Current Topics in Virology
Vol. 13, 2016

Short Communication

Ultrastructure and morphogenesis of PEDV in PEDV-infected Vero-76 cells

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ABSTRACT

Porcine epidemic diarrhea virus (PEDV) infects pigs of all ages but causes severe diarrhea and even death in young nursery piglets, which often results in significant economic losses to the swine industry worldwide. PEDV belongs to the alphacoronavirus genus and is closely related to porcine transmissible gastroenteritis virus (TGEV). PEDV has been adapted to grow in Vero-76 cells (ATCC CRL-1587). Here we describe the ultrastructure and morphogenesis of PEDV in Vero-76 cells. Large vacuoles with stacked membranes are observed and mainly located in the perinuclear space in infected cells. Immature virus particles of different shapes and sizes at different stages of assembly are mostly associated with large vacuoles and arranged in a wheel-like structure. Mitochondria adjacent to the virus-containing vacuoles appear to be swelling and disorganized. Mature viruses with an estimated diameter of 70 nm are mostly dissociated from large vacuoles and diffusely distributed throughout cytoplasm containing dark granular materials. Mature PEDV does not appear to be present in smooth-walled vesicles as described for other coronaviruses. Surface projections or corona-like structures are not apparent in intracellular viruses. Overall, PEDV shows some unique features on ultrastructure and morphogenesis in Vero-76 cells.

KEYWORDS: PEDV, ultrastructure, morphogenesis, Vero cells

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INTRODUCTION

Porcine epidemic diarrhea virus (PEDV) was first described in Europe in the 1970s [1] and has recently emerged in the US [2, 3]. PEDV causes acute diarrhea and dehydration in neonatal piglets, which often results in high morbidity and significant economic losses. PEDV belongs to the *Coronaviridae* family and has a positive-sense single-stranded RNA genome of approximately 27 kb [4, 5]. The amino acid sequence homology analysis reveals that PEDV is closely related to other members of the alphacoronavirus such as porcine transmissible gastroenteritis virus (TGEV) and human coronavirus (HCoV)-229 [5]. PEDV has been adapted to grow in Vero-76 cells *in vitro* [6, 7]. Like other coronavirus, PEDV readily causes syncytia in Vero-76 cells [8]. Abundant viral antigens and virus particles are detected in the epithelial cells of small intestines of piglets infected with PEDV [9, 10, 11]. The ultrastructure and morphogenesis of the prototype European PEDV strain, CV777, in infected intestinal epithelial cells *in vivo* have been reported [9, 10]. In this study, we examined the ultrastructure and morphogenesis of the newly emerged US strain, PEDV NVSL-CO (PEDV USA/Colorado/2013, GenBank accession number KF272920), in infected Vero-76 cells. We observed several unique features of PEDV-CO in Vero-76 cells. Immature viruses at various stages of assembly are typically located in large vacuoles. Mature viruses are mostly dissociated from large vacuoles and diffusely located in the cytoplasm with homogeneous dark granular materials. Virus surface projections are not apparent in intracellular viruses. To our knowledge, this is

the first detailed description on the ultrastructure and morphogenesis of PEDV in infected Vero-76 cells.

MATERIALS AND METHODS

Vero-76 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin-Glutamine (PSG, Gibco) Solution. Prior to virus infection, cells were resuspended in freshly prepared DMEM media containing 1% PSG and 1.3 μg/ml N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-treated trypsin (Sigma). Cells were infected with PEDV-CO at a multiplicity of infection of 1. At 24 h and 48 h after virus infection, cells were washed with phosphate buffered saline (PBS) and fixed in phosphate-buffered 2.5% glutaraldehyde. Cells were then processed for thin sectioning as described previously [12]. The thin sections were placed onto copper grids, stained with 1.0% uranyl acetate and lead citrate, and examined and photographed using a Hitachi 7100 transmission electron microscope.

RESULTS

PEDV causes syncytia in Vero-76 cells. This is verified by the electron microscopic image of PEDV-infected Vero-76 cells. As shown in figure 1A, a giant cell containing four nuclei was observed. Large numbers of vacuoles are present in the cytoplasm of the giant cells. Under higher magnification, we observed numerous large vacuoles with multiple layers of stacked membrane in the perinuclear space adjacent to the mitochondria (Figure 1B). In some sections, we observed virus-containing vacuoles located next to the swollen and disorganized mitochondria. Immature viruses of different sizes and shapes are located in large vacuoles and mostly arranged in a wheel-like structure with no clear membrane around them (Figure 2A). Interestingly, mature viruses of approximately 70 nm are more randomly and diffusely located in the cytoplasm in a background of dark granular materials. There was no smooth-walled membrane surrounding the mature virus particles. The surface projections or corona-like structures are not apparent in the intracellular virus particles (Figure 2B). Under lower magnification, it was observed that numerous virus particles are either diffusely present in the cytoplasm or arranged in a wheel-like structure with no clear membrane boundary. No intact organelles are observed. Instead homogenous dark granular materials are present in the cytoplasm (Figure 2C).

DISCUSSION

PEDV has been adapted to grow in Vero-76 cells with the addition of trypsin in the medium [6].

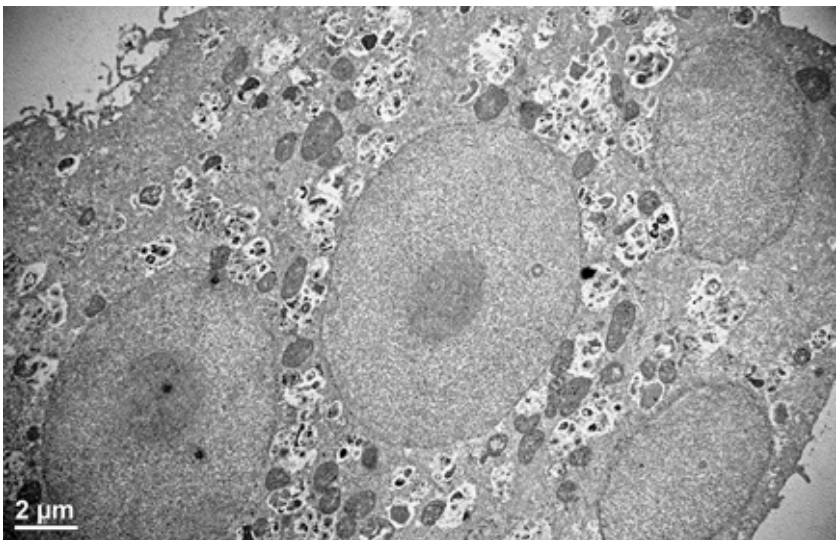

Figure 1A. PEDV infection causes syncytia. A multinucleated cell containing four nuclei is shown in a PEDV-infected Vero-76 cell.

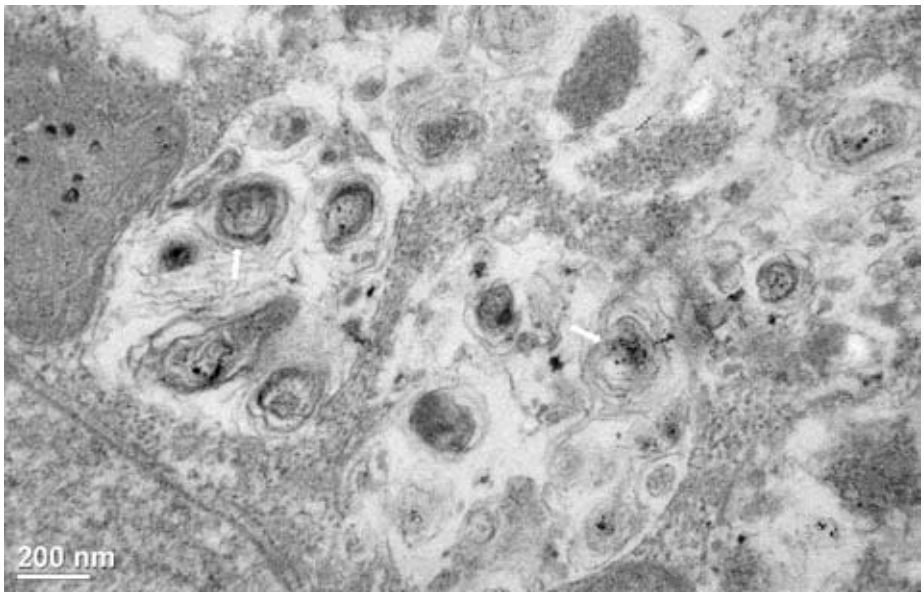

Figure 1B. PEDV infection causes the formation of large vacuoles with stacked membrane in the perinuclear space of a PEDV-infected Vero-76 cell. Arrows indicate the vacuoles with stacked membrane.

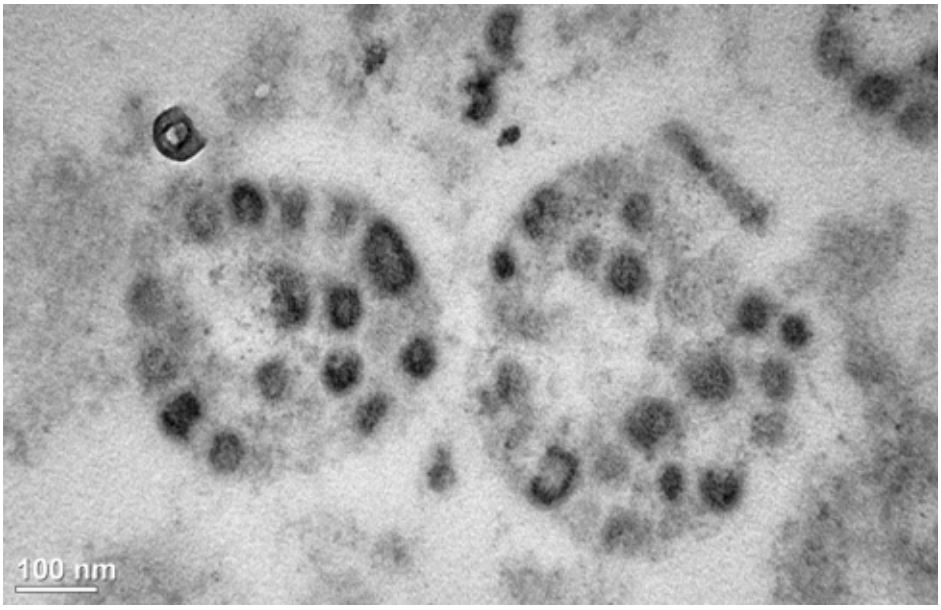

Figure 2A. Immature virus particles of different shapes and sizes at various stages of assembly are mainly located in the large vacuoles and arranged in a wheel-like structure.

Both vacuolation and syncytia are observed in PEDV-infected Vero-76 cells. Vacuolation of intestinal epithelial cells caused by the prototype European PEDV strain CV777 was reported previously [10]. However, syncytia were not described in PEDV-infected intestinal epithelial cells [10]. Similarly, it has been reported that murine hepatitis virus causes syncytia only in continuous cell lines, but not in primary hepatocytes and glial cells [13]. Large vacuoles with stacked membranes located in the

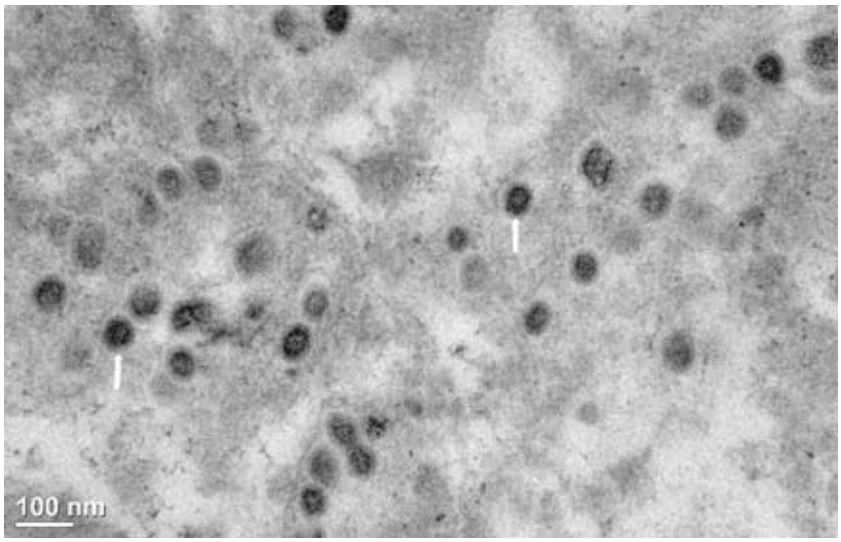

Figure 2B. Mature virus particles of approximately 70 nm in diameter are mostly dissociated from the large vacuoles and diffusely distributed in cytoplasm. Arrows indicate mature virus particles not associated with smooth-walled vacuoles.

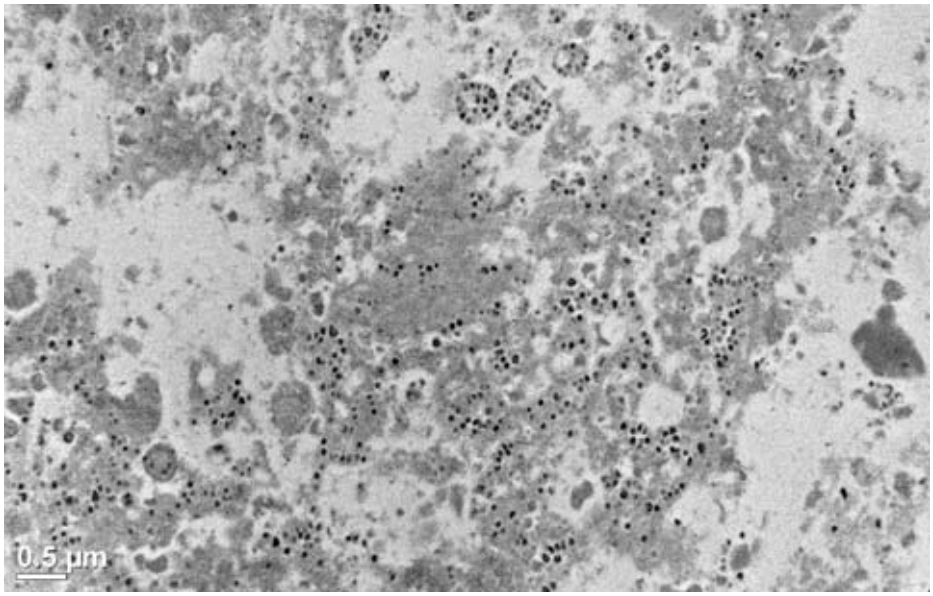

Figure 2C. Both vacuoles-associated and free virus particles are abundantly present in virus-infected Vero-76 cells with little or no intact organelles present. Homogeneous dark granular materials are observed.

perinuclear space are observed in PEDV-CO-infected cells at 24 h after virus infection. These vacuoles are adjacent to the mitochondria. The appearance of these vacuoles seems to be drastically distinct from the double-membrane vesicles (DMV) described for severe acute respiratory syndrome virus (SARS virus) [14, 15]. Similar vacuoles with stacked membrane have been observed in feline infectious peritonitis virus (FIPV)-infected Vero cells [16]. Virus-containing vacuoles are adjacent to

the swollen and disorganized mitochondria. Swollen mitochondria with disrupted cristae were reported in PEDV CV777-infected intestinal epithelial cells [10]. Since both mitochondria and endoplasmic reticulum are involved in the biosynthesis of membrane, it is reasonable to speculate that these stacked membrane vesicles are derived from these organelles. Some viruses such as flock house virus replicates on the outer membrane of mitochondria [17]. The exact mechanism by which PEDV drives the formation of such unique vesicles and their possible role in PEDV replication remains elusive.

Immature virus particles of different shapes and sizes are in a wheel-like structure with no clear membrane surrounding the vacuole. This is consistent with the observation of PEDV CV777-infected small intestinal epithelial cells *in vivo* [10, 18]. Similar nucleocapsid inclusion structures were observed for the assembly and organization of SARS virus particles, a betacoronavirus [18, 19]. We observed that the nucleocapsid inclusion was located next to the mitochondria, suggesting a possible role of mitochondria in the membrane biosynthesis of the virus-containing vacuoles. Interestingly, mature virus particles of approximately 70 nm are more randomly distributed in the cytoplasm. We did not see smooth-walled vesicles surrounding the virus particles. This agrees with the observation in PEDV CV777-infected small intestinal epithelial cells [10]. It is generally believed that mature coronavirus particles are located in smooth-walled vesicles, which transport viruses outside of cells by exocytosis. It is clear that PEDV lack the smooth-walled vesicles. This is distinct from other coronaviruses such as FIPV and avian infectious bronchitis virus [16, 20]. This may also imply that PEDV exit cells not through exocytosis as described for other coronaviruses. More studies are needed to further elucidate the mechanisms by which PEDV exits the infected cells. PEDV destroys all the organelles in PEDV-infected Vero-76 cells at 48 h post-infection. The intact architecture of mitochondria, Golgi apparatus, and endoplasmic reticulum cannot be observed. Instead, numerous individual mature viruses are diffusely distributed in the cytoplasm in a background of homogeneous dark granular materials. This is consistent with the degenerated and translucent intestinal epithelial cells infected with PEDV CV777 [10]. Viruses may be released from infected cells through cell death. Alternatively, PEDV could spread to other cells through cell fusion or phagocytosis of virus-infected cells by neighboring healthy cells as described for FIPV [17].

To our knowledge, this is the first detailed description on morphological and ultrastructure features of PEDV in Vero-76 cells. We speculate that the diffuse distribution nature of PEDV in infected cells may explain the difficulty in growing PEDV into high titers in Vero-76 cells. We suspect that virus release from infected cells may not be very efficient in Vero-76 cells. One study showed the role of trypsin in facilitating virus release from infected cells [7]. We also speculate that the S-protein on the plasma membrane may mediate the fusion of infected cells with adjacent uninfected cells to form multinucleated giant cells, which may facilitate virus spread and its replication in Vero-76 cells.

CONCLUSION

Large vacuoles with stacked membranes are observed in the perinuclear space of infected cells. Immature virus particles are associated with large vacuoles and arranged in a wheel-like structure. Mature virus particles do not appear to be present in smooth-walled vesicles. Surface projections or corona-like structures are not apparent in intracellular viruses.

ACKNOWLEDGMENTS

We thank Ms. Karen Bentley for taking the transmission electron microscopic images. The authors thank Drs. Sabrina Swenson and Melinda Jenkins-Moore of the National Animal Disease Laboratories for providing the cell culture-adapted PEDV-CO isolate. This study was supported by grants from USDA National Institute of Food and Agriculture (2012–35204-05079), Hatch (10001514), Hatch Multi State (1010908), and by the South Dakota Agricultural Experiment Station.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ABBREVIATIONS

PEDV : porcine epidemic diarrhea virus
DMV : double-membrane vesicles
FIPV : feline infectious peritonitis virus
SARS virus : severe acute respiratory syndrome virus
TGEV : transmissible gastroenteritis virus

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