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DISSEMINATION OF SPRING VIRAEMIA OF CARP (SVC) IN SERBIA DURING THE PERIOD 1992-2002.

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An acute contagious viral disease caused by Rhabdovirus carpio, spring viraemia of carp, was described for the first time by Fijan in 1972. Carp is the most important susceptible species in all age categories, although other species of cyprinid fish also contract the disease. As the name suggest, the disease typically appears in spring, when the water temperature increases. The disease is often complicated by the secondary appearance of bacterial and parasitic infections. The clinical symptoms include and presume gathering of infected fish near the sides of the pond, uncoordinated swimming, darkening of body colour, pale gills, petechial bleeding (of the skin, eyes, gills, and fin bases), exophthalmus, and the appearance of pseudofecal expulsions from the infected anus.

Pathoanatomical examination sometimes reveals only oedematous organ enlargement and intestinal inflammation. A varying degree of haemorrhaging is present in the bladder, internal organs, and fluid-containing cavities. Diagnosis is based on isolation and identification of the virus and ELISA in the laboratory.

More than 1664 samples were analysed in the course of the 10-year investigation (from 1992-2002) from different carp hatchery localities in the Republic of Serbia. Rhabdovirus carpio was isolated and identified at more than a third of the tested fish hatcheries (31,57%) infected with the spring viraemia virus.

Although the disease was known earlier, it now represents an urgent problem in many European countries, and has been causing great damage in intensive and semi-intensive carp production in our country during the last few years. Therefore, special attention has been paid to it.

Key words: Carp, CPE (cytopathogenic effect), FHM cell lines, serum neutralizing test, ELISA, hatchery, spring viraemia.

INTRODUCTION

Spring viraemia of carp (SVC) is a virus-caused acute contagious disease of all categories of cyprinid and other kinds of fish. It has been diagnosed in Yugoslavia (Fijan, 1972), Czechoslovakia (Macura *et al*., 1973; Tesarčik *et al*.,

1977), Germany (Ahne, 1982; Bachmann and Ahne, 1974), France (Baudouy, 1975), Austria (Köbl, 1975), the USSR (Rudikov *et.al*., 1975), Hungary (Bekesi and Szabo, 1977), Great Britain (Bucke and Finlay, 1979), Ukraine and Spain (Marcotegiu *et al*., 1972).

Carp are the usual and main victims of spring viraemia. Natural outbreaks of the disease have been recorded in pike-perch (*Stizostedion lucioperca*), perch (*Perca fluviatilis*), pike (*Esox lucius*), and various ides (Fijan, 1974), in bighead (*Aristicthys nobilis*; Rudikov *et al*., 1975), in silver carp (*Hypophthalamicthys molitrix*; Šelkunov *et al*., 1984), in crucian carp (*Carassius carassius*; Köbl, 1975), in grass carp (*Ctenopharyngodon idella*; Ahne, 1975, 1978; Rudikov, 1980) and in young sheatfish (*Siluris glanis*; Fijan *et al*., 1984).

The SVC virus belongs to the family *Rhabdoviridae*, which contains ribonucleic acid. It is readily identifiable from its morphology, which resembles an elongated rifle cartridge. On the basis of its biochemical characteristics, the SVC virus belongs to the genus Vesiculovirus. Since it is similar to the vesicular stomatitis virus, iododeoxyuridine does not inhibit its synthesis, but it is sensitive to the action of ethers, acids and heat. It contains negatively chained ribonucleic acid.

The helicoid nucleocapsid that gives the virus its characteristic shape is enclosed in a lipid-rich envelope. Ribonucleic acid isolated from the virus is not a carrier of infectivity, since it does not contain enzymes capable of replicating it. Moreover, it is incapable of acting until transcribed into a positive chain.

Spring viraemia of carp virus reproduces in vitro and stimulates a cytopathogenic effect (CPE) in carp ovary primary cells and in the following permanent cell lines: the FHM (fathead minnow) line, a cell line of the epithelial type, the EPC (epithelioma papulosum cyprini) line, a cell line derived from tumourous changes on the skin of carp, the PG (pike gonad) line from pike gonads, and the RTG_2 (rainbow trout gonad) line from the gonads of rainbow trout.

The highest titres of the virus are achieved during reproduction in the FHM and EPC cell lines. Apart from cells of fish, Rhabdovirus carpio can reproduce in cells of the fruit fly (*Drosophila melanogaster*), turtle (THI), viper (VSW and VH_2), monkey (VERO), hamster (BHK-21), chicken (CEF), dog (MDCK), man (HPE-2), and swine (SK). Pathogenicity of the virus declines during passage through subcultures of these cell lines.

Serological examination revealed the presence of neutralising antibodies in 95%, 86% and 28% of carp hatcheries in Austria (Köbl and Kainz, 1977), Bavaria (Wizigmann *et al*., 1983), and France (Hattenberger-Baudouy and de Kinkelin, 1987) respectively. All age groups of carp are sensitive to SVC, but 9-12- and 21-24-month-old carp represent the most susceptible groups, suffering the greatest losses.

Both season and temperature exert a significant influence on the virus host interaction. The physiological status of overwintering carp is a determining factor in outbreaks of the disease in spring, which occur at the same temperatures as those prevailing in autumn, when heavy losses never occur. Mortality caused by spring viraemia occurs from November to July, with the peak from April to June.

Outbreaks of the disease rarely occur below 10°C, appear most often between 11 and 17°C, and are very rare between 17 and 20°C. Holding fish in wintering tanks (with a high density of fish lacking food) during the initial spring temperature increase and in the presence of many stress-inducing factors favours development of SVC and high mortality (Jeremić *et al*., 1999). Clinical sickness appears in spring at lower temperatures, when carp are incapable of producing interferon and the antibodies that protect them from the disease. Rapid interferon and antibody production sets in at higher temperatures during the summer. Persistence of antibodies during the fall protects the fish during this period of the year. Long and protracted wintering at low temperatures can cause breakdown of immunoglobulins, so no protective antibodies remain in the spring. Sources of infection are sick fish, fish during incubation, carcasses, disease carriers and other fish (Baudouy, 1980). It is certain that the virus is transmitted by water and sludge (Baudouy, 1980). Mechanical carriers of the disease can be the carp louse (*Argulus foliaceus*; Pfeil-Putzien, 1977, 1978; Pfeil-Putzien and Baath, 1978) and the fish leech (*Piscicola geometra*; Ahne, 1986). The virus remains ineffective for about 35 days in water at 10°C, for about 42 days in mud at 4°C, and for more than four weeks in dried mud at a constant temperature between 4°C and 20°C. Lateral transmission by water, mud, vectors, and equipment is apparently the main route of virus spread.

MATERIAL AND METHODS

Material for isolation of the virus was taken from moribund cyprinid fish of different age categories from 38 fish hatcheries in the Republic of Serbia. Gills and parenchymous organs of the fish were used as material for isolation of the virus. Samples of liver, kidneys, spleen and gills were taken and prepared for virological testing by standard methods, according to the principles set forth by Wolf in 1970. For isolation of *Rhabdovirus carpio*, we used FHM and EPC cell lines at 24^h old (Fig. 1). Inoculated cultures were incubated at 20°C for 7 days and examined daily for the appearance of a cytopathogenic effect (*CPE*). The virus was identified by the serum neutralization test with antiserum prepared by hyperimmunization of carp against *Rhabdivirus carpio*.

The optimal temperature for reproduction in vitro is from 20 to 22°C. One reproductive cycle in FHM cells at 21°C lasts from 8 to 10^h and a CPE usually appears after 24 to 72^h.

Besides standard virological methods, viral antigens were also examined by enzyme-linked immunosorbent assay (ELISA), which enables fast disease detection and thereby contributes to the generation of suitable conditions for the prevention and eradication of spring viraemia of carp (SVC) in an affected area. This method can be used for detection of viral antigens directly from the examined materials and also from cell cultures after inoculation.

For ELISA, parenchymous organs, gills and intestines were prepared as for tissue culture, i.e. the organs were homogenized in 3-5 volumes of phosphate buffered saline (PBS), centrifuged at 2000 g for 5 minutes and the supernatants retained for examination.

Microtitre wells coated with specific antibodies to SVC virus were used for the reaction. If present in the tested sample, viral antigens bind to antibodies. By using antibodies conjugat with enzyme, the virus is marked and visualised after reaction with the substrate. The colour intensity (optical density) is measured precisely on a spectrophotometer (ELISA reader) at a specifically defined wavelength. Virus existence is then confirmed or rejected according to the value obtained.

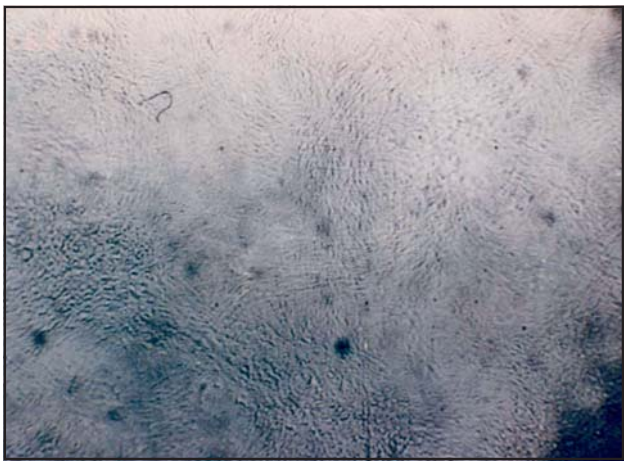

Figure 1. Normal FHM (fathead minnow) cell line 24^{h} old

RESULTS AND DISCUSSION

Sick fish were quiet, and they swam uncoordinatedly. The clinical symptoms included gathering of sick fish near the source of fresh water, floating on the water surface and darkening of body color. The fish did not react to outside stimulations. External examination of the skin, fins, and body cavities, revealed the following changes: increased amount of slimy mass on the skin surface and gills and petechial haemorrhages on the skin of the ventral abdominal part. The abdomen was distended because of ascites (Fig. 2).

The anus was red and with a prolapse. The gills were pale (anaemia) with petechial haemorrhages and oedema, while in some fish there was severe necrosis of the gills. All fish (Fig. 3) exhibited exophthalmus on both sides, which occurred as a consequence of peribulbar oedema and bleeding in the eyes.

Pathoanatomical examination revealed oedema of all internal organs, as well as the wall of the swim bladder. A certain quantity of red fluid and fibrinous peritonitis was present in the abdominal cavity (Fig. 4). Accumulated fibrin caused adhesion of neighboring organs or their parts (Fig. 5). All internal organs showed bleeding, particularly on the internal wall of the swim bladder, gonads, intestines, muscles, kidney, and liver. The spleen was enlarged and differently colored

(Fig. 6). The intestinal wall was oedematous, the lumen was distended, without food but filled with petechial and diffuse bleeding (Fig. 7). Intestinal loops were stuck together. It was difficult to separate them, but they tore apart easily.

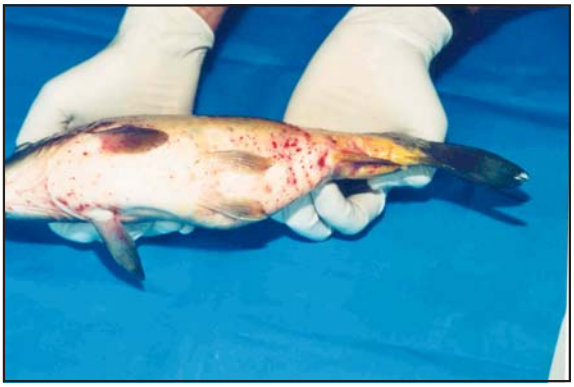

Figure 2. Ascites, petechial haemorrhages on the skin and fins, and red anus

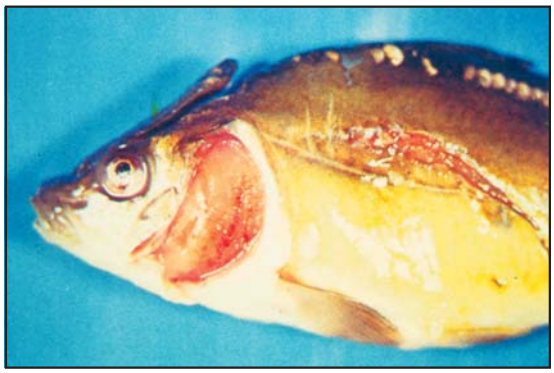

Figure 3. Petechial haemorrhages on the gills associated with necrosis of the tips of the gills

A cytotoxic effect was produced in the first passage during 7 days of incubation of FHM and EPC cells inoculated with a filtrate of pooled samples of the organs of cyprinid species. The control cells were normal, whereas cell cultures inoculated with the reference SVC virus gave a clear CPE in the course of 24 to 72^h in the guise of rounding of EPC and FHM cells and their subsequent falling away from the test tube wall at the end of the incubation period. A strong CPE appeared in the course of 72^h. The control cells were normal, whereas cells with the reference SVC virus also showed a CPE (Fig. 8).

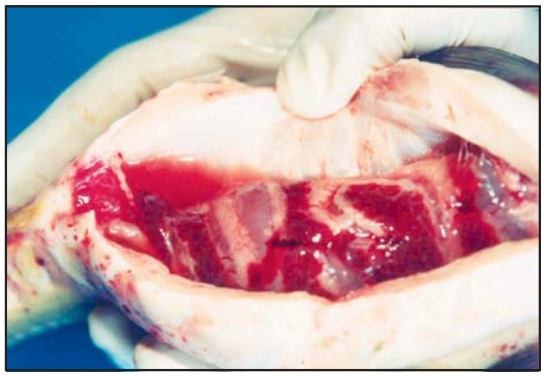

Figure 4. Some red fluid is present in the abdominal cavity

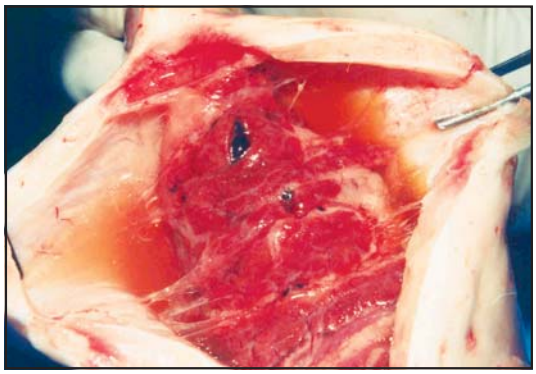

Figure 5. Fibrinous peritonitis. Accumulated fibrin caused adhesion of neighboring organs or their parts

Figure 6. Bleeding of internal organs (swim bladder, intestines, kidneys and liver)

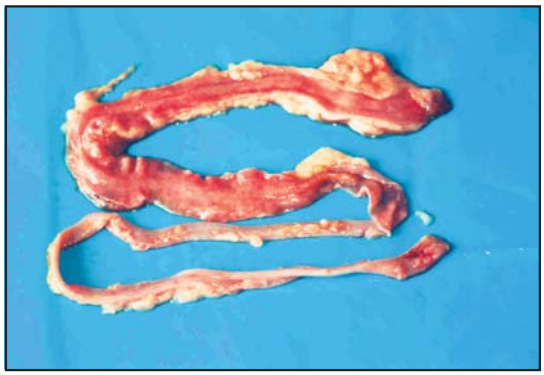

Figure 7. Oedematous and congestive intestinal mucosa as a sign of the inflammatory process

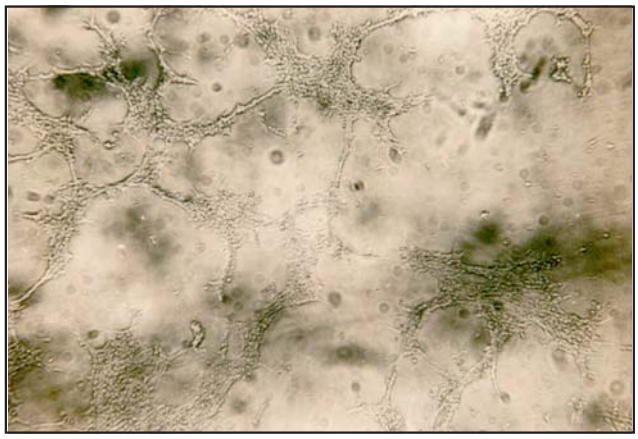

Figure 8. Cytopathogenic effect (CPE) on FHM cell line 72^{h} after inoculation

Material inoculated into cell cultures was simultaneously examined by ELISA and the presence of viral antigen was detected in homogenized organs as well as in infected cell cultures.

The results achieved in cell culture were identical to the ELISA results, which demonstrated or denied the presence of virus in the examined material.

Previous results have shown ELISA to be effective, rapid and technically not demanding. Therefore, it is very effective in routine diagnostics of fish disease.

Virologically positive epidemics of SVC appeared for the first time during the spring of 1992 at water temperatures of 13 and 15°C. The severity of the disease varied from basin to basin at the same hatchery and from hatchery to hatchery and some hatcheries were virologically positive for two successive years.

A picture of the dissemination and frequency of occurrence of the disease can be obtained from data obtained over the 10 year period, during which virological examination of 1,664 specimens of carp from 38 hatcheries in Serbia revealed the presence of *Rhabdovirus carpio* in 224 specimens as follows: 125 specimens in April, 73 in May, 19 in June, four in December, and three in February.

Table 1 gives a summary of the tested and confirmed cases of SVC over the decade. During the winter (February, 1992 and December, 1993) when the water temperature was below 10°C, seven cases of carp death with disease symptoms, pathoanatomical changes, and isolation of the virus were recorded, which agrees with the findings of Baudouy *et al*. (1980).

Table 1. Survey of positive cases of spring viraemia of carp in Serbia (1992-2002)

Year	Number of Samples	Positive Samples	Fish pond	Age
1992	539	109	Uzdin	2 yr. old carp fry
			Srpski Miletić	2 yr. old carp fry
			Iđoš	1 year, 2 yr. old carp fry
			Despotovo	2 yr. old carp fry
			Vrbas	2 yr. old carp fry
1993	157	28	Kovilj	1 year, 2 yr. old carp fry
			Iđoš	2 yr. old carp fry
			Vrbas	2 yr. old carp fry
1994	149	19	Kovilj	2 yr. old carp fry
			Uzdin	2 yr. old carp fry
1995	121	16	Ečka	2 yr. old carp fry
1996	85	0		
1997	128	42	Futog	2 yr. old carp fry
			Iđoš	1 year, 2 yr. old carp fry
			Srpski Miletić	2 yr. old carp fry
			Jazovo	2 yr. old carp fry
1998	185	5	Vrbas	2 yr. old carp fry
			Banatska Dubica	1 year old carp fry
			Iđoš	2 yr. old carp fry
1999	144	3	Banatska Dubica	1 year old carp fry
			Neuzina	2 yr. old carp fry
2000	109	0		
2001	47	2	Sombor	larva
Σ	1664	224		

Thirty-eight hatcheries were encompassed by the 10 year testing. Twelve (31,57%) out of 38 hatcheries were contaminated by the spring viraemia virus, which is very alarming, as it is a threat to carp production.

It is practically impossible to eradicate the disease under the present conditions of carp technology used in Serbia. This is because all our larger carp hatcheries are supplied from open bodies of water inhabited by fish. A small number of infected specimens is present in every natural fish population. Thus, fish in natural waters represent a source of infection for fish in hatcheries. Conversely, the disease is transmitted to natural populations by stocking with fish from contaminated hatcheries. Disease agents enter hatcheries with water and especially with small fish that pass through the vents. On the other hand, when hatchery ponds are emptied, disease agents pass with the water and fish into open waters and are transmitted to the fish there. In many cases, a circle is thereby established that maintains a constant presence of the disease in hatcheries. Moreover, disease spread has been greatly facilitated by inadequate sanitary control of fish transported for various reasons from one hatchery to another.

In suppressing the disease, the "stamping out" method can be successfully used only at small hatcheries that are supplied with water from springs and wells, or are replenished by rainwater (Ghittino *et al*., 1980). Prophylaxis of SVC can be successful, although sanitary control is much harder to implement in fish held in hatchery ponds than in concrete basins (Baudouy, 1980). It is based on zoosanitary measures designed to prevent entry of disease agents into nurseries. By prohibiting the introduction of fish into hatcheries controlled for several years in a row, it should be possible to reduce the number of diseased fish and prevent occurrence of the disease. Infection with the SVC virus during the summer promotes the development of natural immunity, which means that appearance of the disease in the spring is prevented by natural immunization (Köbl and Kainz, 1977). The only successful way of combating SVC is immunoprophylaxis, which is the method of choice at large hatcheries.

By a perennial ban on the input of fishes that are not subject to health control, into the monitored fish population and by regular laboratory examination, the number of infected fishes will be reduced as well as the occurrence of disease.

As a rapid and efficient method, ELISA should be used for confirmation or rejection of virus existence, especially for fishes in quarantine and before transport.

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PODACI O RAŠIRENOSTI PROLEĆNE VIREMIJE ŠARANA (PVŠ) U SRBIJI U PERIODU OD 1992 DO 2002.

JEREMIĆ SVETLANA, JAKIĆ-DIMIĆ DOBRILA i RADOSAVLJEVIĆ V

SADRŽAJ

Prolećna viremija šarana je virusna infekcija šarana i nekih drugih toplovodnih vrsta riba koja postaje iz dana u dan sve značajnije oboljenje za naše ribarstvo.

Mada je bolest poznata još od ranije ona je i danas aktuelna u mnogim evropskim zemljama, a u našoj zemlji u poslednjih nekoliko godina nanosi velike štete intenzivnoj i poluintenzivnoj šaranskoj proizvodnji.

U toku višegodišnjih ispitivanja od 1992. do 2002. godine kojima su obuhvaćeni različiti lokaliteti šaranskih ribnjaka sa preko 1664. uzoraka uspeli smo da izolujemo i identifikujemo *Rhabdovirus carpio* u 224 uzorka šarana, odnosno 31,57% ispitanih ribnjaka bilo je zaraženo virusom prolećne viremije šarana.

U ispitivanjima smo koristili klasične metode virusoloških pregleda od izolacije virusa preko sistema kulture ćelija, identifikacije virusa serum neutralizacionim testom sa anti PVŠ serumom. Takođe smo vršili otkrivanje virusnog antigena ELISA imunoenzimskom metodom koja nam omogućuje brzo otkrivanje bolesti za razliku od klasnih metoda i time doprinosi stvaranju adekvatnih uslova za sprečavanje širenja i eradikaciju na prostoru na kome se pojavila prolećna viremija šarana.

Naša ispitivanja ukazuju da je PVŠ veoma raširena u Republici Srbiji. Iskorenjivanje bolesti u uslovima tehnolgoije gajenja šarana u Srbiji je praktično nemoguće sprovesti.

Pored blagovremene dijagnoze, strogom kontrolom prevoza ribe sa ribnjaka na ribnjak i profilaktičko-sanitarnih mera imunizacija riba je jedan od uspešnijih načina borbe protiv te zaraze.