

INFECTIOUS HEMATOPOIETIC NECROSIS OF RAINBOW TROUT IN MONTANA: A CASE REPORT

Authors: HOLWAY, JAMIESON E., and SMITH, CHARLIE E.

Source: Journal of Wildlife Diseases, 9(4) : 287-290

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-9.4.287>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

INFECTIOUS HEMATOPOIETIC NECROSIS OF RAINBOW TROUT IN MONTANA: A CASE REPORT

JAMIESON E. HOLWAY¹ and CHARLIE E. SMITH, Hatchery Biologists,
U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife
Fish Cultural Development Center, Bozeman, Montana 59715 U.S.A.

Abstract: Infectious hematopoietic necrosis was diagnosed in rainbow fingerlings (*Salmo gairdneri kamloops*) during an epizootic at a hatchery in Montana. Microscopic pathology and effects of the causative virus in cell culture are described.

INTRODUCTION

Infectious hematopoietic necrosis (IHN) was first diagnosed in 1967 in fingerling rainbow trout (*Salmo gairdneri*) at a Canadian hatchery.² The disease has since been diagnosed in rainbow trout at several locations within the United States³ (Amend, personal communication).²

Extensive mortality in kamloops trout (*S. gairdneri kamloops*) fingerlings at a hatchery in Montana prompted histological and virological examination of moribund fish. The high mortality, the extensive histopathological changes, and the absence of any microscopic pathogen suggested viral etiology. Repeated tests for infectious pancreatic necrosis (IPN) virus in cell cultures incubated at 20C were negative. A virus was isolated at 10C and the epizootic was subsequently diagnosed as IHN. The purpose of this report is to describe the epizootic and to document the occurrence of IHN in the State of Montana.

MATERIALS AND METHODS

Moribund fingerling kamloops trout showing signs typical of IHN were selected from fish in the epizootic. Blood smears and imprints of hematopoietic kidney tissue were prepared and stained by the methods of Leishman and

Giemsa.⁷ It was impossible to determine packed erythrocyte volume owing to the small size of the fish.

Fish showing clinical signs of the disease were preserved in Bouin's solution for 24 hours, then transferred to 65% ethanol. Tissue samples were embedded in parafin, sections at 6 μ and stained with hematoxylin and eosin.

Moribund fry were placed in Hank's balanced salt solution and held at 1 C for 2-3 hours until homogenized. Homogenates were filtered through a 0.45 μ pore size membrane filter into monolayer tube cultures of rainbow trout gonad (RTG-2) and fathead minnow (FHM) cells and incubated at 10 C.²

A viral agent was suggested by the progressive cytopathic effect (CPE) noted in tissue culture. To test the infectivity of the virus, ten rainbow trout fingerlings averaging 7.6 cm in length were placed in 2 liters of water containing 5 ml of first passage culture medium. Fish were left in this solution for half an hour and then placed in running water at 11 C.

The following tests were performed to establish some basic characteristics of the virus. Sensitivity to a lipid solvent was tested by exposing the virus to 10% chloroform, with shaking at 15-minute intervals for 2 hours, and subsequent inoculation into RTG-2 cell cultures after

¹ Present address — Hagerman NFH, Idaho 83332, U.S.A.

² Amend, D. F., Western Fish Disease Laboratory, Sand Point Naval Air Station, Bldg. 204, Seattle, Washington 98115.

2 and 48 hours. Glycerin stability was determined in the same manner. Homogenates of internal organs were filtered through a 0.05 μ pore size membrane filter into RTG-2 cell cultures. Temperature sensitivity was determined at 21 C and 10 C. FHM and brown bullhead (BB) cell lines were inoculated with the virus and neutralization tests were performed in which the virus was incubated with antiserum prepared in separate rabbits against IPN and IHN virus and then inoculated into monolayers of RTG-2 cells. Except as indicated, all tests were carried out at 10 C.

RESULTS

Signs of Disease

Grossly infected fish had anemia, lethargy, distended abdomens, exophthalmia, and petechiae. These signs are typical of those described for IHN.^{1,6,10}

Hematology

Stained smears of peripheral blood from diseased fish sampled during the epizootic revealed many abnormalities. Many erythrocytes were immature, they varied in size and shape and several showed cytoplasmic vacuolation. Erythrocyte nuclei also varied in size and shape; some were round and pyknotic, others were swollen and irregular.

The most distinct and consistent changes in blood cell morphology were: bilobed erythrocytes, large numbers of circulating macrophages, many of which contained ingested cellular debris, and degenerating cells and debris circulating freely within the blood.

Microscopic Pathology

With the exception of the degenerative lesions observed in the brain, choroid of the eye and gills, the histopathological changes were the same as those described by others.^{2,6,10} The most consistent and severe lesion was the massive necrosis of hematopoietic tissue of kidney and spleen.

Cell Culture

In the primary isolates, CPE was first noted in RTG-2 cells on the 5th day. The CPE which occurred in focal areas of the cell sheet was typical of IHN.² As the entire cell sheet became infected, the rounded cells sloughed. This CPE was distinctly different from the stringy appearance of that caused by IPN virus.

Rainbow trout fingerlings that were exposed to the virus began to die within 1 week and a viral agent, causing identical CPE in RTG-2 cells, was reisolated. Ten more fish were treated in the same manner and again the virus was isolated in RTG-2 cells.

The results of the laboratory tests in cell cultures are presented in Table 1. The virus was inactivated after 2 hours exposure to 10% chloroform and by exposure to 50% glycerin for 48 hours but not 2 hours. It did not pass a 0.05 μ Millipore filter. It did not grow at 21 C but replicated at 10 C. It replicated poorly in both FHM and BB cell lines. Viral effects in FHM and BB cells were limited to a few focal areas which did not progress. The virus was not neutralized by anti-IPN rabbit serum but was neutralized by anti-IHN rabbit serum.

DISCUSSION

A distinct characteristic of IHN virus is its temperature relationship.¹ In the Montana epizootic, as in other cases (Amend and McDaniel, personal communication),³ the outbreaks occur at water temperatures of 10 C or below. Clinical signs and mortality stopped when the fish with which we were working were moved to 15 C water.

The severe anemia observed in moribund fish was undoubtedly the result of the destruction of hematopoietic tissue.

Increased numbers of macrophages and bilobed erythrocytes were observed consistently in blood and kidney films. This is similar to characteristics of a

³ Dave McDaniel, Springville National Fish Hatchery, Springville, Utah.

TABLE 1. Effects of Laboratory Tests on IHN Virus in Cell Culture.

Test	Test Condition	Sensitivity in RTG-2 Cell Culture	
		IPN	IHN
10% Chloroform	2 hours' exposure	NS	S
	48 hours' exposure	NS	S
50% Glycerin	2 hours' exposure	NS	NS
	48 hours' exposure	NS	S
Passage through	.05 μ diameter	NS	S
Millipore filter	.45 μ diameter	NS	NS
Temperature	21 C	NS	S
	10 C	NS	NS
Serum Neutralization	IPN Antisera	S	NS
	IHN Antisera ^[4]	NS	S

S = Sensitive

NS = Non-Sensitive

virus-like disease of sockeye salmon (*Onchorynchus nerka*) reported by Watson et al.⁸ and Wood and Yasutake⁹ which was probably caused by IHN virus.

The two most distinguishing characteristics of IHN in cell culture are: the distinct CPE and neutralization by IHN antisera but not by IPN antiserum. Contrary to findings of Amend et al.,² in which he isolated the virus both in RTG-2 and FHM cells, we did not observe consistent CPE in FHM cells. However, it has been noted at several laboratories that FHM cells may become refractive to IPN, IHN, and tadpole edema viruses (Wolf, personal communication).^[5] It is quite possible that we were working with FHM cells that were refractory to the IHN virus. Preservation of suspect samples in 50% glycerin should be avoided since IHN virus is destroyed by this method.

Presumptive evidence suggested that the virus was transmitted with the eggs

to the Montana hatchery since eggs were obtained from only one source.

At this time we must assume that survivors of the epizootic are carriers of the virus, as are fish surviving IPN. We know that clinical signs disappeared when the temperature was raised but we did not have an opportunity to inspect these fish again. However, we were not able to isolate the virus from the adult fish held in the effluent water from the infected fry.

Amend and Chambers⁸ reported that morphologically there was no significant difference between IHN, Oregon sockeye disease (OSD) and Sacramento River chinook disease (SRCD) viruses. They suggested that the three may be closely related or perhaps separate isolates of the same virus. Other investigators^{4,5,11} were able to artificially infect rainbow trout with OSD and SRCD viruses. This too supports the postulated close or identical relationship of the viruses.

[4] This test was performed at a later date when IHN antisera became available.

[5] Dr. Ken Wolf, Eastern Fish Disease Laboratory, Leesville, W. Va.

Acknowledgements

The authors wish to thank Dr. Ken Wolf and Mr. Don Amend for reviewing the manuscript, and Mr. W. T. Yasutake for his helpful suggestions.

LITERATURE CITED

1. AMEND, D. F. 1970. Control of Infectious Hematopoietic Necrosis (IHN) virus disease by elevating the water temperature. J. Fish. Res. Bd. Canada 27:265-270
2. AMEND, D. F., W. T. YASUTAKE and R. MEAD. 1969. A hematopoietic virus disease of rainbow trout and sockeye salmon. Trans. Amer. Fish Soc. 98:796-804.
3. AMEND, D. F. and V. C. CHAMBERS. 1970. The morphology of certain viruses of salmonid fishes. I. *In Vitro* studies of some viruses causing hematopoietic necrosis. J. Fish. Res. Bd. Canada 27:1285-1293.
4. KLONTZ, G. W., W. T. YASUTAKE and T. J. PARISOT. 1965. Virus diseases of the salmonids of western United States. III. Immunopathological aspects. Ann. N.Y. Acad. Sci. 126, Article 1:531-542.
5. PARISOT, T. J., W. T. YASUTAKE and G. W. KLONTZ. 1965. Virus diseases of the Salmonidae in western United States. I. Etiology and epizootiology. Ann. N.Y. Acad. Sci. 126, Article 1:502-519.
6. PLUMB, J. 1972. A virus-caused epizootic of rainbow trout (*Salmo gairdneri*) in Minnesota. Trans. Amer. Fish Soc. 101:121-123.
7. SMITH, CHARLIE E. 1967. Hematological changes in coho salmon fed a folic acid deficient diet. J. Fish. Res. Bd. Canada 25:151-156.
8. WATSON, M. E., R. W. GUENTHER and R. D. ROYCE. 1956. Hematology of healthy and virus-diseased sockeye salmon. Zoologica 41:27-38.
9. WOOD, E. M. and W. T. YASUTAKE. 1956. Histopathological changes of a virus disease of sockeye salmon. Trans. Amer. Micro. Soc. 75:85-90.
10. YASUTAKE, W. T. 1970. Comparative histopathology of epizootic salmonid virus diseases. In: *A Symposium on Diseases of Fish and Shellfishes*. S. F. Snieszko, editor. Amer. Fish. Soc., Wash., D.C. Special Pub. No. 5, 100: 341-350.
11. YASUTAKE, W. T., T. J. PARISOT and G. W. KLONTZ. 1956. Virus disease of the Salmonidae in western United States. II. Aspects of pathogenesis. Ann. N.Y. Acad. Sci. 126, Article 1: 520-530.

Received for publication 11 November 1972