NEUTRALIZATION TESTS OF INFECTIOUS PANCREATIC NECROSIS VIRUS WITH POLYVALENT ANTISERUM

Author: LIENTZ, JOSEPH C.

Source: Journal of Wildlife Diseases, 9(2) : 120-124

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-9.2.120

NEUTRALIZATION TESTS OF INFECTIOUS PANCREATIC NECROSIS VIRUS WITH POLYVALENT ANTISERUM

JOSEPH C. LIENTZ[1], U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife Fish Cultural Development Center, Bozeman, Montana, U.S.A. and

JERRY E. SPRINGER[1], Willow Beach National Fish Hatchery, Willow Beach, Arizona, U.S.A.

Abstract: Forty-two isolates of infectious pancreatic necrosis virus were cloned by plaquing, and the clones were used in neutralization tests with a 5-strain polyvalent serum. Seventy-six percent of the strains tested were completely neutralized, 19 percent were partially neutralized and 5 percent were not neutralized. Data provide a measure of the antiserum's diagnostic effectiveness and indicate that at least one more antigenic type should be included in preparation of a definitive polyvalent serum.

INTRODUCTION

Serum neutralization tests provide the most specific identification of virus. Following isolation of Infectious Pancreatic Necrosis Virus (IPNV) it became apparent that there were multiple serologic types; to this day the number is uncertain.

In North America, documentation of serologic diversity is to be found in the reports of Malsberger and Cerini[5] Wolf et al.[7] and MacKelvie and Artsob.[4] Frantsi and Savan[1] showed that two IPNV strains differed in virulence depending upon temperature. In Europe, serologic diversity has been reported for IPNV by Jorgensen and Kehlet.[3] Sano[10] showed that IPNV isolates from Japan were serologically similar to certain North American strains.

International control of fish diseases depends on isolation and identification of the causal agents as well as transmission and prevention. At present, fish health surveillance depends on rigid isolation of viruses if present, and upon prompt serologic identification of such agents. A single serum preparation capable of neutralizing all serotypes of IPNV is a needed diagnostic tool for biologists and others responsible for fish health.

The purpose of our study was to test a polyvalent antiserum against representative isolates of IPN virus from various parts of the world to determine if the product qualified as a definitive antiserum capable of neutralizing any IPN virus strain.

MATERIALS AND METHODS

IPNV strains were obtained at the Eastern Fish Disease Laboratory where diagnostic material has been collected since 1955. Ampoules of crude virus at —80 C were thawed and plaqued under a two-phase (gel-liquid) nutrient overlay using procedures of Moss and Gravell[6] as modified by Wolf and Darlington.[9] All viruses tested for neutralizing effects by the polyvalent antiserum were plaque purified by cloning. The viruses used as antigens for the polyvalent antiserum production were also plaque purified by cloning. Plaques were picked, transferred to fresh cultures and used for growing test stocks. Infectivity of these stocks was stabilized by adjusting the pH to acidity with an excess of CO_2.

Cell Cultures

RTG-2 cells in passage 64 to 73 were grown at 20 C in Eagle's minimal essen-

[1] Authors were trainees at the Eastern Fish Disease Laboratory where the work was completed under the direction of Dr. Ken Wolf, Virologist.

tial medium (Earl's salt solution) plus 10 percent fetal bovine serum, 100 IU penicillin, 10 μg streptomycin and 25 IU nystatin per ml.

Antiserum

The production of IPNV antiserum in rabbits has been reported by Wolf and Quimby.[8] Cloned plaque purified virus isolates used as antigens had the following geographic origins:

1. American Type Culture Collection VR #299
2. Buhl, Idaho, U.S.A.
3. Powder Mill, New Hampshire, U.S.A.
4. Bonnamy, France
5. d'Honnincthun, France

Antiserum was prepared by injecting separate rabbits with each antigen. The antisera were each titrated and then the sera were pooled to make the polyvalent product. Against homologous virus the titers of antisera used to make the polyvalent serum was as follows:

Antiserum	Dilution	% Plaque Reduction	Plaque Forming Units (PFU) in Test
VR #299	1:16,384	86	58
Powder Mill	1:2,048	94	37
Buhl, Idaho	1:8,192	77	64
	(50% endpoint dilutions)		
			$TCID_{50}$
Bonnamy	1:363		793
d'Honnincthun	1:3,467		32

Neutralization Test

The neutralization test employed constant amounts of serum with standard dilutions of virus. Normal rabbit serum was diluted 1:1000 and used as a control. Preliminary work showed that the polyvalent antiserum could be used effectively at a dilution of 1:1000, but it was also tested at dilutions of 1:2500 and 1:5000.

The nominal yield of IPNV in RTG-2 cells at 20 C is about $10^{8.5}$ tissue culture infectious doses — 50 percent endpoint ($TCID_{50}$) per ml. Some strains yield more and others less. The $10^{8.5}$ $TCID_{50}$ was a predicted estimate useable for any IPNV strain. Accordingly, dilutions of $10^{-4.5}$ were expected to contain about 1000 $TCID_{50}$, and a $10^{-5.5}$ dilution to contain about 100 $TCID_{50}$. In order to quantify virus used in the tests, dilutions of $10^{-4.5}$ through $10^{-8.5}$ were prepared and reacted with normal rabbit serum diluted 1:1000. Neutralization tests are usually carried out with standard amounts of virus—100 to 200 $TCID_{50}$. Virus dilutions of $10^{-4.5}$ and $10^{-5.5}$ were each reacted with polyvalent antiserum at 1:1000, 1:2500 and 1:5000.

Equal volumes of diluted virus and serum were reacted for 15 minutes at 20 C after which 0.2 ml of each mixture was inoculated into each of three replicate tube cultures. Replicate cultures remained uninoculated as controls.

Wolf et al.[7] tested VR #299 and found that all the virus had been neutralized within 5 minutes. Against VR #299, homologous antiserum left no non-neutralizable persistent fraction. A 15 minute reaction time was selected as it would safely neutralize the IPNV isolates to be tested.

Eagle's minimal essential medium plus 2 percent fetal bovine serum and antibiotics was used as virus and serum diluent throughout.

Cultures were incubated at 20 C for a minimum of 5 days, examined microscopically, scored for cytopathology or its absence and then discarded. Fifty percent end points were calculated.

Not all virus isolates contained the desired 100 $TCID_{50}$. The loss of titer was due to freezing and thawing of isolates.

TABLE 1. Response of Infectious Pancreatic Necrosis (IPN) Virus Strains to Neutralization by a Polyvalent Antiserum[1].

Designation of Virus Isolate	Quantity of Virus Tested[2] Desired Quantity— 100 $TCID_{50}$	Degree of Neutralization[3],[4]	Remarks
North America			
United States			
Arizona, Page Springs	63	C	P @ 1:2500
Arkansas, Norfork	32	C	P @ 1:2500
Georgia, Chatahoochee	63	P	
Idaho, Buhl	63	P	
Grace	32	C	
Pocatello	16	C	C @ 1:2500 P @ 1:5000
Maine, Dead River	63	C	
Dry Mill	159	C	
West Buxton	32	None	
Massachusetts, Gilbert	32	C	
Michigan, Wolf Creek	316	C	
Nevada, Reno	316	None	
New Hampshire, Berlin	63	P	
Powder Mill	63	C	
New Jersey, Hackettstown	159	C	
New York, Adirondack	316	C	
"Conservation Dept."	159	P	
Cortland	63	C	P @ 1:2500
North Carolina, Pisgah Forest	32	C	C @ 1:2500 & 1:5000
Pennsylvania, Bellefonte	159	P	
Bethlehem	32	P	
Lamar	159	C	
Lamar (coho)	32	C	C @ 1:2500
Rhode Island, Providence	32	P	
South Carolina, Walhalla	32	C	
South Dakota, Gavins Point	6	C	
Tennessee, Erwin	159	C	
Utah, Paradise	316	C	
Virginia, Cousey Springs	6	C	P @ 1:2500 & 1:5000
West Virginia, Leetown	32	C	
VR #299	158	C	
Wisconsin, Langlade	16	C	
Canada, Coaticook	316	P	
Gaspé	316	C	
Madaleine	6	C	
South Esk	159	C	
Tadoussac	63	C	

Europe			
Denmark #1	32	C	
France, Bonnamy	<1	C	
Kerlo	16	C	C @ 1:2500 & 1:5000
d'Honnincthum	2	C	P @ 1:2500 & 1:5000
Great Britain	3	C	C @ 1:2500 & 1:5000
42/4059 = 1c	97		

[1] Polyvalent antiserum comprised of neutralizing antibody produced in rabbits against VR #299; Buhl, Idaho Powder Mill, New Hempshire; Bonnamy, France; and d'Honnincthum, France, strains of IPN virus.

[2] Tissue Culture Infectious Doses — 50% endpoint ($TCID_{50}$)

[3] Antiserum diluted 1:1000

[4] C = complete, P = partial

RESULTS

Forty-two virus isolates representing 37 locations in North America and five in Europe were tested. The mean value of infectivity of virus at the $10^{-5.5}$ dilution was 97 $TCID_{50}$ . . . very close to the desired concentration. Test results of that virus dilution mixed with polyvalent antiserum at 1:1000 showed that 32 isolates (76 percent) were completely neutralized and that eight isolates (19 percent) were partially neutralized (Table 1). Only two IPNV strains, 5 percent of the total, were not neutralized. Polyvalent antiserum at a dilution of 1:2500 neutralized part or all the infectivity of only about one-fourth of the isolates tested and when diluted to 1:5000, effects could be observed on only six isolates (about 14 percent).

DISCUSSION

The results show that as presently constituted, the polyvalent anti-IPN serum will probably completely neutralize a standard quantity (about 100 $TCID_{50}$) of three-fourths of the isolates encountered and that it will probably have a readily detected neutralization of most other isolates. Although comparative testing was not carried out, this probably exceeds the capability of any monovalent antiserum.

The results also indicate that the West Buxton, Maine and Reno, Nevada isolates are likely candidates for inclusion as antigens for production of a definitive polyvalent antiserum which will completely suppress a standard quantity of any IPNV encountered.

LITERATURE CITED

1. FRANTSI, C., and M. SAVAN. 1971. Infectious pancreatic necrosis virus — temperature and age factors in mortality. J. Wildl. Dis. 7: 249-255.
2. JORGENSEN, P. V., and P. C. GRAUBALLE. 1971. Problems in the serological typing of IPN virus. Acta Vet. Scand. 12: 145-147.
3. JORGENSEN, P. E. V., and N. P. KEHLET. 1971. Infectious pancreatic necrosis (IPN) viruses in Danish rainbow trout; their serological and pathogenic properties. Nordisk Vet.-Med. 23: 568-575.
4. MacKELVIE, R. M., and H. ARTSOB. 1969. Infectious pancreatic necrosis virus in young salmonids of the Canadian Maritime Provinces. J. Fish. Res. Bd. Canada. 26: 3259-3262.
5. MALSBERGER, R. G., and C. P. CERINI. 1963. Characteristics of infectious pancreatic necrosis virus. J. Bact. 86: 1283-1287.

6. MOSS, L. H. III, and M. GRAVELL. 1969. Ultrastructure and sequential development of infectious pancreatic necrosis virus. J. Virol. 3: 52-58.
7. WOLF, K., G. L. BULLOCK, C. E. DUNBAR, and M. C. QUIMBY. 1969. (In) Progress in Sport Fishery Research 1968. U.S. Dept. Int., Bur. Sport Fish. and Wildl., pp. 136-141.
8. WOLF, K., and M. C. QUIMBY. 1971. Salmonid viruses: infectious pancreatic necrosis virus: morphology, pathology and serology of first European isolations. Arch. für die gesamte Virusforschung 34: 144-156.
9. WOLF, K., and R. W. DARLINGTON. 1971. Channel catfish virus: a new herpesvirus of ictalurid fish. J. Virol. 8: 525-533.
10. SANO, T. 1971. Studies on viral diseases of Japanese fishes. I. Infectious pancreatic necrosis of rainbow trout: first isolation from epizootics in Japan. Bull. Jap. Soc. Sci. Fisheries 37: 495-498.

Received for publication May 22, 1972