Two instances of large genome profile picobirnavirus occurrence in Argentinian infants with diarrhea over a 26-year period (1977–2002)

Miguel O. Giordano [a], Gisela Masachessi [a,b], Laura C. Martinez [a], Patricia A. Barril [a,c], Leonardo J. Ferreyra [a], María B. Isa [a], Silvia V. Nates [a,*]

[a] Instituto de Virología ''Dr. J.M. Vanella'', Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Enfermera Gordillo Gomez s/n Agencia 4, Ciudad Universitaria, 5000 Córdoba, Argentina
[b] Agencia Córdoba Ciencia, CONICET, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Summary   *Background*: Picobirnavirus' (PBV) association with diarrhea in children is not reliably established and the potential role of pathogenic PBV needs further investigations.
Objective: The aim of this study is to clarify the role of PBV in diarrhea illness in children.
Methods: Between January 1977 and December 2002, 2224 stool specimens were collected from children <3 years old with diarrhea illness. All samples were analyzed by the polyacrylamide gel electrophoresis technique (PAGE) for the presence of bisegmented dsRNA virus genomic pattern. Gels were dried and archived. This study procedure allowed us to keep a laboratory electrophoretic record of each sample assayed. In the present study, all the electrophoretic records were reviewed in order to identify PBV positive samples.
Results: Two out of 2224 (0.09%) stools were positive for large genome profile of PBV. These two positive samples were collected from hospitalized children <1 year old; one of them presenting rotavirus co-infection.
Conclusions: The findings obtained in the present report support strong evidence that large genome profile PBV can be considered more an occasional viral agent rather than an etiological agent associated with diarrheal illness.
© 2008 The British Infection Society. Published by Elsevier Ltd. All rights reserved.

Introduction

Picobirnaviruses (PBV) are unclassified, non-enveloped, small spherical viruses, 35–41 nm in diameter without any apparent surface morphology, showing a characteristic

bisegmented double stranded RNA genome of two electrophoretic types. One is called large electrophoretic genomic profile (range 2.3–2.6 Kbp for segment 1 and 1.5–1.9 for segment 2) and the other one is called small genome profile (1.75 Kbp and 1.5 Kbp for segments 1 and 2 respectively).

Picobirnaviruses were first detected in faecal specimens from humans and rats (*Oryzomys nigripes*) in 1988 by Pereira et al.[1,2] Since then, viruses with similar characteristics have subsequently been detected in faecal samples from domestic and wild animal species[2,3] as well as in stool samples from humans with and without diarrhea.[4] Studies of human PBV in children were conducted in Brazil,[1] Italy,[5] Venezuela,[6] Russia[7] and India.[8,9] Published information on the prevalence of the virus has varied in the different investigations, from a frequency of upto 20% in Brazil[1] to a frequency as low as 0.45% of PBV detection reported in studies in Italy and Venezuela. Data about PBV association with gastroenteritis in children are not conclusive, and the potential role of pathogenic PBV needs further investigations.[5–7] In the same way, prospective evaluations of diarrhea in HIV-infected persons were conducted by different authors[10–12] but until now the role of PBV as a cause of gastroenteritis in the immunocompromised population is not clear. Otherwise, Zhang et al.[13] have recently reported the presence of PBV genome in two stool samples obtained at 6-month intervals, from a healthy individual randomly selected. This might constitute new evidence that PBV can be isolated from asymptomatic individuals. Thus, the probable role of PBV as either a primary diarrheal agent in immunocompetent children,[9] a potential pathogen in immunocompromised individuals[10,11] or an innocuous virus in the intestine cannot be ruled out and needs to be investigated.

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 RT-PCR and the detection of the dsRNA bisegmented genome by PAGE. Based on RT-PCR experiments, which specifically amplified regions of the RNA-dependent RNA polymerase gene of segment 2, PBV strains have been classified into two genogroups. Molecular epidemiological data presented on different reports[8,16,17] show a limited efficacy of the sets of primers available to detect PBV circulating in Argentina, India and US. This suggests that human PBV may present a wide genetic diversity. On the other hand, although the PAGE technique has limited sensitivity, it allows unveiling circulating PBV genotypes independently of PBV genomic sequences. This allows to amplify the PBV range detection compared to that reached by RT-PCR. As a consequence of the limited diagnostic methodology available, PBV is only detected in research laboratories with specific interest in this agent or eventually during rotavirus surveillance by PAGE. Therefore, what is known about the epidemiology of PBV reflects more the ease of laboratory detection than the true epidemiology of infection with the agent itself.

It is well known that in developing countries no etiologic agent is usually identified in approximately 12% of children with diarrhea. Therefore, the potential PBV involved in diarrhea illness is an interesting issue to be defined since PBV is the only detectable enteric pathogen in faecal specimens of watery diarrhea in many of the children with

positive PBV results. To address if PBV could be considered an infrequent but an emerging etiological agent of diarrhea in the childhood population, we conducted a 26-year interval study (1977–2002) of the occurrence of picobirnavirus in hospitalized and ambulatory children with acute gastroenteritis in Córdoba city, Argentina.

Materials and methods

In the present study we took advantage of an unusual situation (ie, the etiologic study of a large hospitalized and ambulatory childhood population with diarrhea during a 26-year period in Córdoba city, Argentina) in which PAGE followed by silver staining (SS) for Group A rotavirus detection was used in tandem and polyacrylamide gels were dried and archived (laboratory gel collection). This study procedure allowed us to keep a laboratory electrophoretic record of each sample assayed. All the electrophoretic records were reviewed in order to identify PBV positive samples.

Geographic localization

Córdoba is the capital city of the inland province of Córdoba. Its population of approximately 1,267,521 inhabitants ranks second to Capital Federal (capital city of Argentina) as the most important capital city in Argentina.

Stool samples analyzed

Between January 1977 and December 2002, 2224 stool samples were collected from hospitalized and ambulatory children <3 years old admitted or requiring medical attention with symptoms of acute gastroenteritis to four children hospitals located in different zones of Córdoba city, Argentina (Town Childhood Hospital, Children Hospital, Pediatric Hospital and Misericordia Hospital) as part of the Annual Collaborative National Survey of Aetiological Agents in Acute Diarrhea in Children and the National Sentinel Hospital Surveillance Program for Rotavirus Diarrhea. During the period 1977–1996, stool collection corresponded to a mixed children population with diarrhea (hospitalized and out patients) and between 1997 and 2002 all samples were collected from hospitalized children.

The patients enrolled in this study fulfilled the following pre-established conditions: they were not referred from other hospital centers, had experienced less than 5 days of diarrhea and stool samples could be collected on the hospital admission or visit day. Diarrhea was defined as the occurrence of three or more unformed (loose or watery) stools within a 24-h period. A single specimen of stool from each child was obtained and stored at −20 °C until tested. All samples were analyzed at the Viral Gastroenteritis Unit, Virology Institute ''Dr. J.M. Vanella'', Córdoba city, Argentina, by the polyacrylamide gel electrophoresis technique for the presence of dsRNA rotaviral genomic pattern.

For polyacrylamide gel electrophoresis 10% stool suspensions were homogenized in 0.02 M Tris–HCl, pH 7.2, and clarified by centrifugation. About 200 µl of supernatant was mixed with an equal volume of extraction buffer

(500 mmol/l ethylenediaminetetraacetic disodium salt—EDTA, and 1% sodium dodecyl sulphate—SDS), and 400 μl of phenol—chloroform 1:1. After incubation for 10 min at 56 °C, the materials were centrifuged at 16,000 × g for 30 min and 200 μl of the aqueous phase was added to 1 ml of ethanol for RNA precipitation (overnight at −20 °C). The pellets obtained after centrifugation (16,000 × g, 30 min) were mixed with 20 μl of sample buffer (62 mmol/l Tris—HCl, pH 6.8, 5% 2-mercaptoethanol, 3% SDS, 0.01% bromophenol blue and 30% glycerol), and loaded on top of 10% polyacrylamide gel as described by Laemmli.[14] Gel running was performed during 24 h at 15 mA. Gels were stained by the silver staining technique according to Herring et al.[15]

After silver staining, each gel was placed between two natural cellophane papers (one attached onto a glass) immersed in a drying solution containing 70% methanol and 1% glycerol. Gels were dried at room temperature for 24—48 h.

Results

Picobirnavirus positive samples were sporadically detected among the childhood population with diarrhea. The PAGE/SS analysis revealed two out of 2224 (0.09%) of the symptomatic children, two distinct segments of large profile pattern characteristic of PBV dsRNA. These samples were designated SC-PBV-78 and DP-PBV-84. Both samples were collected from hospitalized children, one male and one female, aged 2 and 7 months respectively. The SC-PBV-78 positive sample was detected with co-infection of rotavirus. This RNA profile is shown in Fig. 1A (lane 3). The other PBV positive sample (DP-PBV-84) was detected in a negative one for rotavirus and is shown in Fig. 1B (lane 4). No data are available about the presence of other viral, bacterial or parasitic diarrheal etiologic agents in both PBV positive samples.

A comparative frequency study between PBV and rotavirus RNA detection by PAGE analysis among children with acute diarrhea is shown in Table 1. As Table 1 indicates, rotavirus was detected in 18.83% of 2224 fecal specimens (within a range of 6.09—20.17%) from a mixed population (hospitalized and out patients) and within a range of 28.54—37.71% from hospitalized children. In contrast, the overall occurrence of PBV detection was found to be as low as 0.09% (two of 2224 total samples). These data highlight the significantly different frequency of rotavirus and PBV detection in a childhood population with acute diarrheal illness.
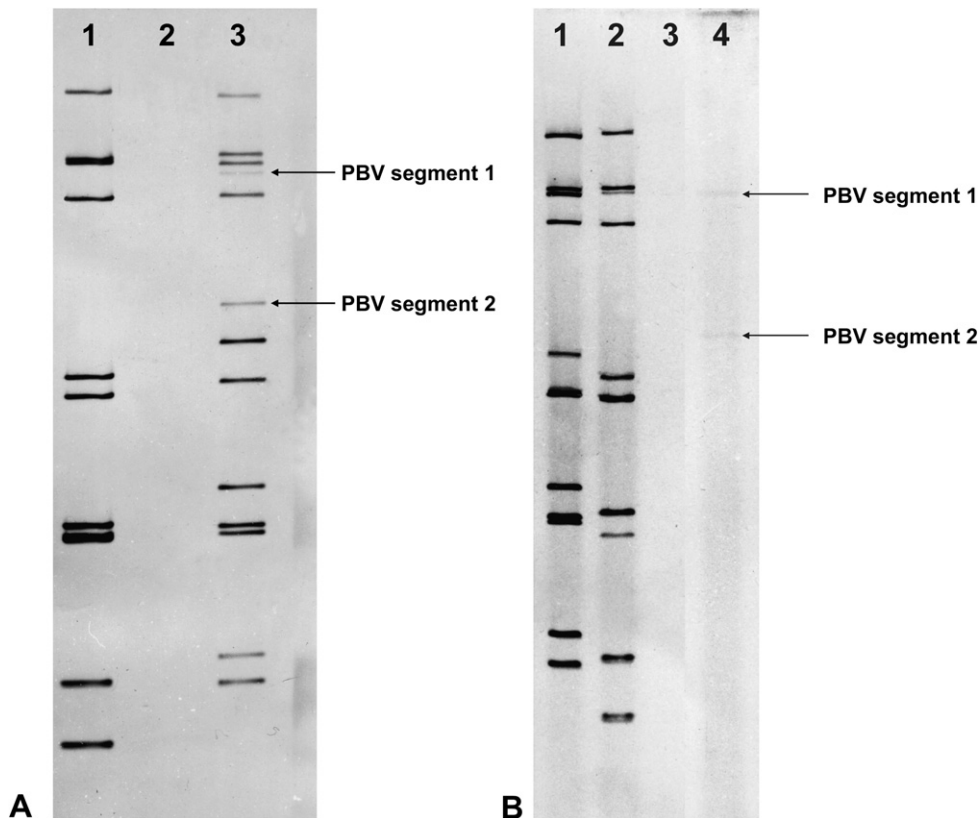
Figure 1 Bisegmented RNA large genome profile of human picobirnavirus as observed by PAGE and silver staining. (A) Lane 1: rotavirus with long electropherotype used to compare PBV genomic segment 1 and 2 migration pattern. Lane 2: negative sample. Lane 3: rotavirus short electropherotype as co-infection in the PBV positive sample (SC-PBV-78). The bisegmented large dsRNA genome profile of PBV is seen below the 3rd and above the 5th segments of the rotavirus genome. (B) Lanes 1 and 2: rotavirus with short and long electropherotypes respectively used to compare PBV genomic segment 1 and 2 migration pattern. Lane 3: negative sample. Lane 4: PBV positive sample (DP-PBV-84). The bisegmented large dsRNA genome profile of PBV is seen very faintly, just at the 3rd and above the 5th segment of the rotavirus genome.

Table 1 Comparative frequency of rotavirus and picobirnavirus positive samples by PAGE technique in children with diarrhea in Cordoba city, Argentina (1977—2002)

Years analyzed	No. of samples tested	Rotavirus positive samples		PBV positive samples	
		n	%	n	%
1977	114	23	20.17	—	—
1978	228	45	19.73	1	0.43
1979	174	26	14.94	—	—
1980	141	14	9.92	—	—
1981	71	8	11.26	—	—
1982	39	4	10.25	—	—
1983	63	11	17.46	—	—
1984	169	28	16.56	1	0.59
1985	62	7	11.29	—	—
1986	123	13	10.56	—	—
1987	117	10	8.54	—	—
1988	82	5	6.09	—	—
1989	103	11	10.67	—	—
1990	45	8	17.77	—	—
1992	32	4	12.50	—	—
1993	127	15	11.81	—	—
1996	21	4	19.04	—	—
1997	114	43	37.71	—	—
1998	106	36	33.96	—	—
1999	14	4	28.57	—	—
2000	124	45	36.29	—	—
2001	72	24	33.33	—	—
2002	83	31	37.34	—	—
Total	2224	419	18.83	2	0.09

Discussion

Our PBV-research was based on the analysis of specimens obtained from children during their first 3 years of life, when they usually undergo most of the primary gastrointestinal infections. Thereby, we expanded the possibility to detect the primary PBV infection event. This target population involved a large diarrheal stool collection during a long-term period of study. In this context, rotavirus was commonly found in fecal specimens from children with acute gastroenteritis in Cordoba city, Argentina. The wide range of rotavirus recorded (ranging from 6.09% to 37.34%) could be ascribed to the different sources of sample collection, ie, hospitalized and out patient children (from 1977 to 1996) and only from hospitalized ones (from 1997 to 2002). In contrast, PBV large genome profile positive samples were sporadically detected (years 1978 and 1984) at a notably low rate (0.43% and 0.59% respectively) among children with acute watery diarrhea. This evidence suggests a lack of etiological relation of large profile PBV with the disease.

Therefore, large profile PBV might not have an etiological relation with diarrhea, but instead many hosts' contributing factors could establish the necessary conditions for viral excretion. Supporting this issue, previous studies of human PBV showed higher PBV detection rates mostly among malnourished children,[8] HIV-infected individuals with immunosuppressive conditions,[10—12,16] and kidney transplanted patients.[18] The results in these studies suggest that an immunosuppressant condition in the host might play a role in establishing PBV excretion. In addition, large genomic PBV strains were also detected along with human norovirus during two outbreaks of gastroenteritis at long-term care facilities for the elderly.[17] These authors reported PBV as a second opportunistic pathogen or an innocuous virus in the intestine. Perhaps in these cases PBV excretion was facilitated by physiological immunosuppressive conditions of old people and as a consequence of high viral replicative cycles in the gut, the virus could assume if any a role of a second opportunistic pathogen. Overall, PBV excretion could not be linked to any pathological situation.

Recently, Bhattacharya et al.[9] reported the co-circulation, in India, of human PBV with small and large genome profiles. These authors suggest that small PBV genome profiles were found to be associated with diarrhea illness. To date the circulation of this PBV electropherotype has been detected only in India. The pathogenic potential of emerging PBV-small genome variants requires a stringent surveillance.

Taking into account the results herein reported and the above published evidences, it could be established that PBV showing large genome profile can be considered more an occasional viral agent in stool samples than an etiological agent associated to diarrheal illness in the childhood population.

References

1. Pereira HG, Fialho AM, Flewett TH, Teixeira JMS, Andrade ZP. Novel viruses in human faeces. *Lancet* 1988;**2**:103—4.
2. Pereira HG, Flewett TH, Candeias JA, Barth OM. A virus with a bisegmented double stranded RNA-genome in rat (*Oryzomys nigripes*) intestines. *J Gen Virol* 1988;**69**:49—54.
3. Haga IR, Martins SS, Hosomi ST, Vicentini F, Tanaka H, Gatti MS. Identification of a bisegmented double stranded RNA virus (picobirnavirus) in faeces of giant anteaters (*Myrmecophaga tridactyla*). *Vet J* 1999;**158**:234—6.
4. Gallimore CI, Appleton D, Green LJ, Brown DWG. Detection and characterization of bisegmented double-stranded RNA viruses (picobirnavirus) in human fecal specimens. *J Med Virol* 1995;**45**:135—40.
5. Cascio A, Bosco M, Vizzi E, Giamanco A, Ferraro D, Arista D. Identification of picobirnavirus from faeces of Italian children suffering from acute diarrhea. *Eur J Epidemiol* 1996;**12**:545—7.
6. Ludert JE, Liprandi F. Identification of viruses with bi- and trisegmented double-stranded RNA genome in faeces of children with gastroenteritis. *Res Virol* 1993;**144**:219—24.
7. Novikova NA, Epifanova NV, Fedorova OF, Golitsyna LN, Kuprilianova NV. Detection of picobirnaviruses by electrophoresis of RNA in polyacrylamide gel. *Vopr Virusol* 2003;**48**(6):41—3.
8. Bhattacharya R, Sahoo GC, Nayak MK, Sur D, Nik TN, Bhattacharya SK, et al. Molecular epidemiology of human picobirnaviruses among children of a slum community in Kolkata, India. *Infect Genet Evol* 2006;**6**:453—8.
9. Bhattacharya R, Sahoo GC, Nayak MK, Rajendran K, Dutta P, Mitra U, et al. Detection of genogroup I and II human picobirnavirus showing small genomic RNA profile causing acute watery diarrhoea among children in Kolkata, India. *Infect Genet Evol* 2007;**2**:229—38.

10. Grohmann GS, Glass RI, Pereira HG, Monroe SS, Hightower AW, Weber R, et al. Enteric viruses and diarrhea in HIV-infected patients. *N Engl J Med* 1993;**329**:14—20.

11. Giordano MO, Martinez LC, Rinaldi D, Güinard S, Naretto E, Casero R, et al. Diarrhea and enteric emerging viruses in HIV-infected patients. *AIDS Res Hum Retroviruses* 1999;**15**:1427—32.

12. Gonzalez GG, Pujol FH, Liprandi F, Deibis L, Ludert JE. Prevalence of enteric viruses in human immunodeficiency virus seropositive patients in Venezuela. *J Med Virol* 1998;**55**:288—92.

13. Zhang T, Breitbart M, Lee WH, Run JQ, Wei CL, Soh SW, et al. RNA viral community in human feces: prevalence of plant pathogenic viruses. *Plos Biol* 2006;**4**(1):108—18.

14. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;**227**:680—5.

15. Herring AJ, Inglis NF, Ojeh CK, Snodgrass DR, Menzies JD. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver stained polyacrylamide gels. *J Clin Microbiol* 1982;**16**:473—7.

16. Martinez LC, Giordano MO, Isa MB, Alvarado LF, Paván JV, Rinaldi D, et al. Molecular diversity of partial-length genomic segment 2 of human picobirnavirus. *Intervirology* 2003;**46**:207—13.

17. Rosen BI, Fang ZY, Glass RI, Monroe SS. Cloning of human picobirnavirus genomic segments and development of an RT-PCR detection assay. *Virology* 2000;**277**:316—29.

18. Valle MC, Martinez LC, Ferreyra LJ, Giordano MO, Isa MB, Paván JV, et al. Viral agents related to diarrheic syndrome in kidney-transplanted patients. *Medicina* 2001;**61**:179—82.