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REVIEW

Picobirnaviruses in animals: a review

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Abstract

Picobirnaviruses (PBVs) are small non enveloped viruses with bi-segmented ds RNA. They have been observed in a wide variety of vertebrates, including mammals and birds with or without diarrhoea, as well as in sewage samples since its discovery (1988). The source of the viruses is uncertain. True hosts of PBVs and their role as primary pathogens or secondary opportunistic agents or innocuous viruses in the gut remains alien. The mechanisms by which they play a role in pathogenicity are still unclear based on the fact that they can be found in both symptomatic and asymptomatic cases. There is a need to determine their tropism since they have not only been associated with viral gastroenteritis but also been reported in the respiratory tracts of pigs. As zoonotic agents with diverse hosts, the importance of epidemiological and surveillance studies cannot be overstated. The segmented genome of PBV might pose a serious public health issue because of the possibility of continuous genetic reassortment. Aware of the growing attention being given to emerging RNA viruses, we reviewed the current knowledge on PBVs and described the current status of PBVs in animals.

Keywords Picobirnavirus · Bi-segmented RNA · Prokaryotic virus · Opportunistic · Zoonotic potential · Emerging viruses

Introduction

Worldwide, gastro-intestinal tract infections are recognized as a major public health issue, as well as one of the major challenges to the livestock industry and human population [1]. Diarrhoea is a common occurrence in all species of animals, and it can result from several factors, including nutritional imbalance, poor management, coccidia, chlamydiae, and viruses. The pathogen species or strains associated with two or more infectious agents may act synergistically and cause greater pathogenesis, increasing the overall disease burden on the host, this may be true both individually and collectively in a herd [2]. There are several causes for the emergence of enteric viruses, which account for over 100 viruses, including re-emerging, emerging, and novel pathogens that affect animals and/or humans at various stages of their lives. Acute gastroenteritis is caused by viruses such as enteric Coronaviruses, Bocavirus, Kobuvirus, Rotavirus as well as later recognized viruses such as Picobirnavirus (PBV) [3].

Discovery

Picobirnavirus (PBV) was accidentally discovered in Brazil in 1988 in faeces from the black footed pigmy rice rat (*Oryzomys nigripes*) [4] and human [5] as 2 bands/segments following polyacrylamide gel electrophoresis (PAGE). The large segment of the genome of rabbit PBV was first sequenced ten years after the virus's discovery and recently, it has been suggested that PBV may exist as a quasispecies [6].

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Taxonomy

Picobirnavirus is the only genus in the *Picobirnaviridae* family placed under the order "*Diplornavirales*". The two species under the genus are Human Picobirnavirus (type species) and Rabbit Picobirnavirus (designated species) by the International Committee on Taxonomy of Viruses (ICTV) in 2008 [7]. The nomenclature of PBV is based on the structural characteristics of the virus: the prefix "Pico" (in Spanish) refers to the small size of the virion (35–40 nm in diameter) and "birna" (bi in Latin) due to the bi-segmented double stranded RNA (dsRNA) of the viral genome [8]. Monopartite genomes of PBV were also reported [9, 10]. It has been reported from the pooled serum samples of six horses. They found the presence of multiple divergent PBV large contigs of 4.2 kb containing both segment 1 and 2. Fused PBV genome (containing segments 1 and 2) was confirmed by nested PCR bridging segments 1 and 2. Upon sequencing, the result confirmed to be a junction between both segments 1 and 2. In addition to the fused genome, 3 complete copies of segment 2 and segment 1 each were also reported [10]. The heterogeneous nature of PBV can be attributed to its segmented genome and remarkable genome flexibility that includes frequent recombination among structural and non-structural genomic regions over long evolutionary timescales, lateral gene transfer among virus and host, gene gain and loss complex genome rearrangements [9].

Family: *Picobirnaviridae.*
Genus: *Picobirnavirus.*
Type species: Human *Picobirnavirus.*
Designated species: Rabbit *Picobirnavirus.*

Virion morphology

The virion is small, non-enveloped and spherical with a higher buoyant density in caesium chloride (CsCl) of 1.38 to 1.4 g/ml [11]. The first description of the icosahedral arrangement of the simple core capsid was made by Duquerroy et al. [12] using 3.4 A° resolution X-ray diffraction crystallography. In *baculovirus* expression studies, they demonstrated the structure of a rabbit PBV as virus like particles (VLPs) encoded by ORF (Open Reading Frame)-3 within segment 1. The coat protein has a 3-dimensional fold, composed of 60 two-fold symmetric dimers. According to their study, PBV is structurally unrelated to Birnaviruses with respect to host, virion size, capsids, RNA polymerase, genome size and organization. In vitro studies have demonstrated the ability of PBV particles to disrupt biological membranes, which indicates the evolution of animal cell invasion properties that result from its 120 subunits capsid. It appears that PBV either has a large genome profile (2.3–2.6 kbp and 1.5–1.9 kbp for segments1 and 2, respectively) or a small genome profile (1.75 and 1.55 kbp for larger and smaller segments, respectively) depending upon the migration pattern of the bisegmented dsRNA in PAGE experiments [7].

Viral genome

The total size of the genome is about 4.2 kb. The nucleotide composition is balanced with a GC content of 46.1%. The 5′ non- coding region is AU rich and the polyadenylation signal (AAUAAA) is absent, and the 5′ end five nucleotides, GUAAA are conserved in both genomic segments [13]. The gene segment-1 of Human PBV genogroup I strain Hy005102 (GenBank accession number AB186897), which is 2525 bp length consists of 2 or 3 open reading frames (ORFs), designated as ORF1, ORF2 and ORF3 from the 5′ end. The ORF 3 begins at position nt 828–830 and terminates at position nt 2484–2486, leaving position nt 2487–2525 untranslated fragments at the 3′ end. As a precursor to the major capsid protein, ORF3 encodes a 552 amino acid protein that undergoes catalytic cleavage to form it. ORF 2 which begins at position nt 157–159 and terminates at UGA position nt 829–831 thus encodes a protein (224 aa in length) of unknown function. The presence of ORF 1 is still in question. The termination codon (UGA) for ORF2 and the initiation codon (AUG) for ORF 3 in segment 1 are overlapped. The gene segment-2 of Human PBV genogroup I strain Hy005102 (GenBank accession number Ab186898) which is 1745 bp length, contains a single large ORF, which begins with AUG position nt 94–96 and terminates with UGA at position nt 1696–1698 which encodes for the RNA-dependent RNA polymerase (RdRp). In total 2 segments encode for a total of 3–4 proteins. RdRp forms a complex with the viral genome during encapsidation [14]. On the basis of the RdRp gene of prototype strains, there are two groups; 4-GA-91 (genogroup II) and 1-CHN-97(genogroup I) [15]. These genogroups contain PBV RdRp sequences that use standard genetic code for translation. The PBV RdRp catalyzes RNA synthesis. Most of the strains belonged to genogroup I and infect a wide range of host species compared to genogroup II. One putative genogroup III was identified in Picobirnaviruses [16] (Fig. 1).

Viral genome of picobirnavirus in some species

Otarine *Picobirnaviruses* The segment 1 of PF080915 strain is 2347 bp long with a GC content of 42.8%. The segment 2 is 1688 bp long with a GC content of 47.45%. 5′ non coding regions of both segments are AU rich. The segment 1 encodes for 2 ORFs while segment 2 encodes for 1 ORF. Segment 1 of strain PF080915 contain 40.9% GC content and 88 bases at 5′ non coding region and 71.4% GC content

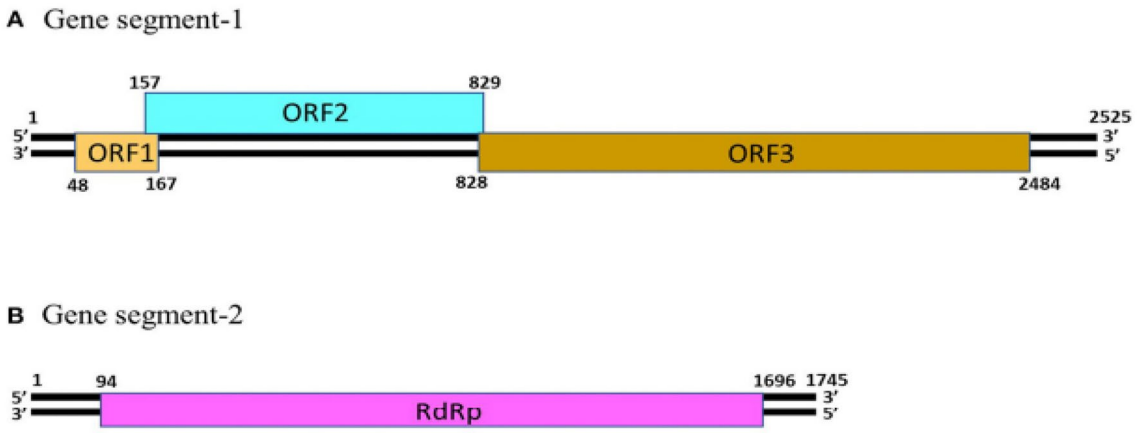

Fig. 1 Organization of genome of human PBV genogroup-I strain Hy005102. **A** There are three putative open reading frames (ORF) in gene segment-1 (GenBank accession number AB186897) of PBV strain Hy005102: ORF1, ORF2 and ORF3. The ORF3 codes for a precursor of the viral capsid protein (GenBank accession number AB186897). **B** Gene segment-2 of PBV strain Hy005102 contains a single ORF that encodes the viral RNA-dependent RNA polymerase (RdRp) (GenBank accession number AB186898) [13]

and 28 bases at 3′ non coding region. Segment 2 of strain PF080915 contain 28.3% GC content and 46 bases at 5′ non coding region and 46.5% GC content and 43 bases at 3′ non coding region [14].

Bovine *Picobirnaviruses* Gene segment 2 of RUBV-P is 1758 bp long with AU rich untranslated 5′ region. 5′- (GUAAA) and 3′- (ACUGC) are conserved in end sequences of gene segment 2 of bovine strain [14].

Lapine *Picobirnaviruses* The length of segment 1 of strain 35,227/89 is 2362 bp. There are 3 ORFs in the segment 1. Because of the presence of 2 stop codons at nucleotides 213–215 and 530–532 there might be 2 frame shifts taking place to produce 1 long protein from nucleotides 51 to 2312 [14].

Nomenclature Typically, the PBV strain name starts with genogroup I or genogroup II, followed by PBV, host species, three letter country code, strain name and isolation year, separated by slashes [6]. Example: Ganesh et al. [13] reported the first incidence of detection and molecular characterization of Porcine PBV in faeces of domestic pigs from India using the human PBV genogroup I specific primer pair (PicoB25 + and PicoB43 −) is designated as genogroup I PBV/Pig/India/BG-Por-2/2010.

Diagnosis of picobirnavirus

Electron microscopy

The PBV's structure may be visualized using electron microscopy. Viruses with an average diameter of 34 nm and uniform morphology were seen in clusters and as single viruses. Most of the particles were spherical with a smooth outline, and in many of them, there was a distinct core, which was narrowly separated from the outer rim. PBVs have an outer rim of about 3 nm thickness [11]. The detection of dsRNA bisegmented genomes based on PAGE (Polyacrylamide Gel Electrophoresis) and silver staining (S/S) is the mainstay of laboratory diagnosis since there is no animal model of infection or disease.

Poly acrylamide gel electrophoresis (PAGE)

Direct visualization of dsRNA can be done by PAGE in conjunction with silver staining. The use of PAGE for the detection of Rotavirus has continued long after commercial antigen detection assays were developed, during the time Picobirnaviruses were accidentally discovered. PAGE and S/S are reliable tests for the detection of PBV since they are simple, economic, fast, and based on the electrophoretic mobility of the viral genomic segments and allow for the differentiation of strains. The PAGE and S/S tests can be used to detect PBV, but since large amounts of viral load are required to visualize the viral genome of PBV, they are relatively insensitive [6, 12]. PBV-positive samples were invariably negative in PAGE once they had been frozen and thawed numerous times, probably because the virus is labile [11].

PAGE and S/S has been used by many researchers for detection of PBV in clinical samples. Correct position of PBV segments can be assessed by comparison with migratory pattern of segments of group A Rotavirus on PAGE. Ghosh et al. reported the presence of PBV (RUBV-P) from a calf via PAGE and noticed that larger segment of PBV appeared to be slightly larger than the segment 2 (VP2 gene) (2684 bp) of rotavirus strain DS-I, while the smaller band of

PBV (1.5–1.9 kbp) of RUBV-P appeared between segment 4 (VP4) (2328 bp) and segment 5 (NSP1) (1461 bp) genes of rotavirus strain DS-I [17]. Malik et al. detected the presence of PBV via PAGE and found larger band of PBV paralleled segment 2 of rotavirus with size of 2.6 kbp, while smaller band of PBV migrated closer to segment 5 of rotavirus (size 1.6 kbp) [18].

A study conducted by Ludert and Liprandi, among children with diarrhoea showed the presence of 3 bands (2.92, 2.37, 1.32 kbp) in PAGE analysis and named it "Picotrirnavirus" [19].". The studies conducted in chicken [20, 21] and dogs in Brazil [22, 23] also reported the presence of Picotrirnavirus. Although it is unclear if these strains are new viral entities with three dsRNA genomic segments or represent a mixed infection with multiple strains [13]. In many studies, researchers have noticed that PBV segments were not detectable by PAGE and S/S but detected by RT-PCR. Periera et al. (Brazil) [24] and Ludert and Liprandi (Venezuela) [19] noticed very low frequency of PBV detection by PAGE suggesting that the dsRNA concentration of the tested sample was below the detection limit of the applied PAGE- S/S, which is plausible given the fact that PAGE is poorly (≈ 100-fold less) sensitive than RT-PCR and detects only dsRNA viruses present with high viral load.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Molecular based test like RT-PCR gained importance in detection for cloning and sequencing of the genome because of the poor sensitivity of PAGE and S/S. The frequency of PBV detection was increased with the usage of RT-PCR amplification techniques and sequencing. Carruyo et al. [25] noticed a PBV detection rate of 60% using RT-PCR which is very high in comparison to PAGE which showed a detection rate of only 27%. Martinez et al. [26] in 2010 also observed similar results i.e., higher detection rates of PBV by RT-PCR than PAGE.

To detect the PBVs, PCR amplification strategies based on single and double primers are used. A single amplification strategy created by Lambden et al. [27] uses, viral RNA and oligonucleotide as a matrix and an adapter, respectively. Viral RNA is then ligated with an oligonucleotide and a complementary adapter primer is used for the cDNA synthesis [17]; Boros et al. [28] used this strategy in their studies. This strategy is further modified by Wakuda et al. [29] to prepare a full-length cDNA of Human PBV genome (strain Hy005102). This strategy is generally used for characterization of full length PBV genome segments. The second strategy of specific amplification uses a pair of primers selected for RdRp gene of the segment 2 of PBV (RT-PCR) assay.

The primers developed by Rosen et al. were widely used for characterization of human PBV and some PBV strains of animals [30]. Narrow specificity of primers restricted the recognition of all circulating PBV strains of human and animals [25] and also failed in amplifying many PBV strains from human and animal species which were earlier detected positive through PAGE and S/S [31]. In order to enhance specificity Malik et al. [32] later developed a primer sequence for the detection of the sequences of genomic segments of PBV (Table 1).

In PAGE-S/S, both genomic profiles (small and large) were found, but only the large profiles were successfully amplified by RT-PCR using the primer pair PicoB25+ and PicoB43- [30]. PBV with a large genome profile was detected most frequently in diarrhoeic sample suggesting that in calves diarrhoea was associated with large genome profile [8].

Metaviromic approaches

The development of bioinformatic tools for sequence analysis and advancement in sequencing techniques have changed the approach to studying viruses. Increasingly, viral metagenomics is being accepted as a method for characterizing viral sequences that is unbiased. By using metaviromics, it is possible to identify viral genomes from samples containing a single or very low number of viral species efficiently and straightforwardly regardless of the presence of non-viral background sequences. There have been many authors who have used this approach [33] [34] [35] [36]. Novel PBV sequences were found from clinical and environmental samples through this approach.

Xiao et al. [33] used metaviromic analysis and investigated faecal, oral, blood and skin samples form 10 lab rabbits. Picobirnavirus was detected from faecal sample, along

Table 1 Primer sequences used for RT-PCR detection of PBV

Primer	Genogroup and strain	Polarity	Nucleotides	Sequence	
PicoB23	Genogroup II (4-GA-91)	+	685–699	CGG TAT GGA TGT TTC	[30]
PicoB24	Genogroup II(4-GA-91)	–	1039–1053	AAG CGA GCC CAT GTA	[30]
PicoB25	Genogroup I(1-CHN-97)	+	665–679	TGG TGT GGA TGT TTC	[30]
PicoB43	Genogroup I(1-CHN-97)	–	850–865	A(GA)T G(CT)T GGT CGA ACT T	[30]
PBV-7F	Genogroup I(GPBV10)	+	754–771	GCN TGG GTT AGC ATG GA	[32]
PBV-7R	Genogroup I(GPBV10)	–	1028–1011	CAY GGN ATG GSA TSB GG	[32]

with other viruses viz., *Polyomaviridae*, *Parvoviridae* and *Microviridae*. Ramesh et al. [34] performed metagenomic next-generation sequencing on 9 swine slurry and 3 environmental samples from a USA farm operation and identified novel viruses. They discovered a total of 1792 viral genome, of which 554 were novel. Among the 1637 Picobirnavirus genome segments, 538 were found to be novel. A total of 638 RdRp and 1033 capsid segments greater than 1 KB were assembled across all 9 slurry samples but no viral genomes would be assembled from the farm environment samples. On phylogenetic analysis of all complete RdRp segments (354/638) identified in this study and all complete PBV genomes from NCBI indicates PBV's are highly diverse and belongs to GGI and GGII [34]. Chauhan et al. [35] investigated the diversity of oral RNA virome from 3 samples of backyard swine oral secretion, using total viral RNA extraction followed by deep sequencing using Illumina HiSeqX. The assembled nucleotide sequences were analysed using the PhyML phylogenetic tree. Sequence analysis identified a high diversity of swine enteric viruses in the saliva samples obtained from backyard wine farm 2 and 3 while only few viruses were identified from farm 1. On characterization of viruses in saliva samples of South African backyard swine, found the presence of multiple PBV species viz., Dog PBV, chicken PBV, Green monkey PBV, Roe deer PBV and Feline PBV suggested the possible interactions of the backyard swine with other wild and domestic animal species [35]. Lojkic et al. (2016) evaluated the faecal virome of juvenile and adult foxes from peri urban areas in central Croatia, and found the presence of fox picobirnavirus and parvovirus. Fox Picobirnaviruses were closely related to porcine and human picobirnavirus than to known fox PBV [36].

Various authors detected the presence of PBV in samples using PAGE-S/S, RT- PCR (Table 2) and metaviromics. As PBV is not isolated yet, these methods provide the only way of detecting its presence in samples. The primers mentioned in Table 1 have been used by various authors to detect PBV from different species of animals. If seeking for PBV in a particular clinical sample, then using a targeted approach would be a better option. Since Picobirnaviruses have flexible segmented genome, it can undergo genetic reassortment, it also has broad host range allowing for genetic recombination between host and viral genome; thereby, leading to the emergence of novel PBV which may or may not be detectable by published primers. The detection of novel PBVs can therefore be made more reliable through metaviromics.

Molecular characaterization of PBV from different animals

Porcine

The molecular characterization of picobirnavirus is done by subjecting the suspected picobirnavirus samples to RNA-PAGE, RT-PCR targeting RdRp gene of segment II and whole genome analysis. The inability to grow/isolate PBV in any cell culture or animal model leaves us with no other option than going for PAGE and RT-PCR. However, the genetic diversity found among PBV strains in a single animal suggest that PBV exists as quasispecies**.** Banyai et al. [37] screened 20 intestinal samples from weaned pigs via PAGE & S/S and RT-PCR which yielded positive results of 2/20 and 13/20 for PBV, respectively suggesting that RT-PCR is sensitive compared to PAGE. Six of thirteen positive RT-PCR samples on cloning revealed that most belonged to genogroup I PBVs and also found genetic relatedness between a porcine PBV to a Hungarian human PBV strain suggesting the genetic diversity found among PBV strains in the pig intestinal tract. Carruyo et al. [25] reported the partial and molecular characterization of genomic segment-2 from porcine isolates. On phylogenetic analysis they found that these porcine isolates were more closely related (78.5%) to human PBV belonging to genogroup I. Stool samples from 7 to 56-day age was collected and subjected to PAGE which showed the prevalence of 10–12%. They developed a specific RT-PCR assay for detection of virus in faeces of porcine. Over the period of 5 years, Martinez et al. [26] in 2010 conducted a study on porcine PBV and determined the prevalence from 265 faecal samples from animals which were grouped based on the physiological status and age. Additional 103 samples collected from follow up studies which were subjected to PAGE & S/S and RT-PCR analysis and concluded that PBV establishes persistent infection with periods of silence and interspersed with periods of low and high viral excretion. High and low PBV excretion levels were detected by PAGE and RT-PCR, respectively. Giordano et al. [38] conducted a study on faecal specimens collected from humans and piglets via RT-PCR which showed that 14 out of 74 samples were positive for PBV. An analysis of phylogenetic relationship revealed similarities between human and porcine PBV strains collected in Argentina, as well as genetic diversity among human and porcine PBV strains from other countries. Presence of closely related human and porcine PBV strains suggests interspecies transmission. Smits et al. [39] in 2011 detected PBV from respiratory tracts of pigs and identified both genogroup I and II explaining the PBV diversity and tropism. The genetic relationship between porcine respiratory and human enteric PBV suggest cross species transmission between pigs and human implying zoonotic aspect of PBV. First reported case study

Table 2 Detection of PBV in domestic animals by PAGE, RT- PCR and Sequence Analysis

Year of publication	Authors	PAGE	% Positive in PAGE	RT-PCR	% Positive in RT-PCR	Sequence Analysis
Porcine						
1989	Gatti et al	✓	106/912 = 11.6%	-	-	NA
1990	Chasey et al	✓	-	-	-	NA
1991	Ludert et al	✓	27/244 = 11.1%	-	-	NA
1993	Alfieri et al	✓	5/75 = 6.7%	-	-	NA
1996	Pongsuwanna et al	✓	2/557 = 0.4%	-	-	NA
2008	Banyai et al	✓	2/20 = 10%	✓	13/20 = 65%	✓
2009	Carruyo et al	✓	39/144 = 27%	✓	87/144 = 60.4%	✓
2010	Martinez et al	✓	56/265 = 21.1%	✓	-	✓
2010	Giordano et al	✓	19/64 = 30%	✓	6/19 = 32%	✓
2011	Smits et al	NA	-	✓	16/60 = 27% for GG I 4/60 = for GG II 3/60 = 5% for both GGI & GG II	✓
2012	Ganesh et al	✓	2/11 = 18.2%	✓	2/11 = 18.2%	✓
2014	Banyai et al	NA	-	✓	1/1 = 100%	✓
2014	Chen et al	NA	-	✓	39/187 = 20.9%	✓
2016	Wilburn et al	NA	-	✓	112/380 = 29.4%	✓
2019	Kylla et al	✓	2/457 = 0.4%	✓	-	NA
2020	Joycelyn et al	NA	-	✓	49/65 = 75.38%	✓
Bovine calves						
1989	Vanopdenbosch and Wellemans	✓	-	NA	-	NA
1991	Villacorta et al	✓	-	NA	-	NA
1997	Chandra	✓	-	NA	-	NA
2003	Buzinaro et al	✓	4/576 = 0.69%	NA	-	NA
2003	Novikova et al	✓	-	-	-	NA
2009	Ghosh et al	✓	1/78 = 0.01%	✓	1/1 = 100%	✓
2011	Malik et al	✓	5/136 = 3.67%	NA	-	NA
2013	Malik et al	✓	-	✓	1/1 = 100%	✓
2013	Mondal et al	✓	4/113 = 3.53%	NA	-	NA
2014	Mondal and Joardar	✓	2/89 = 2.25%	✓	2/2 = 100%	NA
2014	Malik et al	NA	-	✓	1/1 = 100%	✓
2016	Takiuchi et al	✓	24/289 = 8.30%	✓	15/24 = 62.5%	✓
2018	Navarro et al	NA	-	✓	18/77 = 23.4%	✓
2018	Prasad et al	✓		✓	52/408 = 13%	NA
2019	Woo et al	NA	-	✓	3/51 = 5.9% for GG I 1/51 = 1.9% for GG II	✓
2021	Nazaktabar et al	✓	5/485 = 1%	✓	5/5 = 100%	✓
2021	Huaman et al	-	-	-	-	-
2022	Atasoy et al	NA	-	✓	9/127 = 7.08%	✓
Foals (Equine)						
1991	Browning et al	✓	-	NA	-	NA
2011	Ganesh et al	✓	0/7 = 0%	✓	1/7 = 14.3%	✓
Lambs (Ovine)						
1996	Munoz et al	✓	-	✓	-	✓
2018	Kunz et al	✓	5/100 = 5%	✓	62/100 = 62%	✓
Lambs and kids (Ovine and caprine)						
2018	Malik et al	NA	-	✓	143/400 = 35.75%	✓

Table 2 (continued)

Year of publication	Authors	PAGE	% Positive in PAGE	RT-PCR	% Positive in RT-PCR	Sequence Analysis
Birds (Chicken) and turkeys						
1989	Alfieri et al	✓	17/120 = 14.2%	-	-	NA
1990	Leite et al	✓	44/257 = 17.1%	-	-	NA
1991	Monteiro et al	✓	-	-	-	NA
2003	Tamehiro et al	✓	13/378 = 3.4%	-	-	NA
2010	Day et al	NA	-	-	-	✓
2012	Bezerra et al	✓	-	✓	-	✓
2014	Silva et al	✓	13/85 = 15.3%	✓	42/85 = 49.4%	✓
2018	Pankovics et al	NA	-	-	-	✓
2019	Ribeiro et al	✓	3/85 = 3.5%	✓	10/85 = 11.76%	✓
Dogs (Canine)						
2001	Volotao et al	✓	5/1041 = 0.48%	-	-	NA
2004	Costa et al	✓	3/163 = 1.84%	-	-	NA
2009	Fregolente et al	✓	3/349 = 0.85%	✓	2/349 = 0.6%	✓
2017	Navarro et al	NA	-	✓	1/42 = 2.3%	✓

✓ = Attempted, *NA* Not attempted

on Porcine PBV from India was conducted by Ganesh et al. [13] in which 11 faecal samples collected from pigs of different ages were screened for porcine PBV by PAGE & S/S (2/11) and RT-PCR (2/11). On sequence and phylogenetic analysis showed close genetic relatedness between human, porcine as well as murine PBV strains which is in agreement with the findings of Carruyo et al. [25] and Giardano et al. [38]. Chen et al. [40] studied around 187 stool samples from pig over the period of 8 months. Thirty nine out of 187 (20.9%) samples were positive for PBV by RT-PCR, and among the positive samples 84.6% belonged to genogroup I, 38.5% came from genogroup II, rest 23.1% had both genogroup I and II. Nineteen and eleven representative strains from genogroup I and genogroup II, respectively were analyzed phylogenetically which suggested the prevalence of multiple PBV from pigs in China. Wilburn et al. (2016) [41] determined the prevalence and genetic diversity of porcine PBV by studying 380 faecal samples from both diarrhoeic and non-diarrhoeic piglets. PBV was detected in 86 of 265 (32.5%) diarrhoeic and 26 of 115 (22.6%) non-diarrhoeic piglets using RT-PCR for RdRp gene. All these strains show high similarity between them and were also closely related to genogroup I PBV Chinese porcine strain. They concluded that PBV infection is seen irrespective of diarrhoea in faecal samples of piglets. Some researchers like Kylla et al. [2] concluded that PBV coinfects with Salmonella Typhimurium causing piglet diarrhoea. They collected a total of 457 fresh faecal samples from organised (225) and unorganised (232) pig farms from different regions of Northeast region of India and screened them using PAGE & S/S and RT-PCR and noticed higher prevalence of coinfection from unorganised farms and crossbred pigs compared to organised farm and local indigenous pigs with higher detection in summer.

Bovine

The very first report of PBV came from Brazil in 2003 where Buzinaro and coworker [42] while screening for the presence of Rotavirus using PAGE from diarrhoeic and non diarrhoeic faecal samples from calves of age 1–45 days reported the occurrence of bisegmented genome suggesting the presence of PBV. Ghosh et al. [17] reported the presence of bovine genogroup I strain from a month-old diarrhoeic calf on molecular characterization. On sequence and phylogenetic analysis of gene segment 2 revealed low nucleotide identities (51.2–64.9%) with and distant genetic relatedness to other genogroup I suggesting that bovine strain RUBV-P might be different from genogroup I of human and other animals. Over a period of 3 years, Malik et al. in 2011 [43] screened a total of 136 faecal samples for the presence of PBV in buffalo and cattle calves by RNA PAGE. PAGE analysis confirmed 3.67% (5/136) positivity for PBV, suggesting the presence of sporadic infection of PBV in bovine calves. For the first time from western India PBV was reported in 2013 in cattle and buffalo. A total of 113 diarrhoeic faecal samples were screened for the presence of Rotavirus via PAGE during which they also found PBV in four samples [44]. Malik et al. [18] reported the presence of genogroup I of PBV from faecal samples of buffalo. A sequence analysis revealed 44.5% and 45.1% homology, respectively between the human PBV prototype from China and bovine PBV prototype from India. A unique PBV isolate from buffalo

showed a separate lineage from genogroup I and genogroup II PBV sequences suggesting the emergence of new heterogenous group of viruses with a distinct lineage. Mondal and Jaordar [45] screened 89 diarrhoeic faecal samples from cattle calves in West Bengal and found 2/89 (2.25%) positive for PBV using both PAGE and RT-PCR and both the samples belonged to genogroup I. Considering the fact that genogroup II is uncommon, first report of genogroup II of PBV strains in a diarrhoeic bovine calf was done by Malik et al. [15]. Takiuchi et al. [8] screened a total of 289 faecal samples using PAGE and found 24 samples positive for PBV. Among these 24 positive PBV samples, 5 showed small electrophoretic profile which was the first detection of small genome profile of PBV like strains in bovine. Based on phylogenetic analysis the bovine strain identified in Turkey had an 81% nucleotide identity with the bovine strain identified in Turkey. Prasad et al. [46] detected presence of PBV in 52 samples out of 408 diarrhoeic buffalo calf faecal samples, all belonging to genogroup I using RT-PCR and PAGE. None of the samples were positive for genogroup II. Navarro et al. [47] analyzed 77 diarrhoeic and non diarrhoeic faecal samples from bovine in Brazil and found 18/77 = 23.4% positive for genogroup I of PBV using RT-PCR. On phylogenetic analysis high diversity among the sequences were reported at nucleotide level revealing heterogenous phylogenetic clustering profiles. Nazaktabar [48] in 2021 reported the presence of PBV in bovine diarrhoeic faecal samples from Iran. A total of 485 stool samples collected from 1 month old diarrhoeic calves, were subjected to PAGE and RT-PCR. Out of which only 5 samples were positive in PAGE (1%). Nazaktabar-14 strain phylogenetic analysis showed a low similarity with bovine PBV sequences and closer relationship with isolates from other hosts. This is the first report on PBV occurrence in Iran. From Australia, Huaman et al. [49] in 2021 detected the presence of genogroup I and genogroup II of PBV from respiratory tract of wild deer and cattle. They screened for the presence of RdRp gene of segment 2 of PBV in various samples like serum, faeces, spleen, lung, nasal swabs and trachea collected from cattle and wild deer. The presence of PBV in respiratory tract addresses the question regarding its tropism and pathogenicity. Atasoy et al. [50] investigated the frequency of bovine rotavirus (BRV) and bovine coronavirus (BCoV) and PBV in causing gastro enteritis in young calves associated with diarrhoea. Out of 127 diarrhoeic bovine faecal samples screened for 3 viruses, BRV and BCoV had the frequency of 38.58% and 29.92%, respectively whereas 7.08% of bovine calf samples were positive for genogroup I. Sequence analysis of PBV revealed high genetic heterogeneity.

Broiler

The occurrence of PBV dsRNA was detected in chickens from 2 to 7-week-old showing pasty consistency of faecal material Tamehiro et al. [51] screened a total of 378 faecal samples from broiler chicken aged 1–7 weeks using PAGE. They found characteristic migration profile of dsRNA of avian rotavirus (AvRV), reovirus (Arv) and PBV in 32 (8.5%), 7(1.8%) and 13(3.4%) samples, respectively. Silva et al. [52] reported the first gene sequence of avian PBV in Brazilian broiler chickens. Sequencing of these strains demonstrated a considerable RdRp gene heterogeneity that ranged from 56.1 to 100% at the nucleotide level compared with prototypes of different species and water sewage and from 50.3 to 100% among themselves. Around 85 samples were collected and analyzed by PAGE and RT-PCR which showed a positivity of 15.3% (13/85) by PAGE and 49.4% (42/85) by RT-PCR. A novel picobirnavirus was detected in a cloacal sample from broiler breeder chicken by Pankovics et al. [53] using viral metagenomics and molecular techniques. Segment 1 of chicken PBV genome showed low amino acid sequence identity to the corresponding proteins of marmot and dromedary PBV, whereas segment 2 showed higher amino acid sequence identity to a wolf PBV protein sequence. Ribeiro et al. [54] were first to report the presence of PBV -2 in birds which showed high genetic similarity from the isolates obtained from Korea and high diversity was reported with other species of animals (swine, humans, cattle, nonhuman primates). They analyzed 85 samples from chicken faeces, for the presence of PBV using RT-PCR (RdRp) and PAGE followed by sequencing. Out of 85 samples PBV was detected in 10 samples (11.76%) and only 3 samples were positive by PAGE. Seven out of 10 samples were sequenced and analyzed phylogenetically.

Turkey

Day and Zsak [55] developed a diagnostic assay which targets RdRp gene of novel turkey-origin PBV and produces 1135 bp amplicon, on phylogenetic analysis of Turkey PBV, suggested that it is unique because it does not group closely with the recognized PBV circulating in mammalian hosts.

Canine and feline

Costa et al. [23] in 2004 reported the detection of PBV in faecal samples from Rio de Janeiro, Brazil. They collected 163 diarrhoeic faecal samples from dog and 3 samples were positive for PBV by PAGE. Navarro et al. [56] reported molecular characterization of complete genomic segment 2 of PBV strains of cat (K40) and dog (RVC7) on the Caribbean Island of St. Kitts, using non-specific primer-based amplification method and stated that the strains exhibited

a high genetic similarity among themselves and between PBVs from other hosts. PBV strain detected in cats and dogs were characterized molecularly for the complete genomic segment-2 and was reported for the first time.

Small ruminant population

Kunz et al. [57] reported the detection of partial RdRp gene in 100 faecal samples in meat sheep flock from southern Brazil. 62% were found to be positive for PBV by RT- PCR, which showed high genetic variability within the same flock. Malik et al. in 2018 [58] screened 400 faecal samples of small ruminant population (ovine and caprine) in India via RT- PCR assay and found that 143 samples (35.75%) were positive for PBV. Out of 143, 83 belonged to caprine and 60 belonged to ovine. On genogrouping found 38.47% belonged to genogroup I, 3.49% belonged to genogroup II and 38.47% belonged to both genogroup I and genogroup II.

Others

Woo et al. [59] discovered a novel otariine PBV from faecal samples of California sea lions [59]. Yinda et al. [60] in 2018 identified PBV from fruit bats using an alternate mitochondrial genetic code. This was the first report about PBV like sequences in bats. Junior et al. [61] detected the PBV in 1/23 (4.34%) faecal samples from wild birds which belonged to genogroup I of PBV from Brazil. Their findings also reported the circulation of Rotavirus A, Rotavirus D, Rotavirus F, Rotavirus G and PBV suggesting the possible interspecies transmission. Kleyman et al. [62] detected genogroup I PBV in 29 out of 82 (35.3%) non diarrhoeic faecal samples from small Indian mongoose and identified novel RdRp gene sequence that uses alternate mitochondrial code for translation.

Host diversity of PBV

Picobirnaviruses have been detected from prokaryotic and eukaryotic organisms. Moreover, the presence of ribosomal binding site (RBS) sequences in the PBV gene segment, which is generally found in viruses that infect prokaryotes suggest wide host range of PBV.

Picobirnavirus Might be a prokaryotic virus

The Shine-Dalgarno sequence/ Ribosomal binding site sequences which is a conserved hexamer (AGGAGG) or their subsets (4-, 5-, or 6-mer of AGGAGG), has been identified upstream of putative ORF/s in PBV gene segment-1 and -2 sequences, which has a classically defined prokaryotic motif. It has been shown that a number of viruses that infect prokaryotes contain a high proportion of RBS sequences example Cystoviridae having segmented dsRNA genome. Sequences of PBV obtained from animals, humans, and environmental samples have been found to contain the conserved RBS sequence. PBVs showed a higher degree of enrichment for RBS sequences than any other known prokaryotic viral family, suggesting PBVs as prokaryotic viruses. It has been hypothesized that PBVs may have a greater tendency of infecting bacteria having highly conserved RBS sequences for their own genes [14].

To date, PBVs have not been propagated successfully in eukaryotic cell cultures, supporting the hypothesis on prokaryotic hosts. It does not preclude the possibility that PBVs are animal viruses despite the absence of cell culture platform. When attempts were made to grow PBVs in prokaryotic cells by inoculating it into brain heart infusion broth no amplification of PBV through RT-qPCR assays was noticed, even after the culture was grown at both aerobic and anaerobic conditions for 2 weeks. The same study demonstrated the in vivo functionality of RBS-containing segments of PBV segment-1 in *Escherichia coli* using recombinant segments tagged with 6xHis. Additionally, viral RNA has been identified in faeces from several animal species and in persistent faecal shedding by asymptomatic animals, suggesting that PBVs are prokaryotic viruses inhabiting the gut microbiome [28].

Picobirnavirus might be a mitovirus

Mitoviruses generally consist of plus stranded RNA virus-like elements that replicate within the mitochondria of fungus. The viral genome of mitoviruses contains a single long ORF which codes for a protein having conserved motifs of RdRp gene from virus. Analysis of Cameroonian PBV- like sequences revealed mitochondria like genetic code, which was needed to translate the RdRp, absence of PBV like capsid and clustering of these sequences with mitoviruses. The event of these PBV like arrangements without an evident capsid is suggestive to that of mitoviruses [60]. The phylogenetic analysis of viral RdRp sequences from a myriapod, bat and mongoose that makes use of alternate genetic code for translation clustered independently from the PBVs using standard genetic code for translation. Based on all the points discussed PBV's might actually invade the gut microbiome of mammals rather than the cells of mammals themselves [63].

Picobirnavirus is a protozoan virus?

Human faecal samples containing cryptosporidium oocysts also revealed the presence of atypical PBV. They are probably *Cryptosporidia* viruses or PBV that replicate more easily with *Cryptosporidia* present [64].

Evidence of viral persistence

Shedding of PBV in faeces was studied using RNA-PAGE experiments or RT-PCR. Haga et al. in 1999 [65] mentioned that there is a prolonged period of virus shedding in three giant anteaters (*Myrmecophaga tridactyla*) in captivity in Brazilian zoo and claimed that adult animals infected with PBV could be asymptomatic carriers persistently infected, serving as reservoirs of infection. They stated that prolonged virus shedding with chronic infection was the consequence of persistent virus infection and not the result of re-infections in the affected animals. Masachessi et al. [66] in 2007 observed that there was PBV shedding for prolonged periods in animal excreta where Armadillos shed PBV for at least 6 months, and Orangutans shed PBV for 7 months using RNA- PAGE. Martinez et al. [26] in 2010 conducted a follow up study on pigs of different age groups and physiological status. In the early first week after weaning, PAGE and silver staining (PAGE S/S) negative samples were positive by RT-PCR, and two months later PAGE S/S also detected the virus. RT-PCR detected PBV excretion sporadically, followed by almost six months with no virus detection by PAGE S/S, occasionally detected by RT-PCR.

In addition to RT-PCR, PAGE-S/S could also be used to identify virus during 1st gestation and farrowing period which correspond to the virus excretion period. The pattern of PBV excretion was continuous during this period. PBV excretion followed a similar pattern in the third and fourth reproductive cycles, although positive samples were only detected by RT-PCR, suggesting that lower viral loads were shed than during the first cycle. They finally stated that PBV establishes a persistent infection in the host with periods of silence intermingled with periods of low and high viral excretion. Periods of silence may be because of production of some antibody levels. Stress caused by pig farming practices or physiological stress conditions such as lactation and farrowing results in the production of cortisol, which affects the lymphoid cells and reduces the lymphocyte proliferation which in turn decreases the antibody formation. Hence, the resistance to infection is decreased leading to shedding of PBV. This probably suggests the association between excretion levels of virus and immunosuppression or a particular physiological status of the animal. These findings suggest that it is possible to acquire PBV infection early in life and then establish a persistent infection, with periods of high viral activity interspersed with periods of silence.

Pathogenicity

Although some scientists have attempted to associate picobirnavirus with manifestations of gastroenteritis, its pathogenicity is still not well defined since picobirnavirus have been identified in both normal and diarrhoeic animals. Gatti et al. [67] in 1989 stated that PBVs are more frequently found in diarrhoeic animals while Ludert et al. [68] in 1991 stated similar proportions of PBV detection in diarrhoeic and healthy animals.

Zoonotic potential

Banyai et al. [37] (Europe) and Carruyo et al. [27] (Latin America) detected and sequenced genogroup I PBVs from pigs and noticed close relationship to human genogroup I PBVs. Equine strains which were detected followed by sequencing from faeces of domestic foals in Kolkata, India [69] revealed close relationship with human strains [31] and PBV strains of environmental samples from USA [70]. Similarly, the PBVs detected from diarrhoeic children in an urban slum in Kolkata, India showed genetic relatedness to porcine PBV strain reported from Hungary, Venezuela and Argentina. Genetic relatedness between human PBV strains and strains isolated from foxes [36] and also genetic similarity between PBV isolated from human and bats were also reported. Yinda et al. [60] stated that hunting and eating of bats in Cameroon might be the cause of zoonotic transmission of PBVs to human. These findings suggest the mark of zoonotic nature of PBV infections.

Interspecies transmission

Viruses with segmented genomes are potential candidates for segment reassortment, thus explaining the heterogeneity of their genomes [37]. Through evolution, PBVs acquired the capability of interspecies transmission through genetic reassortment which could result to the emergence of virulent progeny [71]. As a result of this reassortment of segments of their genomes, multiple PBVs from different species may simultaneously infect a single cell [72]. Mutation, recombination, genome segment reassortment and combination of these molecular events might lead to viral emergence. Hence, they pose a serious threat to humans and animals.

Conclusion

Picobirnaviruses have been detected in faeces of diarrhoeic and free-living healthy animals, sewage water and broad range of zoo animals and wild birds and not only alimentary tract harbors the PBV but has also been reported from the respiratory tract of wild deer and cattle, suggesting its ubiquitous distribution. The exact role of PBV's in causing gastroenteritis is still not fully understood as we are not able to cultivate/isolate the PBV in any cell culture or any animal model, thus impeding virus isolation and its clinical and pathological studies. There are convincing arguments provided by the various authors suggesting the PBV infecting

prokaryotic cells, but this assumption remains hypothetical unless a host system is identified for PBV propagation. There are findings which suggest a close genetic relatedness between PBVs from different species, which implies the zoonotic aspect of picobirnavirus. Considering the fact, that PBV have segmented genome makes them the potential candidate to undergo genetic reassortment and become potentially more pathogenic, keeping these facts under consideration it is utmost important to have the continuous monitoring of circulating strains from different species and to develop a model for isolation of this virus.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human and animals rights This article does not contain any studies with human participants or animals performed by any of the authors.

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