Hepatitis E virus (HEV; family Hepeviridae, genus Orthohepevirus A) comprises at least 8 distinct genotypes (1). In industrialized countries, swine HEV of the zoonotic genotypes 3 and 4 (HEV-3 and HEV-4) is an emerging foodborne pathogen, transmitted by consumption of raw or undercooked pork (2). Recently, HEV-3 has been detected in human blood donors in the United States (3,4). We previously showed that HEV-3 is present in US swine herds (5) and that a small proportion of commercial pork products, such as liver and chitterlings, from US grocery stores contain infectious HEV (6). However, the current HEV infection status of US market-weight pigs at the time of slaughter, the entry point to the food supply chain, remains unknown. We therefore investigated the presence of HEV RNA and HEV IgG prevalence in 5,033 serum samples from market-weight pigs at 25 slaughterhouses in 10 US states.

The Study
During 2017–2019, a comprehensive set of archived serum samples from 22,940 market-weight pigs from 25 slaughterhouses in 10 US states was collected for an unrelated prevalence study of Toxoplasma and Trichinella. The samples were collected from slaughterhouses processing adult market-weight pigs ≥6 months of age: ≈250-pound market-weight hogs 6 months of age and female pigs >1 year of age. The blood samples were collected on the kill floor at the slaughterhouses, and serum was separated and stored frozen (~20°C) at the US Department of Agriculture–Agricultural Research Service, Beltsville Agricultural Research Center (Beltsville, MD, USA).

For our study, an aliquot of frozen serum samples was sent to Virginia Polytechnic Institute and State University (Blacksburg, VA, USA). From a total of 22,940 samples available, we performed a stratified random selection of 5,033 samples for this study, using the SURVEYSELECT procedure in SAS version 9.4 (https://www.sas.com); a combination of state and ZIP code of origin constituted the strata. To detect HEV RNA, we used an established quantitative reverse-transcription PCR (qRT-PCR) (7) and a nested RT-PCR (5). To detect HEV IgG, we used a commercial PrioCHECK Porcine HEV Ab ELISA kit (https://www.thermofisher.com), according to the manufacturer’s protocol.

Results of qRT-PCR indicated that ≈6.3% (318/5,033; 95% CI 5.6%–7.0%) of the market-weight pigs from US slaughterhouses were viremic for HEV RNA at the time of slaughter (Table). Viral loads ranged from <100 to 10⁶ copies/mL (mean 8,285 copies/mL; 95% CI 6,210.7–25,397.2 copies/mL). The percentage of HEV-viremic pigs varied among slaughterhouses (range 0%–17.4%) and among states (Table). Higher serum HEV RNA positivity was found in pigs from 3 slaughterhouses in Iowa (17.4%, 9.5%, and 8.3%), 2 in Illinois (8.5% and 7.5%), 1 in North Carolina (7.9%), and 1 in Pennsylvania (7.5%). To determine HEV genotype in US slaughterhouse pigs, we further tested the 318 serum samples...
Among 2,007 HEV IgG–positive samples, Of note, HEV seropositivity was higher in serum samples from Tennessee, but only 1.8% of these samples were positive for HEV RNA (Table). We performed the Spearman correlation by using SAS version 9.4 and found no apparent correlation between HEV antibody seropositivity and serum HEV RNA positivity in this study (Spearman correlation R² = 0.07); among 2,007 HEV IgG–positive samples, only 145 were also positive for HEV RNA (7.2%, 95% CI 6.1%–8.3%).

**Conclusions**

HEV-3 and HEV-4 are zoonotic viruses that infect pigs and humans. In this study, we found that ~40% of US slaughterhouse pigs were seropositive for HEV, indicating prior HEV infection of the pigs on the farms, which was consistent with prior estimates for farmed US pigs (8,9). Despite the relatively high seropositivity, however, only a small proportion (6%) of the pigs had detectable HEV viremia, probably because HEV viremia is transient and thus the window for detecting HEV RNA in serum is narrow. In addition, active HEV infection occurs naturally in most farm pigs around 2 months of age (5,9). Therefore, most market-weight pigs >6 months of age at the time of slaughter positive by qRT-PCR by using an established nested RT-PCR (5). We successfully amplified the HEV capsid gene region (348 bp) from 182 samples. Subsequent sequencing and phylogenetic analyses revealed that all 182 HEV sequences belonged to the zoonotic HEV-3 genotype and clustered within group 2 (HEV-3abchij) (Figure). The HEV sequences from slaughterhouse pigs shared ~90%–94% nt sequence identity with the previously reported US HEV-3 isolates (GenBank accession nos. JN837481 and KF719308). We did not detect any HEV-3-efg subgenotype or HEV-4.

We found that the national average of HEV seropositivity among market-weight pigs in US slaughterhouses is ~40% (95% CI 38.5%–41.2%). Seroprevalence varied from slaughterhouse to slaughterhouse and from state to state (range 0%–66.8%) (Table). At 1 slaughterhouse in Pennsylvania, all 50 pigs tested were seronegative. Higher HEV seropositivity was found at slaughterhouses in Iowa (68.8%), Oklahoma (59.1%), Tennessee (57.1%), and North Carolina (55.2%) (Table).

**Table.** Detection of HEV IgG and RNA in serum of market-weight pigs from 25 slaughterhouses in 10 US states, 2017–2019*

| State, slaughterhouse no. | No. samples tested | Positive for HEV IgG by ELISA, no. (%) | Positive for HEV RNA by qRT-PCR, no. (%) | HEV RNA, copies/mL, range |
|--------------------------|--------------------|----------------------------------------|------------------------------------------|--------------------------|
| Oklahoma                 | 455                | 269 (59.1)                             | 24 (5.3)                                 | <100–1 × 10⁴              |
| Tennessee                | 56                 | 32 (57.1)                              | 1 (1.8)                                  | <100                     |
| Virginia                 | 213                | 89 (41.8)                              | 6 (2.8)                                  | <100–4 × 10⁴              |
| Illinois                 |                    |                                        |                                          |                          |
| 1                        | 40                 | 18 (45)                                | 3 (7.5)                                  | 102–3,083                |
| 2                        | 55                 | 22 (40)                                | 3 (5.5)                                  | <100                     |
| 3                        | 40                 | 16 (40)                                | 0                                        | <100                     |
| 4                        | 445                | 118 (26.5)                             | 38 (8.5)                                 | <100–3 × 10⁵              |
| 5                        | 259                | 60 (23.3)                              | 2 (0.8)                                  | 100                      |
| Wisconsin                | 20                 | 9 (45)                                 | 0                                        | <100–1 × 10⁴              |
| Iowa                     |                    |                                        |                                          |                          |
| 1                        | 379                | 135 (35.6)                             | 36 (9.5)                                 | <100–1 × 10⁴              |
| 2                        | 455                | 304 (66.8)                             | 23 (5.1)                                 | <100–3 × 10⁴              |
| 3                        | 70                 | 21 (30)                                | 1 (1.4)                                  | 10⁴                     |
| 4                        | 105                | 39 (37.1)                              | 1 (1)                                    | <100                     |
| 5                        | 180                | 67 (37.2)                              | 15 (8.3)                                 | <100–1 × 10⁴              |
| 6                        | 37                 | 17 (46.0)                              | 0                                        | 0                       |
| 7                        | 453                | 153 (33.8)                             | 79 (17.4)                                | <100–2 × 10⁵              |
| 8                        | 22                 | 5 (22.7)                               | 0                                        | 0                       |
| Minnesota                | 233                | 76 (32.6)                              | 10 (4.3)                                 | <100–1 × 10⁴              |
| North Carolina           |                    |                                        |                                          |                          |
| 1                        | 245                | 61 (24.9)                              | 7 (2.9)                                  | <100–3 × 10⁴              |
| 2                        | 482                | 266 (55.2)                             | 39 (7.9)                                 | <100–1 × 10⁵              |
| Nebraska                 |                    |                                        |                                          |                          |
| 1                        | 223                | 62 (27.8)                              | 2 (0.9)                                  | 100–300                  |
| 2                        | 241                | 66 (27.49)                             | 9 (3.8)                                  | <100–1 × 10⁵              |
| Pennsylvania             |                    |                                        |                                          |                          |
| 1                        | 50                 | 0                                      | 1 (2)                                    | 200                      |
| 2                        | 35                 | 8 (22.9)                               | 1 (2.9)                                  | <100                     |
| 3                        | 240                | 94 (39.2)                              | 18 (7.5)                                 | <100–1 × 10⁴              |
| Total                    | 5,033              | 2,007 (39.9)                           | 318 (6.3)                                | <100–1 × 10⁴              |

*HEV, hepatitis E virus; qRT-PCR, quantitative reverse-transcription PCR.
Figure. Phylogenetic tree of the capsid gene region of reference HEV-1, HEV-2, HEV-3, and HEV-4 strains within species Orthohepeivirus A, representative HEV strains from species Orthohepeviruses B, C, and D, as well as the cutthroat trout virus in the genus Piscibepeivirus. The phylogenetic analysis was performed by using MEGA6 software (http://www.megasoftware.net) and the maximum-likelihood bootstrap method based on the Tamura-Nei model (1). The figure represents a cladogram. The HEV-3abchij sequences belonging to HEV-3 group 2 (HEV-3–2) are highlighted in gray (n = 182 sequences from slaughterhouse pigs in this study; n = 19 reference sequences from the GenBank database). The HEV-3efg sequences belonging to HEV-3 group 1 (HEV-3–1) are shown on a white background (n = 7 reference sequences from the GenBank database). Reference HEV sequences from genotypes 1/2/4 clade of Orthohepeivirus A, and Orthohepeivirus B/C/D clade are also shown as collapsed branches, and the cutthroat trout virus within the genus Piscibepeivirus is shown as a separate clade. The HEV-3 reference sequences used in the phylogenetic analysis include HEV-3a AF082843, HEV-3b AP003430, HEV-3c FJ705359, HEV-3d AF296165–7, HEV-3e AB248521, HEV-3f AB369687, HEV-3g AF455784, HEV-3h JQ013794, HEV-3i FJ998008, HEV-3j JY154888, HEV-3 AB290312. We also included the following other HEV-3 sequences in the phylogenetic analysis, which were some of the top candidates from our initial BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis: AB091394-HEV-3, AB154830-HEV-3, AB670953-HEV-3, DQ860011-HEV-3, FJ426404-HEV-3, FJ527832-HEV-3, JN837481-HEV-3, KF719308-HEV-3, KT718043-HEV-3. HEV, hepatitis E virus.
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About the Author
Dr. Sooryanarain is a research scientist in the Department of Biomedical Sciences and Pathobiology at Virginia Polytechnic Institute and State University, Blacksburg, VA. Her research interest focuses on the mechanism of HEV pathogenesis and epidemiology.

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Address for correspondence: Xiang-Jin Meng, Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, 1981 Kraft Dr, Blacksburg, VA 24061-0913, USA; email: xjmeng@vt.edu