PICOBIRNAVIRUS, A NOVEL GROUP OF UNDESCRIBED VIRUSES OF MAMMALS AND BIRDS: A MINIREVIEW

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Received January 7, 1997

Summary. – Picobirnavirus, a novel group of viruses recently detected in children and several species of animals including chickens, are different from the existing members of the family *Birnaviridae*. Picobirnavirus (PBV) is the tentatively proposed name for the group of these viruses. These viruses are 30 – 40 nm in diameter and have icosahedral symmetry with triangulation number (T) equal to 3. Their buoyant density in CsCl is 1.4 g/ml. Their genome is bi- or trisegmented double-stranded RNA (dsRNA) with segment lengths of 2.6 and 1.9 kbp for bisegmented and 2.9, 2.4 and 0.9 kbp for trisegmented genomes. The electrophoretic migration profile has considerable heterogeneity. PVBs are detected in diarrhoeic as well as non-diarrhoeic animals, hence, their potential needs further investigation.

Key words: picobirnavirus; *Birnaviridae*

Introduction

Viruses with a bisegmented dsRNA genome infecting vertebrates are classified in the family *Birnaviridae* (Brown, 1986). They consist of virions of 60 to 65 nm in diameter with a skew icosahedral symmetry with T = 13 (Ozel and Gelderblom, 1985). In the last 10 years, in the course of testing faecal samples for rotavirus RNA by polyacrylamide gel electrophoresis (PAGE), two sharply defined distinct bands stainable by ethidium bromide or silver nitrate representing a tentatively designated group of viruses known as PBV were demonstrated in the samples from several species of animals and children. The differential features of the two groups of viruses are presented in the Table.

As there is growing interest in these new viruses, an attempt has been made to review the available information on various aspects of these viruses.

Abbreviations: dsRNA = double-stranded RNA; ELISA = enzyme-linked immunosorbent assay; PAGE = polyacrylamide gel electrophoresis; PBV = picobirnavirus; p.i. = post inoculation; T = triangulation number; UKBRV = UK bovine rotavirus

Distribution and prevalence of PBVs in man and animals

Faecal samples from children (Pereira *et al.*, 1988a), rats (Pereira *et al.*, 1988b), guinea pigs (Pereira *et al.*, 1989), rabbits (Gallimore *et al.*, 1993; Ludert *et al.*, 1995), pigs (Gatti *et al.*, 1989; Chasey, 1990; Ludert *et al.*, 1991), calves (Vanopdenbosch and Wellemans, 1989, 1990; Chandra, 1991), foals (Browning *et al.*, 1991) and chickens (Leite *et al.*, 1990; Monteiro *et al.*, 1991) when tested by PAGE, revealed bisegmented genomes. Pereira *et al.* (1988a) observed 0.45% of selected faecal samples from patients with acute gastroenteritis and 20% of unselected samples positive for PBV electropherotype. The faecal samples collected from 11.6% (Gatti *et al.*, 1989) and 11.1% of pigs (Ludert *et al.*, 1991) also showed a similar electropherotypic pattern. In calves, a higher prevalence rate of 50% was recorded (Vanopdenbosch and Wellemans, 1990). While working on the protection afforded by colostrum feeding in calves against rotavirus infection at the Compton Laboratory, U.K., we have also observed 16 out of 108 faecal extracts from 5 calves positive for PBV electropherotype. An examination of rabbit faecal samples revealed the typical electrophero-

Table. Differentiating properties of birna- and picobirnaviruses

Properties of virus	Birnavirus	Picobirnavirus
Host animals	Fish and birds	Mammals and birds
Pathogenicity	Pathogenic for fish and birds	Pathogenic potential not established
Diameter of virus	60 – 65 nm	30 – 40 nm
Buoyant density in CsCl	1.32 – 1.35 g/ml	1.4 g/ml
Size of genome segments	3.3 and 3.8 kbp	1.9 and 2.6 kbp

type in about 11% of samples (Gallimore *et al.*, 1993; Ludert *et al.*, 1995). A very low prevalence of PBV was also detected in foals (Browning *et al.*, 1991).

Morphology of PBVs

The electron microscopic study of CsCl-purified preparations from faeces of different species of animals showed the presence of particles of uniform morphology (Pereira *et al.*, 1988a,b; Vanopdenbosch and Wellemans, 1989, 1990; Chasey, 1990; Gallimore *et al.*, 1993; Ludert *et al.*, 1995) suggestive of icosahedral symmetry of PBV. The virion is non-enveloped (Pereira, 1991; Ludert *et al.*, 1995) with a diameter of 30 – 35 nm (Pereira *et al.*, 1988a,b; Chasey, 1990; Leite *et al.*, 1990; Pereira, 1991; Ludert *et al.*, 1991, 1995) or 40 nm (Vanopdenbosch and Wellemans, 1989, 1990), T of 3, and buoyant density in CsCl gradients of 1.39 to 1.42 g/ml (Leite *et al.*, 1990; Pereira, 1991; Ludert *et al.*, 1991).

Properties of genome of PBVs

The ethanol-precipitated phenol-chloroform extracts of faeces or intestinal contents were not digested by proteinase K (Pereira *et al.*, 1988a), RNAse T_1 and DNAse RQ_1 but digested completely with pancreatic RNAse A indicating that the two bands in PAGE are dsRNA (Pereira *et al.*, 1988a; Gatti *et al.*, 1989; Ludert *et al.*, 1991; Gallimore *et al.*, 1993).

The two segments of PBV genome have different migration profiles in PAGE and the slow and fast migrating bands in rat faeces correspond approximately to segment 2 and segment 5 of simian rotavirus SA 11 genome respectively, with segment lengths of 2.5 – 2.6 and 1.5 kbp (Pereira *et al.*, 1988a,b). Our observations at the Compton Laboratory, U.K., also revealed that the two bands migrated between segment 4 and segment 5 of group A UK bovine rotavirus (UKBRV) and had segment lengths of 2.4 and 1.9 kbp for the slow and fast moving bands, respectively. Leite *et al.* (1990) also demonstrated the lengths of the segments as 2.6 and 1.9 kbp. However, the migration profile detected by several investigators varried within a narrow range of 2.56 –2.12 and 1.61 – 1.33 kbp (Gatti *et al.*, 1989), 2.4 – 2.6 and 1.7 – 1.9 kbp (Ludert *et al.*, 1991), 2.3 – 2.0 and 1.6 – 1.9 kbp (Gallimore *et al.*, 1993), and 2.4 – 2.5 and 1.6 – 1.7 kbp (Ludert *et al.*, 1995).

In chickens, in addition to bisegmented genomes, trisegmented genomes of 2.9, 2.4 and 0.9 kbp were also observed (Leite *et al.*, 1990). The electrophoretic bands had considerable heterogeneity (Gatti *et al.*, 1989; Ludert *et al.*, 1991; Gallimore *et al.*, 1993). Some of the faecal samples tested by us also showed the presence of mixed infections, as two larger and two smaller, well separated segments were detected.

The preparations purified by CsCl isopycnic centrifugation when recycled, resulted in co-sedimentation of the two bands at densities ranging from 1.38 to 1.40 g/ml (Pereira *et al.*, 1988b). In human, rabbit and porcine faeces, the two bands co-sedimented in CsCl gradients with respective PBVs (Pereira *et al.*, 1988b; Ludert *et al.*, 1991; Gallimore *et al.*, 1993) at a buoyant density of 1.415 g/ml (Ludert *et al.*, 1991) or 1.39 g/ml (Gallimore *et al.*, 1993). The buoyant density of bisegmented or trisegmented genomes of PBV of chicken origin was also 1.4 g/ml (Leite *et al.*, 1990).

Isolation and cultivation of PBVs

Vanopdenbosch and Wellemans (1990) isolated an icosahedral and non-enveloped virus with a diameter of 40 nm from bovine faecal samples for the first time in secondary foetal calf kidney cell culture. The isolated virus also produced cytopathic effects in cell culture. However, similar attempts made by us in secondary foetal calf kidney cell culture as well as by Ludert *et al.* (1995) in rabbit kidney cell culture were unsuccessful.

Pathogenic potential and transmissibility of PBVs

The circulation of a novel virus, PBV, possibly of verterbrates, in man and several species of animals was confirmed (Ludert *et al.*, 1991), however, its pathogenic potential was not established. The agent has been detected in animals with or without diarrhoea (Pereira *et al.*, 1988b; Vanopdenbosch and Wellemans, 1990; Ludert *et al.*, 1991; Browning *et al.*, 1991). Although PBVs were detected more in diarrhoeic than non-diarrhoeic calves (Vanopdenbosch and Wellemans,

1990), a specific relationship of the presence of PBV in faeces to a disease did not appear to be obvious, particularly when other pathogenic organisms were also detected (Chasey, 1990), i.e., corona- and/or rotavirus or *Cryptosporidium* (Vanopdenbosch and Wellemans, 1990). Leite *et al.* (1990) also suggested that the source and pathogenic potential of these viruses need further investigation. However, they do not appear to be bacteriophages or mycoviruses as abortive replication of a guinea pig isolate of PBV was demonstrated in several cell lines of vertebrates (Pereira *et al.* 1989).

In experimental studies, the inoculation of rabbits with human (Gallimore *et al.*, 1993) or rabbit PBV (Gallimore *et al.*, 1993; Ludert *et al.*, 1995) resulted in discrete equimolar PAGE bands typical of PBV being detected in several faecal specimens (Gallimore *et al.*, 1993). Although the genome PAGE profile in comparative studies of inoculated and excreted virus differed in one study, indicating that a passage of PBV had not taken place and that the bands were a coincidental finding (Gallimore *et al.*, 1993), the other experiment resulted in a similar electropherotype in inoculated and excreted virus. The shedding of virus was seen from days 11 to 17 with maximum amount of virus on days 13 and 14 post inoculation (p.i.) but with no signs of diarrhoea or detection of antibodies by direct immune electron microscopy or solid phase immune electron microscopy (Ludert *et al.*, 1995). Further studies are required to confirm the multiplication of PBV in vertebrate hosts. This needs gnotobiotic animals to avoid any further confusion.

Immunological response to PBV infection

In an outbreak of diarrhoea in 50 calves, serological tests such as enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence and seroneutralization revealed 40% of the calves seropositive at the start; 30% had low antibody titers and 30% had high passive antibody titers. All 34 seronegative or low seropositive calves seroconverted mostly during the first month. In a serological survey on seven other fattening farms, most calves seroconverted against this virus during the first two months of life (Vanopdenbosch and Wellemans, 1989). Gallimore *et al.* (1993) also found evidence of an immune response against PBV in rabbits, and in pigs the excretion of virus has been associated with a narrow host range group (Ludert *et al.*, 1991): both observations suggest that the agent is able to induce an immune response in its host. We have also failed to detect the presence of PBV genome in the faeces of calves in the presence of faecal antibodies against rotavirus which is suggestive of the presence of specific antibodies against PBV too in immune colostrum-fed calves. This relationship makes it unlikely that the presence of PBV may have resulted from ingestion without involving replication of virus within the host. A failure to detect an immune response (Grohmann *et al.*, 1993; Ludert *et al.*, 1995) may be attributed to the inability of the technique used to detect the low levels of antibodies induced by what appeared to be a slow course of infection.

Future research directions

(1) The relationship between the viruses detected by Vanopdenbosch and Wellemans (1989, 1990) and other investigators, particularly Pereira and his associates (Pereira, 1991; Pereira *et al.*, 1988a,b; 1989) needs be established.

(2) The pathogenic potential of PBV needs further studies in gnotobiotic animals.

(3) Attempts should also be made to isolate PBV in cell cultures so that further characterization of virus may be feasible.

(4) The PBV may be alloted a place as a separate genus entitled *Picobirnavirus* in the family *Birnaviridae* pending final classification based on complete characterization of the virus.

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