Comparative Immunology, Microbiology and Infectious Diseases 45 (2016) 10–15

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Faecal virome of red foxes from peri-urban areas

Ivana Lojkić[a,*], Marina Bidin[b], Jelena Prpić[a], Ivana Šimić[a], Nina Krešić[a], Tomislav Bedeković[a]

[a] Department of Virology, Croatian Veterinary Institute, Zagreb, Croatia
[b] Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form 5 January 2016
Accepted 23 January 2016

Keywords:
Circovirus
Parvovirus
Picobirnavirus
Red fox
Viral metagenome

ABSTRACT

Red foxes (*Vulpes vulpes*) are the most abundant carnivore species in the Northern Hemisphere. Since their populations are well established in peri-urban and urban areas, they represent a potential reservoir of viruses that transmit from wildlife to humans or domestic animals. In this study, we evaluated the faecal virome of juvenile and adult foxes from peri-urban areas in central Croatia. The dominating mammalian viruses were fox picobirnavirus and parvovirus. The highest number of viral reads ($N = 1412$) was attributed to a new fox circovirus and complete viral genome was *de novo* assembled from the high-throughput sequencing data. Fox circovirus is highly similar to dog circoviruses identified in diseased dogs in USA and Italy, and to a recently discovered circovirus of foxes with neurologic disease from the United Kingdom. Our fox picobirnavirus was more closely related to the porcine and human picobirnaviruses than to known fox picobirnaviruses.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Red foxes (*Vulpes vulpes*) are the most abundant and widespread carnivore species in the Northern Hemisphere. Populations of the red fox are well established in peri-urban and urban areas, so they represent a potential reservoir of viruses that transmit from wildlife to humans or domestic animals. Foxes and dogs (*Canis lupus familiaris*) often share the same viral pathogens such as canine parvovirus 2, canine enteric coronavirus, rotavirus and canine distemper [1]. The most important is rabies, which was endemic in Croatia since 1977, but today almost eradicated thanks to the ongoing vaccination campaigns using oral rabies vaccine to target the red fox population in Croatia [2]. Fox carcasses, collected regularly to access the effectiveness or vaccination, can also be used for various laboratory investigations.

Today, high-throughput sequencing followed by viral metagenomic analysis has proved to be powerful tool for exploring and analysing new and existing viruses from variety of human and animal sample types and faeces are commonly studied.

In the present study we evaluated the faecal virome of asymptomatic juvenile and adult red foxes from peri-urban areas in central Croatia using random cDNA synthesis followed by high-throughput Illumina sequencing. Considering the fact that composition of the faecal virome of foxes has been studied recently [3], our results showed different viral profile. We detected a new fox circovirus and other viruses including fox parvovirus and *fox picobirnavirus* but not the astroviruses and hepevirus.

2. Materials and methods

2.1. Sample collection

Twenty four fox carcasses were collected during a regular fox shooting period associated with the nationwide oral rabies vaccination (ORV) of foxes approved by Croatian Ministry of Agriculture. All foxes were collected in central Croatia in two counties: Zagrebačka and Bjelovarsko-Bilogorska (peri-urban area). Fox jaws were subjected to age and tetracycline determination, the key techniques in the evaluation of oral vaccination effectiveness [4] and according to result classified as adult or juvenile. Carcasses were frozen at −80 °C for 1 week and after defrosting, faecal materials were sampled from the rectum of the foxes and frozen at −80 °C until processing. Samples from juvenile foxes were pooled into four samples, two samples per county. The same criterion was applied to the samples from adult foxes.

2.2. Sample preparation and viral nucleic acid extraction

A 10% (wt/vol) mixture of faeces in phosphate-buffered saline (PBS) was prepared and centrifuged. The supernatants were then

* Corresponding author: Tel.: +385 1 612 3605.
E-mail address: ilojkic@veinst.hr (I. Lojkić).

http://dx.doi.org/10.1016/j.cimid.2016.01.005
0147-9571/© 2016 Elsevier Ltd. All rights reserved.

filtered using 0.22 μM filters (Millipore, USA) to remove remaining cell fragments and bacteria. The resulting filtrates were subsequently subjected to nuclease treatment with 100 U of DNase I (New England Biolabs, UK) at 37 °C for 1 h. The resulting virion-enriched samples were used for simultaneous viral RNA and DNA automatic extraction using iPrep viral kit and iPrep instrument (Invitrogen, USA). Ribosomal RNA was depleted from the genomic DNA-depleted samples using 30 μl of RNA, 3 μl of reaction buffer A, 0.5 μl Riboguard Rnase inhibitor (20–40 U/μl, Epicentre Biotechnologies, USA) and 1 μl (1 U/μl)Terminator™ 5′-Phosphate-Dependent Exonuclease (Epicentre). The mixture was incubated at 30 °C for 60 min. Thereafter the samples were subjected to a subsequent round of purification using RNA clean XP (Beckman Coulter, USA) magnetic beads and then used as template for double-stranded cDNA synthesis using random hexamers, random primer FR26RV-N (5′ GCC GGA GCT CTG CAG ATA TCN NNN NN 3′) at a concentration of 0.5 μM and FR40RV-T primer (5′ GCC GGA GCT CTG CAG ATA TC $(T)_{20}$ 3′) at 0.05 μM [5] with cDNA Synthesis System Kit (Roche Diagnostic GmbH) according to the manufacturer's instructions.

2.3. Library construction and Nextera XT Illumina sequencing

The resulting dsDNA was quantified using Qubit fluorimeter (Life Technologies, USA), and diluted to a final concentration at 0.2 ng/uL (1 ng total of each sample). Sequencing libraries were then prepared using Nextera XT sample preparation and Nextera index kits (Illumina, USA) using 5 uL of diluted dsDNA according to manufacturer's instructions and then sequenced using the MiSeq nano 300 cycles kit on MiSeq platform (Illumina). An option for automatic trimming for quality and primer was used.

2.4. Data analysis

The resulting Fastq files for each paired read were subjected to *de novo* contig assembly (CAP3), with criteria of 90% minimum overlap identity and a minimum overlap length of 40 nucleotides; contigs < 200 bp in length were not analysed further. Both, reads and contigs were compared to the GenBank non-redundant protein database using BLASTx with an *E*-value cut-off of 10^{-4} and search was filtered to be restricted to the sequences in the database that correspond to subset Viruses (taxid:10239). The Blast output was used to create a taxonomic classification of the reads and contigs with Megan 5.8.3. [6]. The reference sequences were downloaded from the NCBI (http://www.ncbi.nlm.nih.gov/) for further assemblies using Geneious 5.0.8. The raw sequence data have been submitted to the Sequence Read Archive (SRA) at GenBank with SRA accession number SRP056276. The nucleotide sequences obtained from our study were deposited in GenBank with accession numbers KP941111-KP941114. Recombination analysis of multiple sequence alignments was conducted with RAT [7].

2.5. Phylogenetic analyses

jModelTest V.0.1.1. [8] was used to estimate best-fit model by hierarchical likelihood ratio tests (hLRTs) and approximate Akaike information criterion (AIC). Global alignments, Neighbour-joining (NJ) and maximum likelihood (ML) phylogenetic analyses were generated using MEGA 6 [9]. Reliabilities of phylogenetic relationships were evaluated using nonparametric bootstrap analysis with 1000 replicates for NJ and ML analysis. Bayesian Inference (BI) analysis [10] was performed with MrBayes v3.0b3. [11]. The GenBank accession numbers of the viral sequences used in the phylogenetic analyses are shown on tree figures.

3. Results

3.1. Overview of sequence data

In this study, 8 pooled fox faecal samples of juvenile (marked as 55588, 55591, 55594, 55596) and adult (55539, 55589, 55590, 55592) animals were used for metagenomic analysis by random cDNA synthesis followed by high-throughput sequencing. From the total of 285,838 obtained reads, 9,798 reads showed significant sequence identities to known viruses. Among the viral contigs, 64.4% were bacterial viruses and 22.6% were not assigned to any virus family. The pie charts of assigned viral reads obtained by Megan 5 for all 8 samples are shown in Fig. 1. The ratio between assigned eukaryotic virus sequences *vs.* bacteriophage sequences were 54.8%: 45.2% in the adult foxes and 16.02%: 83.08% in juvenile. The dominating mammalian virus in adult foxes was picobirnavirus.

3.2. Virome of fox faeces

Fox Circovirus. Circovirus sequences were found in three samples, two from juvenile foxes with relatively low number of reads (28 and 30), respectively. From sample 55590 (adult foxes; Fig. 1, Table 1) a single *de novo* assembled genome-sized contig was generated from even 1412 specific reads with mean coverage = 139 (GenBank accession no. KP941114). Phylogenetic analysis of complete nucleotide (nt) sequence (Fig. 2) showed that our sequence clustered with recently identified canine circoviruses (214, H3, Bari/411, UCD2-3216, UCD3-478) and newly described circovirus from fox sera (VS7). The genetic relatedness with circoviruses sequences of other species was low (<50% nt identity with porcine circoviruses). Our fox circovirus genome comprises 2063 nt with a GC content of 52.3%. The genome contains two putative open reading frames (ORFs), on complementary strands in opposite orientation that encode the viral replicase (rep) (303 amino acids (aa)) and capsid (cap) protein (270 aa). It has two intergenic noncoding regions that are 135 and 203 nt long.

In the rep gene, the identity to canine circoviruses was generally high with the highest identity to strain UCD3 and fox isolate VS7100005 (94% aa) and the lowest to the Bari/411–13 (89% aa). In contrast to rep, in the cap protein the highest identity was observed to fox isolate VS7100005 (92%), dog strain Ha13 (91.4%), while identity to strain UCD3 was lower (88.1%). There is no proof for recombination event.

Fox picobirnavirus. We detected sequences with homology to picobirnaviruses in 6 out of 8 samples (Fig. 1). All pooled samples of adult foxes had relatively high number of picobirnaviral sequence reads ($N = 416$); there were only two juvenile samples that had picobirnaviral reads ($N = 22$) (Table 2). Sample 55590 (adult foxes) had 38 contigs matching members of *Picobirnaviridae* assembled from 150 reads (max coverage = 8×), so the whole-size RNA-dependent RNApolymerase (RdRp) sequence has been generated mapping to picobirnavirus consensus sequence. The complete coding sequence of picobirnaviral RdRp gene from the sample 55590 (GenBank accession no. KP941111) consisted of 1650 nt (549 aa) and was longer than recently described fox picobirnavirus F5-1 (GenBank accession no. KF823811; 1620 nt). Phylogenetic analysis of our picobirnavirus nt sequence (Fig. 3) with other RdRp gene sequences of picobirnaviruses of similar length showed that the obtained sequence of the RdRp gene was more closely related to the porcine picobirnavirus than to fox picobirnavirus. Aminoacid sequence alignment and comparison revealed the greatest similarity to porcine picobirnavirus KF861773 (69%), human AB517731 (69.2%) and to fox KF823811 (68%).

Fox Parvovirus. Parvovirus were present in all four adult and one juvenile sample (Fig. 1, Table 2) with the highest number of

Download English Version:

https://daneshyari.com/en/article/2428136

Download Persian Version:

https://daneshyari.com/article/2428136

Daneshyari.com