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LETTER

Ultrastructural features of PPRV infection in Vero cells

Dear Editor,

The peste des petits ruminants virus (PPRV) causes an increasingly important viral disease of livestock that predominantly infects small ruminants such as goats and sheep. It belongs to the *Paramyxoviridae* family and is classified as the fourth member of the genus *Morbillivirus* because of its genetic similarity with other members of this genus, which includes measles virus (MV), rinderpest virus (RPV), canine distemper virus (CDV), and a number of other viruses that infect aquatic mammals (Yin Z, et al., 1997). Morbilliviruses are characterized by a single-stranded, negative-sense RNA genome enveloped within pleomorphic virions that are similar in appearance to other members of the Paramyxoviridae family. Many previous studies have reported on the morphogenesis of MV (Nakai M, et al., 1969; Raine C S, et al., 1969), CDV (Lawn A M, 1970), and RPV (Tajima M, et al., 1971). The morphologies of CDV (Norrby E, et al., 1963) and RPV (Plowright W, et al., 1962) have been studied by negative staining using electron microscopy, revealing the presence of structurally similar virions that resembled those of MV and other paramyxoviruses. Although they were varied in shape and size, most virions were circular or oval and measured between 110 and 300 nm in diameter. Typically, ultrastructural analysis is used to evaluate the virus-cell interaction. However, thus far, a detailed electron microscopic study of the PPRV–cell interaction has not been performed.

Here, we conducted electron microscopy studies on Vero cells infected with PPRV strain Nigeria 75/1 (obtained from the virus bank of the China Institute of Veterinary Drug Control) that were seeded on 75-cm^3 tissue culture flasks. Vero cells were infected with a virus concentration of 10^5 TCID50 mL^{-1} and incubated for 1, 2, 3, 4, 5, 6 and 7 days at 37 °C and 5% CO_2. Cells were detached using a cell scraper and centrifuged at 1500 g/min for 10 min. The samples of the sections were fixed with 2.5% glutaraldehyde in 0.2-mol/L phosphate buffer (pH 7.4), post-fixed with 1% osmium tetroxide, dehydrated in a series of concentrations of 50%, 70%, 80%, 95% and 100% ethanol, and embedded in Epon 812 for observation under a transmission electron microscope (TEM). Ultrathin sections were stained with uranyl acetate followed by lead citrate. Samples for negative staining were subjected to three freeze–thaw cycles at −20 °C/37 °C, followed by precipitation by interaction with a PPRV N monoclonal antibody at 37 °C for 60 min and centrifugation at 6000 g/min for 30 min. The resulting precipitate was resuspended in 50 μL of 0.1-mol/L phosphate buffer (pH 7.2) and negatively stained using 1% phosphotungstic acid (pH 6.5). All samples were observed under a TEM (JEM 1230, Japan).

The negative staining performed in this study revealed that PPRV are similar to MV, CDV (Cornwell H J, et al., 1971; Poste G, 1972; Narang H K, 1982), and RPV (Tajima M, et al.,1971) in many aspects such as virion ultrastructure in which the circular and oval forms were most commonly observed, mode of virus replication in infected cells, and the sequential development of cytopathic effects. PPRV particles are circular or oval and composed of two parts, the core and envelope (Figure 1A). These particles had diameters in the range of 40 to 275 nm. The viral core was uniform electron-dense. The cores were present as three predominant morphological forms, the most common of which were circular and oval, with rod-shaped cores being less frequent. The virus cores measured approximately 254.5 nm across the widest part and 44 nm across the narrower end. The viral particles often possessed envelopes measuring 12 to 32 nm in thickness (Figure 1A) and spikes were occasionally observed on the envelope surface. Presumably, the spikes on the surface of the PPRV particles represent the envelope glycoproteins. Paramyxovirus particles show considerable differences in size, i.e., that of MV range from 180 to 600 nm in diameter, RPV 120 to 300 nm, CDV 110 to 550 nm, Newcastle disease virus (NDV) 100 to 250 nm, and parainfluenza virus 100 to 250 nm (Nakai M, et al., 1969; Yin Z, et al., 1997). In the present study, particles ranged from 40 to 275 nm in diameter. Although the results were similar to those of a previous report, showing they varied between 130 and 390 nm, the particles analyzed in our study were smaller than those reported by Durojaiye et al. (1985).

Paramyxoviruses spread through cell monolayers or through an organ by two mechanisms, successive rounds of virus infection and cell–cell fusion. Thus, infected

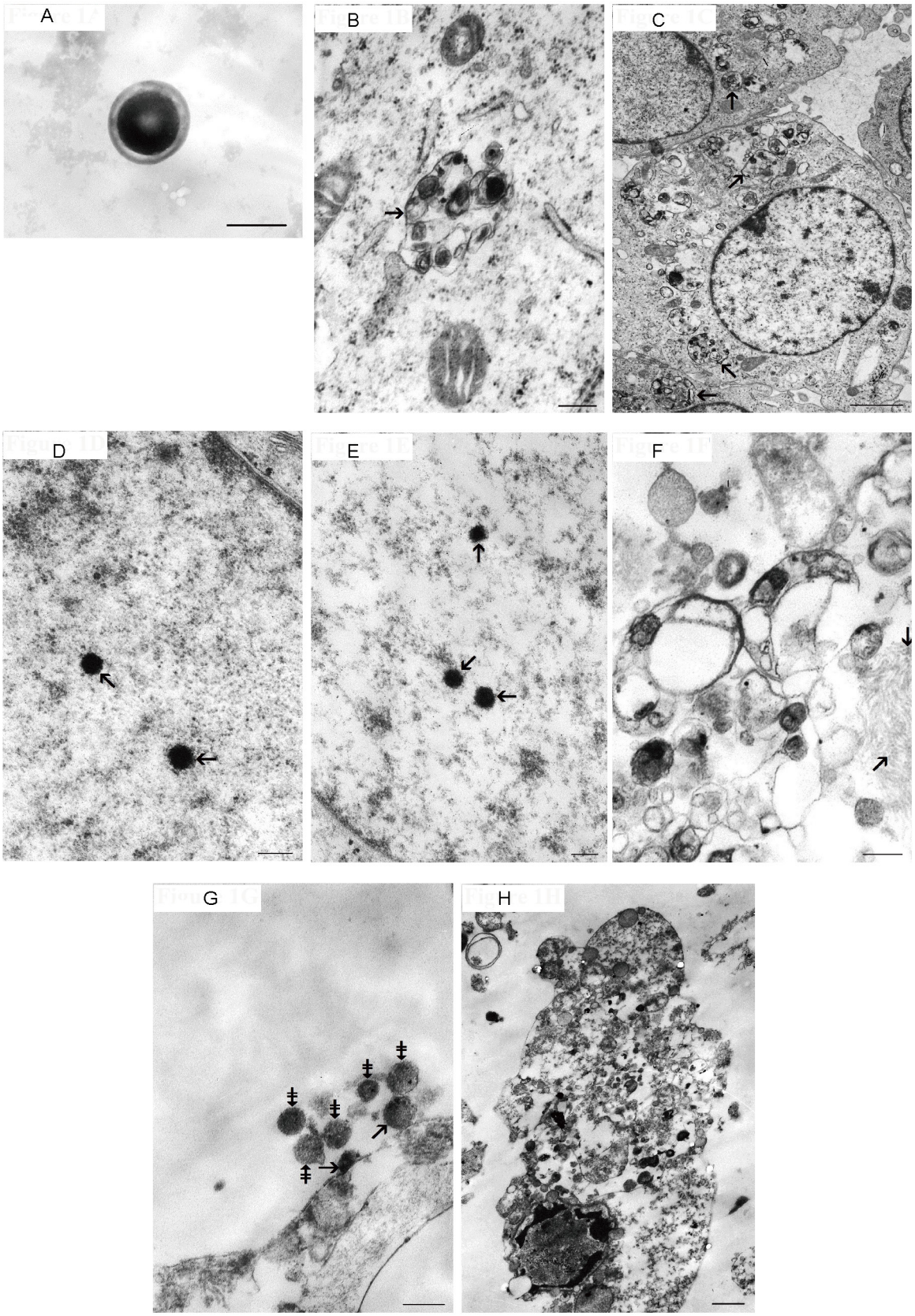
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Figure 1. A: Negative staining and electron microscopic analysis of PPRV. Scale bar=200 nm. B: PPRV-infected Vero cell at 24 h showing marked autophagosome-like vesicles (→). Scale bar=200 nm. C: PPRV-infected Vero cell at 48 h showing marked aggregates of autophagosome-like vesicles (→). Scale bar=2 μm. D: PPRV-infected Vero cell 48 h after infection with the peste des petits ruminants virus showing particles (→) in the cytoplasm. Scale bar=200 nm. E: PPRV-infected Vero cell at 72 h showing particles (→) in the nucleus. Scale bar=200 nm. F: The peste des petits ruminants virus filamentous nucleocapsid (→) in the cytoplasm of a PPRV-infected Vero cell 72 h after infection. Scale bar=200 nm. G: PPRV-infected Vero cell at 72 h showing virus particles released by budding (→) and mature particles (‡).Scale bar=200 nm. H: PPRV-infected Vero cell at 168 h. Scale bar=2 μm.

cell surfaces produce virus particles and also fuse with adjacent cells. Organellar changes were noted in Vero cells infected with PPRV. These changes were especially evident for mitochondria and rough endoplasmic reticulum. Three stages were recognized in the production and assembly of the main morphological components of PPRV, including the formation of the virus nucleocapsid in the cytoplasm and nucleus, alignment of the nucleocapsid below the cell membrane, and maturation of virus particles by budding. Autophagosome-like vesicles and PPRV particles first appeared in the cytoplasm. At 1 day post-infection (dpi), autophagosome-like vesicles were generated in the cytoplasm (Figure 1B), which were similar to that in a previous report (Zhang Y, et al., 2013). For 2 to 3dpi, PPRV particles in individual cells were observed in the nucleus and cytoplasm, and the number of autophagosome-like vesicles and filamentous tubular nucleocapsids gradually increased (Figure 1C–1E). However, in the present study, the tubular strands and "herring bone-like" structures were not observed universally, which was in contrast with that observed in previous studies (Nakai M, et al., 1969; Cornwell H J, et al., 1971; Durojaiye O A, et al.,1985). This may be due to viral particle integrity and the seriality of sections. The aggregates of filamentous forms were observed at 3dpi, at which point the endoplasmic reticulum swelled and disintegrated (Figure1F). As has been reported for MV, CDV, and RPV, mature PPRV particles were released by budding from the cell membrane (Figure 1G). By 6 to 7dpi, vesicles and virus particles filled the cytoplasm, karyopyknosis was noticeable, and abundant of mature virus particles were released by a budding process (Figure 1H). Recently, Liljeroos et al. combined electron cryotomography with subvolume averaging and immunosorbent electron microscopy to characterize the 3D ultrastructure of the MV particle (Liljeroos L, et al., 2011). Therefore, further investigations are warranted to determine the significance of intranuclear inclusions and spikes in the infectious process of PPRV, in addition to revealing the 3D ultrastructures of virions.

FOOTNOTES

All the authors declare that they have no competing interests. The samples were collected under a research protocol approved by the Lanzhou Veterinary Research Institute Clinical Research Biosafety Committee.

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