

HEALTH STATUS OF SALMONIDS IN RIVER SYSTEMS IN NATAL. II. ISOLATION AND IDENTIFICATION OF VIRUSES

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ABSTRACT

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A total of 678 fish from 26 different sites along 8 river systems was examined for the presence of trout viruses. No isolations of any viruses were made from any fish collected from these systems in Natal during this study. No antibodies against any of the trout viruses were detected in the serum collected from these fish.

It would appear from this study that the river systems in Natal are free of any of the known viruses of trout. Infectious pancreatic necrosis virus was, however, isolated from trout from the Natal Parks Board Hatchery at Kamberg Nature Reserve on the Mooi River during June and July of 1988. Neutralising antibodies against the VR 299 serotype of IPN virus were detected in the serum collected from trout at Kamberg in September 1988.

INTRODUCTION

The second part in this series covers the isolation and identification of viruses from salmonids and non-salmonid fish from Natal.

There are four well-characterized viruses which are known to cause serious diseases in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). The first, and most well-characterized of these is infectious pancreatic necrosis (IPN) virus. This virus has an almost world-wide distribution, with Australia being one of the few countries where it has not been found yet (Langdon, Humphrey, Copland, Carolane, Gudkovs & Lancaster, 1986). The VR 299 serotype of IPN virus which has been, isolated a number of times in South Africa (Bragg & Combrink, 1988) was also isolated from a shipment of imported trout ova from the USA (Bragg & Combrink, 1988). There are a number of reports which indicate that this virus is vertically transmitted (Bullock, Rucker, Amend, Wolf & Stuckey, 1976; Mulcahy, Jenes & Pascho, 1984).

The other important viruses of trout include infectious haematopoietic necrosis virus (IHN), viral haemorrhagic septicaemia (VHS) virus and *Herpesvirus salmonis*. None of these viruses have been isolated in South Africa (Bragg & Combrink, 1988) and all appear to have a limited geographical range. There is some evidence that both IHN and VHS viruses may be transmitted via contaminated ova. In both cases, the virus does not occur inside the ova, but is found on the surface of the ova (Pilcher & Fryer, 1980).

As large numbers of trout ova are imported into South Africa annually, the possibilities exist to import IPN virus into the country. If ova are not properly disinfected, IHN and VHS virus may also be introduced into the country. Before 1983, no laboratory in South Africa had the facilities to test trout or ova for the presence of viruses. The only virological survey of trout which had been done here was carried out as a result of the isolation of the VR 299 serotype of IPN virus in 1985 and 1986 (Bragg & Combrink, 1988) and only covered trout produced by certain producers for resale as fingerlings. There has thus never been any in depth investigations of the virological state of salmonids which occur in the

ivers, either through breeding in the river, or through the stocking activities of angling clubs or from trout hatcheries which are not producing fingerlings for resale. The present study is a report on the virological investigation of fish, both salmonid and non-salmonid, from some of the river systems in Natal.

MATERIALS AND METHODS

Collection of fish from rivers, dams and production sites

The methods for the collection of fish for the virological survey were the same as those outlined in Part 1 (Bragg 1991) without any modifications.

Collection of serum from fish

Blood was collected from the fish by cardiac puncture with a 25 gauge needle. The blood was placed in 1 ml tubes and incubated at room temperature for about 15 min. The clot was loosened and left overnight. The serum was removed and placed into sterile 1 ml tubes. The serum was stored at about 15 °C until it was processed in the laboratory.

Sample collection for virus isolation

Samples of liver, spleen and kidney were collected from each fish and placed into sterile phosphate buffered saline (PBS) which contained 240 mg of penicillin/l and 400 mg of streptomycin/l. The viscera of each fish were kept and processed separately.

Isolation procedures

The techniques for the isolation of viruses from the fish were the same as those described by Bragg & Combrink (1988). Samples were homogenized, diluted 1/10 with balanced salt solution and filtered through a 0,45 µm filter. The samples were inoculated onto the RTG2, BF2 and FHM cell lines according to the techniques described by Bragg & Combrink (1988).

Virus neutralization test

The serum that was collected in the field was filtered through a 0,45 µm filter and was frozen until use.

The serum was thawed out and tenfold serial dilutions were made from 10⁻¹ to 10⁻⁶. Samples of the VR 299, Ab and Sp serotypes of IPN virus¹ were

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¹ Supplied by Dr B. J. Hill, Fish Disease Laboratory, Weymouth, England

used for the neutralization test. The virus neutralization test was done according to the methods described by Wolf & Quimby (1969). The virus neutralization test was done on BF2 and RTG2 cells².

RESULTS

Collection of fish

The numbers of fish and locations where the fish were obtained during the survey are listed in Part 1 (Bragg 1991).

Isolation of viruses and detection of neutralizing antibodies

No viruses were isolated from any of the fish collected during this survey. No neutralizing antibodies against the VR 299, Ab or Sp serotypes of IPN virus were detected in any of the serum samples collected from any of the fish collected as part of either survey.

DISCUSSION

IPN virus has been isolated from trout in Natal (Bragg & Combrink, 1988, unpublished data). The trout were obtained from the NPB hatchery at Kamberg in June 1988 and IPN VR 299 was isolated from one group of fish. These fish were retested in July 1988 and IPN VR 299 was re-isolated. High levels of neutralizing antibodies against the VR 299 serotype of IPN virus were detected in the serum collected from fish in September 1988 (Bragg & Combrink, 1988, unpublished data).

Samples 1-17 (Table 1 of Part 1, Bragg, 1991) were collected from various sites in Natal as a result of the isolation of IPN virus from the NPB hatchery at Kamberg. The main objective of this work was to ascertain if the virus which had been isolated from Kamberg had been inadvertently spread to other sites in Natal as a result of the stocking of public water by the NPB. No viruses could be isolated from any of the sites which were tested and no neutralizing antibodies against the VR 299, Ab or Sp serotypes of IPN virus could be detected in the serum from any of the fish examined.

The virological results obtained from samples 18 to 38 (Table 1 of Part 1, Bragg, 1991) confirmed the results obtained in the first group of samples. No viruses were isolated from any of the fish collected from any of the sites. No neutralizing antibodies were detected in the serum from any of the fish examined.

The virological situation for trout in Natal is similar to that obtained when IPN VR 299 virus was isolated in the Transvaal in 1985 and 1986 (Bragg & Combrink, 1988). Some of the sites which were found to be infected with IPN VR 299 in 1985 and 1986 underwent extensive disinfection operations and the virus was not re-isolated from these sites. Other producers, however, made no attempt to disinfect their sites and the virus was also not reisolated from these sites.

From all of these results, it would appear that IPN virus is not capable of surviving for longer than about four months in this country. Although IPN virus has been isolated a number of times in this country, there has never been any clinical outbreak of infectious pancreatic necrosis and all of the isola-

tions made were from asymptomatic fish which had been subjected to routine health certification checks, or from the survey which was carried out as a result of the first isolation of the virus in this country (Bragg & Combrink, 1988). There is, however, no scientific evidence at this stage to prove that the virus cannot successfully establish a carrier state. The high average summer water temperatures in most of the areas where trout are produced in South Africa may play some role in preventing the establishment of carriers. These higher temperatures may in themselves be detrimental to the virus, or may result in a more active immune system in the fish, thus effectively eradicating the virus from the fish. These aspects need further investigation.

CONCLUSION

IPN virus has previously been isolated from trout at the NPB hatchery at Kamberg in Natal. Although the NPB had stocked a number of rivers, dams and production sites with fish from the same population before the detection of the virus and before quarantine measures were subsequently introduced at the hatchery, no viruses could be isolated from any of the fish collected from various sites in Natal during the present study. No neutralizing antibodies could be detected in the serum collected from any of the fish obtained during this survey.

It thus appears that all the fish tested from various rivers in Natal are free of viruses in spite of the fact that IPN virus had been isolated from a hatchery which stocks most of the rivers and dams in Natal. These findings also enhance the theory that IPN virus is incapable of surviving or establishing a carrier state in this country. This theory was formulated when IPN virus was isolated from various hatcheries in the Transvaal and the Cape but could not be reisolated from the same sites, or any other sites four to six months later. It appears possible that the high average prevailing temperatures in the trout producing areas of this country may have some role to play in the apparent inability of IPN virus to establish a carrier state.

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REFERENCES

- BRAGG, R. R. & COMBRINK, M. E., 1988. Isolation and identification of trout viruses in South Africa. *Onderstepoort Journal of Veterinary Research*, 55, 139-143.
- BRAGG, R. R., 1991. Health status of salmonids in river systems in Natal. I. Collection of fish and parasitological examination. *Onderstepoort Journal of Veterinary Research*, 58, 59-62.
- BULLOCK, G. L., RUCKER, R. R., AMEND, D., WOLF, K. & STUCKEY, H. M., 1976. Infectious pancreatic necrosis: transmission with iodine-treated and non-treated eggs of brook trout (*Salvelinus fontinalis*). *Journal of the Fisheries Research Board of Canada*, 33, 1197-1198.
- LANGDON, J. S., HUMPHREY, J. D., COPLAND, J., CAROLANE, R., GUDKOV, N. & LANCASTER, C., 1986. The disease status of Australian salmonids: viruses and viral diseases. *Journal of Fish Diseases*, 9, 129-135.

² Supplied by Sterilab Services, P.O. Box 2021, Kempton Park, 1620.

MULCAHY, D., JENES, C. K. & PASCHO, R., 1984. Appearance and quantification of infectious hematopoietic necrosis virus in female sockeye salmon (*Oncorhynchus nerka*) during their spawning migration. *Archives of Virology*, 80, 171-181.

PILCHER, K. S. & FRYER, J. L., 1980. The viral diseases of fish: A

review through 1978. Part 1. Diseases of proven viral etiology. *Critical Reviews in Microbiology*, 7, 287-363.

WOLF, K. & QUIMBY, M. C., 1969. Infectious pancreatic necrosis: clinical and immune response of adult trout to inoculation with live virus. *Journal of the Fisheries Research Board of Canada*, 26, 2511-2516.