

**CHARACTERISATION OF ASTROVIRUSES  
FROM SELECTED CLINICAL AND  
ENVIRONMENTAL SETTINGS**

by

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DEPARTMENT : MEDICAL VIROLOGY

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### SUMMARY

Astroviruses (AstVs), classified within the family *Astroviridae*, include both animal and human pathogens. Human AstVs (HAstVs) include eight serotypes and after rotaviruses, are the most common pathogen in childhood viral diarrhoea. With the impending licensure of a rotavirus vaccine, the significance of HAstVs in diarrhoeal disease needs to be reassessed. HAstV infection has been reported in all age groups, with the young, elderly and immunocompromised at greatest risk. Transmission occurs via the faecal-oral route and the occurrence of AstVs in water sources has been documented. The significance to humans of AstVs in environmental sources has not been quantified.

The aim of this study was to optimise and apply molecular techniques for the detection and characterisation of AstVs in human stool specimens and water samples. The HAstV serotypes primarily responsible for gastroenteritis in this region of South Africa (SA) could then be established. Animal stools would also be screened for AstVs to obtain a SA reference strain for further characterisation and comparative studies. Nucleotide sequences



of the clinical and environmental isolates could be compared with each other and to AstVs found outside SA. These data would also provide information on the role of water as a source of human infection and of the source of faecal contamination of surface waters.

Human stool specimens, water concentrates and cell culture derivatives of these, were screened for HAstVs by enzyme immunoassay and type-common reverse transcriptase polymerase chain reactions (RT-PCR). AstV isolates were characterised by nucleotide sequence analysis of RT-PCR amplicons, generated in the 5' and 3' ends of the genome, using type-common and type-specific primer pairs.

Of a total of 35 clinical isolates, 22 AstV strains were characterised and compared to 25 environmental strains obtained from 15 surface water and wastewater samples. Cell culture amplification of selected specimens enabled the amplification of isolates present in low titres as well as the isolation of multiple AstVs serotypes from single sewage samples. All AstVs from the stool specimens and water samples were identified as HAstVs. All eight HAstV serotypes were represented in the combined study samples. Phylogenetic analyses of the nucleotide sequences of each of the HAstV isolates and comparisons with isolates from the rest of the world showed that some SA strains formed unique genetic clusters, as has been observed in other studies at other sites. AstVs in some clinical and environmental samples were identical. The existence of HAstVs in water samples highlights the potential health risk posed by these waters used for recreational and domestic purposes. This study also presents new baseline data on the molecular epidemiology of HAstVs in SA.

**KEYWORDS:** Human astroviruses, sewage, multitypes, reverse transcriptase-polymerase chain reaction, molecular characterisation, sequencing, cell culture amplification

## KARAKTERISERING VAN ASTROVIRUSSE VAN GESELEKTEERDE KLINIESE EN OMGEWINGS BRONNE

deur

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### OPSOMMING

Astrovirusse (AstVs), geklassifiseer in die familie *Astroviridae*, bevat beide dierlike en menslike patogene. Daar is agt serotipes van mens astrovirusse (HAstVs) en is na rotavirusse die mees algemene patogeen van virale diarree in kinders. Met die naderende lisensiëring van 'n rotavirus entstof, moet die belangrikheid van HAstVs met betrekking tot diarree herevalueer word. Alhoewel HAstV infeksie in alle ouderdomsgroepe gerapporteer word, is kinders, bejaardes en immuungekompromitteerde individue meer vatbaar. Oordrag is deur die fekaal-orale roete en die voorkoms van AstVs in gekontameneerde waterbronne is al beskryf. Die kliniese betekenis van omgewings AstVs tot mense is onbekend.

Die doel van hierdie studie was om molekulêre tegnieke te optimiseer en toe te pas vir die opsporing en karakterisering van AstVs in mens-stoelgang- en water-monsters. Die HAstV-serotipes wat primêr verantwoordelik was vir gastroenteritis kan dan vasgestel word. Stoelgang monsters van diere sou ook ondersoek word vir AstVs om 'n SA bron van dierlike AstV te verkry vir verder karakterisering en vergelykende studies. Nukleotied basispaar volgorde-bepaling van kliniese en omgewings isolate kan dan



vergelyk word om vas te stel wat die verhouding van SA AstVs tot stamme in die res van die wêreld is. Hierdie data sal ook inligting verskaf tot die moontlik rol van water in menslike infeksie en die bron van fekale kontaminasie van oppervlak water.

Mens stoelgang monsters, water konsentrate en hul selkultuur ekstrakte is ondersoek vir HAstVs deur middel van ensiem immuunbepaling en tipe-algemeen tru-transkripsie polimerase ketting reaksie (TT-PKR). AstV isolate is deur nukleotied basispaar volgorde-bepaling, van die 3' en 5'-kant van die genoom, deur tipe-algemene en tipe-spesifieke voorvoerdes gekarakteriseer.

Uit 'n totaal van 35 kliniese isolate, kon 22 AstV stamme gekarakteriseer en vergelyk word met 25 omgewings stamme geïsoleer uit 15 oppervlak en rioolwater monsters. Deur middel van selkultuur vermeerdering kon isolate teenwoordig in lae titers in geselekteerde monsters geamplifiseer word. Selkultuur amplifikasie het ook die isolasie van veelsoortige AstVs van enkele rioolmonsters bevorder. Alle AstVs vanuit stoelgang en water monsters was geïdentifiseer as HAstVs. Ten minste een van elk van die agt HAstV serotipes is geïdentifiseer uit die totale aantal studie monsters. Filogenetiese analise van die nukleotied basispaar volgorde-bepaling van elk van die HAstV isolate, asook vergelyking met isolate uit die res van die wêreld, het getoon dat geselekteerde SA stamme hul eie unieke groepe vorm, soortgelyk aan patrone gerapporteer in ander studies. Sekere AstV-stamme vanaf kliniese en omgewingsbronne was identies. Die identifisering van HAstVs in water monsters beklemtoon die potensiële gesondheidsrisiko vir individue wat hierdie waterbronne vir huishoudelike en ontspanningsdoeleindes gebruik. Hierdie studie verteenwoordig nuwe inligting oor die molekulêre epidemiologie van HAstVs in SA.

**SLEUTELWOORDE :** Mens astrovirusse, riool, veelsoortige tipes, tru-transkripsie polimerase kettingreaksie, molekulêre karakterisering, nukleotied basispaar volgorde-bepaling, selkultuur vermeerdering

## PUBLICATIONS AND PRESENTATIONS

### Publications

**Nadan S**, Walter JE, Grabow WOK, Mitchell DK, Taylor MB.

Molecular characterization of astroviruses: comparison between clinical and environmental isolates from South Africa. *Applied and Environmental Microbiology* (*submitted*)

### Presentations

**Nadan S**, Grabow WOK, Taylor MB. The molecular detection and characterisation of astroviruses from human stool specimens and sewage [Poster/Presentation]. Faculty Day, Faculty of Health Sciences, University of Pretoria 21-22 August 2001: Pretoria.

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Molecular epidemiology of human astroviruses from the Tshwane area (Pretoria), Gauteng.
- D.3 **ABSTRACT: Nadan S**, JE Walter, Grabow WOK, Taylor MB.  
The molecular detection and characterisation of astroviruses from human stool specimens and sewage.
- D.4 **ABSTRACT:** WB van Zyl, **S Nadan**, JC Vivier, JME Venter, K Riley, EKM Tlale, LR Seautlueng, WOK Grabow, MB Taylor.  
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## ABBREVIATIONS

Å	:	angstrom
aa	:	amino acid
Ab	:	antibody
AMPS	:	ammonium persulfate
AMV	:	avian myeloblastosis virus
Ag	:	antigen
ANV	:	avian nephritis virus
ARC	:	Agricultural Research Council
AstV	:	astrovirus
ATCC	:	American Type Culture Collection
BAstV	:	bovine astrovirus
BK	:	bovine kidney cell line
bp	:	base pair
(C)	:	carboxy terminal
CCC	:	child care centre
CaCo-2	:	colonic carcinoma cell line
cpe	:	cytopathic effect
CsCl	:	caesium chloride
CV	:	caliciviruses
DAstV	:	duck astrovirus
DTT	:	dithiothreitol
EIA	:	enzyme immunoassay
ELISA	:	enzyme-linked immunosorbent assay
EM	:	electron microscopy
E-MEM	:	Eagle's Minimum Essential Medium
EtBr	:	ethidium bromide
FAstV	:	feline astrovirus
FCS	:	foetal calf serum
FEA	:	feline embryo cells
GBEB	:	glycine-beef-extract buffer
g/cm <sup>3</sup>	:	gram per cubic centimeter
g/ml	:	gram per millilitre
HAdV-40/41	:	human adenovirus 40/41
HAstV	:	human astrovirus
HAstV-1	:	human astrovirus serotype 1
HAstV-2	:	human astrovirus serotype 2
HAstV-3	:	human astrovirus serotype 3
HAstV-4	:	human astrovirus serotype 4
HAstV-5	:	human astrovirus serotype 5
HAstV-6	:	human astrovirus serotype 6
HAstV-7	:	human astrovirus serotype 7
HAstV-8	:	human astrovirus serotype 8

HIV	:	Human Immunodeficiency Virus
HEK	:	human embryonic kidney cell line
HEL	:	human embryonic lung fibroblasts
HRP	:	horseradish peroxidase
HRV	:	human rotavirus
HS	:	horse serum
h	:	hour
IEM	:	immune electron microscopy
IF	:	immunofluorescence
ISEM	:	immunosorbent electron microscopy
kb	:	kilobase
kDa	:	kilodalton
L	:	litres
LLCMK2	:	rhesus monkey kidney cell line
MAb	:	monoclonal antibody
MDBK	:	Madin-Darby bovine kidney cell line
min	:	minute
ml	:	millilitre
MW	:	molecular weight
(N)	:	amino terminal
NaCl	:	sodium chloride
NCR	:	non-coding region
NBK	:	neonatal bovine kidney cell line
NIV	:	National Institute for Virology
nm	:	nanometre
nt	:	nucleotide
NV	:	Norwalk virus
OAstV	:	ovine astrovirus
ORF	:	open reading frame
PEG	:	polyethylene glycol
PAGE	:	polyacrylamide gel electrophoresis
PAstV	:	porcine astrovirus
PBK	:	primary bovine kidney cell line
PBS	:	phosphate buffered saline
PCR	:	polymerase chain reaction
RFLP	:	restriction fragment length polymorphism
p.i.	:	post infection
PLC/PRF/5	:	primary hepatoma cell line
RIA	:	radio immunoassay
RNA	:	ribonucleic acid
rRNA	:	ribosomal ribonucleic acid
RT	:	reverse transcription
RT-PCR	:	reverse transcriptase-polymerase chain reaction
SA	:	South Africa

SDS-PAGE	:	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SPIEM	:	solid phase immune electron microscopy
SRV	:	small round virus
SRSV	:	small round structured virus
ss	:	single stranded
TEMED	:	N,N,N',N'-tetramethyl-ethylenediamine
tRNA	:	transfer ribonucleic acid
TYPE-EIA	:	typing enzyme immunoassay
TAstV	:	turkey astrovirus
TAstV-1	:	turkey astrovirus serotype 1
TAstV-2	:	turkey astrovirus serotype 2
TMB	:	3,3',5,5'-tetramethylbenzidine
UK	:	United Kingdom
UNICEF	:	United Nations International Children's Fund
US	:	United States of America
UTR	:	untranslated region
UV	:	ultraviolet
WT	:	wild type