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Article

Detection of human parechovirus A in respiratory, gastrointestinal, and neurological clinical samples of hospitalized patients in Panama

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Abstract:
Human parechoviruses, officially known as Parechovirus A (PeV-A), from the family *Picornaviridae*, genus Parechovirus, are non-enveloped, icosahedral, positive-sense RNA viruses associated with mild gastrointestinal and respiratory illness in young children, however, they may also give rise to Central Nervous System (CNS) infections and neonatal sepsis. While studies have delved into the detection of HPeVs in different populations, the detection of PeV-A in Hispanic populations in Latin American countries is not well-known.
The aim of this study was to characterize PeV-A circulating in the Republic of Panama.
Respiratory, gastrointestinal and neurological samples were collected from 200 Panamanian pediatric patients hospitalized between 2014 and 2015 and were analyzed for the presence of PeV-A by real-time RT-PCR. PeV-A positive samples were sequenced for genetic characterization. These samples followed predetermined inclusion criteria and were negative for viral/bacterial examinations that were requested by the patient's physician when the specimen was sent to the ICGES.
Eight positive PeV-A infections (4%) were detected for the 200 subjects, in gastrointestinal samples.
Human Parechovirus PeV-A was detected and genetically characterized for the first time in the Republic of Panama in samples from 2014 and 2015.

Keywords: human parechovirus; HPeV; PeV-A; Panama; gastrointestinal infection.

1. Introduction

Parechovirus is part of the picornovirus family. They are small, single-stranded, positive-sense RNA viruses, non-enveloped and enclosed in an icosahedral capsid. [*1*] The first strains were identified in 1956 [*2*]. Parechovirus has been divided into eight species, of which Parechovirus A (formerly named Human parechovirus HPeV) is the only one infecting humans. The others are Parechovirus B (formerly named Ljungan virus) [*3*], Parechovirus C (Sebokele virus) [*4*], Parechovirus D (ferret parechovirus) [*5, 6*], Parechovirus E (falcon parechovirus) [*7*], Parechovirus F (gecko parechovirus) [*8*], Manhatten parechovirus [*9*] and Bovine parechovirus [*10*].

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PeV-A has a genome slightly over 7,000 nucleotide bases which encodes for structural proteins, non-structural enzymatic genes, and conservative untranslated regions [*11*]. The genotypification requires the use of the highly variable genetic region that encodes for structural proteins, VP0, VP1 and VP3 [*12*]. To date, Parechovirus A (PeV-A) is subdivided into nineteen genotypes, PeV-A 1 to 19, this classification is based on sequence analysis of VP1.

Clinical manifestation from PeV-A can range from asymtptomatic to severe disease. These viruses can cause gastro-intestinal or respiratory diseases in young children and the transmission mechanisms are associated via fecal–oral route or respiratory route [*1,13*]. In infants, PeV-A can cause upper respiratory lesions and gastrointestinal symptoms that could be complicated or have long-term neurodevelopmental sequelae. There is also the risk that patients may suffer from encephalitis, meningitis, myocarditis, and sepsis [*14,15,16,17*]. Furthermore, the disease depends on the genotype and the age of the patients, severity being higher in young infants and complications being generally associated to PeV-A3.

PeV-A infections are common throughout the world and have caused high-impact epidemics such as the one in Australia between 2017-2018 [*18*]. Although several studies have been published on the epidemiology of PeV-A in Europe, Asia and North America, information is lacking from other regions such as Latin America [*19*]. In South America, PeV-A has been described in Chile, Argentina and Ecuador from retrospective studies [*20*], clinical reports [*14*] and analysis from urban streams [*30*]. In Central America and Panama, PeV-A has not been described yet as a pathogenic agent for acute gastrointestinal, respiratory, or neurological infections. Between 2013 and 2014, unknown viral infections were reported in febrile neonates with clinical sepsis at Hospital del Niño Dr. José Renán Esquivel in Panama city, the author suggested that the cases were related to HPeV-A [*21*]. However, this clinical suspision was not confirmed by laboratory diagnosis through polymerase chain reaction (PCR) molecular detection test, which was not available in hospitals in Panama at that moment. This study evaluates the prevalence and presence of PeV-A in hospitalized patients with acute infections with clinical presentations previously associated to this virus.

2 Materials and methods

The study was conducted at the Gorgas Memorial Institute for Health Studies (ICGES) (Panama City, Panama). Nasopharyngeal swab, cerebrospinal fluid (CSF), stool, serum, eye swab and anal swab samples were collected from hospitalized pediatric patients under 16 years between January 2014 and December 2015. Acute hospitalized patients between the ages of 0 and 16 were evaluated from 18 hospitals and children's clinics throughout Panama.

The investigation aimed at PeV-A mono-infections associated with gastroenteritis, respiratory and/or neurologic symptoms during infancy, childhood and adolescence. Therefore, only samples from hospitalized pediatric patients with the following characteristics were included evaluated for nine viruses with a negative diagnostic test for all of them: enterovirus, cytomegalovirus (CMV), varicella-zoster virus (VZV), herpes simplex virus (HSV), Rotavirus, Echovirus, Poliovirus, Coxackievirus,

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Adenovirus, Influenza A, Influenza B, Human Metapneumovirus, Parainfluenza 1, 2 and 3, Rhinovirus and Respiratory Syncytial Virus. A total of 230 samples, 116 from 2014 and 114 from 2015, were collected from 200 patients who met the inclusion criteria.

Extraction RNA was performed according to the manufacturer's recommendations using QIAamp® Viral Mini Handbook (QIAGEN). For reverse transcription, PeV-A - specific single-target assay was done following previously validated protocols [22] using the AgPath-ID ™ One-Step RT-PCR Reagents (Applied Biosystems Life Technologies) with the Applied Biosystems® 7500 Fast Real-Time PCR System.

For sequencing, the primers designed by [22] were used. Positive amplicons were purified with the QIAquick® PCR Purification Kit and sequenced. The sequencing PCR was performed with an Applied Biosystem® 9600 thermocycler. The amplicons were purified according to the BigDye XTerminator Purification Kit protocol prior to genetic sequencing in the sequencing machine, Applied Biosystems® 3130x/Genetic Analyzer. The nucleotide sequences obtained from the samples were compared with other genomes available at the NCBI GeneBank data base to observe the percent identities.

3 Results

The clinical 200 samples were divided into three groups: 68 neurological samples (34%) (CSF, serum, eye swab, anal swab), 68 gastrointestinal (feces) samples (34%) and 64 respiratory (nasopharyngeal swab) samples (32%). Eight (8) positive HPeV-A infections (4%) were detected from the 200 samples (Table 1). From these, only two were identified with a similarity greater than 90% compared to the strain 2040 / N. Nov / RU / 2008 5 'UTR reported in GenBank accession number JQ437883.1, collected in 2008 in Russia from stoll from a child with acute enteric infection. The other PeV-A sequences identified having a lesser identity with this PeV-A strain, however all PeV-A sequences from Panama were classified as PeV-A1.

The analyzed samples came from all over the country; however, the largest number of cases came from the Hospital del Niño Dr. Jose Renan Esquivel, in Panama city, (64%), which is the main tertiary pediatric public hospital and serves child patients throughout the country (Table 1). In addition, just over 50% of the samples came from children living in the metropolitan area of the province of Panama (Figure 1). We don't find a difference in the proportion of girls than boys that were infected with HPeV-A.

The three PeV-A confirmed respiratory cases, had were rhinorrhea and, while the 4 confirmed cases with gastrointestinal diseases had vomiting and diarrhea, whereas in the only neurological case had symptoms similarly to HSV. Other clinical symptoms were abdominal pain (50%) and to a lesser extent, fever and respiratory distress. The most frequent final clinical diagnosis was Pneumonia (25%) and only one reported case of Bronchopneumonia.

A total of 119 patients (54.9%) were less than two years old. The median age was 5 months, the age range being from one newborn from one day to one year of birth. There was a higher incidence in 2015 (7 patients) and most PeV-A1 infections occurred between the end of the rainy season and the beginning of the dry season, with a predominance in December (2 patients) and January (1 patient); however, two of the reported cases were identified between May and June 2015. In all reported cases, the

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clinical diagnosis was usually confused with Rotavirus (50%) and to a lesser extent with Respiratory Syncytial Virus, Enterovirus and Herpes Virus.

3.1. Figures, Tables and Schemes

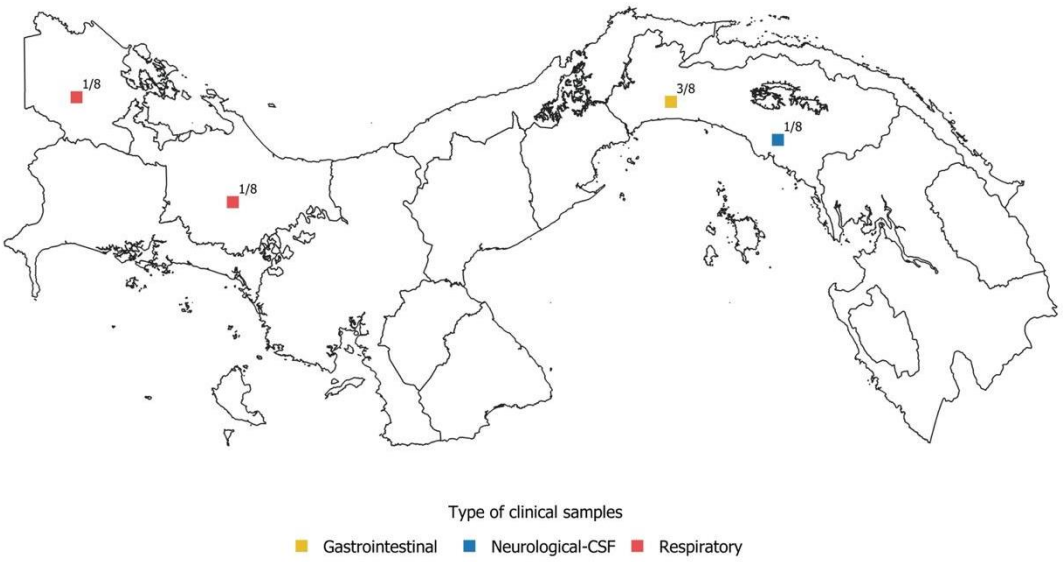

Figure 1. Distribution of PeV-A positive samples in the Republic of Panama according to the type of clinical samples analyzed. Two positive samples had no location information.

Table 1. Characteristics of PeV-A positive hospitalized pediatric cases in Panama, 2014–2015.

year	age	sex	type of sample	symptom	clinical diagnosis	observation
2014	7 m	M	Feces	diarrhea, vomiting, abdominal pain	Rotavirus	Negative for rotavirus
2015	1 day	F	CSF	suspected encephalitis	Herpes Virus 1 and 2	Mother with suspected Herpes Virus Encephalitis; Negative
2015	2 m	M	NP	cough, runny nose, shortness of breath	Respiratory syncytial virus	Negative for: Adenovirus, Influenza A, Influenza B, Human Metapneumovirus, Parainfluenza 1,2,3, Rhinovirus, Respiratory Syncytial Virus
2015	1 m	F	OP, A, E	pneumonia, vomiting, diarrhea	Enterovirus	Negative for enterovirus (echovirus, poliovirus, coxsackievirus
2015	12 m	M	NP	Fever, runny nose, cough, bronchopneumonia	Unknown	Negative for: Adenovirus, Influenza A, Influenza B, Human Metapneumovirus, Parainfluenza 1,2,3, Rhinovirus, Respiratory Syncytial Virus

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2015	48 m	F	Feces	diarrhea, vomiting, abdominal pain	Rotavirus	Negative for rotavirus
2015	UNK	UNK	Feces	diarrhea, vomiting, abdominal pain	Rotavirus	Negative for rotavirus
2015	UNK	UNK	Feces	diarrhea, vomiting, abdominal pain	Rotavirus	Negative for rotavirus

Abbreviations: m, months; F, feminine; M, masculine; CSF, Cerebrospinal fluid; NP, nasopharyngeal swab; OP, oropharyngeal swab; E, eye swab; A, anal swab; UNK, Unknown

4 Discussion

The presence of PeV-A had not been confirmed in Panama despite strong suspicions after outbreaks of viral infections in febrile neonates with clinical sepsis at Hospital del Niño between 2013 and 2014 [*21*]. Therefore, our findings show that PeV-A is present in Panama and that it can cause sepsis and gastroninteestinal, respiratory and neurological acute infections. In addition, Panama with a 4% prevalence rate of PeV-A, is in the previously reported ranges for PeV-A in children with diarrhea, that ranged from 2% to 16.3% [*13*,*23*].

The PeV-A prevalence of 4% was similar to a Nigerian study of children with a similar age range [*24*], but lower than what has been reported in studies carried out in Chile, Spain and Malawi [*19*,*20*,*29*]. Although a higher prevalence of PeV-A was obtained from stool samples, the clinical symptoms of the acute infection could not be directly related to PeV-A, since it has been shown that the duration of the virus in stool samples can last several months [*25*].

Infections were more common in infants under one year of age, regardless of sex, and these had more severe symptoms in infections that could be related to the symptoms reported for PeV-A and are similar to the results obtained in Missouri in 2019 [*26*]. Only a 1-day-old female patient with CSF could be associated with PeV-A3 and that he had some symptoms previously reported by [*27*].

In the current study, we have described a first case of CNS infection in newborns in Panama Confirmation of genotyping, including follow-up for long-term effects, will be required. Although detection and typing is important to ensure better medical care, general epidemiological surveillance and thus also reduce the use of antibiotics. Unfortunately, we do not have the capacity for detection and genotyping in hospitals in the country. Despite having the diagnostic capacity at ICGES,the small number of clinical suspected samples related in the majority to hospitalized patients and not to outpatients, could introduce a biais in the epidemiological surveillance.

In our study, PeV-A infections were more common in infants under one year of age, regardless of sex. The detection rate in young children under 2 years of age was 2.5% for the two years we analyzed, in contrast to results higher than 10% obtained in Germany [*13*]. Our monoinfection study model did not include an evaluation of coinfection, and this should be evaluated in future studies, due to the high rates of coinfections reported in Japan, where 59.2% of PeV-A positive samples were coinfected with other enteric viruses [*28*]. Our results do not mean that there is no coinfection in the country. Another

caveat of our study is that the correlation of PeV-A detection, especially in gastrointestinal stool samples, with the causality of the patients' symptoms was not analyzed.

Our results ~~also~~ demonstrated that PeV-A can be detected in acute samples of hospitalized patients that had an erroneous clinical diagnosis, showing the difficulties to do a differential diagnosis when there are so much similarities in the symptoms associated with other viruses such as Herpes Virus, Enterovirus, RSV, and rotavirus. Thus, your results show the importance to strengthen the surveillance of enteric viruses in children, and this is reinforced by the possible relationships between neurological symptoms and the long-term effects of some PeV-A genotypes.

Author Contributions:

Conceptualization: LA, NS, BM

Methodology: LA, VS, LG

Validation: LA, ZCB, JMP

Formal analysis: EF

Investigation: LA, SLV, NS

Resources: DF, MC, BM

writing—original draft preparation: EF, ZCB

writing—review and editing: LA, SLV, ZCB

supervision: LA, NS, JMP

project administration: LA

funding acquisition: LA, NS

All authors have read and agreed to the published version of the manuscript."

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Conflicts of Interest: Authors have disclosed any conflicts of interest related to this article.

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