

This study was supported by the National Major S & T Project (2012ZX10004-206), the International Science and Technology Cooperation Program of China (2010DFB33270), China National Funds for Distinguished Young Scientists (81225014), Program for Changjiang Scholars and Innovative Research Team in University (IRT13007), and Fondation Mérieux.

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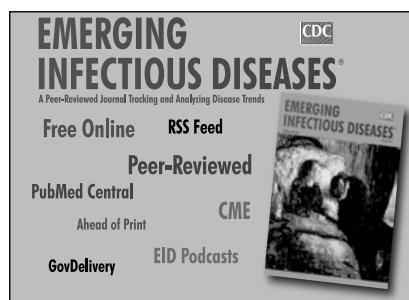
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DOI: <http://dx.doi.org/10.3201/eid2006.131596>

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Bufavirus in Feces of Patients with Gastroenteritis, Finland

To the Editor: For nearly 3 decades, human parvovirus B19 (B19V) was considered to be the only pathogenic parvovirus found in humans. Since 2005, several new human parvoviruses have been found, including human bocaviruses 1–4 and human parvovirus 4 (PARV4) (1–5), and during 2012, metagenomic analysis of fecal samples from children in Burkina Faso with acute diarrhea showed a highly divergent parvovirus, which was named bufavirus (BuV) (6). Its sequence in the coding region showed <31% similarity with known parvoviruses, the closest genera being *Protoparvovirus* and *Amdoparvovirus*. Subsequent studies, on the basis of PCR results, showed that 4% of fecal samples from Burkina Faso (n = 98) and 1.6% from Tunisia (n = 63) harbored either of 2 genotypes of this new virus, which belongs to the species *Primate protoparvovirus 1* of the genus *Protoparvovirus* (6,7; <http://ictvonline.org>).

To assess the occurrence of BuV in northern Europe, we analyzed 629 fecal samples from patients of all ages (median 51.5 years, range 0–99) in Finland who had gastroenteritis. To gain a more complete representation of BuV occurrence, we obtained samples retrospectively from routine diagnostics for bacterial and viral gastroenteritis-inducing pathogens (HUSLAB, Helsinki University Central Hospital Laboratory Division, Helsinki, Finland) and analyzed all samples available during the collection periods.

The samples originally sent to HUSLAB for bacterial diagnosis (bacterial cohort, n = 243) had been analyzed during October 2012–March 2013 for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia*

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spp., *Vibrio cholerae*, and *Escherichia coli* (subtypes enterohemorrhagica, enteropatogena, enterotoxigenic and enteroagregativa) by using culture or PCR (8). In 81 (33.3%) of the samples, ≥ 1 bacterial pathogen was found.

The samples originally sent for viral diagnosis (viral cohort, $n = 386$) had been tested in HUSLAB for norovirus during April–May, 2013 by using reverse transcription quantitative PCR (RT-qPCR) (HUSLAB in-house). Further diagnosis for rotavirus and adenovirus had been requested by physicians from 105 (27.2%) of 386 samples (Diarlex MB antigen detection assay, Orion Diagnostica, Espoo, Finland), and for astrovirus from 33 (8.6%) samples (RT-PCR, HUSLAB in-house). A viral pathogen was discovered in 141 (36.5%) samples; in 139, the pathogen was norovirus.

The samples had been sent from diverse locations within Finland, and thus were not from a few isolated outbreaks. No further information on patients and samples was available for either cohort, and not enough samples were left for retrospective analysis of additional pathogens. The Ethics Committee of the Hospital District of Helsinki and Uusimaa approved the study.

BuV DNA was detected by using a new real-time qPCR with the

following primers and probe: BuV forward, 5'-ACAGTGTAGACAGTG-GATCAAACCTT-3'; BuV reverse, 5'-GTTGTGGTTGGATTGTGGT-TAGTTC-3'; BuV qPCR probe, 5'-FAM-CGGAAGAGATTTTGA-CAGTGCYTAGCAA-BHQ1 3'. The detailed qPCR protocol is shown in the online Technical Appendix (wwwnc.cdc.gov/EID/article/20/6/13-1674-Techapp1.pdf). The analytical sensitivity of the RT-qPCR assay was 5–10 copies per reaction.

Of the 629 fecal samples, 7 (1.1%) were positive for BuV DNA, of which 4 were from the bacterial cohort and 3 from the viral cohort. BuV DNA quantity was low in all samples, ranging from 1.9×10^3 to 3.2×10^4 copies per milliliter of fecal supernatant (Table). In contrast to the original discovery of the virus in children with diarrhea (6), all positive samples were from adults (median age 53 years, range 21–89 years). All BuV DNA-positive results were confirmed by repeated BuV qPCR, by amplifying and sequencing another area of the virus, or by both methods (Table): all sequenced amplicons were more similar to the BuV genotype 1 (online Technical Appendix Figure) (6). Two of the BuV-positive samples were from the same patient, taken 4 days apart, and the latter sample also harbored norovirus. The additional 6 BuV-positive samples

were negative for the other viral or bacterial pathogens tested.

Seven fecal samples collected from adults in Finland contained BuV DNA, indicating that circulation of the virus is restricted neither to children nor to Africa. However, the low DNA loads in all the positive samples suggest that BuV might not be the primary cause of these cases of gastroenteritis. A known gastroenteritis-inducing pathogen (norovirus) was found in 1 of the 7 BuV-positive samples. We did not observe any clustering of the 7 positive samples into a specific period (Table).

Although the association with gastroenteritis seems weak, BuV might cause symptoms of other types. We did not include feces from healthy subjects for comparison. The identified BuV DNA in our samples could originate from previous or current infections unrelated to gastroenteritis, or be associated with prolonged virus secretion in the respiratory or digestive tracts, a phenomenon shown, e.g., for human bocavirus1 (9,10). Acquisition of the virus from a food source cannot be ruled out, although 1 patient harbored the DNA for at least 4 days, during which a 10-fold increase in viral load was observed.

Overall, this study shows that BuV circulates in northern Europe and can be found in the feces of patients with

Table. Samples collected for bacterial and viral testing that were subsequently positive for bufavirus DNA*

Sample no.	Sample cohort	Quantity, copies/mL supernatant	Age, y/sex	Pathogens tested for by HUSLAB†	Other pathogens found	Sampling date	Sequenced region, nt, divergence (%) from JX027295‡
1	Bacterial	5.2×10^3	21/M	Bacteria	0	2012 Dec 4	VP2, 2786–4495, 0.88
2	Bacterial	1.9×10^4	38/M	Bacteria	0	2013 Jan 6	VP2, 2786–4495, 0.71
3§	Bacterial	1.9×10^3	53/M	Bacteria	0	2013 Jan 11	§
4	Bacterial	3.7×10^3	46/M	Bacteria	0	2013 Apr 27	VP2, 2786–4495, 0.76
5	Viral	3.4×10^3	77/M	Norovirus	0	2013 Apr 19	VP2, 2786–4495, 1.60
6¶	Viral	3.6×10^3	89/F	Norovirus	0	2013 Apr 20	Partial NS, 16–1080, 1.13
7¶	Viral	3.2×10^4	89/F	Norovirus	Norovirus	2013 Apr 23	VP2, 2786–4495, 1.36

*VP2, viral protein 2; NS, nonstructural.

†Samples originally sent to HUSLAB (Helsinki, Finland) for bacterial diagnosis were analyzed for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp., *Vibrio cholerae*, and *Escherichia coli* (subtypes enterohemorrhagic, enteropatogena, enterotoxigenic, and enteroagregativa) by using culture or PCR. Bufavirus-positive samples could not be analyzed for the presence of pathogens other than those originally tested for because the samples had been discarded.

‡Sequence divergence analyzed by using the DNA distance matrix in BioEdit (www.mbio.ncsu.edu/BioEdit/bioedit.html). The bufavirus sequences were submitted to GenBank (accession nos. KJ461874–KJ461879).

§This sample was positive for bufavirus by quantitative PCR. However, we were not able to amplify another region of the virus from this sample, likely caused by a low amount of the virus in the sample, which had the lowest copy number among the positive samples.

¶Samples from the same patient, collected 4 days apart.

gastroenteritis. Despite the absence of known pathogens among 6 of 7 BuV-shedding patients, the causative role of BuV in gastroenteritis remains uncertain. Serologic studies will help clarify a possible association between BuVs and diarrhea or other diseases.

This study was funded by the Helsinki Biomedical Graduate Program, the Instrumentarium Foundation, the Research Funds of the University of Helsinki, the Helsinki University Central Hospital Research and Education and Research and Development Funds, the Finnish Medical Foundation, Sigrid Juselius Foundation, and the Academy of Finland (project no. 1122539).

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DOI: <http://dx.doi.org/10.3201/eid2006.131674>

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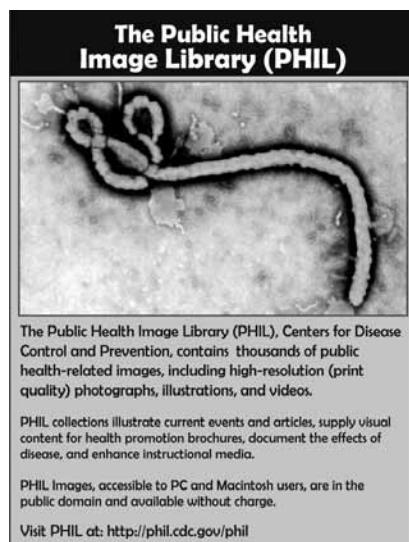
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Human Granulocytic Anaplasmosis Acquired in Scotland, 2013

To the Editor: Human granulocytic anaplasmosis is a tick-borne disease caused by *Anaplasma phagocytophilum*, an obligate intracellular gram-negative bacterium that infects granulocytes. The usual clinical signs and symptoms include nonspecific fever, chills, headache, and myalgia. Infection is usually mild or asymptomatic, but severe systemic complications can occur, leading to a need for intensive care and estimated fatality rates of 0.5%–1.0% (1,2).

A. phagocytophilum was first described in 1932 in Scotland as the causative agent of tick-borne fever in sheep (3). Although some clinical cases of human granulocytic anaplasmosis have been reported in Europe, mostly from Slovenia, Sweden, and Poland (4), most cases have occurred in the United States. This difference cannot be explained by the prevalence of the pathogen in ticks or human exposure to the pathogen because the 3% prevalence of *A. phagocytophilum* among *Ixodes ricinus* ticks in Europe seems to be nearly as high as that among ticks in the United States (2). The median seroprevalence rate for *A. phagocytophilum* infection among humans in Europe is 6.2%, reaching up to 21% (2). This incongruence between seroprevalence rate and number of human cases might be associated with underdiagnosis of cases (2), a high rate of asymptomatic disease (5), or cross-reactivities in serologic tests that might lead to overestimation of seroprevalence rate (5).

In August 2013, an immunocompetent 40-year-old man sought treatment for fever (≈39°C) and other nonspecific symptoms such as malaise, myalgia, and severe headache 3 days after becoming aware of several tick bites received while on a hiking



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Technical Appendix

Bufavirus Quantitative PCR

A hydrolysis-probe based quantitative PCR (qPCR) was designed on the conserved NS1 region of the bufavirus genome, based on the bufavirus sequences published in GenBank (accessed 1st Oct 2012). The following primers and probe were selected (nucleotides corresponding to JX027295): BuV fwd (705-730nt), 5'-ACA GTG TAG ACA GTG GAT TCA AAC TT-3'; BuV rev (830-806nt), 5'-GTT GTG GTT GGA TTG TGG TTA GTT C-3'; BuV qPCR probe (789-762nt), 5'-FAM-CGG AAG AGA TTT TGA CAG TGC YTA GCA A-BHQ1-3'. The qPCR reaction consisted of 1x Maxima Probe qPCR Master Mix (Fermentas, Vilnius, Lithuania), 30 nM of ROX passive reference dye, 0.5 μ M of each primer, 0.2 μ M of BuV qPCR probe, 5 μ l of template and H₂O up to a final volume of 25 μ l. DNA was amplified by the Stratagene Mx3005P machine with the following program: initial denaturation of 10 min at 95°C followed by 45 cycles of 15 s at 95°C and 1 min at 60°C.

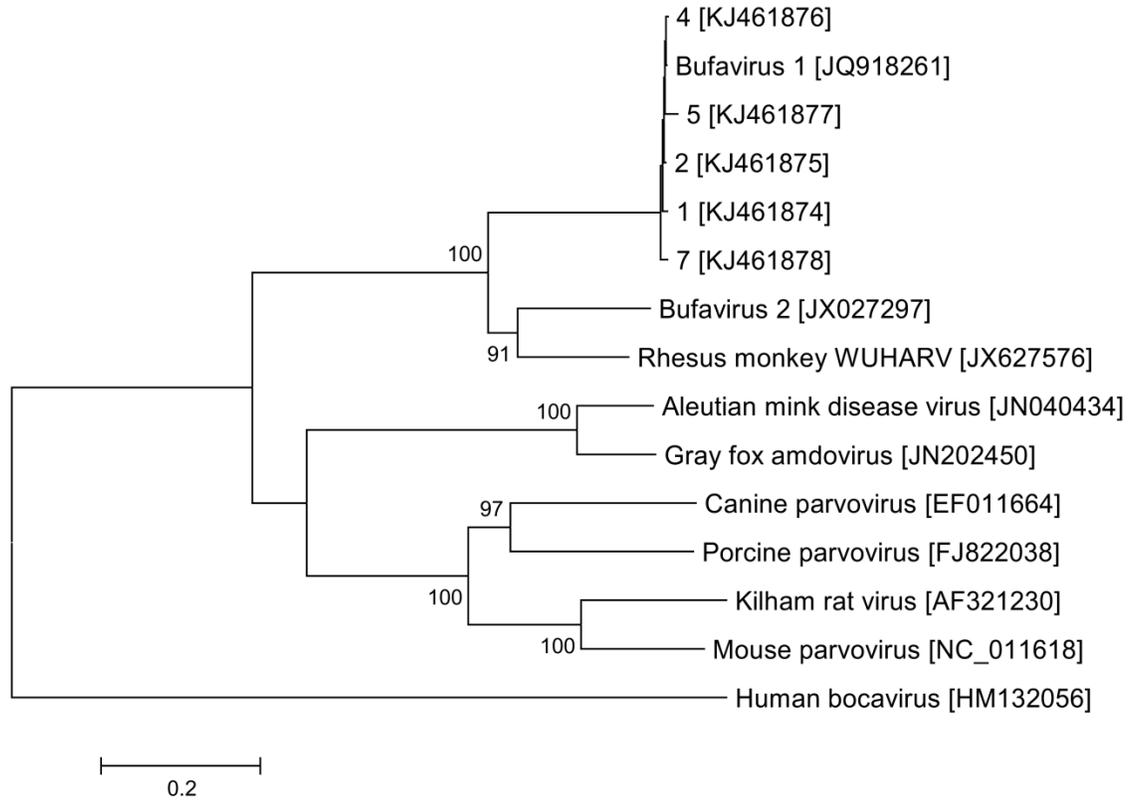
For controls in methods set up and optimization, a 3.5kb piece of BuV1 (16-3577 nt, JX027295) and 830 nt of BuV2 (1-829 nt, JX027297) were amplified from the original fecal supernatants and cloned into the pSTBlue vector (6). These plasmids were used in testing of sensitivity and specificity of the qPCR with and without 500 ng human DNA per reaction (HEK293 cell line DNA) and extracted DNA from fecal samples. Specificity of the assay was tested with other human parvoviruses, human parvovirus B19 (B19V), HBoV1, and PARV4. A 10-fold dilution series of the BuV1 plasmid was used in the generation of a standard curve for quantification.

The analytical sensitivity of the RT-qPCR assay was 5-10 copies per reaction for both of the BuV plasmids, and the assay showed linearity over a range of 50 to $\geq 5 \times 10^6$ copies per reaction. Human DNA or fecal DNA extracts did not interfere with the amplification or cause

false positives. Furthermore, the qPCR did not amplify B19V, HBoV1 or PARV4 DNAs even when using very high copy-number templates. All negative controls, i.e. water, human DNA and negative fecal samples, remained negative in all assays.

DNA Extractions from Fecal Samples

The DNA from the fecal samples in the bacterial cohort was extracted from 900 μ l of 10% fecal suspension with an automated Tecan pipetting robot system (Männedorf, Switzerland) following the NucliSens EasyMag nucleic acid extraction protocol (bioMerieux, Marcy l'Etoile, France). The nucleic acids in the viral cohort were extracted from 200 μ l of a 10% fecal suspension in Tris-NaCl-CaCl₂ buffer with the MagnaPure LC Total nucleic acid isolation kit (Roche Applied Sciences, Mannheim, Germany).



Technical Appendix Figure

Phylogenetic analysis of the viral protein 2 of bufavirus strains in Finland and related parvoviruses. Genetic distances were calculated by the Kimura 2-parameter method (PHYLIP), and a phylogenetic tree with 100-bootstrap resampling of the alignment data sets was generated by using the neighbor-joining method. Scale bar indicates the number of amino acid substitutions per position.