Bufavirus genotype 3 in Turkish children with severe diarrhoea

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Abstract

Recently a parvovirus called bufavirus (BuV) has been implicated as a causative agent of diarrhoea. To further reveal the epidemiology and genetic characteristics of BuV, this study was performed in Turkish children with diarrhoea. BuV was detected in 1.4% (8/583) of stool samples. All stool samples from healthy children (n = 148) were negative for BuV. Diarrhoea in BuV-positive patients was severe and occurred mainly during the colder months of the year. Complete genome sequences were generated from four BuVs. Only BuV3 was found, which was genetically and phylogenetically similar to Bhutanese BuV3, indicating that BuV3 is prevalent in Asian countries.

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Every year, 862 000 to 1 265 000 children are affected by diarrhoea in Turkey. However, the role of emerging viruses in Turkish children with diarrhoea has not been widely investigated [1–5]. Bufavirus (BuV) is a newly identified virus, detected in the diarrhoeal stool samples of patients from Burkina Faso, Bhutan and Finland [6–8]. It belongs to the species Primate protoparvovirus 1 of the genus Protoparvovirus [9]. BuV has an ssDNA genome and is divided into three genotypes, BuV1 to BuV3 [7]. The epidemiology and genetic characteristics of BuVs are obscure; therefore, the present study was performed to further characterize BuV in Turkish children.

From September 2004 to June 2011, a total of 1221 stool samples were collected from children with diarrhoea aged <5 years who received care at the Gazi University Hospital and the Ministry of Health Ankara Training and Education Hospital, Ankara, Turkey. All samples were tested for pathogenic bacteria, parasites, rotavirus [2] and norovirus (NoV) [10]. From February to September 2013, a total of 148 stool samples were collected from age-matched healthy controls assessed at the Well-Child Clinic, Gazi University. Ethical committees of both hospitals approved the study. The climatic values were obtained from http://www.tutiempo.net.

BuV PCR detection and sequencing were performed [6,7]. Rotavirus, adenovirus, human bocavirus (HBoV), astrovirus, NoV, salivirus, cosavirus and Aichi virus were tested for in the BuV-positive samples by PCR [7] (Supplementary Table 1), and NoV genotyping was performed [11]. The phylogenetic tree was constructed using the neighbor-joining method [12], and bootstrap analysis of 1000 replicates was performed. SPSS 10 was used for other statistical analyses (IBM, Armonk, NY, USA).

All 1221 samples were negative for diarrhoea-causing bacteria or parasites [13]. This indicates that effective public health measures are in place in Turkey, where 100% and 97% of the population has access to safe water and sanitary toilets, respectively (http://data.worldbank.org). Excluding the ELISA-positive rotavirus and NoV samples, 583 samples were available for BuV detection. The mean age of these patients was 19.5 months (range, 1–60 months). The male–female sex ratio was 1.5:1. BuV was detected in eight samples (1.4%) (Supplementary Table 2); the male–female sex ratio was 1:1. The mean age was 29.6 months (range, 11–56 months). Three samples were associated with other viruses: one sample was associated with NoV GII.21, another sample with NoV GII.4
and HBoV2 and the third sample with HBoV3. All 148 samples from normal control children, mean age 17.3 months (95% confidence interval (CI), 14.3–20.3), were negative for BuV. These results indicate a trend, although not statistically significant (p 0.15, χ² test), towards a higher detection rate in patients than in controls. The number of diarrhoeal stools per day in BuV-positive patients (mean 7.0; 95% CI, 5.2–8.8) was significantly higher than in BuV-negative patients (mean 5.1; 95% CI, 4.9–5.3; p 0.017), rotavirus-positive patients (mean 5.5; 95% CI, 5.2–5.8; p 0.046) or NoV-positive patients (mean 5.1; 95% CI, 4.7–5.5; p 0.25). In BuV-positive patients, the number of diarrhoeal stools per day was not significantly different (p 0.645) between patients with and without co-infection. The number of those vomiting in BuV-positive patients (mean 0.9; 95% CI, 0.6–2.4) was not significantly different from BuV-negative patients (mean 1.4; 95% CI, 1.2–1.5; p 0.344), rotavirus-positive patients (mean 2.3; 95% CI, 1.9–2.7; p 0.119) or NoV-positive children (mean 1.9; 95% CI, 1.4–2.4; p 0.202).

BuV was detected in two samples during 2007, three during 2008, and three during 2010. In other years, no BuV was detected. The annual fluctuation of BuV infection did not have a statistically significant relationship with annual temperature or other annual climatic values in Ankara. Seasonal prevalence showed that most of the BuV infection occurred in cool months (Supplementary Table 2), which is typical of many viral diarrheas [14].

Nearly complete genome sequences were obtained in four samples (Table 1). The lengths of the open reading frames of NS1, VP1 and VP2 genes were 2022, 2133 and 1719, respectively, except in one sample (AHP-228), where the open reading frame of NS1 was 2019.

The NS1, VP1 and VP2 of the Turkish strains showed 95–98%, 98–99% and 99% nucleotide and 97–99% amino acid identities among themselves. Compared with Bhutanese BuV3, the respective NS1, VP1 and VP2 nucleotide (amino acid) identities of Turkish strains were 96–99% (95–98%), 97–99% (98–99%) and 97–99% (98–99%).

Similar to other BuVs, Turkish BuVs contained an ATP- or GTP-binding Walker loop, phospholipase A₂, two conserved replication initiator motifs and a glycine-rich sequence; they also had the same splice sites. In the Turkish BuVs, the tandem repeats in the 3' untranslated region occurred at a frequency of none or one time for TAGTTGATAAGT; two, three, five or six times for TAGTTTATAAGT; and none, one or two times for TAGTTTATAAAT.

Phylogenetic analysis (Fig. 1) showed that BuVs diverged into distinctly three genotypes, and Turkish BuVs belong to BuV3 genotypes. Turkish strain AHP-740 formed a cluster with Bhutanese strains of BuV3; the remaining Turkish strains formed a separate cluster.

Only a small number of children were infected with BuV; their number of diarrhoea episodes per day was higher than that of other patients. A proportion (3/8) of BuV-positive children were also infected with other viruses, but statistical analysis did not support that co-infection resulted in more severe symptoms than in patients with BuV alone. Two samples initially negative for NoV by ELISA were positive by PCR, indicating the higher sensitivity of the latter method. However, NoV GII.21, detected in the Turkish stool sample with BuV, is an unusual genotype, detected mainly in the environment [15]. However, the association of NoV in BuV diarrheoa needs further investigation because this co-infection is common and was also detected in Bhutanese and Finnish patients [7,8].

Despite Turkey’s geographic proximity to Europe, our analysis showed that BuVs from Turkey and Bhutan have a

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Total</th>
<th>5' UTR</th>
<th>NS1</th>
<th>VP1</th>
<th>VP2</th>
<th>3' UTR</th>
<th>TAGTTGATAAGT</th>
<th>TAGTTTATAAGT</th>
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</table>

BuV, bufavirus; ND, sequence could not be determined; UTR, untranslated region.

*Only partial sequence of 3' UTR regions have been achieved in all strains. Nucleotide sequence of 5' UTR of Turkish bufavirus strains could not be determined. Strain numbers and their corresponding DNA Data-Bank of Japan, European Molecular Biology Laboratory, and GenBank accession numbers are as follows: AB982217 (strain AHP-178), AB982218 and AB982219 (NS1 and 3' UTR of strain AHP-228), AB982220 (strain AHP-368), AB982221 (strain AHP-692), AB982222 (strain AHP-740), and AB982223 and AB982224 (NS1 and 3' UTR of strain AHP-747).
common ancestor, have close genetic identities and belong to genotype 3. A recent study showed that BuVs detected in diarrhoeal stool samples from Thailand [16] belong to genotype 1, as found in Burkina Faso, Finland and the Netherlands [17]. More study is certainly needed to delineate the genotype distribution of BuVs in different countries to identify the pattern of virus spread.

**Transparency Declaration**

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**Appendix A. Supplementary data**

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.06.006.

**References**


