Electron microscopy of bovine virus diarrhoea virus

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Summary: Bovine virus diarrhoea virus (BVDV) has been (tentatively) identified by electron microscopy in purified virus preparations, in infected cell cultures and in tissues and cells from infected animals. These studies have revealed a spherical membrane-bound particle with a diameter of 40-60 nm. The membrane is smooth, bilaminar and surrounds a dense or semi-dense core of 20-25 nm. The core particle may be isometric or hexagonal. In studies of the morphogenesis of BVDV in infected cell cultures, it was found that assembly and maturation of the viral particles occur via a condensation process within membrane-bound vesicular organelles, in which the virions subsequently accumulate. Release of the virus occurs when the cell finally lyses and/or via exocytosis. Thus, both with regard to morphogenesis and to morphology, BVDV bears close resemblance to the Flaviviridae.


INTRODUCTION

Studies of the morphology and morphogenesis of bovine virus diarrhoea virus (BVDV) as well as identification/detection of the virus in situ in cells and tissues have been severely hampered by some of the inherent characteristics of the virus. These include the lack of shut-down of cellular protein synthesis in infected cells, a density of the virus particle similar to that of subcellular constituents of the host cell, growth to comparatively low titres in cell culture, virion fragility in purification procedures and lability of antigenic sites. Many of these factors may have contributed to the late appearance of reports on the morphology of the virus relative to discovery of the bovine virus diarrhoea-mucosal disease (MD) causing agent (25, 28) and to the widely disparate estimates of particle size as determined on negatively stained preparations of purified or partially purified virus. Thus, sizes ranging from 35 to more than 100 nm were reported in early descriptions (17, 18, 20, 21, 29, 30). In the description of the Pestivirus group by the International Nomenclature Committee on Togaviridae (24) a dimension of 40-60 nm was given as characteristic for the group members. As will be reported below, this size range agrees with the findings in most of the recent reports on virus morphology, although exceptions still occur (11, 33).

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Some of the discrepancies may be ascribed to different techniques of sample preparation for electron microscopy (EM), but could also be attributable to the viral envelope (see Fig. 1 and below).

![Image of virus particles](image)

**FIG. 1**

*Virus particles displaying a smooth bilaminar membrane and a dense or semi-dense core*

Granular cellular material can on some occasions be seen binding to the virus membrane. Slightly aberrant forms (b, c) may be due to distortion of the viral membrane during fixation or be a phenomenon occurring during morphogenesis.

Top left: bar = 85 nm; top right: bar = 92 nm.
Bottom left and right: bars = 80 nm

**THE VIRUS**

Based on EM examinations, the virus particles have been described as pleomorphic, mostly spherical, membrane-bound structures. In a closely related virus, hog cholera virus (HCV), the membrane was described as surrounding an isometric or hexagonal electron-dense core structure with a diameter of approximately 27-30 nm (13, 14, 20). This description also seems to apply to BVDV (20). However, in contrast to what has been described for HCV (13), surface projections on the viral membrane cannot be distinguished with certainty on BVDV, either on purified virus preparations or on putative viral particles in cells and tissues (Ritchie as quoted in ref. 34; 6, 22, 30, and author’s unpublished data; see below).
THE VIRUS IN CELL CULTURES OF NON-LYMPHOID AND LYMPHOID ORIGIN

The morphology and morphogenesis of BVDV have been described for various cell culture systems of both non-lymphoid and lymphoid origin (6, 7, 10, 15, 22). In most cases, cultures were infected with a predetermined infectious dose of cytopathic BVDV; however, findings in persistently infected ("contaminated") cell cultures (5, 22) and cell cultures infected by co-cultivation with peripheral blood mononuclear cells from persistently viraemic calves (7) have also been described.

In all cases there seems to be agreement on the morphology of the mature virus particle which is described as approximately 35-60 nm in diameter, with a smooth bilaminar membrane surrounding a dense or semi-dense core of 20-25 nm (6, 7, 10, 15, 22) (Fig. 1). Sometimes a semi-dense space between the core and the membrane can be distinguished.

When the infection was followed by sequential termination and preparation for EM of cell cultures over a period of 0 to 68 hours post-infection, the following features of the morphogenesis could be discerned (6, 7):

1. The initial step involves formation of small membranous structures, probably arising from the trans-Golgi network or the endoplasmic reticulum (9, 16). Similar structures can be seen in normal, non-infected cells, but during BVDV infection their occurrence is significantly accentuated.

2. Their appearance is followed by intermembranous accumulation of viral structural components, resulting in formation of narrow cisternae, sometimes including cytoplasmic constituents, as well as membrane-bound granules with a dense, amorphous content and an inner thickened membrane. These structures are usually found in close proximity to the Golgi region (Fig. 2).

3. De novo formation of viral particles occurs in these granules by accretion of the nucleocapsid and envelope proteins. This process may encompass de novo formation of long membranous profiles, often seen lying in parallel arrays. Whether the latter phenomenon only occurs when membranous components are in excess is still not known.

As virus maturation proceeds, the amorphous content of the granules is replaced by densely packed viral particles. Often these particles appear to retain a fuzzy coating derived from the granular material (Fig. 3). If the viral structural components are not present in equimolar amounts, the maturation process may go partly astray, resulting in the formation and accumulation of various membranous structures as well as granular material. In addition, a few mature virions are usually seen in these vesicles. The whole structure often bears a resemblance to a (auto-)phagosome and may indeed in some cases be of this nature (7).

The majority of the particles appear spherical, but occasionally a faceted outline may appear. Other slightly abnormal forms, such as particles exhibiting a translucent centre or elongation, can also be observed, especially at later stages of in vitro replication (6).

Simultaneously with the replication of cytopathic virus strains, slowly evolving degenerative changes occur and give rise to the characteristic cytopathic effect observed
FIG. 2

Electron micrograph of a BVDV-infected cell

Membrane-bound granules (arrows) with a dense, amorphous content and viral particles, 45-55 nm in diameter, seen in the vicinity of the Golgi region (G). Bar = 0.1 µm
FIG. 3

Densely packed virions in membrane-bound vesicle in cultivated cell (MDBK) infected with BVDV by co-cultivation with peripheral blood mononuclear cells from a persistently infected calf

Bar = 0.1 μm

in cell cultures at the light microscopic level. These comprise vacuolation, rounding up of the cells, membrane rupture and partial detachment of cells. However, intact virion-loaded vesicles can still be detected in necrotic cells. This most probably indicates that the majority of viral particles are released following complete cell disruption, although evidence of an exocytotic process, whereby the virus containing vacuoles fuses with the plasma membrane and expels their contents, has occasionally been observed (6). Budding of mature virus from the cell surface membrane has never been observed in any of the studies on pestiviruses reported to date (6, 7, 10, 15, 22, 27).

The maturation process, as tentatively described here, resembles the events described for the morphogenesis of flaviviruses (34) and differs significantly from that of the alpha togaviruses (19, 23). Thus, although conclusive evidence for the interpretation of the replication events is still lacking, the tentatively deduced morphogenesis of BVDV corroborates other molecular results and points to a warranted reclassification of BVDV (and other pestiviruses) under the Flaviviridae (12). A complete resolution of the ultrastructural localisation of the various steps in the morphogenesis of BVDV may have to await a better understanding of the biology of the virus, including its biochemical characteristics. The development and employment of monoclonal antibodies with specificity for the various viral proteins
and a circumvention of the inherent problems in the immuno-EM approach, should help us attain a more definite knowledge of the temporal sequence of events in BVDV replication and their subcellular localisation in the infected host cell (7).

BVDV replication and morphology have also been studied in leukocyte cultures derived from non-infected and persistently infected calves (1, 6, 7, 31, 32). In vitro infection of mononuclear leukocytes from immune or non-immune cattle appears to result in a relatively low frequency of infected cells and the duration of the infection may be short (31; author’s unpublished data). In contrast, in persistently infected calves, up to 30% of the circulating mononuclear leukocytes may be infected at any particular time (3, 8). Among these, only a few cells produce and contain retrievable infectious particles at any given time (8). However, when purified, peripheral blood mononuclear leukocytes (PBML) from such animals are co-cultivated with BVDV-free and susceptible MDBK cells, not only is the virus transmitted to the MDBK cells, but the viral replication in the PBML is also significantly enhanced (7). Using this approach all of the above morphologic and morphogenic features described for infection of continuous cell lines could be found in the PBML although to a much less conspicuous extent (6, 7).

Macrophage cultures derived from peripheral blood monocytes can be maintained under in vitro conditions for months on end. Such cultures derived from persistently infected calves continue to produce infectious virus, and viral particles of the type described above can be detected by EM throughout cultivation, although with time it may become increasingly difficult to distinguish the true viral particles from elements of multivesicular bodies and (auto-)phagosomes (Bielefeldt Ohmann and Bloch, unpublished data).

THE VIRUS IN TISSUES

Despite the widespread occurrence of BVDV antigens in tissues of persistently infected, clinically normal cattle (4), it appears to be very difficult to detect changes consistent with virus replication as seen in cell culture (see above). Mature virus particles are most easily detected in circulating mononuclear leukocytes (1, 7, 8). Productively infected cells occur and are mainly T lymphocytes, monocytes and “null cells” (i.e., a non-T-, non-B-, non-macrophage mononuclear cell subset). In these cells, BVDV-like particles, 45-55 nm in diameter, are found in small cytoplasmic vesicles (Fig. 4) (7, 8).

In animals suffering from MD, virus particles are most easily detected in the intestinal crypt epithelial cells (1, 6). Virions with a diameter of 45-55 nm can be detected in various sized membrane-bound vesicles, often embedded in a finely floccular material (Fig. 5). As was found in the cell culture systems, the virus particles apparently are released when the cell eventually disintegrates following desquamation into the crypt-lumen (Fig. 6). All stages of the putative morphogenesis, as described above, can be found in intact and in situ epithelial cells (6) although here their temporal relationship cannot be deduced.

In contrast to the evidence of widespread and substantial infection in the lymphoid tissues of animals succumbing to MD (2, 3) and in clinically normal, persistently
infected animals (4), EM observations of mature virus particles are rare. This may partly be due to the confounding problem of differentiating the true virus particles from organellar material, as described above for macrophage cultures, and partly because of a relatively low replication rate per se as indicated also by the much smaller, particle-containing vesicles compared to what can be found in the enterocytes (6; author's unpublished data) and the titre of re-isolatable infectious virus (author's unpublished data). Nevertheless, the findings in cattle are similar to those reported in tonsils from young pigs experimentally infected with HCV (26).
FIG. 5

Electron micrographs of intact intestinal crypt-epithelial cells from a calf suffering from mucosal disease

Large virion-containing vesicles are visible in some of these cells (arrows)

(a) Bar = 650 nm; (b) bar = 147 nm

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Electron micrograph of (a) desquamated crypt-epithelial cell from the ileum of a calf with mucosal disease, with (b) enlargement of a virus-containing vesicle from the same cell. Arrows indicate virus-containing vesicles. (a) Bar = 606 nm; (b) bar = 83 nm

**FIG. 6**

MICROSCOPIE ÉLECTRONIQUE DU VIRUS DE LA DIARRHÉE VIRALE BOVINE.
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**Résumé** : Le virus de la diarrhée virale bovine (BVD) a été identifié (provisoirement) par microscopie électronique dans des préparations virales purifiées, dans des cultures cellulaires infectées et dans des tissus et cellules d’animaux infectés. Ces études ont mis en évidence une particule sphérique, liée à une membrane d’un diamètre de 40 à 60 nm. La membrane, d’aspect lisse, est formée d’une bi-couche et entoure un noyau dense ou semi-dense de 20 à 25 nm. La particule du noyau peut être isométrique ou hexagonale. Les études sur la morphogenèse du virus BVD dans les cultures cellulaires infectées ont montré que l’assemblage et la maturation des particules virales s’effectuent par un processus de condensation à l’intérieur d’organelles vésiculeuses liées à une membrane dans lesquelles les virions s’accumulent par la suite. La libération du virus a lieu lorsque la cellule est finalement lysée et/ou par exocytose. Ainsi, par sa morphogenèse comme par sa morphologie, le virus BVD présente une forte ressemblance avec les Flaviviridae.
FIG. 6 (contd.)


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MICROSCOPÍA ELECTRÓNICA DEL VIRUS DE LA DIARREA VIRAL BOVINA. – H. Bielefeldt Ohmann.

Resumen: El virus de la diarrea viral bovina (BVD) ha sido identificado (provisoriamente) por microscopía electrónica en preparaciones virales purificadas, en cultivos celulares infectados y en tejidos y células de animales infectados. Estos estudios permitieron detectar una partícula esférica, ligada a una membrana de 40 nm a 60 nm de diámetro. La membrana, de aspecto liso, está formada por una capa doble y rodea un núcleo denso o semidenso de 20 nm a 25 nm. La partícula del núcleo puede ser isométrica o hexagonal.
Los estudios sobre la morfogénesis del virus BVD en cultivos celulares infectados mostraron que la reunión y maduración de las partículas virales se efectúan mediante un proceso de condensación dentro de organelas vesiculares ligadas a una membrana en las cuales se acumulan a continuación los viriones. La liberación del virus tiene lugar cuando la célula está finalmente lisada y/o por exocitosis. Así, tanto por su morfogénesis como por su morfología, el virus BVD presenta un fuerte parecido con los Flaviviridae.

PALABRAS CLAVE: Flaviviridae - Microscopía electrónica - Morfogénesis - Morfología - Partículas virales - Virus de la diarrea viral bovina.

**REFERENCES**


