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Co-circulation of classic and novel astrovirus strains in patients with acute gastroenteritis in Germany

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ABSTRACT

Objectives: In order to analyze the molecular epidemiology of human astroviruses (HAstV) in Germany, a retrospective long-term study was performed to characterize circulating human astrovirus in patients with acute gastroenteritis in Germany.
Methods: A total of 2877 stool samples, collected between January 2010 and December 2015 from sporadic cases and outbreaks of acute gastroenteritis were retrospectively analyzed for astrovirus. A two-step PCR algorithm was developed and used to identify and characterize human astrovirus infections.
Results: Overall, 143 samples were astrovirus-positive (5.0%). Astrovirus infection was most frequently detectable in samples from children of 3–4 years (15%) followed by children of 1–2 years (8.6%), detection rates in adults were lower (1%–3.6%). A high number (71.3%) of co-infections, mainly with noro- or rotaviruses, were identified. Genotyping revealed that at least ten genotypes from all four human MAstV species were circulating in the study population. HAstV-1 was predominant in different age groups. Novel HAstV (MLB and VA genotypes) were also circulating in Germany.
Conclusion: Our findings give new insights into the circulation and genetic diversity of human astroviruses in patients with acute gastroenteritis. The novel HAstV-MLB and -VA genotypes could be characterized firstly in Germany while the analysis showed that these viruses have been dispersed in Germany since 2011 as a causative agent of acute gastroenteritis.

Introduction

Non-bacterial acute gastroenteritis (AGE) is one of the most common public health problems worldwide and can be caused by various viral pathogens, e.g. norovirus (NV), rotavirus (RV), sapovirus (SaV), adenovirus (AdV), and astrovirus (AstV).[1–4]

Astroviruses belong to the family of *Astroviridae*. The virus particles are non-enveloped containing a single-stranded positive-sense RNA. Using electron microscopy astroviruses appear as small round particles (28–30 nm) with a characteristic star-like structure in around 10% of virions (reviewed in Bosch et al (2014)[5]). The RNA genome is 6.2–7.8 kb in size and consists of a 5′ untranslated region with an attached genome-linked protein (VPg protein) and a poly(A) tail. Three open reading frames (ORF) are described for human astroviruses. ORF1a and ORF1b encode the non-structural proteins involved in RNA transcription and replication, e.g. RNA-dependent RNA polymerase, viral protease and VPg protein (reviewed in Bosch et al (2014)[5]). ORF2 encodes the capsid protein. An additional ORF (ORFx) with an alternative start codon overlapping the 5'-end of ORF2 has been proposed with unknown function.[6]

Astroviruses are classified into 2 genera, *Mamastrovirus* (MAstV) and *Avastrovirus* (AAstV), infecting several mammals (e.g. bovine astrovirus, ovine astrovirus, feline astrovirus, mink astrovirus) and birds (e.g. duck astrovirus and turkey astrovirus), respectively (reviewed in De Benedictis et al (2011)[7]). The official 2015 ICTV release (https://talk.ictvonline.org/taxonomy/) distinguishes 19 species within the genus of Mamastrovirus (MAstV-1 to 19) and three species within the genus of Avastrovirus (AAstV-1 to 3). To date, four MAstV species (MAstV-1, MAstV-6, MAstV-8 and MAstV-9) have been identified in human. MAstV-1 consists of the classic human pathogenic genotype 1 to 8 (HAstV). Recently, two new clades of astroviruses have been discovered (MLB and VA), which are highly genetically divergent from previously known strains and more closely related to animal astroviruses. The MLB clade with the strains MLB1, MLB2, and MLB3 was assigned to the MAstV-6 species. MLB1 was first detected in a diarrheic child in Australia.[8] After the recent detection of astroviruses in rats from Hong Kong which are phylogenetically related to the MLB strains a common ancestor has been suggested.[9] Furthermore, a second group of novel HAstV was described in 2009

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named VA/HMO.[10,11] The strains VA-2, VA-4 and VA-5 were grouped in MAstV-8, whereas VA-1 and VA-3 belong to MAstV-9 species.[5]

The transmission of HAstV occurs through the fecal–oral route mainly by person-to-person contact, as well as food- or waterborne transmission.[12,13] HAstV gastroenteritis has been reported worldwide, especially in infants, children and immunocompromised patients[14] with the typical symptoms, e.g., watery diarrhea, and less commonly vomiting, headache, fever, abdominal pain and anorexia. The symptoms are usually milder compared to infections with noroviruses or rotaviruses.[15] The infection is self-limiting within two to four days, but persistent infections in immunocompromised patients[16,17] as well as asymptomatic infections in children have been described.[2,18] A recent report showed that infections with MAstV-1 can spread systemically leading to lethal infections in highly immunocompromised children.[19] The novel described astroviruses (MLB and VA) have been associated with neuronal disorder or encephalitis in immunosuppressed patients.[20–23]

Little is known about the diversity of classic and novel described astroviruses in the German population. However, only four studies on genetic diversity of acute gastroenteritis pathogen including human astroviruses were performed previously in water samples and hospitalized children in Germany.[24–27] To identify recent molecular epidemiological trends regarding astroviruses in Germany, a retrospective long-term study was performed to identify detection rates, seasonal distribution, viral co-infections and genetic diversity of human astrovirus infections in children and adults with acute gastroenteritis for a six-year period in Germany.

Results

Human astrovirus detection and epidemiology

Between 2010 and 2015 a total of 2877 fecal samples were analyzed retrospectively for human astrovirus (HAstV) infections. Of these, 143 samples were HAstV-positive, with an overall detection rate of 5.0%.

Information on the patients' gender was available for 2108 of 2877 samples. In total, samples from 940 male and 1168 female patients were analyzed. The detection rate of astroviruses in males and females of the study population was 44/940 (4.7%) and 64/1168 (5.5%), respectively. No significant difference between the gender and infection rate was found (p = 0.4873).

In 2010 six astrovirus infections were identified in 303 tested samples, resulting in a detection rate of 2.0% (Table 1). In the following three years, the detection rate increased to a maximum of 12.1% in 2013 with 47/387 HAstV-positive samples. Thereafter the detection rate decreased to 2.9% in 2015.

The study population included samples from children as well as from adults. Information about the patients' age was available for 1966 out of 2877 samples. The mean age of patients with astrovirus infections was 17.5 years (3 months to 102 years, median age was 2.1 years). In the age group of 3–4 years the most frequent human astrovirus-positive samples (15/100, 15.0%) were detected followed by children of age 1–2 years with 50/582 (8.6%) (Fig. 1). In the age groups of adult patients (20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and >80 years) the detection rate ranged between 1% in adults of 70–79 years (1/103) and 3.6% in 50–59 year old adults (3/83). In patients of 10–19 years no astrovirus infection was found.

Table 1
Detection rates of human astroviruses (HAstV) infection among children and adults with acute gastroenteritis in Germany from 2010 to 2015.

Year	Number of HAstV-positive samples/number of samples investigated/detection rate	Number of HAstV-positive samples in male/female patients/unknown gender
2010	6/303/2.0%	1/1/4
2011	27/501/5.4%	13/10/4
2012	14/311/4.5%	7/6/1
2013	47/387/12.1%	10/25/12
2014	22/449/4.9%	6/11/5
2015	27/926/2.9%	7/11/9

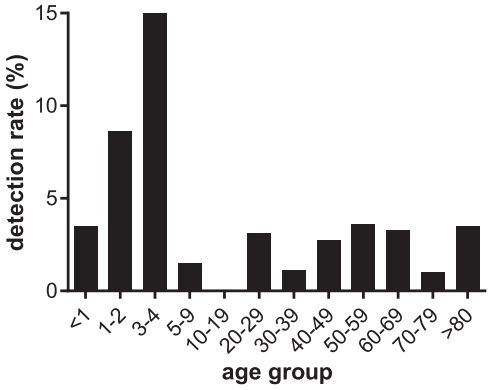

Fig. 1. Detection rates of human astrovirus infection in different age groups between 2010 and 2015 in Germany.

Nearly all age groups were affected by human astroviruses in 2013, where the detection rate was more than twice as high as compared to the previous and following seasons. Detection of astroviruses was most frequent in infants of 1–2 years (8/47 PCR-positive samples) and in patients of >80 years (9/47 PCR-positive samples). Only the age groups between 5–9 years and 20–29 years were HAstV-negative in 2013 which changed in 2014 and 2015 where both age groups were also affected by astroviruses.

Seasonality of human astrovirus infections

Little is known about the seasonality of human astrovirus infections due to the small numbers of positive samples reported in previous studies. In the present study the date of collection was available for 1747 of the 2877 tested samples, among these 104 samples were tested positive for human astrovirus and 1643 were negative. During the six-year period (2010–2015) of this study, the highest detection rates were detected between October and May, with the exception of January, and ranged between 5.3% in October and 11.0% in February. Within this timeframe 86.5% of all human astrovirus infections were detected. The detection rate decreased in the summer months from June to September and ranged between 2.0% and 3.5% (Fig. 2).

Detection of mixed viral infections

All 2877 samples investigated in this study were pretested for the presence of noroviruses or rotaviruses and in a second step they were retrospectively analyzed for astroviruses and sapoviruses. Of the 143 human astrovirus-positive samples, 41 samples (28.7%) showed mono-infection. A total of 102 astrovirus-positive samples were also positive for at least one other enteric virus (71.3%). From the norovirus-positive samples, 49 samples were also positive for astrovirus (49/1103, 4.4%). Altogether 44 samples showed mixed infections of astrovirus and rotavirus, representing 5.3% of all 828 rotavirus-positive samples. A mixture of sapovirus and human astrovirus was detected in one sample.

In seven patients mixed infections with three different viral gastroenteritis pathogens were detected. Two samples showed co-infections with astrovirus, norovirus and rotavirus. Five patients were positive for astrovirus, norovirus and sapovirus. One sample was