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Mini review

Transmission of tick-borne pathogens between co-feeding ticks: Milan Labuda's enduring paradigm

Sarah E. Randolph*

Dept. of Zoology, University of Oxford, Tinbergen Building, South Parks Road, Oxford OX1 3PS, UK

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ABSTRACT

During the 1990s, Milan Labuda's experimental results established a new paradigm for the study of tick-borne viruses that has since been strengthened by its demonstrated effectiveness in explaining the epidemiology of tick-borne encephalitis (TBE). This brief review summarizes the essential features of the transmission of tick-borne pathogens such as TBE virus. Leukocytes migrate between tick feeding sites, bearing infective virions and providing a transport route for the virus between co-feeding ticks independent of a systemic viraemia. Such tick-borne pathogens are thus transmitted from tick to tick via vertebrates; the ticks are the reservoirs as well as the vectors, while the vertebrate is the transient bridge. The aim is to bring the related but non-synonymous terms (co-feeding and non-systemic) to the attention of workers who use simple PCR screening to identify additional vertebrate reservoir hosts of vector-borne pathogens that are not in fact maintained in nature through systemic transmission.

The elegant and detailed work pioneered by the late Milan Labuda (Institute of Zoology, Slovak Academy of Sciences) underpins our current understanding of the processes of transmission of tick-borne viruses (Labuda et al., 1993a,b,c,d, 1996, 1997b), and the resulting distribution of endemic regions of tick-borne encephalitis (Labuda and Randolph, 1999; Randolph et al., 1999, 2000; Randolph, 2000). His work in the 1990s catapulted the words "non-systemic transmission" and "co-feeding ticks" into the acarological literature, where they now occur regularly in papers concerned with the ecology and epidemiology of tick-borne pathogens (TBPs). Yet these terms are too commonly used incorrectly and therefore, by implication, are not clearly understood. This brief review aims to clarify the relationship between these non-synonymous phenomena. A second aim, in the face of recent publications, is to ensure that this pioneering work, a rare example of something that really does deserve to be labelled a paradigm shift, is not buried beneath myths nor overlooked by successive new generations of vector-borne disease biologists whose literature searches are limited to the 21st century.

Throughout most of the history of the study of vector-borne pathogens, the underlying biological concept was that an infected vector delivered an inoculum of the pathogen to a susceptible host during the blood meal. If this host was receptive and also transmission-competent, this inoculum developed into a systemic infection as pathogens multiplied and spread to many parts of the body, from where vectors could subsequently acquire infections as they fed. Indeed, the standard way to assess the role of a particular vertebrate species in transmission cycles was to look for a certain, putative threshold, level of viraemia/bacteraemia/parasitaemia and/or virus infection in host tissues taken more or less anywhere from the body. Labuda and his colleagues showed that viraemia or a generalized (systemic) infection was not only unnecessary for successful transmission, but could also be negatively correlated with transmission potential.

We were first alerted to the demise of the old conventional wisdom when Jones et al. (1987) showed that the African tick, *Rhipicephalus appendiculatus*, could transmit Thogoto virus (family Orthomyxoviridae) more efficiently via non-viraemic guinea pigs than via highly viraemic hamsters, as long as uninfected ticks fed simultaneously with (even though physically separated from) infected ticks. Similar results followed for tick-borne encephalitis virus (TBEV) (family Flaviviridae) via non-viraemic guinea pigs, with transmission achieved equally efficiently using native European tick species, *Ixodes ricinus* or *Dermacentor reticulatus*, or an exotic species, *R. appendiculatus* (Alekseev and Chunikhin, 1990; Labuda et al., 1993b). Evidently, none of these particular tick species possesses certain intrinsic biological features conferring greater transmission efficiency for TBEV than any other in the laboratory, although ecological features determine differential contributions to transmission in the field (Labuda and Randolph, 1999). Nevertheless, for other pathogens some specificity of competence amongst vector species has been demonstrated in the laboratory (Nuttall and Labuda, 2008).

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* Tel.: +44 1865 271241; fax: +44 1865 271240.
E-mail address: sarah.randolph@zoo.ox.ac.uk

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Table 1
The degree of viraemia established when 2 infected adult ticks (*Ixodes ricinus*) fed in cell 1 on a range of natural vertebrate hosts, and the yield of infected ticks when co-feeding in cells 1 or 2 (see text). Data from Labuda et al. (1993d).

Vertebrate species	Viraemia		% ticks infected		Ticks fully fed (%)	Total yield: no. infected ticks (%)
	Titre	Systemically infected hosts	Cell 1	Cell 2	Cells 1 and 2	Cells 1 and 2
Field mouse: *Apodemus flavicollis*	<1–1.5	3/6	80	51	185/240 (77)	121/240 (50)
Bank vole: *Myodes glareolus*	1.3–3.5	8/8	33	25	130/280 (46)	38/280 (13.5)
Pine vole: *Pitymys subterraneus*	3.7–4.8	3/3	92	20	17/120 (14)	9.5/120 (8)
Hedgehog: *Erinaceus europaeus*	No virus detected		10	0	48/80 (60)	2.4/80 (3)
Pheasant: *Phasianus colchicus*	No virus detected		0	0	97/300 (32)	0

During the 1990s, Labuda's primary experimental work continued to focus primarily on the Western subtype of TBEV, medically the most important arbovirus in Europe, as he transferred his attention to natural vector-host associations to dissect the mechanisms operating in nature. He used the same basic procedure to test for transmission. Two cells were fixed to the host's back. Into cell 1, one or two infected females were introduced, together with males to ensure mating and therefore engorgement by the females, and approximately 20 uninfected nymphs. In cell 2 were another approximately 20 uninfected nymphs. Assays for viraemia and virus infection in host tissues and infection in nymphs showed that some free-living mammals, hedgehogs (*Erinaceus europaeus*) and goats, and some birds, pheasants (*Phasianus colchicus*) and blackbirds (*Turdus merula*), did not support natural systemic TBEV infections and could not transmit the virus back to ticks (Labuda et al., 1993d). Other hosts (notably pine voles, *Pitymys subterraneus*) developed very high levels of virus in their blood and internal organs, but also suffered high mortality. They commonly died before most of the ticks could feed to repletion and so, even though the infection prevalence in any fed ticks was high, very few infected ticks were returned. The greatest numbers of infected ticks were obtained from *Clethrionomys* (now *Myodes*) *glareolus* and even more from *Apodemus flavicollis*, even though these species had very low levels of viraemia or virus in various organs, even undetectable in the latter (Table 1).

Crucially, Labuda also revealed the cellular basis for the empirical patterns (Labuda et al., 1996), which depends on the very mechanisms by which the vertebrate tries to defend itself against feeding ticks. When ticks fed in two cells a few centimetres apart on the back of *A. flavicollis* or (*C.*) *M. glareolus*, virus transmission from infected to uninfected co-feeding ticks within the same cell and between cells was correlated with infection in the skin site of tick feeding; virus was recruited preferentially to the site where ticks were feeding rather than to uninfested skin sites, and waned somewhat with distance between co-feeding ticks, i.e. to ticks in the neighbouring cell (Labuda et al., 1993d, 1996). This selective trafficking of virus was shown to depend on exploitation of the host's immune system and its response to tick saliva. Skin explants of tick feeding sites contained numerous leukocytes that migrated into culture medium; migratory Langerhans cells and neutrophils contained viral antigen, and migratory monocyte/macrophages produced infectious virus. These events were captured visually with two-colour immunocytochemistry. It was clear that the "local skin site of tick feeding is an important focus of viral replication early after TBE virus transmission by ticks" (Labuda et al., 1996). Cellular movement into tick feeding sites and subsequent migration away from such sites, evidently towards other tick feeding sites, provides a transport route between co-feeding ticks independent of a systemic viraemia. Furthermore, immunization of wild rodents by TBEV-infected tick bite and the presence of neutralizing antibodies to TBEV reduced, but did not eliminate virus transmission between co-feeding ticks (Labuda et al., 1997b), which would allow the population of transmission-competent hosts to be maintained in nature.

The role of tick saliva in promoting transmission had already been established (Jones et al., 1989; Nuttall et al., 1994). This is one of a burgeoning list of ways in which pathogens are now known to enhance their transmission by exploiting the immunomodulation of the host immune defences by the tick's salivary proteins for the sake of its own blood feeding (Bowman and Nuttall, 2008). The mechanism of saliva-assisted transmission (SAT) appears to operate at the interaction of the pathogen with the interface between vector and hosts. This interface is "highly complex, remarkably specific, and considerably variable" (Nuttall and Labuda, 2008). The greater transmission efficiency of mice than voles for TBEV, for example, may reflect a greater susceptibility of mice to SAT factors (Labuda et al., 1996).

Thus, we can draw a clear distinction between non-systemic infections, as the cell-based mechanism of viral survival and transport within the host, and transmission between co-feeding ticks, as the outcome with significant quantitative consequences. Tick saliva acts to lubricate the cogs of this complex machine at many points. This route of transmission, therefore, is not an optional extra, a minor additional route, but is the major route for these particular viruses. It is important to see such tick-borne pathogens as being transmitted from tick to tick via vertebrates; the ticks are the reservoirs as well as the vectors, while the vertebrate is the transient bridge. The duration of infectivity to ticks (as distinct from residual viral infection – see below) in the vertebrate is similarly brief, 2–3 days, whether infection is systemic or non-systemic (Kožuch et al., 1967, 1981; Chunikhin and Kurenkov, 1979; Labuda et al., 1996), while the adaptive significance lies in host survival times relative to tick feeding periods (typically 2–4 days for immature stages). Contrary views are still appearing in the literature. Recent papers, even some that include clear accounts of co-feeding transmission, nevertheless imply that this route is supplemental to the principal route of transmission from systemically infected vertebrates by explicitly identifying certain rodent species as "reservoir hosts" based on levels of viraemia (Mansfield et al., 2009; Pfeffer and Dobler, 2010) or prevalence of PCR-detected TBE virus (Achazi et al., 2011). These "reservoirs" are held responsible for maintaining and spreading infections, rather than merely providing useful markers of the presence of natural virus circulation (Achazi et al., 2011).

Clearly, a necessary condition for transmission of any short-lived infection is for infective (donor) and infectible (acquiring) ticks to feed together on the competent host individual; this is the significance of co-feeding. Co-feeding in space is more or less the default situation, especially for ticks feeding on rodents, because ticks show marked spatial aggregations on certain parts of the host body. About 90% of immature stages (larvae and nymphs) of many species of ticks that feed on rodents attach to the ears or around the eyes or on the snout, ensuring that at least approximately 45% of feeding ticks are within ~1 cm of others. At the population level, this is exacerbated by the usual pattern of aggregated distributions of ticks amongst their hosts: Most hosts carry very few ticks, but a small proportion (typically ~20%) carries the majority (~80%) of ticks (indeed of parasites of many types) (Randolph et al., 1999).