

## Changed Behavior of the Porcine Hemagglutinating Encephalomyelitis Virus (Betacoronavirus) in Respiratory Epithelial Cells

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**Abstract :** Porcine hemagglutinating encephalomyelitis virus (PHEV) is a betacoronavirus that has been studied in the past as a cause of vomiting and wasting disease (VWD) in young piglets (<3 weeks). Nowadays, the virus is still circulating on most farms in Belgium, but there are no descriptions anymore of VWD. Therefore, we are interested in differences between the old and new strains. We compared the replication kinetics of the old well-studied strain VW572 (1972) and the recent isolate P412 (2020) in a susceptible continuous cell line (RPD cells) and in primary porcine respiratory epithelial cells (PoRECs). The RPD cell line was inoculated with each PHEV strain at an m.o.i. of 1 the supernatant was collected, and the cells were fixed at different time points post-inoculation. The supernatant was titrated (extracellular virus titer), and the infected cells were revealed by immunofluorescence staining and quantitated by fluorescence microscopy. We found that VW572 replicated better in the RPD cell line at earlier time points when compared to P412. Porcine respiratory epithelial cells (PoREC) were isolated, grown at air-liquid interphase in transwells and inoculated with both strains of PHEV at a virus titer of 106.6TCID<sub>50</sub> per 200 µl either at the apical side or at the basal side of the cells. At different time points after inoculation, the transwells were fixed and stained for infected cells. VW572 preferentially infected the epithelial cells via the basolateral side of porcine nasal epithelial cells, whereas P412 preferred the apical side. These findings suggest that there has been an evolution of PHEV in its interaction with the respiratory epithelial cells. In the future, more virus strains will be enclosed and the tropism of the strains for different neuronal cell types will be examined for the change in virus neurotropism.

**Keywords :** porcine hemagglutinating encephalomyelitis virus (PHEV), primary porcine respiratory epithelial cells (PoRECs), virus tropism, vomiting and wasting disease (VWD)

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