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NEUTRALIZATION OF CHANNEL CATFISH VIRUS BY SERUM OF CHANNEL CATFISH

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Abstract: Sera from 71 adult channel catfish *(Ictalurus punctatus)*, with a history of channel catfish virus (CCV) association, were assayed for CCV neutralization activity. Sixty-seven serum samples had positive CCV neutralization indices. Sera from 10 fish with no known history of CCV exposure, showed no evidence of virus neutralization activity. Viable CCV was not isolated from 232 organs or excretory products of studied fish. Serum from channel catfish experimentally immunized with viable CCV reached peak neutralization indices 60 days after virus injection. Detection of CCV serum neutralization activity can afford a possible method of identifying channel catfish populations with a previous exposure to CCV.

INTRODUCTION

Channel catfish virus disease (CCVD) is one of the most recently reported virus diseases of fish.[5,6] The disease affects fry and fingerling channel catfish on numerous fish farms throughout the southern United States during summer months when susceptible fish are abundant and high water temperatures are common. Mortality in infected populations may be as high as 95%. The etiological agent of CCVD is a herpesvirus[14] which produces a general viremia that affects most organs and tissues of diseased fish.[8,11] Channel catfish virus has been isolated only from sick fingerlings during epizootics and an acceptable method of identifying CCV carrier fish is not available.

Channel catfish virus disease was reported from channel catfish fry at the Mammoth Spring National Fish Hatchery, Mammoth Spring, Arkansas.[6] This paper describes the detection of a CCV neutralizing agent in the sera of adult channel catfish that produced CCV diseased fry at Mammoth Spring NFH. The experimental stimulation of a CCV neutralizing substance in fish under laboratory conditions is also described.

MATERIALS AND METHODS

Fish

A group of 71 (37 males and 34 females), 2 to 5-year-old adult channel catfish at the Mammoth Spring National Fish Hatchery, Mammoth Spring, Arkansas was tested for virus and assayed for CCV neutralization activity. The fish averaged 1.4 kg (0.45 to 2.7 kg). A second group of 10 (5 males and 5 females) 3-year-old channel catfish (0.73 to 1.16 kg) from the Fisheries Research Unit, Alabama Agricultural Experiment Station (Auburn University) was similarly studied and used as controls. Twenty 2-year-old channel catfish which averaged 0.15 kg (0.1 to 0.2 kg) from Auburn University were inoculated with CCV and periodically sampled for measurements of CCV neutralization activity.

Cell cultures

Monolayer cultures of brown bullhead (BB) *(Ictalurus nebulosus)* cells (A.T.C.C. Certified Cell Line 59) were used for virus assay. The cultures were grown in 16 x 125 mm tubes using Eagles minimum essential medium (EMEM) supplemented with 10% fetal calf serum and incubated at 25 C.

Virus assay of Mammoth Spring fish

Conventional assay methods used to detect virus carriers in trout were followed.[2,15] Different groups of Mammoth Spring fish were assayed for virus during three sampling periods: July 1970 (28 fish); September, 1970 (22 fish); and July, 1971 (21 fish). Fish were anesthetized in 20 ug/ml quinaldine sulfate, and urine and fecal samples were collected. Blood samples were collected from the hemal artery in the area of the caudal peduncle. While the fish were completely anesthetized internal organs were removed, individually homogenized, and placed on ice. Twenty-four hours after collection the fecal and urine samples were diluted 1:10 with Hank's balanced salt solution (BSS) and filtered through a membrane filter (0.45 nm). Organ homogenates were diluted 1:100 with BSS before filtering. Most filtrates were immediately inoculated into triplicate BB cultures (0.1 ml/tube).

Filtrates not immediately assayed were stored at —80 C for up to 36 days. A total of 232 specimens from the Mammoth Spring fish was assayed for CCV, including 52 kidney, 30 liver, 30 spleen, 30 intestine, 32 gonad, 38 fecal, and 20 urine samples.

Virus neutralization tests of adult fish

Virus titrations were determined by inoculation of 0.1 ml quantities of serial, ten-fold dilutions of CCV in BSS into triplicate BB cultures. Titers were calculated by the method of Reed and Muench[12] and expressed as tissue culture infectious doses — 50% endpoint ($TCID_{50}$). One virus titration was made for each group of five serum samples.

CCV neutralization indices (NI)[4] were determined on sera from Mammoth Spring fish using sera from the 3-year-old Auburn fish as control. Sera were diluted 1:4 with BSS and filtered. A volume of each serum was combined with an equal volume of each 10-fold dilution of virus and incubated for 30 minutes at 25 C. Then 0.2 ml of each virus-serum mixture was inoculated into triplicate BB cultures. The tubes were examined for cytopathic effect after 5 days and the titer determined. The NI of each test serum was obtained by subtracting the log of its serum-virus titer from the log of the control serum-virus titer. Values less than 1.0 were considered negative; 1.0-1.6 questionable, and greater than 1.6 positive for CCV[4]. Statistical comparisons were made on the NI between sexes and sampling periods using an analysis of variance. Some sera were heated to 56 C for 30 minutes and titrated to determine if the neutralizing agent was heat labile.

Experimental Immunization

Ten fish were injected intraperitoneally with 1.5×10^5 $TCID_{50}$ of CCV and divided into two groups of five fish each (groups I and II). Five additional fish were injected with virus that had been heated at 60 C for 1 hour (group III). Five other fish were injected with EMEM and used as controls (group IV). After 7 days, each group received a second injection of the respective inocula. Test fish were held at 22-24 C and fed daily with a commercial fish food. Serum samples were collected from each fish prior to injection and at 30 day intervals up to 180 days after initial injection and the NI determined as described. On day 120 three virus inoculated fish from group I were given a booster injection of approximately 1.5×10^5 $TCID_{50}$. After each handling the fish were given a prophylactic bath of acriflavin at a concentration of 10 ug/ml for 1 hour.

RESULTS

Virus assay of Mammoth Spring fish

Channel catfish virus was not isolated from any of the 232 samples from the Mammoth Spring broodstock.

Neutralization tests of adult fish

The mean virus-serum titers of Auburn males and females differed from the corresponding virus titer by less than one $\log_{10}$, therefore, these sera were considered free of CCV neutralizing activity.

The neutralization indices of the Mammoth Spring brood fish are summarized in Table 1. A wide variation of NI was present in the sera from the Mammoth Spring fish but the differences between the male and female fish or between sampling periods were not significant at the 5% level. There was no difference in NI noted between heated and non-heated sera from the same fish.

Positive CCV neutralization indices ($\geq$1.6) were obtained from 67 of the 71 Mammoth Spring serum samples tested; two sera were in the questionable range (1-1.6) and two were negative (0-1.0). All of the questionable and negative values came from males. Eighty percent of the NI from all fish were above 3.0.

Experimental immunization

Clinical signs of CCVD[6] did not appear in any of the injected Auburn fish. The calculated neutralization indices of groups I, II, and III are presented in Figure 1. Prior to injection, the mean NI of groups I, II, and III was 0.28, 0.24, and 0.28 respectively, therefore all groups were considered to be free of CCV neutralization activity. Thirty days after inoculation the mean NI of each group was as follows: group I, 2.31; group II, 2.72; and group III, —0.4. Primary peak NI was reached in the virus injected fish 60 days after inoculation (Figure 1). Fish in group III showed no increase in virus neutralizing activity during the first 60 days of the study, therefore sampling of these fish was discontinued. Neutralization activity of groups I and II decreased to the original level 120 days after inoculation but a booster injection at that time resulted in a rapid and strong anamnestic response, followed by a seemingly more rapid decline than had occurred after the first peak.

DISCUSSION

The inability to isolate CCV from the adult channel catfish agrees with the absence of viable virus in artificially infected adult fish for more than 12 days after injection (Plumb, unpublished data). The fact that CCV, which is a herpesvirus, has been isolated only from fingerling channel catfish during an epizootic,[9,10] agrees with the epidemiological characteristics of herpesviruses of higher vertebrates[1] in that the virus often can be isolated only during the early stage of active infections. This phenomenon makes the application of the methods currently used to detect carriers of salmonid borne viruses[2,15] impractical for identification of possible CCV carrier catfish.

The presence of CCV neutralization activity in the sera of adult fish which have a history of CCV suggests that this may afford a means by which CCV exposed populations of catfish can be identified. It is significant that nearly 95% of the adult channel catfish at Mammoth Spring NFH had positive neutralization indices against CCV and that 80% were above 3.0. However, since the vertical passage of CCV from fish with positive NI to their offspring has not been demonstrated, any such tests cannot be relied on at this time to suggest a carrier state but only as indicative of previous viral exposure.

It is possible that the neutralizing substance in the sera of the adult Mammoth Spring fish occurs naturally. This is doubtful, however, since the agent was not heat labile, it was not present in the sera from the Auburn fish which had no history of CCVD and sera from other groups of tested channel catfish (Plumb, unpublished data) have had no virus neutralizing activity.

The immune responses to experimental injection did not conform to the neutralization indices of sera from the naturally exposed adult channel catfish at Mammoth Spring. Neutralization indices of naturally infected adults were on the average 1 to 2 logs higher and remained stable for a period of 1 year. Inoculated fish lost their neutralization activity in 30 to 60 days after reaching a peak. This could indicate that repeated exposure to CCV via natural routes of infection resulted in higher and more stable levels of neutralization activity.

TABLE 1. Mean channel catfish virus neutralization indices of sera from adult channel catfish from Mammoth Spring NFH. Range of values are in parentheses.

Date of Serum Collection	Mean Virus Titer, ①,②	Male			Female		
		No. of Fish	Mean Virus-Serum Titer ②	Mean Neutralization Index ③	No. of Fish	Mean Virus-Serum Titer	Mean Neutralization Index ③
July, 1970	5.63 (5.25-6.25)	19	2.58 (1.81-4.81)	3.30 (1.07-4.51)	9	2.58 (1.81-3.79)	3.27 (2.06-4.04)
Sept., 1970	6.0 (5.25-6.25)	9	2.41 (1.5-4.85)	3.47 (1.30-4.38)	13	2.05 (1.5-2.85)	3.80 (3.0-4.35)
July, 1971	6.35 (6.25-6.5)	9	2.51 (0.22-5.5)	3.27 (0.38-5.66)	12	1.77 (0.5-4.0)	4.05 (1.85-5.35)
Control ④ Sept., 1970	6.75	5	5.88 (5.67-6.5)	—	5	5.85 (5.5-6.26)	—

① Mean of 3 to 5 CCV titrations.

② Expressed as exponent ($\log_{10}$) of inefectivity ($TCID_{50}$) in BB cell cultures.

③ Difference between exponents ($\log_{10}$) of control and test serum titrations.

④ Sera from 3-year-old channel catfish from Fisheries Research Unit, Agriculture Experiment Station, Auburn University.

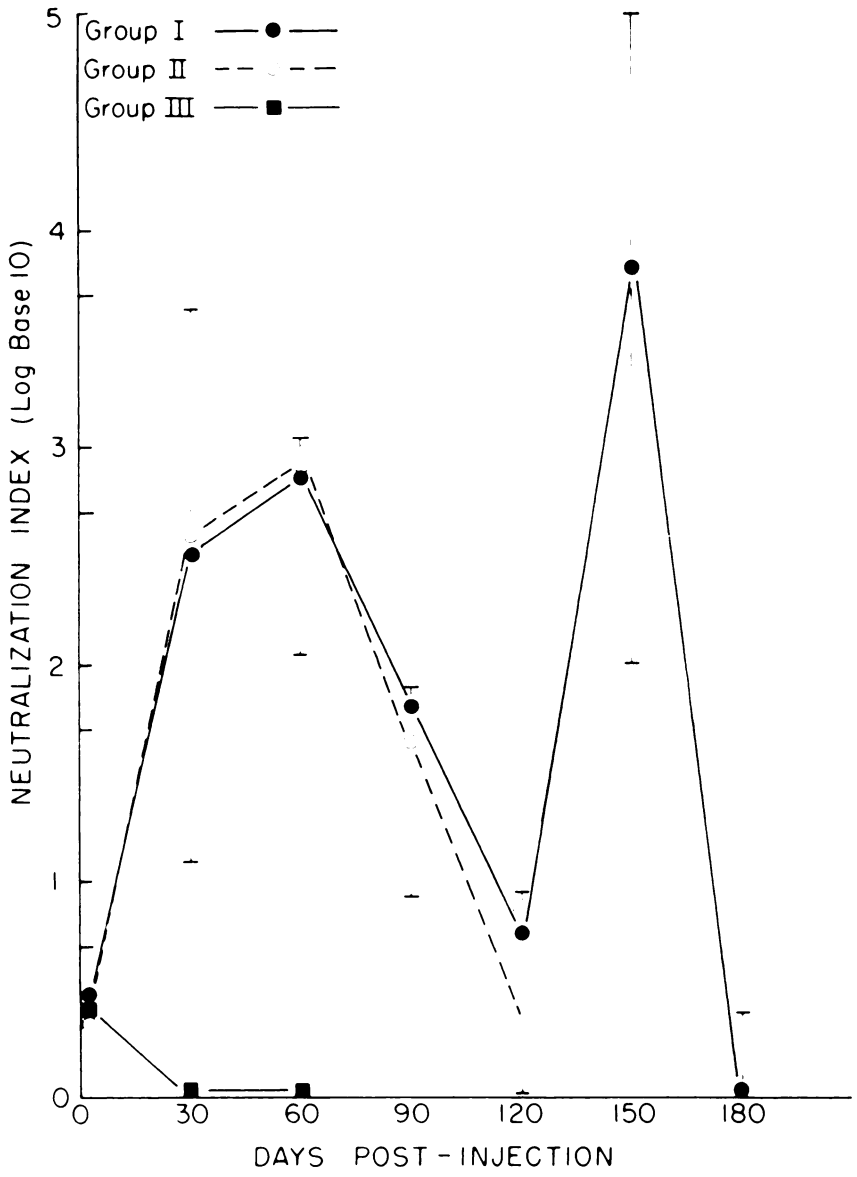

FIGURE 1. Neutralization indices (log base 10) of 2-year-old channel catfish injected with CCV. Groups I and II were inoculated with infectious virus and Group III was inoculated with heat killed virus. Group I was given a booster injection 120 days after the initial inoculation.

The immune response of CCV infected channel catfish did not reach maximum titer after initial injection as rapidly as was reported by McGlamery, et al.,[7] when bovine serum albumin and vesicular stomatitis virus were used. Temperature in the present study ranged from 22-24 C in the troughs, as compared to 22-28 C in ponds for the previous study, which could partially explain the difference in the rapidity of the response. Bissett[3] and Sniezko[13] noted that the immune response of fish is temperature correlated and will develop more rapidly at higher temperatures.

It is concluded that the techniques presently used for detecting carriers of trout viruses are probably not applicable to CCV. The use of immunological tests that can detect CCV neutralizing agents in the sera of adult channel catfish can provide a possible means of determining potential virus carrier populations. These populations in which CCV neutralizing serum is present could be avoided as broodstock, thus possibly reducing the spread of the disease.

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