

# Susceptibility of Swine to Hepatitis E virus and its Significance to Human Health

K.B. Platt, professor of veterinary microbiology,

K-J. Yoon, assistant professor of veterinary microbiology and

J.J. Zimmerman associate professor of veterinary microbiology  
College of Veterinary Medicine

## ASL-R1511

### Summary and Implications

Previous reports indicate that swine can be experimentally infected with Asian isolates of human hepatitis E virus (HEV), which supports epidemiological data indicating that domestic swine can serve as a reservoir for the virus in parts of Asia and as such have the potential to transmit the virus to humans by the fecal-oral route or through contact with pork products. The increasing incidence of human HEV infections in the western hemisphere raises the question of whether or not pigs can play a role in the transmission of this virus in the Americas. Accordingly the susceptibility of swine to a New World isolate of the human hepatitis E virus, Mexico 14, was evaluated. No evidence of infection was detected in experimental pigs. However a high herd and individual prevalence rate for seroreactivity to recombinant HEV antigen was detected in Iowa swine during the selection of experimental pigs. These observations suggests that swine vary in their susceptibility to human HEV isolates. Whether or not swine are susceptible to other New World isolates of HEV and can serve as a reservoir for human infection remains to be determined. The significance of the high rate of seroreactivity of swine to recombinant antigen with respect to human and swine health is not known. An epidemiological study currently in progress should help answer this important question.

### Introduction

HEV causes acute to inapparent infections in humans and is characterized by high mortality rates in pregnant women. The virus is enterically transmitted and is a significant public health problem in Mexico and developing countries in Africa and Asia. The virus is currently classified as a calicivirus (4). A recent phylogenetic analysis of caliciviruses based on the hypervariable region of the capsid gene suggests that the family can be divided into five distinct subfamilies with HEV representing one subfamily.

In 1990, Balayan et al. (1) reported that pigs could be infected with a Russian isolate of HEV. Subsequently Innis et al. (5) infected four weanling pigs with a Nepalese

HEV isolate. The infected pigs failed to show any clinical signs or increases in hepatic transaminase levels. However, HEV antigen was detected in the livers of infected animals up to but not beyond 14 days post infection. Necrosis was only observed in rare, scattered individual hepatocytes. In response to these reports and the increasing prevalence of human HEV infections in the western hemisphere preliminary experiments were conducted to determine if New World isolates of HEV also could also infect pigs. As a result we discovered that potential experimental pigs from several herds with no previous history of unusual disease occurrence strongly seroreacted in the ELISA with recombinant HEV capsid antigen.

The following report summarizes initial experiments designed to determine whether or not swine are susceptible to the New World HEV isolate, Mexico 14, and the significance of the seroreactivity of swine serums to recombinant HEV antigen in the Iowa swine population with respect to human and swine health.

### Materials and Methods

*Susceptibility of swine to a Mexican isolate of HEV.* The infectivity of HEV Mexico 14 was assessed in 5 SPF weanling pigs. Pigs were selected for experimentation by assessing pre-virus-challenged liver biopsies for normality, liver transaminase levels (i.e. AST, ALT) and by the absence of seroreactivity in the ELISA to the 62 kD recombinant HEV capsid protein encoded by ORF 2 of HEV Burma. Test and control pigs were randomly selected and housed separately in BL3 quarters. Test pigs were challenged i.v. with 1,800 CID 50s of virus. Serums and fecal specimens were collected at 2 to 3 day intervals beginning 14 days prior to virus challenge and extending to the conclusion of the experiment on day 94. Serums were assayed for, AST and ALT, antibody to the recombinant HEV antigen and the presence of a HEV viremia. Feces were collected throughout the experiment and archived. One control was killed on the day of challenge (day 0) and tissues were collected from the liver and other organs for use as comparative controls for, reverse transcriptase polymerase chain reaction (RT-PCR) analysis, FA examination of frozen tissue sections, electronmicroscopic examination, and standard histopathological examination. On day 50, liver biopsies were taken from both virus challenged pigs and one control pig. Test pigs were again inoculated with HEV-Mexico 14 as before with the exception that half the inoculum was given i.v. and half subcutaneously in the snout and tongue. All pigs were killed between days 85 and 91.

A second experiment was done with two test pigs and two controls. Pigs were inoculated with 1,800 CID 50s of HEV Mexico 14 with half of the inoculum given i.v. and half given subcutaneously in the snout and tongue. Baseline liver transaminase values were established and liver biopsies taken prior to virus challenge and on day 19. Serums were collected as above. Pigs were killed and necropsied on day 59.

*Prevalence of seropositive pigs in Iowa.* Three hundred and ninety-eight serums representing 31 Iowa farms were collected during the spring of 1996. Twenty farms represented commercial sow herds, one a SPF herd and 10 represented finisher operations. Serums were analyzed by ELISA using recombinant HEV capsid protein as antigen.

### Results and Discussion

No significant evidence of infectivity by HEV Mexico 14 was detected in either group of experimental pigs. Liver biopsies of control pigs were normal. No evidence of inflammation or necrosis was observed in liver biopsies of virus challenged pigs in both experiments. However, diffuse areas of swollen, pale hepatocytes were seen in the liver biopsies taken on day 50 from virus challenged pigs of the first experiment. Similar changes were not observed in pigs of the second experiment that were biopsied on day 19. The hepatic transaminase levels of challenged and control pigs of both experiments remained within normal limits. There was no seroconversion in any of the four virus-inoculated pigs nor any evidence of virus infection in the pigs as determined by RT-PCR. No gross abnormalities or histological changes suggestive of virus infection were observed at necropsy. The apparent resistance of swine to infection by HEV Mexico 14 as demonstrated in the current study and the susceptibility of swine to infection by at least two Asian isolates of HEV suggest that the susceptibility of swine to HEV varies with virus isolate. Whether or not other New World isolates exist that are infective for pigs remains to be determined.

Results of the serological survey of Iowa swine are summarized in Figure 1. These data reveal a widespread prevalence of antibody in swine that reacts with the recombinant HEV antigen used in the assay. When a conservative OD cutoff value of 1.000 is used for a 1:100 serum dilution as is used for testing human sera, the herd and individual prevalence is 80.6% and 39.6%, respectively. When an OD cutoff value of 0.300 is used that was calculated using serums from SPF swine the herd and individual prevalence increases to 90.3% and 87.5%, respectively. The significance of this seroreactivity is not clear. The HEV is a calicivirus and as such the seroreactivity may represent cross-reactivity between members of the same virus family. This is a very likely possibility in view of a recent report by Meng et al. (6) who demonstrated the presence of a likely viral candidate by RT-PCR. They used a series of degenerate primers to analyze archived serums collected from young pigs prior to seroconversion to HEV recombinant antigen and demonstrated the presence of genetic material that shared a nucleotide homology of approximately 80% with ORF 2 and 3 of human HEV strains.

The significance of their finding with respect to public health and swine health remains to be determined. An ongoing retrospective survey of producers and veterinarians associated with swine herds with high and low individual prevalence rates is being conducted by this laboratory. To date this survey has not revealed any unusual pattern of swine health problems that may be due

to an agent or agents capable of inducing cross-reactive antibody. A stratified study of serums collected from humans associated and not associated with the swine industry is also in progress and may reveal whether or not the agent or agents responsible for HEV seroreactivity in swine have the potential to infect humans. At this point in time there is no justifiable reason to suspect that any virus that is in the Iowa swine population and that is capable of inducing an antibody response to the HEV recombinant antigen poses a threat to public health.

### References

1. Balayan, M.S. R.K. Usmanov, N.A. Zamyatina, D.I. Djumalieva, and R.F. Karas. 1990. Brief report: experimental hepatitis E infection in domestic pigs. *J. Med. Virol.* 32:58-59.
2. Berke, T., B. Golding, X. Jiang, D. Cubitt, M/Wolfaardt, A.W. Smith, D.O. Matson. 1997. Phylogenetic analysis of the caliciviruses. *J. Med. Virol.* 52:419-424.
3. Clayson E.T., B.L. Innis, K.S.A. Myint, S. Narupiti, D.W. Vaughn, S. Giri, P. Ranabhat and M. Shrestha. 1995. Detection of hepatitis E virus infections among domestic swine in the Kathamandu Valley of Nepal. *Am. J. Trop. Med. Hyg.* 53:228-232.
4. Cubitt, W.D., D. Bradley, M. Carter, S. Chiba, M. Estes, L. Saif, F. Schaffer, A.W. Smith, M.J. Studdert, H. J. Thiel. 1995. Caliciviridae In: *The sixth Report of the International Committee for Taxonomy of viruses* ed. by Murphy F.A., Fauquet C.M., Bishop D.H.L., Ghabrial S.A., Jarvis A.W., Martel G.P., Mayo M.A., Summers M.D.. *Arch. Virol.* (suppl) 10:359-363.
5. Innis B.L., K.D. Corcoran, K.S.A. Myint, E.T. Clayson, M. Ngampochjana, G.D. Young, D. Mongkolsirichaikul, S. Narupiti, C. Manomuth, P. Hansukjariya, K.B. Platt. 1993. Experimental transmission of hepatitis E virus to swine. *Proc., Abstr. of 1993 Intrnl. Symp.*
6. Meng, X-J., R.H. Purchell, P.G. Halbur, J.R. Lehman, D.M. Webb, T.S. Tsareva, J.S. Haynes, B.J. Thacker, S.U. Emerson, 1997. A novel virus in swine is closely related to the human hepatitis E virus. *Proc. Natl. Acad. Sci., U.S.A.* 94:1-6.

Figure 1. Prevalence of antibody in Iowa swine that reacts in the ELISA with recombinant hepatitis E virus. The optical density cut-off point is 1.000. The bottom boundaries of the shaded boxes represent the 25th percentile. The upper boundaries represent the 75th percentile. The line within each box is the median. Error bars above and below individual boxes represent the 95th and 5th percentile within the herd.

