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FISH VIRUSES: SEROLOGIC COMPARISON OF THE GOLDEN SHINER AND INFECTIOUS PANCREATIC NECROSIS VIRUSES

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Abstract: High titer neutralizing antiserum to golden shiner virus (GSV) was produced in a rabbit. The GSV antiserum had significant neutralization indices in tests with the original strain of GSV and with another more recent isolate. Neutralization indices were not significant when two strains of infectious pancreatic necrosis virus (IPNV) were reacted with GSV antiserum, and neither isolate of GSV showed significant reactivity in neutralization tests with polyvalent IPNV antiserum. Our results indicate that IPNV and GSV are serologically unrelated.

INTRODUCTION

The recently discovered golden shiner virus (GSV)[5,8] shares many morphological, biochemical and biophysical characteristics with infectious pancreatic necrosis virus (IPNV).[1,4,9] Extensive research on the serology of IPNV has been conducted; several strains have been identified and all show some degree of cross-reactivity. To cope with the diversity of the IPNV strains, a 5-strain polyvalent antiserum was developed and subsequently found to neutralize 95% of 42 isolates that were tested.[3] The polyvalent IPNV antiserum that we used incorporated two additional strains of the virus and neutralized all of the known isolates (K. Wolf, pers. commun.). The purpose of our study was to determine whether or not IPNV and GSV were serologically related.

MATERIALS AND METHODS

Virus Production

Growth medium was Eagle's minimum essential medium (MEM) plus 10% fetal bovine serum, 100 IU/ml penicillin, 100 ug/ml streptomycin and 50 ug/ml gentamicin. The MEM was drained from two Blake bottles of FHM cells and each culture was inoculated with 1.5×10^6 tissue culture infectious doses— 50% end point ($TCID_{50}$) of GSV II (Southeastern Cooperative Fish Disease Laboratory case # AL-77-131) passage 6. Virus was absorbed for 30 min at 30 C, then maintenance medium (MEM plus 2% fetal bovine serum and antibiotics) was added. Cell sheets showed massive viral effects within 48 h at 30 C and virus was harvested. The cell sheet was removed, triturated, resuspended in MEM-2 and clarified by centrifugation at 13,500 g for 20 min at 24 C. The supernatant fluid was collected and centrifuged at 109,000 g for 4 h at 4 C. The resulting pellet was suspended in one thirtieth the original volume of Hanks' balanced saline solution (HBSS). Virus was titrated in microculture plates[2] prior to use as the antigen.

GSV Antiserum Production

Preimmunization blood was drawn from a rabbit and screened for GSV neutralizing antibody. Untreated virus was given intravenously (IV), but it was homogenized with an equal volume of Freund's complete adjuvant for intramuscular (IM) injections. Initial in-

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TABLE 1. Results of a serological comparison of golden shiner and infectious pancreatic necrosis viruses.

Virus	Neutralization Index	
	Anti-GSV II	Polyvalent Anti-IPNV
Golden Shiner Virus I	100,000	1
Golden Shiner Virus II	63,000	0
Infectious Pancreatic Necrosis Virus		
Strain West Buxton	0.2	10,000
Strain VR#299	0	630,000

oculation consisted of 0.2 ml given IV and 0.4 ml administered IM. Subsequent IV injections were given at 4, 8, 12 and 16 days. On the 20th day, the rabbit received final injections of 0.2 ml IV and 0.4 ml IM. It was bled out 13 days later and the serum frozen.

Viruses

Two strains of IPNV (VR#299 and West Buxton[2]) were propagated in RTG-2 cells grown in MEM-10 at 20 C. At 5 days, when CPE was extensive, virus was harvested and clarified by filtration through a 0.45 μm membrane.

Two GSV isolates from the Southeastern Cooperative Fish Disease Laboratory (Arkansas 77-4 (GSV I) and Alabama 77-131 (GSV II)) were propagated in FHM cells grown at 30 C. Virus was harvested after 2 days and clarified by filtration through a 0.45 μm membrane.

Serum Neutralization

The anti-GSV and polyvalent anti-IPNV sera[3] were tested for neutralizing activity against the four virus isolates. We used the alpha procedure, described by Rovozzo and Burke.[6]

The IPNV and serum mixtures were incubated for 30 min at 20 C, but the GSV and serum mixtures were incubated for 30 min at 30 C. Titrations of reaction mixtures were carried out in microculture plates (Linbro) using a procedure employing simultaneous seeding of susceptible cells (FHM for GSV and RTG-2 for IPNV).

Microculture plates of RTG-2 cells were incubated for 5 days at 20 C and FHM cells for 2 days at 30 C. Cultures were then fixed with formalin and stained with crystal violet. Plates were scored and the titers calculated.[7] Neutralization indices (NI) were calculated for each virus-serum mixture according to Rovozzo and Burke.[6]

RESULTS AND DISCUSSION

Cross-neutralization tests showed no evidence of a serologic relationship between the golden shiner virus and two strains of infectious pancreatic necrosis virus (Table 1). The GSV anti-serum had high neutralization indices to both isolates of GSV. The original or homologous isolate had a slightly lower NI than the heterologous isolate, 63,000 and 100,000, respectively. However, when reacted against the GSV antiserum, the two IPNV strains had NI of less than 10 and were therefore not significant.

[2] IPNV isolates were provided by Dr. Bruce Nicholson, Department of Microbiology, University of Maine at Orono.

[3] Obtained from the National Fish Health Research Laboratory, Kearneysville, West Virginia.

The polyvalent IPNV antiserum had highly significant NI when reacted with the two component strains of IPNV. The VR#299 IPNV strain had a NI of 630,000 and the West Buxton isolate had a NI of 10,000. In contrast, the polyvalent IPNV antiserum did not have a significant NI with either isolate of GSV.

The two isolates of GSV tested were serologically indistinguishable and the polyvalent IPNV antiserum neutralized the West Buxton and the VR#299 strains of IPNV. It was also shown that IPNV and GSV have heterologous serological properties. It was concluded that GSV is not a strain of IPNV.

LITERATURE CITED

1. COHEN, J., A. POINSARD and R. SCHERRER. 1973. Physico-chemical and morphological features of infectious pancreatic necrosis virus. J. Gen. Virol. 21: 485-498.
2. GRATZEK, J.B., M.H. MC GLAMERY, D.L. DARVE and T. SCOTT. 1973. Microcultures of brown bullhead *(Ictalurus nebulosus)* cells: their use in quantitation of channel catfish *(Ictalurus punctatus)* virus and antibody. J. Fish. Res. Board Can. 30: 1641-1645.
3. LIENTZ, J.C. and J.E. SPRINGER. 1973. Neutralization tests of infectious pancreatic necrosis virus with polyvalent antiserum. J. Wildl. Dis. 9: 120-124.
4. MALSBERGER, R.G. and C.P. CERINI. 1963. Characteristics of infectious necrosis virus. J. Bacteriol. 86: 1283-1287.
5. PLUMB, J.A., P.R. BOWSER, J.M. GRIZZLE and A.J. MITCHELL. 1979. Fish virus: a double stranded RNA icosahedral virus from a North American cyprinid. J. Fish. Res. Board Can. 36: 1390-1394.
6. ROVOZZO, G.C. and C.N. BURKE. 1973. *A Manual of Basic Virological Techniques.* Prentice-Hall Inc., Englewood Cliffs, New Jersey. 287 pp.
7. REED, L.J. and H. MUENCH. 1938. A simple method for estimating fifty percent end points. Am. J. Hyg. 27: 493-497.
8. SCHWEDLER, T.E. 1980. Characteristics of a new virus of golden shiners *Notemigonus crysoleucas.* Ph.D. Dissertation. Auburn University. 70pp.
9. WOLF, K. and M.L. QUIMBY. 1971. Salmonid virus: infectious pancreatic necrosis virus: morphology, pathology and serology of first European isolations. Arch. gesamt. Virusforsch. 34: 144-x56.

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