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Serological Screening of Channel Catfish Virus

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ABSTRACT—Disease-free channel catfish, Ictalurus punctatus, *were used to study the pathogenesis of channel catfish virus disease and to determine a median lethal dose for exposed 30-day-old hatched fry. We were unable to establish an* LD_{50} *using fertile eggs and 30-day-old fry which were maintained under disease-free conditions. This data suggests that the high mortality seen in infected catfish under natural conditions is not due solely to the virus but results from concurrent infections and environmental conditions.*

In an effort to study in depth the pathogenesis of channel catfish virus (CCV) we attempted to: 1) Obtain disease-free fish, 2) develop the methodology for characterizing the infecting virus, and 3) assay the host response.

Our first need was a source of fish free of active or latent CCV and/or adventitious agents and diseases. We also wanted to raise these fish under laboratory conditions. The starting virus pool had to be characterized and purified. We obtained a pool of CCV from John Plumb[1] and used the plaque technique to clone the virus.

Our problems began when we tried to interpret our serological data and to establish disease and lethality patterns for the plaque-purified virus pool. We needed to choose: 1) A screening method to serologically define a population of fish with statistical validity, and 2) an assay technique to find latent infections and evidence of previous exposure to CCV.

SCREENING PROCEDURE

All serum samples were screened at a 1:8 dilution against a calculated challenge of 10 tissue culture infective doses ($TCID_{50}$) of CCV in a tube system using a minimum of three tubes per point. The design was based on our experience with a number of mammalian herpesviruses which we routinely screen. We have used this test for the last 3 years. As a part of this investigation, we screened a number of serum samples obtained from adult channel catfish, *Ictalurus punctatus,* from the Ft. Worth, Tex. area. These specimens were provided by J. P. McCraren[2] as part of a cooperative study. The acceptability of the results of the serological tests reported by our laboratory were questioned because our serum neutralization (SN) method was not in agreement with the 1974 recommendations of the CCVD Technical Procedures Committee.[3]

The criteria set up at the Denver meeting of the CCVD team for identifying a positive antibody response specified the use of a 1:100 serum dilution inoculated with 100-$TCID_{50}$. I think the test is not sensitive enough for most herpesviruses. We did not get that kind of antibody response normally, not even in fish that we hyperimmunized against CCV using virulent CCV in a water-in-oil adjuvant (Tween 80 - Arlacel-sterile mineral oil)[4]. The maximum antibody titers obtained ranged from 1:64 to 1:128.

Table 1 shows the results of our CCV antibody screening technique, and the data demonstrates the potential of the screening method. As can be seen in the table, all samples registered by the tube test as positive gave significant log neutralization indices (LNI) when tested in this manner. One sample, fish serum No. 44 tested only by the plaque reduction test neutralized 4 $\log_{10}$ of virus (90 percent of the plaque forming units present) as compared to the control titration. The test errs in the plus-minus area where significant LNI were obtained in two of the three specimens recorded as suspicious.

Table 1.—Comparison of tube serum neutralization screen test to log neutralization index test.

Fish serum	Tube screen	Interpretation[1]	LNI[2]
18	[3]2/3	–	0.5
19	0/3	+	4.0
24	0/3	+	5.4
26	0/3	+	3.0
27	2/3	–	0.0
32	0/3	+	3.0
41	0/3	+	3.0
43	0/3	+	5.0
44	0/3	+	[4]4.0
46	0/3	+	1.5
47	1/3	±	1.5
48	0/3	+	5.0
50	0/3	+	5.0
59	2/3	–	0.5
52	1/3	±	2.5
55	1/3	±	3.0

[1] – = no antibody; ± = suspicious; + = antibody.
[2] LNI = log neutralization index.
[3] Number of tubes with cytophathic effect (CPE) over total.
[4] Tested by the plaque reduction (90 percent) method.

[1] Auburn University, Auburn, Ala.

[2] U.S. Fish and Wildlife Service, San Marcos, Tex.

[3] CCVD Technical Procedures Committee Meeting, Denver, Colo. 1974. (American Fisheries Society, Fish Health Section Meeting, U.S. Fish and Wildlife Service, 1975.)

[4] Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

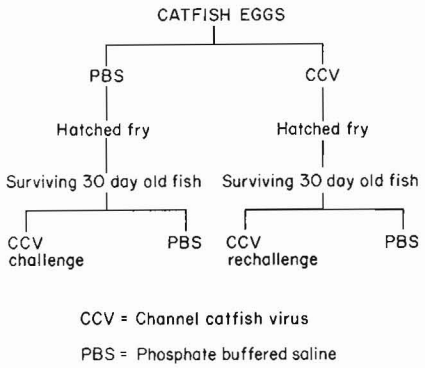

Figure 1.—Schematic of egg and fish hatch and channel catfish virus (CCV) challenge. Challenge and control (phosphate buffered saline, PBS) exposures—immersion in beakers for 1 hour at 25°C.

Table 2.—Results of egg channel catfish virus challenge studies.

Group	Sample	Challenge dose[1]	Virus reisolation[2]	Fish sick or dead on hatch
				All dead within
1	Eggs	3.5 logs	No	5 days
2	Eggs	2.5 logs	No	None + +[3]
3	Eggs	3.5 logs	No	None + +
Controls (1-3)	Eggs	PBS	No	None + +

[1]3.5 logs = 3,160-$TCID_{50}$/0.1ml; 2.5 logs = 316-$TCID_{50}$/0.1 ml; PBS = phosphate buffered saline—also used as virus diluent; TCID = tissue culture infected doses.
[2]Days sampled = 1 through 7.
[3]+ + = approximately 75 percent of all hatched fish survived. No difference between CCV-challenged eggs and PBS controls.

Table 3.—Results of channel catfish virus studies on 30-day-old catfish.

Group[1]	Sample[2]	Challenge dose	Virus	Fish sick or dead
2A	fish	PBS	no	none
2B	fish	2.5 logs	no	none
2C	fish	2.5 logs	yes[3]	yes
2D	fish	PBS	no	none
3A	fish	PBS	no	no
3B	fish	3.5 logs	yes[4]	yes
3C	fish	3.5 logs	yes[5]	yes
3D	fish	PBS	no	no

[1]A = control (PBS); B = egg control, 1st challenge; C = CCV egg, rechallenge; D = CCV egg, PBS control.
[2]Daily sample size = 8-10 fry.
[3]Days 4, 7, and 8 postinfection.
[4]Day 3 postinfection.
[5]Days 3, 4, and 6 postinfection.

ATTEMPTS TO ESTABLISH AN LD_{50}

Another problem was to attempt to establish an LD_{50} for cloned CCV in laboratory-raised fish. Establishment of an LD_{50} is important for the performance of back-challenge studies.

We screened a sizable number of fish from a hatchery with no known history of CCV. We found no evidence of CCV antibodies in the fish sampled. We obtained eggs from the hatchery and transported them to our laboratory tanks where equipment and hatching water were checked bacteriologically for freedom from contaminating organisms in an effort to establish and develop disease-free fish.

Lethality and pathogenesis were studied using two modes: 1) virulent CCV challenge of egg clutches, and 2) virulent CCV challenge of sac fry obtained from these egg clusters. A flow diagram for the CCV challenge is illustrated in Figure 1.

Three separate spawns of eggs were challenged with CCV using an immersion period of 1 hour. The first and third spawns were challenged with 1,000 $TCID_{50}$ of virus, and the second spawn was challenged with 100 $TCID_{50}$. After exposure to the virus, fluid was decanted, the eggs returned to a holding aquarium, and the batch was allowed to proceed. Samples of CCV and phosphate buffered saline (PBS) exposed eggs were collected immediately after exposure and at 24-hour intervals thereafter for 7 days.

The first phase of the study examined the susceptibility of fertilized eggs to CCV. The estimated number of eggs per group was 1,300 (CCV) and 1,300 (PBS) for group 1; 2,600 (CCV) and 3,600 (PBS) for group 2; and 2,400 (CCV) and 3,000 (PBS) for group 3. The number of hatched fish were: Group 1, 1,000 and 1,100; Group 2, 1,950 and 2,700; and Group 3, 1,800 and 2,250 with an average hatching survival of 75 percent.

Exposure or fertilized eggs to CCV by submerging them in either a 100 $TCID_{50}$/ml (group 2) or 1,000 $TCID_{50}$/ml (group 1 and 3) virus suspension did not result in the expression of overt disease, nor interfere with the resultant fry. The results of these challenge studies are shown in Table 2. No egg sample or resultant sac fry collected during the experiments yielded infectious virus on reisolation efforts, nor were we able to reisolate virus from the aquatic environment. All samples assayed were subpassaged three times before being considered negative for CCV.

In the second phase of the study the hatched fry (groups 2 and 3) were subdivided into four subsets and half of them challenged with the same dose of CCV as used for the egg clutch studies. For easy reference they were subdivided as follows: Progeny from the PBS egg control group were divided into a control subset (A) and inoculated subset (B) while progeny from the previously challenged group were divided likewise into a control subset (D) and into a rechallenged subset (C). The results are shown in Table 3.

Daily samples of randomly collected fry (8-12/day) were negative for virus in three of the four subsets in group 2. Virus was isolated, however, from apparently healthy fish in subset C collected on days 4 and 7.

Group III subset C registered virus on days 3, 4, and 6. The samples assayed on the third and fourth day sampling periods were positive for virus although no significant death pattern had occurred up to this period. Interestingly the death of 100 fry occurred in this group between days 4 and 5 postchallenge and more specifically between 7 and 16 hours postsampling on day 4. In addition, virus was also isolated from Group III subset B (day 3), however no overt clinical disease was evident nor were any virus-associated deaths observed. All virus isolates were subsequently identified as CCV by the SN test. However, the mortalities were not consistent and pathogenesis studies with these fish were discontinued.

Later, additional fish from the same supplier and the same age hatch were purchased and stabilized in the laboratory. We attempted to determine an LD_{50} for our cloned CCV by 1) immersion of the fish in log dilutions (1 through 7) of virus (1 hour at 27°C) and maintenance of these challenged fish at the same temperature; and 2) by intraperitoneal (IP) inoculation (0.05 ml) of log dilutions of the same virus pool.

LD_{50} DETERMINATION RESULTS

We were unable to establish an LD_{50}. Our results can be summarized as follows.

Trial 1: immersion for 1 hour at 27°C (12/point).

A) Held 17 days with two deaths, one on day 6 and one day 7, both at 10^{-2}. Virus reisolated and identified by SN [(10^7/ml)—challenge titer].

B) Reexposed a second time to the same virus dilution, observed 10 days longer; no deaths resulted (titer 10^9/ml).

Trial 2: IP, 0.05 ml, 10^0 through 10^{-3}. All died within 24 hours, cause undetermined.

Trial 3: IP, 0.05 ml 10^{-5} through 10^{-8}.

A) Titer of pool $10^{5.5}$/ml (12 point). No deaths in 7 days.

B) Reinoculated the same groups on day 8 using 10^{-2} through 10^{-5} dilutions. One death on day 6 at 10^{-3} dilution.

C) Fifteen days after intitial exposure. Flumethasone 0.5 mg (an anti-inflammatory adrenocortical steroid) was added to the 10^{-2} tank and to the PBS control tank. These were observed for 12 days. No disease. (Note: Each tank had 12 gallons of water when the corticosteroid was added.)

D) Challenge these fish with a one pass field isolate 29 days after initial exposure. Observed an additional 30 days with no death or illness seen. (Titer $10^{6.5}$/ml.)

E) Blood was collected from caudal vein of these fish, pooled, and allowed to clot. Serum extracted from this blood was assayed for antibody to CCV. Pooled sera showed significant levels of antibody by the log neutralization index test.

DISCUSSION

Perhaps our challenge techniques have been insensitive. Immersion times of longer than 1 hour or injections of greater than 0.05 ml may precipitate a more consistent mortality. We found at this size and age any quantity of fluid in excess of 0.05 ml either just leaked out or, if great enough, the fish were blown apart from fluid pressure. Regardless, we were unable in any of our trials to establish an LD_{50} with our pools of CCV.

Failure to establish an LD_{50} may be attributed either to: A) Genetic resistance of the fish used; B) the fish may have had latent infections, thus creating a resistance to reinfection; C) modification of the virulence of cloned CCV; or D) high mortality in naturally infected catfish due to another infection rather than to CCV alone.

Genetic resistance in catfish had been suggested earlier and this feature merits further study. We may have modified the virulence of our virus by plaque purification, but comparative studies with field isolates indicates little if any strain differences exist. The development of SN antibody confirms an exposure to the virus accompanied by an immune response albeit without clinical evidence of illness and a low mortality index.

We must define a way to establish the presence of latent infections in fish. My contention is that the high mortality seen in infected catfish under natural conditions is not due solely to CCV, but results from intercurrent infections plus environmental stresses. In many instances we have attempted to isolate virus from so-called outbreaks of CCV and have failed. We have never failed to isolate a number of other contaminating organisms.

ACKNOWLEDGMENTS

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Portions of the material in this paper will be included in a dissertation by Jack Austen as part of the requirements for the degree of Doctor of Philosophy.

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