RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Assessment of the Zoonotic Risk of Swine Hepatitis E Virus Infection in Swine Veterinarians in the United States - **NPB# 01-004**

Investigator: Xiang-Jin Meng, M.D., Ph.D.

Institution: Virginia Polytechnic Institute and State University

Date Received: 5/7/2002

I: Abstract:

Hepatitis E virus (HEV), the causative agent of human hepatitis E, is endemic in many developing and some industrialized countries. It has been hypothesized that animals may be the source of infection. The identification of swine hepatitis E virus (swine HEV) in U.S. pigs and the demonstration of its ability to infect across species have led credence to this hypothesis. To assess the potential risk of zoonotic HEV infection, we tested a total of 468 veterinarians working with swine (including 389 U.S. swine veterinarians) and 400 normal U.S. blood donors for IgG HEV antibodies (anti-HEV). Recombinant capsid antigens from a U.S. strain of swine HEV and from a human strain of HEV (Sar-55) were each used in the ELISA. The anti-HEV prevalence assayed with the swine HEV antigen showed 97% concordance with that obtained with the human HEV antigen (K=92%). Among the 295 swine veterinarians tested from the eight U.S. States (Minnesota, Indiana, Nebraska, Iowa, Illinois, Missouri, North Carolina and Alabama) from which normal blood donor samples were available, 26% were positive with Sar-55 antigen and 23% with swine HEV antigen. In contrast, 18% of the blood donors from the same eight U.S. states were positive with Sar-55 antigen and 17% were positive with swine HEV antigen. Swine veterinarians in the eight States were 1.51 times when tested with swine HEV antigen (95% confidence interval [1.03-2.20]) and 1.46 times when tested with Sar-55 antigen (95% confidence interval [0.99-2.17]) more likely to be anti-HEV positive than normal blood donors. We did not find a difference in anti-HEV prevalence between veterinarians who reported having had a needle stick or cut and those who had not, or between those who spent more time (≥80% of time) and those who spent less time (≤20% of time) working with pigs. Similarly, we did not find a difference in anti-HEV prevalence according to four veterinary job categories (academic, practicing, student and industry veterinarians). There was a difference in anti-HEV prevalence in both swine veterinarians and blood donors among the 8 selected states, with subjects from Minnesota (a major swine state) 6 times more likely to be anti-HEV positive than those from Alabama (a traditionally non-swine state). Age was not a factor for the observed differences from state to state. Anti-HEV prevalence in swine veterinarians and normal blood donors was

age-specific and paralleled increasing ages. The results suggest that swine veterinarians are at higher risk of HEV infection than are normal blood donors.

II. Introduction:

Human hepatitis E is an important public health disease in many developing countries and is also endemic in industrialized countries such as the United States. The disease generally affects young adults. The mortality rate associated with HEV infection in infected pregnant women is reportedly as high as 20%. The causative agent of hepatitis E, hepatitis E virus (HEV), is a single-stranded positive-sense RNA virus without an envelope. HEV is generally transmitted by the fecal-oral route. The genomic RNA of HEV is about 7.5 kb and contains 3 open reading frames (ORFs). ORF1 is predicted to encode viral nonstructural proteins, ORF2 encodes the putative capsid protein and ORF3 encodes a cytoskeleton-associated phosphoprotein. HEV remains unclassified (Kabrane-Lazizi et al., 1999; Emerson et al., 2001).

Swine hepatitis E virus (swine HEV) was first identified by Meng et al in 1997 from a pig in Illinois (Meng et al., 1997). Swine HEV is ubiquitous in pigs in the United States (Meng et al., 1997, 1999). Later studies revealed that swine from other countries such as Australia, Thailand, Vietnam, Taiwan, Korea, China, Canada and Spain were also infected with HEV (Meng et al, 1997, 1999; Hsieh et al., 1999; Meng, 2000a, 2000b, 2002; Wu et al., 2002; Fang et al, 2002). The swine HEV strain isolated from a pig in Illinois is genetically very closely related to two U.S. strains of human HEV (Meng et al., 1998a, 1998b). Similarly, the swine HEV strains isolated from pigs in Taiwan are closely related to Taiwanese strains of human HEV. Interspecies transmission of HEV has been experimentally demonstrated: swine HEV infected non-human primates and a U.S. strain of human HEV infected pigs (Meng et al., 1998a; Halbur et al., 2001). These data suggested that HEV infection of humans through contact with pigs may be possible, and that swine veterinarians and other pig handlers may be at risk of zoonotic infection.

In a preliminary study, we tested a very limited number of pig handlers from two countries with endemic HEV (Meng et al., 1999). We found that 11 of 11 swine veterinarians from China and 5 of 7 swine veterinarians from Thailand were positive for IgG anti-HEV. However, 17/31 (55%) normal blood donors in China were also positive for anti-HEV. A conclusion as to whether swine handlers have a higher risk of HEV infection could not be drawn from our preliminary study because of the limited number of swine handlers tested and because of the high anti-HEV background level in normal blood donors from endemic countries. A much larger number of subjects, preferably in industrialized countries where hepatitis E is rare, was needed to determine the risk of transmitting HEV from pigs to humans. Therefore it is important to evaluate the potential risk and risk factors of HEV infection in U.S. swine handlers such as swine veterinarians. The information gained from this study will help pork producers, swine veterinarians and other swine handlers prevent potential HEV zoonosis.

III. Objectives:

(1). To assess the prevalence of anti-HEV antibody in the U.S. swine veterinarians and normal blood donors. (2). To identify potential risk factors associated with HEV zoonosis in swine veterinarians.

IV. Procedures:

Collection of human serum samples. Serum samples were taken from a total of 468 swine veterinarians attending the 1999 Annual Meeting of the American Association of Swine Practitioners. Participants' background information was obtained, including age,

percentage of time working with pigs, state of residence, job category (practicing veterinarians, industry, academic veterinarians, and veterinary students), history of needle stick, or cut with blood to blood contact. About 85% of the participants were from the U.S. or Canada. About 6% were from other regions of the world including Australia, Denmark, Italy, Japan, Mexico, Philippines, Spain and South America. The remaining 9% of the participants did not provide geographic information. From the 8 U.S. States (Iowa, Minnesota, Illinois, Indiana, North Carolina, Nebraska, Missouri, and Alabama) where most of the veterinarians resided, 400 control sera were collected from normal blood donors by Millennium Biotech, Inc. The blood donors' age and sex were also recorded. All samples were coded and tested blindly. The study was approved by the Institutional Review Board of Virginia Polytechnic Institute and State University.

Production of swine HEV capsid (ORF2) protein. The putative capsid gene (ORF2) of swine HEV was amplified by RT-PCR with a set of swine HEV specific primers: forward primer, 5'-TTCGGATCC**ATG**CGCCCTAGGGCTGTTCTGTTGTTGCTC-3'; reverse primer, 5'-CAACTCGAG**TCATTA**AGATTCCCGGGTTTTACCTACCTT-3'. The expected PCR product was purified from an agarose gel with a GeneClean kit and sequenced. The sequence was identical to that of the published sequence of swine HEV (Meng et al., 1997, 1998a). The putative capsid gene (ORF2) of swine HEV was subsequently cloned into a baculovirus expression vector and expressed in insect cells essentially as described previously for the capsid protein of the human HEV strain Sar-55 (Meng et al., 1997, 1998b, 1999; Robinson et al., 1998). The recombinant capsid protein of swine HEV, purified by anion-exchange and subsequent gel filtration chromatography as described previously (Robinson et al., 1998), was used in an ELISA.

Standardization of ELISA for detecting anti-HEV antibodies in humans. The recombinant capsid protein of the Sar-55 strain of human HEV is broadly reactive for the detection of anti-HEV antibodies, and was used as the antigen in one ELISA. Our earlier studies have shown that the human HEV Sar-55 antigen reacts well with antibodies to swine HEV (Meng et al., 1997, 1999; Kasorndorkbua et al., 2002). The similarly prepared recombinant capsid protein of swine HEV was used in a second ELISA. The ELISA protocol, standardized to detect anti-HEV in humans, has been described previously. Convalescent sera from a chimpanzee experimentally infected with HEV and preinoculation chimpanzee sera were included as positive and negative controls, respectively. Briefly, capture plates were prepared by adding 100 µl of purified swine HEV antigen or human HEV Sar-55 antigen to wells of flat bottom polystyrene 96-well plates (Linbro/Titertek) at 0.05 µg/well. The plates were incubated overnight at room temperature. The coated plates were washed twice with PBS-0.02% Tween-20, super-coated with 120 µl of blocking solution (0.5% gelatin, 0.03M NaCl, 10% fetal bovine serum) and incubated for 1 hour at 37ºC to reduce non-specific binding. All serum samples were tested in duplicate at a dilution of 1:100 both with the Sar-55 antigen and with the swine HEV antigen. Goat anti-human IgG (KPL, Gaithersburg, MD) was used as the secondary antibody. Azino-diethylbenzotyazol-sulfonate (ABTS) was used as the substrate for the development of a colorimetric reaction. The plates were read at an absorbance of 405 nm.

All ELISAs were calibrated against an anti-HEV antibody standard recently proposed by the World Health Organization (WHO). Four five-fold dilutions of a well-characterized IgG anti-HEV secondary antibody standard (0.250, 0.050, 0.010 and 0.002 WHO units) were tested with each plate. The standard used in this study was calibrated to the WHO

anti-HEV antibody standard preparation 95/584 (100 units/ml) which is available from the National Institute for Biological Standards and Control, Hertfordshire, England. The proposed WHO standard is a lyophilized human serum preparation that, when re-suspended with 0.5 ml of distilled water, yields 100 units/ml of anti-HEV. Based on previous comparisons, the 0.010 WHO unit standard served as a reliable cutoff point for both the Sar-55 human HEV and swine HEV ELISAs, as determined by end-point dilution studies. A serum sample with an OD value equal to or above this cutoff was considered positive. Samples that were positive at 1:100 were confirmed by re-testing, and were further titrated at 1:1,000 and 1:10,000 dilutions.

Statistical analyses. Results were analyzed from a total of 868 subjects (864 for whom the age was known and 825 for whom the geographic location was available). Samples with both geographic location and age information were obtained from 295 swine veterinarians and 400 normal blood donors from the 8 selected states. Information about potential risk factors was complete for 412 swine veterinarians. All variables were first evaluated by univariate analysis using PROC FREQ and PROC GENMOD of SAS® (SAS®, release 8.01, 2000. SAS Institute, Cary, NC). Variables with model p-values <0.20 were selected for further analysis by multivariate logistic regression using PROC GENMOD. The best model fit was found by a combined forward- and backward-selection process in which the likelihood-ratio test was used to test the significance of adding to or subtracting one variable at a time from the model. Potentially relevant 2- and 3- way interactions were evaluated by the forward-selection process.

V. Results:

Detection of anti-HEV antibodies with recombinant HEV capsid antigens from a swine HEV and a human HEV. Our previous studies showed that the Sar-55 human HEV antigen reacted well with anti-HEV in sera from pigs and primates experimentally infected with swine HEV (Meng et al., 1997, 1998a; Halbur et al., 2001; Williams et al., 2001) and with anti-HEV in sera of chickens experimentally infected with the newly identified avian HEV (Haqshenas et al., 2001a, 2001b, 2002). In this study, we expressed the putative capsid protein of the swine HEV from recombinant baculoviruses in insect cells and used the purified antigen for comparison with the human HEV Sar-55 antigen in ELISA. All sera were tested in duplicate with both recombinant antigens. The results obtained with the human HEV Sar-55 antigen show 97.4% concordance with those obtained with swine HEV antigen for a kappa value of 0.92, indicating excellent agreement. This is not surprising since the putative capsid protein of swine HEV shares about 92% amino acid sequence identity with that of the Sar-55 strain of human HEV, and our previous studies demonstrated that the human HEV Sar-55 antigen cross-reacted well with antibodies to swine HEV. Among the 109 of 468 swine veterinarians positive with Sar-55 antigen and 97 swine veterinarians positive with swine HEV antigen, 95 were positive with both antigens. There were 2 sera positive with swine HEV antigen but negative with Sar-55 antigen and 14 sera positive with Sar-55 antigen but negative with swine HEV antigen. Similarly, among the 73 of 400 normal blood donors positive with Sar-55 antigen and 66 normal blood donors positive with swine HEV antigen, 66 were positive with both antigens. There were 7 sera positive with Sar-55 but negative with swine HEV antigen, and 0 sera positive with swine HEV but negative with Sar-55 antigen. Thus, the Sar-55 antigen was slightly more sensitive than the swine HEV antigen for detecting anti-HEV in both populations.

Prevalence of IgG anti-HEV antibodies in swine veterinarians from the United States and other countries. The veterinarians tested in this study all reported having contact with swine, ranging from 1% to 100% of time working with swine. Among all the 468 swine veterinarians tested, 109 (23%) were positive for anti-HEV when tested with Sar-55 antigen and 97 (21%) were positive when tested with swine HEV antigen. Among the 295 swine veterinarians from the 8 U.S. states from which normal blood donor data were available, 78 (26%) were positive for anti-HEV with Sar-55 antigen and 68 (23%) were positive with swine HEV antigen (Table 1). In contrast, 73 of 400 (18%) normal blood donors from the same eight U.S. states were positive with Sar-55 antigen and 66 (16%) were positive with swine HEV antigen (Table 1). Swine veterinarians in these eight states with blood donor controls were 1.51 times more likely to be anti-HEV positive than were normal blood donors when tested with swine HEV antigen (95% confidence interval [1.03-2.20]) and 1.46 times more likely to be anti-HEV positive when tested with Sar-55 antigen (95% confidence interval [0.99-2.17]). There was a difference in anti-HEV prevalence in both swine veterinarians and blood donors among the 8 selected states, with subjects from Minnesota 6 times more likely to be anti-HEV positive than those from Alabama. Age was not a factor for the observed differences from state to state. Except for Alabama, the other 7 states are considered major pork-producing states in the U.S. (data from National Animal Health Monitor System 2000 study) with North Carolina joining the ranks only in the last 2 decades. Since age was not a factor for the observed differences among states, it is possible that geography might be a risk factor. However, since many swine veterinarians practice in multiple states and since there exist other potential animal reservoirs for HEV, a definitive conclusion as to whether individuals from states with higher pig populations have higher risks could not be drawn. Fifteen of 93 (16%) swine veterinarians from 21 other U.S. states from which normal blood donors were not available were also positive for IgG anti-HEV. IgG anti-HEV was also detected in 8 of 37 (22%) swine veterinarians from other countries (Table 1).

Assessment of potential risk factors associated with HEV infection in swine veterinarians. In an attempt to identify potential risk factors that may be associated with HEV infection in swine veterinarians, we compared anti-HEV serological data with the available exposure history of the swine veterinarians (Table 2). Multiple variant analyses showed that there was no significant difference in anti-HEV prevalence between swine veterinarians who had reported having a history of needle stick or cut with blood to blood contact and those who did not (Table 2). There was also no difference in anti-HEV prevalence between those who spent a greater percentage of time (≥80% of time) and those who spent less time (≤20% of time) working with pigs (Table 2). These findings are not surprising since, in swine HEV-infected pigs, viremia lasts only about 1 to 2 weeks and virus shedding in feces also lasts only a few weeks. Acute HEV infection occurs primarily in young pigs 2 to 3 months of age. Therefore, it may be that the age of the pigs rather than the percentage of time spent with them is important for zoonotic HEV infection. The veterinary students had the lowest anti-HEV prevalence among the 4 job categories (industrial veterinarians, academic veterinarians, practicing veterinarians and veterinary students). However, the students were <30 years of age and the low prevalence in students was largely due to the age factor since multivariate analyses did not find a difference in anti-HEV prevalence among the four different job categories. There was an association between age and prevalence of anti-HEV both in swine veterinarians and in blood donors (Table 2).

Age-specific prevalence of IgG anti-HEV in swine veterinarians and in normal blood donors. To determine the interaction between age and geography, we analyzed the

serological data derived from different age groups (<30, 30-39, 40-49, 50-59 and ≥60 years old) of the 295 swine veterinarians and 400 normal blood donors from 8 states (Table 3). Anti-HEV prevalence in both swine veterinarians and normal blood donors increased with age, which is consistent with other HEV seroepidemiological studies in humans (Meng, 2000a, 2000b). In the 8 states from which blood donors were available, about 39% (Sar-55 antigen) or 29% (swine HEV antigen) of the swine veterinarians over 60 years of age were positive for anti-HEV compared to only about 13% (Sar-55 antigen) or 7% (swine HEV antigen) of the swine veterinarians younger than 30 years of age. A similar pattern was also found in the normal blood donors. Swine veterinarians and blood donors over 60 years of age were 4.0 times (Sar-55) or 4.3 times (swine HEV antigen) more likely to be positive for anti-HEV than those younger than 30 years of age (Table 3). This parallelism of anti-HEV prevalence with age was independent of state residence.

Summary of the knowledge of immediate or future benefit to pork producers:
(1). Pigs should be considered a reservoir for HEV. Swine veterinarians and other pig handlers are at increased risk of zoonotic HEV infection. (2). The results from this study suggested that individuals from major swine-producing states might have higher risk of zoonotic HEV infection than those from traditionally non-swine states. However, more studies are needed in order to draw a definitive conclusion. (3). Age and geography appeared to be potential risk factors for zoonotic HEV infection. However, no association was found for other factors such as percentage of time working with pigs, job category, history of needle stick, or cut with blood to blood contact, etc. (4). As HEV is transmitted fecal-orally, the most effective measure to prevent HEV zoonosis is to wash hands after handling pigs and avoid drinking contaminated water. Education of pork producers about this disease is the key to effectively prevent zoonotic transmission since there is no vaccine available against HEV.

References:

Emerson SU, Zhang M, Meng X.J., St. Clair M, Nguyen H, Huang Y, and Purcell RH (2001). Recombinant hepatitis E virus genomes infectious for primates: importance of capping and discovery of a cis-reactive element. *Proceedings of the National Academy of Sciences USA* 98:15270-15275.

Halbur, P.G., C. Kasorndorkbua, C. Gilbert, D. Guenette, M. B. Potters, R. H. Purcell, S. U. Emerson, T.E. Toth, X.J. Meng (2001). Comparative pathogenesis of infections of growing pigs with hepatitis E viruses recovered from a swine and a human. *Journal of Clinical Microbiology*. 39:918-923.

Haqshenas, G., H.L. Shivaprasad, P. Woolcock, D. Read, X.J. Meng (2001a). Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-spleenomegaly syndrome. *Journal of General Virology*. 82(10):2449-2462.

Haqshenas G. and X.J. Meng (2001b). Determination of the nucleotide sequences of the extreme 3' and 5' ends of the swine hepatitis E virus genome. *Archives of Virology*. 146:2461-2467.

Haqshenas G., F.F. Huang, M. Fenaux, D.K. Guenette, F.W. Pierson, H.L. Shivaprasda, T.E. Toth and X.J. Meng (2002). The putative capsid protein of the newly identified avian hepatitis E virus shares antigenic epitopes with that of swine and human hepatitis E viruses and the chicken big liver and spleen disease virus. *Journal of General Virology.* In Press.

Hsieh, S.-Y., X.J. Meng, Y.-H. Wu, A.W. Tam, S.-T. Liu, D.-Y. Lin, and Y.-F. Liaw (1999). Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group

with the Taiwan isolates of human hepatitis E virus. *Journal of Clinical Microbiology*. 37:3828-3834.

Huang, F.F., G.Haqshenas, D.K. Guenette, P.G. Halbur, S.Schommer, F.W. Pierson, T.E. Toth and X.J. Meng (2002). Detection by RT-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the U.S. *Journal of Clinical Microbiology*. 40:1326-1332.

Kabrane-Lazizi, Y., J.B. Fine, J. Elm, G.E. Glass, H. Higa, A. Diwan, C.J. Gibbs Jr, X.J. Meng, S.U. Emerson, R.H. Purcell (1999a). Evidence for wide-spread infection of wild rats with hepatitis E virus in the United States. *American Journal of Tropical Medicine and Hygiene*. 61:331-335.

Kabrane-Lazizi, Y., X.J. Meng, R.H. Purcell, S.U. Emerson. Evidence that the genomic RNA of hepatitis E virus is capped (1999b). *Journal of Virology*. 73:8848-8850.

Kasorndorkbua, C., P.G. Halbur, D.K. Guenette, T.E. Toth, X.J. Meng (2002). Use of a swine bioassay and a RT-PCR assay to assess the risk of transmission of swine hepatitis E virus in pigs. *Journal of Virological Methods*. 101:71-78.

Meng, X.J., R.H. Purcell, P.G. Halbur, J.R. Lehman, D.M. Webb, T.S. Tsareva, J.S. Haynes, B.J. Thacker, and S.U. Emerson (1997). A novel virus in swine is closely related to the human hepatitis E virus. *Proceedings of the National Academy of Sciences USA* 94:9860-9865.

Meng, X.J., P.G. Halbur, M. Shapiro, S. Govindarajan, J.D. Bruna, I. K. Mushahwar, R.H. Purcell, and S.U. Emerson (1998a). Genetic and experimental evidence for cross-species infection by the swine hepatitis E virus. *Journal of Virology*. 72:9714-9721.

Meng, X.J., P.G. Halbur, J.S. Haynes, T.S. Tsareva, J.D. Bruna, R.D. Royer, R.H. Purcell, and S.U. Emerson (1998b). Experimental infection of pigs with the newly identified swine hepatitis E virus, but not with human strains of HEV. *Archives of Virology*. 143:1405-1415

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

Meng, X.J (2000a). Novel strains of hepatitis E virus identified from humans and other animal species: Is hepatitis E a zoonosis? *Journal of Hepatology* 33:842-845.

Meng, X.J. (2000b). Zoonotic and xenozoonotic risks of the hepatitis E virus. *Infectious Disease Review*. 2(1):35-41.

Meng, X.J. (2002). Swine hepatitis E virus: cross-species infections into humans. *In*: C. Wilson and D. Salamon (eds) *Current Topics in Microbiology and Immunology: Xenotransplantation*. Springer-Verlag, NY. In Press.

Robinson, R.A., W.H. Burgess, S.U. Emerson, R.S. Leibowitz, S.A. Sosnovtseva, S. Tsarev, and R.H. Purcell. 1998. Structural characterization of recombinant hepatitis E virus ORF2 proteins in baculovirus-infected insect cells. *Protein Expr. Purif*. 12:75-84.

Williams, T., Kasorndorkbua C., Halbur P.G., Guenette D., Toth T.E., X.J. Meng (2001). Evidence for extrahepatic sites of replication of the hepatitis E virus in a swine model. *Journal of Clinical Microbiology*. 39(9):3040-3046.

Wu, J.C., CM Chen, TY Chiang, WH Tsai, WJ Jeng, IJ Sheen, JY Chen, CC Lin, X.J. Meng (2002). Potential Subclinical spread of HEV among swine, swine handlers and different countries: a longitudinal study. *Journal of Medical Virology*. 66:488-492.

Table 1. Prevalence of IgG anti-HEV in swine veterinarians and normal blood donors from different geographic regions

Location	Human HEV (Sar-55) No. pos. /No. tested (%) Swine vets	Blood donors	OR[d]	95% confidence interval	Swine HEV No. pos. /No. tested (%) Swine vets	Blood donors	OR[d]	95% confidence interval
USA[a]	78/295(26.4)	73/400(18.3)			68/295(23.1)	66/400(16.5)		
MN	21/47(44.7)	14/50(28.0)	6.33	[2.30;17.43]	17/47(36.2)	14/50(28.0)	5.13	[1.85;14.25]
IN	5/30(16.7)	18/50(36.0)	4.64	[1.64;13.17]	5/30(16.7)	17/50(34.0)	4.25	[1.49;12.09]
NE	6/27(22.2)	12/50(24.0)	3.63	[1.25;10.53]	4/27(14.8)	12/50(24.0)	3.00	[1.02;8.79]
IA	26/90(28.9)	8/50(16.0)	3.40	[1.25;9.30]	24/90(26.7)	5/50(10.0)	2.72	[0.99;7.52]
IL	11/37(29.7)	9/50(18.0)	3.25	[1.14;9.31]	9/37(24.3)	7/50(14.0)	2.40	[0.82;7.02]
MO	1/19(5.3)	7/50(14.0)	1.73	[0.53;5.65]	1/19(5.3)	7/50(14.0)	1.69	[0.52;5.51]
NC	5/22(22.7)	3/50(6.0)	1.50	[0.46;4.89]	5/22(22.7)	2/50(4.0)	1.26	[0.38;4.23]
AL	3/23(13.0)	2/50(4.0)			3/23(13.0)	2/50(4.0)		
USA[b] Other State	15/93(16.1)		2.62[e]	[0.90;7.57]	15/93(16.1)		2.62[e]	[0.90;7.57]
Non-USA[c]	8/37(21.6)		0.97[f]	[0.43;2.15]	8/37(21.6)		0.84[f]	[0.38;1.88]

[a] Swine veterinarians from eight U.S. states from which normal blood donors were available. Compared to normal blood donors, swine veterinarians were 1.46 times (p=0.06, 95% confidence interval: [0.99;2.17]) more likely to be positive for anti-HEV when tested with Sar-55 antigen and 1.51 times (p=0.03, 95% confidence interval: [1.03;2.20]) more likely to be positive when tested with swine HEV antigen.

[b] Swine veterinarians from 21 other U.S. states from which blood donors were not available: 12 from KS, 11 from OH, 9 from MI, 8 from KY, 8 from WI, 7 from PA, 7 from SD, 5 from OK, 4 from CO, 4 from GA, 1 from each of eleven states (AR, AZ, CT, MD, MS, ND, NJ, NY, TN, VA, WY), and 7 without location information.

[c] Swine veterinarians from other countries: 11 from Mexico, 10 from Canada, 4 from Spain, 2 each from Denmark and Japan, 1 each from Australia, Belgium, Brazil, Italy, Philippines, Sweden and United Kingdom. One respondent listed South America.

[d] Odds ratio. Odds of seropositive test for pooled swine veterinarians and blood donors of each state to odds of Alabama subjects. There was no location x profession or location x age interaction in the multivariate model.

[e] Odds of seropositive test in other U.S. states' swine veterinarians to odds in AL subjects; separate analysis.

[f] Odds of seropositive test in 789 US subjects to odds in non-U.S. swine veterinarians; separate analysis.

Table 2. Risk factors associated with HEV infection in veterinarians working with swine

		Human HEV (Sar-55)				Swine HEV			
	No. tested	**No. positive**	**(%)**	**OR [a]**	**95% confidence interval**	**No. positive**	**(%)**	**OR[a]**	**95% confidence interval[a]**
Reported needle stick[b]									
yes	351	87	(25)	1.90	[0.90;4.02]	78	(22)	1.89	[0.86;4.15]
no	61	9	(15)			8	(13)		
Reported cut with blood-blood contact[c]									
yes	337	82	(24)	1.40	[0.74;2.64]	73	(22)	1.32	[0.69;2.53]
no	75	14	(19)			13	(17)		
Percentage of time working with swine[d]									
80+	180	45	(25)	1.02	[0.56:1.83]	41	(23)	1.25	[0.66:2.35]
60-79	32	4	(13)	0.44	[0.14:1.38]	4	(13)	0.61	[0.19:1.96]
40-59	47	15	(32)	1.43	[0.65:3.11]	15	(32)	1.99	[0.88:4.46]
20-39	64	10	(16)	0.56	[0.25:1.29]	10	(16)	0.69	[0.29:1.67]
0-19	89	22	(25)			22	(25)		
Veterinarians' job category[e]									
Industry	108	30	(28)	6.15	[0.78;48.46]	28	(26)	5.60	[0.71;44.19]
Practicing	210	48	(23)	4.74	[0.61;36.67]	43	(20)	4.12	[0.53;31.94]
Academic	77	17	(22)	4.53	[0.56;36.69]	14	(18)	3.56	[0.43;29.08]
Student	17	1	(6)			1	(6)		
Age[f]									
60+ years	27	10	(37)	5.29	[1.58;17.74]	7	(26)	5.48	[1.29;23.38]
50-59 years	62	21	(34)	4.61	[1.59;13.35]	18	(29)	6.41	[1.77;23.27]
40-49 years	149	40	(27)	3.30	[1.22;8.91]	40	(27)	5.75	[1.69;19.51]
30-39 years	124	20	(16)	1.73	[0.61;4.90]	18	(15)	2.66	[0.75;9.47]
<30 years	50	5	(10)			3	(6)		

[a] **OR** and P values from univariate analyses. Inclusion in multivariate logistic regression of needle stick, cut with blood-to-blood contact, percentage of time working with swine or job category, either separate or combined, to age, or interaction with age, did not improve the model fit (Sar-55 antigen, P=0.23; swine HEV antigen, P=0.21).

[b] Sar-55: X^2_{1df}=3.19, P=0.07; Swine-HEV: X^2_{1df}=2.86, P=0.09

[c] Sar-55: X^2_{1df}=1.15, P=0.28; Swine-HEV: X^2_{1df}=0.72, P=0.40

[d] Sar-55: X^2_{4df}=6.90, P=0.14; Swine-HEV: X^2_{4df}=7.18, P=0.13

[e] Sar-55: X^2_{3df}=5.05, P=0.17; Swine-HEV: X^2_{3df}=4.97, P=0.17

[f] Sar-55: X^2_{4df}=16.94, P=0.002; Swine-HEV: X^2_{4df}=17.74, P=0.0014

Table 3. Age-specific IgG anti-HEV prevalence in swine veterinarians and normal blood donors from 8 U.S. states

	Swine Veterinarians			Blood Donors				
Age (years)	**No. tested**	**No. pos.**	**(%)[c]**	**No. tested**	**No. pos.**	**(%)[c]**	**OR[d]**	**95% confidence interval**
Human HEV (Sar-55)[a]								
60+ *	18	7	(39)	55	16	(29)	4.00	[1.77;9.03]
50-59 *†	48	18	(38)	65	16	(25)	3.06	[1.43;6.54]
40-49 †¶	117	31	(27)	104	21	(20)	2.30	[1.32;4.71]
30-39 †¶	82	18	(22)	95	13	(14)	1.74	[0.82;3.70]
<30 ¶	30	4	(13)	81	7	(9)		
Swine HEV[b]								
60+ *	18	5	(28)	55	14	(25)	4.34	[1.76;10.73]
50-59 *†	48	15	(31)	65	16	(25)	3.92	[1.68;9.12]
40-49 †¶	117	31	(27)	104	18	(17)	3.13	[1.40;6.98]
30-39 †¶	82	15	(18)	95	12	(13)	2.12	[0.91;4.92]
<30 ¶	30	2	(7)	81	6	(7)		

[a]ELISA with human HEV Sar-55 recombinant antigen

[b]ELISA with swine HEV recombinant antigen

[c]Odds of seropositivity for swine veterinarians or blood donors. Rows with different subscript symbols (*, †, ¶) differ (P<0.02 for Sar-55 antigen; P<0.04 for swine HEV antigen).

[d]OR for swine veterinarians and blood donors combined. Multivariate model including profession (odds of seropositive test in 295 veterinarians to odds in 400 control subjects from 8 U.S. states: $OR_{Sar\text{-}55}$= 1.46, 95% confidence interval [0.99;2.17]; $OR_{Swine\text{-}HEV}$= 1.51, 95% confidence interval [1.03;2.20]), state and age. There was no age x profession interaction.