INTRODUCTION

Hepatitis E virus (HEV) was recognized as a cause of acute, enteric, viral hepatitis distinct from hepatitis A in the 1980s and underwent extensive molecular characterization in the 1990s. It is the leading cause of enteric, viral hepatitis in developing countries of the Eastern Hemisphere and isolated outbreaks have also been reported in Mexico. Endemicity and outbreaks are generally attributed to fecally contaminated drinking water. In the industrialized world, as well as in developing countries with an adequate sanitation infrastructure, disease caused by HEV is very infrequent and usually caused by strains of the virus returning with travelers from endemic areas.

Recently, however, HEV strains isolated from three autochthonous cases in the United States have been shown to be closely related to an HEV strain in U.S. swine. Cross-species infectivity for both of these strains has been demonstrated. In Taiwan, strains infecting humans and swine, respectively, have been shown to form a distinct, monophyletic group. These discoveries, along with data from healthy U.S. adults demonstrating an overall 1–3% HEV antibody (anti-HEV) prevalence, have lead to the insight that HEV circulates and human HEV infection is endemic in the United States and other industrialized countries, albeit without causing much clinical disease. Recent animal experimentation indicates that clinical expression of HEV infection in macaques depends upon receipt of a sufficiently high dose of inoculum, but that a lesser dose results in viremia and fecal excretion of transmissible virus, but no transaminasemia. Whether inoculum size or strain-specific virulence is more important in explaining the near absence of clinically expressed human disease in industrialized areas, despite significant virus circulation, remains to be determined.

The apparent presence of significant animal reservoirs (swine, cattle, sheep, rodents, etc.) of HEV throughout the world, along with disease occurrence in the humans associated with them, has naturally raised the question of whether hepatitis E is a zoonosis. Reports have now been published of elevated anti-HEV levels among swine handlers in Taiwan (26.7% versus 8% in controls) and Moldova (51.1% versus 24.5% in controls). Since no reports investigating anti-HEV prevalence among domestic animal workers in the United States have appeared, we decided to conduct such a study of swine workers in North Carolina along with the swine, rat, and mouse associated with their workplaces.

MATERIALS AND METHODS

The eastern North Carolina “hog belt” was chosen as the venue for this survey; it is the second largest swine producing area in the United States, after Iowa. From August to December 1999, serum specimens were collected in this region from swine workers, commercial swine, and muids (rats and mice of the family Muridae). Our objective was to collect representative serum samples from workers occupationally exposed to swine, as well as from the swine with which they worked and from the rodents associated with their workplaces. To accomplish this, we visited two commercial and three academic swine production sites (obtaining 7 and 11 samples, respectively). We also recruited commercial and non-commercial (academic or state employed) swine workers (119 and 28 samples, respectively) at two swine worker educational seminars conducted in the study area. After informed consent (approved by the Chairman of the Human Use Review Committee at Walter Reed Army Institute of Research [WRAIR] and the Internal Review Board for Human Subjects in Research at North Carolina State University), volunteers were asked to complete a 30-item questionnaire and a blood sample was drawn. Swine were bled at three of the same five farm sites, as well as at one additional site; murids were trapped and bled at the same three sites and at three additional sites.

From November 1999 to December 2000, serum specimens were collected from, and the questionnaire administered to, a human control (non-swine worker) population living in the same eastern North Carolina counties as our swine worker subjects.

The administered questionnaire queried demographic data (age, gender, country of birth, race, Hispanic or not), any history of jaundice, hepatitis, or other major medical problems, swine kept at home, degree of animal contact other than swine (dogs, cats, cows, sheep, goats, chickens, turkeys, horses), current and past employers, nature of job duties (ani-
mal handling, equipment maintenance, pen cleaning, veterinary work, etc.), degree of physical occupational swine contact (graded 0–4), degree of physical contact with swine in specific occupational areas (also graded and broken down into gestation/breeding, farrowing, nursery, grow-out, transport, slaughter, and rendering), swine inventory (number) at work site, duration of occupational exposure to swine, age (and calendar year) at first significant swine contact, and a detailed history of any travel exceeding two weeks duration outside the United States and Canada.

Blood samples were centrifuged within a few hours of collection and all sera were stored on dry ice at a temperature of approximately –80°C while awaiting and during shipment. Anti-HEV levels were measured with a quantitative enzyme-linked immunosorbent assay (ELISA) detecting immunoglobulin to the HEV capsid (open reading frame [ORF] 2) protein of the Sar55 (Sargoda, Pakistan) HEV strain13 by an indirect technique.14 This assay15 detects bound anti-HEV using a goat antibody (IgM + IgG + IgA for humans and murids, IgG only for swine)-horseradish peroxidase conjugate. Quantitation of human antibodies was done by comparison with an HEV immunoglobulin standard containing 940 WR U/ml. This standard was made by pooling equal aliquots of convalescent serum from four donors in Nepal, who during a six-month study in 1988 acquired an antibody to HEV detectable using a first-generation test (Genelabs Diagnostics, Singapore). Cross-reactivity with both the U.S. human and swine HEV strains has been established (Tsarev SA, unpublished data), as it has with previously reported ELISAs using the same antigen.11,16 Conjugates were created separately for the swine and murid assays and different standard curves were calculated using the appropriate hyperimmune sera and negative controls.

Based on previous experience with these assays,17 interpretation of results for human sera is as follows: < 20 U/ml is consistent with no past infection, 20–40 U/ml is consistent with probable past infection, > 40 U/ml is consistent with definite past infection, and > 250 U/ml is consistent with infection during the preceding 1–2 years.

Specific pathogen-free (SPF) swine and laboratory mice, not injected (or prior to injection) with the protein, were used as negative controls for the respective animal assays. In this study, a cutoff of 20 U/ml is used for both human and swine sera. For murid sera, ≥ 5 mouse U/ml is considered positive. These cut-off values were established from normal curves representing titers from the uninjected laboratory mice and SPF piglets.

The titers and data from the questionnaire were combined on a database using SPSS® Version 10.0 statistical software (SPSS Inc., Chicago, IL). Pearson’s chi-square test, Fisher’s exact test, and Pearson’s correlation coefficient were used as appropriate.

RESULTS

Exposed subjects. Sera were collected from 165 swine workers of which 18 (10.9%) were seropositive. (A cutoff of 40, rather than 20, U/ml would yield nine of 165 [5.5%].) Selected mean titers and seroprevalences for some risk factors are shown in Table 1. Mean and median antibody titers were 10.4 and 1.9 U/ml, respectively (SD = 32.6), with values ranging from 0.1 to 280.8 U/ml. Of all samples, 135 (81.8%) were from men and 30 (18.2%) were from women; seropositivity was not significantly associated with gender. The mean and median age were 36.1 and 35.0 years, respectively (range = 18–64); there was no statistical correlation between antibody titer and age (Pearson’s r = 0.016). Racial self-descriptions were white (155), black (3), American Indian (3), Asian (3), and other (1); this was not sufficiently heterogeneous to analyze for differences according to race. Workers born in the United States numbered 133 (81%); the remainder reported origins in 12 different countries. Non-U.S.-born workers were more likely to be seropositive (9 of 32 [28.1%]) than were U.S.-born workers (9 of 133 [6.8%]; P = 0.001). Of all the foreign-born, the largest component (19 of 32) was Mexican and six of them (31.6%) were seropositive; country of birth was a statistically significant risk factor (P = 0.003). The closely related (and partially overlapping) risk factor of having been a traveler or resident outside the United States or Canada for at least two weeks was also statistically significant (P = 0.014), but this significance for travel disappears (P = 0.826) for the U.S.-born workers analyzed separately. Thirty workers (18.2%) designated themselves “Hispanic.” Of these, seven (23.3%) were seropositive, whereas only 11 of the 135 “non-Hispanics” (8.1%) were seropositive (P = 0.016).

Mean and median duration (years) of occupational exposure to swine (excluding gaps in the work period) were 13.14 and 10.25, respectively (SD = 10.43) and ranged from 0.08 to 50. There was no statistical correlation between this duration of exposure and antibody titer (Pearson’s r = –0.073). All subjects reported working for a large corporate farm, a small family-owned farm, or an academic or state-owned swine research facility. The top four responses were three different large corporate swine producers (21.9%, 8.9%, and 6.5%) and a state university swine farm (12.4%). The remaining 50.3% was divided among 30 smaller corporations or family farms and three smaller research agencies. No statistical association between antibody titer and any specific employer or workplace (past or present) could be detected.

Only two volunteers had a history of jaundice or hepatitis (one case each of hepatitis B and C) and both of these were anti-HEV seronegative. Forty-nine of the 165 subjects (29.7%) had a history of keeping pigs at home at some point in the past. Neither this nor any degree of reported contact

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean titer</th>
<th>Median titer</th>
<th>Number seropositive*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>8.99</td>
<td>1.90</td>
<td>15/135 (11.1%)</td>
</tr>
<tr>
<td>Women</td>
<td>16.69</td>
<td>2.15</td>
<td>3/30 (10.0%)</td>
</tr>
<tr>
<td>U.S.-born</td>
<td>7.76</td>
<td>1.90</td>
<td>9/133 (6.8%)</td>
</tr>
<tr>
<td>Travelers</td>
<td>3.52</td>
<td>1.90</td>
<td>8/115 (7.0%)</td>
</tr>
<tr>
<td>Non-travelers</td>
<td>8.36</td>
<td>1.90</td>
<td>1/18 (5.6%)</td>
</tr>
<tr>
<td>Non-U.S.-born</td>
<td>20.95</td>
<td>3.30</td>
<td>9/32 (28.1%)</td>
</tr>
<tr>
<td>Mexicans</td>
<td>12.73</td>
<td>3.05</td>
<td>6/19 (31.6%)</td>
</tr>
<tr>
<td>Hispanics</td>
<td>18.25</td>
<td>2.75</td>
<td>7/30 (23.3%)</td>
</tr>
<tr>
<td>Non-Hispanics</td>
<td>8.65</td>
<td>1.80</td>
<td>11/135 (8.1%)</td>
</tr>
<tr>
<td>Seropositives</td>
<td>72.60</td>
<td>40.45</td>
<td>18/18 (100%)</td>
</tr>
<tr>
<td>Seronegatives</td>
<td>2.78</td>
<td>1.60</td>
<td>0/147 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>10.39</td>
<td>1.90</td>
<td>18/165 (10.9%)</td>
</tr>
</tbody>
</table>

* >20 WR U/ml.
with any of the eight other domestic animals was statistically correlated with seropositivity. No association with seropositivity was evident for specific type of job duties, degree of physical occupational swine contact, degree of physical contact with swine in specific occupational areas, swine inventory (either according to three age categories or in aggregate), duration of occupational exposure to swine, or age or calendar year at first swine contact.

Of the nine subjects who had HEV titers > 40 WR U/ml, seven were males and two were females (mean age = 38.4 years). Five were U.S.-born, one was a U.S. military dependent born in Japan, one was a U.S. military dependent born in Uruguay. Nearly half (4 subjects) had previously or currently kept pigs at home (mean duration of occupational exposure to swine = 12.5 years). There was no distinguishing pattern of specific work experience, non-swine animal exposure, or workplace affiliation, except that two subjects were veterinarians working at different facilities.

Control (unexposed) subjects. Sera were collected from 127 control volunteers, none of whom had had past occupational exposure to swine. Additionally, it was ensured that all were U.S.-born and were North Carolina residents for a minimum of five years. Control subjects were recruited up to one year after swine worker recruitment and from the same rural communities of eastern North Carolina. Twenty-one control volunteers were recruited during home visits to swine workers, 87 during rural community church visits, and 19 from agricultural machinery factory workers.

Of the 127 control subjects, three (2.4%) were seropositive. (A cutoff of 40, rather than 20, U/ml would yield 1 of 127 [0.08%].) Selected mean titers and seroprevalences are shown in Table 2. Mean and median antibody titers were 4.1 and 2.3 U/ml, respectively (SD = 7.47), with values ranging from 0.0 to 73.2 U/ml. Of all controls, 66 (52.0%) were men and 39 (46.5%) were women. The mean and median ages were 43.5 and 43.0 years, respectively (range = 18–75). Racial self-descriptions were white (92), black (33), American Indian (1), Asian (1), and other (1). No control subject self-designated as “Hispanic.”

No control subjects had a history of hepatitis or unexplained jaundice. Nineteen (15%) reported past travel outside the United States or Canada exceeding two weeks duration. Only one control subject had an HEV titer > 40 WR U/ml (73.2); this was a 61-year-old white woman who denied significant exposure to any domestic animals as well as any overseas travel.

The difference between the mean anti-HEV titer of swine workers and that of controls (10.4 and 4.1 U/ml, respectively) is statistically significant ($P = 0.006$, by Pearson’s chi-square test). Significance is still retained when the 30 “Hispanic” swine workers are excluded ($P = 0.043$), but not when the 32 non-U.S.-born swine workers (23 of them among the “Hispanics”) are excluded ($P = 0.116$).

Sera were collected from 84 swine (Sus scrofa domestica) at four farm sites. All swine were more than two months old. Mean and median titers (swine U/ml) were 53.2 and 6.7, respectively (SD = 142.6, range = 0.1–947.2). Overall, 29 of 84 swine (34.5%) were seropositive. Mean titers and seroprevalences according to farm site are shown in Table 3. Farm sites 1, 2, and 3 operated on an all-in/all-out production concept (i.e., all animals of a given age cohort being moved progressively to new, empty, and sanitized facilities at each growth stage). Site 4, however, used a continuous flow production concept (i.e., smaller numbers of animals were moved to join new cohorts as they reached certain designated weights, thus making facility sanitization less effective since the units are never completely empty). In addition, site 4 differed in having stricter biosecurity (e.g., shower-in/shower-out) practices. No overt signs of hepatitis in any of the sampled portions of the herds were noted at the time of serum collection.

Sera were also collected from 61 wild-caught murids in or near swine-houses (58 house mice [Mus musculus domesticus] and three Norway rats [Rattus norvegicus norvegicus]). None of these murid samples was seropositive for anti-HEV.

**DISCUSSION**

Our determination of an anti-HEV seroprevalence of 11% for all 165 swine workers and of 7% for 133 U.S.-born swine workers contrasts with that of 2.4% in the 127 U.S.-born, non-swine working controls. It also contrasts with previously reported surveys indicating a 1–3% prevalence among healthy U.S. blood donors.\(^6\) Although these previous studies used assays different from ours, our own group has previously reported a series\(^7\) of U.S. soldiers and civilians (n = 461) tested with our assay and showed an anti-HEV seropositivity of less than 1%.

The North Carolina swine worker population seems to be at increased risk for infection with HEV. This is presumably due to occupational swine exposure and is similar to the findings recently reported for swine workers in Taiwan\(^1\) and Moldova.\(^2\) Our population’s anti-HEV prevalence is similar to the 8.7% reported for a convenience sample of U.S. State Department employees who had taken up residence overseas in areas endemic for HEV,\(^3\) as well as to the 10% reported among U.S. Peace Corps workers returning from Africa.\(^4\) (As with our swine workers, no history of jaundice was re-
called by any of these former expatriates.) The lack of any association between HEV titer and either age (Pearson’s $r = 0.016$) or duration of occupational exposure (Pearson’s $r = -0.073$) in our swine workers most likely relates to the relatively low seroprevalence in our population compared with surveys in developing countries where the former, at least, has been documented. This is somewhat difficult to interpret, since the published data on the duration of IgG anti-HEV persistence are conflicting. The finding of lower mean anti-HEV titers among international travelers than among nontravelers (for both subjects and controls) is interesting. (The difference is not statistically significant with our data but, if real, may be related to higher socio-economic status among such travelers.)

The seroprevalence (31.6%) in our smaller Mexican swine worker subgroup is approximately double the 16% reported among Mexican migrant workers in California and approximately triple the 10.5% reported in a community-based study from Mexico restricted to individuals less than 30 years old. In contrast, the seroprevalence in our U.S.-born swine worker population (6.8%) was much smaller than that reported for the same occupation in Taiwan (26.7%) or Moldova (51.1%); these differences may reflect varying levels of hygiene and local sanitation infrastructures. In the Moldova study, for example, seropositivity was significantly associated with absence of a running water supply at home.

None of our swine data conflict with previous reports in which the majority of U.S. swine less than three months old were seropositive and in which the prevalence of anti-HEV in swine increases with age. Moreover, the large variability in seropositivity among the four herds tested is quite consistent with other recent studies of swine herds in the United States and elsewhere. However, the strikingly higher titers and seropositivity at site 4 (where one different type of heightened bioscience precaution [shower-in/shower-out] was in place along with a less stringent procedure [continuous flow]) raises questions regarding the degree to which transmissibility may be enhanced by these commercial production measures. Specifically, it may be that transmission of HEV occurs more readily when age and weight cohorts are not kept separate with rooms disinfected between populations. This is consistent with the “isowean principle” (preventing pathogen transmission by isolating cohorts) of swine veterinary practice in the United States, which is the basis of modern multiple-site swine production systems.

In light of recent reports of high anti-HEV prevalences among rodents in the United States, the complete absence of antibodies in the 58 mice (*M. m. domesticus*) that we trapped in association with swine farms was unexpected. In the report of Favorov and others, two of seven *M. musculus* from Georgia were seropositive, although all seven from Alabama and Texas were negative. We were generally unsuccessful in trapping adult rats (*Rattus* spp.) from swine farms (our three specimens of *R. r. norvegicus* were very young adults). It may be that various murid species and subspecies are highly heterogeneous in their abilities to generate and maintain antibody levels after HEV infection. (In one clearly successful attempt at experimental infection of 27 laboratory *R. norvegicus* [Wistar strain] no anti-HEV IgM or IgG could be detected.) This finding does not support recent speculation regarding the possible cross-species transmission of HEV between domestic animals and peri-domestic rodents.

In conclusion, our findings are compatible with previous speculation that the presence of anti-HEV in healthy adults without history of jaundice may reflect subclinical infection of humans with swine HEV, even in developed areas where clinical HEV is rare or nonexistent. Although we were unsuccessful in finding serologic evidence suggestive of HEV exchange between rodents and swine, our data do add to the growing evidence that hepatitis E may be a zoonosis and specifically to the concept of it as an occupational infection of livestock workers. Proof of this awaits the isolation of genetically identical viruses from a livestock worker (with or without clinically apparent disease) and a closely associated animal.

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