Studies on coronaviruses causing enteric infections in domestic animals in Japan

（日本における家畜の下痢原因コロナウイルス
に関する研究）

Abstract of Doctoral Thesis

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Abstract

Coronaviruses cause a wide range of diseases in farm and domestic animals, some of which are a threat to the farming industry and give serious issue on the economy. In 2013, a huge porcine epidemic diarrhea (PED) outbreak has occurred in Japan after a period of 7 years of absence, which causes high morbidity and mortality in piglets. On the other hand, bovine torovirus (BToV) causes mild to moderate diarrhea in claves. BToV isolated from diarrheal specimens has the full length hemagglutinin-esterase (HE) gene; however, the viruses lose the HE protein as a result of mutation of the HE gene following several passages in cultured cells. In the present study, phylogenetic and antigenic characterization of newly isolated porcine epidemic diarrhea virus (PEDV) in Japan is described. Moreover, biological activity of the HE protein of BToV has been explored.

To evaluate the mechanism by which a large outbreak of PED occurred in Japan, where the majority of sows are vaccinated, we isolated two new strains of PEDV from the intestines of piglets and found that they showed greater similarity to US isolates (group II PEDV) than to the Japanese vaccine strain (group I PEDV). We compared the antigenicity of the vaccine type strain and newly isolated strains by means of neutralization test using sera from a number of pigs from various farms and showed that they are antigenically similar. This is the first report of the similarity of group I and II viruses using sera from individual pigs vaccinated with group I virus. These data suggest that the large outbreak of PED in Japan may not be attributed to inefficient vaccination but may be due to the extremely high virulence of the newly appearing viruses.

BToV, which causes diarrhea in calves, contains the HE protein on the viral envelope when isolated from the host, although HE is often lost from the virion after multiple

passages in cultured cells. This suggests that HE protein may be important for replication or pathogenesis in infected animals, but is not indispensable for the replication in cultured cells. In the present study, we explored the biological functions of the HE protein. We isolated the BToV Niigata-3 (Nig-3) from diarrheal specimen of the cattle and cloned Nig-3-3 with HE (HE+) and Nig-3-8 without HE (HE-) using human rectal tumor (HRT-18) cells and compared their growth in cultured cells. Nig-3-8 (HE-) grew more efficiently than Nig-3-3 (HE+), suggesting the possibility that HE inhibits viral growth in culture. It was found that the interferon (IFN)-α reduced the infection of HE- virus in cells, but not that of HE+ viruses, whereas IFN-β had no influence on their growth. HE protein expressed in human embryonic kidney 293T (HEK 293T) cells were examined whether HE works as IFN-α antagonist by using Sindbis virus. The infection of Sindbis virus in HEK 293T cells expressing HE was not affected by the IFN-α treatment, though the infection was depressed in cells expressing the truncated HE after treatment of IFN-α. This indicated that HE protein acts as an IFN-α antagonist. These results collectively suggest that HE plays an important role in the pathogenesis in BToV infections as an IFN antagonist to innate immunity.