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# Prevalence of Antibodies to Hepatitis E Virus in Veterinarians Working with Swine and in Normal Blood Donors in the United States and Other Countries

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**Hepatitis E virus (HEV) is endemic in many developing and some industrialized countries. It has been hypothesized that animals may be the source of infection. The recent identification of swine HEV in U.S. pigs and the demonstration of its ability to infect across species have lent 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 immunoglobulin G anti-HEV. Recombinant capsid antigens from a U.S. strain of swine HEV and from a human HEV strain (Sar-55) were each used in an enzyme-linked immunosorbent assay. The anti-HEV prevalence assayed with the swine HEV antigen showed 97% concordance with that obtained with the human HEV antigen ($\kappa = 92\%$). 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% were positive 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 more likely when tested with swine HEV antigen (95% confidence interval, 1.03 to 2.20) and 1.46 times more likely when tested with Sar-55 antigen (95% confidence interval, 0.99 to 2.17) 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 the time) and those who spent less time (≤20% of the time) working with pigs. Similarly, we did not find a difference in anti-HEV prevalence according to four 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 eight selected states, with subjects from Minnesota six times more likely to be anti-HEV positive than those from Alabama. Age was not a factor in the observed differences from state to state. Anti-HEV prevalence in swine veterinarians and normal blood donors was age specific and paralleled increasing age. The results suggest that swine veterinarians may be at somewhat higher risk of HEV infection than are normal blood donors.**

Hepatitis E is an important public health problem in many developing countries. The disease generally affects young adults (2, 3, 5, 11, 28–31, 34, 35). Although the overall mortality rate associated with HEV infection is low, it is reportedly as high as 20% in infected pregnant women (13, 16, 31, 34, 35). The causative agent of hepatitis E, hepatitis E virus (HEV), is a single-stranded positive-sense RNA virus without an envelope (34, 35). HEV is generally transmitted by the fecal-oral route. The genomic RNA of HEV is about 7.5 kb and contains three 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 (34, 35, 47). HEV was originally classified as a calicivirus, but recent data showed that HEV does not share some important features with caliciviruses (18, 20). Therefore, HEV was recently declassified from the *Caliciviridae* family and remains unclassified (33).

Balayan et al. (4) first demonstrated that domestic pigs could be experimentally infected with a human HEV isolate. Clayson et al. (7) subsequently detected HEV RNA and antibodies in pigs in Nepal, but the virus was not characterized. A unique swine HEV was first isolated in 1997 (24). Swine HEV is ubiquitous in pigs from the midwestern United States. Later studies revealed that swine from other countries, such as Australia, Thailand, Vietnam, Taiwan, Korea, China, Canada, and Spain, were also infected with HEV (6, 7, 15, 27, 32, 40, 46). The swine HEV strain isolated from a pig in Illinois is genetically very closely related to two U.S. strains of human HEV (8, 24, 26, 37). Similarly, the swine HEV strains isolated from pigs in Taiwan are closely related to Taiwanese strains of human HEV (14, 15, 46). Interspecies transmission of HEV has been experimentally demonstrated: swine HEV infected nonhuman primates, and a U.S. strain of human HEV infected pigs (12, 26). These data suggested that HEV infection of humans

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through contact with pigs may be possible and that swine veterinarians and other pig handlers may be at risk of zoonotic infection (28–30).

In a preliminary study, we tested a very limited number of pig handlers from two countries with endemic HEV (27). We found that 11 of 11 swine handlers from China and 5 of 7 swine handlers from Thailand were positive for immunoglobulin G (IgG) anti-HEV. However, 17 of 31 normal blood donors in China (55%) 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 countries where HEV is endemic. Clearly, 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. Here we report the results of a larger seroepidemiological study of HEV in normal blood donors and swine veterinarians mostly from the United States.

## MATERIALS AND METHODS

**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 veterinarians, academic veterinarians, and veterinary students), and history of needle stick or cut with blood-to-blood contact. About 85% of the participants were from the United States or Canada. About 6% were from other regions of the world, including Australia, Denmark, Italy, Japan, Mexico, the Philippines, Spain, and South America. The remaining 9% of the participants did not provide geographic information. From the eight 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' ages and sexes 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.

**Amplification of the putative capsid gene (ORF2) of swine HEV.** The putative capsid gene (ORF2) of swine HEV was amplified from swine bile (25) by reverse transcription-PCR with the following set of swine HEV-specific primers: forward primer, 5′-TTCGGATCC**ATG**CGCCCTAGGGCTGTTCTGTTGTTGCTC-3′; reverse primer, 5′-CAACTCGAG**TCATTA**AGATTCCCGGGTTTTACCTAC CTT-3′. The introduced restriction sites in the primers (*Bam*HI in the forward primer and *Xho*I in the reverse primer) are underlined, and the ORF2 start codon (ATG) and stop codons (TAA and TGA) are shown in boldface. The expected PCR product was purified from an agarose gel by the glass milk procedure with a GeneClean kit (Bio 101, Inc.) and sequenced. The sequence was identical to that of the published sequence of swine HEV.

**Production of swine HEV ORF2 protein.** The putative capsid gene (ORF2) of swine HEV was 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 (36). The recombinant capsid protein of swine HEV, purified by anion-exchange chromatography and subsequent gel filtration chromatography as described previously (36), was used in an enzyme-linked immunosorbent assay (ELISA).

**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 (12, 15, 19, 24–27, 41, 42) 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 (12, 24–26). 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 (24, 26, 41, 42). Convalescent-phase serum from a chimpanzee experimentally infected with HEV and preinoculation chimpanzee serum 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 phosphate-buffered saline–0.02% Tween 20, supercoated with 120 μl of blocking solution (0.5% gelatin, 0.03 M NaCl, 10% fetal bovine serum), and incubated for 1 h at 37°C to reduce nonspecific 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 (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was used as the secondary antibody. Azino-diethylbenzotyazol-sulfonate (ABTS) was used as the substrate for the development of a colorimetric reaction. Absorbance was read at 405 nm.

All ELISAs were calibrated against an anti-HEV standard recently proposed by the World Health Organization (WHO). Four fivefold dilutions of a well-characterized IgG anti-HEV secondary 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 standard preparation 95/584 (100 U/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 resuspended with 0.5 ml of distilled water, yields 100 U of anti-HEV per ml. 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 optical density equal to or above this cutoff was considered positive. Samples that were positive at a 1:100 dilution were confirmed by retesting and were further titrated at 1:1,000 and 1:10,000 dilutions.

**Statistical analyses.** We analyzed results from a total of 868 subjects (the ages were known for 864 and the geographic location was available for 825 of these subjects). Samples with both geographic location and age information were obtained from 295 swine veterinarians and 400 normal blood donors from the eight 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 (release 8.01 [2000]; SAS Institute, Cary, N.C.). Variables with model $P$ values of $<0.20$ 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 or subtracting one variable at a time to or from the model. Potentially relevant two- and three-way interactions were evaluated by the forward-selection process.

## RESULTS

**Comparison of anti-HEV detection with two different recombinant HEV capsid antigens.** The ELISA antigen used in our earlier studies was the baculovirus-expressed recombinant capsid protein from the human Sar-55 strain of HEV (36). Previous studies showed that the Sar-55 antigen reacted well with anti-HEV in sera from pigs and primates experimentally infected with swine HEV (12, 24–27). 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. 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 seven sera positive with Sar-55 but negative with swine HEV antigen and zero 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.

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

| Location | Human HEV (Sar-55) | | | | Swine HEV | | | |
|---|---|---|---|---|---|---|---|---|
| | No. positive/no. tested (% positive) | | OR[a] | 95% confidence interval | No. positive/no. tested (% positive) | | OR | 95% confidence interval |
| | Swine veterinarians | Blood donors | | | Swine veterinarians | Blood donors | | |
| United States | | | | | | | | |
| States with blood donors[b] | | | | | | | | |
| Total | 78/295 (26.4) | 73/400 (18.3) | | | 68/295 (23.1) | 66/400 (16.5) | | |
| Minnesota | 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 |
| Indiana | 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 |
| Nebraska | 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 |
| Iowa | 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 |
| Illinois | 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 |
| Missouri | 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 |
| North Carolina | 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 |
| Alabama | 3/23 (13.0) | 2/50 (4.0) | | | 3/23 (13.0) | 2/50 (4.0) | | |
| Other states[c] | 15/93 (16.1) | | 2.62[e] | 0.90–7.57 | 15/93 (16.1) | | 2.62[e] | 0.90–7.57 |
| Other countries[d] | 8/37 (21.6) | | 0.97[f] | 0.43–2.15 | 8/37 (21.6) | | 0.84[f] | 0.38–1.88 |

[a] OR, odds ratio, i.e., odds of seropositive test for pooled swine veterinarians and blood donors of each state to odds of Alabama subjects. There was no location-profession or location-age interaction in the multivariate model.

[b] 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 to 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 to 2.20) more likely to be positive when tested with swine HEV antigen.

[c] Swine veterinarians from 21 other U.S. states from which blood donors were not available: 12 from Kansas, 11 from Ohio, 9 from Michigan, 8 from Kentucky, 8 from Wisconsin, 7 from Pennsylvania, 7 from South Dakota, 5 from Oklahoma, 4 from Colorado, 4 from Georgia, 1 from each of 11 states (Arizona, Arkansas, Connecticut, Maryland, Mississippi, North Dakota, New Jersey, New York, Tennessee, Virginia, and Wyoming), and 7 without location information.

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

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

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

**Prevalence of IgG anti-HEV in swine veterinarians from the United States and other countries.** The veterinarians tested in this study all reported having contact with swine, spending from 1 to 100% of their time working with swine. Among all 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 eight 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 normal blood donors (18%) 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 to 2.20) and 1.46 times more likely to be anti-HEV positive when tested with Sar-55 antigen (95% confidence interval, 0.99 to 2.17). There was a difference in anti-HEV prevalence in both swine veterinarians and blood donors among the eight selected states, with subjects from Minnesota six times more likely to be anti-HEV positive than those from Alabama. Age was not a factor in the differences observed from state to state. Fifteen of 93 swine veterinarians (16%) 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 swine veterinarians (22%) 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). There was no significant difference in anti-HEV prevalence between swine veterinarians who had reported having a history of a needle stick or a 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 their time) and those who spent less time (≤20% of their time) working with pigs (Table 2). The veterinary students had the lowest anti-HEV prevalence among the four 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 to 39, 40 to 49, 50 to 59, and ≥60 years old) of the 295 swine veterinarians and 400 normal blood donors from eight states (Table 3). Anti-HEV prevalence in both swine veterinarians and normal blood donors increased with age. In the eight 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

TABLE 2. Risk factors associated with HEV infection in veterinarians working with swine[a]

| Risk factor | No. tested | Human HEV (Sar-55) | | | Swine HEV | | |
|---|---|---|---|---|---|---|---|
| | | No. (%) positive | OR | 95% confidence interval | No. (%) positive | OR | 95% confidence interval |
| 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) | | |
| % 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) | | |
| Veterinary 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] (yr) | | | | | | | |
| ≥60+ | 27 | 10 (37) | 5.29 | 1.58–17.74 | 7 (26) | 5.48 | 1.29–23.38 |
| 50–59 | 62 | 21 (34) | 4.61 | 1.59–13.35 | 18 (29) | 6.41 | 1.77–23.27 |
| 40–49 | 149 | 40 (27) | 3.30 | 1.22–8.91 | 40 (27) | 5.75 | 1.69–19.51 |
| 30–39 | 124 | 20 (16) | 1.73 | 0.61–4.90 | 18 (15) | 2.66 | 0.75–0.47 |
| <30 | 50 | 5 (10) | | | 3 (6) | | |

[a] OR (odds ratio) and *P* values are from univariate analyses. Inclusion in multivariate logistic regression of incidences of needle sticks, cuts with blood-to-blood contact, percentages of time working with swine, or job category, either separately with or combined 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, $\chi^2_{1df} = 3.19$ and $P = 0.07$; swine HEV, $\chi^2_{1df} = 2.86$ and $P = 0.09$.
[c] Sar-55, $\chi^2_{1df} = 1.15$ and $P = 0.28$; swine HEV, $\chi^2_{1df} = 0.72$ and $P = 0.40$.
[d] Sar-55, $\chi^2_{4df} = 6.90$ and $P = 0.14$; swine HEV, $\chi^2_{4df} = 7.18$ and $P = 0.13$.
[e] Sar-55, $\chi^2_{3df} = 5.05$ and $P = 0.17$; swine HEV, $\chi^2_{3df} = 4.97$ and $P = 0.17$.
[f] Sar-55, $\chi^2_{4df} = 16.94$ and $P = 0.002$; swine HEV, $\chi^2_{4df} = 17.74$ and $P = 0.0014$.

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.

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

| HEV antigen | Age (yr) | Swine veterinarians | | Blood donors | | OR[d] | 95% confidence interval |
|---|---|---|---|---|---|---|---|
| | | No. tested | No. (%)[c] positive | No. tested | No. (%)[c] positive | | |
| Human (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[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 symbols (*, †, or ‡) differ ($P < 0.02$ for Sar-55 antigen; $P < 0.04$ for swine HEV antigen).
[d] OR (odds ratio) for swine veterinarians and blood donors combined. The multivariate model included profession (odds of seropositive test in 295 veterinarians to odds in 400 control subjects from eight U.S. states: Sar-55 OR = 1.46 and 95% confidence interval, 0.99 to 2.17; swine HEV OR = 1.51 and 95% confidence interval, 1.03 to 2.20), state, and age. There was no age-profession interaction.

## DISCUSSION

Hepatitis E is endemic and occasionally epidemic in many developing countries in Asia and Africa, and explosive waterborne epidemics are the most dramatic form of HEV infection in these countries (2, 3, 5, 11, 29–31, 34, 35, 44, 45). In industrialized countries, although anti-HEV has been detected in normal blood donors (5, 17, 23, 31, 32, 34, 35, 39, 48), sporadic cases of hepatitis E not associated with traveling to regions of endemicity have only rarely been reported (8, 14, 15, 17, 37, 38, 46, 49). The source of HEV infection in industrialized countries is not known, but increasing evidence supports the hypothesis of a zoonotic infection (28–30). Many different serological tests have detected anti-HEV in animals. Anti-HEV has been detected in about 33% of domestic swine in Nepal, where hepatitis E is endemic (7). In the United States, anti-HEV has been detected in more than 80% of pigs older than 3 months of age but in very few animals younger than that age (24). Anti-HEV also has been detected in wild-caught rats in the United States and other countries (10, 19, 22, 43). The prevalence of anti-HEV increased in parallel with the estimated age of the rats. In Vietnam, where hepatitis E is endemic, anti-HEV has been detected in 44% of chickens, 36% of pigs, 27% of dogs, and 9% of rats (40). Recently, Favorov et al. (9) found anti-HEV in about 29 to 62% of cows from three countries where HEV is endemic (Somalia, Tajikistan, and Turkmenistan) and in 12% of cows in a country where HEV is not endemic (Ukraine). In Turkmenistan, about 42 to 67% of the sheep and goats were also found to be positive for IgG anti-HEV. Naturally acquired anti-HEV was also detected in rhesus macaques (1, 41). These serological data strongly suggest that these animal species have been exposed to HEV (or a related agent).

Our recent isolation of swine HEV from pigs in the United States (24) and the demonstrated ability of swine HEV to infect across species (12, 26) prompted us to assess the potential risk of zoonotic HEV infection in swine veterinarians. In this study, we found that swine veterinarians from the eight U.S. states from which normal blood donors were also available had an increased risk of HEV infection compared to the normal U.S. blood donors. In Taiwan, where HEV was not considered to be endemic, Hsieh et al. (15) found that about 27% of Taiwanese pig handlers were positive for anti-HEV, compared to only about 8% of control subjects. Taken together, these seroepidemiological data suggest that swine veterinarians and other pig handlers may be at potential risk of zoonotic HEV infection. The source of the relatively high anti-HEV prevalence in normal U.S. blood donors is not known. Previously, Thomas et al. (39) reported that IgG anti-HEV was detected in about 21% of normal blood donors from Baltimore, Md. Our data confirm that such high rates occur and, in fact, are common in many states. In Japan, another country where HEV is not endemic, the prevalence of IgG anti-HEV in healthy individuals was found to range from 1.9 to 14.1%, depending on the geographic location (21).

Previous seroepidemiological studies were all conducted with recombinant antigens from human strains of HEV. In this study, recombinant swine HEV capsid antigen and human HEV capsid antigen were tested in parallel in an ELISA. We found that the rates of anti-HEV prevalence obtained with swine HEV antigen and the Sar-55 antigen were generally in agreement (97% concordance; $\kappa = 92\%$). 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 (24), and previous studies demonstrated that the human HEV Sar-55 antigen cross-reacted well with antibodies to swine HEV (12, 24–27).

To identify potential risk factors associated with possible zoonotic HEV infection in swine veterinarians, we compared serological data with exposure histories. There was no significant difference in the prevalence of anti-HEV between swine veterinarians with or without needle sticks and with or without cuts with blood-to-blood contact. There was also no difference in anti-HEV prevalence between swine veterinarians who spent $\geq$80% of their time working with swine and those who spent only $\leq$20% of their time working with swine. 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 (25). Acute HEV infection occurs primarily in young pigs 2 to 3 months of age (24, 27). 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. There was no difference in anti-HEV prevalence among practicing veterinarians, academic veterinarians, industrial veterinarians, and veterinary students other than that related to age: we found that anti-HEV prevalence in both swine veterinarians and normal blood donors increased with age. This finding is consistent with other HEV seroepidemiological studies with humans (2, 3).

State-to-state differences in anti-HEV prevalence in both swine veterinarians and normal blood donors were noted, with subjects from Minnesota six times more likely to be anti-HEV positive than subjects from Alabama (Table 1). Except for Alabama, these states are considered major pork-producing states in the United States (unpublished 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 in 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.

HEV appears to be ubiquitous in pigs worldwide, and at least one strain can infect across species barriers. The results from the present study suggested that swine veterinarians are at increased potential risk of zoonotic HEV infection. However, the high prevalence of anti-HEV in a number of other animal species, coupled with a high prevalence of anti-HEV in human populations not at apparent risk of exposure to HEV, suggests that multiple sources of exposure to HEV may exist in the general U.S. population, as well as in specialty populations such as swine veterinarians.

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