AN ANTIGENIC COMPARISON BETWEEN INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (OSV STRAIN) AND THE VIRUS OF HAEMORRHAGIC SEPTICAEMIA OF RAINBOW TROUT (Salmo gairdneri) (DENMARK STRAIN) BY CROSS NEUTRALIZATION 1

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AN ANTIGENIC COMPARISON BETWEEN INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (OSV STRAIN) AND THE VIRUS OF HAEMORRHAGIC SEPTICAEMIA OF RAINBOW TROUT *(Salmo gairdneri)* (DENMARK STRAIN) BY CROSS NEUTRALIZATION[1]

P. E. McALLISTER, J. L. FRYER and K. S. PILCHER

Abstract: The infectious hematopoietic necrosis virus (OSV strain) and the virus of haemorrhagic septicaemia of rainbow trout *(Salmo gairdneri)* (Denmark strain) were examined for possible antigenic relationship by plaque neutralization using homologous and heterologous antisera. No neutralization of either virus was observed on exposure to heterologous antiserum. This indicates that there is no antigenic relationship between these two viruses as determined by this method.

INTRODUCTION

To date four viruses having a bullet shaped morphology have been isolated from salmonid fishes. These are infectious hematopoietic necrosis virus (IHNV), Oregon sockeye salmon virus (OSV), Sacramento River chinook disease virus (SRCV), and the virus of haemorrhagic septicaemia of rainbow trout *(Salmo gairdneri)* (VHS) (Egtved virus). Investigation of the antigenic relationships between IHNV, OSV, and SRCV demonstrated that the first two were indistinguishable and the third was closely related.[1] This evidence and the observation that these three viruses are apparently morphologically identical[1,2] seems to indicate that they are strains of the same viral agent.

The possibility of an antigenic relationship between IHNV and VHS has not been adequately investigated. Preliminary investigations employing neutralization and fluorescent antibody tests using VHS antiserum have indicated that no close serological relationship could be demonstrated between IHNV and VHS.[4] The experiments reported here were designed to detect a possible antigenic relationship between IHNV (OSV strain) and VHS (Denmark strain) using the cross plaque neutralization assay technique with homologous and heterologous antisera.

MATERIALS AND METHODS

Viruses and cell cultures

The IHNV (OSV strain) was isolated in 1958 by one of us (J.L.F.) during a natural epizootic among young sockeye salmon at the Willamette River hatchery in Oregon. Since the time of isolation, this virus has been propagated in salmonid cell cultures. The virus used in the experiments reported here was plaque purified, propagated, and assayed in the CHSE-214 cell line which was derived from chinook salmon *(Oncorhynchus tshawytscha)* embryos.

The VHS (Denmark strain) was kindly provided by Dr. K. E. Wolf of the Eastern Fish Disease Laboratory, Kearneysville, West Virginia, U.S.A. This virus was propagated and assayed in the STE-137 cell line derived from steelhead trout *(Salmo gairdneri)* embryos.

[1] From the Department of Microbiology, Oregon State University, Corvallis, Oregon 97331, U.S.A.
Oregon Agricultural Experiment Station, Technical Paper No. 3599.

Both cell lines were initiated by the method of Fryer[3] and are heteroploid. Cell cultures were maintained and virus propagated as previously described.[6]

Preparation of antisera

Antisera for each virus was prepared by injecting rabbits with concentrated virus, which was titrated to insure that the virus content was in excess of 1 x 10^9 plaque forming units (pfu) per ml.

Two rabbits for each virus were injected according to the following schedule. For the first injection series, each rabbit was injected intravenously with 1.0 ml of undiluted virus concentrate and intramuscularly with two 1.0 ml injections of a sterile emulsion consisting of equal volumes of virus concentrate and Complete Freund's adjucant. At 2 and 4 weeks after the first series of injections, each rabbit received two 1.0 ml intramuscular injections prepared as described above. Rabbits were bled at 10 and 17 days after the last injection.[6]

Plaque neutralization assay

Immediately before each neutralization test, a sample of each antiserum was heated at 56 C for 30 minutes. Normal rabbit serum was treated in the same manner.

Each antiserum was tested for virus-neutralizing activity against homologous and heterologous virus. Five 2-fold antiserum dilutions were made which were known to cause between 0 and 100% plaque neutralization of the homologous virus. Equal volumes of diluted standardized virus stock containing 100 pfu/0.15 ml and diluted antiserum were mixed and incubated at 18 C for 1 hour. At the end of this time interval, each virus-antibody mixture was assayed for unneutralized virus using the monolayer plaque assay technique.[6] Normal rabbit serum was used as a control. The dilution of the normal rabbit serum was the same as the lowest dilution of the antiserum being tested. The percent plaque neutralization for each antiserum dilution was calculated. The 50% end point was estimated by the method of Reed and Meunch.[7]

RESULTS AND DISCUSSION

The results of plaque neutralization experiments with IHNV (OSV strain) antiserum and with VHS (Denmark strain) antiserum are shown in Table 1.

TABLE 1. Plaque neutralization of IHNV and VHS by their homologous and heterologous antisera.

Reciprocal of serum dilution	Percent plaque reduction [1]	
	IHNV	VHS
IHNV antiserum		
100	99[2]	0
200	71	0
400	0	0
VHS antiserum		
100	0	73[3]
200	0	53
400	0	23
Normal serum		
100	0	0

[1] Figures in the table represent mean values from 2 experiments.
[2] Interpolated serum dilution giving 50% plaque reduction is 1:245.
[3] Interpolated serum dilution giving 50% plaque reduction is 1:215.

No neutralization of IHNV or VHS was observed on exposure to heterologous antiserum. These results indicate that there is no antigenic relationship between these two viruses as determined by this method. With the homologous antiserum, a 50% plaque reduction with IHNV was estimated by interpolation at a serum dilution of 1:245.[7] For VHS the comparable dilution of homologous antiserum was 1:212.

The evidence presented here indicating that the two viruses are not serologically related accentuates the differences observed in other *in vitro* investigations. Thin section electron micrographs have indicated that IHNV (OSV strain) has dimensions of 170 to 188 nm in length by 70 nm in diameter[2] (McAllister, unpublished data) while VHS (Denmark strain F_1) has average dimensions of 240 nm in length by 75 nm in diameter.[5] Optimum growth with highest virus yields occurred at 18 C with IHNV,[8] but at 14 C with VHS.[5]

Differences in *in vivo* host range and susceptibility have been noted, but investigations are not complete.[9]

All of these differences together with the evidence reported here clearly indicate that these two viruses are separate and unrelated agents.

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