



Title	PROPERTIES OF A SMALL VIRUS ASSOCIATED WITH INFECTIOUS CANINE HEPATITIS VIRUS AND ELECTRON MICROSCOPIC STUDY OF THE MULTIPLICATION OF INFECTIOUS CANINE HEPATITIS VIRUS IN CYTOPLASM
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## INFORMATION

Hokkaido University granted the degree of Master of Veterinary Medicine to the following 10 graduates of the Post-Graduate School on March 25, 1969.

The authors' summaries of their theses are as follows:

### **PROPERTIES OF A SMALL VIRUS ASSOCIATED WITH INFECTIOUS CANINE HEPATITIS VIRUS AND ELECTRON MICROSCOPIC STUDY OF THE MULTIPLICATION OF INFECTIOUS CANINE HEPATITIS VIRUS IN CYTOPLASM**

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Properties of a small virus associated with infectious canine hepatitis virus

The associated small virus (ASV) contained in infectious canine hepatitis virus (ICHV) Matsuda was studied. In the culture fluid of dog kidney cells (DKC) infected with Matsuda, the ratio of ICHV particles to ASV particles was from 1:60 to 1:70, as observed through the electron microscope. Infectivity titer of Matsuda, containing ASV, was distinctly lower than that of another ICHV, FD, which contained no ASV. ASV virus alone inoculated in DKC caused no CPE and did not multiply. Multiplication of ASV was found only when ICHV was coinfecting. From the above findings, ASV detected for the first time from ICHV, was found to be a member of adeno-associated satellite virus. Ultra thin section studies suggested that this ASV multiplied not only in the nucleus but also in the cytoplasm.

Electron microscopic study of the multiplication of infectious canine hepatitis virus in cytoplasm

Electron microscopic study of the multiplication of ICHV in DKC cultures was carried out. Resulting multiplication in the nucleus was the same as previously reported. But as described below, the multiplication in the cytoplasm also became clear for the first time. Eighteen hours after inoculation two types of formation were seen in the cytoplasm: (1) crystalline array, (2) scattered distribution. The virus particles in the cytoplasm were found to be different from the virus particles discharged from the nucleus and enclosed in the phagocytic vacuole. Therefore these virus particles were considered to have multiplied in

the cytoplasm.

Furthermore, lamella-like structures and dense spheroid structures, which were specific for ICHV infected cells, were observed.

## **STUDIES ON CULTURAL AND SEROLOGICAL PROPERTIES AND VIRULENCE OF LEPTOSPIRA**

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It has been more than 50 years since *Leptospira* appeared in microbiology, nevertheless there are many unknown problems.

The author was interested in the investigations on 1) cultivation of *Leptospira* in a chemically defined medium, 2) effect of the cultivation on leptospiral virulence, and 3) antigen analysis of *Leptospira* by precipitin reaction in gels. The results obtained were summarized as follows:

Some strains of *Leptospira* did not grow in SHENBERG's chemically defined medium. However, cultivation of these strains in the boiled serum medium easily produced the mutants, which could grow in SHENBERG's chemically defined medium. The virulence of the mutants was slightly decreased after 21 serial cultivations in the defined medium. The antigenicity of the mutants was unchanged after the same serial cultivations.

By applying precipitin reaction in gels it was found that sodium deoxycholate extract of leptospiral cells contained both genus and type specific antigens. Genus specific antigens were heat stable while type specific antigens were heat labile. It was considered that precipitin reaction in gels should be used for the classification of *Leptospira* in parallel with the agglutination test.