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Novel bisegmented virus (picobirnavirus) of animals, birds and humans

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PEER REVIEW

Peer reviewer

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Comments

The distinctions (similarity and dissimilarity) among the animal, bird and human picobirnaviruses are not clear. The authors have attempted to bring all the research findings of PBVs systematically in this review.
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ABSTRACT

Picobirnaviruses (PBVs) are novel group of small, nonenveloped, bisegmented and double stranded RNA viruses. PBVs have been identified in the faeces of a broad range of hosts by several international research groups. Since attempts to culture PBV *in vitro* have not been made to date and no animal model of infection and disease exists. Laboratory diagnosis relies upon electron microscopy, the detection of the double stranded RNA bisegmented genome by polyacrylamide gel electrophoresis and reverse transcription polymerase chain reaction. PBVs have been identified in both normal and diarrhetic faeces. Although their pathogenicity is still unclear, their potential needs further investigation.

KEYWORDS

Picobirnavirus, Double stranded RNA, Electron microscopy, Polyacrylamide gel electrophoresis, Reverse transcription polymerase chain reaction

1. Introduction

Picobirnavirus (PBV) is a double stranded bisegmented RNA virus under the family Picobirnaviridae. Picobirnaviridae is composed of only one viral genus, picobirnavirus. The species in this genus are human PBV and rabbit PBV, and the human PBV is designated as a type species[1]. The name of the virus was proposed based on its characteristics. The prefix “pico” denotes the small diameter of the viral particle (35 nm), and “birna” indicates a genome composed of two segments of double-stranded RNA (dsRNA)[2]. The large segment encodes a capsid precursor that self-assembles form a single-shell capsid[3], and the small segment encodes the RNA-dependent-RNA polymerase (RdRp).

They consist of non enveloped, icosahedral virion, with triangulation number (T) equal to 3 (T=3) symmetry. Their buoyant density in CsCl is 1.4 g/mL. The virion consists of a simple core and capsid. PBVs were demonstrated in the faecal samples of mammals and birds[4]. PBVs have been identified in both normal and diarrhetic faeces, although their pathogenicity is still unclear. This article describes the biology, epidemiology, diagnosis, interspecies transmission and zoonotic potentiality of PBV.

2. Genome profile

PBVs are novel group of small viruses whose genome is

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composed of two segments of dsRNA, ranging in size from 2.4 to 2.6 kbp and 1.5 to 1.8 kbp (Figure 1) for the slow and fast migrating segments, respectively[5]. The segmented dsRNA linear genome segments encode for a total of 3–4 proteins. Genome's total size is about 4 kb. The partial molecular characterization of human and animal strains revealed that PBVs are highly variable, and at least two distinct genogroups have been recognized[6]. PBVs have been classified into two genotypes, namely Genotype I and Genotype II. PBV has large and small genome profile depending on the size of two segments of dsRNA. PBV with small genome profile has two genomic segments of 1.75 and 1.55 kbp for segment 1 and 2, respectively[6]. Large genome profile picobirnavirus has two genomic segments of 2.3–2.6 kbp for segment 1 and 1.5–1.9 kbp for segment 2[7].

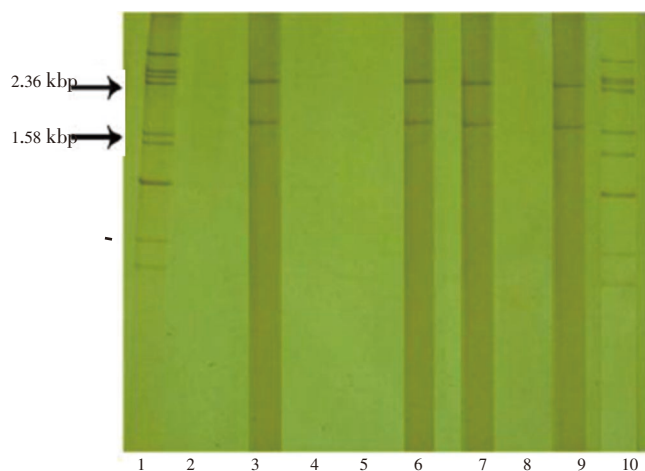


Figure 1. Electrophoretic analysis of RNA genome segments of rotavirus and PBV isolated from bovine and buffalo calves.

Lane 1: cattle rotavirus; Lane 10: buffalo rotavirus; Lane 3: cattle PBV; Lanes 6, 7, and 9: buffalo PBV; Lanes 2, 4, 5, and 8: negative samples.

3. Gene expression

The genomic segment 1 encodes for a polyprotein (open reading frame 1) that self-cleaves to yield a mature coat protein and a large peptide. It also encodes another protein (open reading frame 2)[6]. The genomic segment 2 encodes for the RdRp. The larger RNA segment, or segment 1, contains two open reading frames encoding 224 and 552 amino acids. The small RNA segment, or segment 2, contains a single open reading frame encoding 534 amino acids[6]. Because of its genomic and morphological characteristics, this novel group of viruses is a likely candidate for a new taxon. Like many non-enveloped animal viruses, coat protein undergoes an autoproteolytic cleavage, releasing a post-translationally modified peptide that remains associated with nucleic acid within the capsid[3]. PBV particles are capable of disrupting biological membranes *in vitro*, indicating that its simple 120-subunits capsid has evolved animal cell invasion properties[3].

4. Pathogenic potentiality

PBVs have been shown to cause gastroenteritis in immunocompromised individuals, *viz.* HIV infected patients or kidney transplanted patients[8,9]. They indicated that an immunosuppressive condition in the host might play a role in establishing PBV infection. Gastrointestinal symptoms are common in HIV-infected patients in advanced disease conditions[8]. HIV itself might play a role in the pathogenesis of diarrhea by infecting cells of the intestinal mucosa, but also both common and unusual infectious pathogens have been shown to cause acute and chronic diarrhea, suggesting that one or more etiologic agents can be identified in most episodes of diarrhea in these patients[8].

5. Epidemiology

PBV was first described by Pereira *et al.* who detected two bands of bisegmented dsRNA genome by polyacrylamide gel electrophoresis (PAGE) in faecal samples from children[2]. It is unclear whether the epidemiology of PBVs is influenced by host-species restriction or whether animals may act as reservoirs of infection for humans. Since then, PBVs have been detected in faecal samples of different animals including rats[2], avians[9,10], guinea pigs[11], pigs[12], rabbits[13], cattle[14–16] and giant anteaters (*Myrmecophaga tridactyla*)[17]. PBVs have been frequently detected as coinfections together with rotaviruses, caliciviruses and astroviruses[7,18,19]. Genetic diversity was also observed among swine PBV strains in mixed infection[20]. Single point mutations and deleterious mutations within highly related strains suggested that PBVs exist as quasispecies in the swine alimentary tract[6]. Information on the epidemiology of PBVs has been based primarily on the detection of viral dsRNA by PAGE followed by silver staining and by electron microscopy[6]. The detection of PBV by these assays has been difficult because of the low titres of the virus in many clinical samples[21]. Mondal tested 38 diarrhoeic buffalo calves for viral gastroenteritis and found 3 (7.89%) samples positive for PBV in Mumbai Region, Western India[22]. Ghosh *et al.* detected bovine PBV from a 1-month old diarrhoeic calf in Eastern India[23]. Mondal *et al.* reported 3.53% (4/113) incidence of bovine PBV for first time in Western India[14]. Ganesh *et al.* identified PBV in the faeces of a 10-month old weaned female foal with diarrhoea from Kolkata, India[24]. Malik *et al.* detected 3.67% (5/136) positivity for PBV in bovine and buffalo calves from foothills of Himalaya and Central India[15]. Ganesh *et al.* reported PBVs as mixed infection from a 43-month old male child who had severe diarrhoea and was passing loose stool more than six times a day[6]. Bhattacharya *et al.* detected PBVs with bisegmented small RNA genome profile (1.75 and 1.55 kbp for segment 1 and 2,

respectively) from 1999 to 2003 in faecal specimens of acute watery diarrhoea cases, largely children ($n=20$) and an adult in Kolkata, India[7]. They found that PBV was associated with rotavirus ($n=3$) or astrovirus ($n=3$) and with both in one case. Gallimore *et al.* studied the effect of oral feeding of human and rabbit PBVs in rabbits[13]. On PAGE analysis of nucleic acid extracted from faecal samples collected from orally inoculated rabbits, they demonstrated the presence of discrete equimolar bands, typical of picobirnaviruses, in several specimens. Buzinaro *et al.* analyzed 576 samples obtained from calves aged 1–45 d with and without diarrhea, on PAGE and identified four picovirnavirus positive samples from two diarrheic and two non-diarrheic calves[16]. Tamehiro *et al.* analyzed a total of 378 faecal samples from 1–7 weeks old chicken by PAGE and found migration profile characteristic in 13 (3.4%) samples[25]. Costa *et al.* screened a total of 163 faecal samples from dogs with diarrhea by RNA-PAGE and they found that two out of three samples were from dogs less than six months of age and one of them was also positive for canine parvovirus[5]. Banyai *et al.* described the molecular analysis of porcine PBVs identified in the intestinal content of dead pigs[26]. They found 13 positive picobirnavirus samples. Fregolente *et al.* screened a total of 487 faecal specimens from dogs, snakes and rats by PAGE and reported the first detection of PBVs in snakes (8.5%) [27]. Pereira *et al.* detected picobirnavirus by PAGE in 0.45% of human faecal samples[28]. Gallimore *et al.* detected PBV with an atypical genome profile by PAGE in 37% (20/54) of human faecal samples[21]. Giordano *et al.* collected a total of 224 stool samples from HIV infected or uninfected patient with or without diarrhea[8]. PBV was detected in 14.63% of 82 HIV infected patients, but it was detected neither in those without diarrhea nor in the uninfected HIV patients. Martinez *et al.* examined a total of 82 faecal samples from HIV-infected patients who had been assisted at the Rawson Hospital (Ministry of Health, Cordoba Province, Argentina) for persistent diarrhea, of which 13 faecal specimens were positive for PBV by PAGE[29]. Recently, a novel otarine PBV was discovered in faecal samples of California sea lions[30]. Prevalence of simian genogroup I PBVs was reported in 48% (44/92) of the faecal specimens of monkey by reverse transcriptase polymerase chain reaction (RT-PCR)[31]. PBVs were detected in four species of animals, *viz.*, *Panthera leo*, *Panthera onca*, *Puma concolor* and *Oncifelis geoffroyi*, representing new PBV-susceptible hosts. All strains belonged to genogroup II[32]. Recently, Bodewes *et al.* detected PBV in red foxes (*Vulpes vulpes*)[33].

6. Interspecies transmission and zoonotic potentiality

The highly heterogenous nature of human PBVs is explained due to their segmented genomes[27], and the

chances of segment reassortment either *in vivo* or *in vitro* may lead to emergence of virulent progeny[34]. The reports from Hungary[26], Venezuela and Argentina[35] showed a number of PBV strains with complete sequence identities originating from different animals and suggested effective and easy animal to animal transmission of the virus. Although it remains to be determined whether PBVs are enteric pathogens or innocuous agents of the intestine[4,19], recent studies have reported high genetic diversity and emergence of strains exhibiting inter-species relatedness[7,18,26,34]. Segmented viruses have the tendency to reassort, and often, such reassortments lead to emergence of virulent progeny[31]. Therefore, surveillance studies on detection and molecular characterization of PBVs from humans, animals and birds should be continued. Ganesh *et al.* provided the evidence for genetic relationship between human and equine PBVs[24]. Ganesh *et al.* discussed about circulation of picobirnavirus between human and pig population[6]. As the PBVs have 2 genome segments, there is a theoretic possibility that genome segment reassortment may permit a large variety of the RdRp and capsid gene combinations to rise. Accordingly, gathering information on the genetic diversity of animal PBVs is critical to generate a more precise picture of the ecology of PBVs in humans. Banyai *et al.* reported genetic relationships between human and animal PBVs[20]. Porcine PBV strains are genetically diverse, related to human strains and cause frequent infections among young pigs[35]. The genogroup I PBV detected in pigs in parts of Europe[18] and Latin America[36] were closely related to human genogroup I PBV, suggesting the zoonotic potential of PBV strains.

7. Diagnosis

Since attempts to culture PBVs *in vitro* have not been made to date and no animal model of infection and disease exists. Laboratory diagnosis relies upon electron microscopy and the detection of the dsRNA bisegmented genome by PAGE. Rosen *et al.* have developed a RT-PCR detection assay with two pairs of primers targeted to the genomic segment 2 of 4-GA-91 and 1CHN-97 PBV strains, isolated in the USA and China, respectively[19]. The two different sets of primer pairs of Banyai *et al.* and Rosen *et al.* were used *viz.*, (i) PicoB25 [+] and PicoB43 to amplify the 201 bp fragment of the RdRp gene, related to picobirnavirus strain 1-CHN-97 (Genogroup I), (ii) PicoB23[+] and PicoB24 to amplify the 369 bp fragment of the RdRp gene of strains related to strain 4-GA-91 (Genogroup II) (Table 1)[18,19]. When RT-PCR results are available, the PBV genogroup should be identified before the strain name using GI or GII for genogroup I and II, respectively. The PBVs that are not amplified by these described primer pairs should be identified as non-I and non-II. In future, if other genogroups

are determined using newly designed primers, the present order of genogroup numbers should be maintained.

Table 1

Primer sequences for picobirnavirus identification and genogroup definition.

Primer	Reference strain	Polarity (nucleotide)	Position	Sequence (5'–3')	Genogroup
PicoB25	1–CHN–97	+	665–679	TGG TGT GGA TGT TTC	Genogroup I
PicoB43		–	850–865	A(GA)T G(CYT) GGT CGA ACT T	
PicoB23	4–GA–91	+	685–699	CGG TAT GGA TGT TTC	Genogroup II
PicoB24		–	1039–1053	AAG CGA GCC CAT GTA	

At present, no cell culture and animal model exists for PBVs. Well-structured epidemiological studies are still the only alternative to demonstrate the potential etiological role of PBVs in acute gastroenteritis or other diseases[37]. The PBVs are distributed worldwide that infect a broad range of vertebrate species. Phylogenetic studies have indicated that PBVs show extensive genetic diversity[31]. Segmented viruses have the tendency to reassort, and often, such reassortments lead to emergence of virulent progeny[34]. PBVs are an emerging group of RNA viruses whose epidemiology and virion composition are beginning to be understood. However, much remains need to be known about the biology of the virus and the adaptation of the virus to grow in tissue culture being essential to this end.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The authors reviewed the PBVs, the novel group of small, non-enveloped, bisegmented, dsRNA viruses, that have been identified in the faeces of a broad range of hosts (animals, birds and humans), with emphasis on their biology, pathogenicity, epidemiology, diagnosis, interspecies transmission and zoonotic potentiality. As they are considered as emerging pathogens, there is a need to understand the above aspects.

Research frontiers

PBVs are an emerging group of RNA viruses whose epidemiology and virion composition are not fully understood and much remains need to be known about the pathobiology of these viruses.

Innovations and breakthroughs

The authors have attempted to all bring all the research findings of PBVs systematically in this review.

Applications

It is a good piece of collection of research findings on the emerging PBVs and a useful source of information for researchers.

Peer review

The distinctions (similarity and dissimilarity) among the animal, bird and human PBVs are not clear. The authors have attempted to bring all the research findings of PBVs systematically in this review. As the PBVs infect a broad range of vertebrate species, and their pathogenicity is still unclear, the information provided in this review would be of much use to further understand this novel bisegmented virus.

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