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John A. Plumb
Auburn University Main Campus

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CHANNEL CATFISH VIRUS DISEASE¹

John A. Plumb

Cooperative Fish Disease Project
Department of Fisheries and Allied Aquacultures
Alabama Agricultural Experiment Station
Auburn University, Alabama 36849

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¹Revision of Fish Disease Leaflet 18, same title, by T. J. Wellborn, N. N. Fijan, and J. P. Naftel (1969), and J. A. Plumb (1972; 1977).
Introduction

Channel catfish virus disease (CCVD) is an acute infection of cultured fry and fingerling channel catfish (Ictalurus punctatus). The disease occurs primarily during summer and, with few exceptions, in fish less than 4 months old. The causative agent, the channel catfish virus (CCV), is a member of the herpesvirus group. Since its first identification (Fijan 1968), it has been isolated from infected fish in most areas of the United States where channel catfish are cultured.

Diagnosis and Identification

A sudden increase in morbidity among young channel catfish is the first indication of the disease. Infected fish swim erratically or convulsively, sometimes rotating about their longitudinal axis. Victims sink to the bottom, become quiescent, respire weakly but rapidly, and then die.

Clinical signs of disease vary and some or all of the following may be present: distention of the abdomen due to the accumulation of a clear straw-colored fluid in the peritoneal cavity, exophthalmia, pale or hemorrhagic gills, and hemorrhagic areas at the bases of fins and throughout the skin—particularly on the ventral surface. Although the liver and kidneys may be pale, a general hyperemia occurs throughout the visceral cavity. The spleen is generally enlarged and dark red. The stomach and intestine are devoid of food but filled with a mucoid secretion. In fingerling channel catfish, the clinical signs may resemble those of enteric septicemia, a bacterial disease caused by Edwardsiella ictaluri.

Histopathologic changes are similar in natural and experimental CCV infections (Wolf et al. 1972; Plumb et al. 1974; Major et al. 1975). Renal hematopoietic tissue is edematous, and extensive areas of necrosis and cellular dissolution occur, coupled with an increase in macrophages. The liver develops regional edema, necrosis, and hemorrhages, and hepatic cells have eosinophilic intracytoplasmic inclusions. Pancreatic acinar cells are necrotic. The submucosa of the digestive tract is edematous and has focal areas of macrophage concentration and hemorrhage. The spleen becomes congested with erythrocytes and lymphoid tissue becomes greatly reduced. Virus particles have been seen in electron micrographs of the liver, kidneys, and spleen of infected fish (Plumb et al. 1974).

A generalized viremia is established within 24 h after experimental infection. The kidneys, liver, spleen, and intestine become involved in virus replication 24-48 h after infection, and virus can sometimes be isolated from brain tissue after 48 h. Virus titers are highest in the kidneys and intestine 72 h after infection, and in the spleen, brain, and liver after 96 h. Virus titers in the muscle are comparatively low.

The virus is frequently found in fish with concurrent Aeromonas hydrophila or Flexibacter columnaris infections. Thus it is imperative that virological examination of affected catfish be coupled with bacteriological examination.

Although the appearance of syncytia in inoculated cell cultures provides presumptive identification of CCV, positive virus identification is determined by serum neutralization assay.

Cause of the Disease

The etiological agent of the disease is a herpesvirus. Enveloped virions have a diameter of 175-200 nm. The outer viral capsid is 95-105 nm in diameter (Wolf and Darlington 1971). Viral infectivity is inactivated by treatment with 20% ether or 5% chloroform. Channel catfish virus is heat labile at 60°C for 1 h, is unstable in seawater, and is inactivated by ultraviolet light in 20-40 min (Robin and Rodrique 1980). It survives for less than 24 h on dried concrete chips, and less than 48 h on dried fish netting or glass cover slips. It retains infectivity in pond water for only about 2 days at 25°C, but for 28 days at 4°C. In dechlorinated tap water, infectivity is retained for 11 days at 25°C and for more than 2 months at 4°C. Under experimental conditions, infectivity is immediately destroyed in pond bottom mud. Infectious virus could not be isolated 48 h after death from decomposing victim fish at 22°C; however, it was recoverable for as long as 14 days from iced fish, for 162 days from fish frozen at -20°C, and for 210 days from fish frozen at -80°C (Plumb et al. 1973).
Channel catfish virus replicates in cell culture at 10-35°C (30-33°C is the optimum). Although brown bullhead (BB) cells are susceptible to CCV, the channel catfish ovary (CCO) cell line is preferred for virus assays because of its higher sensitivity to the virus (Bowser and Plumb 1980). When inoculated CCO cells are incubated at 30°C, cytopathic effect develops within 12 h. Cells derived from the walking catfish kidney (K1K) are also susceptible to CCV (Noga and Hartmann 1981). Other poikilothermic and mammalian cell systems are refractory to CCV. In susceptible cell cultures, the virus induces the formation of characteristic giant multinucleated (syncytium) cells.

Source of Infection

Channel catfish virus has been isolated during epizootics from fry and fingerling channel catfish showing clinical signs of the disease. These fish are a source of infection for other fish. Although adults have been considered a source of CCV infection for transmission of the virus to offspring by way of the reproductive products, this mode of transmission has not been conclusively proven. Bowser et al. (1985) and Wise et al. (1985) isolated CCV from adult channel catfish collected in January, when water temperature was 8°C—suggesting that CCV may lie dormant in adult fish much of the year.

Mode of Transmission

Under experimental conditions, virus from infected moribund or dead fish can be transmitted to healthy fish by way of the water. The virus can also be transmitted by intramuscular or intraperitoneal injection, by incorporating the virus into feed, or by swabbing the gills with a saline solution containing virus. Circumstantial evidence points to vertical transmission of the virus from adult to offspring.

Incubation Period

The incubation period is inversely related to water temperature. Experimental infection at 30°C is followed by clinical signs in 32-72 h, and the first deaths occur several hours later; at 20°C the incubation period is 10 days. At 25-30°C, healthy channel catfish fingerlings develop the disease within 72-78 h after exposure, and most die within 6 days. All of a group of naturally infected 21-day-old fry held at 28°C developed clinical signs of CCVD and died within 72 h.

Detection

The most reliable method for detecting CCV is by isolating the virus in cell culture. Detection of CCV carrier fish is more difficult. Plumb (1973) demonstrated that CCV antibodies could be detected in adult channel catfish that were exposed to the virus and suggested that antibody detection may be used to separate exposed from unexposed fish. Amend and McDowell (1984), who used serum neutralization tests to separate possible adult CCV carrier catfish, demonstrated that 7 of 17 major brood populations that were tested showed positive CCV serum neutralization; CCV was subsequently isolated from fingerlings of two of the seven positive populations. Plumb et al. (1981) showed that CCV nucleic acid could be detected in gonads of adult fish by immunofluorescence, and Bowser et al. (1985) isolated CCV from adult channel catfish—proving that fish other than fingerlings can develop active infections. Wise and Boyle (1985) and Wise et al. (1985), using a CCV-specific DNA probe, clearly demonstrated the presence of CCV nucleic acid in livers of adult channel catfish.

Period of Communicability

Infectious channel catfish virus can be detected from the time when the first clinical signs appear until soon after death. In surviving experimentally infected fingerlings, the virus begins to disappear after 120 h. Isolating virus by routine procedures is difficult or impossible once the clinical signs—enlarged abdomen, exophthalmia, and hemorrhage—have passed.

Susceptibility and Resistance

The channel catfish is clearly the principal species affected by CCV. Experimental infection
was induced in fingerling blue catfish (*Ictalurus furcatus*) and in hybrid channel catfish × blue catfish by injection, but not by oral exposure nor by cohabitation with virus-infected channel catfish fingerlings (Plumb and Chappell 1978). Brown bullheads (*I. nebulosus*) or yellow bullheads (*I. natalis*) could not be infected by injecting or feeding virus. Plumb et al. (1985) demonstrated that the European catfish (*Silurus glanis*) is resistant to CCV. Feeding virus to different strains of channel catfish fry has indicated variation in susceptibility (Plumb et al. 1975). The young resulting from the crossing of different strains of channel catfish were more resistant to CCV than were pure strains. Channel catfish up to 1 year old or 10–15 cm long were susceptible under experimental conditions. Injected virus killed fish weighing up to 50 g. McDowell and Hedrick (1986) reported clinical signs of CCVD and virus isolation from 2.5-year-old adult channel catfish 1 week following waterborne exposure to the virus. The growth of survivors of CCV epizootics is sometimes stunted (McGlamery and Gratzek 1974).

### Range

Channel catfish virus has been reported in most Southern States and from culture systems in most other areas of the United States where channel catfish are grown. It has also been isolated from fry shipped from the United States to Honduras, Central America. The isolation of CCV from wild channel catfish has not been reported.

### Occurrence

Cases of CCVD have been diagnosed in the period from June to September. A number of disease outbreaks occurred after handling and when water temperatures were above 25°C. Laboratory experiments and field studies indicated that temperatures of 25 to 30°C favor the development of CCVD. The occurrence of epizootics at channel catfish farms increases during years of high water temperature and in fingerling ponds that are heavily stocked. Although Bowser et al. (1985) isolated CCV from adult channel catfish during January, the virus was not considered detrimental to the fish.

### Methods of Control

There is no known chemotherapeutic treatment for CCVD or other viral diseases of fishes. The only practical control measures are avoidance, isolation, and sanitation. The incidence of CCVD is closely correlated to temperature. In laboratory experiments, mortality decreased significantly when, after infection, the water temperature was reduced from 28°C to 19°C or less. Although this procedure has only limited application, it may be useful when cool water is available.

Channel catfish virus becomes attenuated when passaged frequently and rapidly in K1K (walking catfish kidney) cells (Noga and Hartmann 1981). When channel catfish are injected with or bathed in the attenuated virus they become immune to challenge with wild type (virulent) CCV. This response suggests that vaccination against CCV is possible.

Surveying brood stocks for CCV neutralizing antibody or testing stocks of fish during winter may also be feasible as steps toward avoiding the disease.

Ponds from which diseased fish are removed should be drained or disinfected with 40 ppm chlorine. Survivors of CCV epizootics may be grown to a marketable size, but the fish should be held in ponds that are separated from ponds containing unexposed channel catfish. Under no circumstances should survivors of CCV epizootics be stocked in noninfected waters, nor should they be used as brood stock.

### Annotated Bibliography


Problems discussed concern the use of serum neutralization assays to determine prior exposure to channel catfish virus (CCV), and seasonal fluctuations of serum neutralization titers. Population history and mortality patterns are not reliable for determining previous exposure to CCV. Although there was a good correlation between detection of serum neutralization assays in brood fish and the presence of CCV in fry, more information is needed before serum neutralization assay tests can be used to predict the presence of CCV in channel catfish populations.

Comparisons were made between brown bullheads and channel catfish ovary cells for detecting CCV and measuring CCV neutralizing antibody; the cells of brown bullheads were the less sensitive; therefore, it was suggested that brown bullhead cells be used for serum neutralization tests because they enable detection of a lower concentration of virus. Serum neutralization tests were standardized by mixing equal volumes of CCV containing 100 TCID<sub>50</sub> with sera diluted 1:25 for 1 h at 25°C, and assaying the mixture in a 96-well microtiter plate. When this procedure was used, serum neutralization antibody to CCV was detected in brood populations at 7 of 17 farms and CCV was later isolated from fingerlings at 2 of these 7 farms.


In this first reported isolation of CCV from adult channel catfish, the virus was isolated from the kidneys of a moribund adult male in January 1984, when water temperature was 8°C. After immunosuppression, CCV was isolated from leucocytes and kidneys of 7 of 15 seemingly normal adults from the same population.


A cell line was established from ovaries of a healthy, juvenile channel catfish. By 1980, these ovary cells had been passaged about 130 times. The cells grew well in Eagle’s MEM-10 at 25 to 30°C. The species of origin of the cells was confirmed by a cytotoxic dye exclusion test. Although the channel catfish ovary line replicated CCV, it was refractory to four other fish viruses.


Growth rates of a new cell line from channel catfish were determined at temperatures of 10 to 35°C. Growth was most rapid at 35°C but the viral yield was highest at 30°C; cell numbers declined at 10°C. The cells survived storage at −70°C for as long as 6 months.


In a comparison of cell lines from the channel catfish ovary and the brown bullhead, that from the channel catfish ovary showed cytopathic effect more rapidly and detected CCV at higher dilutions. Replication of channel catfish virus was more rapid in channel catfish ovary cells than in brown bullhead cells, but the peak titers were not significantly different.


The first published account of CCVD. Mortalities occurred about 1 week after fish were exposed to low oxygen concentration; total morbidity was about 40%. Clinical signs were briefly described.


Clinical signs of CCVD are described in detail. The disease had a peracute to acute course, and environmental stress preceded most epizootics. A heat-labile virus smaller than 0.22 µm was isolated from diseased fish on primary channel catfish ovary cell cultures. River’s postulates were fulfilled and CCV was established as the cause of four epizootics. The virus multiplied and produced cytopathic effects in cultures of brown bullhead cells, inducing the formation of syncytia in affected cultures.


Inoculation of channel catfish with Chondrococcus columnaris cells and CCV resulted in a rapid primary immune response to both antigens. Analysis of total serum proteins and selected isozyme systems by acrylamide gel electrophoresis revealed differences between normal and experimental fish sera in the number of protein components. Specific antibody activity of the immune serum occurred in the macroglobulin fraction. Partial antibody characterization indicated that specific channel catfish immunoglobulins are macroglobulins, with characteristics like those of most fish immunoglobulins.


Several approaches were taken to detect CCV in adult fish. It was not isolated from explanted tissues, by co-cultivation with cell lines that permitted CCV replication, or through prolonged culture and chemical induction. Enzyme linked immunosorbent assays were not effective in detecting CCV. Molecular hybridization was not useful for the detection of latent CCV in
seropositive fish. In infected cells, CCV induced high levels of thymidine kinase that displayed novel electrophoretic mobility.


Two groups of fingerling channel catfish, one experimentally infected with CCV by transmission through water and the other collected during an epizootic, were examined histologically. Previously unreported pathologic changes of pancreatic and brain tissue are described.


Disease-free channel catfish were used to study the pathogenesis of CCVD and to determine a median lethal dose for 30-day-old fry. An LD50 was not established when fertile eggs and 30-day-old fry were used, suggesting that high mortality under natural conditions is not due solely to the virus but also to concurrent infections and environmental conditions.


A brief description of the response of 2.5-year-old channel catfish to waterborne exposure to CCV. The immune response and isolation of CCV is also reported.


Stunting was reported in channel catfish that survived a channel catfish virus infection under experimental conditions.


Cell lines were established from apparently normal gill, gonad, and kidney tissue of adult walking catfish. The gill and kidney cells were the first to be established from these tissues in fish. The cells were propagated in a modified Ham's F-12 medium at 25°C but were capable of growing at 37°C. All three cell lines were susceptible to CCV as shown by cell fusion and other marked cytopathic effects. A live attenuated vaccine strain of CCV was produced by repeated passage of virulent virus in kidney cell cultures. The vaccine strain was less virulent than the wild-type CCV and protected fingerling catfish against challenge with wild-type virus.


Channel catfish virus disease is caused by a specific virus that has been isolated from fish in 23 epizootics reported from nine States. Clinical signs, mortality patterns, and susceptibility of fish of different ages and sizes to CCV are described. Recommended practices for controlling the disease are presented.


The kidneys, liver, intestine, brain, and muscle from live infected channel catfish were assayed for CCV in channel catfish gonad cell cultures. Tissues were sampled at 24-h intervals for 120 h. The virus was detected first in the kidneys of channel catfish, 24 h after inoculation, and later was isolated from the intestine, liver, brain, and muscle.


Mortality differed among lots of channel catfish fingerlings held at different temperatures 24 h after injection with CCV; it was 24% when the water temperature was reduced from 28°C to 19°C, 94% in fish held at a constant temperature of 28°C, and 14% in fish held at 19°C; it was 78% when the temperature was reduced to 19°C after the first deaths had occurred at 28°C (64–72 h after injection). The potential value of temperature manipulation in reducing the effects of CCVD under cultural conditions is discussed.


When sera from 71 adult channel catfish known to have been exposed to CCV were assayed for CCV neutralization activity, 67 showed positive channel catfish neutralization indices. Sera from 10 fish with no known history of exposure to CCV showed no evidence of virus neutralization activity. Virus was not isolated from 232 samples of organs or excretory products from the fish studied. Serum from channel catfish experimentally immunized with viable CCV showed peak neutralization indices 60 days after virus injection. Detection of channel catfish virus serum neutralization activity may identify channel catfish populations that have been exposed to CCV.

This review of viral diseases of fish in the Southern United States includes limited original data on the inactivation of CCV. Virus was inactivated in less than 1 day when held on concrete chips, in 1.5 to 2 days on dried net and glass, and in 1 day in pond mud. Virus in pond water was inactivated in 7 days at 25°C and in 56 days at 4°C.


A summary of the known information on the epizootiology of CCV.


An immunofluorescence technique was used to detect CCV in CCO cells 4 h postinfection. The refractiveness of young brown, black, and yellow bullheads to CCV by various methods of virus exposure was reported.


The susceptibility of blue catfish and reciprocal channel catfish × blue catfish hybrids to CCV was determined by using several methods of exposure. Blue catfish and hybrids of channel catfish × blue catfish were as susceptible as channel catfish to intraperitoneal injection of CCV, but were refractory to infection when the gills were swabbed, the fish were dipped into a virus solution, or the fish were held with channel catfish having CCVD.


Sequential virus titrations were made on kidneys, intestine, liver, spleen, brain, and blood of fish injected with CCV. Corresponding sequential histopathogenesis is described for each tissue except blood. Virus titers were highest in kidneys, intestine, and liver; virus production peaked 3–5 days after exposure. Histopathology was characterized by necrosis, hemorrhage, and edema.


A histologic and electron microscopic study was made of selected organs from channel catfish fingerlings that were experimentally infected with CCV. Histopathology was characterized by necrosis and hemorrhage in kidneys and liver, and hemorrhage in the spleen and gastrointestinal tract. Virus replication occurred in nuclei of cells in the kidneys, liver, and spleen. Intranuclear inclusion bodies, consisting of geometric crystalline arrays and lamellar structures, were associated with virus replication.


Experimental infections were made by feeding CCV to six strains of 1-, 2-, and 3-month-old channel catfish from different geographical areas and to two crossbred strains. Although there was no significant difference in mortality among age groups, mortality rates differed significantly between the various strains, mean mortality ranged from 10 to 71% among infected fish. Mortality was lowest in hybrid strains.


European catfish were tested for susceptibility to CCV. Fingerlings (weight, 2–4 g) supported CCV replication at 2 days after intraperitoneal injection of $10^5$ TCID$_{50}$. Homogenates of liver, kidneys, and spleen contained $10^5$ TCID$_{50}$ per gram of tissue 2 days after inoculation, but the titer decreased to $10^2$ TCID$_{50}$ per milliliter at 4 days. Bathing European catfish in CCV yielded only one positive sample with a titer of $10^1.83$ TCID$_{50}$ per gram of tissue. No clinical signs of CCVD developed, and no virus-related deaths occurred.


A review paper of CCVD, with sections on history, geographical distribution, etiology, epizootiology, diagnosis, and methods for prevention and control of the virus.


When assays were performed on suspected CCV carrier fish, serum neutralization titers did not change from prespawning levels. No CCV was isolated from internal organs, eggs, or fry, nor were virus-like particles seen in electron micrographs of ovarian tissue.
Indirect fluorescent antibody tests demonstrated fluorescent inclusions in the first passage ovarian cell cultures, and focal areas of fluorescence were present in frozen ovarian sections from the suspected CCV carriers treated with two levels of Betamethasone. No fluorescence was observed in either primary cell cultures or tissue sections from fish free of CCV.


Channel catfish virus survived for only 2 days in decomposing channel catfish, but it survived for 14 days in iced fish, and for more than 200 days in fish frozen at −20 °C and −80 °C.


Channel catfish virus was purified by precipitation with PEG-6000 and isopycnic centrifugation in a metrizamide gradient. The final recovery of infectivity was about 30%, and only 0.09% and 0.05%, respectively, of the host DNA and the host proteins were recovered at the position of the viral band. Electron microscopy showed mostly intact viral particles.


Channel catfish virus was attenuated by frequent passage in walking catfish (K1K) cells. Waterborne administration of the attenuated CCV provided protection against lethal challenge with virulent wild type CCV. Other cell culture characteristics of the attenuated CCV are given.


Two populations of channel catfish were examined for CCV by using a nucleic acid probe. In one population with a history of CCV, co-cultivation of the leucocytes with catfish tissue cells identified virus in 10 of 22 fish, whereas the DNA of CCV was found in all 22 fish when the DNA probe was used. In a second population, the use of the DNA probe led to detection of viral DNA in 11 of 14 fish that had survived a CCV epizootic.


A nucleic acid probe for CCV was constructed by using recombinant DNA techniques. The probe—a specific viral DNA fragment generated by digestion of channel catfish virus DNA with the restriction enzyme EcoRI—was used to detect viral DNA isolated from tissues of fish that had been injected with CCV. The sensitivity of detection was one viral DNA per cell.


Channel catfish virus was studied in ictalurid fish cell culture, the only cell culture system of fish, amphibian, avian, and mammalian origin that was susceptible. Channel catfish virus infection resulted in intranuclear inclusions and extensive syncytium formation. Replication occurred at 10 to 33 °C, but best growth was at 25 to 33 °C. The amount of virus released nearly equaled the amount that remained cell-associated. The virus contained essential lipids, and indirect determinations with labeled precursors and a metabolic inhibitor showed evidence of DNA. Electron microscopy showed progeny virus, about 100 nm in diameter, in various stages of development in cell nuclei by 4 h after infection. Nuclear masses of exceptionally electron-dense lamellar material were also present. Virus was enveloped at the nuclear membrane and in cytoplasmic vacuoles, resulting in virions having a diameter of 175 to 200 nm. Negative staining demonstrated icosahedral symmetry and 162 capsomeres. The data indicated that the CCV is a herpesvirus.


Histologic examination of fry experimentally infected with CCV showed hemorrhagic and necrotic disease; the target organs were the kidneys, liver, digestive tract, and skeletal muscles. The results agreed with clinical signs of the disease and with published data on quantifications of virus in specific organs.

NOTE: Use of trade names does not imply U.S. Government endorsement of commercial products.