Comparison of the Sequential Viral Antigen Distribution between Viral Deformity Virus (VDV)-and Yellowtail Ascites Virus (YAV)-Infected Yellowtail Fingerlings Following Immersion Infection

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Viral deformity (VD) of yellowtail *Seriola quinqueradiata* fingerlings, which is caused by viral deformity virus (VDV), is characterized by abnormal swimming behavior and deformity of the body ^{1),2)}. Yellowtail ascites virus (YAV) is the causative agent of viral ascites (VA) which is characterized by marked accumulation of ascites in yellowtail fingerlings ³⁾. These viral diseases have resulted in economic loss to yellowtail culture in Japan. The two viruses are members of the Birnaviridae and are closely related in serological analysis and in reaction patterns of monoclonal antibodies against VDV or YAV ^{4),5)}. However, the comparison of the ds RNA genome segments and virus-specific polypeptides of these two viruses revealed that these viruses were different ⁶⁾. In the present study, yellowtail fingerlings were experimentally infected with VDV or YAV by immersion, and the sequential distribution of viral antigen in brain and visceral organs was studied using an immunofluorescence (IF) test.

The BN-1 strain of VDV and the YAV-1 strain of YAV were cultured in CHSE-214 cells at 20°C in minimum essential medium supplemented with 10% (V/V) fetal bovine serum and antibiotics (penicillin 100 unit/ml and streptomycin 100 µg/ml). For experimental infection with VDV, 80 yellowtail fingerlings were exposed by immersion infection to 10⁵ TCID₅₀/ml of VDV for 1 hr at 20°C. Thirty fish were kept in a 60 l tank, and used for recording the mortality pattern, and other 50 fish were kept in a separate tank and used for the detection of viral antigen and histological observations. For experimental infection with YAV, 80 fish were exposed to immersion infection with 10⁶ TCID₅₀/ml of YAV for 1 hr at 20°C. The fish were divided into groups of 30 and 50 fish and used as in the former experiment. In both experiments, 30 control fish were exposed to water containing cell culture medium without virus. The body weight of fish used in each experiment ranged 9.4–12.2 g and 1.8–4.2 g, respectively. Three fish from the VDV experiment and 2 from the YAV experiment were sampled at 0, 1, 2, 3, 4, 5, 7, 10 and 14 days post-infection (dPI). The tanks were supplied with running seawater maintained at 20°C during the experiments. From each fish, the liver, kidney, spleen, pan-

creas, and brain were collected and stored in liquid nitrogen until sectioned in a microtome-cryostat. Tissue sections of 4 µm thickness were fixed with cold acetone for 10 min, and served for the IF test using primary (anti-VDV or anti-YAV rabbit serum) and secondary antibody (fluorescein isothiocyanate-conjugated swine anti-rabbit Ig serum). The remaining portions of tissues were fixed in phosphate buffered formalin, and embedded in paraffin wax for histological examination.

In fish challenged with VDV or YAV, the mortalities occurred between 4 and 11 dPI, and between 4 and 8 dPI, respectively. The cumulative mortality was 40% in VDV infected fish and 60% in YAV infected fish. No mortality was observed in control fish in the both experiments.

Sequential detection of VDV or YAV antigen in the brain and visceral organs is shown in Table 1. In the brain of fish infected with VDV, the first detection of viral antigen was at 1 dPI. At 2 and 3 dPI, the viral antigen was demonstrated in 2 of 3 fish sampled. IF positive cells were detected in all of the fish sampled at 4 (Fig. 1), 5 and 7 dPI. Thus, viral antigen

Table 1. Detection of VDV or YAV antigen from the tissues of experimentally infected yellowtail fingerlings using an immunofluorescence test.

	Tissue	0*1	1	2	3	4	5	7	10	14	(day post infection)
VDV infected fish	Liver	0/3*2	0/3	0/3	1/3	3/3	1/3	2/3	2/3	1/3	
	Kidney	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	
	Spleen	0/3	0/3	0/3	1/3	3/3	1/3	1/3	1/3	0/3	
	Pancreas	0/3	0/3	0/3	0/3	0/3	1/3	0/3	1/3	0/3	
	Brain	0/3	2/3	2/3	2/3	3/3	3/3	3/3	2/3	3/3	
YAV infected fish	Liver	0/2	0/2	2/2	2/2	1/2	2/2	1/2	2/2	1/2	
	Kidney	0/2	0/2	2/2	0/2	0/2	0/2	0/2	0/2	0/2	
	Spleen	0/2	2/2	2/2	2/2	1/2	2/2	2/2	1/2	1/2	
	Pancreas	0/2	0/2	0/2	2/2	1/2	2/2	1/2	1/2	0/2	
	Brain	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	

*1 Tissues were collected immediately after fish were exposed by immersion.
*2 Number of immunofluorescence positive fish/number of fish tested.

Fig. 1. Brain tissue shows immunofluorescence positive reactions in an experimentally VDV infected fish at 4 dPI. Scale bar=20 µm

was continuously detected by IF test in the brain of VDV infected fish. Histologically, the brain of moribund fish exhibited congestion in the valvula cerebelli and hemorrhages in the cavity of the optic tectum (data not shown). Viral antigen was observed in a small number of pancreatic acinar cells in VDV infected fish at 5 and 10 dPI. The degeneration of pancreatic acinar cells was found in two fish, but those changes were confined to small areas. Foci of specific fluorescence occurred in the hepatic tissue of one among VDV infected fish at 3 dPI. In all fish sampled at 4 dPI, viral antigen was easily detected and occurred in extensive areas of the liver. IF positive cells were still detected in the hepatic tissue of fish sampled at 14 dPI. In the spleen, viral antigen was seen with low intensity between 3 and 10 dPI.

In contrast to VDV infected fish, no viral antigen or histological lesion was found in the brain of the fish infected with YAV throughout the experiment. But viral antigen was observed in the pancreas of YAV infected fish at 3 dPI, and continuously observed until 10 dPI. Pancreatic necrosis was also evident at 3, 4, and 5 dPI. The viral antigen was detected in the liver of YAV infected fish at 2 dPI, and the marked and broad distribution of viral antigen appeared in fish sampled at 3, 4, 5, and 7 dPI. IF positive hepatocytes were easily observed during the course of the experiment. Viral antigen was found in the spleen of YAV infected fish, but the distribution of the antigen was limited to small areas of parenchymal tissue. No or little viral antigen was detected in the kidney of VDV or YAV infected fish during the experimental period. Neither viral antigen nor histological changes were found in control fish at the beginning and end of each experiment.

In the present study, the distribution of viral antigen in the brain and pancreas was different between VDV and YAV infected fish, but similar distributions of viral antigen were found in the liver of fish infected with VDV or YAV. In the spleen of YAV infected fish, the appearance of the viral antigens were earlier than those of VDV infected fish. However, the antigens were observed in restricted small area of splenic tissue of YAV or VDV infected fish during the experiment. The appearance of VDV antigens in the brain occurred at early stage of infection

(1dPI) and continued throughout the experimental period. In contrast, YAV antigen was not detected in the brain throughout the experiment. In the histopathological study, the fish intraperitoneally injected with VDV showed congestion and hemorrhage in the brain²⁾, while no change was found in the brain of YAV infected fish⁷⁾. Histological observations of the brain in the present study were consistent with these studies. These results suggest that VDV has a specific tissue tropism for the brain. Therefore the abnormal swimming behavior and deformity in fish with VDV infection may be caused by the lesions in the central nervous system. In the pancreas of YAV infected fish, there was marked concentration of viral antigen, although viral antigen was scarcely observed at this site in fish infected with VDV. Histological features in the pancreas of the fish experimentally infected with YAV in the present study agree with those reported by Egusa and Sorimachi (1986). However, how these changes related to the ascites accumulation requires further study.

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