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Short communication

High diversity of human parechovirus including novel types in stool samples from Ghanaian children

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ABSTRACT

Background: Little is known on human parechovirus (HPeV) infections in Africa.
Objectives: We aimed to determine the prevalence, genetic diversity, and association with diarrhea of HPeV in Ghanaian children.
Study design: A total of 682 stool samples from a pediatric case-control study on causes of diarrhea collected in 2007–2008 were used. Laboratory analysis included HPeV real-time RT-PCR and sequencing partial viral protein (VP) 1 gene region of HPeV. In addition, data on co-infections using the xTAG Gastrointestinal Pathogen Panel were available.
Results: Overall, a prevalence of 24% was found and 14 different HPeV types were detected. Phylogenetic analysis of the VP1 region indicated a novel type tentatively designated as HPeV-18. No association with diarrhea was found (OR = 0.8; 95% CI: 0.5–1.1), and HPeV viral concentrations were not different among cases and controls. No seasonal pattern was observed. HPeV-positive cases displayed a slightly higher chance of co-infections.
Conclusions: A high prevalence and genetic diversity of HPeV including novel types was found by sequencing partial VP 1 region. HPeV was not associated with diarrheal disease in this pediatric population and the high number of co-infection suggests transient colonization without clinical relevance.

1. Background

Human parechoviruses (HPeV) belong to the family *Picornaviridae* and circulate worldwide, but information on the prevalence and diversity of HPeV in sub-Saharan Africa is limited [1]. At least 17 HPeV genotypes have been described to date, most of them only recently (www.picornaviridae.com/parechovirus/hpev/hpev.htm). In general, infection caused by HPeV-1 and −2 remains asymptomatic or causes mild enteric or respiratory symptoms. For HPeV-3, however, severe clinical courses with sepsis-like illness or encephalitis have been described [2]. The clinical association of HPeV types 4–17 is only ill-defined and in particular HPeV types 8–17 have been detected only occasionally. In light of the pathogenic potential, possibly in combination with other infections, data on HPeV in Africa is crucial for individual and public health. Molecular surveillance is also warranted to detect emerging HPeV variants or recombinants which might spread worldwide [3].

2. Objectives

We aimed to determine the rate and diversity of HPeV types in stool samples in a cohort of children in rural Ghana and to unravel a possible association with diarrhea and gastrointestinal co-infections.

3. Study design

Stool samples collected in the framework of a case-control study on causes of diarrhea in children were analysed. All study participants were recruited at the childreńs Outpatients Department (OPD) of the Agogo Presbyterian Hospital in the Ashanti region of Ghana between

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June 2007 and October 2008 [4]. Samples were collected from children < 6 years. Children with diarrhea were defined as cases with at least three loose stools within the last 24 h. Children attending the OPD without diarrhea served as controls. From this study group 341 age-matched case-control pairs were selected. Each study participant provided one fecal sample.

Nucleic acid preparation and HPeV real-time reverse transcription PCR (rRT-PCR) were done as described elsewhere [5]. Molecular typing of HPeV positive samples was done by amplifying and sequencing the nearly entire viral protein 1 (VP1) [1]. Assignment to reference HPeV types was conducted by phylogenetic analysis including a 690 base pair fragment (nucleotide position 2336–3025 according to HPeV-1 prototype Harris L02971) using the Neighbor joining algorithm with 1000 replications. Evolutionary distances were calculated with the maximum composite likelihood (MCL). Data on gastrointestinal co-infections were obtained from our previous study using the xTAG Gastrointestinal Pathogen Panel (Luminex, Austin, TX, USA) [6].

Descriptive statistics were applied and differences between cases and controls assessed with the Mantel-Haenszel Odds Ratio (OR). Ethical approval for this study was provided by the Committee on Human Research Publication and Ethics, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Informed consent was obtained from parents or guardians of all children.

4. Results

Table 1 summarizes characteristics of study participants. Overall, 162 (24%) of 682 samples tested HPeV rRT-PCR positive [73/341 (21%) cases and 89/341 (26%) controls]. Amplification and sequencing of the VP1 region was successful in 114/162 (70%) of HPeV-positive samples [52 (46%) cases and 62 (54%) controls]. In the phylogenetic analysis the HPeV strains clustered with different reference strains (Fig. 1). The majority was HPeV-1 (30/114, 26%), followed by HPeV-4 (16/114, 14%), and HPeV-8 (12/114, 11%) (Table 2). Less frequently detected strains included: HPeV-5 (11/114, 10%), HPeV-7 (10/114, 9%), HPeV-14 (7/114, 6%), HPeV-2 (6/114, 5%), HPeV-9 (6/114, 5%), and HPeV-17 (6/114, 5%). Given the minimum of 75% nucleotide identity between different HPeV types [7], one strain showed borderline nucleotide identity to the closest reference types: GhanaA68 had 76% identity to type 10 (GenBank accession no. GQ402516). Three samples contained a novel HPeV type assigned to HPeV-18 by the picornaviridae study group (R. Zell, pers. comm.): GhanaA06, GhanaA36, and GhanaA29 (Fig. 1). All sequences were submitted to GenBank (accession numbers KY931547–KY931660).

No association of HPeV with diarrhea was observed (OR = 0.8; 95% CI: 0.5–1.1). HPeV detections occurred throughout the study period without a clear seasonal pattern. However, HPeV infections were more often seen during the rainy months (OR = 1.5; 95% CI: 1.0–2.1) (Fig. 2). Finally, we analysed the number of gastrointestinal co-infections using the xTAG Gastrointestinal Pathogen Panel, which is capable to identify 19 pathogens (Supplemental Table). The overall median number of co-infections was 2 (IQR: 2–3), while up to 7 co-infections per person were detected. HPeV-positive cases had a slightly higher

Table 1
Characteristics of cases and controls, Ghana, 2007–2008.

Patient characteristic	Cases, n = 341	Controls, n = 341
Male sex, n (%)	183 (54)	199 (58)
Age in months; median (IQR)	19 (10–34)	18 (10–32)
HPeV rRT-PCR positive; n (%)	73 (21)	89 (26)
HPeV rRT-PCR Ct-value; median (IQR)	31 (28–34)	31 (27–34)
Median number of co-infections in cases and controls		
HPeV rRT-PCR positive; median (IQR)	3 (2–4)	
HPeV rRT-PCR negative; median (IQR)	2 (1–3)	

IQR, interquartile range; Ct-value, cycle threshold-value.

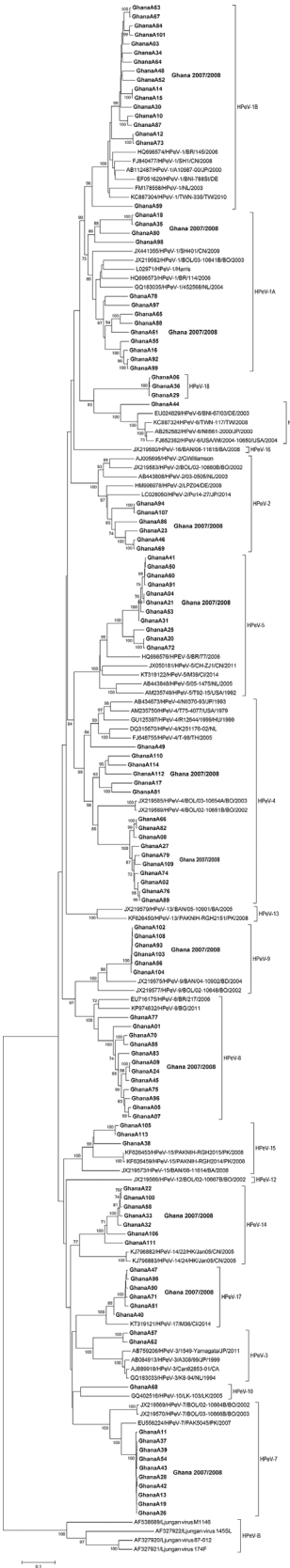

Fig. 1. Phylogenetic analysis of the viral protein VP1 nucleotide sequences of human parechoviruses detected in Ghana, June 2007–October 2008, compared with representative reference strains. Neighbor joining algorithm was used and reference sequences were obtained from GenBank. Bootstrap values > 70 are shown at the branch nodes. Bar indicates substitutions per site. Strains identified in this study are shown in boldface.