

**Hepatitis E Virus in South Africa: Seroprevalence of Anti-HEV IgG in
Swine and Detection of the Virus in Swine Faecal Specimens and
Domestic Sewage Samples**

by

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"Ek verklaar dat die verhandeling wat ek hiermee aan die Universiteit van Pretoria vir die MSc (Geneeskundige Virologie)-graad voorlê, my eie werk is en nie vantevore deur my aan enige ander tersiêre inrigting vir enige graad voorgelê is nie."

"I certify that the thesis hereby submitted to the University of Pretoria for the degree of MSc (Medical Virology) is my own work and has not previously been submitted by me in respect of a degree at any other tertiary institution."

Signature:



Date:

2004.11.10

This thesis is dedicated to my parents

"The success of current scientific theories is no miracle. It is not even surprising to the scientific (Darwinist) mind. For any scientific theory is borne into a life of fierce competition, a jungle red in tooth and claw. Only the successful theories survive - the ones which in fact latched on to the actual regularities in nature."

Bas Van Fraassen (b. 1941)

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LIST OF ABBREVIATIONS

aa	Amino acid
ALT	Alanine aminotransferase
Anti-HEV	Antibodies to hepatitis E virus
Anti-HAV	Antibodies to hepatitis A virus
AST	Aspartate aminotransferase
BEVS	Baculovirus expression vector system
bp	Base pair
CDC	Centers for Disease Control and Prevention
cDNA	Complimentary DNA
CI	Confidence interval
CO	Cut-off
CPE	Cytopathic effect
<i>E. coli</i>	<i>Escherichia coli</i>
EIA	Enzyme immuno assay
ELISA	Enzyme-linked immunosorbent assay
IFM	Immuno fluorescence microscopy
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCl	Hydrochloric acid
HEV	Hepatitis E virus
HEVAg	Hepatitis E virus target antigen
H ₂ SO ₄	Sulphuric acid
IEM	Immuno electron microscopy
IV	Intravenous
kb	Kilobase

KCl	Potassium chloride
kDa	Kilo-Dalton
MgCl ₂	Magnesium chloride
mM	Millimolar
mRNA	Messenger RNA
NS	Non-structural
nm	Nanometre
OD	Optical density
ORF	Open reading frame
PCR	Polymerase chain reaction
pmol	Picomole
RdRp	RNA-dependant RNA polymerase
rHEV	Recombinant hepatitis E virus
RNA	Ribonucleic acid
RT-PCR	Reverse transcription-polymerase chain reaction
SD	Specimen diluent
SNC	Standard negative control
SPC	Standard positive control
UK	United Kingdom
US	United States
UTR	Untranslated regions
UV	Ultraviolet
VLP	Virus-like particles

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None

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SUMMARY

Hepatitis E virus (HEV) is a major cause of enterically transmitted hepatitis in areas with a tropical or subtropical climate and poor sanitary conditions. Hepatitis E virus is responsible for both waterborne epidemics and sporadic cases of acute hepatitis in many developing countries world-wide, but rarely causes epidemics in more industrialised countries. Hepatitis E occurs predominantly in the adult population, causing mild symptoms that usually resolve within 2 weeks. However, the disease poses a great threat to pregnant women, specifically during the third trimester, with a mortality rate of up to 25% due to liver failure. Many different animal species world wide, especially swine have been shown to have antibodies against HEV. Hepatitis E virus strains isolated from animals showed a remarkable resemblance to human HEV strains, raising the suspicion that these viruses may be zoonotic. Recently direct evidence supporting this suspicion

was provided by reports of foodborne transmission of HEV from animals to humans in Asia.

Seroprevalence studies conducted in South Africa have suggested that HEV may be endemic in some areas of the country, especially in low socio-economic communities. Only a few clinical cases of hepatitis E, mainly associated with travelling to HEV endemic countries, have been reported. Similar results have been reported in other predominantly non-endemic countries such as Spain, the UK and the US. Since at least some animal strains of HEV are considered to be zoonotic, it is suggested that these strains are being transmitted to humans via water sources contaminated with animal wastes, which results in the eliciting of an anti-HEV response.

The objective of this study was, therefore, to assess the seroprevalence of HEV in swine, as well as to determine what strains of HEV are circulating in South Africa in order to establish the role which swine may play in the transmission of the virus in the country. The seroprevalence of anti-HEV in selected swine populations in this study indicated that HEV is widespread throughout the swine population in South Africa. This is in agreement with results obtained in similar studies conducted in other non-endemic countries. In addition, this study reported the detection and characterisation of four novel isolates of HEV in domestic sewage samples and swine faecal specimens. The two HEV strains isolated from sewage were closely related to strains of genotype I, which includes human-related strains from Asia, Europe, Africa and the Middle East. This indicated that HEV is most likely circulating in the human population in South Africa, despite the absence of clinical disease. The two swine HEV strains detected in the swine faecal specimens showed a relationship with genotypes III and IV respectively, which raises concern, as the strains of these HEV genotypes have shown to cross the species barrier to cause disease in the human population.

Future research on HEV in South Africa includes determining the prevalence and characterisation of the virus in the human, swine and other animal populations throughout

the country in order to cast more light on the potential health impact they may have on the human population.

HEPATITIS E VIRUS IN SUID-AFRIKA: DIE VOORKOMS VAN ANTI-HEV IgG IN VARKE EN DIE DETEKSIE VAN DIE VIRUS IN VARK STOELGANGE EN RIOOL MONSTERS

deur

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OPSOMMING

Hepatitis E virus (HEV) is die hoof oorsaak van enteries-oordraagbare hepatitis in tropiese en subtropiese gebiede en waar daar swak sanitêre toestande heers. Hepatitis E virus is verantwoordelik vir beide water-gedraagde epidemies en sporadiese gevalle van akute hepatitis in verskeie ontwikkelende lande wêreldwyd en veroorsaak selde epidemies in ontwikkelde lande. Hepatitis E kom hoofsaaklik in die volwasse populasie voor waar dit matige simptome veroorsaak wat gewoonlik binne 2 weke opklaar. Daarteenoor is die siekte lewensgevaarlik vir swanger vroue, spesifiek gedurende die derde trimester, met 'n mortaliteitsyfer van tot 25% as gevolg van lewerversaking. Daar is wêreldwyd bewys dat verskeie dierspesies, veral varke, HEV teenliggame bevat. Daar word bespiegel dat HEV soönoties oorgedra kan word as gevolg van die ooreenkoms

tussen HEV stamme wat onderskeidelik in mense en diere voorkom. Hierdie bespiegeling is onlangs bewys deurdat voedsel-oordrag van HEV vanaf diere na mense in Asië gerapporteer is.

Vorige studies wat die voorkoms van HEV teenliggame in Suid Afrika nagevors het, het voorgestel dat HEV endemies is in sommige dele van die land, veral in lae sosio-ekonomiese gemeenskappe. Die paar kliniese gevalle van hepatitis E wat wel aangemeld word, was gewoonlik geassosieer met 'n reis-geskiedenis na 'n endemiese land. Soortgelyke gevalle is ook aangemeld in ander lande, soos Spanje, die Verenigde State van Amerika en die Verenigde Koninkryk, waar HEV nie endemies is nie. Aangesien dit wil voorkom asof ten minste sommige HEV stamme soönoties oorgedra kan word, word daar vermoed dat hierdie HEV stamme oorgedra word na mense via waterhulpbronne wat gekontamineer is met dierlike afval, wat aanleiding kan gee tot 'n HEV teenliggaam reaksie in mense.

Die doel van hierdie studie was om die voorkoms van HEV teenliggame in varke te bepaal, asook om vas te stel watter HEV stamme in Suid Afrika sirkuleer, om sodoende die rol wat varke mag speel in die oordrag van die virus vas te stel. Die voorkoms van HEV teenliggame in geselekteerde vark populasies in hierdie studie het aangedui dat HEV wydverspreid is in die vark populasie in Suid Afrika. Hierdie resultate is in ooreenstemming met soortgelyke studies wat in ander nie-endemiese lande gedoen is. Hierdie studie beskryf ook die opsporing en karakterisering van vier nuwe HEV isolate vanuit riool en varkmis. Die twee HEV isolate wat uit riool geïsoleer was, stem ooreen met mens HEV stamme van genotipe I, wat stamme van Asië, Europa, Afrika en die Midde Ooste insluit. Dit dui aan dat HEV moontlik in die mens-populasie van Suid-Afrika sirkuleer, ongeag die feit dat geen kliniese gevalle voorkom nie. Die twee HEV isolate vanuit varkmis stem ooreen met stamme van genotipe III en IV onderskeidelik, wat kommerwekkend is, aangesien hierdie HEV genotipes die vermoë het om verskillende spesies te infekteer, wat aanleiding kan gee tot die oordrag van die virus na die menslike populasie.

Toekomstige navorsing op HEV in Suid Afrika sluit in die opsporing en karakterisering van die virus in die mens, vark en ander dier-populasies regoor die land om sodoende meer uitsluitel te kry oor die potensiële gesondheidsimpak wat HEV mag hê.