

# Routes of Transmission of Swine Hepatitis E virus in Pigs

## A.S. Leaflet R1981

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### Summary and Implications

Hepatitis E virus (HEV) is believed to be transmitted by the fecal-oral route in pigs. To date, HEV has been transmitted experimentally by the intravenous or intrahepatic route. To assess the possible route (s) of HEV transmission, tonsil/nasal secretions, use of contaminated needles, or fecal-oral exposures were simulated in a swine bioassay. Three positive-control pigs were inoculated intravenously with swine HEV and served as the source of biological samples (tonsil/nasal secretion swabs, blood-contaminated needles, or pooled fresh feces) to be used as inocula in the swine bioassay. Three uninoculated pigs were placed with the positive-control pigs and served as contact controls. Weekly fecal and serum samples from each exposed pig were tested for anti-HEV antibodies and HEV RNA to confirm HEV infection. All positive-control and contact-control pigs became infected. One of three fecal-oral exposed pigs shed the virus in feces and seroconverted. Three pigs exposed to the tonsil/nasal secretion swabs and the three pigs exposed to the contaminated needles remained negative. The fecal-oral route of transmission was confirmed in pigs. It is less likely that HEV is transmitted via contaminated needles or tonsil/nasal secretions.

### Introduction

Hepatitis E virus (HEV) is an important cause of enterically transmitted, non-A, non-B hepatitis in humans. Serological surveys of pig veterinarians and handlers showed an increased prevalence of anti-HEV antibodies suggesting potential pig-to-human transmissions. Direct evidence of zoonotic HEV transmission has recently been reported in Japanese patients following the consumption of uncooked pig livers or of raw meat from wild deer.

The natural route (s) of swine HEV transmission in pigs remains unknown. Swine HEV is transmitted experimentally via direct contact. Pigs housed in the same pen are exposed to saliva, nasal secretions, urine, and feces of multiple pen mates repeatedly each day. Repeated use of needles for drug administration or vaccination is commonly practiced in swine health management. The objective of the

present study was to determine if swine HEV transmission occurs via exposure to (i) tonsil and nasal secretions from infected pigs (ii) repeated use of contaminated needles, and (iii) oral consumption of feces from infected pigs.

### Materials and Methods

Twenty seven SPF pigs were separated into nine groups of three pigs. Positive-control pigs were inoculated intravenously with swine HEV and served as the source of HEV for the 3 exposure groups. Uninoculated contact pigs were placed in the positive-control group. On three consecutive days, the naïve pigs were inoculated using samples collected from the positive-control pigs at 9, 10, and 11 days postinoculation (dpi). The tonsils and nasal mucosa of each positive-control pig were swabbed and that swab was used to rub the tonsils, nasal and ocular mucosa of one naïve pig. The positive-control pigs were also injected with a commercial bacterin, and the same needle was used to immediately inject the 3 naïve pigs. Three sham groups were added for each exposure. Feces were collected from positive controls and fed by oral gavage to naïve pigs. Weekly fecal and serum samples from each pig were tested for anti-HEV antibodies and HEV RNA. The HEV found in the inocula was quantified by real-time RT-PCR.

### Results and Discussion

All positive-control pigs inoculated intravenously with swine HEV shed large amounts of HEV in feces during the time the feces were used for exposure of naïve pigs (9 to 11 dpi). Viremia was detected, and these pigs developed anti-HEV antibodies by the end of the study. The tonsil and nasal secretion swabs collected from the positive-control pigs were negative for HEV RNA (Table 1).

The findings of the present study demonstrate the first report of experimental fecal-oral transmission of swine HEV in pigs. Efficient transmission of swine HEV in pigs via the fecal-oral route may require repeated exposure and high doses. Evidence of HEV transmission through tonsil/nasal secretions was lacking. It is unlikely that the repeated use of needles represents a common means of transmission in pigs. The ubiquitous nature of swine HEV in the swine population and the presence of HEV in pig feces for a longer period than the length of HEV viremia also favor the fecal-oral route as the main route of transmission.

**Table 1. Detection of anti-HEV serum antibodies by ELISA and of HEV RNA by RT-PCR in pigs inoculated with biological samples from HEV-infected pigs**

Group	Inoculation Route	No. of positive pigs ( $n = 3$ ) <sup>a</sup>		
		Seroconversion <sup>b</sup>	HEV RNA in feces	HEV RNA in serum
1	Swine HEV positive control <sup>c</sup>	2/3	3/3	2/3
2	Direct contact exposure to positive control	2/3	3/3	1/3
3	Tonsil and nasal secretions exposure	0/3	0/3	0/3
4	Needle exposure	0/3	0/3	0/3
5	Fecal-oral exposure	1/3	1/3	0/3
6	Negative control	0/3	0/3	0/3
7	Sham tonsil and nasal secretions exposure	0/3	0/3	0/3
8	Sham needle exposure	0/3	0/3	0/3
9	Sham fecal-oral exposure	0/3	0/3	0/3

<sup>a</sup> Number of positive pigs of three pigs tested.

<sup>b</sup> All pigs were tested on 56 dpi.

<sup>c</sup> Inoculated intravenously with swine HEV.