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BRIEF COMMUNICATION

The First Molecular Detection of Aichi Virus 1 in Raw Sewage and Mussels Collected in South Africa

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Abstract
Aichi virus 1 (AiV-1) has a worldwide distribution and is associated with gastroenteritis in humans. In this study, raw sewage and mussel samples were analyzed for the presence of AiV-1 using reverse transcription-PCR (RT-PCR). Amplification and sequencing of the 3CD and VP1 genomic regions followed by phylogenetic analysis using selected genome sequences revealed the presence of AiV-1, genotype B. The results highlight the importance of further screening to evaluate the prevalence and epidemiology of this clinically important virus in South Africa.

Keywords Aichi virus 1 · Kobuvirus · Mussels · Waste water · Picornaviridae

Introduction

Aichi virus (AiV, genus Kobuvirus, family Picornaviridae), is a small non-enveloped positive-sense single stranded RNA (+ssRNA) virus with a genome of ~8400 nucleotides (nt) (Yamashita et al. 1998; Reuter et al. 2011; Kitajima and Gerba 2015). AiV was first isolated in 1989 following outbreaks of oyster-associated gastroenteritis in Japan (Yamashita et al. 1991) and the full genome was sequenced in 1998 (Yamashita et al. 1998). AiV-1, the causative agent of human gastroenteritis, belongs to Aichi virus host species A (Yamashita et al. 2000; Ambert-Balay et al. 2008). Reverse transcription-PCR of the genetically diverse nucleotide sequences encoding the VP1 capsid protein is typically used to identify and classify picornaviruses (Oberste et al. 1999). More commonly, AiV-1 is classified using the conserved nucleotide sequences of the 3C and 3D (3CD) junction region, into three genetically distinct genotypes (A, B, and C) (Yamashita et al. 2000; Ambert-Balay et al. 2008).

AiV-1 is transmitted by the fecal-oral route through contaminated water, raw and treated sewage, and shellfish consumption (Kitajima and Gerba 2015). The virus has been reported from several regions of the world including Asia (Pham et al. 2008; Yang et al. 2009; Kitajima et al. 2011; Saikruang et al. 2014; Yip et al. 2014; Chuchaona et al. 2017), Europe (Oh et al. 2006; Le Guyader et al. 2008; Reuter et al. 2009; Kaikkonen et al. 2010; Di Martino et al. 2013; Lodder et al. 2013), South Americas (Oh et al. 2006; Alcalá et al. 2010), and Africa (Sdiri-Loulizi et al. 2008, 2009). The frequency with which AiV-1 is detected in environmental samples, the association of the virus with water and food borne gastroenteritis and its widespread geographic distribution suggest that this virus is a globally important emerging pathogen (Kitajima and Gerba 2015).

In South Africa, waterborne infection remains a major cause of death in lower socio-economic groups (Revelas et al. 2012). Interestingly, several "candidate" viruses have been documented in this region: rotavirus (Schoub et al. 1976, 1977; Steele et al. 1988; Knox et al. 2012), norovirus (NoV) (Taylor et al. 1996; Smit et al. 1999), human adenovirus (HAdV) (Vos and Knox 2017), human bocavirus (HBoV) (Smuts and Hardie 2006; Venter et al. 2011; Subramoney et al. 2018), and more recently, sapovirus (Page et al. 2016). Despite its worldwide distribution, to date, no published reports of AiV-1 prevalence and its potential association with gastroenteritis in South Africa exist. In an attempt to initiate screening studies, we aimed to recover and detect AiV-1 in raw sewage and mussel samples using sequence analysis of the 3CD and VP1 genomic regions.

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Materials and Methods

During May 2016, raw sewage was collected by swabbing influent separation grids at the Makana Wastewater Treatment Plant in Grahamstown, Eastern Cape, South Africa. 120 swab samples were collected, pooled into groups of 10 and processed as described by Di Martino et al. (2013). Mussels were collected from the Swartkops River in Port Elizabeth, Eastern Cape, South Africa (coordinates: 33°51′36.7″ S, 25°37′12.0″ E) and pooled into groups of 5. Mussel collection was conducted under the license of C McQuaid (Department of Zoology and Entomology, Rhodes University), field permit RES2014/12, issued by the Department of Agriculture, Forestry and Fisheries of South Africa. Preparation of mussel samples was performed following a method described by Pina et al. (1998) with some modification. Briefly, 10 g of tissue was homogenized and eluted in 20 ml 0.25 N glycine buffer (pH 10) containing NP-40 with a final concentration of 1%. Debris was removed by centrifugation at 9000×*g* for 3 min and the supernatant was sonicated at 60 kHz for 15 s prior to filtration

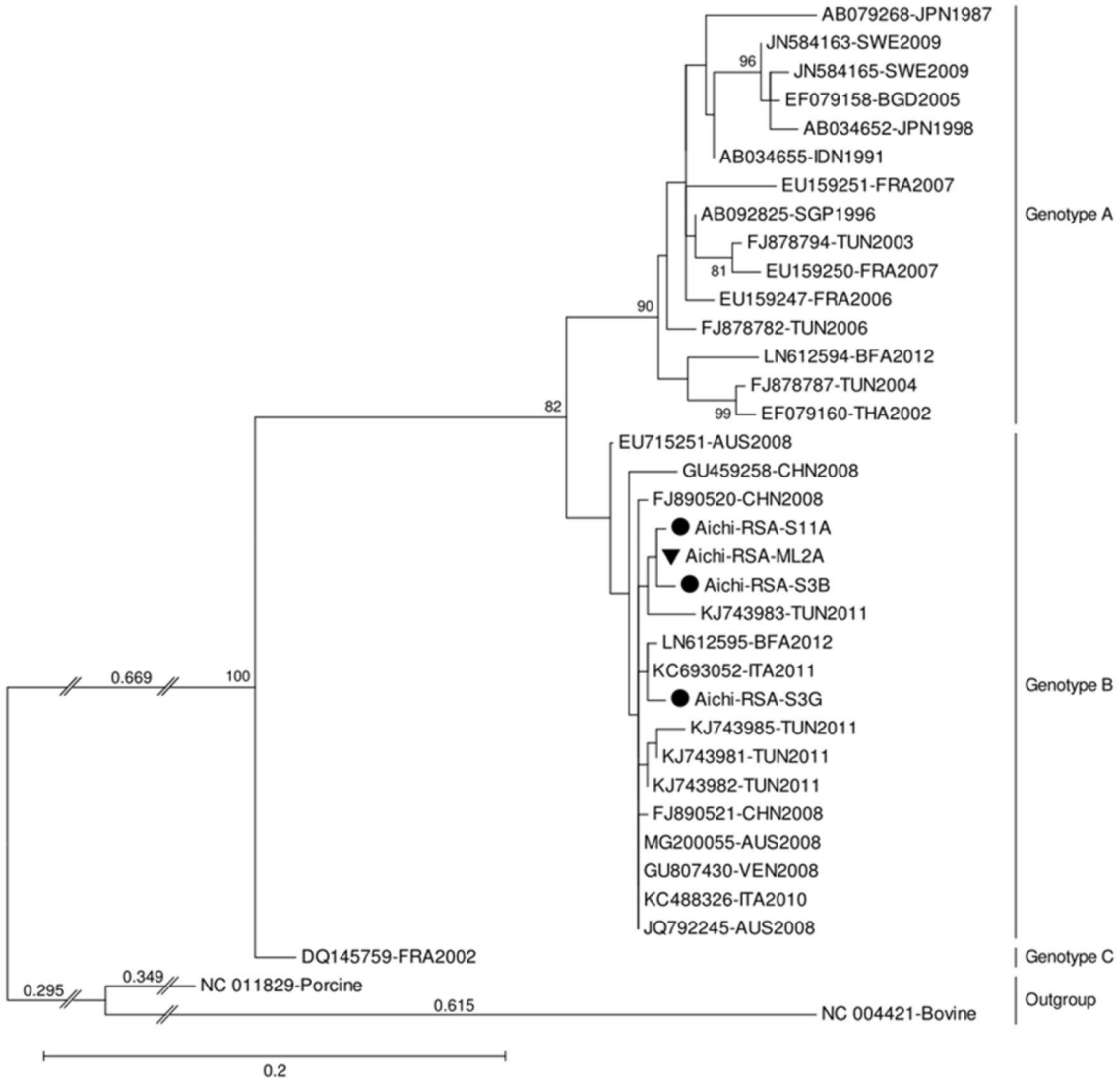

Fig. 1 Phylogenetic analysis of AiV-1 detected in sewage and mussel samples from South Africa, based on a 263-nt segment of the 3CD junction. The tree was constructed using the Maximum Likelihood method based on the Kimura 2-parameter model, implemented in the package MEGA 7. A total of 1000 bootstrap replicate trees were generated with the Neighbor-Join and BioNJ algorithms. Only bootstrap values ≥ 70% are shown. Scale bar represents the genetic distance (nucleotide substitutions per site). Where branches have been truncated//genetic distances are indicated above. The South African AiV-1 strains detected in three sewage samples are indicated by filled circles. Filled triangle represents AiV-1 sequences from all mussel samples and one sewage sample that shared 100% sequence similarity. Abbreviations in isolate names: AUS—Australia; BFA—Burkina Faso; BGD—Bangladesh; CHN—China; FRA—France; IDN—Indonesia; ITA—Italy; JPN—Japan; RSA—South Africa; SGP—Singapore; SWE—Sweden; THA—Thailand; TUN—Tunisia; VEN—Venezuela

through a 0.22 µM membrane (GVS filter technology, USA). Viral RNA was extracted from processed swab and mussel samples using the QIAamp® Viral RNA Mini Kit (Qiagen, USA) following manufacturer's instructions. Molecular detection of AiV involved RT-PCR amplification of a 266 bp region of the 3CD junction using primers AiV-C94b and AiV-246K (Yamashita et al. 2000). To increase sensitivity, a semi-nested RT-PCR assay producing a 472 bp product of the VP1 region was then applied using primers (first round) CapE (Pham et al. 2008) and VP1-IR 5′-GGGATGGAAAAGGAGACCAT (this study) and primers (second round) AiV-VP1-F2 (Lodder et al. 2013) and VP1-IR. The Verso 1-Step RT-PCR Hot-Start Kit (Thermo Scientific, USA) was used according to manufacturer's instructions. PCR products were cloned into pJET 1.2/blunt (CloneJET™ PCR Cloning Kit; Thermo Scientific, USA) prior to commercial sequencing (Inqaba Biotechnical Industries Pty. Ltd, Pretoria, South Africa). Resultant nucleotide sequences were trimmed and aligned (ClustalW) (Thompson et al. 1994), and phylogenetic relationships determined using MEGA 7 software (Kumar et al. 2016).

Results and Discussion

Out of 12 pooled sewage and mussel samples analyzed, 10 and 8 were positive for AiV-1 respectively, using primers targeting the 3CD region of the genome. Two additional positive sewage samples were obtained by RT-PCR amplification of the VP1 region. Selected amplicons were cloned and sequenced. Phylogenetic analysis of partial 3CD and VP1 nucleotide sequences revealed that all South African sequences were within genotype B. Based on the partial 3CD sequences; AiV-1 detected in some samples formed a tight cluster together and were most closely related to a Tunisian strain. AiV-1 from one sewage sample (Aichi-RSA-S3G) clustered with strains from Burkina Faso and Italy (Fig. 1). Based on the analysis of the 472-nt segment of VP1, AiV-1 detected in sewage samples (AiV-VP1-RSA-S11 and AiV-VP1-RSA-S9) formed a tight cluster together and were mostly related to a Vietnamese strain (Fig. 2). Sequence analysis showed that 3CD from all mussel samples and one

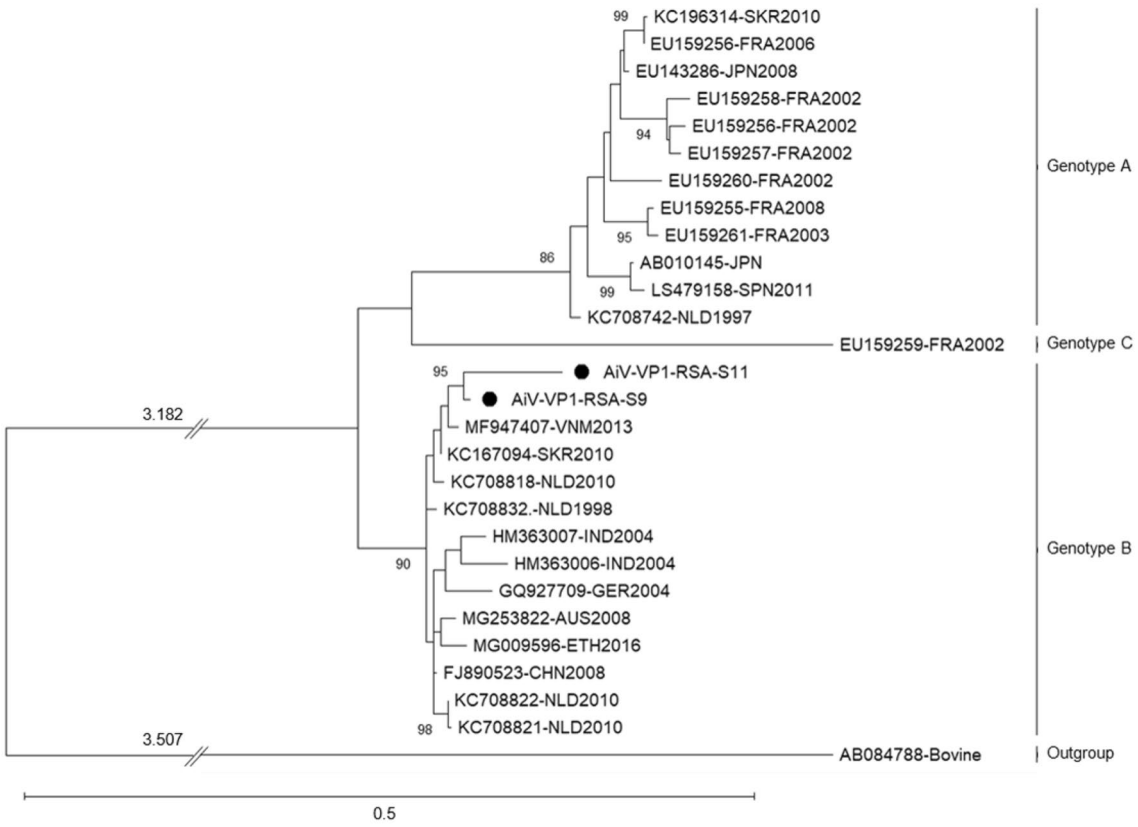

Fig. 2 Phylogenetic analysis of AiV-1 detected in sewage samples from South Africa, based on a 472-nt segment of VP1. The tree was constructed using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model, implemented in the package MEGA 7. A total of 1000 bootstrap replicate trees were generated with the Neighbor-Join and BioNJ algorithms. Only bootstrap values ≥ 70% are shown. Scale bar represents the genetic distance (nucleotide substitutions per site). Where branches have been truncated//genetic distances are indicated above. The South African AiV-1 strains detected in two sewage samples are indicated by filled circles. Abbreviations in isolate names: AUS—Australia; CHN—China; ETH—Ethiopia; FRA—France; GER—Germany; IND—India; JPN—Japan; NLD—The Netherlands; RSA—South Africa; SKR—South Korea; SPN—Spain; VNM—Vietnam

sewage sample shared 100% sequence identity (represented by Aichi-RSA-ML2A), while sequences from the other sewage samples shared 98.1–99.8% identity. The identity between the two VP1 sequences from sewage samples was 94%. The nucleotide sequences were deposited in the GenBank database (MK059396–MK059401).

The results of this study support others in providing further evidence for the circulation of diverse and related AiV-1 strains (notably genotype B) on a global scale [see for example, Alcalá et al. (2010), Kitajima et al. (2011), Di Martino et al. (2013), Lodder et al. (2013)]. Published reports of AiV-1 in Africa are limited to Tunisia (Sdiri-Loulizi et al. 2008, 2009, 2010), Ethiopia (Altan et al. 2018), and more recently in Burkina Faso where genotypes A, B, and C were detected in stool samples from diarrhoeic children (Ouédraogo et al. 2016). This study does not preclude the presence of genotype A (or even C) in South Africa; the small number of clones selected for sequencing undoubtedly limited the detection of other AiV-1 genotypes that may be circulating in the region. The presence of AiV-1 in mussels from the Swartkops river estuary is cause for concern. The ecology of this region is continuously threatened by wastewater effluent from industry and raw sewage from nearby informal settlements (Odume et al. 2012) and our previous studies have reported the presence of other enteric viruses such as HAdV-D17 (Vos and Knox 2017), HAdV C and F, HBoV and NoV in the same samples (data not shown). Such contamination is a serious threat to human health given that this is an area where recreational activities take place.

In conclusion, this preliminary study presents the first report on the detection of AiV-1 in South Africa, furthering our knowledge about the global distribution of genotype B on the continent. Although the introduction of the monovalent rotavirus vaccine, into the South African national immunization program in 2009 has significantly decreased diarrhoeal hospitalisations in the country, a large percentage of diarrhoeal cases (~40%) remain undiagnosed (Groome et al. 2016). Additional studies are therefore warranted to investigate the significance of the results reported in this study and to understand the clinical importance of this and other unidentified enteric viruses in the region.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics Approval Ethics approval for this work was not required as the study only involved invertebrates.

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