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A STUDY OF TWO SPECIES OF FISH INOCULATED WITH SPRUCE BUDWORM NUCLEAR POLYHEDROSIS VIRUS

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Abstract: Preliminary studies conducted on rainbow trout *(Salmo gairdneri)* and white suckers *(Castostomus commersoni)* exposed to spruce budworm nuclear polyhedrosis virus revealed minor histopathologic changes in various organs of each fish which were interpreted as not being significant. However, an evaluation (mean values) of the total changes in groups of fish suggested that a relationship may exist in the suckers exposed to either purified polyhedra or virions. A more extensive and definitive study must be done before any conclusions are warranted.

INTRODUCTION

Aerial spraying of forest areas with the spruce budworm nuclear polyhedrosis virus and insecticides effectively controls or reduces spruce budworm *(Choristoneura fumiferana)* populations to density levels below those necessary to produce significant damage to spruce trees.[4,5] However, aerial spraying exposed the ecosystem in the area to unusually high concentration of the virus and raises the question of the possible effects it may have on vertebrates in the area.[6] Our report summarizes the results of a preliminary laboratory study of the possible effects of the spruce budworm nuclear polyhedrosis virus on two species of fish.

MATERIALS AND METHODS

Rainbow trout *(Salmo gairdneri)* weighing between 24-124 gm were purchased as near yearlings from a commercial hatchery, and white suckers *(Castostomus commersoni)* weighing between 40-96 gm were purchased from a commercial bait fisherman. The fish were held for a two-week acclimatization period in large holding tanks in an animal isolation unit.

The agents[2] tested were:

1. Nuclear polyhedrosis virus infected larvae, supplied as freeze-dried powder containing 5×10^9 polyhedra/g
2. Purified polyhedra supplied as a freeze-dried powder containing 5×10^{10} polyhedra/g
3. Virions of nuclear polyhedrosis virus supplied in physiological saline solution with a particle count equivalent to 1.7×10^{10} polyhedra/ml
4. Healthy uninfected larvae supplied as freeze-dried powder.

The test agents were kept frozen at -25 C or -70 C until 48 h prior to use, at which time they were thawed and suspended in physiological saline at concentrations equivalent to what might be fed to a 70 kg man.[3] The actual suspension or slurry of each was calculated and diluted so

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[2] Supplied by Dr. Basil M. Arif of the Forest Pest Management Institute of the Department of Environment Canada, Sault Ste. Marie, Ontario.

[3] Based on the World Health Organization recommendation as the amount administered in the form of a spray over 20 acres which might be ingested by a 70 kg man, ($20 \times 50 \times 10^9$ polyhedra).

that it could be administered at the rate of 0.025 ml/g of body weight of the individual fish.

Groups of nine trout and 10 suckers were anesthetized, [4] weighed, and measured amounts of the appropriate inoculum were introduced directly into the stomach of each fish by intubation. Each group of fish was then returned to separate 20 l aquaria. They were fed three times a day and observed for abnormalities in behaviour or appearance. Following a 28-day observation period, the fish were killed, and tissue samples collected for further evaluation. Samples designated for virus isolation studies [5] were placed in individual vials and stored at -70 C. Duplicate samples were fixed in 10% buffered formalin and stained with hematoxylin-eosin. The stained sections were examined for pathological changes and each organ was scored from 0 to 4, normal to widespread degenerative or inflammatory changes, respectively. Mean values were calculated for each group of fish.

RESULTS

White suckers refused to ingest any of the food offered, but there were no other observable abnormalities in the appearance or behavioural patterns among the suckers or the trout during the 28-day experimental period. There was an average weight loss of 4.0 g among the suckers and a weight gain of 5.4 g among the trout. Examination of the stained tissue sections revealed only minor changes such as increased hemosiderin, slight infiltration of mononuclear cells or focal degeneration of some cells in one or more of the organs of each fish, but rarely warranting a score of greater than two per organ. The total scores per fish ranged from 0 to 6 among the trout and from 2 to 10 among the suckers. However, when mean values were calculated on the basis of groups of fish receiving a particular inoculum, differences became apparent (Table 1). The mean values (i.e. the histologic changes) were higher among the groups of trout that received the purified polyhedra or uninfected larvae than among those inoculated with infected larvae or the saline controls. Among the white suckers (Table 1), those groups inoculated with virions or purified polyhedra had significantly higher scores than the saline controls. In addition, each of the three groups of suckers that had been exposed to either virions, purified polyhedra or infected larvae, had mean scores higher than the group that received the uninfected larvae. [6]

DISCUSSION

The nuclear polyhedrosis and granulosis viruses are classified as Baculoviruses and all evidence indicates that members of this group of viruses are highly host specific. They are reported to be neither infective, mutagenic nor oncogenic in mammalian cell cultures or animals [2,3,7] and, therefore, may be used safely as viral insecticides to control crop and forest damage caused by insect pests. Our experiments failed to indicate any clinical illness among the groups of inoculated fish. The average weight loss of 4.0 g among the suckers can be explained by their failure to feed throughout the course of the experiment. The histopathologic changes noted in the

[4] A 1:1000 dilution of Ethyl-m-aminobenzoate methanesulfonate (MS222) Eastman Chemicals, Rochester, New York, USA.

[5] Detection and/or isolation of nuclear polyhedrosis virus from the fish tissues was conducted by the Forest Pest Management Institute, Department of Environment, Sault Ste. Marie, Ontario P6A 5M7.

[6] The Forest Pest Management Institute reported no evidence of viable virus was found in any of the organs tested. Viable virus was detected in some fecal samples from trout 1, 2 and 3 days post inoculation.[1]

TABLE 1. Analysis of data per group of inoculated fish.

Inoculated Group	Trout	Suckers
Saline Controls	2.22* ± .536	3.37 ± .500
Virions	2.62 ± .480	5.14 ± .346
Purified Polyhedra	3.40 ± .364	4.75 ± .600
Infected Larvae	2.00 ± .132	4.33 ± .610
Uninfected Larvae	3.57 ± .516	3.00 ± .156

*Mean values ± two times the standard error.

organs of individual fish were slight to mild and could be regarded as insignificant. However, when these changes were evaluated according to groups, differences were evident among the trout receiving purified polyhedra or uninfected larvae, while among the suckers the groups inoculated with virions, purified polyhedra or infected larvae had significantly higher scores than the group inoculated with uninfected larvae. Thus, although the histopathologic changes recorded in individual organs could be regarded as insignificant, the total results per group of fish may be significant. Admittedly the preliminary nature of this study with small numbers of fish per group and the fact that the suckers were not feeding during the course of the experiment, plus the changes recorded among the groups exposed to saline or uninfected larvae, preclude any definitive evaluation, or conclusion, but the results obtained indicate the need for more extensive studies with fish.

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