodes ricinus* high-incidence habitats and a tick-borne encephalitis risk assessment using satellite data. Exp. Appl. Acarol. 22 (1998) 417–433

8. *Dizij, A.* and *K. Kurtenbach: Clethrionomys glareolus*, but not *Apodemus flavicollis*, acquires resistance to *Ixodes ricinus* L., the main European vector of *Borrelia burgdorferi*. Parasite Immunol. 17 (1995) 177–183
9. *Gould, E., P. M. Zanotta,* and *E. C. Holmes:* The genetic evolution of flaviviruses. In: Factors in the Emergence of Arbovirus Diseases (*J. F. Saluzzo* and *B. Dodet*, eds.), pp. 51–63. Elsevier, Paris (1997)
10. *Gresikova, M.* and *C. H. Calisher:* Tick-borne encephalitis. In: The Arboviruses: Epidemiology and Ecology (*T. P. Monath*, ed.). CRC Press Inc., Boca Raton, Florida (1988) 177–202
11. *Holmegren, E. B.* and *M. Forsgren:* Epidemiology of tick-borne encephalitis in Sweden. A study of 1116 cases. Scand. J. Infect. Dis. 22 (1990) 287–295
12. *Immuno AG:* Tick-borne encephalitis (TBE) and its immunoprophylaxis. Vienna, Austria (1997)
13. *Johnson, D. D. P., S. I. Hay,* and *D. J. Rogers:* Contemporary environmental correlates of endemic bird areas derived from meteorological satellite sensors. Proc. R. Soc. Lond. B 265 (1998) 951–959
14. *Jones, L. D., C. R. Davies, G. M. Steele,* and *P. A. Nuttall:* A novel mode of arbovirus transmission involving a nonviraemic host. Science 237 (1987) 775–777
15. *Jones, L. D., W. R. Kaufman,* and *P. A. Nuttall:* Modification of the skin feeding site by tick saliva mediates virus transmission. Experientia 48 (1992) 779–782
16. *Kääriäinen, L., E. Hirvonen,* and *N. Oker-Blom:* Geographical distribution of diphasic tick-borne encephalitis in Finland. Ann. Med. exp. Fenn. 39 (1961) 316–328
17. *Korenberg, E. I.,* and *Y. V. Kovalevskii:* A model for relationships among the tick-borne encephalitis virus, its main vectors, and hosts. Adv. Dis. Vector Res. 10 (1994) 65–92
18. *Krivanec, K., J. Kopecky, E. Tomková,* and *L. Grubhoffer:* Isolation of tick-borne encephalitis virus from the tick *Ixodes hexagonus*. Folia Parasitol. 35 (1988) 273–276
19. *Kunz, C.:* Tick-borne encephalitis in Europe. Acta Leidensia 66 (1992) 1–14
20. *Labuda, M., J. M. Austyn, E. Zuffová, O. Kozuch,* and *P. A. Nuttall:* Importance of localised skin infection in tick-borne encephalitis virus transmission. Virology 219 (1996) 357–366
21. *Labuda, M., L. D. Jones, T. Williams, V. Danielová,* and *P. A. Nuttall:* Efficient transmission of tick-borne encephalitis virus between co-feeding ticks. J. Med. Entomol. 30 (1993) 295–299
22. *Labuda, M., L. D. Jones, T. Williams,* and *P. A. Nuttall:* Enhancement of tick-borne encephalitis virus between co-feeding ticks. Med. Vet. Entomol. 7 (1993) 193–196
23. *Labuda, M., O. Kozuch,* and *J. Lysy:* Tick-borne encephalitis virus natural foci in Slovakia: ticks, rodents, and goats. In: 4th International Potsdam Symposium on Tick-borne Diseases: Tick-borne encephalitis and Lyme Borreliosis, February 1997 (*J. Süss* and *O. Kahl*, eds.), pp. 34–46. Pabst Science Publishers, Lengerich (1997)
24. *Labuda, M., O. Kozuch, E. Zuffová, E. Elecková, R. S. Hails,* and *P. A. Nuttall:* Tick-borne encephalitis virus transmission between ticks co-feeding on specific immune natural rodent hosts. Virology 235 (1997) 138–143
25. *Labuda, M., P. A. Nuttall, O. Kozuch, E. Eleèková, T. Williams, E. Zuffová,* and *A. Sabó:* Non-viraemic transmission of tick-borne encephalitis virus: a mechanism for arbovirus survival in nature. Experientia 49 (1993) 802–805
26. *Nosek, J., O. Kozuch, E. Ernek,* and *M. Lichard:* The importance of goats in the maintenance of tick-borne encephalitis in nature. Acta virol. 11 (1967) 470–472

27. *Nosek, J., O. Kozuch, E. Ernek,* and *M. Lichard:* Übertragung des Zeckenencephalitis-Virus (TBE) durch die Weibchen von *Ixodes ricinus* und die Nymphen von *Haemaphysalis inermis* auf die Rehkitze (*Capreolus capreolus*). Zbl. Bakt. 1. Orig. 203 (1967) 162–166
28. *Nuttall, P. A.* and *M. Labuda:* Tick-borne encephalitis subgroup. In: Ecological Dynamics of Tick-borne Zoonoses (*D. E. Sonenshine* and *T. N. Mather*, eds.). pp. 351–391 Oxford University Press, New York, Oxford (1994)
29. *Nuttall, P. A.* and *M. Labuda:* Tick-borne encephalitides. In: Zoonoses (*S. R. Palmer, Lord Soulsby,* and *D. I. H. Simpson*, eds.), pp. 469–486. Oxford University Press, Oxford, New York, Tokyo (1998)
30. *Perez-Eid, C.:* Les relations tiques – petits mammiferes dans le foyer Alsacien d'encéphalite tiques. Acarologia 31 (1990) 131–141
31. *Perez-Eid, C., C. Hannoun,* and *F. Rodhain:* The Alsatian tick-borne encephalitis focus: presence of the virus among ticks and small mammals. Europ. J. Epidemiol 8 (1992) 178–186
32. *Randolph, S. E.:* Density-dependent acquired resistance to ticks in natural hosts, independent of concurrent infection with *Babesia microti*. Parasitology 108 (1994) 413–419
33. *Randolph, S. E., L. Gern,* and *P. A. Nuttall:* Co-feeding ticks: epidemiological significance for tick-borne pathogen transmission. Parasitol. Today 12 (1996) 472–479
34. *Randolph, S. E., D. Miklisová, J. Lysy, D. J. Rogers,* and *M. Labuda:* Incidence from coincidence: patterns of tick infestation on rodents facilitate transmission of tick-borne encephalitis virus. Parasitology 118 (1999) 177–186
35. *Randolph, S. E.* and *D. J. Rogers:* A generic population model for the African tick *Rhipicephalus appendiculatus*. Parasitology 115 (1997) 265–279
36. *Randolph, S. E.* and *K. Storev:* Microclimate determines relative infestations of immature tick stages (Acari: Ixodidae) on rodents: implications for parasite transmission. J. Med. Ent. (submitted) (1999)
37. *Stanko, M.:* Ecology of *Ixodes ricinus* immatures on *Apodemus agrarius* (Muridae) in the lowland ecosystem conditions. Wiad. Parazytol. 41 (1995) 329–336
38. *Streissle, G.:* Versuche zur Übertragung des Virus der Frühsommer-Meningoencephalitis durch Lederzecken. Zbl. Bakt. 1. Abt. Orig. 182 (1961) 159–169
39. *Tongeren van, H. A. E.:* Central European encephalitis – epidemiology and vectors. Proc. 6th Int. Congr. Trop. Med. Malar., Lisbon, 5–13 September 1958, Vol. 5 (1962) 174–179
40. *Wahlberg, P., P. Saikku,* and *M. Brummer-Korvenkontio:* Tick-borne viral encephalitis in Finland. The clinical features of Kumlinge disease during 1959–1987. J. Internal Med. 225 (1989) 173–177
41. *Wikel, S. K.* and *D. Bergman:* Tick-host immunology: Significant advances and challenging opportunities. Parasitol. Today 13 (1997) 383–389
42. *Zeipel von, G.:* Isolation of viruses of the Russian spring-summer encephalitis – louping ill group from Swedish ticks and from human cases of meningoencephalitis. Arch. Virusforsch. 9 (1959) 460–469

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