but, if they are subjected to an appropriate environmental stress, they may quickly succumb to the infection. Secondly, *Aeromonas salmonicida* is often present in a large percentage of fish in a carrier state which can again be triggered into an active infection by a stressful environment. It is our feeling, therefore, that any significant deep organ infection (particularly the brain) is of major consequence to the fish population.

In the future, I believe that additional anaerobic bacterial fish pathogens will be discovered. Much of the groundwork is set, and, as more people begin to look for this group of organisms, more will be found.

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MFR PAPER 1334

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Recent Developments in Channel Catfish Virus Research

J. A. PLUMB and P. R. BOWSER

Channel catfish virus (CCV) is well established as a highly virulent pathogen in some cultured channel catfish fingerling populations. This highly contagious disease of juvenile channel catfish occurs during the warm summer months. Although most epizootics have occurred in the southern states, the virus has been isolated from channel catfish fingerlings in several northern and western states. Mortality rates of CCV-infected populations may approach 100 percent. CCV is caused by herpesvirus of ictalurids (Wolf and Darlington, 1971). The virus has been isolated only from fish when fry or fingerlings are actually dying. Epizootics appear to be associated with some form of stress.

The brown bullhead (BB) cell line is normally used for isolation of CCV. A cell line is being developed from channel catfish ovaries (CCO) which is now in the 75th passage and approximately 20 months old. CCO cells appear to be more sensitive to CCV than the BB cells; CCO cells develop initial microscopic cytopathic effect (CPE) 12-24 hours before the BB cells, and total CPE occurs 24-48 hours in CCO cells before it does in BB cells. Also, the CCV titers in CCO cells are 0.5-1.0 log (base 10) higher than in the BB cells.

A fluorescent antibody (FA) technique has been developed for detecting CCV in vitro. Rabbit anti-CCV serum was conjugated with fluorescein isothiocyanate (FITC) using the method of Cherry (1974). CCO cultures in Leighton tubes were infected with CCV, and, at 2-hour intervals after infection, coverslip cultures were removed, fixed in acetone, and incubated with FITC conjugate. Infected CCO cells fluoresced at 4 hours postinfection (PI) with focal cytoplasmic fluorescence. Fluorescence increased with larger focal areas through 6 and 8 hours PI, until at 10 hours the entire cell sheet was fluorescing. The FA technique has not been applied to in vivo infections.

Confirmed CCV epizootics have been found only in farm-raised channel catfish populations. Different strains of channel catfish vary in their susceptibility to CCV (Plumb et al., 1975); however, data on susceptibility of other species of ictalurids to CCV are lacking. Young brown, black, and yellow bullhead catfish were exposed to CCV by incorporating the virus into the feed, intraperitoneal (IP) injection, and by cohabitation with CCV-infected channel catfish. Although some mortality occurred in all of these bullheads in each method of exposure, CCV was not isolated from any of the exposed fish. It is interesting to note that the BB cells support CCV replication but that the fish itself apparently does not.

In another study, blue and channel catfish fingerlings and their reciprocal crosses were compared for CCV

susceptibility by IP injection. All groups were similar in sensitivity to the virus. The titer in channel catfish was $1\times10^{3.29}$ LD_{50}, blue catfish was $1\times10^{4.5}$ LD_{50}, channel female × blue male was $1\times10^{4.87}$ LD_{50}, and blue male × channel female was $1\times10^{4.08}$ LD_{50}.

In a CCV titration in blue catfish, the initial mortality of those injected with 3.16×10^{5} $TCID_{50}$ occurred 36 hours after injection, and, after 60 hours, 100 percent of these fish were dead. Blue catfish injected with 3.16×10^{4} $TCID_{50}$ had 100 percent mortality after 84 hours. Approximately 80 percent of those fish injected with 3.16×10^{3} or 3.16×10^{2} $TCID_{50}$ died; 70 percent of those injected with 3.2×10^{1} $TCID_{50}$ died; and 20 percent of the fish injected with 3.2×10^{0} $TCID_{50}$ died. No control fish were lost.

Intraperitoneal injection was the only successful method of infecting blue catfish. Feeding virus and cohabitation with infected channel catfish were unsuccessful in establishing infection in blue catfish, although these methods worked well for infected channel catfish. CCV replication reached a peak in blue catfish 42 hours PI when $1\times10^{5.8}$ $TCID_{50}$/gm of viscera were isolated. The channel female × blue male fingerlings reached a similar peak of replication 72 hours PI; however, the channel catfish fingerlings reached a peak of $1\times10^{2.7}$ $TCID_{50}$/gm of viscera 72 hours PI.

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MFR PAPER 1335

Viral Diseases of the Blue Crab, *Callinectes sapidus*

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To date, 13 viruses have been found in crustaceans (Bang, 1971; Bazin et al., 1974; Bonami and Vago, 1971; Bonami et al., 1971; Chassard-Bouchaud et al., 1976; Couch, 1974; Federici and Hazard, 1975; Johnson, 1976a, b; Johnson and Bodammer, 1975; Vago, 1966). Eleven of them are from decapods, and 4 of the 11 occur in the blue crab.

Known viruses of the blue crab include: 1) a *Baculovirus* that infects the hepatopancreas (Fig. 1); 2) a reolike virus found in hemopoietic tissue, hemocytes, certain other mesodermal cells, and some ectodermal cells, including the general epidermis and probably the glia of the central nervous system (Fig. 2); 3) a herpeslike virus that is mainly confined to hemocytes (Fig. 3); and 4) a picornalike virus that attacks ectodermal elements, including neurosecretory cells, epidermis, and the bladder epithelium (Johnson[1]) (Fig. 4). Occasionally, the latter also infects hemocytes and hemopoietic tissues. A fifth entity which has many characteristics of the Rhabdoviridae has been found in crabs also infected with any of the last three viruses (Fig. 2), and it may represent a virus that manifests itself mainly in stressed animals (Johnson, footnote 1).

The *Baculovirus* apparently does not cause overt disease in its host. It usually infects only scattered cells, and, since tissue replacement is occurring constantly in the hepatopancreas, the damage caused usually must be minimal. The *Baculovirus* occurs in all

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[1]Johnson, P. T., Oxford Laboratory, Northeast Fisheries Center, National Marine Fisheries Service, NOAA, Oxford, MD 21654. Unpublished observations.