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Purification and characterization of recombinant bovine viral diarrhea virus particles with epitope-tagged envelope proteins

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Bovine viral diarrhea virus (BVDV) is a member of the genus *Pestivirus* within the family *Flaviviridae*. The virions contain a lipid membrane of cellular origin and the three glycosylated viral proteins E^{rns}, E1 and E2 are supposed to be part of this. Because of the absence of an efficient purification method for BVDV, detailed studies of virus assembly and morphology do not exist at the moment. In this study, infectious BVDV with N-terminally FLAG-tagged E^{rns} or E2 proteins were generated. For efficient propagation of the virus with the tagged E^{rns} protein an insertion of an amino acid upstream of the FLAG-epitope and an amino acid substitution within the E^{rns} protein were necessary. In contrast, the virus with the tagged E2 protein was growing efficiently without further modifications. After all, the generated recombinant viruses replicated efficiently in bovine cells and showed similar growth characteristics as the parental virus. The expression of the FLAG-epitope could be shown in immunofluorescence as well as in Western blot experiments. Furthermore, we could establish a highly efficient affinity tag purification protocol for the isolation and concentration of infectious BVDV that allowed for the first time electron microscopical studies of a high number of virus particles. In the preparations with a titre of $10^{8.75}$ $TCID_{50}$ ml^{-1}, particles with a diameter of about 43 to 58 nm (mean: 48 nm) could be detected by negative contrast electron microscopy and immunogold labelling located both E^{rns} and the E2 protein at the lipid membrane of these virions.