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Channel Catfish Virus Research at Auburn University[1]

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CHANNEL CATFISH VIRUS DISEASE (CCVD) was first diagnosed in 1968 by Fijan et al. (2) and has now been diagnosed in 11 epizootics from six Southern States. It may cause up to 90 per cent mortality among infected, susceptible, fingerling channel catfish, thus is a serious disease in some catfish operations. The degree of mortality depends upon the condition of the fish, water temperature and probably other environmental factors.

Primary interest at Auburn University is in learning more about the virus disease and its association with the host. The areas of research are: (1) age and size of fish affected, (2) other affected species of catfish, (3) what the reservoir is and how the disease is passed from one individual to another, (4) methods of detecting carrier fish, (5) determination of primary target tissues and, (6) determination of the immune response stimulated by the virus. Although some data have been accumulated in all these areas, these comments will be restricted to those of greatest importance to the catfish producer.

METHODS OF IDENTIFYING CARRIER BROODSTOCK

Before arriving at any meaningful conclusion about the extent of the distribution of CCVD, methods of detecting carrier or reservoir fish must be developed. This is dependent upon the theory that the disease is carried by the parent fish and can be passed from the adult to the offspring via the reproductive products. Evidence for such a method of vertical transmission is only circumstantial. However, it is known that the disease can be transmitted horizontally from infected fish to uninfected fish via the water or by contact Fijan et al. (2).

The geographical range of CCVD has been determined by isolating the virus from infected fish in active epizootics. It appears that only a small percentage of the outbreaks is recognized as many are not revealed because of the reluctance on the growers' part to report them. Such attitudes further complicate any hopeful measures of reducing the effects of the disease. This in itself has probably led to its increased distribution because there is circumstantial evidence to indicate that survivors of epizootics may be carriers of the virus.

Methods used in identifying carrier fish with infectious pancreatic necrosis (IPN) virus (Wolf and Quimby (5)), and viral hemmorrhagic septicemia (VHS) (Hoffman et al. (3)) of trout have been tried experimentally with CCV, however, none of these methods have afforded satisfactory results. Internal organs, peritoneal wash or fecal samples from fish artificially infected with virus and sampled several weeks after injection did not yield virus. IPN, or VHS of trout may be detected in carrier fish by at least one of these methods. However, the only method of detecting carriers that holds any promise for CCV at present is immunological, that is to demonstrate a previous exposure to the disease. Two-year old channel catfish that were injected with CCV demonstrated an immune response to the virus several weeks after injection. Also virus neutralizing antibodies have been found in channel catfish broodstock that produced CCV diseased offspring 2 out of 3 previous years.

The attempts to isolate CCV from the artificially infected fish was done in the laboratory by injecting 2-year-old channel catfish (200 g. to 400 g.) with virus and holding them in tanks with water temperature at 75° F to 80° F. These fish were checked periodically by taking fecal and peritoneal wash samples and gill swabs, then assaying these samples for virus in brown bullhead (BB) cell cultures. The only sam-

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ple that was positive for CCV was the peritoneal wash, 12 days post injection, when 3 of 7 sampled fish were positive for virus. No other subsequent samples were positive, therefore, it is thought that the recovered virus was actually residual particles from the inoculations.

In July, 1970, a federal fish hatchery decided to liquidate their channel catfish broodstock because these fish had produced CCV diseased offspring. These fish were made available to Auburn University for study and with the cooperation of The Division of Fish Hatcheries, U.S. Bureau of Sport Fisheries and Wildlife some interesting information is emerging from the study. It must be added, however, that so far much of the information is negative. Initially 30 fish were studied in July and an additional 22 in September, 1970. The sampling techniques were divided into two catagories: (1) sampling techniques without sacrificing the fish, and (2) sampling techniques by sacrificing the fish, Table 1.

TABLE 1. TISSUES, ORGANS, AND PRODUCTS ASSAYED FOR CHANNEL CATFISH VIRUS FROM ADULT CHANNEL CATFISH

Techniques without sacrificing the fish	Techniques by sacrificing the fish
Blood	Liver*
Kidney-blood*	Intestive*
Gill-swab	Kidney
Urine	Spleen
Feces	Ovaries

* Not checked for virus in September.

Sera were also collected from the blood of each fish to determine if CCV specific neutralizing antibodies were present.

RESULTS

Virus was not isolated from any of the samples from the fish in July or September. There was poor survival of virus in the July samples where CCV was added with the exception of one urine sample where the survival was good, Table 2.

A positive CCV neutralization index (Casals *(1)*) was found in the serum of each of the 30 fish examined in July, however, the antibody level for females differed from that of the males, Table 3. The average neutralization index for 21 females was 20,559 (range ≥56 to ≥177,800), whereas the average for 9 males was 55,503 (range 103 to ≥177,800).

TABLE 3. CCV NEUTRALIZATION INDEX OF SERUM FROM CHANNEL CATFISH BROODSTOCK SUSPECTED OF CARRYING CCV

Sex	Number	Neutralization index		
		Max	Min	Mean
Female	21	≥177,800	≥56	≥20,859
Male	9	≥177,800	103	≥55,503

The data collected thus far are primarily negative, although they do reveal some interesting facts. It is assumed that the CCV infected channel catfish fingerlings in question contacted the disease from the broodstock, however, no viable virus was isolated in surveys of the adult fish during July and September, 1970. Several factors may contribute to the lack of recovered virus: (1) the broodstock may have lost their infection, during the year, (2) the infection is so slight that the level of viable virus is below our detection methods, (3) the virus released from infected cells may be neutralized by the high level of circulating antibody in the blood, or (4) viable virus may only be released during certain metabolic cycles such as during spawning activity, or (5) there may be some disease reservoir other than channel catfish. Any of these factors will present problems in virus detection and continued work during the next spawning season may answer some of these questions.

TABLE 2. SURVIVAL OF CCV IN TISSUE SAMPLES AND EXCRETORY PRODUCTS FROM ADULT CHANNEL CATFISH

Fish number	Days post inoculation	Virus survival[1] (expressed as $TCID_{50}$ per ml.)								
		Feces	Urine	Kidney-blood	Gill swab	Kidney	Liver	Spleen	Intestine	CCV neutralization index (serum)
6	11	Neg.	--------	Neg.	--------	Neg.	Neg.	1,778	Neg.	660
12	17	Neg.	--------	--------	177	Neg.	Neg.	Neg.	354	316
18	24	Neg.	--------	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	≥31,620
24	36	Neg.	--------	Neg.	18	Neg.	Neg.	Neg.	112	≥177,800
30	38	Neg.	≥1,000	Neg.	56	Neg.	Neg.	Neg.	Neg.	17,780

[1] 17,380 $TCID_{50}$ virus were added per ml. of sample.

Blood was taken with a 10 ml. syringe from the dorsal aorta in the caudal peduncle; kidney-blood was secured by injecting physiological saline into the kidney and withdrawing a sample; gill-swabs were taken by swabbing the gill with a Q-tip soaked in saline; urine was aspirated from the urinary bladder with a Pasteur pipette; and feces were taken by squeezing intestinal content into a test tube. Internal organs were obtained by sacrificing the fish and removing the organs. These samples were homogenized, diluted, filtered (0.45 u), and assayed in BB cell cultures. These samples were not assayed for 48 hours after being taken, therefore, a known amount of CCV was added to the samples from 1 out of 6 fish to determine the survival of virus in the samples.

REPLICATION OF CCV IN INTERNAL ORGANS

The purpose of this study was to determine from which organs of infected channel catfish could one most likely isolate CCV. Eight-month-old channel catfish weighing 5 g. each were injected intraperitoneally with CCV and held in aquaria at 80°F. At 24-hour intervals for a period of 5 days, two samples of fish (each sample consisting of 2 fish) were removed from the aquaria for virus assay. The kidney, liver, intestine, brain, and a sample of skeletal muscle were removed from the fish in each sample and the individual organs combined. These samples were then homogenized and serially diluted 10 fold to 10^{-2} from which cell free filtrates were prepared (0.45 u.) The filtrates were then further serially diluted 10 fold to 10^{-5}. Triplicate tubes of channel catfish gonad (CCG) cells were inoculated with 0.1 ml. each of the dilutions. The CCG cells were incubated at 25° C and after 7 days were scored for cytopathic effect (CPE) and the 50 per cent end point ($TCID_{50}$) determined by the Reed-Muench method (Reed and Muench *(4)*).

CCV was isolated from all organs and tissues assayed but at different levels and different frequencies, Table 4. In live fish the kidney was the initial organ demonstrating virus replication where virus was recovered 24 hours after inoculation. Initial CCV was isolated from the other organs as follows: intestine—48 hours; liver—72 hours; brain and muscle—96 hours.

TABLE 4. REPLICATION OF CHANNEL CATFISH VIRUS IN ORGANS AND TISSUES OF LIVING CHANNEL CATFISH FINGERLINGS INJECTED WITH CCV

Hours post injection	Sample number[1]	Tissue culture infectious doses$_{50}$ ($TCID_{50}$) per ml. of tissue				
		Kidney	Intestine	Liver	Brain	Muscle
24	1	≧175	-------	-------	-------	------
	2	-------	-------	-------	-------	------
48	1	562	-------	-------	-------	------
	2	5,620	1,000	-------	-------	------
72	1	31,620	562	17,400	-------	------
	2	10,000	3,162	174	-------	------
96	1	17,400	1,000	57,540	-------	------
	2	100,000	31,620	3,981	3,162	316
120	1	468	316,000	3,162	≧10,000	316
	2	3,162	5,623	≧100,000	≧10,000	58

[1] Two fish in each sample.

It can be concluded from this study that the kidney is the initial organ affected by virus and therefore is prime tissue for study, however, the intestine and liver are only slightly less desirable.

Investigations into the effects of CCV and its impact on the host have many unanswered questions. It is hoped that continued research at Auburn University and other institutions will provide solutions to some of these problems.

SUMMARY

Channel catfish virus (CCV) research at Auburn University Agricultural Experiment Station primarily involves development of methods for detecting the disease in carrier populations. Data collected to date indicate that methods used in detection of infectious pancreatic necrosis (IPN) virus and viral hemorrhagic septicemia (VHS) of trout are not applicable to CCV. High CCV neutralization indices were found in 52 channel catfish broodstock suspected of being virus carriers but no viable virus was isolated. The principal organs involved in fingerling channel catfish infected with CCV are kidney, liver and intestine although the muscle and brain yielded detectable virus.

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