

Rapid Diagnosis of Porcine Epidemic Diarrhea Virus Infection by Polymerase Chain Reaction

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ABSTRACT. The diagnosis of porcine epidemic diarrhea virus (PEDV) infection in the laboratory is rather fastidious because of difficulties in virus propagation. The feasibility of virus propagation *in vivo* is also limited by the handling of a number of samples at the same time. In this study, the detection of PEDV by reverse transcription polymerase chain reaction (RT-PCR) is described. The RT-PCR could detect up to 10^4 TCID₅₀/ml of PEDV and did not show any cross reaction with transmissible gastroenteritis virus or porcine rotavirus. Using this method, the detection of PEDV in experimentally inoculated piglets was possible as early as one day after inoculation. These results suggest that the RT-PCR could be applicable for a rapid diagnosis of PEDV infection. — **KEY WORDS:** porcine epidemic diarrhea virus, rapid diagnosis, RT-PCR.

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Porcine epidemic diarrhea virus (PEDV), a coronavirus, is the etiological agent of entero-pathogenic diarrhea in swine [1, 4, 8–10]. Since the clinical symptoms of PEDV infections are similar to transmissible gastroenteritis virus (TGEV) infection, differential diagnosis is necessary to identify the causative agent in the laboratory [8]. Although PEDV infection can be diagnosed by the isolation of the virus in a cell culture, the virus can only be replicated in Vero cells and several blind passages are usually required in the presence of trypsin [6, 7].

For the reverse transcription polymerase chain reaction (RT-PCR) of PEDV, three primers within the membrane protein (M) gene of PEDV were selected, based on the sequence information of Durate *et al.* [5]: P1 (20 mer), 5'-GGACACATTCTTGGTGGTCT-3' for PCR; P2 (24 mer), 5'-GTTTAGACTAAATGAAGCACTTTC-3' for PCR; P3 (25 mer), 5'-GCCATAAAGTTTCTGTTTAGACTAA-3' for the synthesis of complimentary DNA (cDNA) (Fig. 1).

In order to reveal the sensitivity, the cell-adapted KPEDV-9 strain at 93 passages in Vero cells was diluted in distilled water and tested for amplification by RT-PCR [2]. Commercially available kits were used for the extraction of RNA (Ultraspec-3, U.S.A.), and the first strand cDNA synthesis (Boehringer Mannheim, Germany).

PCR reactions were performed with P1 and P2 primers under the following conditions: 1 cycle of 2 min at 94°C, 2 min at 58°C, and 2 min at 72°C; 40 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C; and 1 cycle of 2 min at 94°C, 2 min at 58°C, and 2 min at 72°C in a thermal cycler (Perkin Elmer, U.S.A.).

In this experiment, the result indicated that an amplified DNA fragment with a size of 377 bp could detect up to 10^4 TCID₅₀/ml of PEDV (Fig. 2).

Detection of PEDV by the RT-PCR was also conducted from the feces of piglets experimentally infected with a wild type of PEDV before adaptation in Vero cells. The wild type of PEDV was directly prepared from the small intestine of a neonatal pig, which showed a positive result in an immunofluorescence assay [3]. The small intestine was ground in phosphate buffered saline (PBS, pH 7.4). A

10% suspension of the ground intestine was then filtered using 0.2 μ m membrane filter (Acrodisk, Gelman) and passaged three times in Vero cells for the titration of infectivity [8].

A group of six 3-days old piglets was orally fed with 1 ml of the filtered suspension of intestine, containing roughly 10^4 TCID₅₀/ml of PEDV. After artificial infection, the piglets were supplied with dairy milk. Clinical signs of diarrhea were observed daily until the death of the piglets. Fecal specimens were obtained daily from all the inoculated piglets, pooled and stored at -70°C until use for the experiment. These crude feces were diluted to 1:10 in distilled water and tested by the RT-PCR under the conditions described above. A mixture of cell culture adapted TGEV (Pyung-tak strain) and porcine rotavirus

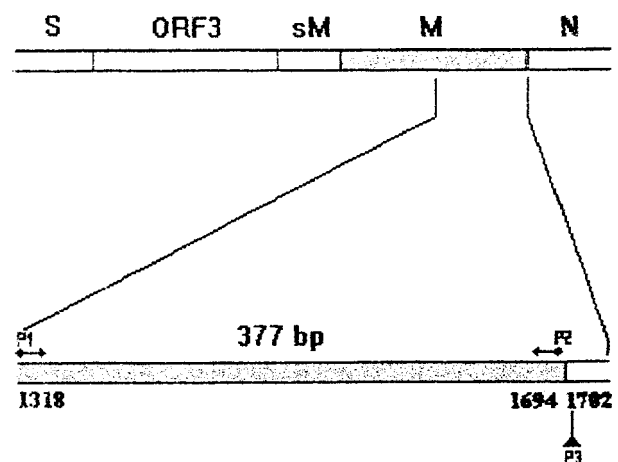


Fig. 1. Outline of RT-PCR for the detection of 377 bp of PEDV M gene. The upper part shows the genomic organization of the PEDV genome. The lower part indicates the position of the primers for PCR (P1 and P2) with the directions of the extension and cDNA synthesis (P3). The numbers are the numerical position on the PEDV genome region between the S and N genes designated by Durate *et al.* [5].

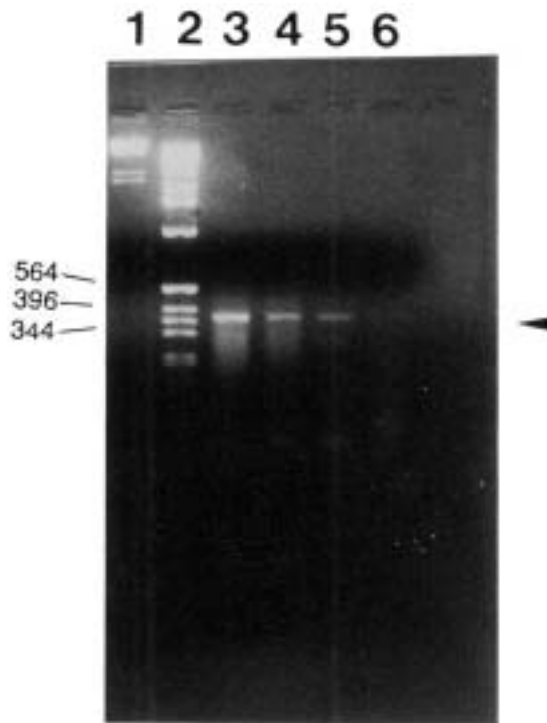


Fig. 2. Sensitivity of RT-PCR with cell culture-adapted PEDV. Lane 1, *Hind*III digested λ DNA size marker; lane 2, 1 kb DNA ladder (Gibco-BRL, U.S.A.); lane 3–6, RT-PCR products from cell adapted PEDV supernatant of titer 10^6 (3), 10^5 (4), 10^4 (5) and 10^3 (6) TCID₅₀/ml, respectively. DNA sizes in bps are indicated on the left.

(OSU strain), titer 10^6 TCID₅₀/ml each, was tested for cross reaction at the same time in this experiment. The results, as shown in Fig. 3, revealed that a positive DNA band was detectable from the sample collected at 24 hr post-infection without any cross reaction with TGEV and porcine rotavirus. Signs of mild diarrhea were seen on the second day after inoculation, and from the third day, watery diarrhea was observed in all the inoculated piglets and continued until the death of half of the inoculated piglets, 6 days after artificial inoculation.

In conclusion, the results of this study indicate that RT-PCR can detect the expected signal from fecal specimens and be applied as a tentative screening method for the rapid diagnosis of PEDV infection in the field.

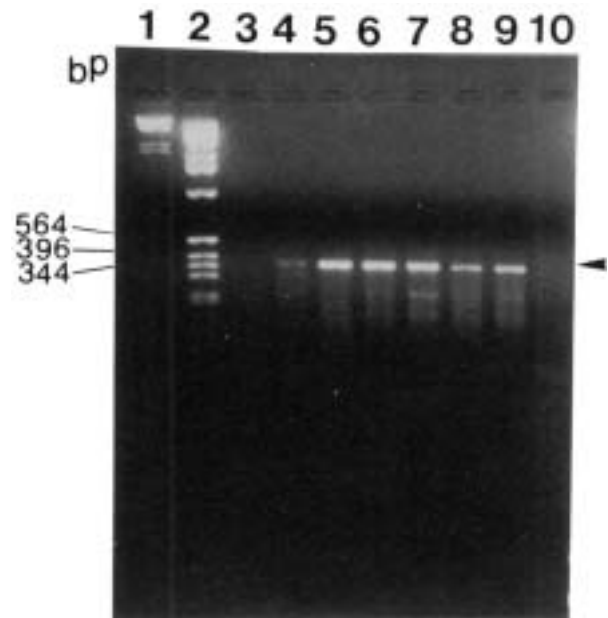


Fig. 3. RT-PCR detection of PEDV from fecal samples of the experimentally inoculated piglets. Lane 1, *Hind*III digested λ DNA size marker; lane 2, 1 kb DNA ladder (Gibco-BRL, U.S.A.); lane 3–9, days after inoculation, 0 day (3), 1 day (4), 2 days (5), 3 days (6), 4 days (7), 5 days (8) and 6 days (9), respectively; lane 10, mixture of transmissible gastroenteritis virus and porcine rotavirus.

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