Supplementary information to

Hepatitis E virus seroprevalence in pets in the Netherlands and the permissiveness of canine liver cells to the infection

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Supplementary Table 1.

Table 1. qRT-PCR primer sequences

| Primer ID            | Sequence (5’-3’)                  |
|----------------------|-----------------------------------|
| HEV sense            | GGTGGTCTTCTGGGTGAC                |
| HEV anti sense       | AGGGTTGGTGATGAA                   |
| Human GAPDH sense    | GTCTCCTCTGACTTCAACAGCG            |
| Human GAPDH antisense| ACCACCTGTTGCTGAGGACCA             |
| Dog GAPDH sense      | GATGGGCCTGAAACCAGGAG              |
| Dog GAPDH antisense  | TCATGAGGCCCTCCACGAT               |

Supplementary Figure 1.

Supplementary Figure 1. qRT-PCR determined standard curve. HEV plasmid based standard curve is generated by plotting the log copy number versus the cycle threshold (CT) value.
Supplementary Figure 2. Potent anti-HEV activity of ribavirin and interferon-α (IFN-α) in Huh7.5 cell model. Treatment of ribavirin or IFN-α for 24, 48, 72 or 96 hours in the subgenomic model determined by luciferase activity (mean ± SEM, n = 5. CTR, non-treatment control.), and in the infectious model determined by viral RNA (mean ± SEM, n = 4. CTR, non-treatment control). *P < .05; **P < .001; ***P < .0001.

Supplementary Figure 3.
Supplementary Figure 3. Immunofluorescence staining of viral protein ORF2 (red) in BDE cells, upon infection of 24h, 48h, 72h and 96h. BDE-HEV cells incubated with the matched IgG control antibody serves as negative control, and HEV infected Huh7.5 cells serves as positive control. DAPI (blue) was applied to visualize nuclei. (40× oil immersion objective; Scale bar, 200 μm).

Supplementary Methods

Electroporation of HEV RNA. The BDE cells were collected and centrifuged for 3 minutes at 1500 rpm, 4°C. The supernatant was removed and the cells were washed with 4 mL Opti-MEM (Thermo Scientific, The Netherlands) by centrifuging for 5 minutes at 1500 rpm, 4°C, 3 times. The cell pellet was resuspended in 100 μL Opti-MEM and mixed with p6 full-length HEV RNA or p6-Luc subgenomic RNA. Electroporation was performed with Bio-Rad’s electroporation systems using the protocol of a designed program (600 volts, pulse length 0.5, number 1, 4 mm cuvette).

Immunofluorescence. Cells grown on coverslips were fixed with 4% (w/v) paraformaldehyde (PFA) for 10 min at RT. After three washes with PBS buffer, cells were permeabilized with 0.1% (v/v) Triton X-100 for 10 min and washed three times with PBS. Block for 1h at Room Temperature with blocking solution (5% Normal Donkey Serum, 1% Bovine Serum Albumin, 0.2% TRITON X in 1x PBS). Cells were then incubated with primary anti-HEV ORF2 (1:200) antibody (aa 434-457, clone 1E6, Millipore, Amsterdam-Zuidoost, the Netherlands) at 4°C overnight. The control group was incubated with the matched mouse IgG 2b antibody (1:200) (InvivoGen) at 4°C overnight. Remove excess primary antibodies and wash the cells for three times with PBS, inoculate the cells with Anti-mouse-Alexa Fluor® 594-Conjugate antibody (Cell Signaling Technology) (1:5000) for 1 hour at room temperature. Nucleus was stained with 4,6-diamidino-2-phenylindole (DAPI) for 10 min at room temperature. Images were detected with confocal electroscope (lens: 40×).

Statistical analysis. Statistical analysis was performed using the nonpaired, nonparametric test with the Mann-Whitney test (GraphPad Prism version 5.01; GraphPad Software). P-values less than 0.05 were considered statistically significant.