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Prevention of a Mortal Disease of Carps Induced by the Carp Interstitial Nephritis and Gill Necrosis Virus (CNGV) in Israel

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Massive mortality of Koi and Common carp - *Cyprinus carpio* species - was observed in many farms throughout Israel, resulting in severe financial losses.

Fig. 1. The spread of the carp viral disease in Israel between 1998 and 2000.

----- Area of infected farms in 1998
----- Area of infected farms in 1999
----- Area of infected farms in 2000

This lethal disease is highly contagious and extremely virulent, but morbidity and mortality are restricted to Koi and Common carp populations (Fig. 1).

We isolated a carp nephritis and Gill necrosis virus (CNGV), which is the etiologic agent of this disease. The virus propagates and induces severe cytopathic effects five days post infection in fresh Koi fin cell cultures (KFC). The virus harvested from KFC cultures induced the same disease with mortality of 75-95% upon inoculation of naive Koi and Common carp (Pikarsky *et al.*, 2004).

Electron microscopy revealed viral cores with icosahedron morphology of 100-110 nm resembling the herpes virus. Electron micrographs of purified pelleted CNGV sections, together with sensitivity to ether and Triton x 100 suggest that it is an enveloped virus. However, the genome of the isolated virus is a double-stranded DNA molecule of 250-300 Kbp, larger than that of known *Herpesviridae* members. The viral DNA seems highly divergent and bears only small (16-45 bp) fragments similar to the genomes of several DNA viruses. We suggest, therefore, that the etiologic agent of this disease may represent as yet unclassified virus species endemic to cyprinids (Hutoran *et al.*, 2004).

Carps, exposed to the virus at 23 ℃ for 3-5 days and then transferred to the non-permissive temperature of 30 ℃, became resistance to a challenged infection and their

sera demonstrated a high level of virus specific antibodies (Fig. 2).

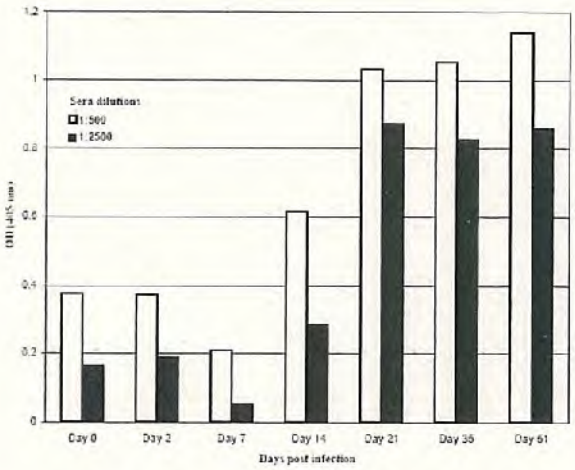

Fig. 2. Induction of anti-CNGV antibodies in carp sera following infection by cohabitation with sick fish.

We have isolated attenuated non-pathogenic viruses that render virus-vaccinated carps resistant to the disease (Fig. 3) (Ronen *et al.*, 2003). Furthermore, vaccinated fish developed high levels of antibodies against the virus. We suggest, therefore, that this attenuated virus could be used as a live vaccine for the eradication of the mortal disease afflicting common and ornamental carp fisheries in many countries (Perelberg *et al.*, 2005).

We examined the pathobiology of this disease in carp using immuno histochemistry. We found large amounts of the virus in the kidneys of sick fish, and lesser amounts in liver and brain. A rapid increase in the viral load in the kidneys was detected using both immuno-fluorescence and semi-quantitative PCR. Histological analyses of fish at various times after infection revealed signs of interstitial nephritis as early as 2 days post-infection, which increased in severity up to 10 days post-infection. There was severe gill disease evidenced by loss of villi with accompanying inflammation. Minimal focal inflammation was noted in livers and brains.

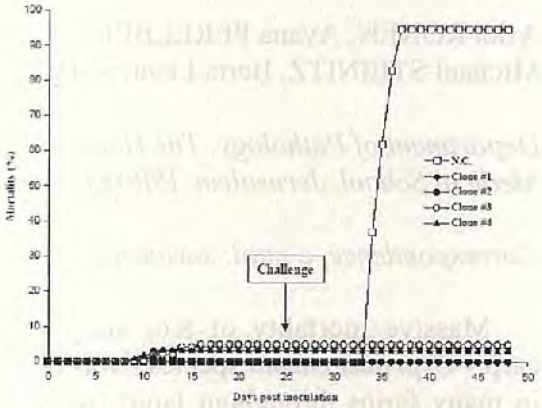

Fig. 3. Mortality rate in vaccinated fish following a challenge infection.

Two diagnostic methods for identifying the CNGV in live fish are in use in our laboratory: Using PCR with authentic primers is applied on blood, kidney or gills DNA samples and immunological diagnostic kit. The immunological kit is designed to be a simple, rapid and low cost diagnostic kit, which will be appropriate for retailers as well as hobbyists to identify the CNGV in presymptomatic fish. We believe that these means will be instrumental in preventing the distribution of sick fish world wide.

Conclusion

1) The causative agent of the disease in common carp and koi population is an enveloped DNA virus with a core of 110 nm with an electron dense region.

2) We have named the virus Carp Nephritis and Gill necrosis Virus (CNGV) according to its pathological manifestations.

3) There are some morphological similarities between CNGV and herpes viruses.

4) The CNGV genome is composed of a ca~ 277Kbp DNA molecule, larger than the other *herpesviridae* members.

5) The viral DNA sequences resolved so far suggest that the viral genome is highly divergent from that of *herpesviridae* members, and is not similar to other DNA viruses.

6) We have selected an attenuated viral strain following multiple transfers in tissue culture. The attenuated virus is non-pathogenic, induces humoral response in vaccinated fish and renders them protected against challenge infections with the wild type virus.

References

Ronen A., Perelberg A., Abramowitz J., Hutoran M., Tinman S., Bejerano I., Steinitz M. and Kotler M., 2003: Efficient vaccine against the virus causing a lethal disease in cultured *Cyprinus carpio*. *Vaccine*, **21**, 4677-4684.

Pikarsky E., Ronen A., Abramowitz J., Levavi-Sivan B., Hutoran M., Shapira Y., Steinitz M., Perelberg A., Soffer D. and Kotler M., 2004: The pathogenesis of the acute viral disease in fish induced by the carp interstitial nephritis and gill necrosis virus. *J. Virol.*, **78**, 9544-9551.

Hutoran M., Ronen A., Perelberg A., Ilouze M., Dishon A., Bejerano I., Chen N. and Kotler M., 2004: Description of an as Yet Unclassified DNA Virus from Diseased *Cyprinus carpio* Species. *J. Virol.*, **79 (4)**, 1983-1991.

Perelberg A., Ronen A., Hutoran M., Smith Y. and Kotler M., 2005: Protection of cultured *Cyprinus carpio* against a lethal viral disease by an attenuated virus vaccine. *Vaccine* 2005, pp. 8 (In press).