SUSCEPTIBILITY OF BLUE CATFISH TO CHANNEL CATFISH VIRUS

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Abstract: The susceptibility of blue catfish, (*Ictalurus furcatus*), and reciprocal channel (*I. punctatus*) x blue catfish hybrids to channel catfish virus (CCV) was determined through several methods of exposure. Mortalities of blue catfish, when injected intraperitoneally with CCV, were similar to what would be expected with channel catfish. Histopathology of CCV-injected blue catfish did not deviate from that of similarly infected channel catfish. Infection of blue catfish and the hybrids by swabbing the gills, dipping fish in a virus solution, or by cohabitation with CCV-diseased fish were primarily ineffective. It is concluded that blue catfish and hybrids of channel catfish x blue catfish are as equally susceptible as channel catfish to injection of CCV but the blue catfish are refractive to the virus by more natural routes of transmission.

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Channel catfish virus is an infectious disease of hatchery-reared channel catfish fry and fingerlings (Fijan, et al. 1970; Plumb 1971a). The virus has not been isolated from a hatchery-reared species other than channel catfish, although blue catfish have occasionally been in proximity to CCV-infected channel catfish populations. It has been demonstrated that white catfish (*I. catus*) are only slightly susceptible to CCV, but blue catfish are more susceptible by experimental infection (N. N. Fijan, personal communication; Plumb 1971a). However, the means of infection and effect of CCV on blue catfish as well as other species of catfish have not been reported. This paper describes the efficacy of several routes of infection on blue catfish, comparative susceptibility of channel, blue, and reciprocal blue x channel catfish hybrids to CCV, and rate of CCV replication in blue catfish.

METHODS AND MATERIALS

Susceptibility of channel catfish, blue catfish, and reciprocal channel x blue hybrids to channel catfish virus (CCV) was compared in 2 experiments. In Experiment I the susceptibility to CCV was determined only in blue catfish by various methods of infection. In Experiment II blue, reciprocal channel x blue hybrids and channel catfish fingerlings were utilized.

The Auburn strain of channel catfish virus ($CCVA_{la}$) was used in all tests and the brown bullhead (BB) cell line (ATCC-59) was employed in growing CCV for inoculation and virus assay of fish. All virus assays and virus titrations were made by serial 10-fold virus dilutions in Hanks balanced salt solution (HBSS) and inoculations of BB cell monolayers in microtiter plates (Gratzek et al. 1973). Infectivity of the 10° dilution (titer) was calculated by the Reed and Muench (1938) method and expressed as tissue culture infectious doses -50% end point ($TCID_{50}$).

All experimental infections of fish were carried out in static 57 l aquaria filled with 40 l of dechlorinated municipal water and aerated with compressed air. Water temperature was 27 ± 1 C.

Experiment I

Blue catfish fingerlings, obtained from the Southeastern Fish Cultural Laboratory, U.S. Department of Interior, Marion, AL, were used in Experiment I. They were approximately 6 weeks old, 5 cm (4.8 to 5.6 cm) in length and averaged 1.9 g each. Thirty fish were injected intraperitoneally with 0.1 ml of each of 10-fold serial virus dilutions from 10^{-1} through 10^{-6} of CCV with an in vitro titer of $10^{6.5}$ $TCID_{50}$/0.1 ml. Fish injected with the respective dilutions were divided equally into 3 aquaria and maintained for 14 days. Two aquaria were stocked with 10 control fish each that were injected with 0.1 ml

HBSS. Deaths were collected daily, and samples of moribund fish 72 hours post injection (PI) were assayed for virus according to the method of Plumb (1971b). Four moribund, virus injected fish were removed from the 10^{-3} or 10^{-4} infected groups 3 days PI, the abdomen opened, and preserved in Bouins fluid. From each of 4 infected and 2 control fish, the liver, posterior and anterior kidney, spleen, brain, and intestine were removed, embedded in paraffin, sectioned and stained with Harris' hematoxylin-eosin. Three moribund blue catfish from aquaria injected with 10^{-3} and 10^{-4} virus dilutions were placed in an aquarium with 24 healthy blue catfish fingerlings to test horizontal CCV transmission.

Virus dilutions of freshly harvested tissue culture medium from CCV infected BB cells were made in 1 liter of dechlorinated tap water so that the respective dilutions contained $10^{3.5}$, $10^{2.5}$, $10^{1.5}$, and $10^{0.5}$ $TCID_{50}$ per ml of water. Twenty blue catfish were placed into each aerated virus concentration for 4 hours, and 20 fish were placed in a bucket with 1 liter of noncontaminated water. Fish in each virus dilution and the controls were divided equally into separate aquaria providing 2 replicates of 10 fish each for each treatment. The gills of 30 additional fish were bathed with a cotton swab dipped in a virus dilution containing $10^{5.5}$ $TCID_{50}$/ml and the fish were divided into 10 fish groups in 3 aquaria.

Ten blue catfish fingerlings were placed in each of 3 aquaria and fed a commercial No 3 trout granule on demand until they readily accepted the feed. Filtered (.45 μm) tissue culture medium with a titer of $10^{5.5}$ $TCID_{50}$/0.1 ml from CCV infected BB cells was fed to the fish on 3 consecutive days. Fish in 2 additional aquaria were fed a virus free ration. Fish in each aquarium were fed 1.5 g (3% of body weight) per day. Three ml of solution containing virus were mixed with the feed which was equally divided among the 3 aquaria; so that the calculated dose of virus suspension was 0.1 ml/fish. The remaining virus solution was refrigerated (4 C) and used on the 2 subsequent days. The study was terminated after 15 days.

Experiment II

Channel and blue catfish fingerlings and both reciprocal hybrids were obtained from breeding experiments of the Fisheries Research Unit, Auburn University. The channel catfish averaged 8.7 cm (8.2-9.2) and 5.8 g, the blue catfish averaged 9.5 cm (9.0-10.0) and 6.9 g, channel ♀ x blue ♂ averaged 9.1 cm (8.0-9.5) and 6.7 g and the blue x channel ° averaged 10.1 cm (9.0-11.0) and 9.8 g. Each group was identified by clipping a particular fin.

Triplicate lots of 5 fish each from the different species or hybrids were inoculated intraperitoneally with 0.1 ml from each dilution of a particular 10-fold serial dilution of CCV with a 10° titer of $10^{5.5}$ $TCID_{50}$/ml. Each replicate was placed in an aquarium along with 5 marked fish from each of the other groups inoculated with the same virus dilution. In this way 3 replicate titrations were obtained for each test group. Dead fish were removed daily. The lethal dose-50% end point (LD_{50}) for each group of fish was determined at 14 days by the Reed and Muench (1938) method. HBSS injected controls from each group were maintained at the same stocking density. Moribund or fresh dead fish were assayed for CCV in BB microtiter plates.

A second group of fish was inoculated with CCV to determine replication rate of CCV. Twenty-five blue, channel, and channel x blue ° hybrids were inoculated IP with 0.1 mm of a CCV dilution containing $10^{4.5}$ $TCID_{50}$/0.1 ml and were placed in separate aquaria. Three living (some moribund) fish were removed 6 hours PI and then at 24 hour intervals for 4 days, and pooled viscera of 3 fish were assayed for CCV and titrated (Plumb 1971b).

RESULTS AND DISCUSSION

Experiment I

Blue catfish injected with $10^{5.5}$ $TCID_{50}$ exhibited clinical signs of CCVD 24-36 hours PI and the first deaths occurred at 48 hours PI. By 56 hours 100% of this group was dead. The mortality in other groups followed a progressive pattern (Fig. 1); with increased dilution initial mortality was delayed and the total mortality was less. Final mortalities of other groups injected with the respective virus levels were: $10^{4.5}$ $TCID_{50}$ - 100%, $10^{3.5}$ $TCID_{50}$ - 80%, $10^{2.5}$ $TCID_{50}$ - 83%, $10^{1.5}$ $TCID_{50}$ - 70%, and $10^{0.5}$ $TCID_{50}$ - 23% while the control group had 6.7% mortality. A high rate of mortality (70%) may develope when even a small number of virions (32 $TCID_{50}$) penetrate the blue catfish fingerling.

Severe histopathology, similar to that in CCV infected channel catfish (Wolf, et al. 1972; Plumb et al. 1974), occurred in blue catfish which were injected with CCV. Hemorrhage, focal necrosis of tubules, but little edema, were present in the posterior kidney. The anterior kidney was characterized by hemorrhage, necrosis, edema, and enlarged cells. Massive hemorrhage occurred in the spleen which contained enlarged cells and possibly some accumulation of hemosiderin. Liver was hemorrhaged and epithelial cells were necrotic. The stomach had little injury, but the lumen of the intestine was filled with cellular debris, possibly epithelial sloughing (Wolf et al. 1972) and red blood cells. The epithelium of the intestinal folds was necrotic and in some areas completely destroyed. Extensive hemorrhage was evident in the intestine. The brain appeared normal, but hemorrhage was present in cranial nerve ganglia near the brain.

Healthy blue catfish fingerlings exposed to moribund fish dying with CCV were held for 30 days, during which time no deaths occurred in the healthy fish, therefore horizontal transmission was not attained. A few mortalities occurred in the fish dipped in the $10^{3.5}$ $TCID_{50}$/ml virus solution. The first fish died the second day after exposure and no mortality occurred after 6 days. CCV was isolated from moribund fish which died 5 and 6 days PI. respectively, but not from specimens collected 2 and 4 days. No control fish died and none of the fish infected by swabbing the gills died. The blue catfish readily ate the CCV contaminated feed, but no deaths occurred in any of the tanks in this study.

Experiment II

The LD_{50} of channel, blue and reciprocal hybrids of the 2 species indicated that the channel catfish used here were less sensitive to the injected CCV than either the blue catfish or the 2 hybrids (Table 1). CCV was reisolated from moribund specimens of all groups inoculated with $10^{4.5}$ and $10^{3.5}$ $TCID_{50}$ during the first 3 or 4 days after injection and from all groups injected with $10^{2.5}$ and $10^{1.5}$ $TCID_{50}$ except the channel catfish. CCV was not isolated from any fish injected with $10^{0.5}$ $TCID_{50}$. The control groups which were injected with HBSS suffered from 33 to 47% mortality which correlates to mortalities in the test groups injected with $10^{1.5}$ and $10^{0.5}$ $TCID_{50}$, however, no virus was isolated from the control fish.

Sequential virus titrations were obtained from viscera of channel catfish, blue catfish and channel♀x blue♂hybrids (Fig. 2). The replication rates were very similar in the blue catfish and the hybrids, both reaching peak virus levels at 48-72 hours PI, and then dropping rapidly at 96 hours. The maximum titers of $10^{5.5}$ $TCID_{50}$/g for the hybrid and $10^{5.66}$ $TCID_{50}$/g for the blue are very similar to that reported for channel catfish (Plumb 1971b; Plumb and Gaines 1975). The peak virus levels in channel catfish in this study were 3 logs less than that of the other groups; it reached a peak of $10^{2.5}$ $TCID_{50}$/g at 72 hours PI, which is below the titers of over 10^5 $TCID_{50}$/g previously reported from various organs (Plumb and Gaines 1975). This phenomenon of lower virus replication in the channel catfish might be expected when considering that this group of channel catfish also exhibited low susceptibility to CCV.

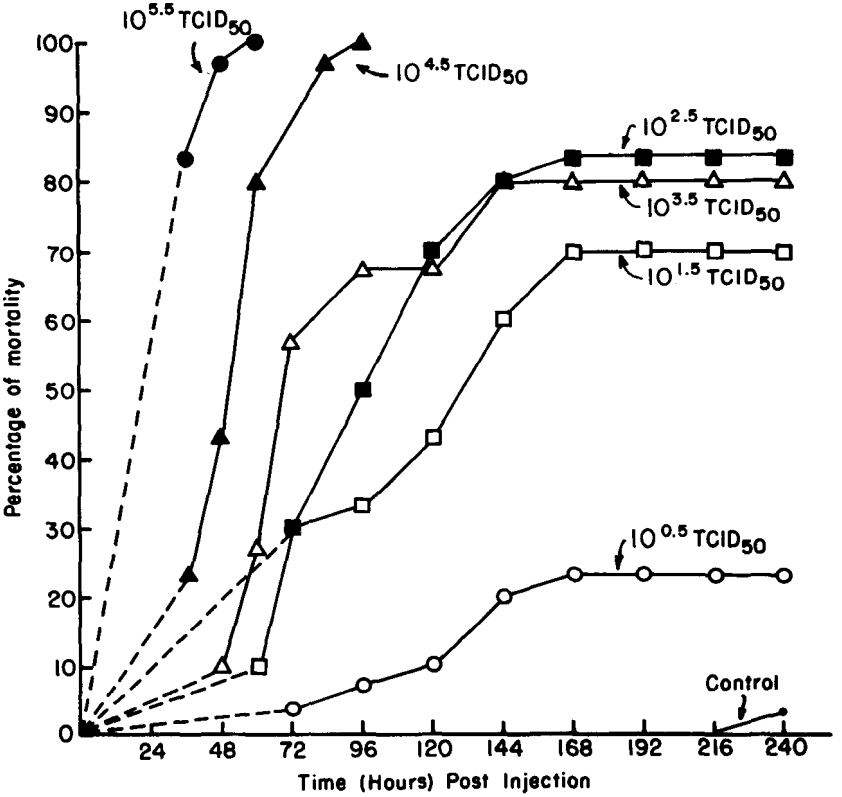

Fig. 1. Mortalities of blue catfish fingerlings at 27 C injected with serial 10-fold dilutions of channel catfish virus. Titer of the 10° virus suspension was $10^{6.5}$ TCID$_{50}$ per ml.

Table 1. LD$_{50}$ of CCV in fingerling channel catfish, blue catfish, and 2 reciprocal hybrids of these species. In vitro titer of injected virus in BB cell cultures was $10^{5.5}$ TCID$_{50}$/0.01 ml.

Broodstock pairing	*Replicate LD$_{50}$*[a]			*Composit LD$_{50}$*
	1	*2*	*3*	
Channel x Channel	$10^{2.73}$	$10^{2.75}$	$10^{4.6}$	$10^{3.29}$
Blue x Blue	$10^{4.36}$	$10^{3.82}$	$10^{5.55}$	$10^{4.5}$
Channel ♀ x Blue ♂	$10^{5.16}$	$10^{4.52}$	$10^{4.88}$	$10^{4.87}$
Blue ♀ x Channel ♂	$10^{4.2}$	$10^{2.88}$	$10^{4.7}$	$10^{4.08}$

[a]Reciprocal of the negative log of dilution at which 50% of injected fish died (LD$_{50}$) as determined by the Reed and Muench (1938) method.

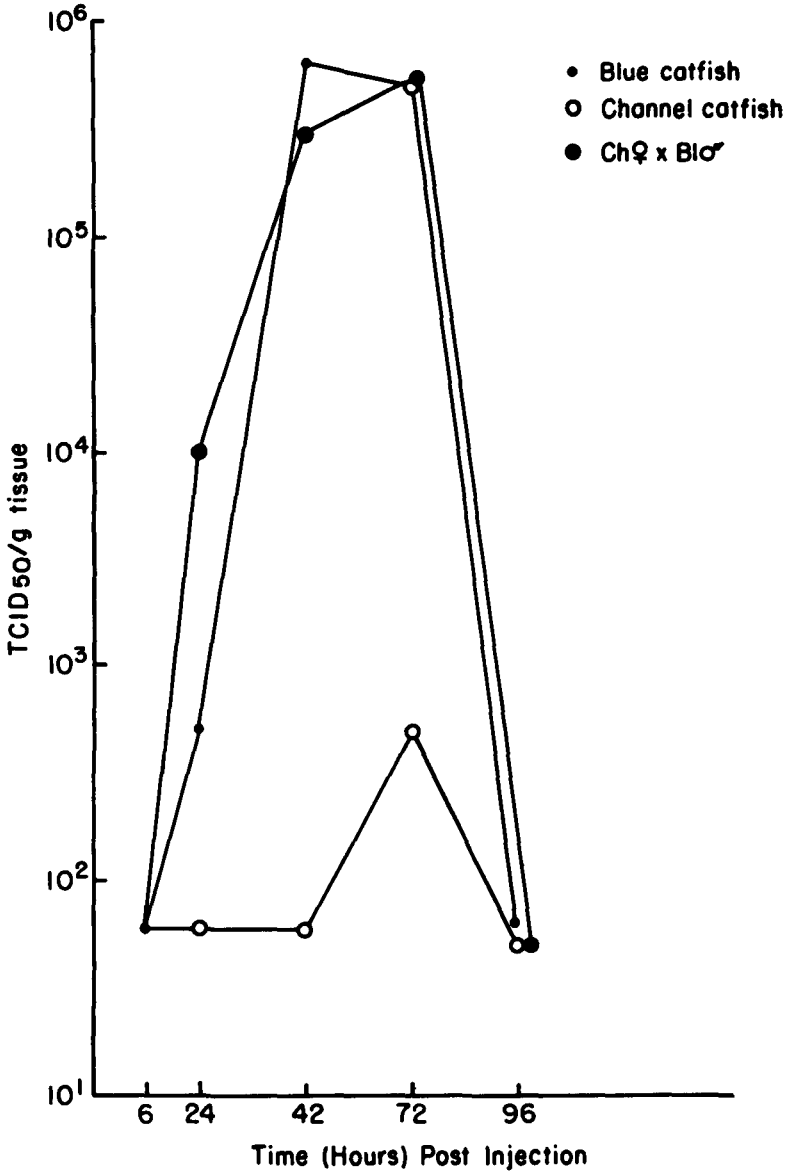

Fig. 2. Sequential virus titer of CCV isolated from virus injected channel, blue and female channel x male blue hybrid catfish fingerling. Each fish was injected with 0.1 ml of CCV containing $10^{4.5}$ $TCID_{50}$.

CONCLUSIONS

Of the infectivity routes investigated in the present study, injection was the only one which resulted in a severe CCV infection in blue catfish. Deaths did occur as result of dipping fish in water containing CCV, but the mortalities were inconsistent. Swabbing the gills with virus or administering them orally were not successful in transmitting CCV to blue catfish. In comparing susceptibility of hybrid channel x blue catfish, the hybrids appear to be as susceptible to CCV by injection as channel catfish. At farms which have an indigenous problem with CCV that severely affects their production, managers may want to consider substituting blue catfish for the more popular, but more susceptible, channel catfish.

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