To the Editor: Porcine epidemic diarrhea virus (PEDV) was first detected in pigs in the United States in May 2013 (1). Since then, according to the American Association of Swine Veterinarians (https://www.aasv.org, see link to number of new cases reported), PEDV has spread to 41 states, and as of October 15, 2014, 8,622 confirmed cases of PEDV infection have been reported in swine. PEDV (family Coronaviridae, genus Alphacoronavirus) is an enveloped, positive-sense, single-stranded RNA virus (2). The virus replicates in epithelial cells of small and large intestines and causes highly contagious infection in pigs. The disease is characterized by watery diarrhea, vomiting (leading to subsequent dehydration), and high rates of death, especially in young piglets; thus, outbreaks cause substantial economic losses to the swine industry (1). Variants of the original virulent PEDV have recently been isolated in the United States, making development of a vaccine to protect against this devastating disease even more challenging (3). Vero cells are used for the isolation of virus from clinical samples and for virus propagation and titration and virus neutralization studies. The addition of exogenous trypsin in culture medium is a prerequisite for efficient replication of PEDV in Vero cells (4): trypsin cleaves the spike protein of PEDV into 2 subunits that mediate cell-to-cell fusion and virus entry into the cells (5).

We examined PEDV replication in a newly established immortalized duck intestinal epithelial cell (MK-DIEC) line, which was generated from the intestinal tissues of a 19-day-old white Pekin duck embryo. MK-DIECs are cuboidal (characteristic of epithelial cells), express epithelial marker (pan-cytokeratin), and show extensive proliferation in culture. Several coronaviruses, including PEDV, use aminopeptidase N (APN) as the cellular receptor for attachment to cells (6). As a first step, we used a rabbit polyclonal anti-human APN antibody (Abcam, Cambridge, MA, USA) in an indirect immunofluorescence assay (IFA) to examine whether MK-DIECs express APN. We found that nearly 100% of the cells expressed APN on their surface (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/21/3/14-1658-Techapp1.pdf).

Next, we examined PEDV replication in MK-DIECs. The cells were cultured in medium containing equal amounts of Dulbecco modified Eagle medium; Mammary Epithelial Growth Medium (Lonza, Walkersville, MD, USA) supplemented with bovine pituitary extract (70 µg/mL), human epidermal growth factor (5 ng/mL), insulin (5 µg/mL), and hydrocortisone (0.5 µg/mL); and 2% fetal bovine serum. Near confluent cells were infected with PEDV at a multiplicity of infection of 0.1. The Colorado strain of PEDV (obtained from the National Veterinary

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**Figure.** Replication of porcine epidemic diarrhea virus (PEDV) in a newly established immortalized duck intestinal epithelial cell line (MK-DIEC) infected with PEDV at a multiplicity of infection of 0.1 in the presence of different concentrations of trypsin. A) Twenty-four hours after infection, PEDV nucleoprotein in infected cells was detected by immunofluorescence assay using fluorescein isothiocyanate–labeled nucleoprotein-specific monoclonal antibody. B) PEDV-induced cytopathic effect in MK-DIEC cells 36 h after infection.
Lack of Effect of Lamivudine on Ebola Virus Replication

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To the Editor: The unprecedented number of Ebola virus disease (EVD) cases in western Africa has compelled the world to consider experimental and off-label therapeutics to mitigate the current outbreak. For clinicians, approved drugs are an attractive solution because of known safety profiles and availability.

Oral lamivudine (GlaxoSmithKline, Brentford, UK), a US Food and Drug Administration–approved anti-HIV drug, has been suggested as a possible antiviral agent against Ebola virus (EBOV). In September 2014, a Liberian physician, Dr. Gorbee Logan, reported positive results while treating EVD with lamivudine (1). Thirteen of 15 patients treated with lamivudine survived presumed EVD and were declared virus free. Clinical confirmation of EVD in these cases remains to be verified.

Our laboratory had previously assessed this antiretroviral compound in drug screens against EBOV and observed no discernable antiviral activity. However, given

References


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Porcine Epidemic Diarrhea Virus Replication in Duck Intestinal Cell Line

Technical Appendix

Technical Appendix Figure 1. Expression of aminopeptidase N (APN) on immortalized duck intestinal epithelial cell line (MK-DIEC). APN expression was detected on MK-DIEC cell surfaces by immunofluorescence assay using rabbit polyclonal anti-human APN as primary antibody and fluorescein isothiocyanate–labeled goat anti-rabbit as secondary antibody.
Technical Appendix Figure 2. Quantification of released progeny virus in porcine epidemic diarrhea virus–infected supernatant of immortalized duck intestinal epithelial cell line MK-DIEC cells, as measured by titration in Vero cells. Each bar represents mean (±SD) virus titer at each time point from 3 independent experiments. TCID$_{50}$, 50% tissue culture infectious dose.