CHAPTER 1

Introduction

Hepatitis E virus (HEV), the etiological agent of hepatitis E, was first discovered in the late 1970's after it had become evident that there was a hepatitis virus other than hepatitis A virus and hepatitis B virus (Wong *et al.*, 1980). Hepatitis E virus was initially classified in the family *Caliciviridae*, but is currently recognised as the type species of the genus *Hepatitis E-like viruses* (Pringle, 1999; Berke and Matson, 2000).

Hepatitis E virus is transmitted via the faecal-oral route and is an important cause of acute epidemic viral hepatitis in some developing countries (Favorov *et al.*, 2000; Schlauder and Mushahwar, 2001; Van der Poel *et al.*, 2001). Outbreaks of hepatitis E are primarily associated with faecally contaminated drinking water (Favorov *et al.*, 2000; Schlauder and Mushahwar, 2001; Van der Poel *et al.*, 2001), and have been reported from countries in Asia, Africa and the Middle East (Byskov *et al.*, 1989; Mushahwar *et al.*, 1993; Swanepoel *et al.*, 1995; Hunter, 1997; Van der Poel *et al.*, 2001; Grabow, 2002).

Hepatitis E occurs predominantly in the young adult population, while it tends to be asymptomatic in the younger age groups (Van der Poel *et al.*, 2001). The onset of disease follows an incubation period of one to eight weeks (mean of 40 days) (Purcell, 1996; Van der Poel *et al.*, 2001). In areas where the disease is endemic, HEV is an important cause of death due to liver failure, especially in pregnant woman during the third trimester, with mortality rates of up to 25% (Grabow *et al.*, 1996; Van der Poel *et al.*, 2001). Chronic HEV infections have not been observed and the disease is usually mild and resolves within 2 weeks (Van der Poel *et al.*, 2001).

Hepatitis E virus may be endemic in certain areas of South Africa (Tucker and Kirsch, 1994; Tucker *et al.*, 1996; Grabow *et al.*, 1996). It was thought that clinical cases of hepatitis E in South Africa were limited to a small number of imported cases, but it would appear that there might be more local cases of clinical disease (Grabow *et al.*,

1994; Grabow, 1997; South African Virus Laboratories Surveillance Bulletin, 2003). The rare diagnosis of clinical cases of hepatitis E in South Africa is therefore surprising, since outbreaks have been recorded in the neighbouring countries Namibia and Botswana (Byskov *et al.*, 1989; Swanepoel *et al.*, 1995; South African Virus Laboratories Surveillance Bulletin, 2001).

Although a reservoir of HEV has not yet been established, indirect evidence has suggested that at least some strains of HEV may be zoonotic (Meng *et al.*, 1997; Hsieh *et al.*, 1999; Kabrane-Lazizi *et al.*, 1999; Tei *et al.*, 2003). An increase in HEV infection among persons with occupational exposure to swine has been reported (Meng *et al.*, 1999; Drobeniuc *et al.*, 2001). Recently a few cases of foodborne HEV have been reported after the consumption of raw pig liver and deer meat, which provides direct proof of zoonotic transmission of HEV (Matsuda *et* al., 2003; Tei *et al.*, 2003; Yazaki *et al.*, 2003; ProMed, 2004).

Rare cases of hepatitis E without a travel history have been reported in non-endemic countries, in which case the transmission of the virus remains uncertain (Hsieh *et al.*, 1998; Erker *et al.*, 1999; Schlauder *et al.*, 1999; Zanetti *et al.*, 1999; Favorov *et al.*, 2000). It has been established, however, that the genomic sequences of the HEV strains detected in these patients were more related to the swine HEV strains prevalent in the swine population of the same area than to human HEV strains (Wu *et al.*, 2000). These swine HEV strains may undergo genetic reversion to HEV strains that cause clinical disease in humans similar to that in many parts of the world where hepatitis E has major public health implications (Meng *et al.*, 1998; Hsieh *et al.*, 1999).

The detection of anti-HEV IgG in swine populations from both developing and industrialised countries has been documented (Clayson *et al.*, 1995; Meng *et al.*, 1997; Chandler *et al.*, 1999; Hsieh *et al.*, 1999; Meng *et al.*, 1999; Pina *et al.*, 2000). It was found that swine herds from developing and industrialised countries contained many pigs that were seropositive for HEV, which suggests that HEV may be enzootic in swine regardless of whether HEV is endemic in the human population (Meng *et al.*, 1999).

Since the waterborne transmission of HEV is well established and animal strains of HEV are closely related to the human strains, it would appear to be possible that the virus may be transmitted from animals to humans via water sources polluted with animal wastes. It is, therefore, important to determine what the prevalence of HEV is in the swine population of South Africa as well as the role that swine may play in the transmission of the disease. The purpose of this study was to determine the seroprevalence of anti-HEV IgG in swine sera and to detect HEV strains in swine faecal specimens and domestic sewage samples. This could cast light on the prevalence of the virus in selected areas of South Africa, as well as the possible relationship of these viruses with known strains of HEV from other parts of the world.

The objectives of this study were to:

1. Validate and optimise an enzyme-linked immunosorbent assay (ELISA) for the detection of anti-HEV IgG in swine serum samples.
2. Assess the seroprevalence of anti-HEV IgG in swine from selected areas of the Gauteng and Limpopo Provinces of South Africa by means of the ELISA.
3. Validate and optimise a reverse transcriptase-polymerase chain reaction (RT-PCR) procedure for the detection of HEV RNA.
4. Attempt to detect HEV RNA in swine faecal specimens by means of the RT-PCR.
5. Analyse selected domestic sewage samples for the presence of HEV by means of the RT-PCR.
6. Determine the relationship of HEV viruses detected in swine excreta and sewage to those described in other parts of the world using nucleotide sequence analysis.

References:

Berke T and Matson DO (2000). Reclassification of the *Caliciviridae* into distinct genera and exclusion of hepatitis E virus from the family on the basis of comparative phylogenetic analysis. *Archives of Virology* **145**, 1421-1436.

Byskov J, Wouters SM, Sathekge TJ and Swanepoel R (1989). An outbreak of suspected waterborne epidemic non-A, non-B hepatitis in northern Botswana with high prevalence of hepatitis B carriers and hepatitis delta markers among patients. *Transactions of the Royal Society for Tropical Medicine and Hygiene* **83**, 110-116.

Chandler JD, Riddell MA, Li F, Love RJ and Anderson DA (1999). Serological evidence for swine hepatitis E virus infection in Australian pig herds. *Veterinary Microbiology* **68**, 95-105.

Clayson ET, Innis BL, Myint KS, Narupiti S, Vaughn DW, Giri S, Ranabhat P and Shrestha MP (1995). Detection of hepatitis E virus infections among domestic swine in the Kathmandu Valley of Nepal. *American Journal of Tropical Medicine and Hygiene* **53**, 228-232.

Drobeniuc J, Favorov MO, Shapiro CN, Bell BP, Mast EE, Dadu A, Culver D, Iarovoi P, Robertson BH and Margolis HS (2001). Hepatitis E virus antibody prevalence among persons who work with swine. *Journal of Infectious Diseases* **184**, 1594-1597.

Erker JC, Desai SM, Schlauder GG, Dawson GJ and Mushahwar IK (1999). A hepatitis E virus variant from the United States: molecular characterisation and transmission in cynomolgus macaques. *Journal of General Virology* **80**, 681-690.

Favorov MO, Kosoy MY, Tsarev SA, Childs JE and Margolis HS (2000). Prevalence of antibody to hepatitis E virus infection among rodents from the United States. *Journal of Infectious Diseases* **181**, 449-455.

Grabow WOK (1997). Hepatitis viruses in water: Update on risk and control. *Water SA* **23**, 279-386.

Grabow WOK (2002). Enteric hepatitis viruses. In: Guidelines for Drinking Water Quality, Second Edition, Addendum: Microbiological agents in drinking water. World Health Organization, Geneva. p. 18-39

Grabow WOK, Favorov MO, Khudyakova NS, Taylor MB and Fields HA (1994). Hepatitis E seroprevalence in selected individuals in South Africa. *Journal of Medical Virology* **44**, 384-388.

Grabow WOK, Taylor MB and Webber LM (1996). Hepatitis E virus in South Africa. *South African Journal of Science* **92**, 178-180.

Hsieh SY, Yang PY, Ho YP, Chu CM and Liaw YF (1998). Identification of a novel strain of hepatitis E virus responsible for sporadic acute hepatitis in Taiwan. *Journal of Medical Virology* **55**, 300-304.

Hsieh SY, Meng XJ, Wu Y, Lui S, Tam AW, Lin D and Liaw YF (1999). Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. *Journal of Clinical Microbiology* **37**, 3828-3834.

Hunter PR (1997). Viral hepatitis. In: *Waterborne Disease: Epidemiology and Ecology*. John Wiley & sons Ltd, Chichester. pp. 215-221.

Kabrane-Lazizi Y, Fine JB, Glass GE, Higa H, Diwan A, Gibbs CJ, Meng XJ, Emerson SU and Purcell RH (1999). Evidence for widespread infection of wild rats with hepatitis E virus in the United States. *American Journal of Tropical Medicine and Hygiene* **61**, 331-335.

Matsuda H, Okada K, Takahashi K and Mishiro S (2003). Severe hepatitis E virus infection after ingestion of uncooked liver from a wild boar. *Journal of Infectious Diseases* **188**, 944.

Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ and Emerson SU (1997). A novel virus in swine is closely related to the human hepatitis E virus. *Proceedings of the National Academy of Science in the USA* **94**, 9860-9865.

Meng XJ, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Purcell RH and Emerson SU (1998). Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *Journal of Virology* **72**, 9714-9721.

Meng XJ, Dea S, Engle RE, Friendship R, Lyoo YS, Sirinarumitr T, Urairong K, Wang D, Wong D, Yoo D, Zhang Y, Purcell RH and Emerson SU (1999). Prevalence of antibodies to the hepatitis E virus in pigs from countries where hepatitis E is common or is rare in the human population. *Journal of Medical Virology* **59**, 297-302.

Mushahwar IK, Dawson GJ, Bile KM and Magnius LO (1993). Serological studies of an enterically transmitted non-A, non-B hepatitis in Somalia. *Journal of Medical Virology* **40**, 218-221.

Pina S, Muti M, Cotrina M, Piella J and Girones R (2000). HEV identified in serum from humans with acute hepatitis and in sewage of animal origin in Spain. *Journal of Hepatology* **33**, 826-833.

Pringle CR (1999). Virus Taxonomy-1999. The universal system of virus taxonomy, updated to include the new proposals ratified by the International Committee on Taxonomy of viruses during 1998. *Archives of Virology* **144**, 421-429.

ProMed (2004). Hepatitis E virus, wildlife to humans-Japan (Nagasaki). ProMed mail: *promed@promed.isid.harvard*.edu, 14 May.

Purcell RH (1996). Hepatitis E virus. In: Fields BN, Knipe DM and Howley PM (ed.) *Fields Virology*. Lippencott-Raven, Philadelphia. pp. 2831-2843.

Schlauder GG, Desai SM, Zanetti AR, Tassopoulos NC and Mushahwar IK (1999). Novel hepatitis E virus (HEV) isolates from Europe: Evidence for additional genotypes of HEV. *Journal of Medical Virology* **57**, 243-251.

Schlauder GG and Mushahwar IK (2001). Genetic heterogeneity of Hepatitis E virus. *Journal of Medical Virology* **65**, 282-292.

South African Virus Laboratories Surveillance Bulletin (2001). Hepatitis, October, p. 2. National Institute for Virology, Johannesburg.

South African Virus Laboratories Surveillance Bulletin (2003). Hepatitis, June, p. 6. National Institute for Virology, Johannesburg.

Swanepoel R, Sim JGM and Van Niekerk ABW (1995). Confirmed outbreaks of hepatitis E in southern Africa. *Proceedings of the Vth International Congress on the Impact of Viral Diseases in the Developing World*, 9-14 July, Johannesburg.

Tei S, Kitajima N, Takahashi K and Mishiro S (2003). Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet* **362**, 371-373.

Tucker TJ and Kirsch RE (1994). Hepatitis E. *South African Medical Journal* **84**, 548-550.

Tucker TJ, Kirsch RE, Louw SJ, Isaacs S, Kannemeyer J and Robson SC (1996). Hepatitis E in South Africa: evidence for sporadic spread and increased seroprevalence in rural areas. *Journal of Medical Virology* **50**, 117-119.

Van der Poel WHM, Verschoor F, Van der Heide R, Herrera M, Vivo A, Kooreman M and De Roda Husman AM (2001). Hepatitis E virus sequences in swine related to sequences in humans, the Netherlands. *Emerging Infectious Disease* **7**, 970-976.

Wong DC, Purcell RH, Screenivasan MA, Prasad SR and Parvi KM (1980). Epidemic and endemic hepatitis in India: evidence for non-A, non-B hepatitis virus etiology. *Lancet* **2**, 876-879.

Wu J, Chen C, Chiang T, Sheen I, Chen J, Tsai W, Huang Y and Lee S (2000). Clinical and epidemiological implications of swine hepatitis E virus infection. *Journal of Medical Virology* **60**, 166-171.

Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y and Okamoto H (2003). Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *Journal of General Virology* **84**, 2351-2357.

Zanetti AR, Schlauder GG, Romano L, Tanzi E, Fabris P, Dawson GJ and Mushahwar IK (1999). Identification of a novel variant of hepatitis E virus in Italy. *Journal of Medical Virology* **57**, 356-360.