ORIGINAL ARTICLE

# Diversity of viruses in *Ixodes ricinus*, and characterization of a neurotropic strain of Eyach virus

**S. Moutailler[1], I. Popovici[1,2], E. Devillers[1], M. Vayssier-Taussat[1] and M. Eloit[3,4]**

1) *UMR Bipar, INRA, Anses, ENVA, Maisons-Alfort, France,* 2) *Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Department of Public Health, Iasi, Romania,* 3) *PathoQuest SAS and* 4) *Institut Pasteur, Biology of Infection Unit, Inserm U1117, Pathogen Discovery Laboratory, Paris, France*

## Abstract

Ticks transmit more pathogens—including bacteria, parasites and viruses—than any other arthropod vector. Although the epidemiological status of many tick-borne bacteria is very well characterized, tick-borne viruses are still relatively under-studied. Recently, several novel tick-borne viruses have been isolated from human febrile illnesses following tick bites, indicating the existence of other potential new and unknown tick-borne viruses. We used high-throughput sequencing to analyse the virome of *Ixodes ricinus*, the main vector of tick-borne pathogens in Europe. The majority of collected viral sequences were assigned to two potentially novel *Nairovirus* and *Phlebovirus* viruses, with prevalence rates ranging from 3.95% to 23.88% in adults and estimated to be between 0.14% and 72.16% in nymphs. These viruses could not be isolated from the brains of inoculated immunocompromised mice, perhaps indicating that they are unable to infect vertebrates. Within the *I. ricinus* virome, we also identified contigs with >90% identity to the known Eyach virus. Initially isolated in the 1980s, this virus was indirectly associated with human disease, but had never been extensively studied. Eyach virus prevalence varied between 0.07% and 5.26% in ticks from the French Ardennes and Alsace regions. Eyach virus was successfully isolated following intracerebral inoculation of immunocompromised mice with Eyach virus-positive tick extracts. This virus was also able to multiply and persist in the blood of immunocompetent mice inoculated by intraperitoneal injection, and caused brain infections in three of nine juveniles, without any obvious deleterious effects.



**Keywords:** Bunyaviridae, Eyach virus, Flaviviridae, *Ixodes ricinus*, metagenomics, Next Generation Sequencing, Reoviridae, Rhabdoviridae, tick, virus

**Original Submission:** 8 January 2016; **Revised Submission:** 26 February 2016; **Accepted:** 26 February 2016

**Article published online:** 5 March 2016

**Corresponding authors**: M. Eloit, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France and M. Vayssier-Taussat, UMR Bipar, INRA, Anses, ENVA, 14 Rue Pierre et Marie Curie, Maisons-Alfort, France

**E-mails:** marc.eloit@pasteur.fr **(M. Eloit),** mvayssier@vet-alfort.fr **(M. Vayssier-Taussat)**

## Importance

Due to environmental changes, the global incidence of tick-borne disease is increasing worldwide, thus tick-borne diseases have been listed alongside diseases with high emergence risk. Several tick-borne pathogens have already emerged in certain geographical regions, such as tick-borne encephalitis virus and Crimean–Congo haemorrhagic fever virus, and novel tick-borne pathogens are continually being discovered. A proportion of these viruses are zoonotic (severe fever with thrombocytopenia syndrome virus in China, the Heartland virus and the Bourbon virus in the USA) but the pathogenicity of many others is poorly documented. Consequently, it is extremely important to determine the pathogenicity of these viruses, and their potential involvement in undiagnosed animal or human febrile illness or encephalitis.



http://dx.doi.org/10.1016/j.nmni.2016.02.012

## Introduction

Ticks are widespread throughout Europe and are the primary arthropod vector of both human and domestic animal disease agents [1]. In terms of public health, the most important European tick is *Ixodes ricinus*, the vector of the Lyme borreliosis bacteria [2,3]. *Ixodes ricinus* can transmit many varieties of pathogens, including bacteria, parasites and viruses, due to specific biological adaptations and its capacity to feed on numerous different animal species. The most prevalent tick-borne disease transmitted by *I. ricinus* is Lyme borreliosis, with over 85 000 new European cases annually [2,3]. However, patients bitten by ticks can also be infected by many other zoonotic pathogens, including parasites, viruses and other bacteria [4,5]. Some of these pathogens were initially identified in ticks decades before their association with human disease (such as *Borrelia miyamotoi*), whereas others have only been discovered very recently (such as the Bourbon virus). The global incidence of tick-borne disease is increasing worldwide as the result of environmental changes, so tick-borne diseases are now highlighted as having significant emergence risk [6–9]. Several tick-borne pathogens have already emerged in specific geographical regions, such as the tick-borne encephalitis virus (TBEV), louping ill virus, Powassan virus, deer tick virus, severe fever with thrombocytopenia syndrome virus and Crimean–Congo haemorrhagic fever virus [10–14], whereas novel tick-borne pathogens are continually being discovered [15–18]. These factors highlight the importance of studying viral epidemiology in tick populations.

Although ticks have the potential to transmit many different viruses, most studies surveying tick-borne pathogens in Europe have focused on bacterial and/or parasitic pathogens. Numerous reports detailing parasitic or bacterial prevalence in either European ticks or animal reservoirs are published every year [19–23]. For viruses however, the situation is completely different. For instance, even though several tick-borne encephalitis cases have been reported in France [24,25], recent data on TBEV prevalence in *I. ricinus* or animal reservoirs in France do not exist. Moreover, no data are available regarding the prevalence of other tick-borne viruses in Europe. This lack of knowledge and relevant data within ticks is surprising, but may be explained by the fact that it is far easier to detect bacterial and parasitic DNA, because they possess the conserved rRNA genes that are most often targeted by broad-range molecular testing. Subsequently, many laboratories, including ours, have focused their research on bacteria and parasites rather than viruses.

In this study, we aimed to describe the global picture of viruses carried by French *I. ricinus*, using RNA deep sequencing to identify and better characterize both DNA and RNA viruses that replicate in ticks. We identified 545 assembled contigs related to eukaryotic viruses. The vast majority of hits mapped to families known to include arboviruses, and the greatest number of contigs probably designated a novel *Nairovirus* and a new *Phlebovirus*. The only known virus identified in the ticks was the Eyach virus, first isolated in Europe in the 1980s, and which has since been forgotten. Here, we demonstrated its capacity to multiply and persist in the blood of OF1 mice, and its ability to colonize murine brains.

## Methods

### Tick collection and extract preparation

Questing *I. ricinus* female ticks (268 in Ardennes), male ticks (228 in Ardennes) and nymphs (285 and 1455 in Ardennes and Alsace, respectively) were collected by flagging from northeastern France (Ardennes in 2012 49.574177, 4.802835; Alsace in 2010 47.918066, 7.146111). All collected ticks were washed as previously described [26], nymphs were pooled into groups of 15 individuals (116 pools in total) and adults were individually treated. Before RNA extraction, tick samples were crushed in 300 µL Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. A pathogen-free colony was obtained as follows; females from the field were engorged on rabbits and allowed to lay eggs. DNA and RNA samples were extracted from female ticks and PCRs were performed to test for the presence of *Borrelia* spp., *Bartonella* spp., *Anaplasma* spp., *Rickettsia* spp., *Francisella* spp. and *Coxiella* spp. Only larvae from 'pathogen-free' female ticks were conserved and maintained in our colony before use in high throughput sequencing (HTS) experiments.

### Animal and ethical issues

Newborn (72 h old) type I interferon receptor knock-out mice ($IFNAR^{-/-}$, genetic background: A129SvEvBrd) [27]) kindly provided by Dr Damien Vitour (Virology Unit, Animal Health Laboratory, ANSES) were used for viral isolation from tick extracts. Females (4–5 weeks old) and newborn OF1 mice (72 h old) (Charles River Company, Wilmington, MA, USA) were used for studying the course of the Eyach virus infection. Animal experiments were carried out in strict accordance with appropriate animal care practices as recommended by European guidelines. Protocols were approved by the ANSES-ENVA-UPEC Ethics Committee for Animal Experimentation (Agreement Number: 13-021).

### RNA extraction

Crushed ticks (individual adults or pooled nymphs) were divided into two equal samples. One half was directly frozen