SHORT REPORT

A novel parvovirus, Roe deer copiparvovirus, identified in *Ixodes ricinus* ticks

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Abstract

The family *Parvoviridae* contains diverse viruses that are capable of infecting a wide range of hosts. In this study, metagenomic sequencing of *Ixodes ricinus* ticks harvested in 2016 on red deer (*Cervus elaphus*) and European roe deer (*Capreolus capreolus*) in Belgium detected a new 6296-bp parvoviral genome. Phylogenetic and sequence analyses showed the new virus belongs to a new species within the *Copiparvovirus* genus. PCR screening of 4 pools of 10 serum samples from both deer species identified the new copiparvovirus DNA only in roe deer sera. Together, these results are the first evidence of a copiparvovirus in a deer species. Besides its potential pathogenicity to roe deers, the detection of this new virus in ticks raises questions about the possible transmission of parvoviruses by ticks. This report further increases the current knowledge on the evolution and diversity of copiparvoviruses.

Keywords Ticks · Copiparvovirus · European roe deer

Introduction

The family *Parvoviridae* contains two subfamilies: *Parvovirinae* (in vertebrates) and *Densovirinae* (in invertebrates). *Parvoviridae* are small non-enveloped ssDNA viruses with a ≈ 5–6 kb genome. The *Parvovirinae* subfamily is further subdivided into eight genera (ICTV Taxonomy release 2018: https://talk.ictvonline.org/taxonomy/), including the *Copiparvovirus* genus. Currently, two species are recognized within this genus: *Ungulate copiparvovirus 1* (Bovine parvovirus 2) and *Ungulate copiparvovirus 2* (Porcine parvovirus 4). In addition, other members of the *Copiparvovirus* genus have been described in horses (Horse parvovirus-CSF and Equine parvovirus-hepatitis) and in sea lions (Sesavirus) [1–3].

Ixodes ricinus ticks are present in a wide area covering Europe, Western Russia, and North Africa. These ticks require a high relative humidity, restricting them to regions with moderate to high rainfall and adapted vegetation, primarily in deciduous or mixed forests [4]. The complete cycle (larva, nymph, and adult) of *I. ricinus* is performed thanks to feeding on a broad range of mammalian, avian, or reptilian hosts. *I. ricinus* ticks are involved in the transmission of a series of viral and bacterial infections [4]. These ticks are by far the dominant tick species found on deer species in Western Europe.

Next-generation sequencing (NGS) proved to be an efficient tool for viral discovery in a large variety of sample types [5]. We successfully applied the method to mammalian, avian, and insect samples [6–8]. In order to determine the virome of deer ticks in Belgium, we screened pools of *I. ricinus* ticks harvested in 2016 on red deer (*Cervus elaphus*) and European roe deer (*Capreolus capreolus*) using NGS-based viral metagenomics. We unexpectedly found reads of a new viral species related to *Ungulate copiparvovirus 1*. The full genome was obtained by de novo assembly and subsequent investigations by PCR revealed that the virus was largely present in roe deer, but not red deer, sera. We propose the name "*Ungulate copiparvovirus 3*", strain "Roe deer copiparvovirus" for this new virus.

Results and discussion

Two pools of 30 *I. ricinus* ticks collected in 2016 from red and roe deer from southern Belgium (provinces of Liège, Luxembourg and Namur) were submitted to a viral metagenomics analysis by NGS, as in [6–8]. Blastx (https://blast.ncbi.nlm.nih.gov/Blast.cgi) alignment to GenBank viral sequences permitted the identification of a few reads with a low amino acid identity to Bovine parvovirus 2 (*Ungulate copiparvovirus 1*). Subsequent de novo assembly using Geneious v10.0.9 (Biomatters, New Zealand) allowed the reconstruction of a 6296-nucleotide (nt) genome, based on 2529 reads out of 4,785,610. The full genome sequence was deposited in GenBank under accession number MK091524. The genome displays two putative open reading frames (ORFs) typical of members of the *Parvovirinae* subfamily, the first (nts 670–2430) coding for the putative non-structural (NS) protein and the second (nts 2828–6109) coding for the putative capsid protein (Fig. 1a). Given the relative proximity to *Ungulate copiparvovirus 1* and the fact that ticks had been collected on deer species, we hypothesized that this new virus was picked up by ticks from their deer host. To determine the possible presence of DNA from this new copiparvovirus in red and roe deer, primers were designed in ORF2 using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/): DCoPV F: GTATTCTGGCGACACAGCAA, DCoPV R: TCTTTT TGGCTGCTTGGTCT (amplicon size 188 bp). Total DNA was extracted from 4 pools of 10 sera of red deer (*C. elaphus*) and 4 pools of 10 sera of roe deer (*C. capreolus*) collected in 2016, using NucleoMag® VET kit (Macherey–Nagel, Düren, Germany), according to the manufacturer's instructions. Conventional end-point PCR was performed using the Luna® Universal Probe qPCR Master Mix kit (New England Biolabs, Ipswich, Massachusetts, USA), with the following cycling conditions: 95 °C for 5 min, followed by 45 cycles at 95 °C for 20 s, 55 °C for 30 s and 72 °C for 1 min, and a final elongation at 72 °C for 2 min. The four pools of roe deer serum revealed to be positive for the presence of new copiparvovirus DNA, while all red deer sera were negative (data not shown). As a consequence, we propose to call this new virus "*Ungulate copiparvovirus 3*", strain "Roe deer copiparvovirus". In addition, the amplicons obtained from Roe deer sera by PCR were sequenced by Sanger sequencing and showed a nt identity ranging from 98.4 to 100% to the sequence obtained by NGS in ticks (data not shown). This sequence variation tends to exclude the hypothesis of an endogenous parvovirus.

Sequence alignment of the NS protein sequence of Roe deer copiparvovirus and representative strains of *Copiparvovirus*, *Tetraparvovirus*, *Protoparvovirus*, *Amdoparvovirus*, *Erythroparvovirus*, *Dependoparvovirus*, and *Bocaparvovirus* genera was performed using ClustalW implemented in Geneious v10.0.9. A maximum-likelihood phylogenetic analysis was performed using MEGA7 (http://www.megasoftware.net) [9] and the LG substitution model, as determined by a model selection analysis (Fig. 1b). Bootstrap values ($\geq$ 70 %, based on 1000 replicates) for each node are given. The phylogenetic analysis confirmed that the new virus clustered with the members of the *Copiparvovirus* genus. Species demarcation criteria of the ICTV for *Parvoviridae* consist in a sequence nt identity < 85% for the NS gene. Roe deer copiparvovirus NS gene showed $\approx$ 55% nt identity to its closest relative, the *Ungulate copiparvovirus 1* (GenBank accession number KP264971), clearly indicating it belongs to a new viral species.

The full genome of Roe deer copiparvovirus (6296 nt) is similar in length to that of Porcine parvovirus 6 [10] and longer than the genome of Bovine parvovirus 2 (*Ungulate copiparvovirus 1*) and *Porcine parvovirus 4* (*Ungulate copiparvovirus 2*). The putative NS protein of Roe deer copiparvovirus contains the typical ATP/GTP-binding site motif A (P-loop) conserved in parvoviruses (GDPQTGKS) [11], including *Ungulate copiparvovirus 1* [12]. A 271-nt 5′ and a 156-nt 3′ palindromic terminal repeats, similar to the terminal repeats observed in other parvoviruses and thought to be involved in the genome replication, are present (Fig. 1a). Putative splicing sites were predicted using NNSPLICE version 0.9 (http://www.fruitfly.org/seq_tools/splice.html; Fig. 1c).

Although the pathogenic potential of copiparvoviruses is still a matter of debate, Porcine parvovirus 6 was the sole pathogen found in a series of swine aborted fetuses in China [10] and Bovine parvovirus 2 was suspected to be involved in respiratory disease in cattle [13]. In addition, Equine parvovirus-hepatitis was demonstrated to be a cause of acute hepatitis in horses [3] and Horse parvovirus-CSF has been identified in the cerebrospinal fluid of a horse with neurological signs [1]. Roe deer copiparvovirus was detected in *I. ricinus* ticks and subsequently in serum samples from roe deer without evidence of any associated lesions. The pathogenicity of this new copiparvovirus and its possible presence in other wild and domestic ruminant species and in other tick species remain to be determined. Since all 4 pools of 10 roe deer sera that we tested were positive by PCR for the presence of this virus, its prevalence is believed to be relatively high in the roe deer population.

The detection of this copiparvovirus in engorged ticks raises questions about the possible role of mechanical or even biological vector that such ticks might play in the transmission of the virus. This mode of transmission has not been documented for parvoviruses so far and deserves further investigations. In this study, no attempt has been made to
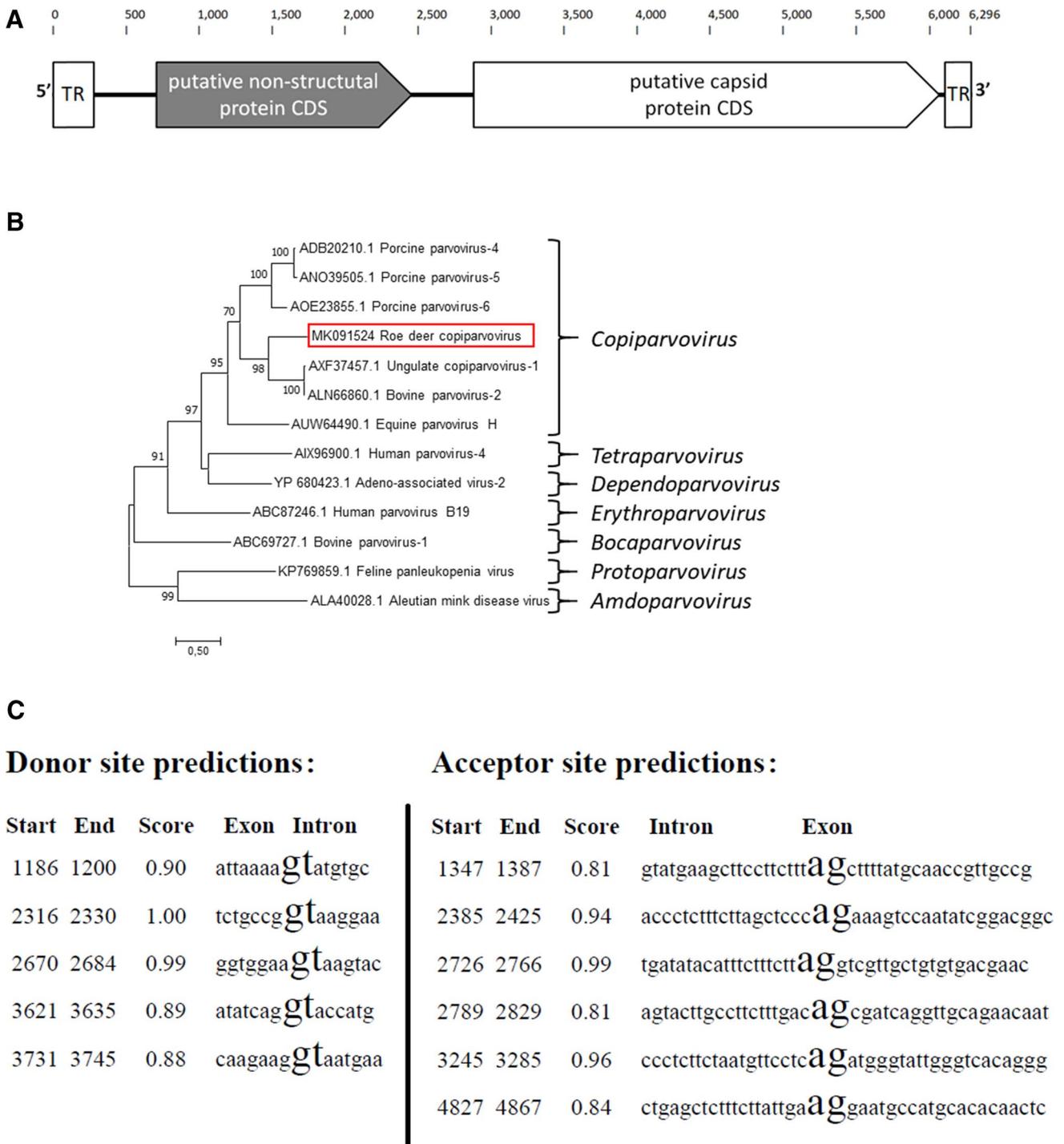
Fig. 1 a Genome structure of the novel copiparvovirus (GenBank accession number MK091524) identified in *Ixodes ricinus* ticks in Belgium. The linear ssDNA genome is approximately 6 kb in length and contains two ORFs, encoding for the non-structural protein (Rep) and the structural (or VP) protein. **b** Evolutionary relationships of the novel *copiparvovirus* identified in *Ixodes ricinus* ticks in Belgium (box) compared to representative members of the subfamily *Parvovirinae*, based on the maximum-likelihood phylogeny of the non-structural protein. The phylogenetic analysis was performed using the LG substitution model. Bootstrap percentages > 70% (1000 resamplings) are indicated at the nodes. GenBank accession numbers are indicated for each strain. Scale bar indicates amino acid substitutions per site. **c** Putative splicing sites in the genome of Roe deer copiparvovirus, predicted using NNSPLICE version 0.9 (http://www.fruitfly.org/seq_tools/splice.html)

isolate the new virus or to assess the possible role of ticks as vectors. The presence of the virus has not been assessed in other ticks species in Belgium. Beside the potential risk of tick-mediated transmission, a long persistence has been experimentally demonstrated for Canine parvovirus 2 in *Haemaphysalis longicornis* ticks [14], which could explain the detection of Roe deer copiparvovirus in engorged *I. ricinus* ticks by NGS in the present study. Similarly, bocaparvovirus sequences have been detected in ticks in China (unpublished data; GenBank accession numbers KY807998 to KY808033).

In this study, we report the detection of a new copiparvovirus in *I. ricinus* ticks collected on red deer and roe deer in southern Belgium and its full genome characterization. We showed the virus was present in roe deer, but not red deer, sera and belonged to a different species according to ICTV species demarcation criteria. We propose the name "*Ungulate copiparvovirus 3*", strain "Roe deer copiparvovirus" for this new virus. The host range, distribution, tissue tropism, and pathogenic potential of this new virus remain to be determined.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Statement on the welfare of humans/animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent concerns are not applicable.

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