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# SPRING VIREMIA OF CARP

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# INTRODUCTION

Dating back nearly 50 years and possibly even to the Middle Ages, European pond culture of carp *(Cyprinus carpio)* and perhaps other cyprinid fishes has been plagued with a contagious disease of great importance. Variously known as infectious dropsy, infectious ascites, hemorrhagic septicemia, or rubella, the disease is probably the most serious cause of losses among these fishes; nearly 500 reports have been published on the subject.

Despite the great mass of literature, the cause of the disease has been controversial. Some authorities consider it to have a bacterial etiology because gram-negative organisms such as *Aeromonas punctata, A. hydrophila,* or *Pseudomonas fluorescens* have been implicated in major outbreaks. Moreover, several antimicrobials are reported to have been successfully used either prophylactically or therapeutically, thus suggesting bacterial involvement. In addition, the administration of bacterins has been reported as being beneficial.

On the other hand, certain circumstantial evidence supports a viral etiology; filtrates of homogenized diseased tissues induced signs of dropsy and mortality. Adding to the complexity of interpreting early literature is the fact that a mixed etiology of both virus and bacteria has also been proposed. Authoritative reviews of infectious dropsy are included in the texts of Reichenbach-Klinke (1966), Schäperclaus (1954), and Bullock et al. (1971).

One aspect of the conflicting information concerning viral etiology was recently resolved by Fijan et al. (1971), who isolated a rhabdovirus from a case of acute infectious dropsy and additionally fulfilled Rivers' postulates. It is apparent that the presence or absence of virus in fish that were involved in epizootics reported before 1971 cannot be determined with any degree of confidence. Fijan et al. (1971) proposed that the newly identified viral disease be recognized as the previously known acute infectious dropsy and that the so-called chronic form be termed erythrodermatitis. We believe that there is ample justification for a complete separation of these two diseases, and consequently have restricted our presentation to the disease that results from infection with the virus. Accordingly, we employ exclusively the term first proposed by Fijan et al. (1971)--spring viremia of carp (SVC)--as the disease caused by the agent they named *Rhabdovirus carpio.*

In Europe, swimbladder inflammation of carp is a contagious disease that results in significant mortality. Its etiology is still a matter of controversy among workers who have reported the condition. In 1971, a rhabdovirus, the 10/3 strain, was isolated from carp with the acute form of the disease (Ahne 1973). Since

this isolate shares similar, if not identical, properties with *R. carpio*, and since there is complete cross-neutralization between strain 10/3 and *R. carpio*, it is considered that SVC and the acute stage of swimbladder inflammation have a common etiology and at best are variations in the expression of the infection.

## DEFINITION

Spring viremia of carp is an acute contagious viral disease of carp *(Cyprinus carpio)* and perhaps other cyprinids. It usually occurs and causes high mortality at temperatures of 13 to 22 C, typically as temperatures rise in spring.

## PATHOLOGICAL CHANGES

The behavior changes of infected carp consist of reduced respiratory rate and in some cases fibrillation of skeletal muscle. Affected fish collect at the water inlet, lose their balance, and swim in an uncoordinated manner or lie on their side.

Externally, infected carp are darker than normal, show slight exophthalmia, abdominal distension, and an inflamed and edematous vent. Their gills are pale and they have petechial hemorrhages both in the gills and in the skin. Dermal lesions are usually absent in epizootics of SVC.

Peritonitis, ascites, and a catarrhal or hemorrhagic enteritis are the usual internal signs. Viscera are edematous, and petechial hemorrhages occur in the heart, liver, kidneys, intestine, internal wall of the swimbladder, and in skeletal muscle (Fig. 1).

Carp experimentally infected with strain 10/3 of SVC also show marked hemorrhaging of the swimbladder (Fig. 2).

## IDENTIFICATION

Spring viremia of carp can best be diagnosed by isolating the causal virus and identifying it serologically. The diagnosis should include three steps: (1) determination of a possible history of the disease, (2) macroscopic and microscopic examination for pathologic alterations of tissues and organs, and (3) isolation and serological identification of *Rhabdovirus carpio*.

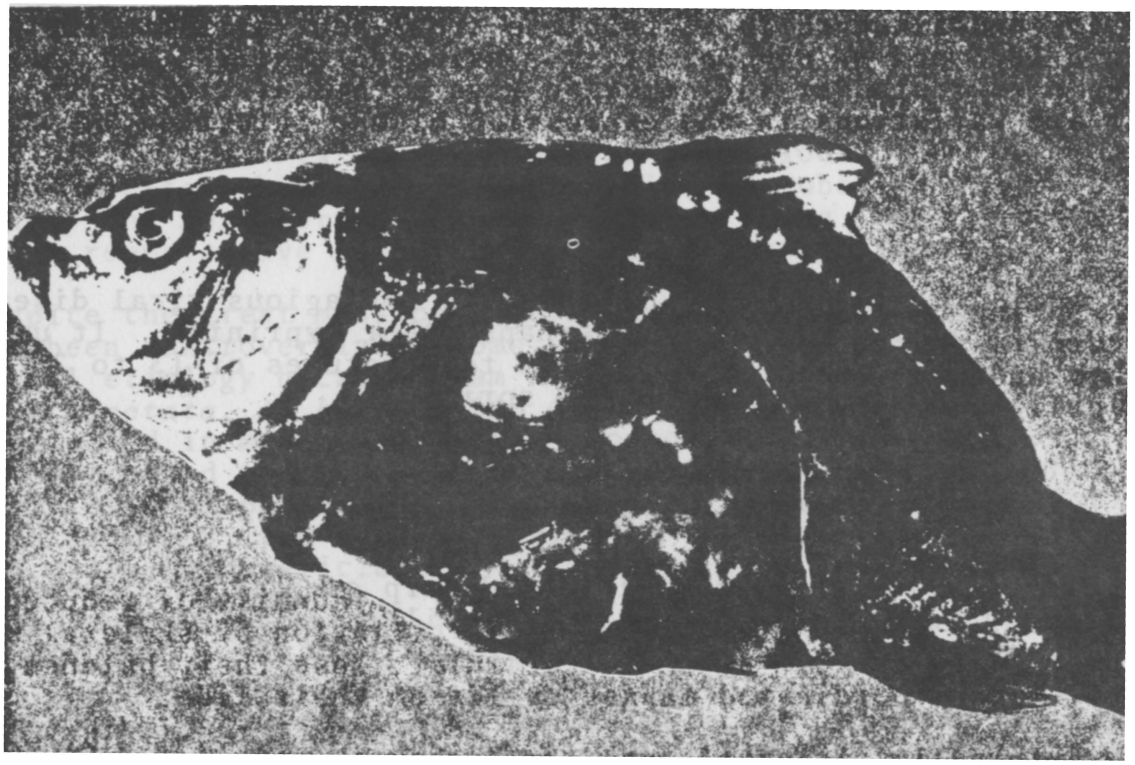

Fig. 1. Carp that succumbed to experimental infection with *Rhabdovirus carpio* shows a generally hemorrhagic visceral mass.

Fig. 2. A typical finding in spring viremia of carp is widespread petechiation of the swimbladder.

Fig. 3. In fathead minnow cells (FHM) infection with *Rhabdovirus carpio* shows pronounced margination of chromatin before lysis. Hematoxylin and eosin stain.

(Photos by W. Ahne)

On the basis of the amount of virus found in various infected tissues (Table 1), the liver, kidneys, and spleen should be used for virus assay. The virus has also been detected in circulating blood, thus indicating viremia (W. Ahne, unpublished report).

Table 1. Quantification of *Rhabdovirus carpio* from various tissues.

| Tissue | Amount of virus ($log_{10}$ $TCID_{50}$/g) | |
|---|---|---|
| | Natural infection[a/] | Experimental infection[b/] |
| Liver | 6.5 | 6.8 |
| Spleen | 3.8 | 5.5 |
| Kidney | 5.8 | 5.2 |
| Brain | 4.3 | 4.5 |
| Gills | 3.5 | no data |

a/ From Fijan et al. 1971
b/ From Ahne 1973

*Rhabdovirus carpio* produces plaques (2-3 mm) in fathead minnow (FHM) cells (CCL-42) within 3 days. The plaques show features distinctive from those produced by Egtved virus, infectious hematopoietic necrosis virus (IHNV), and infectious pancreatic necrosis virus. Plaques produced by pike fry rhabdovirus are similar to those induced by *R. carpio*.

*Rhabdovirus carpio* is also distinguishable from the other fish rhabdoviruses (Egtved, IHNV, pike fry rhabdovirus) by its optimum growth temperature (20 to 22 C) and by its host range.

Rabbit antisera against Egtved virus, IHNV, and pike fry rhabdovirus do not neutralize *R. carpio*.

## CAUSE OF THE DISEASE

*Rhabdovirus carpio* is typical bullet-shaped and measures 90-180 X 60-90 nm. It is ether-, acid-, and heat-labile and has a buoyant density of 1.195-1.200 g/$cm^3$ in CsCl. The virus passes through membrane filters of 220-nm average pore diameter, but is retained on 100-nm filters. It contains five structural proteins which are similar to those of pike fry rhabdovirus, but distinct from those of Egtved and infectious hematopoietic necrosis virus. *Rhabdovirus carpio* replicates in the cytoplasm of four fish cell lines--BB, EPC, FHM, and RTG-2-- and in chick embryo fibroblasts, BHK-21 and certain other mammalian cells, and in some reptilian cells.

Optimum replication in FHM cells takes place at 20 to 22 C. Cytopathic effects appear at varying times of infection, depending on the temperature and cell line used. Granulation and rounding of infected cells lead to a complete lysis of the cell sheet, usually 24 h after infection. Staining of infected cells shows margination of the nuclear chromatin (Fig. 3).

The replication cycle of *R. carpio* requires 8 to 9 h in FHM cells at 20 to 22 C. Progeny virus is first evident in 4 to 6 h, cell-associated virus reaches a peak titer in 8 to 9 h, and released virus peaks 22 to 24 h after infection.

*Rhabdovirus carpio* is stable to freezing in tissue culture medium containing 5% serum (Table 2).

Table 2. Stability of *Rhabdovirus carpio* during storage for 180 days in Eagle's minimum essential medium (MEM), with or without serum, at different temperatures (Ahne 1976).

| Temperature and medium (S = MEM with 5% serum, NS = MEM with no serum) | | Virus titer ($log_{10}$ $TCID_{50}$/ml) | |
|---|---|---|---|
| | | Before storage | After storage |
| 4 C | S | 7.8 | 4.2 |
| | NS | 6.5 | 0.0 |
| -20 C | S | 7.8 | 5.8 |
| | NS | 6.5 | 2.8 |
| -74 C | S | 7.8 | 7.5 |
| | NS | 6.5 | 4.1 |

## SOURCE AND RESERVOIR OF INFECTION

Diseased and dead carp are sources of infection. Persistent infections with SVC have been reported, and outbreaks may result from stress due to fluctuations of environmental factors, handling, crowding, drug treatment, and unfavorable management practices.

## MODE OF TRANSMISSION

The natural route of transmission has not been determined. Healthy carp can be infected by simple cohabitation; the virus

apparently is waterborne and infection probably occurs via the gills or the gastrointestinal tract. Spring viremia of carp exists in the carrier state, and *Rhabdovirus carpio* has been found in fry. The virus is probably transmitted vertically or on the surface of eggs, as has been postulated for other fish viruses.

## INCUBATION PERIOD

The incubation period of SVC depends on water temperature, species, age, condition of the fish, quantity of virus, and on certain environmental conditions.

In ponds, the disease typically occurs in spring when temperatures are 15 to 20 C, but it also occurs at temperatures near 10 C (W. Ahne, unpublished report).

The influence of temperature on development of SVC was studied by Fijan et al. (1971), who found an optimum temperature of 16 to 17 C, where 90% of experimentally infected carp died between 5 and 17 days post-infection. Only 30% died at 17 to 26 C. The disease developed more slowly at 11 to 15 C, but a 90% mortality occurred within 2 to 3 weeks.

## SUSCEPTIBILITY

Most of the documented information on susceptibility to *Rhabdovirus carpio* is limited to the carp. Other fishes, especially cyprinids, may be susceptible to the virus, but careful experimentation will be needed to determine which sustain disease, which are merely infected, and which are refractory.

Experimental infections show that guppies *(Poecilia reticulata)*, fry of northern pike *(Esox lucius)*, and grass carp *(Ctenopharyngodon idella)* are susceptible to *R. carpio*.

Inoculation of carp with *R. carpio* by several routes showed the fish to be most susceptible when inoculation was intracranial; however, clinical signs were less severe than in cases of natural infections (Table 3). In contrast, intraperitoneal injection resulted in typical signs of SVC.

Table 3. Response of carp to *Rhabdovirus carpio* inoculated experimentally by various routes (Krunoslava and Fijan 1973).

| Route of inoculation | Amount of virus ($log_{10}$ $TCID_{50}$/g) | Time to death (days) | Typical signs |
|---|---|---|---|
| Oral | 6.1 | --- | - |
| Intracranial | 2.6 | 12.9 | ± |
| Intraperitoneal | 3.7 | 12.8 | + |
| Swimbladder | 4.5 | 8.9 | + |

## IMMUNITY

Carp that have survived experimental infection are resistant to later virus challenge. Carp infected with *Rhabdovirus carpio* develop antibody near 20 C, and several globulin fractions can be demonstrated in the serum. Serum of carp held under natural conditions sometimes contains specific antibody to *R. carpio*. This fact could reflect an enzootic situation, and serology might prove helpful both in diagnostics and in epizootiological studies.

## RANGE

To date, SVC has been virologically confirmed only in Czechoslovakia, France, Germany, Yugoslavia, and the Soviet Union. Fijan (1972) postulated that it was probably present wherever the acute form of infectious dropsy of carp has been observed.

## METHODS OF CONTROL

Where water supplies are free of virus and brood stock are healthy, the only certain method of control is avoidance. Therapy has yet to be developed. Immunization with attenuated live virus vaccine has promise, as does the selection of resistant strains of fish.

General prophylactic measures should be taken, and one should consider that the virus might be introduced into clean hatcheries by infected fish, water, or contaminated persons, water, animals, or equipment.

## ANNOTATED BIBLIOGRAPHY

Ahne, W. 1973. Cell cultures from different tissues of freshwater teleosts and investigations of the etiology of swimbladder inflammation of the carp. (Zellkulturen aus verschiedenen Süsswasserteleosteergeweben und Untersuchungen über die Ätiologie der Schwimmblasenentzündung der Karpfen). Ph.D. Thesis, Univ. of Munich, Federal Republic of Germany. 172 pp. (in German)

The work concerns clarification of the etiology of swimbladder inflammation of carp. It documents the isolation and characterization of a rhabdovirus from an acute case of the disease. The virus shares similar if not identical properties with *Rhabdovirus carpio*. Light and electron micrographs are included. The thesis also describes preparation of primary tissue cultures from carp and trout. Graphic and tabular data are presented.

Ahne, W. 1976. Investigations on stability of the carp pathogenic virus strain 10/3. (Untersuchungen über die Stabilität des karpfenpathogenen Virusstammes 10/3). Fisch und Umwelt 2:121-127. (in German)

This paper deals with the stability of virus strain 10/3 during storage at different temperatures, its stability in water, and the effects of heat and of pH 3.0.

Bachman, P. A., and W. Ahne. 1974. Biological properties and identification of the agent causing swimbladder inflammation in carp. Arch. gesamte Virusforsch. 44:261-269.

A report on some properties of the rhabdovirus isolated from an acute case of swimbladder inflammation. Carp infected with the isolate showed clinical signs, after 3 to 4 days, that were similar to those of SVC. Additionally, there was strong involvement of the swimbladder. In serum neutralization tests, complete cross-reaction occurred between the isolate from swimbladder inflammation and *Rhabdovirus carpio*. The common etiology of SVC and swimbladder inflammation is discussed.

Bootsma, R. 1973. An outbreak of carp (*Cyprinus carpio* L.) erythrodermatitis caused by a myxobacterium. Aquaculture 2:317-320.

Myxobacteria belonging to the Cytophaga family *(Flexibacter)* have been isolated from an outbreak of carp erythrodermatitis. The disease could be transmitted experimentally; lesions of typical erythrodermatitis and mortality were produced in carp.

Bullock, G. L., D. A. Conroy, and S. F. Snieszko. 1971. Bacterial diseases of fishes. Book 2A (151 pp.) in S. F. Snieszko and H. R. Axelrod, eds. Diseases of Fishes. T.F.H. Publications, Inc., Neptune, N. J.

The book describes bacterial diseases of fishes, and methods of detection, identification, and control; infectious dropsy is included.

Fijan, N. 1972. Infectious dropsy in carp--a disease complex. Pages 39-51 in L. E. Mawdesley-Thomas, ed. Diseases of fish. Symp. Zool. Soc. Lond. No. 30. Academic Press, London.

The author proposes that the acute form of infectious dropsy of carp, which is caused by *Rhabdovirus carpio*, should be named spring viremia of carp and that the chronic form of infectious dropsy of carp, which does not involve *R. carpio* should be named carp erythrodermatitis. The cause, pathology, and diagnoses of both diseases are described.

Fijan, N., Z. Petrinec, D. Sulimanovic, and L. O. Zwillenberg. 1971. Isolation of the viral causative agent from the acute form of infectious dropsy of carp. Vet. Arh. 41:125-138.

This is a landmark paper in which the authors isolated virus from carp with infectious dropsy and showed the agent to be capable of causing an acute disease course. Rivers' postulates were fulfilled. The paper is well illustrated and includes both light and electron micrographs. Characteristics of the virus, including requirements for growth and isolation, are described.

Krunoslava, V., and N. Fijan. 1973. Susceptibility of carp to *Rhabdovirus carpio* at various routes of inoculation. (Osjetljivost šarana prema rhabdovirus carpio pri raxnim načinima inokulacije). Vet. Arh. 43:271-276. (in Jugoslavian)

The report provides results of investigations of the routes of experimental infections of carp with *Rhabdovirus carpio*. Intracranial, intraperitoneal, and swimbladder inoculation routes led to the development of typical signs of SVC. In contrast, oral inoculation of *R. carpio* caused neither signs of SVC nor mortality.

Reichenbach-Klinke, H. H. 1966. Diseases and injuries of fish. (Krankheiten und Schädigungen der Fische). Gustav Fischer Verlag, Stuttgart (Federal Republic of Germany). 389 pp.

This is a well-illustrated guide to the recognition and treatment of diseases and injuries of fishes. General fish

pathology, infectious diseases, fish disorders of nonparasitic origin, and environmental and pollution problems are also described. Numerous microphotographs and macrophotographs are given. Five pages deal with infectious dropsy of carp.

Schäperclaus, W. 1954. Fish Diseases (Fischkrankheiten). Akademie Verlag. Berlin. 708 pp. (in German)

This is one of the first books on fish diseases that describes causal agents, diagnosis, pathology, and treatment. The valuable work includes tables and graphs as well as macrophotographs and microphotographs. The author was the first investigator of the cause of infectious dropsy of carp and has published about 20 papers on the condition. Fifty pages of the book are devoted to this disease.