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Maintenance of picobirnavirus (PBV) infection in an adult orangutan (*Pongo pygmaeus*) and genetic diversity of excreted viral strains during a three-year period

Gisela Masachessi [a,b,*], Balasubramanian Ganesh [c], Laura C. Martinez [a], Miguel O. Giordano [a], Patricia A. Barril [a,b], Maria B. Isa [a], Giorgio V. Paván [a], Carlos A. Mateos [a], Silvia V. Nates [a]

[a] Virology Institute "Dr. J. M. Vanella", School of Medical Sciences, National University of Córdoba, Córdoba, Argentina
[b] National Scientific and Technical Research Council (CONICET), Argentina
[c] Division of Virology, National Institute of Cholera and Enteric Diseases (NICED), P-33, C.I.T. Road, Scheme-XM, Beliaghata, Kolkata 700 010, West Bengal, India

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ABSTRACT

The present work provide data about the maintenance of picobirnavirus (PBV) infection during adulthood in a mammalian host. For this purpose PBV infection was studied in an adult orangutan (*Pongo pygmaeus*) by PAGE/SS, RT-PCR and nucleotide sequencing. PBV infection in the animal was asymptomatic and was characterized by interspaced silent and high/ low active viral excretion periods. The PBV strains excreted by the studied individual were identified as genogroup I and revealed a nucleotide identity among them of 64–81%.

The results obtained allowed to arrive to a deeper understanding of the natural history of PBV infection, which seems to be characterized by new-born, juvenile and adult asymptomatic hosts which persistently excrete closely related strains in their feces. Consequently, picobirnaviruses could be considered frequent inhabitants of the gastrointestinal tract, leaving the question open about the molecular mechanisms governing persistent and asymptomatic coexistence within the host and the potential host suitability to maintain this relationship.

1. Introduction

Picobirnaviruses (PBVs) are a new group of viruses that belong to the genus Picobirnavirus, family *Picobirnaviridae* (ICTV, 2008). Virions are isometric, non-enveloped, 35 nm in diameter and they present a bi-segmented double-stranded RNA (dsRNA) genome (Duquerroy et al., 2009; Nates et al., 2011; Pereira et al., 1988b). The large genome segment (segment 1) is 2.3–2.6 kb in size and encodes the capsid protein and a polypeptide of unknown function. The small genome segment (segment 2) is 1.5–1.9 kb and encodes the viral RNA-dependent RNA polymerase (RdRp) (Wakuda et al., 2005). Based on the sequences of the small segment, PBVs are classified into genogroup I (prototype strain, 1-CHN-97) and II (prototype strain, 4-GA-91) (Bányai et al., 2003; Rosen et al., 2000). Recently a PBV genogroup III was reported (Smits et al., 2014).

After being reported in the year 1988 (Pereira et al., 1988b), PBVs have been detected in stools samples of several species of animals and from different countries, including rabbits (Gallimore et al., 1993; Ludert et al., 1995), dogs (Costa et al., 2004), cattle (Buzinaro et al., 2003; Malik et al., 2014; Vanpodenbosch and Wellemans, 1990), foals (Ganesh et al., 2011b), pigs (Bányai et al., 2008; Carruyo et al., 2008; Ganesh et al., 2012; Martínez et al., 2010), guinea pigs (Pereira et al., 1989), rats (Pereira et al., 1988a), monkeys (Wang et al., 2007), giant anteaters (Haga et al., 1999), orangutans, armadillos (Masachessi et al., 2007), Pantheraleo, Pantheraonca, Puma concolor, *Oncifelis geoffroyi* (Gillman et al., 2013), snakes (Fregolente et al., 2009), chickens (Tamehiro et al., 2003), geese, pheasants, pelicans (Masachessi et al., 2007) and humans (Gallimore et al., 1995; Ganesh et al., 2010, 2011a; Giordano et al., 1998; Grohmann et al., 1993; Pereira et al., 1988b). PBVs have been detected in feces from animals with or without diarrhea (Bhattacharya et al., 2006, 2007; Gatti et al., 1989). Nowadays, they could be considered as

* Corresponding author at: Virology Institute "Dr. J. M. Vanella", Enfermera Gordillo Gómez s/n, Ciudad Universitaria, Córdoba, Córdoba, Argentina. Tel.: +54 3514334022.
E-mail addresses: gmasachessi@fcm.unc.edu.ar (G. Masachessi), ganeshvirologist@yahoo.co.in (B. Ganesh), lcm@jrp.com.ar (L.C. Martinez), mogiordno@hotmail.com (M.O. Giordano), patricia_barril@yahoo.com.ar (P.A. Barril), mbisa@yahoo.com (M.B. Isa), jorgevpavan@gmail.com (G.V. Paván), mateos.andres@gmail.com (C.A. Mateos), snates@fcm.unc.edu.ar (S.V. Nates).

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opportunistic diarrheagenic agents (Bányai et al., 2003; Gallimore et al., 1995; Ludert et al., 1991; Rosen et al., 2000).

The host-PBV interaction as well as the factors that facilitate higher production of viral progeny in mammals and birds (particularly in porcine and rhea animal models) are now better understood (Martínez et al., 2010; Masachessi et al., 2012). The results demonstrated that PBV infection is characterized by asymptomatic and persistently infected carriers which acquire PBV infection very early in their lives and hold the viral infection at least until the beginning of adulthood.

So far, there are no reports about the maintenance of the PBV infection during adulthood in individuals infected by PBV.

The aim of this study is to provide the first data about the progression of PBV infection during adulthood in a mammalian host, an orangutan (Pongo pygmeus), kept in captivity in a zoo of Córdoba city, Argentina, as well as to reveal the intra-host PBV strain genetic diversity excreted by the animal during a three-year period and to study the relationship of these PBV strains with the strains circulating in other hosts.

The data obtained in the present study would reinforce the knowledge about the natural history of PBV circulation in nature.

2. Materials and methods

2.1. Background

In 2003 we identified PBV in the stool samples of an orangutan kept in captivity in a single cage. The orangutan had no contact with other animals and received food and water individually in a zoo of Córdoba city, Argentina (Masachessi et al., 2007). This finding expanded the knowledge of the host range of PBV. There was no reference about the moment in which the orangutan acquired the PBV infection due to the fact that the animal was adult (approximately 28 years old) at the time of sampling. In addition, we had no information about the animal health status during the period of sampling (infections with any other virus/pathogen), but in general, the animal neither showed any symptoms of diarrhea nor other visible illness.

2.2. Stool sample collection

A follow-up study was carried out during 3 years (from June-2005 to June 2008) with the same orangutan. A total of 117 stool samples were collected (2–5 times a month), immediately after defecation.

The specimens collected were classified as diarrheic or normal on the basis of their consistency. Fecal specimens were stored at −20 °C until analysis.

Samples from the follow-up study were analyzed by PAGE/SS ($n = 117$) and a number of them ($n = 80$) were also assayed by RT-PCR using a genogroup-I-specific primer pair (Pico B25/Pico B43) derived from genomic segment 2 of the 1-CHN-97 prototype strain (Rosen et al., 2000).

2.3. Viral RNA extraction

Nucleic acids were extracted directly from 10% stool suspensions in 0.2 M Tris–HCl, pH 7.2, and clarified by centrifugation at 2000×g for 10 min. Nucleic acids were extracted as described by Perry et al. (1972). Briefly, approximately 400 µl of the supernatant were mixed with an equal volume of extraction buffer (10 mM EDTA disodium salt, 500 mM LiCl and 1% SDS) and 800 µl phenol chloroform (1:1). After incubation for 10 min at 56 °C, samples were centrifuged at 16,000×g for 30 min, and 800 µl of the

Table 1
Follow-up study of PBV excretion in the adult orangutan (*Pongo pygmaeus*) followed for a period of 3 years.

Orangutan																								
Date	Jun-05				Jul-05			Aug-05				Sep-05		Oct-05			Nov-05							
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
PAGE	–	+	–	+	–	–	+	–	–	+	–	–	–	–	–	+	+	–	–	+	–	–	–	–
RT-PCR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	+	+
Orangutan																								
Date	Dec-05		Jan-06			Feb-06			Mar-06					Apr-06						May-06				
Sample	25(1)	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42(2)	43	44	45	46	47	48
PAGE	–	+	+	+	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	+	–	–	–	+
RT-PCR	+	+	–	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	+	+	+	–	–	+	–
Orangutan																								
Date	Jun-06				Jul-06			Aug-06					Sep-06					Oct-06					Nov-06	
Sample	49(3)	50	51	52	53	54	55	56	57(4)	58(5)	59	60	61	62	63	64	65	66	67	68	69	70	71	72
PAGE	+	–	–	–	–	–	–	–	+	+	–	–	–	–	–	+	+	–	–	–	–	+	+	–
RT-PCR	+	–	–	–	–	+	+	–	+	+	–	–	–	–	–	+	+	+	–	–	–	–	+	+
Orangutan																								
Date	Dec-06		Jan-07			Feb-07		Mar-07		Apr-07			May-07			Jun-07		Jul-07		Aug-07		Sep-07		
Sample	73	74(6)	75	76	77	78	79	80	81	82(7)	83	84	85	86	87	88	89	90	91	92	93	94	95	
PAGE	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	
RT-PCR	+	+	–	+	+	–	+	+	+	+	–	–	–	–	–	–	+	+	+	+	+	+	+	
Orangutan																								
Date	Oct-07		Nov-07			Dec-07		Jan-08		Feb-08		Mar-08				Apr-08			May-08		Jun-08			
Sample	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117		
PAGE	–	+	–	–	–	–	+	–	–	+	–	+	–	+	+	–	–	+	+	–	+	+		
RT-PCR	–	–	–	–	–	–	+	–	–	+	–	+	+	+	+	+	+	+	nd	nd	nd	nd		

Note: (a) PAGE: polyacrylamide gel electrophoresis and silver staining. (b) RT-PCR: reverse transcription and polymerase chain reaction. (1);(2);(3), (4); (5); (6); (7) Amplicons sequenced. nd: not done.