Comparison of PCR and a Swine Bioassay to Detect Hepatitis E Virus in Pig Tissues and Feces

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Abstract
Swine hepatitis E virus (HEV) was detected by a semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) in liver tissue and feces but not in skeletal muscle, pancreas, or heart from pigs experimentally infected intravenously with swine hepatitis E virus (swine HEV). Homogenates of liver tissue and suspensions of feces prepared from swine HEV-infected pigs were inoculated intravenously into naïve pigs and induced infection. There was no evidence of transmission of swine HEV to pigs by intravenous route of inoculation with heart or pancreas, or oral route with skeletal muscle homogenates or fecal suspensions prepared from HEV-infected pigs.

Results indicate that there is potential for transmission of swine HEV to naïve pigs, and potentially to humans, via pig liver or liver cell xenotransplantation. Failure to detect HEV by RT-PCR in muscle tissue and failure to transmit swine HEV via oral inoculation of muscle tissue suggests that the risk of transmission of HEV in pork meat products is minimal. The route of natural transmission of HEV is thought to be fecal-oral so the failure to transmit HEV via feces suggests that a very high infectious dose is necessary, or there are other routes of transmission. The semiquantitative RT-PCR assay correlates well with that of in vitro swine bioassay.

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Comparison of PCR and a Swine Bioassay to Detect Hepatitis E Virus in Pig Tissues and Feces

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Summary and Implications
Swine hepatitis E virus (HEV) was detected by a semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) in liver tissue and feces but not in skeletal muscle, pancreas, or heart from pigs experimentally infected intravenously with swine hepatitis E virus (swine HEV). Homogenates of liver tissue and suspensions of feces prepared from swine HEV-infected pigs were inoculated intravenously into naïve pigs and induced infection. There was no evidence of transmission of swine HEV to pigs by intravenous route of inoculation with heart or pancreas, or oral route with skeletal muscle homogenates or fecal suspensions prepared from HEV-infected pigs.

Results indicate that there is potential for transmission of swine HEV to naïve pigs by inoculation with tissues (liver, heart, pancreas, or skeletal muscle) and feces prepared from known HEV-infected pigs. The second objective was to evaluate the application of an in vitro semiquantitative RT-PCR assay and its correlation with an in vivo swine bioassay in detecting swine HEV infection.

Introduction
HEV is a major cause of enterically transmitted non-A, non-B hepatitis in humans in several regions worldwide, including Asia, Africa, and Mexico. In 1997, a novel virus designated as swine hepatitis E virus (swine HEV) was discovered in pigs in Illinois (1). Sporadic cases of HEV infection in human patients with acute hepatitis have recently been reported in the United States. These cases were determined to be caused by strains of HEV that are very closely related to the swine HEV (2,3). Recent experimental results provide evidence of interspecies transmission of swine HEV to nonhuman primates, and human HEV to swine (2). The pig is considered a potential reservoir of HEV (4). The pig also may be a source for HEV infection of humans through transplantation of pig tissues or organs (such as liver, pancreas, and heart) to humans (xenotransplantation) (3). Swine and human HEV induce subclinical infection in pigs and mild-to-moderate hepatitis lesions. Pigs experimentally inoculated with swine or human HEV shed virus in feces for 3 to 4 weeks (5).

The first objective of this study was to assess the risk of transmission of swine HEV to naïve pigs by inoculation with tissues (liver, heart, pancreas, or skeletal muscle) and feces prepared from known HEV-infected pigs. The second objective was to evaluate the application of an in vitro semiquantitative RT-PCR assay and its correlation with an in vivo swine bioassay in detecting swine HEV infection.

Materials and Methods
Seventy-five, 2-week-old pigs were randomly separated into 24 groups of 3 to 4 pigs and inoculated with tissue homogenates (liver, heart, pancreas, or skeletal muscle) or a suspension of feces collected from swine HEV-infected pigs at 3, 7, 14, 20, 27, or 55 days post inoculation (DPI). Tissues or feces collected at 3 and 7 (3/7), 14 and 20 (14/20), and 27 and 55 (27/55) DPI from pigs in a previous study (4) were used as inocula. Each inoculum was prepared as a 10% suspension (w/v) in PBS buffer and tested by semiquantitative RT-PCR for swine HEV RNA and by a swine bioassay. The inoculation route was intravenous for liver, heart, and pancreas and orally via stomach tube for skeletal muscle and fecal suspensions. Homogenates of tissues and suspensions of feces collected from normal SPF pigs served as negative controls. As positive controls, three groups of pigs were inoculated with 10^{4.5} 50% PID_{50} of swine HEV in fecal suspension from experimentally infected pig via oral drop, stomach tube, or intravenous route. Blood samples were collected weekly and at necropsy at 56 DPI. Sera were tested for anti-HEV antibodies to determine whether infection had occurred.

Results and Discussion
There was no evidence of clinical disease in any of the groups. The liver homogenates and feces collected at 3/7 DPI and 14/20 DPI were positive for swine HEV RNA by RT-PCR. The RT-PCR titer, semiquantitatively measured as genome equivalents (GE) per millileter of the inocula was as follows: 10^4 GE/ml for the 3/7 DPI liver homogenates, 10^2 GE/ml for the 14/20 DPI liver homogenates, 10^3 GE/ml for both the 3/7 and 14/20 DPI feces. Swine HEV RNA was not detected by RT-PCR in heart, pancreas, or skeletal muscle. The intravenously
inoculated positive control pigs and the pigs inoculated with liver homogenates collected at 3/7 DPI and 14/20 DPI developed anti-HEV serum antibodies between 3 and 8 weeks post inoculation. Other groups remained seronegative throughout the study.

The data indicate that there is a potential risk of transmission of swine HEV via liver tissue or pig feces from infected pigs in the early stages (3-20 DPI) of infection. The data also suggest that infection of pigs with swine HEV by oral route may require a considerably higher dose than by intravenous route. The *in vivo* swine bioassay for swine HEV correlates well with the *in vitro* semiquantitative RT-PCR assay. Thus, RT-PCR can be used for risk assessment of swine HEV infection.

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**References**