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Short communication

Salivirus type 1 and type 2 in patients with acute gastroenteritis, Germany

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ABSTRACT

Background: Salivirus (SaV-A) is a novel member of the family Picornaviridae and has been associated with acute gastroenteritis. Recently, a second type of SaV-A, SaV-A2, was identified in a sewage sample from Bangkok, Thailand. No information is available on the prevalence of SaV-A in Western Europe.
Objectives: Stool samples from patients with symptoms of acute viral gastroenteritis were analyzed for SaV-A and the clinical course of SaV-A-positive individuals was evaluated.
Study design: A total of 3019 fecal samples collected during 2012–2013 from 1941 hospitalized patients with acute gastroenteritis were screened for SaV-A by a newly designed real-time reverse transcription polymerase chain reaction targeting a conserved sequence in the 5′-untranslated region. Positive results were verified by sequencing the viral capsid protein 1 gene also allowing typing of the virus. Medical records of SaV-A-infected patients were reviewed for clinical features and laboratory data.
Results: SaV-A was detected in five patients. Viral RNA concentrations ranged from 7.1×10^6 to 7.2×10^8 copies/g feces. The viruses from four patients were classified as SaV-A1 while SaV-A2 was present in one patient. After reviewing the medical records, SaV-A could not be considered as the sole possible cause of gastroenteritis symptoms given the presence of other plausible causes in all five patients.
Conclusion: SaV-A infection can be detected in Germany, Western Europe, albeit at low levels. The detection of SaV-A2 in Europe suggests wider spread of SaV-A2. Presence of SaV-A, even at high concentrations, in a stool sample provides no conclusive evidence that SaV is the major cause of the patient's gastroenteritis symptoms.

1. Background

Salivirus (SaV-A) is a recently identified member of the family Picornaviridae and the only species within the novel picornaviral genus *Salivirus* [1] (http://www.picornaviridae.com/). The same virus was also reported the same year as klassevirus 1 [2,3]. SaV-A RNA was found at frequencies ranging from 0.1 to 8.7% in fecal samples from patients, mostly children, with gastroenteritis, with highest prevalences in Asian countries [1,3–7]. Thus, the virus was suspected to cause acute gastroenteritis [1,5,6,8]. Recently, a second type of SaV-A (SaV-A2) was identified in a sewage sample from Thailand [9]. The nucleotide similarity of the candidate second SaV-A type to previously identified SaV-A1 strains is 81–82% and the pairwise protein identity in the viral capsid precursor protein is 85–87%. SaV-A IgG-antibodies have been detected in 8.7% and 21.8% of pediatric and adult serum samples, respectively, collected in Missouri, U.S.A. [4] suggesting that SaV-A infection is not uncommon.

Up to now, there is little information on the prevalence of SaV-A in Europe except for one Danish birth cohort study in small children between 6 and 15-months-of-age [8], reporting a possible causality between SaV-A and acute gastroenteritis due to a higher detection rate among ill children.

2. Objectives

To investigate the frequency and impact of SaV-A in feces from patients with symptoms suspicious of acute gastroenteritis.

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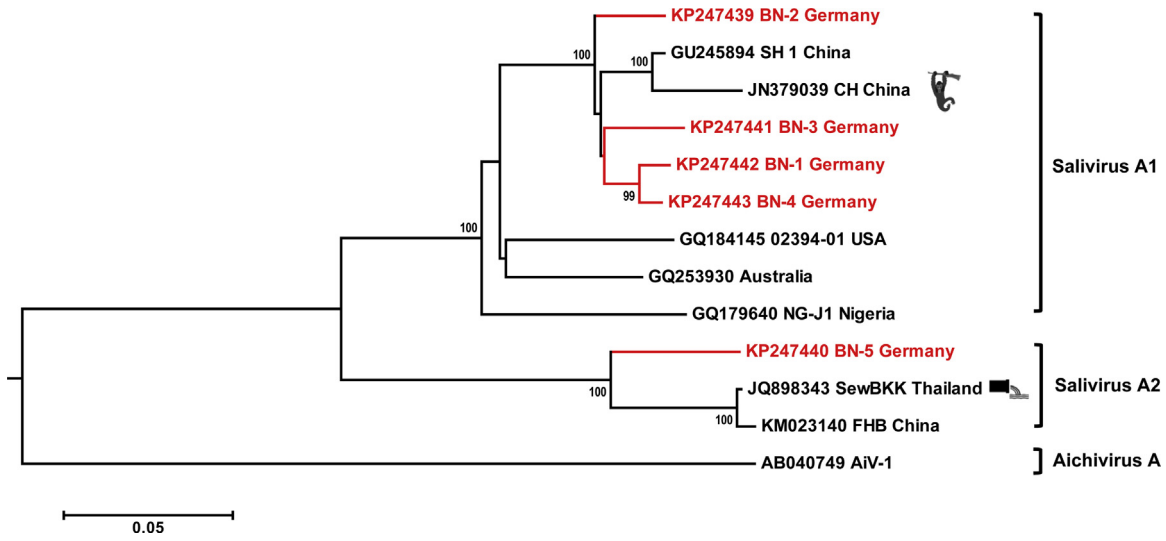

Fig. 1. Phylogenetic tree of salivirus A based on the viral capsid protein 1 (VP1). The tree was constructed in MEGA v6.0 software package ([16]; http://www.megasoftware.net) by using the neighbor-joining method with p-distance and 1000 bootstrap replicates. Bootstrap values of at least 70 are shown at the nodes. Novel viruses from this study are presented in red, previously published saliviruses are shown in black. All salivirus sequences available in the NCBI database covering the whole VP1 gene (nts 2825–3652, numbering according to strain NG-J1, GenBank accession number GQ179640) were used for phylogenetic analysis (last GenBank search, August 20th, 2015). Viruses are given with the GenBank accession numbers, strain names (if available), and countries of origin. Aichivirus 1 was used as outgroup. The icons right to two viruses denote a chimpanzee or sewage as source of the respective virus. Except for these two viruses, all other viruses were from humans. Scale bar indicates percentage uncorrected nucleotide distance.

2.1. Study design

A total of 3019 fecal samples from 1941 hospitalized patients with symptoms of acute gastroenteritis were analyzed. Samples were routinely collected during January 2012–December 2013 at the University Hospital Bonn. Samples were screened by (RT-)PCR for human norovirus, sapovirus, astrovirus, rotavirus, and adenovirus. In total, 774 samples tested positive (for more details, see Supplementary material). The patients' ages were as follows: <1 year, $n = 365$ (18.8%); 1–4 years (y), $n = 257$ (13.2%); 5–9y, $n = 106$ (5.5%); 10–19y, $n = 172$ (8.9%); 20–29y, $n = 83$ (4.3%); 30–39y, $n = 82$ (4.2%); 40–49y, $n = 128$ (6.6%); 50–59y, $n = 167$ (8.6%); ≥60y, $n = 555$ (28.6%); unknown, $n = 26$ (1.3%). The male:female ratio was 1.19:1. Additionally, fecal samples from 204 healthy individuals (76 children/adolescents, 128 adults) were tested (for details, see Supplementary material).

RNA was extracted from 10% fecal suspension in phosphate-buffered saline using the QIAamp Viral RNA Kit (Qiagen). A SaV-A real-time RT-PCR was designed targeting a conserved nucleotide sequence in the 5′-untranslated region. Primers and probe were SaV-F, 5′-CTCTGCTTGGTGCCAACCTC-3′ (nucleotides [nts] 360–379, positions according to GenBank GQ179640), SaV-R, 5′-CTGGTCTGGGACAGCGGAAC-3′ (nts 486–467), SaV-P, FAM-5′-GCGCGCTGCGGGAGTGCTCTTCCC-3′-BHQ-1 (nts 388–411). Reactions were performed using the OneStep RT-PCR Kit (Qiagen) in a volume of 25 μL (for details, see Supplementary material). Thermocycling conditions were 30 min at 50 °C followed by 15 min at 95 °C and 45 cycles of 15 s at 95 °C and 30 s at 60 °C. The sensitivity (LoD_{95}) of the assay was nine copies/reaction ($CI_{95\%}$, 7–14). For SaV-A RNA quantification a standard curve was generated using photometrically quantified *in-vitro*-RNA transcripts of the target region. SaV-A typing was done by sequencing of the virus capsid protein 1 (VP1) gene and the full-length genome (primer sequences available upon request).

3. Results

SaV-A RT-PCR was positive in five stool samples from five patients. No epidemiological connection could be established between the five patients and there was no travel history outside Germany within the preceding three months. Amplification and nucleotide sequencing of the VP1 gene followed by comparison with nucleotide sequences in the NCBI database classified all five virus strains, termed BN-1–BN-5, as SaV-A. The identity of the VP1 sequences with the VP1 sequence of prototype strain NG-J1 was 87.1–88.0% for strains BN-1–BN-4, and 77.4% for strain BN-5. SaV was not detected in healthy individuals.

The phylogenetic relationship between the five SaV-A strains of this study and published saliviruses is depicted in Fig. 1. Strains BN-1–BN-4 showed clustering with two SaV-A1 strains detected in China, one in human and one in chimpanzee feces (unpublished). In contrast, strain BN-5 clustered in a second, more basal branch with SaV-A2 strain SewBKK identified in sewage in Bangkok in 2012 [9] and with a second SaV-A2 strain recently reported in a Chinese child [14].

To verify VP1 typing, we analyzed the full-length genome of one SaV-A1 strain (BN-2) and the SaV-A2 strain BN-5 (BN-2, 7978 nts, BN-5, 7998 nts). Full-length genome analysis confirmed classification and phylogenetic analysis based on VP1 (data not shown).

Virus concentrations in the SaV-A-positive samples ranged between 7.1×10^6 and 7.2×10^8 copies/g feces. None of the five SaV-A-positive feces was positive for the common gastroenteritis viruses tested. From two SaV-A-positive patients, a second stool sample was included in the study, testing SaV-A-negative. From three patients, specimens other than feces were collected at the same day as the SaV-A-positive stool sample, also all testing SaV-A-negative (for details, see Table 1).

The clinical characteristics of the SaV-A-positive patients are shown in Table 1. In patient #1, a bacterial infection was diagnosed which could also explain the clinical features. In patients #2 and #5, gastrointestinal symptoms were explained by non-infectious causes. In patients #3 and #4, respiratory symptoms were present and more severe than gastroenteritis symptoms. In patient #3, the SaV-A-positive stool as well as the second feces specimen collected 20 days after the SaV-A-positive stool contained coxsackievirus B4, able to elicit respiratory symptoms with accompanying gastroenteritis [enterovirus screening and typing according to [10,11]]. In patient #4, a sputum, collected at the same day as the SaV-A-