Virus Research 146 (2009) 125–129

Contents lists available at ScienceDirect

Virus Research

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

Short communication

Detection of a novel porcine boca-like virus in the background of porcine circovirus type 2 induced postweaning multisystemic wasting syndrome

Anne-Lie Blomström[a,b,*], Sandor Belák[a,b], Caroline Fossum[c], John McKillen[d], Gordon Allan[d], Per Wallgren[e], Mikael Berg[a,b]

[a] Department of Biomedical Sciences and Veterinary Public Health, Division of Microbiology and Food Safety, Swedish University of Agricultural Sciences, Uppsala, Sweden
[b] Joint Research and Development Division in Virology of the National Veterinary Institute (SVA) and the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden
[c] Department of Biomedical Sciences and Veterinary Public Health, Section of Immunology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[d] Queens University Belfast and Agri-Food and Biosciences Institute, Belfast, Northern Ireland, United Kingdom
[e] National Veterinary Institute (SVA), Uppsala, Sweden

ARTICLE INFO

Article history:
Received 26 June 2009
Received in revised form 1 September 2009
Accepted 3 September 2009
Available online 11 September 2009

Keywords:
Postweaning multisystemic wasting syndrome
Large-scale sequencing
Bocavirus
Multiple displacement amplification

ABSTRACT

Porcine circovirus type 2 (PCV-2) has been found to be the causative agent of postweaning multisystemic wasting syndrome (PMWS). However, PCV-2 is a ubiquitous virus in the swine population and a majority of pigs infected with PCV-2 do not develop the disease. Different factors such as age, maintenance, the genetics of PCV-2, other pathogens, etc. have been suggested to contribute to the development of PMWS. However, so far no proven connection between any of these factors and the disease development has been found. In this study we explored the possible presence of other so far unknown DNA containing infectious agents in lymph nodes collected from Swedish pigs with confirmed PMWS through random amplification and high-throughput sequencing. Although the vast majority of the amplified genetic sequences belonged to PCV-2, we also found genome sequences of Torque Teno virus (TTV) and of a novel parvovirus. The detection of TTV was expected since like PCV-2, TTV has been found to have high prevalence in pigs around the world. We were able to amplify a longer region of the parvovirus genome, consisting of the entire NP1 and partial VP1/2. By comparative analysis of the nucleotide sequences and phylogenetic studies we propose that this is a novel porcine parvovirus, with genetic relationship to bocaviruses.

Postweaning multisystemic wasting syndrome (PMWS) was first described in Canada in 1991. It is now considered to be a worldwide pig disease that has been reported in European countries as well as in other parts of the world. The most prominent sign of PMWS is wasting in postweaning pigs of about 2–4 months of age (Allan and Ellis, 2000). The disease is also characterized by dyspnea, enlarged lymph nodes and occasional jaundice (Harding and Clark, 1998; Harding et al., 1998).

Porcine circovirus type 2 (PCV-2) is the infectious agent causing PMWS, and has been found at high titres in different lesions (Allan et al., 1998; Ellis et al., 1998; Rosell et al., 1999). PCV-2 is a small non-enveloped circular DNA virus belonging to the family *Circoviridae*. The genome is less than 1800 nucleotides long and contains 4–6 open reading frames (ORFs) (Meehan et al., 1992; Tischer et al., 1982). PCV is divided into two genotypes, PCV-1 and PCV-2. PCV-1 was first discovered as a contaminant virus in a porcine cell line (Tischer et al., 1974) and has so far not been shown to cause disease. PCV-2 on the other hand is frequently detected not only in sick animals but also in healthy ones. Considering the common presence of this virus other factors are thought to influence the development of PMWS as well. Some of these potential factors are the genetic features of the PCV-2 virus, maintenance factors, co-infection with other pathogens, etc. (Madec et al., 2008).

Random amplification methodologies have been used to discover new viruses in humans as well as in animals (Ambrose and Clewley, 2006; Delwart, 2007), and with the new sequencing technologies like the 454, SOLID and Solexa this is becoming a more and more used tool to study different metagenomic issues (Rothberg and Leamon, 2008; Shendure and Ji, 2008; ten Bosch and Grody, 2008).

In the current study the overall presence of viral DNA was investigated in lymph nodes obtained from pigs suffering from PMWS using random multiple displacement amplification (MDA) and large-scale sequencing. By using these technologies we have discovered a novel porcine boca-like parvovirus in pigs.

The lymph nodes were obtained at autopsy of two pigs included in a field study of naturally occurring PMWS in Swedish finishing

* Corresponding author at: Department of Biomedical Sciences and Veterinary Public Health, Division of Microbiology and Food Safety, Swedish University of Agricultural Sciences, Box 7036, S-750 07, Uppsala, Sweden. Tel.: +46 18 674617; fax: +46 18 674669.
E-mail address: Anne-Lie.Blomstrom@bvf.slu.se (A.-L. Blomström).

0168-1702/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2009.09.006

herds (unpublished data). Both pigs showed clinical symptoms of PMWS and the diagnosis was confirmed by typical macroscopic lesions at autopsy and demonstration of PCV-2 in close association to the lesions by immunohistochemistry.

In order to reduce the DNA background of the host and thereby increase the percentage of viral DNA the sample was pre-treated before DNA extraction. This was done by mechanical homogenization of the lymph nodes in 1× DNase buffer (Roche), followed by centrifugation at 4000 rpm for 10 min. The supernatant was filtrated through a 0.45 μM syringe filter (Millipore) before treated with DNase and RNase for 2 h at 37 °C. Thereafter the DNA was extracted and eluted in 50 μl AE buffer using the QIAamp DNA Mini Kit (Qiagen) according to the Blood and Body fluid spin protocol provided by the manufacturer. The DNA was randomly amplified through MDA during 1.5 h using the illustra Genomiphi v2 DNA amplification kit (GE Healthcare) according to the manufacturers instructions. The product was analyzed on a 0.8% agarose gel before purified and eluted in 100 μl 2× TE using the QIAamp DNA Mini Kit (Qiagen) supplement protocol: purification of REPLI-g amplified DNA using the QIAamp DNA Mini Kit. The amplified products from the two lymph nodes were pooled to make one sample for the large-scale sequencing. Sequencing of the pooled sample was done using GS-FLX 454 technology (Roche) at the Royal Institute of Technology (Stockholm, Sweden) according to their standard of operation protocol. Around 9000 unique sequences were obtained from the 454 run. These sequences were assembled using Lasergene v.8 (DNASTAR) giving 145 contigs consisting of one to 2900 sequences. The created contigs and singeltons were blasted against GenBank NCBI using blastn and blastx (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and the results are presented in Table 1. The vast majority of sequences were as expected PCV-2, only about 30 sequences had another origin. Only one of these sequences was of porcine origin, showing that the pre-treatment worked well but also indicating a huge amount of DNA from the different viruses in the samples. Eighteen sequences did not show any similarities neither on protein nor on nucleotide level to any sequence found in the GenBank. This might be due to the short nature of some of these sequences or to the fact that the origin of them is still unclear. A total of 12 sequences were of viral origin apart from PCV-2. These sequences came from Torque Teno virus (TTV) and parvovirus and were studied more closely.

Table 1
Results of blastn and blastx run of all the sequences obtained by the 454 run.

Blast hit	% of sequences
Viruses	
PCV-2	99.545
TTV	0.107
Boca-like virus	0.076
Other	0.015
Pig	0.015
No hit	0.243

The five parvoviral sequences (between 59 and 138 bp) found showed their closest relationship to human bocavirus (HBoV), canine minute virus (CMV) and bovine parvovirus (BPV). Primers were designed based on the sequences obtained from the 454 run and a longer region of about 2000 bp (Fig. 1a) was amplified with Phusion High-Fidelity DNA polymerase (Finnzymes) according to the following conditions; 1× GC buffer, 0.3 mM dNTP, 0.5 U Phusion hot start, 1 M Betaine (Sigma), 0.4 mM of each primer 138b (TTG TGG TGC TTA CCT GCC TC) and 93b (CTG TCA TTA TGT ACC CAT CCA) and 2 μl DNA; 98 °C for 3 min and 35 cycles of 98 °C for 30 s, 58 °C for 30 s, 72 °C for 45 s before the program was ended with 72 °C for 10 min. The PCR products were purified using QIAquick PCR Purification Kit (Qiaqen) according to the manufactures instruc-

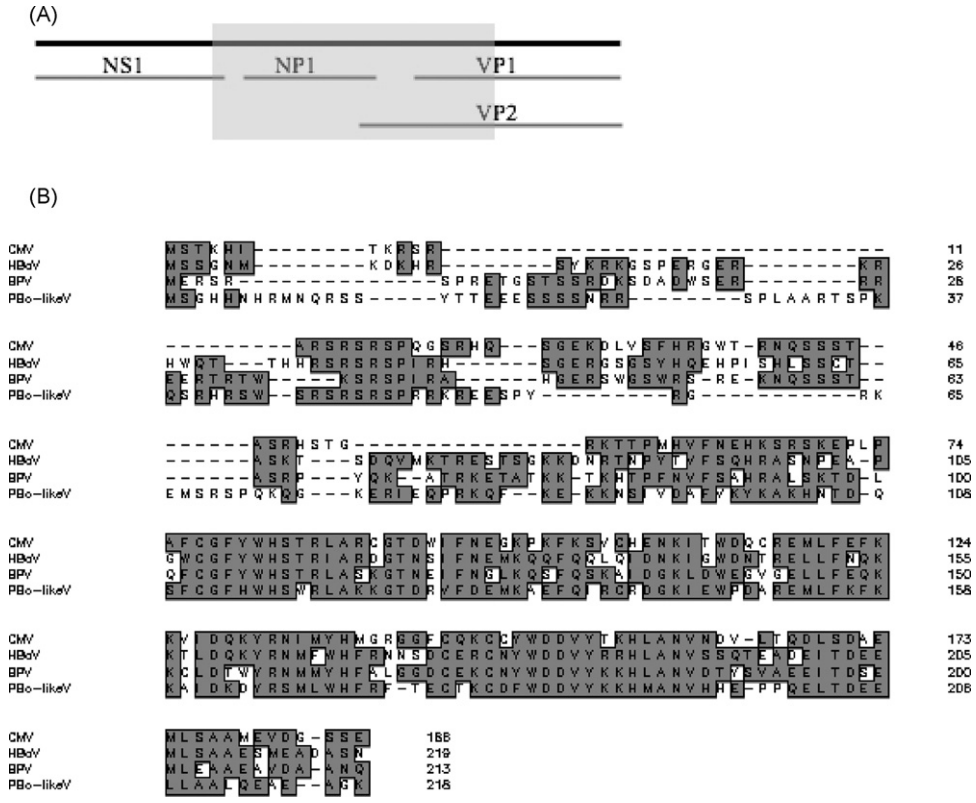

Fig. 1. (A) The genomic organization of bocaviruses showing the different coding regions: Non-structural protein 1 (NS1), Nuclear protein 1 (NP1), Virus protein 1 (VP1) and Virus protein 2 (VP2). The grey shaded box indicates the sequenced region. (B) A multiple alignment of NP1 from different bocaviruses: CMV, HBoV, BPV and the porcine boca-like parvovirus (PBo-likeV).