

Ultrastructure of duck Tembusu virus observed by electron microscopy with negative staining

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Summary. – Duck Tembusu virus (DTMUV) is a newly emerging enveloped flavivirus. This study shows the ultrastructure of DTMUV using viral purification, negative staining and electron microscopy. Electron microscopic examinations revealed mature DTMUV particles with 50 to 75 nm in diameter and typical enveloped flavivirus structure that consists of the internal nucleocapsid, an inner layer of lipid bilayer and an external layer of E glycoprotein ectodomain. Particles appear to be mostly spherical. In particular, RNA core is deep colored and dense, both capsid and lipid bilayer are clearly visible, the capsid forms regular hexagon, and E glycoprotein ectodomain forms a fringe instead of visible spikes. Thus, this report about the clear ultrastructure of the DTMUV particles will be the major driving forces behind structural biology of DTMUV.

Keywords: duck; Tembusu virus; ultrastructure; electron microscopy; allantoic fluid

The duck Tembusu virus (DTMUV) is an enveloped, single-stranded positive-sense RNA virus belonging to the family *Flaviviridae* responsible for severe economic losses in domestic poultry (Wang *et al.*, 2011; Yan *et al.*, 2011). First DTMUV infecting china ducks was reported in 2010. The virus can also infect geese, chickens and birds in succession. Indeed, some studies have shown that DTMUV antibodies were detected in mice and even humans (Liu *et al.*, 2013; Tang *et al.*, 2013). It is suggested that DTUMV spreads fast and has a wide susceptible range. In our lab, we have isolated and identified a novel DTMUV strain (CQW1) isolated from the liver tissue of a sick duck, analyzing genetic and phylogenetic characterization (Zhu *et al.*, 2015). Although structural and functional characterization of the DTMUV was done before, there have been few reports about information on the DTMUV ultrastructure, which are still insufficient to

elucidate the fine structures of complete virions. In this study, we have applied electron microscopy to obtain the clear and complete viroids, which provide an important basis to further study morphological classification of Tembusu virus.

We have used the duck Tembusu virus strain CQW1 (GenBank: KM233707.1). Nine-day-old embryonated duck eggs were inoculated with a suspension of the virus, incubated for 3 days at 37°C. Supernatant after centrifugation of the allantoic fluid, was pelleted by ultracentrifugation at 100,000 x g for 3 h at 4°C and resuspended in phosphate-buffered saline (PBS, pH 7.0). The suspension was then purified on a 30, 45, 60% discontinuous sucrose gradient by ultracentrifugation and then diluted with PBS. Finally, the purified virus was pelleted by ultracentrifugation to remove sucrose. Purified DTMUV was applied to grids coated with carbon and discharged with glow, stained with 20 µl of 3% phosphotungstic acid for 5 min and air dried. Samples were observed with transmission electron microscope (TEM, JEOL JEM-100CX) operating at 100 keV.

In the electron microscopy images, individual duck Tembusu virions do not form clusters, but are solely scattered (Fig. 1). They appear as spherical or elongated particles

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Abbreviations: DTMUV = duck Tembusu virus

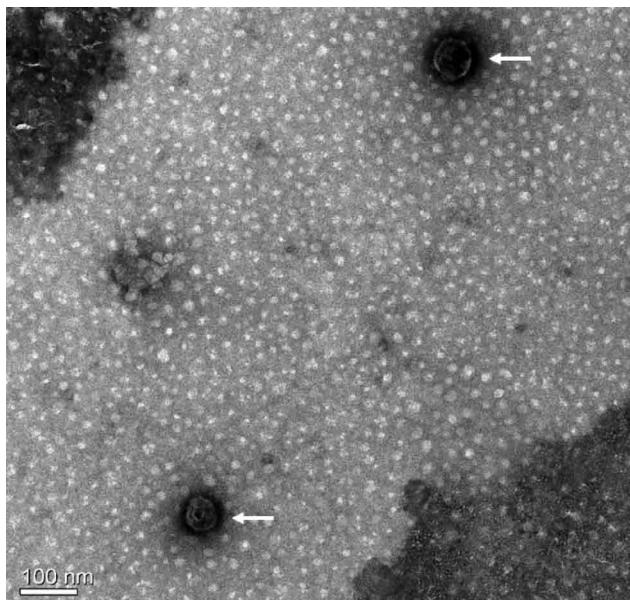


Fig. 1

DTMUV particles

Dissociative DTMUV particles (arrow) purified from duck embryo allantoic fluid. Bar, 100 nm.

which have the typical enveloped flavivirus ultrastructure consisting of E glycoprotein ectodomain, the lipid bilayer, and the internal nucleocapsid (Fig. 2). These viruses have a diameter range of 50–75 nm, or in some viroids even over 100 nm, and an average diameter of 60 nm. Density on sucrose gradient was 45–60%. In general, the virus size can act as one of the criteria of the virus classification. However, DTMUV diameter size has a slight variation between our data and early reports which stated the size to be 30–45 nm from an ultrathin section of infected DEF and DF-1 cells (Su *et al.*, 2011; Yun *et al.*, 2012). There are many possibilities to explain this discrepancy, which may be due to the methods of specimen processing. Compared to DTMUV morphology examined by direct observation of infected cells, DTMUV purified from allantoic fluid can show more primitive ultrastructure. When viruses utilize the endocytic pathway to enter cells, endosomal carrier vesicles (ECVs) fuse with virus particles in early to intermediate endosomes, which can change viral functions and structure (Gruenberg, 2001; Nour *et al.*, 2013). Therefore, in our data, virus infected cells reflect pathogenicity but not the morphological changes caused by biological properties of the virus. In addition, viroids observed by negatively stained technique usually present true size, but ultrathin sections induce cell contraction especially when embedded.

In detail, the duck Tembusu viroids RNA cores are usually spherical and electron-dense particles which measure 15–35 nm (mean, 20 nm) in diameter. The viral capsids

demonstrate low density hexagon image with clear boundaries and a diameter range of 22–45 nm (mean, 30 nm). The lipid bilayers show approximately equal thick and light stained rings in outer layer of viroids. Shallow-stained virus capsid and lipid bilayer are about 12 nm apart. E glycoprotein ectodomains have an average center-to-center spacing of 35 nm with blurred high-dense staining and invisible glycoprotein spikes. Negatively stained viroids which can be

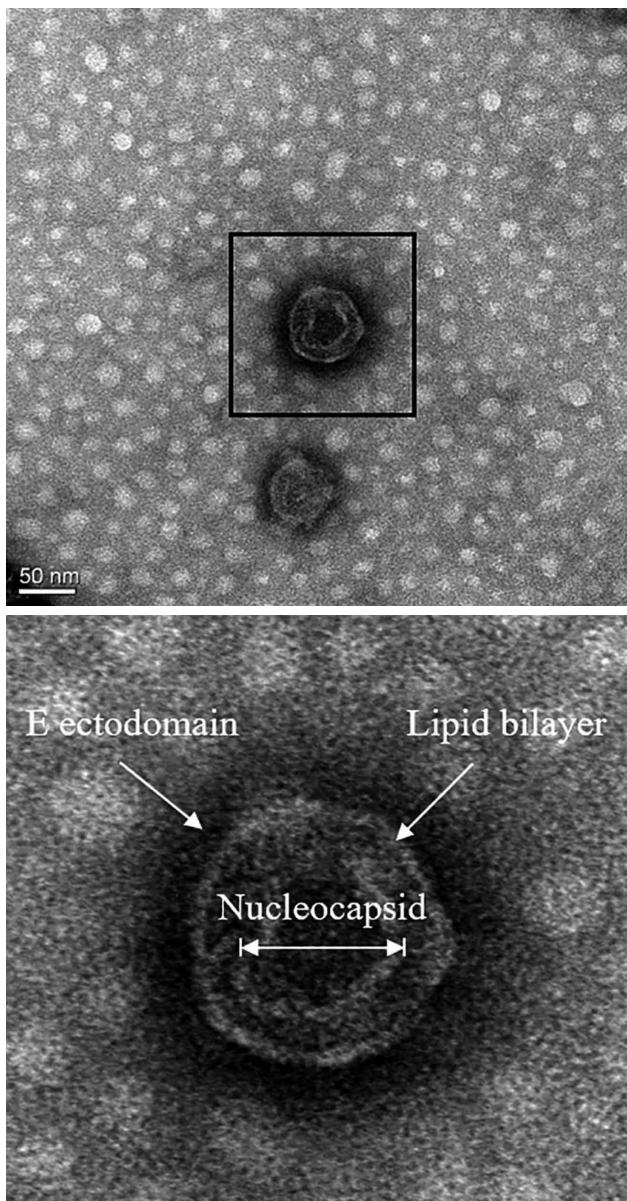


Fig. 2

Ultrastructure of DTMUV

Ultrastructure of DTMUV consisting of E glycoprotein ectodomain, the lipid bilayer, and the internal nucleocapsid. Bar, 50 nm.

affected with surface tension appear in various shapes. In our study, DTMUV particles mostly appeared spherical due to surface enveloped membrane that plays a supporting role against surface tension. As shown in the figure, glycoprotein ectodomains of duck Tembusu virions form a blurred fringe instead of visualized spikes. The reason is that the whole particles are penetrated with stain and the lenses capture more complex elements of viroids. Only when penetration of the stain into the particles is weaker, viral spikes can be seen on the virus particles (Nermut, 1972).

In conclusion, we obtained the ultrastructure of DTMUV using TEM imaging, with complete internal and external structures. We will further study the viral morphology, which will provide better information about DTMUV pathogenesis.

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