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Evidence of closely related picobirnavirus strains circulating in humans and pigs in Argentina

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Summary *Background:* On the basis of the published literature, it is still difficult to draw conclusions as to whether picobirnavirus (PBV) circulation is influenced by host species restriction.
Objective: To provide data regarding the genetic relatedness between porcine and human PBV strains present in Argentina as a means of defining the host range and epidemiology of these viruses.
Methods: Fecal specimens ($n = 74$) collected from kidney transplant patients ($n = 55$) and piglets ($n = 19$) were analyzed by RT-PCR using primers designed to amplify the porcine PBV genomic segment 2. Amplified sequences were further examined phylogenetically.
Results: By RT-PCR amplification 14 of 74 samples rendered amplicons of the expected 282 base pair size (8 detected from humans and 6 from pigs). Eleven amplicons (5 from humans and 6 from pigs) were selected for sequencing and subjected to phylogenetic analysis. The eleven amplicons revealed similarities between human and porcine viral sequences that ranged between 94.7 and 100% in identity. Phylogenetic analysis identified these 11 strains as PBV genogroup I-related strains and showed that they grouped as a single separate clade distinct from other PBV strains detected in humans and porcine from other countries.
Conclusions: The present study suggests that closely related PBV strains infect both pigs and humans in Argentina and that the epidemiology of PBVs is not species restricted.

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Introduction

Picobirnaviruses (PBVs) were recently classified as members of a new virus family named Picobirnaviridae by the International Committee of Taxonomy on Viruses. These viruses are small, non-enveloped viruses with a bisegmented double-stranded RNA (dsRNA) genome.The large genome segment is 2.2—2.7 kbp (kilo base pairs) long and encodes the capsid protein, while the small genome segment is 1.2—1.9 kbp long and encodes for the viral RNA-dependent RNA polymerase (RdRp).[1] PBVs were initially discovered in humans by the finding of the two segments of dsRNA by PAGE, extracted from a small number of fecal specimens during investigation of gastroenteritis in children in 1988.[2] Since then, viruses with similar characteristics have been detected in fecal samples from domestic and wild animal species[3—6] as well as in stool samples from humans with and without diarrhea.[7—17] At present, PBV excretion is not definitely linked to any pathological situation. Moreover, many studies conducted in children suggested that PBV might not be etiologically linked to episodes of diarrhea.[9—11,17] However, studies conducted in humans infected with the human immunodeficiency virus (HIV) suggested that PBV may be an opportunistic pathogen that might cause diarrhea in immunosuppressed patients.[14,15] Thus, the possibility that PBVs are secondary opportunistic pathogens or innocuous viruses in the intestine cannot be excluded.

At present the epidemiology of PBVs has not been well defined. The limited genomic sequence data of virus strains identified from fecal samples from different animal species has not enable investigators to establish whether PBV circulation is influenced by host species restriction.

The aim of the present study was to obtain information regarding the genetic relatedness between Argentinean porcine and human PBV strains as a means of defining the host range and epidemiology of these viruses.

Materials and methods

Porcine sample collection

Porcine fecal samples were collected from piglets ages 60—150 days old housed at a breeding farm situated on the outskirts of Cordoba City, Argentina between November 2003—August 2006, as part of a collaborative study aimed at investigating the reactivity of primers developed by Carruyo et al. for the detection of porcine PBV.[18] Fecal specimens from 64 healthy piglets were initially examined by polyacrylamide gel electrophoresis (PAGE) for PBVs detection. This analysis identified 19/64 (29.6%) PBV-positive samples. These PBV-positive were amplified by RT-PCR and the resulting amplicons sequenced and used for phylogenetic analysis.

Human sample collection

A previous study was conducted in our laboratory as part of a collaborative effort aimed at investigating diarrheic syndrome related to viral agents in kidney transplant individuals. PAGE analysis of nucleic acids extracted from fecal samples identified some PBV-positive samples (2.3%).[16] Since PAGE analysis likely underestimated the frequency of PBV present in the samples examined, the samples collected from these patients were re analyzed by RT-PCR. A total of 55 stool samples, obtained between December 1998 and August 2001 were collected from ambulatory and hospitalized kidney transplant patients (with or without diarrhea post transplantation), treated at the Private Hospital of Cordoba, Argentina. Samples were analyzed by RT-PCR for the presence of PBV and amplicons obtained were sequenced and subjected to phylogenetic analysis. A single stool specimen from each patient was obtained and stored at −70 °C until tested. This study was submitted and approved by the internal ethics review board of the Private Hospital of Cordoba.

Detection of PBV by RT-PCR amplification

Nucleic acid extraction

Nucleic acids were directly extracted from 10% stool suspensions in 0.02 M Tris—HCl, pH 7.2, and clarified by centrifugation at 2000 × g for 10 min. Nucleic acid were extracted as described by Perry et al..[19] Briefly, approximately 400 μl of the supernatant was mixed with an equal volume of extraction buffer (10 mM EDTA disodium salt, 500 mM LiCl and 1% SDS) and 800 μl phenol—chloroform (1:1). After incubation for 10 min at 56 °C, samples were centrifuged at 16,000 × g for 30 min, and 800 μl of the 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 dried under laminar flow for approximately 1 h. After drying, the pellets were resuspended in 20 μl RNase-free water.

cDNA synthesis and PCR amplification

RT-PCR was carried out using the primer pair PBV2-19/PBV2-281 directed towards a conserved domain of the porcine RdRp region associated with a Venezuelan PBV strain, as previously described by Carruyo et al.,[18] with minor modifications. Reverse transcription was carried out using random primers.[20] A mixture that consisted of 0.5 μl random hexamers (1 μg/μl), 1 μl 10 mM dNTPs, and 5.5 μl RNAse-free water was added to 5 μl of extracted RNA, which had previously been heated at 97 °C for 5 min to denature dsRNA, and yielded a total volume of 12 μl. The mixture was incubated for 5 min at 65 °C and chilled on ice for 2 min. Seven microlitres of a mixture consisting of 4 μl of 5X first-strand buffer (250 mM Tris—HCl, pH 8.3, 375 mM KCl, 15 mM MgCl2), 2 μl 0.1 M DTT, and 1 μl RNAsin® Ribonuclease Inhibitor (10 U/μl), was incubated at 37 °C for 2 min. Finally, 1 μl of M-MLV (Moloney Murine Leukemia Virus) reverse transcriptase (200 U/μl) was added,resulting in a total volume of 20 μl. Reverse transcription was carried out at 25 °C for 10 min, followed by incubation at 37 °C for 50 min. The reaction was terminated by incubated the samples at 70 °C for 15 min. PCR amplification was carried out in a 25 μl reaction volume that contained 2.5 μl 10X PCR buffer, 1 μl 50 mM $MgCl_2$, 1 μl 10 mM dNTPs, 1.25 μl of