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BRIEF REPORT

Aichi virus infection in elderly people in Sweden

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Abstract Aichi virus (AiV), genus *Kobuvirus*, family *Picornaviridae*, is associated with gastroenteritis in humans. Previous studies have shown high seroprevalence but low incidence (0.9-4.1%) in clinical samples. We report here the first detection of AiV in Sweden. Two hundred twenty-one specimens from hospitalized patients with diarrhea, who were negative for other enteric viruses, were included in the study. AiV were detected in three specimens, all from elderly patients. Phylogenetic analysis revealed that the three Swedish isolates belonged to genotype A and were genetically closest to European and Asian strains of AiV.

Keywords Kobuvirus · Aichivirus · Sweden · Gastroenteritis

Acute gastroenteritis is one of the most common diseases and causes of death worldwide. Rotaviruses, caliciviruses, adenoviruses and astroviruses have been recognized as the main viral agents responsible for this condition. There is, however, a large diagnostic gap, resulting in the etiologic agent not being identified in approximately a third of all specimens, and it has been suggested that other viruses, including picornaviruses, are involved [1].

Aichi virus (AiV) was first discovered in 1989 during a search for an infectious agent in an outbreak of oyster-associated non-bacterial gastroenteritis [2]. The isolated virus was later classified as the first member of a novel genus, *Kobuvirus*, of the family *Picornaviridae* [3]. Kobuviruses have since then been associated with diarrhea not only in humans but also in cattle [4, 5] and swine [6, 7].

Since its discovery, AiV has been detected with an incidence of 0.9-4.1%, primarily when studying outbreaks of diarrhea in children or young adults [8–12]. AiV seems to be circulating worldwide, and detection of AiV have been reported in Asia [13], South America [14], Europe [8–10, 14] and Africa [11]. Previous studies have suggested oysters, or other seafood, to be the source of AiV infections [2, 8, 15–17], but its occurrence has also been reported in sewage [15, 18], which indicates that kobuviruses might use the fecal-oral route of infection, like several other picornaviruses. Studies in Japan [19], European countries [14, 20, 21] and Tunisia [22] show high seroprevalence, with antibodies against AiV in at least 80% of the adult population, a result that stands in contrast with the low incidence reports. The incongruity between rare isolation and high seroprevalence suggests that the virus might circulate without causing any symptoms or might cause weakly symptomatic infections that do not require medical attention. It is also possible that it co-circulates with other enteric viruses that cause gastroenteritis and has therefore not been detected during screening of patients [8, 11, 14]. The high seroprevalence data suggest a general exposition of the virus, but the importance of AiV in human diseases needs to be further studied.

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Studies of AiV have primarily been performed using reverse transcription followed by PCR of a 519-bp fragment from the 3CD junction, a genomic region coding for the viral protease $3C^{pro}$ and the RNA polymerase $3D^{pol}$ [23].

In this paper, we report the first detection of AiV in Sweden. In addition, this is the first paper describing the presence of AiV in elderly patients.

RNA was isolated from 221 stool samples from hospitalized patients suffering from diarrhea at Linköping University Hospital, Sweden, who were negative for adenovirus, calicivirus and rotavirus. The isolation was done from January to June 2009 without taking the age of the patients into consideration. Nucleic acid was extracted from 200 µl of a 10% particle-cleared fecal suspension using a MagAttract Viral RNA M48 kit (QIAGEN) according to the manufacturer's instructions and eluted in a volume of 75 µl. Extracted viral RNA was reverse transcribed by random priming using an Applied Biosystems TagMan reverse transcriptase kit according to the manufacturer′s protocol. The screening for AiV was performed using a nested PCR, first amplifying 519 bp in the 3CD junction with primers 6261 (5′-ACACTCCCACCTCCCG CCAGTA-3′) and 6779 (5′-GGAAGAGCTGGGTGTCA-AGA-3′) as described previously [23]. Next, a nested PCR amplifying a 223-bp segment within the 3CD junction was carried out using primers C94b (5′-GACTTCCCCGGAGT CGTCGTCT-3′) and 246 k (5′-GACATCCGGTTGACGT TGAC-3′) [13]. Additional PCR to amplify VP1 was performed using primers CapE (5′-CTAGTCGGACCCACAC CGC-3′) [24] and R3636 (5′-GCAAGAGAGCTGGAAGT-3′) [8] on samples positive for AiV. The nucleotide sequences of the PCR products were determined using an ABI Prism 3130 automated sequencer and BigDye chemistry (Applied Biosystems). The sequences obtained were analyzed using the Sequencher, version 4.6, software package (Gene Codes Corporation). AiV sequences were deposited in GenBank with the accession numbers JN584163-65 for the 3CD junction and JN584162 for the VP1 sequence. Nucleotide sequences were aligned using ClustalW [25], and phylogenetic signals were evaluated by likelihood mapping [26]. The phylogenetic relationships between the different AiV strains based on the 3CD junction and VP1 were constructed using the maximum-likelihood method as implemented in PhyML [27] and evaluated by non-parametric bootstrap analysis consisting of 1000 pseudo-replicates [28]. The general time-reversible (GTR) substitution, determined by the Modeltest program, version 3.7 [29], with gamma-distributed rate of heterogenicity among nucleotide positions was used for the analysis. The phylogenetic relationship was also examined by the neighbor-joining method as implemented in MEGA, version 4.0 [30]. Trees were visualized in MEGA.

In this report, we describe the first cases of AiV in Sweden and, to our knowledge, the first report of AiV associated with diarrhea in elderly people. Our findings that 1.4% of the samples were positive for AiV are in accordance with previous observations [8–10, 31].

AiV belongs to the genus *Kobuvirus*, a growing genus in the family *Picornaviridae*, whose members infect not only humans but also cattle [4, 5], swine [6], canines [32], sheep [33] and mice [34]. Since the discovery of AiV in 1988, the virus, or antibodies against it, has been detected worldwide, although knowledge about its epidemiology is still limited. Seroprevalence studies show that 80-95% of the population in their thirties has antibodies against AiV [14, 20, 21], which indicates a general and frequent circulation of the virus among humans. The reported incidence of AiV is in general low, but is significantly higher in outbreaks of gastroenteritis associated with shellfish [8, 11]. However, the high seroprevalence, even in the very young population, indicates the possibility of other transmission routes. AiV has recently been detected in sewage and sewage-polluted water in Venezuela [18] and Tunisia [15], which indicates a circulation of AiV in the environment.

AiV has been associated with gastroenteritis in children and young adults in Germany [14], France [8], Hungary [10], Tunisia [35], Pakistan [12], China [31] and Finland [9], but also in different age groups where gastroenteritis has most often been associated with food consumption [8, 12, 14, 16, 36]. The age distribution of the patients included in the present report shows that a vast majority of the 221 patients studied were over 65 years of age (Fig. 1; positive samples are indicated by arrows). All patients were

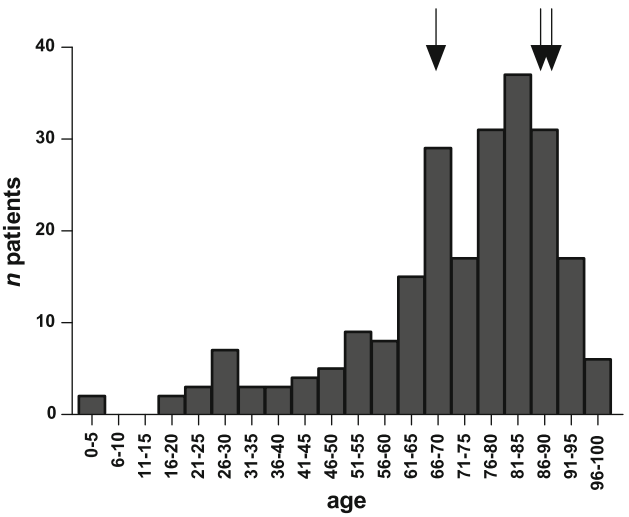

Fig. 1 Age distribution of patients included in the screening of AiV. Two hundred twenty-one samples that previously had been reported to be negative for rotaviruses, caliciviruses and adenoviruses were screened for AiV using a nested PCR in the 3CD junction. Arrows indicate patients who were positive for AiV

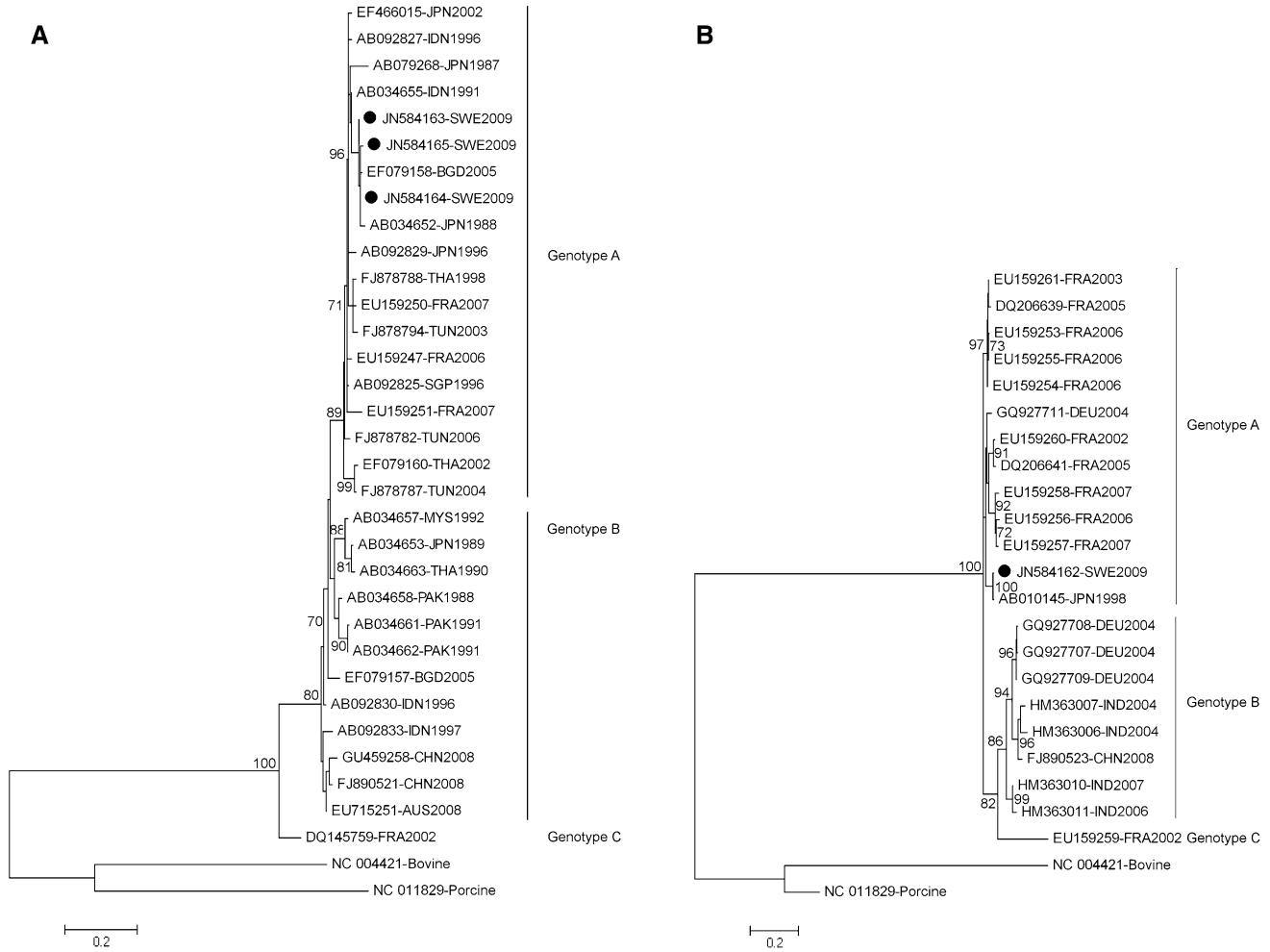

Fig. 2 Phylogenetic trees representing the relationship of Aichi virus strains. (**A**) Phylogram based on the ML method using a 225-nt sequence from the 3CD junction (GenBank accession numbers JN584163-65 for isolates detected in this study) (**B**) Phylogeny of 500 nt of the VP1 gene (GenBank accession number JN584162 for the isolate presented in this study). Both ML trees were evaluated by non-parametric bootstrap analysis with 1,000 pseudo-replicates. Only bootstrap values $\geq$70% are shown. Scale bars represent the genetic distance (nucleotide substitutions per site). The Swedish AiV isolates are indicated by black dots. Abbreviations in isolate names: AUS, Australia; BGD, Bangladesh; CHN, China; DEU, Germany; FRA, France; IDN, Indonesia; IND, India; JPN, Japan; PAK, Pakistan; SGP, Singapore; SWE, Sweden; THA, Thailand; TUN, Tunisia; and VNM, Vietnam

hospitalized and had diarrhea, and standard screening for enteric viruses gave negative results. The majority of previous studies have been performed on children and young adults, which are age groups that are rarely hospitalized with diarrhea in Sweden. Therefore, the majority of the samples in this study originate from elderly people. Similar to our study, Drexler et al. [36] recently presented a German study of patients with approximately the same distribution of age as in this paper. AiV was detected in 2% of the patients, with age ranging from children to adults in their forties.

In this report, we identified three Swedish samples that were positive for AiV, all from elderly people. From patient one, an 88-year-old male, we were able to generate sequence data from the 3CD junction as well as the gene coding for the structural protein VP1. This patient was hospitalized because of deep venous thrombosis with secondary lung embolism. After three days of diarrhea and fever, a fecal sample was sent for viral diagnostics. Patient two was a 68-year-old female hospitalized in the intensive care unit because of trauma with multiple fractures, pneumothorax and splenectomy. She was initially constipated and treated with laxatives; a fecal sample was taken because of diarrhea secondary to this treatment. Hence, this patient had a plausible reason for diarrhea other than that of viral origin. Patient three, an 88-year-old male with diabetes type 2 and cardiovascular disease, was hospitalized because of fever, diarrhea, vomiting and skin eruptions two

to three days prior to hospitalization. He developed high fever and was diagnosed with generalized herpes zoster and sepsis caused by Gram-negative bacteria.

Yamashita et al. [23] has suggested that AiV isolates can be divided into two groups, genotype A and B, based on sequence analysis of a 519-bp sequence in the 3CD junction. In addition to these groups, a third genotype, genotype C, has been suggested [8], comprising only one isolate so far. The genotype classification has been confirmed, and it has been shown that there is a good correlation between phylogenetic trees from both the well-conserved 3CD and VP1, which has higher genetic diversity [8, 37]. The AiV sequences available indicate that the genotype classification can be based on either genomic region. However, molecular typing of the VP1 sequence is the golden standard used for the classification of picornavirus (sero) types [38–40]. The capsid region of genotype B includes three nucleotide insertions within the VP0 region that are not present in genotype A [3, 14, 24]. Genetic and phylogenetic analysis of the three Swedish AiV strains identified in this study showed that they were members of genotype A (Fig. 2), which is the most common genotype found in Europe and Japan [8]. All isolates were genetically close to each other and to previously reported Asian strains.

In conclusion, AiV was detected in three elderly hospitalized patients with diarrhea in Sweden with the same incidence as was previously described for children and young adults in other countries. Our results indicate that AiV may be associated with a limited number of diarrhea cases even in elderly patients. The discrepancies between the high seroprevalence and low incidence of detection could be due to AiV infections being mainly asymptomatic, a lack of appropriate detection assays, or a lack of detection due to infections with other viruses. AiV remains rare, and the significance of AiV as a human pathogen is a question that remains to be further investigated.

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Conflict of interest The authors state that no financial or personal relationships exist with other people or organizations that could inappropriately influence the present report.

References

1. Glass RI, Bresee J, Jiang B, Gentsch J, Ando T, Fankhauser R, Noel J, Parashar U, Rosen B, Monroe SS (2001) Gastroenteritis viruses: an overview. Novartis Found Symp 238:5–19 (discussion 19–25)
2. Yamashita T, Kobayashi S, Sakae K, Nakata S, Chiba S, Ishihara Y, Isomura S (1991) Isolation of cytopathic small round viruses with BS-C-1 cells from patients with gastroenteritis. J Infect Dis 164(5):954–957
3. Yamashita T, Sakae K, Tsuzuki H, Suzuki Y, Ishikawa N, Takeda N, Miyamura T, Yamazaki S (1998) Complete nucleotide sequence and genetic organization of Aichi virus, a distinct member of the Picornaviridae associated with acute gastroenteritis in humans. J Virol 72(10):8408–8412
4. Yamashita T, Ito M, Kabashima Y, Tsuzuki H, Fujiura A, Sakae K (2003) Isolation and characterization of a new species of kobuvirus associated with cattle. J Gen Virol 84(Pt 11):3069–3077
5. Reuter G, Egyed L (2009) Bovine kobuvirus in europe. Emerg Infect Dis 15(5):822–823
6. Reuter G, Boldizsar A, Kiss I, Pankovics P (2008) Candidate new species of Kobuvirus in porcine hosts. Emerg Infect Dis 14(12):1968–1970
7. Reuter G, Boldizsar A, Pankovics P (2009) Complete nucleotide and amino acid sequences and genetic organization of porcine kobuvirus, a member of a new species in the genus Kobuvirus, family Picornaviridae. Arch Virol 154(1):101–108. doi:10.1007/s00705-008-0288-2
8. Ambert-Balay K, Lorrot M, Bon F, Giraudon H, Kaplon J, Wolfer M, Lebon P, Gendrel D, Pothier P (2008) Prevalence and genetic diversity of Aichi virus strains in stool samples from community and hospitalized patients. J Clin Microbiol 46(4):1252–1258. doi:10.1128/JCM.02140-07
9. Kaikkonen S, Rasanen S, Ramet M, Vesikari T (2010) Aichi virus infection in children with acute gastroenteritis in Finland. Epidemiol Infect 138(8):1166–1171. doi:10.1017/S0950268809991300
10. Reuter G, Boldizsar A, Papp G, Pankovics P (2009) Detection of Aichi virus shedding in a child with enteric and extraintestinal symptoms in Hungary. Arch Virol 154(9):1529–1532. doi:10.1007/s00705-009-0473-y
11. Sdiri-Loulizi K, Hassine M, Gharbi-Khelifi H, Sakly N, Chouchane S, Guediche MN, Pothier P, Aouni M, Ambert-Balay K (2009) Detection and genomic characterization of Aichi viruses in stool samples from children in Monastir, Tunisia. J Clin Microbiol 47(7):2275–2278. doi:10.1128/JCM.00913-09
12. Yamashita T, Sakae K, Kobayashi S, Ishihara Y, Miyake T, Mubina A, Isomura S (1995) Isolation of cytopathic small round virus (Aichi virus) from Pakistani children and Japanese travelers from Southeast Asia. Microbiol Immunol 39(6):433–435
13. Pham NT, Khamrin P, Nguyen TA, Kanti DS, Phan TG, Okitsu S, Ushijima H (2007) Isolation and molecular characterization of Aichi viruses from fecal specimens collected in Japan, Bangladesh, Thailand, and Vietnam. J Clin Microbiol 45(7):2287–2288. doi:10.1128/JCM.00525-07
14. Oh DY, Silva PA, Hauroeder B, Diedrich S, Cardoso DD, Schreier E (2006) Molecular characterization of the first Aichi viruses isolated in Europe and in South America. Arch Virol 151(6):1199–1206. doi:10.1007/s00705-005-0706-7
15. Sdiri-Loulizi K, Hassine M, Aouni Z, Gharbi-Khelifi H, Sakly N, Chouchane S, Guediche MN, Pothier P, Aouni M, Ambert-Balay K (2010) First molecular detection of Aichi virus in sewage and shellfish samples in the Monastir region of Tunisia. Arch Virol 155(9):1509–1513. doi:10.1007/s00705-010-0744-7
16. Le Guyader FS, Le Saux JC, Ambert-Balay K, Krol J, Serais O, Parnaudeau S, Giraudon H, Delmas G, Pommepuy M, Pothier P, Atmar RL (2008) Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. J Clin Microbiol 46(12):4011–4017. doi:10.1128/JCM.01044-08
17. Hansman GS, Oka T, Li TC, Nishio O, Noda M, Takeda N (2008) Detection of human enteric viruses in Japanese clams. J Food Prot 71(8):1689–1695

18. Alcala A, Vizzi E, Rodriguez-Diaz J, Zambrano JL, Betancourt W, Liprandi F (2010) Molecular detection and characterization of Aichi viruses in sewage-polluted waters of Venezuela. Appl Environ Microbiol 76(12):4113–4115. doi:10.1128/AEM.00501-10
19. Yamashita T, Sakae K, Ishihara Y, Isomura S, Utagawa E (1993) Prevalence of newly isolated, cytopathic small round virus (Aichi strain) in Japan. J Clin Microbiol 31(11):2938–2943
20. Goyer M, Aho LS, Bour JB, Ambert-Balay K, Pothier P (2008) Seroprevalence distribution of Aichi virus among a French population in 2006–2007. Arch Virol 153(6):1171–1174. doi:10.1007/s00705-008-0091-0
21. Ribes JM, Montava R, Tellez-Castillo CJ, Fernandez-Jimenez M (2008) Buesa J (2010) Seroprevalence of Aichi virus in a Spanish population from 2007 to. Clin Vaccine Immunol 17(4):545–549. doi:10.1128/CVI.00382-09
22. Sdiri-Loulizi K, Hassine M, Bour JB, Ambert-Balay K, Mastouri M, Aho LS, Gharbi-Khelifi H, Aouni Z, Sakly N, Chouchane S, Neji-Guediche M, Pothier P, Aouni M (2010) Aichi virus IgG seroprevalence in Tunisia parallels genomic detection and clinical presentation in children with gastroenteritis. Clin Vaccine Immunol 17(7):1111–1116. doi:10.1128/CVI.00059-10
23. Yamashita T, Sugiyama M, Tsuzuki H, Sakae K, Suzuki Y, Miyazaki Y (2000) Application of a reverse transcription-PCR for identification and differentiation of Aichi virus, a new member of the Picornavirus family associated with gastroenteritis in humans. J Clin Microbiol 38(8):2955–2961
24. Pham NT, Trinh QD, Khamrin P, Nguyen TA, Dey SK, Phan TG, le Hoang P, Maneekarn N, Okitsu S, Mizuguchi M, Ushijima H (2008) Sequence analysis of the capsid gene of Aichi viruses detected from Japan, Bangladesh, Thailand, and Vietnam. J Med Virol 80(7):1222–1227. doi:10.1002/jmv.21193
25. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22(22):4673–4680
26. Strimmer K, von Haeseler A (1997) Likelihood-mapping: a simple method to visualize phylogenetic content of a sequence alignment. Proc Natl Acad Sci USA 94(13):6815–6819
27. Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52(5):696–704 (pii:54QHX07WB5K5XCX4)
28. Felsenstein J (1985) Confidence limits on phylogenies: an approach using bootstrap. Evolution 39(4):783–791
29. Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14(9):817–818 (pii:btb117)
30. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24(8):1596–1599. doi:10.1093/molbev/msm092
31. Yang S, Zhang W, Shen Q, Yang Z, Zhu J, Cui L, Hua X (2009) Aichi virus strains in children with gastroenteritis, China. Emerg Infect Dis 15(10):1703–1705
32. Li L, Pesavento PA, Shan T, Leutenegger CM, Wang C, Delwart E (2011) Viruses in diarrhoeic dogs include novel kobuviruses and sapoviruses. J Gen Virol 92(Pt 11):2534–2541. doi:10.1099/vir.0.034611-0
33. Barry AF, Ribeiro J, Alfieri AF, van der Poel WH, Alfieri AA (2011) First detection of kobuvirus in farm animals in Brazil and the Netherlands. Infect Genet Evol 11(7):1811–1814. doi:10.1016/j.meegid.2011.06.020
34. Phan TG, Kapusinszky B, Wang C, Rose RK, Lipton HL, Delwart EL (2011) The fecal viral flora of wild rodents. PLoS Pathog 7(9):e1002218. doi:10.1371/journal.ppat.1002218
35. Sdiri-Loulizi K, Gharbi-Khelifi H, de Rougemont A, Chouchane S, Sakly N, Ambert-Balay K, Hassine M, Guediche MN, Aouni M, Pothier P (2008) Acute infantile gastroenteritis associated with human enteric viruses in Tunisia. J Clin Microbiol 46(4):1349–1355. doi:10.1128/JCM.02438-07
36. Drexler JF, Baumgarte S, de Souza Luna LK, Eschbach-Bludau M, Lukashev AN, Drosten C (2011) Aichi virus shedding in high concentrations in patients with acute diarrhea. Emerg Infect Dis 17(8):1544–1548. doi:10.3201/eid1708.101556
37. Pham NT, Trinh QD, Nguyen TA, Dey SK, Phan TG, le Hoang P, Khamrin P, Maneekarn N, Okitsu S, Mizuguchi M, Ushijima H (2009) Development of genotype-specific primers for differentiation of genotypes A and B of Aichi viruses. J Virol Methods 156(1–2):107–110. doi:10.1016/j.jviromet.2008.11.005
38. Caro V, Guillot S, Delpeyroux F, Crainic R (2001) Molecular strategy for 'serotyping' of human enteroviruses. J Gen Virol 82(Pt 1):79–91
39. Casas I, Palacios GF, Trallero G, Cisterna D, Freire MC, Tenorio A (2001) Molecular characterization of human enteroviruses in clinical samples: comparison between VP2, VP1, and RNA polymerase regions using RT nested PCR assays and direct sequencing of products. J Med Virol 65(1):138–148. doi:10.1002/jmv.2013
40. Oberste MS, Maher K, Kilpatrick DR, Pallansch MA (1999) Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. J Virol 73(3):1941–1948