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Supplementary Issue: Evolution of the Insect Virome

ABSTRACT: While ticks have been known to harbor and transmit pathogenic arboviruses for over 80 years, the application of high-throughput sequencing technologies has revealed that ticks also appear to harbor a diverse range of endogenous tick-only viruses belonging to many different families. Almost nothing is known about these viruses; indeed, it is unclear in most cases whether the identified viral sequences are derived from actual replication-competent viruses or from endogenous virus elements incorporated into the ticks' genomes. Tick cell lines play an important role in virus discovery and isolation through the identification of novel viruses chronically infecting such cell lines and by acting as host cells to aid in determining whether or not an entire replication-competent, infective virus is present in a sample. Here, we review recent progress in tick-borne virus discovery and comment on the actual and potential applications for tick cell lines in this emerging research area.

KEYWORDS: tick cell line, arbovirus, endogenous virus, endogenous viral element

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Ticks have been recognized as vectors of pathogenic protozoa and bacteria for over 120 years.^{1–3} The first confirmed tick-borne viruses were identified in the early 1930s causing disease in sheep: the flavivirus louping ill virus, transmitted by *Ixodes ricinus* in Scotland,⁴ and the nairovirus Nairobi sheep disease virus, transmitted by *Rhipicephalus appendiculatus* in Kenya.⁵ Since then, ticks have been incriminated as vectors and/or reservoirs of numerous viruses of medical and/or veterinary importance,^{6,7} including the emerging pathogens Alkhumra hemorrhagic fever virus, severe fever with thrombocytopenia virus, Heartland virus, and Bourbon virus.^{8–11} In addition, many apparently nonpathogenic arboviruses have been isolated from ticks.^{6,7}

Research on tick-borne viruses has benefited greatly from the availability of tick cell and tissue cultures. Almost as soon as techniques were developed for repeatedly producing primary tick cell cultures,^{12,13} they were put to use for experiments on arthropod-borne and other viruses, confirming the ability to replicate in tick cells and helping to clarify their host range.^{14–17} Continuous cell lines, first derived from ticks in the 1970s,^{18,19} have since been applied to many aspects of arbovirus research,^{11,20–25} including clarification of actual and potential vector capacity.^{11,14,15,21,22,26–28} Replication was demonstrated in tick cells of viruses carried by ixodid and argasid ticks, mosquitoes, sand flies and midges as well as some viruses with

no known vector.^{15,20,29–31} Surprisingly, there are few reports of tick cells being used for the isolation of tick-borne viruses from field samples; primary tick cell cultures were found to be at least as sensitive as chick embryo cell cultures for the isolation of tick-borne encephalitis virus (TBEV) from vertebrate blood and brain suspension and tick suspension.^{32,33} An uncharacterized RNA virus, Cascade virus, was isolated from frozen triturated *Dermacentor occidentalis* ticks into a *Dermacentor variabilis* cell line (RML-15); Cascade virus also replicated well in amphibian (XTC-2) cells but poorly or not at all in mosquito cells, most vertebrate cells, and suckling mice.³⁴ The *Rhipicephalus microplus* cell line BME26 was used recently alongside Vero and BHK-21 cells for the isolation of a putative novel flavivirus, Mogiana tick virus from *R. microplus* ticks in Brazil.³⁵

Until recently, all the viruses detected in ticks had one characteristic in common—they could all replicate in vertebrate cells and indeed were initially isolated into vertebrate cells. However, it is now recognized that ticks themselves harbor a diverse microflora of vertically transmitted endosymbiotic bacteria and endogenous viruses^{36–40} that may not cycle between ticks and their vertebrate hosts. While endosymbiotic bacteria have been reported to occur in various different tick species for nearly a century,^{3,41–44} very little is known about endogenous viruses of ticks.⁴⁵ An



electron microscope study revealed the presence of virus-like particles in the salivary glands of adult female *R. microplus* ticks feeding on cattle⁴⁶; the presence of large numbers of the particles was associated with possible abnormal tick-feeding behavior. A single apparently tick-only virus has been comprehensively described, sequenced, and characterized: the orbivirus St Croix River virus (SCRV)⁴⁷ was detected in, and replicates in, tick cell lines but has so far failed to infect or replicate in any of the vertebrate or insect cell lines tested.⁴³ Originally, SCRIV was detected in the IDE2 cell line derived from the tick *Ixodes scapularis*,^{47,48} suggesting that this prostrate tick was its natural host; subsequent detection of SCRIV in two cell lines (RA243 and RA257) derived from developing adult *R. appendiculatus* ticks established nearly 20 years previously in a laboratory on a different continent^{18,49} suggests that the virus may have originated from the latter metastriate tick species (Table 1). Electron microscopy revealed the presence of many more putative and potentially tick-only viruses in the majority of over 50 cell lines derived from 14 ixodid and two argasid tick species (Table 1).^{45,49,50} These tick cell lines represent an abundant, easily accessible resource for virus discovery, as well as contributing to studies on virus ancestry and evolution. Studies on the evolution of SCRIV and other tick-borne orbiviruses^{51–53} suggested that SCRIV is the oldest orbivirus identified to date and provided evidence of coevolution of orbiviruses and their respective arthropod host/vector. More recently, the *Amblyomma americanum* cell line AAE12⁵⁴ was found to contain novel orbivirus sequences (H. Attoui, P. Alberdi, C. Sharp, and L. Bell-Sakyi, unpublished data, 2012) (Table 1); if these sequences belong to

a replication-competent virus, it will be interesting to determine its relationship to SCRIV and other orbiviruses.

In the past few years, high-throughput molecular screening and deep sequencing approaches applied to various tick species^{36,38,40,55,56} have revealed tantalizing hints of many more potentially tick-only viruses belonging to diverse families, but for such putative viruses, if they cannot infect and replicate in vertebrate cells, tick cell lines will be needed to establish whether the sequences belong to intact, replication-competent viruses or to endogenous viral elements (partial or full-length DNA copies of the RNA genomes integrated into the genome of the vector/host; EVE) incorporated into the tick genomes. A proteomics informed by transcriptomics approach applied to RNAseq data from uninfected and TBIV-infected tick cells²⁴ revealed RNA sequences with similarity to picorna-like viruses and rhabdoviruses in the *I. scapularis* cell line IDE8 and rhabdoviruses and the iflavivirus deformed wing virus in the *I. ricinus* cell line IRE/CTVM19 (D. Matthews, S. Weisheit, J. de la Fuente, and L. Bell-Sakyi, unpublished data, 2013) (Table 1). Rhabdovirus-like (weakly grouped with lyssaviruses), orthomyxovirus-like (related to quarjviruses), and bunyavirus-like (related to Hazara and Uukuniemi viruses) endogenous virus elements were identified in the genome of *I. scapularis*,⁵⁷ the only tick species with a completely sequenced genome (<https://www.vectorbase.org/>).⁵⁸ RNA sequences with similarity to genes of insect densovirus, hepatitis E virus and the tick-borne pathogen African swine fever virus were detected in transcriptomes of *R. microplus* larvae and/or “tick gut” (unspecified life cycle stage) available on the CattleTickBase website (<http://cattletickbase.ccgapps.com.au/>). These data are not published,

Table 1. Viruses and viral RNA sequences detected in tick cell lines. The tick species and instar from which each cell line was derived have been presented previously (49).

VIRUS OR VIRAL RNA SEQUENCES	TICK CELL LINE	REFERENCE
St Croix River virus	IDE2	(47)
	IDE8, RA243, RA257	(49)
Virus-like particles seen by electron microscopy	RA243	(50)
	AAE2, AAE12, ANE58, AVL/CTVM13, AVL/CTVM17, BDE/CTVM14, BME/CTVM5, BME/CTVM23, BME/CTVM30, DALBE3, DVE1, HAE/CTVM9, IDE2, IDE8, IRE11, IRE/CTVM19, IRE/CTVM20, ISE6, ISE18, RAE/CTVM1, RAN/CTVM3, REE/CTVM28, REE/CTVM29, REE/CTVM31, RSE8	(49)
	CCE1, CCE2, OME/CTVM21, OME/CTVM22, OME/CTVM24, OME/CTVM25, OME/CTVM26, OME/CTVM27	(45)
Novel orbivirus sequences	AAE12	H. Attoui, P. Alberdi, C. Sharp and L. Bell-Sakyi, unpublished data, 2012
RNA sequences with similarity to picorna-like viruses and rhabdoviruses	IDE8	D. Matthews, S. Weisheit, J. de la Fuente and L. Bell-Sakyi, unpublished data, 2013
RNA sequences with similarity to rhabdoviruses and the iflavivirus deformed wing virus	IRE/CTVM19	D. Matthews, S. Weisheit, J. de la Fuente and L. Bell-Sakyi, unpublished data, 2013

and it is unclear whether they represent authentic viral transcripts or EVE transcripts. It is noteworthy that reverse transcriptase activity, as well as retrotransposons, was detected in three of the *I. scapularis* embryo-derived cell lines (IDE2, IDE8, and ISE6) (H. Attoui and F. Mohd Jaafar, unpublished data, 2006). Reverse transcriptase activity may explain why DNA copies of RNA virus genomes occur in the *I. scapularis* genome. Interestingly, it has been shown that TBEV infection of Hep2 human cells resulted in the production of DNA copies of the viral genome and the stable integration of this DNA into the cellular genome backbone⁵⁹ and that TBEV DNA was found integrated into brain cells during progressive infection.⁶⁰ Whether or not a similar process occurs in TBEV-infected tick cells remains to be determined.

In mammalian genomes, endogenous retroviruses have been shown to exert essential regulatory gene expression functions,⁶¹ when integrated in specific positions of the mammalian genome. A decade ago, we identified a DNA form of a virus related to cell fusing agent virus in the *Aedes albopictus* mosquito cell line C6/36, including a 1557 amino acid open reading frame containing the NS1 to NS4B.⁶² Antibodies raised against recombinant NS1 and NS3 of this virus helped to identify expression of its NS3 in C6/36 cells (H. Attoui and F. Mohd Jaafar, unpublished data, 2006). The Dicer-2 of C6/36 cells is nonfunctional as a result of mutations,⁶³ making them highly permissive to a range of arboviruses; expression of viral protein such as NS3 (viral protease) from EVE may also enhance permissivity of cells to viral infections. Similar situations may exist in tick cells in which EVE are expressed.

In conclusion, the present portfolio of cell lines derived from different ixodid and argasid tick species, and new cell lines under development from additional species, constitutes a huge and hitherto underexplored and underutilized resource for the discovery, detection, isolation, and propagation of novel arthropod-borne viruses. It is likely that the application of molecular technologies such as those used in the studies mentioned previously,^{35,36,38,40,55,56} and novel pipelines for metagenomic analysis,⁶⁴ will very soon start to reveal the secrets of tick-borne viruses and endogenous viral elements harbored by tick cell lines and, by inference, the ticks from which they were derived.

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Composed, reviewed, and approved the final article: LB-S and HA.

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