

Susceptibility of Mice to Porcine Epidemic Diarrhea Virus

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Abstract: The swine enteric disease, porcine epidemic diarrhea is caused by Porcine Epidemic Diarrhea Virus (PEDV). The disease continues to be economically significant in the swine industry especially in outbreak events. One of the prerequisites in disease management and epidemiological investigations is knowledge of factors underlying disease transmission and spread. The susceptibility of mice to PEDV was investigated as vectors in transmission. To prove this possibility, specific pathogen free mice were inoculated through oral, intranasal and intraperitoneal routes and investigate the presence of virus in tissues. As per results, PEDV was not able to replicate in the mice and there was no antibody reaction indicating that they are implausible vectors in PEDV transmission.

Key words: Mice, porcine epidemic diarrhea virus, susceptibility, transmission, swine industry, intraperitoneal routes

INTRODUCTION

Swine enteric disease, Porcine Epidemic Diarrhea (PED) is caused by a coronavirus (PEDV) of the family Nidovirales. It is characterized by vomiting, diarrhea and dehydration. PEDV belongs to group 1b with human coronavirus (HCoV 229) but segregated from serologically different transmissible gastroenteritis virus of group 1a coronaviruses. It was first described in 1971 in UK and later in the decade in both UK and Belgium (Penseart and De Bouck, 1978; Pospischil *et al.*, 2002). The virus has since been reported in Canada, Asia and other European countries (Penseart and Yeo, 2006). PEDV impacts heavy losses to the swine industry as exemplified in the Belgium outbreak where swine of all ages suffered from diarrhea with 50% reported mortality in 1 week old piglets (Penseart and De Bouck, 1978). Equally devastating, PEDV killed all newborns in Thailand and while PEDV outbreaks seem to have subsided in Europe, there continues to be severe epidemics in Asia (Pospischil *et al.*, 2002). Mice and rats are natural reservoirs of pathogens. Presence of rodents in piggeries has been associated with encephalomyocarditis virus disease in pigs and *Toxoplasma gondii* infection in mice was correlated to increased transmission in sows (Weigel *et al.*, 1995). Likewise, porcine circovirus replicated in experimentally infected mice and the virus

was transmitted from mouse to mouse (Kiupel *et al.*, 2001). This study aimed at evaluating the susceptibility of mice to PEDV as potential vectors.

MATERIALS AND METHODS

Virus: The Korean PEDV isolate, KPEDV-9 was propagated in Vero cells and purified by ultra centrifugation at 100,000×g for inoculum preparation. The final inoculum titer was 10^{7.2} TCID₅₀/mL (TCID-tissue culture infectious dose).

Mice: Sixteen, 6 week old specific pathogen free ICR mice were purchased (Samtako bio-Korea). They were housed ten mice per cage in an animal facility and were provided with pellets and water *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee, Chungnam National University, Korea.

Experimental design: The mice were divided into four groups with 16 mice each; Control (C), Oral (O), Intranasal (IN) and Intraperitoneal (IP). Groups O, IN and IP were inoculated with 100, 30 and 400 μL of virus, respectively while C remained uninfected. From each group, two mice were sacrificed at 24, 48, 72, 96 and 120 h post inoculation (p.i.) and blood, intestines, spleen, kidneys and lung samples were collected. These were used for periodic

monitoring of the virus at 24 h intervals for 5 days consecutively. The remaining 6 mice from each group were kept for 2 weeks for serology.

Molecular test: Tissue samples were homogenized and viral RNAs extracted using viral RNA extraction kit (Intron biotechnology). For Polymerase Chain Reaction (PCR), two primers for the nucleocapsid gene were designed (sense, 5'-ACA AGT CTC GTA ACC AGT CC-3' and anti-sense, 5'-GTA TCA CCA CCA TCA ACA GC-3'; GenBank Accession Number: AF353511.1) and used for PEDV detection. Conditions for reverse transcription PCR were 30 min of cDNA synthesis at 45°C followed by 5 min of RTase inactivation at 94°C. Forty cycles of denaturation, annealing and extension ensued at 94°C for 30 sec, 55°C for 30 sec, 72°C for 40 sec, respectively and a final extension for 5 min.

Serology: For serology, Enzyme-Linked Immunosorbent Assay (ELISA) was performed following modified method of Callebaut *et al.* (1982). Briefly, primary mice Immunoglobulin G antibodies (IgG) were captured by PEDV antigens previously immobilized on 96-well plates. Goat anti-mouse IgG, peroxidase conjugated secondary antibodies (Santa Cruz) were used (1:3000 dilution) with their binding loci detected using TMB (3,3', 5,5'-tetramethylbenzidine) substrate reagent. Optical densities were read at 450 nm.

RESULTS AND DISCUSSION

RT-PCR results demonstrate none of blood, lung, kidneys, spleen, intestines and fecal samples was positive for the virus (Table 1). As was in control, presence of PEDV was not detected the inoculated mice groups. Virus presence was tested daily with a minimum of 3 replicates. Results remained negative in the 5 days of study (Table 2). Sera obtained at 2 weeks p.i. was analyzed by ELISA with mouse hyper-immune sera as positive control. No antibodies against PEDV were detected (Fig. 1). Rodents are considered reservoirs and important transmission vectors of diverse pathogens. This fact combined with their wide distribution, agility and abundant populations make them particularly important in both human and veterinary disease transmission and spread (Jay *et al.*, 1985). Further, mice have in experimental studies demonstrated susceptibility to other infections that are not naturally established thus displaying potential of harboring more pathogens once exposed (Kiupel *et al.*, 2001). In swine industry especially, it is worth noting the potential risks posed by the small mammals because pig stys are attractive environments

Table 1: RT-PCR results per issue samples and feces investigated in each mice group^a

Organs	C ^b	O ^c	IN ^d	IP ^e
Blood	-	-	-	-
Intestine	-	-	-	-
Lung	-	-	-	-
Spleen	-	-	-	-
Kidney	-	-	-	-
Feces	-	-	-	-

^a- = Negative, C^b = Control, O^c = Oral, IN^d = Intranasal, IP^e = Intraperitoneal

Table 2: 24 h periodic monitoring of virus in the mice groups up to 5 days^a

Groups	Day 1	Day 2	Day 3	Day 4	Day 5
C ^b	-	-	-	-	-
O ^c	-	-	-	-	-
IN ^d	-	-	-	-	-
IP ^e	-	-	-	-	-

^a- = Negative, C^b = Control, O^c = Oral, IN^d = Intranasal, IP^e = Intraperitoneal

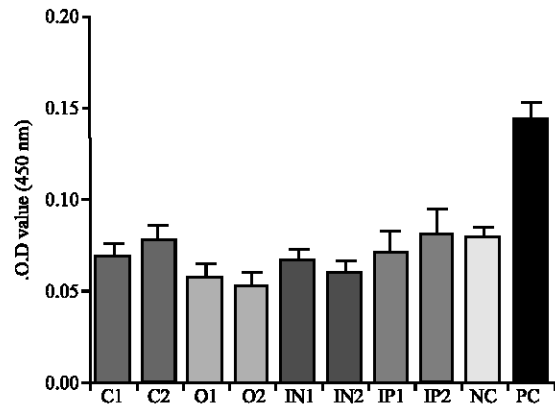


Fig. 1: Detection of anti-PEDV antibodies by ELISA. Data expressed as mean±SD of results of quadruplicate tests. All mice groups were negative for antibodies against PEDV with O.D. values being comparable to the Negative Control (NC) and relatively lower than the Positive Control (PC), C: Control, O: Oral, IN: Intranasal, IP: Intraperitoneal

for the mice and rats. *Treponema hyodysenteriae* has been shown to transmit from mice to pigs as well as from mice to mice through exposure to infected feces and while mice remained asymptomatic, pigs developed swine dysentery within 2 weeks (Joens, 1980). Similarly, porcine circovirus replicated in BALB/c mice and even transmitted vertically to fetuses. Interestingly, no clinical signs have been reported whereas the virus is wasteful among swine (Kiupel *et al.*, 2001). Avian/swine reassortant H2N3 viruses isolated from diseased swine caused disease in both swine and mice on first exposure. The results on the contrary demonstrated mice as being incompetent hosts for PEDV. No symptoms were observed and the virus was not detected in the choice of tissue samples. Similarly, Hooper *et al.* (1994) documented

that the porcine reproductive and respiratory syndrome virus is not harbored by mice and rats. Serological data also concurred with molecular tests with no antibodies being detected.

CONCLUSION

This study investigated the possibility of PEDV transmission by mice. In summary mice are not susceptible to infection with PEDV and therefore, are unlikely vectors of the pathogen. In epidemiology and transmission studies, other factors may account for PEDV transmission.

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