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## Analysis of penetration mechanism of bovine herpesvirus 1 into host cells

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Envelope viruses penetrate into their host cells either by membrane fusion at neutral pH with the plasma membrane or by fusion at acidic pH with the endosomal membrane after endocytosis. Herpes simplex virus 1 is believed to penetrate through fusion with the plasma membrane at neutral pH. To provide information on the mechanism of penetration of bovine herpesvirus 1 (BHV1) into host cells, dequenching assay using octadecylrhodamine B (R18)-labeled BHV1 virion was carried out.

Fusion of viral envelope with cellular membrane was monitored by increase in fluorescence intensity of R18 as a result of relief of self-quenching. R18-labeled BHV1 was incubated with MDBK cells at 4°C for 30min. After washing, the cells were exposed to different pHs and incubated at 37°C. Increase of fluorescence intensity was found at pH 5.2 and 7.2. Preincubation of the cells with unlabeled virus as well

as antibodies against BHV1 prevented R18-labeled virus from dequenching. These findings indicate that the viral envelope fuses the plasma membrane at pH 5.2 and 7.2. Anti-gD antibodies also interfered with dequenching at pH 5.2, suggesting that BHV1 gD plays a role in the fusion of viral envelope with cellular membrane at acidic pH.

Since BHV1 showed membrane fusion at acidic pH, effect of endosomal pH raising was examined on BHV1 infection. The use of bafilomycin A, a specific inhibitor of H<sup>+</sup>-pump in the endosome, reduced yields of BHV1 by 1% at a concentration of 20nM. This finding suggests that acidic pH induces BHV1 envelope fusion in the endosome.

The present results suggest that BHV1 penetrates into the host cell by fusion with the endosomal membrane as well as by fusion with the plasma membrane.

## Detection of antibodies in the sera specific to the hemagglutinin of avian influenza viruses

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Pandemic strains of influenza A virus arise by genetic reassortment between avian and human viruses. Such reassortants are generated in the epithelial cells lining the upper respiratory tract of pigs. Seroepidemiological

studies of pigs in southern China, where new pandemic strains emerge, to detect antibodies specific to the hemagglutinin (HA) of influenza viruses should provide information on new pandemic strains.