 doi:10.20944/preprints202203.0187.v2

Detection of human Parechovirus A in respiratory, gastrointestinal, and neurological clinical samples of pediatric patients from Panama

Lizette Gutiérrez [1,2°], Viridiana Sáenz [1,2°], Danilo Franco [2], Marlene Castillo [2], Brechla Moreno [2], Ediner Fuentes-Campos [2], Zeuz Capitan-Barrios [3], Juan Miguel Pascale [2], Sandra López-Vergès [2], Néstor Sosa [2,4] and Leyda Ábrego [2,3*]

[1] The University of Texas at El Paso (UTEP), El Paso, TX 79968, US

[2] Gorgas Memorial Institute for Health Studies, Department of Research on Virology and Biotechnology, Panama

[3] University of Panama, Faculty of Exact Natural Sciences and Technology, Department of Microbiology and Parasitology, Panama

[4] University of New Mexico Hospital, Division of Infectious Diseases, New Mexico, US.

° L.G. and V.S. contributed equally to this article.

* Correspondence: labrego@gorgas.gob.pa; +5075274815

Abstract:

Human Parechoviruses, officially known as Parechovirus A (PeV-A), is 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 PeV-A 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 determine the presence of PeV-A in respiratory, gastrointestinal, and neurological clinical samples of pediatric patients in Panama. Two hundred samples of pediatric patients with a negative diagnosis for the main

 doi:10.20944/preprints202203.0187.v2

respiratory viruses, rotavirus and neurological viruses such as herpesvirus, enterovirus and cytomegalovirus, collected between 2014 and 2015, were analyzed by real-time RT-PCR.

Eight positive PeV-A infections were detected, 2 in respiratory samples, 5 in stool samples and one detected in cerebrospinal fluid. This is the fisrt report of PeV-A in Panamá.

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

1. Introduction

Parechoviruses are 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).

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.

 doi:10.20944/preprints202203.0187.v2

Clinical manifestation from PeV-A can range from asymtptomatic to severe disease. These viruses can cause gastrointestinal 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 (14–17). 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 (21). 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, and the author suggested that the cases were related to HPeV-A (22). 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 presence of PeV-A in

 doi:10.20944/preprints202203.0187.v2

samples from 2014 and 2015 from pediatric patients with acute infections with clinical presentations previously associated to other viruses.

2 Materials and methods

The study was conducted at the Gorgas Memorial Institute for Health Studies (ICGES) (Panama City, Panama). The study protocol was submitted and approved by the Gorgas Memorial Institute Bioethics Committee (no. 981/CBI/ICGES/16). Nasopharyngeal swab, cerebrospinal fluid (CSF), feces, serum, ocular and anal swab samples were collected from pediatric patients under 16 years old analyzed. This samples were sent to the Institute to be analyzed for viral suspicion between January 2014 and December 2015 and corresponded to pediatric patients under 16 years of age. In total, 200 samples from different regions of the country were analyzed. 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 pediatric patients with a negative diagnostic test for these nine viruses: enterovirus, cytomegalovirus (CMV), varicella-zoster virus (VZV), herpes simplex virus (HSV), Rotavirus, Enterovirus, Adenovirus, Influenza A, Influenza B, Human Metapneumovirus, Parainfluenza 1, 2 and 3, Rhinovirus and Respiratory Syncytial Virus were included in this study. A total of 200 samples were collected and analized. RNA extraction 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 (23) using the AgPath-ID ™ One-

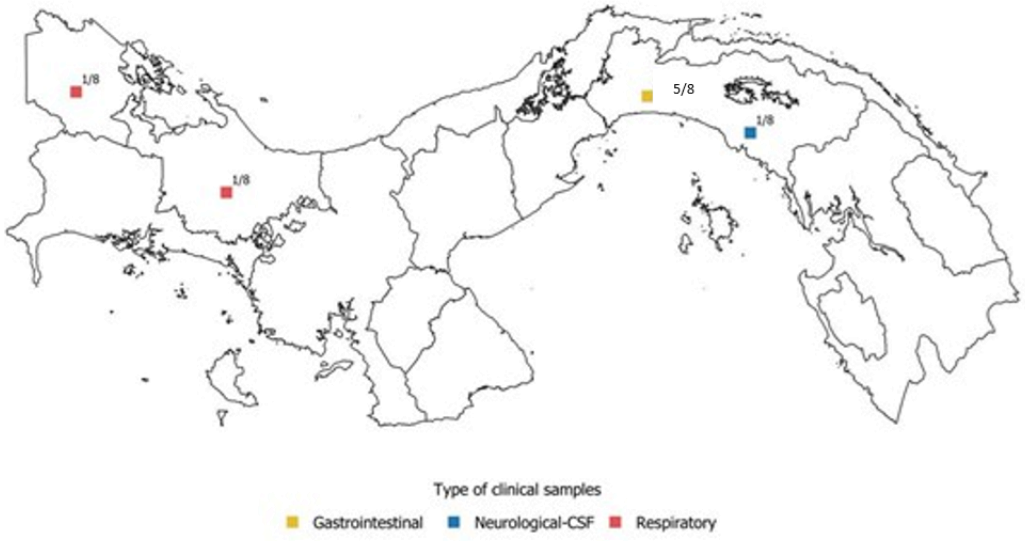
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 April 2022 doi:10.20944/preprints202203.0187.v2

Step RT-PCR Reagents (Applied Biosystems Life Technologies) with the Applied Biosystems® 7500 Fast Real-Time PCR System.

3 Results

The 200 clinical samples corresponded to three type of samples: 68 neurological (CSF, serum, eye swab, anal swab) samples (34%), 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)

The analyzed samples came from all over the country. 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). However, just over 50% of the samples came from children living in the metropolitan area of the province of Panama (Figure 1). There no significant difference in the proportion of girls and boys that were infected with HPeV-A.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 April 2022 doi:10.20944/preprints202203.0187.v2

Figure 1. Geographical distribution of PeV-A positive samples collected between 2014-2015in the Republic of Panama according to the type of clinical samples analyzed (yellow: gastrointestinal, blue: neurological CSF, red: respiratory). Two positive samples had no location information.

Table 1. Characteristics of PeV-A positive 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
2015	12 m	M	NP	Fever, runny nose, cough, bronchopneumonia	Unknown	Negative for Adenovirus, Influenza A, Influenza B, Human Metapneumovirus, Parainfluenza

 doi:10.20944/preprints202203.0187.v2

						1,2,3, Rhinovirus, Respiratory Syncytial Virus
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, Un-known

The two PeV-A confirmed respiratory cases had rhinorrhea cough, while the five confirmed cases with gastrointestinal diseases had vomiting and diarrhea, whereas the only neurological case had suspected for encephalitis caused by HSV (Table 1). Other clinical symptoms were abdominal pain (50%) and to a lesser extent, fever and respiratory distress.

A total of 119 patients (54.9%) were less than two years old. The median age from this age group was 5 months, the age range being from one day old newborn to one year old. There was a higher incidence in 2015 (7 patients) and most PeV-A 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

 doi:10.20944/preprints202203.0187.v2

2015, one in the neurological sample and the other in a respiratory sample. In all reported cases, the clinical diagnosis was usually confused with Rotavirus (50%) and to a lesser extent with Respiratory Syncytial Virus, Enterovirus and Herpes Virus.

4 Discussion

This is the first confirmation of the presence of PeV-A in Panama despite strong suspicions after outbreaks of viral infections in febrile neonates with clinical sepsis at Hospital del Niño between 2013 and 2014 (22). Therefore, our findings show that PeV-A can cause sepsis and gastrointestinal, respiratory and neurological acute infections in Panama. In addition, the largest number of positive samples for PeV-A corresponded to gastrointestinal samples (feces and anal swab), with a frequency percentage of 4%, being within of the ranges previously reported for PeV-A in children with diarrhea, that corresponded from 2% to 16.3% (13, 24). This percetage of 4% was similar to a Nigerian study of children with a similar age range (25), but lower than what has been reported in studies carried out in Chile, Spain and Malawi (19, 20, 26). 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 (27).

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 (28). Only a 1-day-old female patient with CSF could be associated with PeV-A and that he had some symptoms previously reported (29).

 doi:10.20944/preprints202203.0187.v2

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 could reduce the use of antibiotics. Unfortunately, the capacity for detection and genotyping in hospitals in the country need to be developed in the future. Despite having the diagnostic capacity at ICGES, the small number of clinical suspected samples received, related in the majority to hospitalized patients and not to outpatients, could introduce a biais in the epidemiological surveillance.

The detection rate in young children under 2 years of age was 2.5% for the years analyzed (2014-2015), 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, because of the high rates of coinfections reported in Japan, where 59.2% of PeV-A positive samples were coinfected with other enteric viruses (30). 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 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

 doi:10.20944/preprints202203.0187.v2

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.

Funding: This work was supported by the National Institute on Minority Health and Health Disparities (NIMHD) [grant numbers Nº 5T37MD001376-2012].

Institutional Review Board Statement: The research protocol was approved by ICGES IRB, Note N ° 981 / CBI / ICGES / 16 on September 16, 2016.

Acknowledgments: We thank all the members of the Department of Research in Virology and Biotechnology and the Clinical research unit of ICGES. JMP, SLV, LEA are members of the Sistema Nacional de Investigación (SNI) of SENACYT, Panama.

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

References

1. Westerhuis B, Kolehmainen P, Benschop K, Nurminen N, Koen G, Koskiniemi M, Simell O, Knip M, Hyöty H, Wolthers K, Tauriainen S. 2013. Human parechovirus seroprevalence in Finland and the Netherlands. J Clin Virol Off Publ Pan Am Soc Clin Virol 58:211–215.

2. WIGAND R, SABIN AB. 1961. Properties of ECHO types 22, 23 and 24 viruses. Arch Gesamte Virusforsch 11:224–247.

3. Lindberg AM, Johansson S. 2002. Phylogenetic analysis of Ljungan virus and A-2 plaque virus, new members of the Picornaviridae. Virus Res 85:61–70.

4. Joffret ML, Bouchier C, Grandadam M, Zeller H, Maufrais C, Bourhy H, Despres P, Delpeyroux F, Dacheux L. 2013. Genomic characterization of Sebokele virus 1 (SEBV1) reveals a new candidate species among the genus Parechovirus. J Gen Virol 94:1547–1553.

5. Smits SL, Raj VS, Oduber MD, Schapendonk CME, Bodewes R, Provacia L, Stittelaar KJ, Osterhaus ADME, Haagmans BL. 2013. Metagenomic analysis of the ferret fecal viral flora. PLoS One 8:e71595.

6. Zhang C, Sheng J, Zhao Z, Yan C, Tu C, He B. 2019. Genomic Characterization of the First Parechovirus in Bats. Virol Sin https://doi.org/10.1007/s12250-019-00108-1.

7. Pankovics P, Boros Á, Mátics R, Kapusinszky B, Delwart E, Reuter G. 2017. Ljungan/Sebokele-like picornavirus in birds of prey, common kestrel (Falco tinnunculus) and red-footed falcon (F. vespertinus). Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis 55:14–19.

8. Shi M, Lin X-D, Chen X, Tian J-H, Chen L-J, Li K, Wang W, Eden J-S, Shen J-J, Liu L, Holmes EC, Zhang Y-Z. 2018. The evolutionary history of vertebrate RNA viruses. Nature 556:197–202.

9. Firth C, Bhat M, Firth MA, Williams SH, Frye MJ, Simmonds P, Conte JM, Ng J, Garcia J, Bhuva NP, Lee B, Che X, Quan P-L, Lipkin WI. 2014. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal Rattus norvegicus in New York City. MBio 5:e01933-14.

10. Kawasaki J, Kojima S, Tomonaga K, Horie M. 2021. Hidden Viral Sequences in Public Sequencing Data and Warning for Future Emerging Diseases. MBio 12:e0163821.

11. Hyypiä T, Horsnell C, Maaronen M, Khan M, Kalkkinen N, Auvinen P, Kinnunen L, Stanway G. 1992. A distinct picornavirus group identified by sequence analysis. Proc Natl Acad Sci U S A 89:8847–8851.

 doi:10.20944/preprints202203.0187.v2

12. Nix WA, Maher K, Johansson ES, Niklasson B, Lindberg AM, Pallansch MA, Oberste MS. 2008. Detection of all known parechoviruses by real-time PCR. J Clin Microbiol 46:2519–2524.

13. Baumgarte S, de Souza Luna LK, Grywna K, Panning M, Drexler JF, Karsten C, Huppertz HI, Drosten C. 2008. Prevalence, types, and RNA concentrations of human parechoviruses, including a sixth parechovirus type, in stool samples from patients with acute enteritis. J Clin Microbiol 46:242–248.

14. Fox B, Sabio Paz V, Incardona MA, Elisiri ME, Gonzalez Fraga S, Solana CL, Fernández-Canigia L. 2021. Rapid syndromic molecular testing and human parechovirus infection in children: A report of three cases in Argentina. Rev Argent Microbiol https://doi.org/10.1016/j.ram.2021.02.003.

15. Sridhar A, Karelehto E, Brouwer L, Pajkrt D, Wolthers KC. 2019. Parechovirus A Pathogenesis and the Enigma of Genotype A-3. Viruses 11.

16. Harvala H, Calvert J, Van Nguyen D, Clasper L, Gadsby N, Molyneaux P, Templeton K, McWilliams Leitch C, Simmonds P. 2014. Comparison of diagnostic clinical samples and environmental sampling for enterovirus and parechovirus surveillance in Scotland, 2010 to 2012. Euro Surveill Bull Eur sur les Mal Transm = Eur Commun Dis Bull 19.

17. Kadambari S, Harvala H, Simmonds P, Pollard AJ, Sadarangani M. 2019. Strategies to

improve detection and management of human parechovirus infection in young infants. Lancet Infect Dis 19:e51–e58.

18. Khatami A, Burrell R, McMullan BJ, Rawlinson W, Givney RC, Kok J, Alexandersen S, Jones CA, Macartney KK, Britton PN. 2020. Epidemic and Inter-epidemic Burden of Pediatric Human Parechovirus Infection in New South Wales, Australia, 2017-2018. Pediatr Infect Dis J 39:507–511.

19. Brouwer L, Karelehto E, Han AX, Thomas X V, Bruning AHL, Calis JCJ, van Hensbroek MB, Westerhuis BM, Amarthalingam D, Koekkoek SM, Rebers SPH, Phiri KS, Wolthers KC, Pajkrt D. 2019. High frequency and diversity of parechovirus A in a cohort of Malawian children. Arch Virol 164:799–806.

20. Gutiérrez V, Martínez-Valdebenito C, Montecinos L, Alarcón R, Gárate C, Ferrés M. 2016. [Parechovirus as etiologic agent of meningitis and/or sepsis like illness in infants]. Rev Chil Infectol organo Of la Soc Chil Infectol 33:380–388.

21. Guerrero-Latorre L, Romero B, Bonifaz E, Timoneda N, Rusiñol M, Girones R, Rios-Touma B. 2018. Quito's virome: Metagenomic analysis of viral diversity in urban streams of Ecuador's capital city. Sci Total Environ 645:1334–1343.

22. Ximena Norero. 2015. Infecciones virales en neonatos febriles con sepsis clínica. Hospital del Niño. Panamá Diciembre 2013- Junio 2014. Pediátr Panamá 44:12–22.

 doi:10.20944/preprints202203.0187.v2

23. Benschop K, Minnaar R, Koen G, van Eijk H, Dijkman K, Westerhuis B, Molenkamp R, Wolthers K. 2010. Detection of human enterovirus and human parechovirus (HPeV) genotypes from clinical stool samples: polymerase chain reaction and direct molecular typing, culture characteristics, and serotyping. Diagn Microbiol Infect Dis 68:166–173.

24. Yip CCY, Lo K-L, Que T-L, Lee RA, Chan K-H, Yuen K-Y, Woo PCY, Lau SKP. 2014. Epidemiology of human parechovirus, Aichi virus and salivirus in fecal samples from hospitalized children with gastroenteritis in Hong Kong. Virol J 11:182.

25. Osundare FA, Opaleye OO, Akindele AA, Adedokun SA, Akanbi OA, Bock C-T, Diedrich S, Böttcher S. 2019. Detection and Characterization of Human Enteroviruses, Human Cosaviruses, and a New Human Parechovirus Type in Healthy Individuals in Osun State, Nigeria, 2016/2017. Viruses 11.

26. Martín Del Valle F, Calvo C, Martinez-Rienda I, Cilla A, Romero MP, Menasalvas AI, Reis-Iglesias L, Roda D, Pena MJ, Rabella N, Portugués de la Red MDM, Megías G, Moreno-Docón A, Otero A, Cabrerizo M. 2018. [Epidemiological and clinical characteristics of infants admitted to hospital due to human parechovirus infections: A prospective study in Spain]. An Pediatr 88:82–88.

27. Kapusinszky B, Minor P, Delwart E. 2012. Nearly constant shedding of diverse enteric viruses by two healthy infants. J Clin Microbiol 50:3427–3434.

 doi:10.20944/preprints202203.0187.v2

28. Sasidharan A, Harrison CJ, Banerjee D, Selvarangan R. 2019. Emergence of Parechovirus A4 Central Nervous System Infections among Infants in Kansas City, Missouri, USA. J Clin Microbiol 57.

29. van Hinsbergh TMT, Elbers RG, Hans Ket JCF, van Furth AM, Obihara CC. 2020. Neurological and neurodevelopmental outcomes after human parechovirus CNS infection in neonates and young children: a systematic review and meta-analysis. Lancet Child Adolesc Heal 4:592–605.

30. Pham NTK, Thongprachum A, Shimizu Y, Shiota I, Hoque SA, Khamrin P, Takano C, Trinh QD, Okitsu S, Komine-Aizawa S, Shimizu H, Maneekarn N, Hayakawa S, Ushijima H. 2021. Genetic diversity of Parechovirus A in infants and children with acute gastroenteritis in Japan during 2016-2018. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis 90:104776.