Picobirnavirus in Captive Animals from Uruguay: Identification of New Hosts

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Key Words

Picobirnavirus • Captive animal • Uruguay

Abstract

The Picobirnaviruses (PBVs) have been detected in several species of animals from different countries worldwide, including in South America. The host range of these viruses has increased in recent years; thus, in order to contribute to the knowledge in this topic we analyzed samples from captivity animals from Uruguay. We found the presence of PBVs in four species of animals, *Panthera leo*, *Panthera onca*, *Puma concolor* and *Oncifelis geoffroyi*, representing new PBV-susceptible hosts. All strains belonged to genogroup I.

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The Picobirnaviruses (PBVs) were classified in 2008 by the International Committee of Taxonomy on Viruses as a new genus belonging to the new virus family *Picobirnaviridae* [1]. PBVs are small, nonenveloped viruses, of about 35 nm in diameter, icosahedral with two double-stranded RNA genomes of about 2.6 and 1.5 kb in size [2].

Polyacrylamide gel electrophoresis (PAGE) from total extracted RNA and reverse transcription-polymerase

chain reaction (RT-PCR), with specific primers designed by Rosen et al. [3], are commonly used for the detection of PBVs. The primers amplify a conserved region within the smaller segment, encoding the RNA-dependent RNA polymerase. Two primer pairs, PicoB25/PicoB43 and PicoB23/PicoB24, were designed in order to classify PBVs into genogroups I and II, respectively [3, 4]. Several publications have showed that genogroup I of PBV is more frequent than genogroup II [3–6].

Up to now, the presence of PBVs has been described in human feces [3–6], and in several captive or wild life animals such as rat [2], pig [7], chicken [8], guinea pig, hamster [9], calf [10], foal [11], giant anteater [12], rabbit [13], dog, snake [14], monkey [15], choique, Chinese goose, American ostrich, pelican, donkey, orangutan, armadillo and gloomy pheasant [16].

PBVs have been associated with a few episodes of diarrhea and are occasionally detected together with other enteric pathogens (bacteria, parasites and/or others enteric viruses). However, PBV has been detected from individuals without symptoms of gastroenteritis and persistent infections in humans and captive animals have also been reported [13, 16]. For these reasons, it is proposed that this virus could be an opportunistic pathogen or a harmless virus in the intestine [17].

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The aim of this study is to contribute to the knowledge of host range of PBVs by detecting their presence in feces of some captive animals in Villa Dolores Zoo in Montevideo, Uruguay. In addition, we account for the molecular characterization of the first set of animal PBV detected in our country.

Fifty fecal samples from 30 species or groups of species of animals were collected from the Villa Dolores Zoo between May 2009 and March 2010. Samples were gathered from the floor after a short deposition time and kept at -80° until processed. Based on their consistency all of these samples were classified as nondiarrheal.

For viral RNA extraction, a suspension of 20% of every fecal sample was prepared in Tris-HCl 0.02 M pH 7.2 buffer, mixed by vortexing and clarified by centrifugation at 5,000 rpm for 15 min. The viral nucleic acid was extracted by the Boom et al. [18] method and stored at -20° until processing.

In order to detect both genogroups I and II PBVs, an RT-PCR was performed as previously described [4]. The RT-PCR products were purified using a commercial kit (NucleoSpin[®] 64 Extract II, Macherey-Nagel, Germany) and sequenced directly by MacroGen Sequencing Service, Korea. The sequences were analyzed primarily by the method of comparison of nucleotide on page <http://blast.ncbi.nlm.nih.gov/> with the BLASTn program for quick identification. Those similar to PBV sequences were edited manually by using the BioEdit Sequence Alignment Editor (version 7.0.9.0) program. For comparing the Uruguayan sequences with PBVs sequences present in public sequences databases, the alignment was constructed with a BioEdit program. Phylogenetic analysis was performed using the MEGA 4 program in combination with the neighbor-joining method with the Jukes-Cantor model as previously published [3, 4, 6, 19]. The nucleotide sequence data reported in this paper have been submitted to the European Nucleotide Archive and have been assigned the accession numbers HE575079, HE575080, HE575081 and HE575082.

In four of the processed samples, an RT-PCR amplicon of about 200 bp in length was obtained, which was subsequently sequenced with genogroup I specific primers.

Fig. 1. Phylogenetic tree constructed by the neighbor-joining method with the Jukes-Cantor model based on a 170-bp fragment within the smaller segment of the PBV genome. Reference PBV Genogroup II strain 4-GA-91 was used as an out-group. The sequences published in this work are underlined. hu = Human; po = porcine.

Table 1. Animal species positive for PBV

Common name	Scientific name	Number of individuals per cage	Collection date	Collection place	Country	PBV nomenclature
Jaguar	<i>Panthera onca</i>	1	10/06/2009	Villa Dolores Zoo	Uruguay	Jaguar/URU/2009
Geoffroy's cat	<i>Oncifelis geoffroyi</i>	2	03/10/2009	Villa Dolores Zoo	Uruguay	Geoffroy's cat/URU/2009
Lion	<i>Panthera leo</i>	2	25/03/2010	Villa Dolores Zoo	Uruguay	Lion/URU/2010
Puma	<i>Puma concolor</i>	3	25/03/2010	Villa Dolores Zoo	Uruguay	Puma/URU/2010

The primary analysis of these sequences with the BLASTn program showed high nucleotide similarity with PBV isolates belonging to genogroup I deposited in public sequences databases. Table 1 shows PBV-positive species, detailing their common and scientific name and number of individuals per cage.

The phylogenetic tree in figure 1 shows the Uruguayan sequences in a cluster with high statistical support belonging to the genogroup I of PBV, these data are consistent with the RT-PCR product obtained of about 201 bp [3].

The presence of genogroup I PBV in *Panthera leo*, *Panthera onca*, *Puma concolor* and *Oncifelis geoffroyi* gives direct evidence of the same PBV genogroup circulating in different hosts. Moreover, this clearly suggests that genogroups are not restricted to a specific host. This finding was also observed in studies with pigs [20, 21], dogs, snakes and rats [14].

Noticeably, all positive animals have no episodes of diarrhea, which is in agreement with previous research on animals in captivity [16] and humans [22].

The results of this study constitute evidence of circulation of genogroup I of PBV in captive animals from Uruguay, in species representing new hosts. Three species (*P. onca*, *O. geoffroyi* and *P. Concolor*) of the four positive animals are autochthonous.

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Disclosure Statement

The authors declare that they have no conflict of interest.

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