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NOTE

Detection of infectious pancreatic necrosis virus in pelleted cell and particulate components from ovarian fluid of brook trout *Salvelinus fontinalis*

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ABSTRACT: Ovarian fluids from 2 populations of spawning brook trout *Salvelinus fontinalis* were assayed to determine the carrier prevalence of infectious pancreatic necrosis virus (IPNV) and to quantify virus in the supernatant fluid and cell and particulate components separated by centrifugation. Overwhelmingly, the pellet contained the greater amount of virus; accordingly, the cell and particulate component should be assayed when surveying for IPNV in ovarian fluids.

Infectious pancreatic necrosis virus (IPNV) is a nearly ubiquitous fish virus. During epizootics of IPNV, mortality is generally high in salmonids less than 4 mo old but negligible in older fish (Dorson & Torchy 1981, McAllister & Owens 1986). Survivors of infection can remain lifelong asymptomatic carriers of the virus, and these carriers serve as reservoirs of infection, shedding virus in their urine, feces, and reproductive products.

The principal non-destructive method for detection of virus carrier adult fish is the assay of reproductive products. For virus assay, untreated fluids associated with eggs (ovarian fluid) or milt (sperm) are inoculated onto cell cultures, or the fluids are clarified by low speed centrifigation and the supernatant fluids inoculated onto cell cultures.

In this study we compared the soluble (supernatant) and particulate (pelletable) fractions of ovarian fluid for their virus content. The particulate fraction usually yielded by far the higher titer of virus and therefore was the sample of choice for determining IPNV prevalence.

Ovarian fluids were collected from 2 populations (Groups 1 and 2) of brook trout *Salvelinus fontinalis* that carried IPNV. Eggs were stripped into individual paper cups and the ovarian fluid recovered from each egg mass was stored on ice. Within 4 h after collection, ovarian fluid samples (4 ml) were centrifuged at 1500 × *g* for 20 min at 4 °C. The pelleted component was suspended in 0.1 M phosphate-buffered saline (PBS) at pH 7.2 in one-tenth the ovarian fluid sample volume and disrupted, unless otherwise specified, by sonication at 75 to 100 W with ten 1 s bursts. Supernatant and pellet components were freed of bacteria using antibiotics (penicillin 100 I.U ml^{-1} and streptomycin 100 µg ml^{-1}) and were then assayed for infectious virus on CHSE-214 cells by the plaque method of Wolf & Quimby (1973). Virus titer was expressed as plaque-forming units per milliliter (PFU ml^{-1}) and was corrected for volume changes that occurred during processing so that the virus titer of supernatant and pelleted components could be directly compared.

In a preliminary study on Group 1, we found that the sonicated pellet component consistently had the greater concentration of virus (Table 1). The corrected data showed that the pellet component contained a 44- to 468-fold greater virus concentration. No differences

Table 1. *Salvelinus fontinalis*. Recovery of IPN virus from ovarian fluids of brook trout of Group 1

Sample no.	Corrected virus titer (PFU ml^{-1})*		Factor increase
	Supernatant	Sonicated pellet	
1	7.3×10^5	3.2×10^7	44
2	3.0×10^4	1.9×10^6	63
3	7.9×10^2	3.7×10^5	468

* Virus titers were corrected for volume changes that occurred during processing so that titers can be directly compared

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Table 2. *Salvelinus fontinalis.* Recovery of IPN virus from ovarian fluids of brook trout of Group 2

Sample no.	Corrected virus titer (PFU ml^{-1})* Supernatant	Sonicated pellet
1	nd	3.0×10^2
2	nd	1.0×10^2
3	nd	5.9×10^1
4	1.0×10^1	1.3×10^2
5	2.4×10^6	$> 10^7$
6	nd	2.2×10^2
7	5.6×10^5	$> 10^7$
8	nd	6.5×10^1
9	5.0×10^5	$> 10^7$
10	1.3×10^2	6.0×10^0
11	$> 10^7$	$> 10^7$
12	3.5×10^1	4.0×10^1
13	5.0×10^0	5.0×10^{-1}
14	2.5×10^1	1.0×10^1
15	nd	1.5×10^1
16	nd	3.0×10^0
Virus carrier prevalence	56 %	100 %

* Virus titers of supernatant and pellet components were corrected for volume changes that occurred during processing so that titers can be directly compared
nd: no virus was detected when 0.1 ml of undiluted sample was inoculated onto each of 2 monolayer cultures of CHSE-214 cells. Plaque assay sensitivity is 5 PFU ml^{-1}

Table 3. *Salvelinus fontinalis.* Effect of sample processing on recovery of IPNV from ovarian fluids

Sample type	Corrected virus titer (PFU ml^{-1})* Sonication	Sample 1	Sample 2
Whole fluid	+	5.9×10^4	4.0×10^3
	–	9.3×10^2	nd
Supernatant	+	2.0×10^2	nd
	–	1.0×10^2	nd
Pellet	+	1.9×10^5	4.9×10^4
	–	1.8×10^4	7.3×10^3

* Virus titers were corrected for volume changes that occurred during processing so that titers can be directly compared
nd: no virus was detected when 0.1 ml of undiluted sample was inoculated onto each of 2 monolayer cultures of CHSE-214 cells. Plaque assay sensitivity is 5 PFU ml^{-1}

were seen in the virus titers between washed and unwashed pellet components (data not shown).

In a trial with a second population of brook trout (Group 2), assay of the supernatant fluid indicated a prevalence of carrier fish of 56 %, whereas assay of the sonicated pellet component showed a prevalence of 100 % (Table 2). In 75 % of the samples, virus titers were greater in the sonicated pellet component than in the supernatant fluid. We detected infectious virus in the sonicated pellet component at a corrected titer as low as 0.5 PFU ml^{-1}.

In a third trial we examined the effect of sonication on the recovery of IPNV (Table 3). Two ovarian fluid samples were assayed for virus in the whole fluid, supernatant component, and pellet component. In all cases, sonicated samples yielded higher virus titers than their unsonicated counterparts. However, in both sonicated and unsonicated samples the highest titers were found in the pellet component and the lowest titers occurred in the supernatant component. Virus in the supernatants appeared to occur largely unaggregated because the titers in sonicated and unsonicated supernatants were essentially the same.

Whether sonication can be used to enhance the sensitivity of assays for other viruses such as the enveloped viruses (e.g. the rhabdoviruses and herpesviruses) in ovarian fluid pellets has not been adequately tested. One might expect that the delicate envelopes of these viruses could be destroyed (and the sensitivity of the virus assays decreased) by sonication. However, this did not appear to hold true for the one rhabdovirus (the infectious hematopoietic necrosis virus) recently tested with sonication (McAllister unpubl. data). In assaying particulate (cell-containing) samples for sonication-susceptible viruses, one might use mechanically disrupted samples, or, as was done in the present study with unsonicated pellet and whole fluid samples (see Table 3), assay the samples undisrupted. The latter technique, known as the cocultivation procedure (Aguis et al. 1982, Yu et al. 1982) is reported, for example, to be more sensitive than the standard assay method based on filtered, homogenized samples (Aguis et al. 1982).

Cytological examination indicated that the predominant cell types in the ovarian fluid pellets were erythrocytes, lymphocytes, and macrophages (R. L. Herman pers. comm.). However, we have not yet identified or visualized virus in specific cells in the ovarian fluid pellets.

Enzmann (1980) and Yu et al. (1982) showed that salmonid lymphocytes harbor and can support replication of various fish viruses. In a recent study of infectious hematopoietic necrosis virus, a spontaneous conversion of ovarian fluid samples from virus-negative to virus-positive after storage was attributed to release of virus from unidentified cells in the ovarian fluid (Mulcahy 1986).

Our results clearly show that the pelleted cells and particulates of ovarian fluid harbor far more virus than the supernatant fluids, and should therefore be the material assayed for IPNV.

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