TISSUE DISTRIBUTION OF CHANNEL CATFISH VIRUS 1

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TISSUE DISTRIBUTION OF CHANNEL CATFISH VIRUS[1]

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Abstract: The kidney, liver, intestine, brain, and muscle of live infected channel catfish were assayed for channel catfish virus in channel catfish gonad cell cultures. Sampling was done at 24-hour intervals for 120 hours. The virus was first detected in the kidneys of channel catfish 24 hours after inoculation. Channel catfish virus was also isolated at sequential time intervals from the intestine, liver, brain, and muscle. Virus was detected in the kidney, liver, and intestine but not the brain of the fish that died 70 hours after infection.

INTRODUCTION

Channel catfish virus (CCV)[2,6] is the etiologic agent of channel catfish virus disease. A highly contagious disease of cultured channel catfish *(Ictalurus punctatus)*, it is endemic throughout much of the Southern United States. The virus causes high mortality among susceptible fingerlings, however, it is thought by many that the extent of mortality depends upon the condition, size, and age of the fish, and environmental factors such as temperature and "stress".

Use of internal organs is routine in assaying cultured trout populations for presence of infectious pancreatic necrosis virus[1] and viral hemorrhagic septicemia.[3] There is available little quantitative information about the recovery of virus from organs of infected fish; however, Dr. K. E. Wolf (personal communications; unpublished data) determined the amount of infectious pancreatic necrosis virus found in internal organs of five naturally infected rainbow trout. Klontz et al.[4] reported Sacramento River Chinook virus recovery, on a daily basis, from whole fish, but individual organs were not assayed. The purpose of this paper is to describe the quantitative distribution of virus in internal organs and tissues of channel catfish artificially infected with CCV.

MATERIALS AND METHODS

Channel catfish virus was harvested in tissue culture medium from infected monolayer cultures of channel catfish gonad (CCG) cells prepared by the method of Wolf et al.[7] The medium was filtered through 0.45 μ membrane and frozen in 2 ml ampules at —80 C until needed for fish injection.

Channel catfish gonad cell cultures in the 13th to 16th passage were grown in 16 x 125 mm tubes seeded with 1.0 ml cell suspension. Growth medium was Eagle's minimum essential medium in Hanks' balanced salt solution (HBSS) and supplemented with 10% fetal bovine serum, 100 IU penicillin G and 100 μg streptomycin per ml.

On the day fish were to be injected, ampules of virus were thawed and the titer determined by the Reed - Muench method[5] in CCG cells. The virus titer of the stock material was $10^{4.75}$/ml. Forty-five, 8-month-old channel catfish fingerlings averaging 5 g each were injected intraperitoneally with 0.1 ml HBSS containing $10^{2.75}$ 50% tissue culture infectious doses ($TCID_{50}$). Inoculated fish were held in two 40 liter aquaria filled with dechlorinated water; temperature of the water was maintained at 28 C and oxygen was supplied by bubbling compressed air into the aquaria. At 24-

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hour intervals post-inoculation (PI) four live catfish were randomly selected, divided into two groups and killed. The fingerlings that had not been taken in the earlier samplings all died after 120 hours PI and were discarded without testing for virus. The kidneys, livers, intestines, brains, and skeletal muscle taken from the lateral musculature dorsal to the abdominal area, were removed from the two fish of each group and the individual organs were pooled. The intestines were opened and their contents removed by a single washing with HBSS. The pooled tissue samples were homogenized by grinding with a mortar and pestle, then serially diluted with HBSS in two, 10-fold steps. Samples were then filtered through a 0.45 μ porosity membrane and further serially diluted with HBSS until a 10^{-5} dilution of tissue was obtained.

Three channel catfish that died 70 hours PI were also assayed for virus. The kidneys, intestines, livers, and brains were processed similarly to the samples from live fish, however, the organs from each fish were kept separate.

One- or two-day old CCG cell cultures were inoculated in triplicate with 0.1 ml of each dilution from each organ sample without changing the culture medium. The cultures were incubated at 25 C and examined daily for 12 days for cytopathic effect. Titers of virus isolated from tissue samples were determined by the Reed-Muench method.[5]

RESULTS

CCV was isolated from all organs and tissues (Table 1). At 24 hours PI virus was recovered from one of two kidney samples. After 48 hours PI, virus was isolated from both kidney samples and one intestine; this was followed at 72 hours PI with virus isolations from the liver and finally at 96 hours from the brain and skeletal muscle.

The titer of CCV in the kidney of live fish increased from 24 hours PI until the highest titer was found at 96 hours PI (Figure 1).

The virus titrations from dead fish indicated that the kidney was the organ primarily affected (Table 2); the three kidneys averaged 4.7 x 10^4 $TCID_{50}$/0.1 ml of tissue. Virus was also found in the livers and intestines of all three fish but not in the brain.

TABLE 1. Distribution of channel catfish virus in organs and tissues of living injected channel catfish fingerlings.[1]

Hours post-injection	Sample Number[2]	Organ ($TCID_{50}$)				
		Kidney	Intestine	Liver	Brain	Skeletal muscle
24	1	175	—	—	—	—
	2	—	—	—	—	—
48	2	562	—	—	—	—
	2	5,620	1,000	—	—	—
72	1	31,620	526	17,400	—	—
	2	≤10,000	3,162	174	—	—
96	1	17,400	1,000	57,540	—	63
	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] Expressed as tissue culture infectious doses, 50% end point/0.1 ml. of tissue.
[2] Two fish represented in each pooled sample.

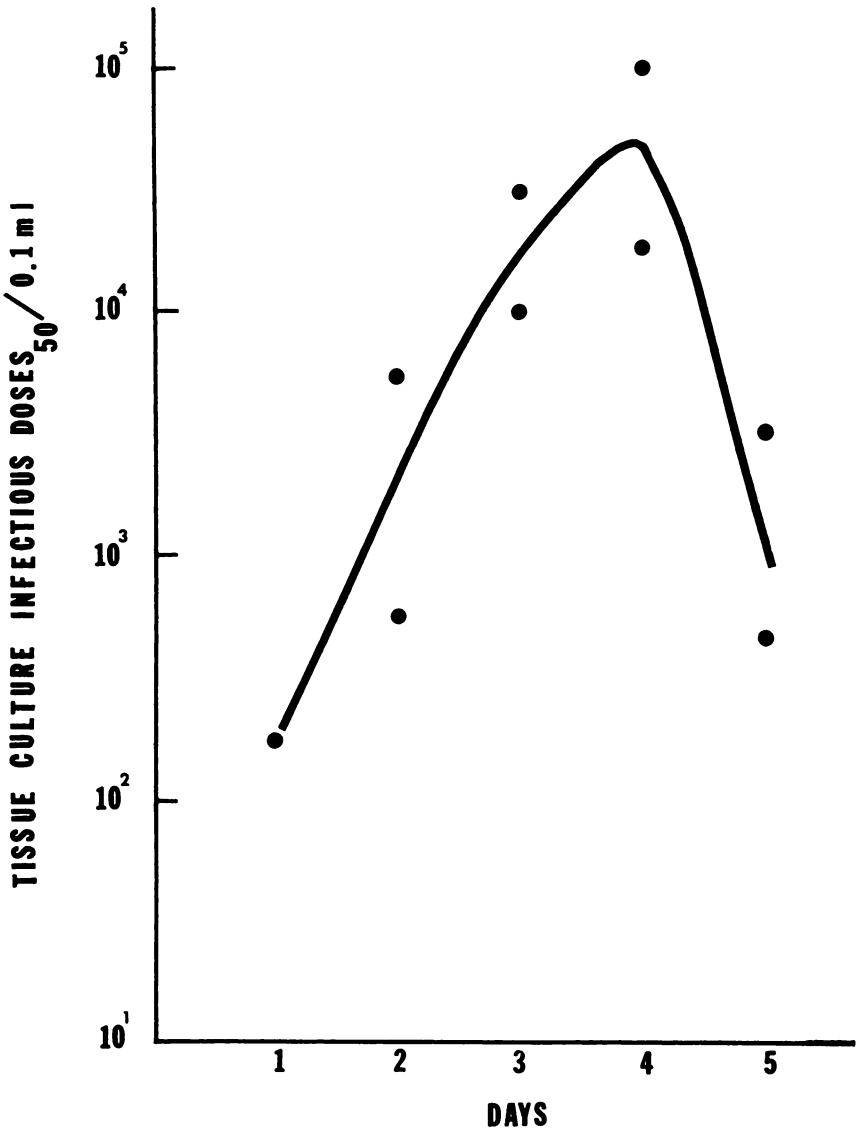

FIGURE 1. Recovery of channel catfish virus from kidneys of artificially infected fish.

TABLE 2. The titer of virus found in organs from fingerling channel catfish that died 70 hours after injection with CCV.[1]

Fish number	Organ ($TCID_{50}$)			
	Kidney	Intestine	Liver	Brain
1	$\geq$ 100,000	31,620	1,746	$\leq$ 100
2	10,000	5,623	316	$\leq$ 100
3	31,620	3,162	316	$\leq$ 100
Mean	47,200	13,470	759	$\leq$ 100

[1] Expressed as tissue culture infectious doses ($TCID_{50}$)/0.1 ml of tissue.

DISCUSSION

The data from this study indicate that the kidney was the primary organ affected by CCV. In the first 4 days following CCV infection the kidney of living fish yielded the greatest amount of virus (Figure 1); the kidneys of fish which died 70 hours PI also yielded a higher amount of virus than did other organs. That the intestine and liver also probably support replication of CCV is shown by the fact that they both yielded high titers of virus. It appeared that the central nervous system may have been affected in the later stages of infection. Virus was not detected in the brain until 96 hours PI and virus was not recovered from brain tissues of fish which died 70 hours after injection. Signs of central nervous system disorders (erratic swimming and some whirling) did not appear until three to four days PI; this correlates with the isolation of virus from brain tissue at 96 hours PI. In contrast to internal organs, skeletal muscle yielded small amounts of virus, suggesting that the virus did not replicate in the skeletal muscle.

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