O. GARKAVENKO , A. OBRIADINA , J. MENG , D.A. ANDERSON, H.J. BENARD,
B.A. SCHROEDER, Y.E. KHUDYAKOV, H.A. FIELDS, M.C. CROXSON

Virology Laboratory, Diatranz Ltd., Auckland, New Zealand;
Hepatitis Branch, A-33, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia;
Hepatitis Research Unit, Macfarlane Burnet Centre for Medical Research, Fairfield, Victoria, Australia;
National Centre for Disease Investigation, Ministry of Agriculture and Forestry, Upper Hutt, New Zealand;
Virology and Immunology Laboratory, Auckland Hospital, Auckland, New Zealand

DETECTION AND CHARACTERISATION OF SWINE HEPATITIS E VIRUS IN NEW ZEALAND

Abstract
The objectives of the present study were to establish the presence of hepatitis E virus (HEV) in New Zealand pigs, first by testing for HEV antibody in pig herds throughout New Zealand to measure the herd prevalence, then by attempting to amplify HEV genomic sequences by PCR. Antibody was measured by two independently designed ELISA serology tests. HEV RNA fragments were amplified by RT-PCR of nucleic acid extracted from faeces of 10-12-week-old piglets using primers targeting ORF1, ORF2, and ORF2/3. PCR products were subject to phylogenetic analysis. Antibody to HEV was found throughout New Zealand pig herds as well as in the different age groups within the herds. Twenty herds from 22 tested were positive for HEV antibody (91% herd prevalence). Phylogenetic analysis of the amplified sequences placed this New Zealand strain of HEV closest to the human European strain It-1 (AF 110390) and U.S. swine strain (AF 082843) with 88% and 83% similarity respectively in ORF1. It was concluded that HEV is widely distributed in the New Zealand pig population. Phylogenetic analysis shows that this is a new HEV strain, grouping most closely with the United States/European cluster, which includes HEV strains of both human and swine origin.
Key words: *hepatitis E epidemiology; zoonoses; phylogenetic analysis*

INTRODUCTION

Human hepatitis E (HEV) is rarely diagnosed in developed countries and, when reported, has usually been found in individuals recently returned from HEV endemic areas [Bradley, 1992; Purdy and Krawczynski, 1994; Krawczynski, 1998]. However, some cases described in Western Europe [Pavia et al., 1998; Pina et al., 1998; Sylvan et al., 1998; McCrudden et al., 2000], Greece [Dalecos et al., 1998; Mateos et al., 1998], United States [Schlauder et al., 1998; Tsang et al., 2000], Taiwan [Wu et al., 2000], Australia [Bowden, 1994], and two cases in New Zealand [Chapman, 1993; unpublished data, 1999], had no identifiable association with endemic regions. In the United States, antibodies to HEV can be found in a significant proportion in high-risk populations [Khuroo, 1980], with up to 23% seropositive among injection drug users [Thomas et al., 1997]. According to other data, the seroprevalence of HEV antibody can be as high as 55% among blood donors in nonendemic countries [Meng X-J et al., 1999]. Some studies have demonstrated a high prevalence of HEV antibody in samples collected at hemodialysis units [Dalecos et al., 1998; Arankalle and Chobe, 1999], indicating a possible transmission route for HEV other than faecal-oral. It should be noted that seroprevalence of HEV antibody in different human populations is an open question since there is low concordance between different serology tests [Ghabrah et al., 1998].

HEV isolates from contiguous geographical regions tend to be closely related to each other and distinct from those in more remote geographical areas [Tsarev et al., 1999]. The observed correlation between the evolutionary tree and geography is consistent with the hypothesis of an

animal reservoir of HEV, restrained within geographically distinct areas [Tsarev et al., 1999]. This hypothesis was directly supported by Clayson et al. [1995] when HEV was detected by polymerase chain reaction (PCR) in Nepalese pigs. It was later shown that human HEV from Nepal was closely related to that detected in Nepalese pigs [unpublished data; cited in Tsarev et al., 1999].

Similar data were reported for swine and human HEV strains from the United States [Meng X-J et al., 1997, 1998a] and Taiwan [Wu et al., 2000]. Furthermore, under experimental conditions it has been shown that human HEV is capable of infecting other species such as pigs [Balayan et al., 1990], rats [Maneerat et al., 1996], sheep [Usmanov et al., 1994], and nonhuman primates [Meng X-J et al., 1998b]. Recently the high seroprevalence of antibody to HEV has been established among rodents in the United States [Kabrane-Lazizi et al., 1999; Favorov et al., 2000]. Where studied, very high seroprevalence of HEV antibodies in pig populations has been reported around the world, with up to 95% in U.S. pigs [Meng X-J et al., 1997], 45% among 6-month-old piglets in Canada [Meng X-J et al., 1999], and up to 90% in Australian pigs [Anderson et al., 1999, Chandler et al., 1999]. No antibody studies have been done in New Zealand pigs. However, two human cases of acute hepatitis have been reported as HEV antibody positive [Chapman, 1993; and unpublished data, 1999], and each year there are many cases of non-A-C acute hepatitis.

The aim of the present study was to check the assumption that hepatitis E virus infection is wide spread in domestic animals (pigs) and thus human infection can represent, in part, a zoonosis.

MATERIALS AND METHODS

Sample Collection

Swine serum samples were obtained from sows (n=24), 1-week-old (n=23), and from 20-week-old (n=25) pigs from one herd. Additionally, sow serum samples from the blood bank were used to check the herd prevalence of HEV antibody throughout New Zealand. It was estimated that 11 herds from the North Island and 11 herds from the South Island would represent the New Zealand pig population, and testing three serum samples for HEV antibody per herd would identify a herd prevalence of 25% with a 95% confidence level, assuming a minimum “within-herd” prevalence of 80%. Thus, 66 blood-bank sera from sows of average age 3 years were screened as “herd prevalence” samples. Additionally, sera were collected from six 6-month-old feral pigs from the Auckland Islands, a sub-Antarctic island group to the south of New Zealand. This population of pigs has been isolated from other pigs for approximately 200 years. Faecal samples from 21 10-week-old, 7 12-week-old, and 17 7-week-old piglets from two herds were collected and processed on the day of collection.

ELISA for Detecting HEV Antibody

Pig IgG HEV antibody was measured using an enzyme-linked immunosorbent assay (ELISA) kit [Anderson et al., 1999] in which wells are coated with a recombinant GST-ORF2.1 antigen. Alternatively IgG HEV antibody was measured with an enzyme immunoassay (EIA) in which three fragments of the ORF2-encoded protein of the HEVBurma strain [Meng J et al., 1999, 2000], namely pN393 (L4), pN421 (L5), or pN452 (L6), were used. Wells of Immulon II EIA microtiter plates (Dynatech Lab., USA) were coated with proteins at a concentration of 5-10 mg/ml dissolved in carbonate-bicarbonate buffer (CBB), pH 9.6, and incubated overnight at 4°C. After extensive washing the plates were incubated for 1 hr at 37°C with 200 ml of 1% nonfat milk in CBB. Specimens were diluted 1/200 in phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBST), 5% NGS, and 1% nonfat milk and incubated at 37°C for 30 min. After extensive washing the antibodies bound to the antigens were detected by adding 100 ml of horseradish peroxidase (HRP)-labeled rabbit anti-pig IgG (Sigma) diluted 1:8,000 in PBST with 5% normal goat serum (NGS), and 1% nonfat milk and incubated for 30 min at 37°C. The antibody complexes were detected by the addition 100 ml of orthophenylendiamine (Abbott, USA) for 10 min. The cutoff was statistically established as the mean of the optical density (OD) value at 492 nm of known negative samples plus 4.0 standard deviations of the mean.

RNA Extraction and Amplification

RNA from the faeces was extracted with TRIZOL reagent (GIBCO-BRL, Gaithersburg, MD), either from 100 μl of a 20% faecal suspension or directly from faeces (30 mg) in accordance with the manufacturer's protocol. cDNA was generated using 2 U M-MLV reverse transcriptase (Life Technologies, BRL) according to the manufacturer's protocol. RNA was reversetranscribed in the presence of random primers at 42°C for 1 hr. PCR was performed with cDNA encompassing one-fifth of the total reaction volume (50 μl) in the presence of 200 ng/reaction of primers to the ORF1, ORF2, or ORF2/3 [Erker et al., 1999; Schlauder, 1999], 2.U Taq polymerase (Life Technologies, BRL) 200 μM (each) dNTP, 10 mM Tris buffer (pH 8.3), and 1.5 mM MgCl2. The degenerate ORF1 primers, HEVORF1-sl positioned at nucleotides 56-79 (Burmese isolate numbering) and HEVORF-al, positioned at nucleotides 473-451, amplify a product of 418 base pairs. The degenerate primers from ORF2, HEVORF2-sl, positioned at nucleotides 6298-6321 and HEVORF2-al, positioned at nucleotides 6494-6470 produce a product of 197 base pairs. The degenerate primers from the overlap of ORF2 and 3, HEVORF2/3-sl positioned at nucleotides 5093-5118 and HEVORF2/3-al positioned at nucleotides 5305-5330 produce a product of 237 base pairs. No positive controls were used at the commencement of the study to avoid any possible contamination with HEV RNA. Amplification cycles were as described previously [Erker et al., 1999; Schlauder et al., 1999]. Amplified products were separated on a 1.8% agarose gel and examined for the presence of PCR products of the appropriate size. PCR products were purified using the Highly Purified PCR Product Purification Kit (Boehringer) according to the manufacturer's protocol, and sequenced utilising an ABI 373A sequencer (Center for Gene Technology, Auckland University). Computer analysis was performed by BLASTN and DNASTAR programs.

RESULTS

Antibody to HEV was found throughout New Zealand pig herds using the ELISA ORF2.1 test. Twenty herds from twenty-two tested were positive for HEV antibody (91% herd prevalence). The same assay was used to identify the seroprevalence of HEV in one particular High Health status herd. The highest seroprevalence within the herd was found in 20-week-old pigs (94%). Sows had a reactivity of 73%. One-week-old piglets had a reactivity of 52.1% (Table I).

TABLE I. **HEV Seroprevalance in Different Age Groups of Pigs From High Health Status Herd**

Age groups	No. tested	No. positive (%)
Sows	24	18 (73)
20-week-old	25	24 (94)
1-week-old	23	12 (52.1)
Total	72	54 (75)

Pigs from the Auckland Islands were nonreactive. These data have been confirmed with a second ELISA serology test that uses three recombinant proteins from open reading frame 2 (ORF2) of the HEV Burma strain: L4 393-660 aa, L5 421-660 aa, L6 452-660 aa. The concordance of the two tests is about 84%. Auckland Islands pigs were negative in both tests.

All faecal samples (n=17) from 7-week-old piglets tested negative for HEV RNA. Ten from 21 faecal samples of 10-week-old piglets, and seven from seven tested faecal samples from 12-week-old piglets, were positive for HEV RNA with primers for three regions of viral genome. Seven amplimers were sequenced and compared. The nucleic acid sequence identity between different New Zealand isolates is 99.4% for ORF1, and 97-99% for ORF2. Only one amplimer has been sequenced for overlap of ORF2/3. Amplimers of isolate N1 for ORF1 and ORF2, and ORF2/3 have been compared with the homologous regions of human HEV isolates from different geographic areas and with a swine isolate from the United States (Figs. 1-3) and was found to segregated with human HEV strains from nonendemic areas (Figs. 1,2,3), in

accordance with previously observed data on phylogenetic analysis of different HEV strains [Tsarev et al., 1999]. Thus the New Zealand swine strain showed 88% similarity in ORF1 region, 91% similarity in ORF2 region, and 93% similarity in ORF2/3 with the European strain It-1(AF110390), and 83% similarity in ORF1, 90% similarity in ORF2, and 91% similarity in ORF2/3 with the U.S. swine HEV-strain (AF035437).

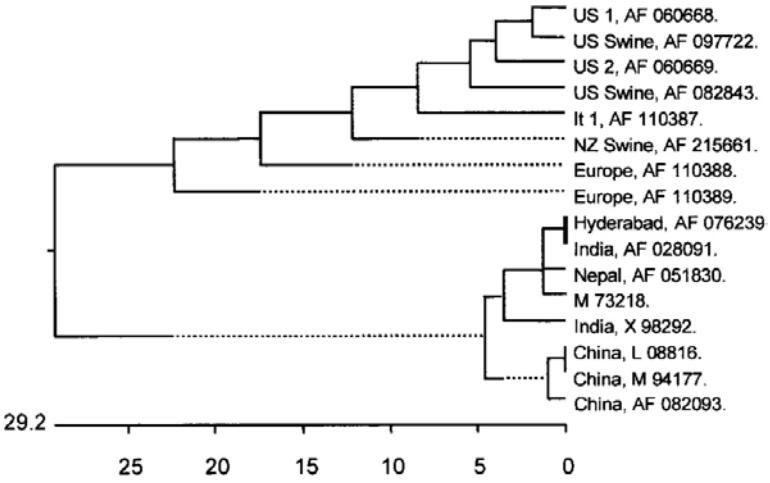

Fig. 1. **Phylogenetic tree of ORF1 region of HEV isolates.**

As previously reported, there was little distinction between groups in the ORF2 fragment, presumably due to the short region used for comparison and the high level of conservation observed in this region [Erker et al., 1999; Schlauder et al., 1999]. ORF1 appears to be the most divergent region. The New Zealand sequences for ORF1, ORF2, and ORF2/3 have been submitted to the GenBank database and have the corresponding accession numbers: AF 215661, AF 200704, and AF 215662.

DISCUSSION

HEV is transmitted primarily by the faecal-oral route, frequently by faecal contamination of the drinking water supply. The possibility of zoonotic infections from pigs to humans was originally postulated after experimental infection of pigs with a human strain of HEV [Balayan et al., 1990] and subsequently by Meng X-J et al. [1998a]. Studies from other domestic livestock and from feral rodents have supported the existence of an animal reservoir for HEV [Balayan et al., 1990; Usmanov et al., 1994; Maneerat et al., 1996; Meng X-J et al., 1998b; Anderson et al., 1999; Kabrane-Lazizi et al., 1999; Favorov et al., 2000]. The identification of HEV-related sequence from swine herds in the United States [Meng X-J et al., 1997b], and Taiwan [Wu et al., 2000] and the observation that the sequences are most closely related to the human isolates from the United States [Schlauder et al., 1998] and Taiwan, respectively, suggested that zoonotic infection between pigs and humans may occur.

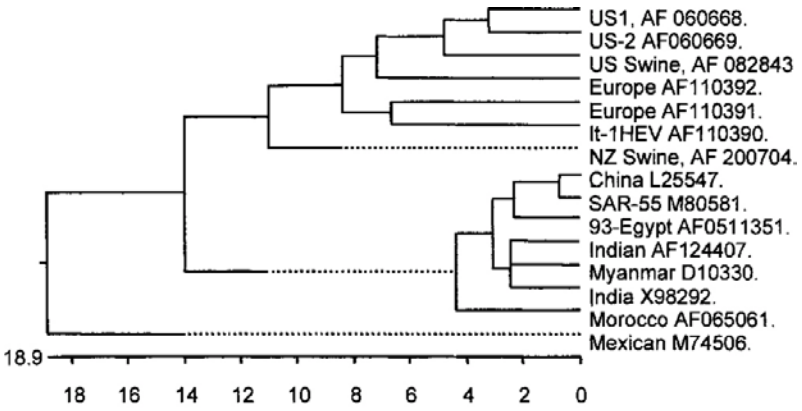

Fig. 2. **Phylogenetic tree of ORF2 region of HEV isolates.**

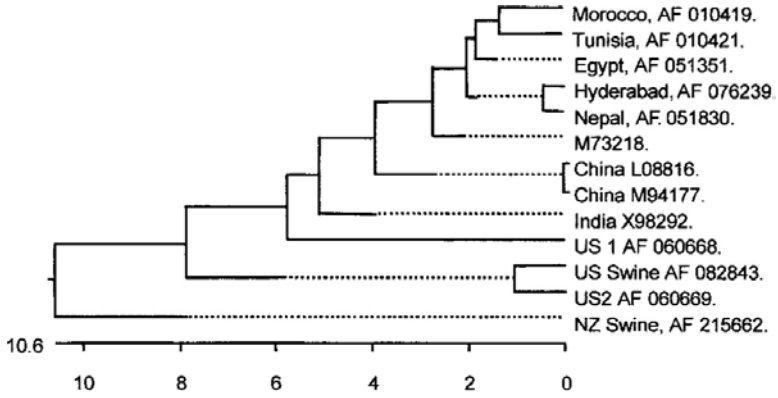

Fig. 3. **Phylogenetic tree of ORF2/3 region of HEV isolates**.

The relatedness between swine and human isolates from the United States and Taiwan, as well as the identification of unique isolates from nontravellers in Italy and Greece [Schlauder et al., 1999] and from the United Kingdom [McCrudden et al., 2000] suggest that human and animal HEV strains may also coexist in other nonendemic areas.

The discovery of novel HEV variants from geographic areas not considered endemic for HEV is important in understanding the worldwide distribution of HEV infection as well as for increasing awareness of HEV as a cause of acute hepatitis in these areas. The existence of zoonotic strains of HEV in so-called nonendemic areas may be responsible for some human cases of acute hepatitis that are currently not linked to infection with this virus. For instance, four cases of sporadic acute hepatitis E were reported in the United Kingdom. Individuals who presented with acute hepatitis had no links between one another and had not travelled in endemic areas [McCrudden et al., 2000]. Similarly, cases of HEV in Australia [Bowden et al., 1994] and New Zealand [Chapman, 1993] had no link with travel to endemic areas. Similar results were reported for patients from Greece and Italy [Schlauder et al., 1999]. Viral RNA has been isolated from serum of some of these patients. Phylogenetic analysis indicated that the Italian and two Greek isolates represent three new genotypes of HEV, distinct from the other described genotypes. The discovery of HEV in geographic areas not considered endemic for the virus can explain the unexpected prevalence of antibody to HEV in these areas [Meng X-J et al., 1999] despite the rarity of HEV-associated hepatitis cases.

The epidemiology of HEV in New Zealand is unclear. There was no prior data on the prevalence of HEV either in human or in pig populations. Only two cases of acute hepatitis in humans have been reported as HEV antibody positive [Chapman, 1993, unpublished data, 1999], although a number of non-A-C acute hepatitis cases are recognised each year. Presented data strongly suggest that HEV is widespread in New Zealand pigs, with a pattern of seroprevalence remarkably similar to that previously described in Australia [Anderson et al., 1999; Chandler et al., 1999]. It is well known that anti-HEV reactivity is highly variable and dependent on the assay used and the concordance between available commercial tests is low [Mast et al., 1998]. Thus we confirmed the seroprevalence pattern in the pig herds by assaying with two independently designed tests.

For isolation of HEV the group of postweaned piglets younger than 20 weeks was targeted. By deliberately eschewing a positive control we avoided any possibility of contamination. Furthermore, no amplification or isolation of HEV RNA had previously been done in the laboratory. The results clearly showed that HEV was present in the herd and was preferentially isolated from 12-week-old piglets. Sequencing of the virus revealed that the New Zealand swine strain segregates with human HEV strains from nonendemic areas (Figs. 1,2,3), in accordance with previously observed data on phylogenetic analysis of different HEV strains [Schlauder et al., 1999; Tsarev et al., 1999]. Recently, a unique HEV strain was recovered from a human case of hepatitis E possibly acquired in Thailand, a nonendemic area for HEV. The sequence of the

virus was most similar to, but not identical with, the New Zealand swine strain presented in this study [Kabrane-Lazizi et al., 2000].

The results from this study suggest that HEV is widespread in the New Zealand pig population. The observation that HEV infection is endemic in swine in remote countries such as Australia and New Zealand, which have had a long history of animal quarantine, suggests that HEV has been present in an animal reservoir for many years. Interestingly, the pig population from the Auckland Islands is apparently free of HEV. This population has been isolated from other pig populations for about 200 years and is only very infrequently exposed to humans.

The reported prevalence of past infection in human populations of nonendemic countries varies from 2% in Australia [Anderson et al., 1999] to 25% in the United States [Thomas et al., 1997] using assay systems, each of which detected almost 100% prevalence in the swine populations of the respective countries [Meng X-J et al., 1997; Chandler et al., 1999]. Future studies will be required to determine the true incidence of human (zoonotic) HEV infection and disease in nonendemic countries. Additional studies on the prevalence of HEVrelated sequences and HEV-reactive antibodies in swine and other animal species would be important for understanding the potential impact they may have on sporadic HEV in nonendemic countries.

ACKNOWLEDGMENTS

The authors thank Diatranz Ltd. for financing the study in New Zealand.

REFERENCES

Anderson DA, Li F, Riddel MA, Howard T, Seow H-F, Torresi J, Perry G, Sumarsidi D, Shrestha SM, Shrestha IL. 1999. ELISA for IgG-class antibody to hepatitis E based on a highly conserved conformation epitope expressed in Escherichia coli. J Virol Methods 81:131-142.

Arankalle VA, Chobe LP. 1999. Hepatitis E virus: can it be transmitted parenterally? J Viral Hepatol 6:161-164.

Balayan M, Usmanov R, Zamyatina N. 1990. Brief report: experimental hepatitis E infection in domestic pigs. J Med Virol 32:58-59.

Bowden F. 1994. Hepatitis E in the Northern territory: a locally acquired case and preliminary evidence suggesting endemic disease. CDI 18:2-3.

Bradley DW. 1992. Hepatitis E: epidemiology, aetiology and molecular biology. Rev Med Virol 2:19-28.

Chandler JD, Riddell MA, Li F, Love RJ, Anderson DA. 1999. Serological evidence for swine hepatitis E virus infection in Australian pig herds. Vet Microbiol 68:97-107.

Chapman BA. 1993. Community acquired viral hepatitis in New Zealand: a case of sporadic hepatitis E virus infection. Aust NZ J Med 23:722-723.

Clayson ET, Myint KSA, Snitbhan R, Vaugh DW, Innis BL, Chan L, Cheung P, Shrestha M. 1995. Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E. J Infect Dis 172:927-933.

Dalecos GN, Zervou E, ElisafM, Germanos N, Galanakis E, Bourantas K, Siamopoulos KC, Tsianos EV. 1998. Antibodies to hepatitis E virus among several populations in Greece: increased prevalence in an hemodialysis unit. Transfusion 38:589-595.

Erker JC, Desai SM, Mushahwar IK. 1999. Rapid detection of hepatitis E virus RNA by reverse transcription-polymerase chain reaction using universal oligonucleotide primers. J Virol Methods 81:109-113.

Favorov MO, Kosoy MY, Tsarev SA, Childs JE, Margolis HS. 2000. Prevalence of antibody to hepatitis E virus among rodents in the United States. J Infect Dis 181:449-455.

Ghabrah TM, Tsarev SA, Yarbough PO, Emerson SU, Strickland GT, Purcell RH. 1998. Comparison of tests for antibody to hepatitis E virus. J Med Virol 55:134-137.

Kabrane-Lazizi Y, Fine JB, Elm J, Glass GE, Higa H, Diwan A, Gibbs CJ Jr, Meng X-J, Emerson SU, Purcell RH. 1999. Evidence for widespread infection of wild rats with hepatitis E virus in the United States. Am J Trop Med Hyg 61:331-335.

Kabrane-Lazizi Y, Emerson SU, Miller KD, Davey RT, Purcell RH. 2000. Acute hepatitis E caused by swine HEV-like virus in a traveller returning from Thailand [abstract 028]. In: 10th International Symposium on Viral Hepatitis and Liver Disease, April 9-13, 2000, Atlanta. p 16.

Khuroo MS. 1980. Study of an epidemic of non-A, non-B hepatitis: possibility of another human hepatitis virus distinct from posttransfusion non-A, non-B type. Am J Med 68:818-824.

Krawczynski K. 1998. Hepatitis E - current status. Advances in therapeutic hepatology: a world view. International Association for the Study of the Liver (IASL), Nov. 6, 1998, Chicago, Nov 23-31.

Maneerat Y, Clayson ET, Myint KSA, Young GD, Innis BL. 1996. Experimental infection of laboratory rats with the hepatitis E virus. J Med Virol 48:121-128.

Mast EE, Alter MJ, Holland PV, Purcell RH. 1998. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E virus antibody serum panel evaluation group. Hepatology 27:857-861.
Mateos ML, Camarero C, Lasa E, Teruel JL, Mir N, Baquero F. 1998. Hepatitis E virus: relevance in blood donors and other risk groups.Vox Sang 75:267-269.
McCrudden R, O'Connell S, Farrant T, Beaton S, Iredale JP, Fine D. 2000. Sporadic acute hepatitis E in the United Kingdom: an underdiagnosed phenomenon? Gut 46:732-733.
Meng X-J, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ, Emerson SU. 1997. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci USA 94:9860-9865.
Meng X-J, Halbur PG, Haynes JS, Tsareva TS, Bruna JD, Royer RL, Purcell RH, Emerson SU. 1998a. Experimental infection of pigs with the newly identi®ed swine hepatitis E virus (swine HEV), but not with human strains of HEV. Arch Virol 143:1405-1415.
Meng X-J, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Purcell RH, Emerson SU. 1998b. Genetic and experimental evidence for cross-species infection by the swine hepatitis E virus. J Virol 72:9714-9721.
Meng J, Cong M-E, Dai X, Pillot J, Purdy MA, Fields HA, Khudyakov YE. 1999. Primary structure of open reading frame 2 and 3 of the hepatitis E virus isolated from Morocco. J Med Virol 57:126-133.
Meng X-J, Dea S, Engle RE, Friendship R, Lyoo YS, Sirinarumitr T, Urairong K, Wang D, Wong D, Yoo D, Zhang Y, Purcell RH, 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. J Med Virol 59:297-302.
Meng J, Dai X, Rak J, Saboraud A, Lorareva E, Chang JC, Purdy MA, Fields HA, Khudyakov YE. 2001. Mapping of neutralizing antigenic epitope(s) of the hepatitis E virus. (in press).
Pavia M, Iiritano E, Veratti MA, Angelillo IF. 1998. Prevalence of hepatitis E antibodies in healthy persons in southern Italy. Infection 26:32-35.
Pina S, Jofre J, Emerson SU, Purcell RH, Girones R. 1998. Characterization of a strain of infectious hepatitis E virus isolated from sewage in an area where hepatitis E is not endemic. Appl Environ Microbiol 64:4485-4488.
Purdy MA, Krawczynski K. 1994. Hepatitis E. Gastroenterol Clin North Am 23:537-546.
Schlauder GG, Dawson GJ, Erker JC, Kwo PY, Knigge MF, Smalley DL, Rosenblatt JE, Desai SM, Mushahwar IK. 1998. The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. J Gen Virol 79:447-456.
Schlauder GG, Desai SM, Zanetti AR, Tassopoulos NC, Mushahwar IK. 1999. Novel hepatitis E virus (HEV) isolates from Europe: evidence for additional genotypes of HEV. J Med Virol 57:243-251.
Sylvan SP, Jacobson SH, Christenson B. 1998. Prevalence of antibodies to hepatitis E virus among hemodialysis patients in Sweden. J Med Virol 54:38-43.
Thomas DL, Yarbough PO, Vlahov D, Tsarev SA, Nelson KE, Saah AJ, Purcell RH. 1997. Seroreactivity to hepatitis E virus in areas where the disease is not endemic. J Clin Microbiol 35:1244-1247.
Tsang TH, Denison EK, Williams HV, Venczel LV, Ginsberg MM. 2000. Acute hepatitis E infection acquired in California. Clin Infect Dis 30:618-619.
Tsarev SA, Binn LN, Gomatos PJ, Arthur RR, Monier MK, Van Cuyck-Gandre H, Longer CF, Innis BL. 1999. Phylogenetic analysis of hepatitis E virus isolates from Egypt. J Med Virol 57:68-74.
Usmanov RK, Balayan MS, Dvoinikova OV, Alymbayeva DB, Zamyatina NA, Kazachkov YA, Belov VI. 1994. Experimental hepatitis E infection in lambs. Vopr Virusol 39:165-168.
Wu JC, Chen CM, Chiang TY, Sheen IJ, Chen JY, Tsai WH, Huang YH, Lee SD. 2000. Clinical and epidemiological implications of swine hepatitis E virus infection. J Med Virol 60:166-171.

J. Med Virol. 2001 Nov; 65(3):525-529