

**RHABDOVIRUS CARPIO AS A CAUSATIVE AGENT OF DISEASE IN RAINBOW TROUT
(ONCORHYNCHUS MYKISS – WALBAUM)**

JEREMIĆ SVETLANA, IVETIĆ V and RADOSAVLJEVIĆ V

The Institute of Veterinary Medicine of Serbia, Belgrade

(Received 10. March 2006)

High mortality of 1-year old rainbow trout occurred on a fish farm in the spring season, with clinical symptoms typical for acute septicemia. Histological examination revealed inflammatory changes and necrosis of the internal organs. The causative agent was isolated on RTG-2 and EPC cell lines and identified by serum neutralisation, enzyme-linked immunosorbent assay (ELISA) and immunofluorescence (IFAT) as Rhabdovirus carpio.

Experimental infection using the virus isolated from rainbow trout caused disease in carp fry, as well.

This is the first case of disease of rainbow trout caused by Rhabdovirus carpio, and the first reported isolation of Rhabdovirus carpio from rainbow trout in Serbia. Also, this is the first successful infection of carp with Rhabdovirus carpio isolated from rainbow trout.

Key words: Rhabdovirus carpio, Spring viremia of carp virus, isolation, identification, rainbow trout, carp, experimental infection.

INTRODUCTION

Spring Viraemia of Carp (SVC) is a contagious viral disease of all varieties of common carp and several other cyprinid species, with very high mortality among infected fishes. The causative agent, Spring Viremia of Carp Virus (SVCV) or *Rhabdovirus carpio*, is a member of the family *Rhabdoviridae*, genus *Vesiculovirus* (Wolf, 1988; Wunner and Peters, 1991). The genome of SVCV has been shown to consist of single stranded RNA of approximately 11000 nucleotides with negative polarity (Hoffmann *et al.*, 2002).

SVC 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), USSR (Rudikov *et al.*, 1975), Hungary, (Bekesi and Szabo, 1977), Great Britain (Bucke and Finlay, 1979) and Spain (Marcotegiu *et al.*, 1972). More recently, SVC has been reported in North America (Goodwin, 2002).

Common carp (*Cyprinus carpio*) is the main species of fish affected by SVC. Natural outbreaks have been recorded in pike-perch (*Stizosteidon lucioperca*), perch (*Perca fluviatilis*), pike (*Esox lucius*), bighead carp (*Aristichthys nobilis*);

Rudikov *et al.*, 1975), silver carp (*Hypophthalmichthys molitrix*; Šelkunov *et al.*, 1984), crucian carp (*Carassius carassius*; Kobl, 1975), grass carp (*Ctenopharyngodon idella*; Ahne, 1975, 1978; Rudikov, 1980) and sheatfish (*Siluris glanis*; Fijan *et al.*, 1984). Recently, russian scientists have isolated SVCV from rainbow trout (ECRL, 2005)

In this paper, clinically manifested disease of rainbow trout fry caused by *Rhabdovirus carpio* was described, as well as pathological changes present in the internal organs in experimentally infected carp fry.

MATERIALS AND METHODS

Case history

A high rate of mortality of one-year old rainbow trout was detected in a fish farm at the end of May. A sample of 39 affected fish, with mildly distended abdomen, haemorrhaging in anterior eye chamber, dark-coloured skin and ataxia and a sample of 10 clinically healthy fish were taken for laboratory examination.

Samples of gills, kidney, spleen, liver and intestines were taken into 10% formol buffered saline for subsequent histological examination. Samples were processed histologically and sections were stained with haematoxylin and eosin according to the method described by Roberts (2001).

For virus isolation, gills and parenchymous organs were used. Samples of parenchimatous organs and gills were taken and prepared for virological testing by standard methods, according to the principles set forth by Wolf in 1970.

Pools of parenchimatous organs and gills were ground with sterile sand in minimum essential medium (MEM) and centrifuged at 2500 g for 20 min. These supernatants were inoculated onto 24^h old monolayers of the RTG-2 (*rainbow trout gonad*) and EPC (*epithelioma papulosum cyprini*) cell lines. Thereafter, both cell lines were incubated at 20°C for 7 days. Cultures were examined daily for the presence of viral-induced cytopathic effect (CPE). In the absence of CPE, samples were given further two passages.

The virus was identified by the serum neutralization with anti-SVCV serum, by enzyme-linked immunosorbent assay (ELISA, Test-Line), and immunofluorescence (IFAT, Bio-X Diagnostics).

Experimental infection

Carp fry (average weight 10-13 g) with no previous exposure to SVCV was maintained at Institute of Veterinary Medicine of Serbia, Belgrade, Serbia, in aquaria with aerated static water at 11°C. A group of 20 fish were infected intraperitoneally (i./p.) with 0.2 ml of a viral suspension of isolate containing about 10⁷ c.f.u./ml. Fish were inspected daily for signs of disease and mortality.

RESULTS AND DISCUSSION

Mortalities of rainbow trout began to first appear in late May, when water temperature rose to 12°C. Affected fish gathered at the pond's edges and near the

bottom. Occasionally, moribund fish »hung« from the water surface. Other external signs included dark coloration, exophthalmos, abdominal distension and haemorrhages in the anterior eye chamber. Internally, blood-tinged ascitic fluid was present in the abdominal cavity. The liver was slightly enlarged, anaemic with petechial haemorrhages. Histopathological examination showed that the microarchitecture of the hepatic tissue was almost destroyed. Small parts of healthy tissue were visible in certain places, while the remaining tissue was atrophied due to pressure of the cellular infiltrate.

The kidney was congested, oedematous and grey in color. Perivascular, inter- and peritubular infiltrate was present in the kidney, and in some areas a small number of extravasated erythrocytes was also visible. The spleen was enlarged with the loss of sharp edges. The intestine was filled with mucous yellowish fluid.

Virological examination

The virus was isolated from the parenchymatous organs and gills of diseased rainbow trout. A cytotoxic effect appeared in the first passage during 7 days of incubation of RTG-2 and EPC cells inoculated with a filtrate of pooled samples. The control cells were normal, whereas cell cultures inoculated with the reference SVC virus gave a clear cytopathic effect (CPE). After subcultivation, the cytopathic effect appeared after 48 to 72^h in the form of focal patches of round refractile cells, with the destruction of the entire monolayer after 72^h. The control cells were normal, whereas cells with the reference SVC virus showed a CPE.

Identification of the obtained viral isolate was done by the serum neutralization test with anti-SVCV serum, SVCV Ag ELISA and immunofluorescence (IFAT).

Experimental infection

In carp, disease symptoms and mortality started on the 8th day after i/p infection. Petechial and diffuse haemorrhaging was detected on the skin in moribund and dead carp. The gills were pale with petechial haemorrhages and swelling of the gill filaments. Dissection revealed peritonitis and bloody transudate in the abdominal cavity. The liver was light-pink in color, with petechial haemorrhages. The spleen was enlarged, with round edges. The kidney was oedematous, with petechial haemorrhages. Inflammation of the intestine was present, and the lumen was filled with yellow mucous fluid.

Rhabdovirus carpio was isolated from all dead and moribund fish, as well as from the kidneys and liver of fish surviving at the end of the experiment. These results differ from previous experiment, where Stone (2004) experimentally produced 70% mortality in i.p. injected rainbow trout fry, but no mortality in i.p. injected yearling carp, using russian SVCV isolate from trout.

Pathomorphological changes in experimentally infected carp fry

Histopathological changes were observed in carp with significant hydrops and fibrinous peritonitis. Changes were detected on liver, kidneys, spleen and intestines.

The pathoanatomical section reveals oedema of all internal organs as well as of the wall of swim-bladder. A certain quantity of red fluid and fibrinous peritonitis were present in the abdominal cavity. Accumulated fibrin caused adhesion of neighbouring organs or their parts. The viscera were oedematous and petechial haemorrhages occurred in the internal wall of air bladder, kidney, liver, intestines and skeletal muscles. The spleen was enlarged and marbled in appearance. The intestinal wall was oedematous, covered with petechial and diffuse bleedings, the lumen distended, filled with yellow mucous fluid. Intestinal curves were stuck together, and teared apart easily. Sulimanović *et al.* (1986) divided histopathological changes in two groups: acute necrotic alterations with an inflammatory reaction in the first group, and prolonged course with hydrops and a powerful host response. Fatty infiltration of liver cells was present. In the hepatocytes, bright fatty vacuoles of different sizes were detected. They almost completely pushed back the nucleus on the periphery of the cell. Vacuoles may conflate, causing interruption of the cell membrane, with formation of large bright spaces. Also, mononuclear cell infiltration (hepatitis) and erythrocyte extravasation were present. Our results are not in agreement with the findings of Roberts (1978) and Sulimanović *et al.* (1986), who did not find fatty cells in the liver. They observed severe necrosis of hepatocytes along blood vessels or by the organ surface. In advanced cases, the authors described the regeneration process with concurrent infiltration of macrophages. Similar histopathological changes, with fatty infiltration in the liver, were described by Amlacher (1961). He described hydrops in the abdominal cavity with lipoid degeneration before the beginning of liver cells necrosis.

Epithelium of the renal tubules was intact. The lumen of renal tubules was visible and completely empty. In the interstitium, intertubular or perivascular infiltration of mononuclear cells was present, with a tendency to confluence, comprising in this way large parts of kidney tissue. Necrotic alterations were not present in the kidney tissue, which is not in agreement with the findings of Sulimanović *et al.* (1986). Their findings suggest that focal necrosis and macrophage infiltration is present in the haematopoietic tissue of kidneys, as well as in the liver. Excretory parts of the kidney were without any changes, except that tubular connective tissue was in some cases slightly edematous.

With the exception of reticular hyperplasia and discrete blood extravasate, there were not other microscopical changes in the spleen tissue. These results are in agreement with the conclusions of Sulimanović *et al.* (1986).

The results of microscopical examination of the intestines showed that *mucosa propria* was infiltrated with inflammatory cell elements. Along the intestinal epithelium, multiplied epithelial cells were visible. The intestinal villi were hypertrophic with a tendency to join together. Sulimanović *et al.* (1986) concluded that in the intestines prevail desquamation of the epithelium, proliferation of mononuclear cells in *tunica propria* and oedema of the muscular tissue.

This is the first case of disease of rainbow trout caused by *Rhabdovirus carpio* and the first reported isolation of *Rhabdovirus carpio* from rainbow trout in Serbia. Also, this is the first successful infection of carp with *Rhabdovirus carpio* isolated from rainbow trout.

Address for correspondence:
Jeremić Svetlana,
The Institute of Veterinary Medicine of Serbia,
Vojvode Toze 14,
11000 Belgrade, Serbia

REFERENCES

1. Ahne W, 1975, A rhabdovirus isolated from grass carp (*Ctenopharyngodon idella* Vat.), *Arch Virol*, 48, 181-5.
2. Ahne W, 1982, Vergleichene Untersuchungen über die Stabilität von vier fischpathogenen Viren (VHSV, PER, SVCV, IPNV), *Zbl Vet Med*, B, 29, 457-76.
3. Bachmann PA, Ahne W, 1974, Biological properties and identification of the agent causing swimbladder inflammation in carp, *Arch Ges Virusforsch*, 44, 261-9.
4. Baudouy AM, 1975, Viremie printaniere de la carpe: premiers isolement du virus en France, *Bull Off Int Epiz*, 83, 717-22.
5. Bekesi L, Szabo E, 1977, First isolation of *Rhabdovirus carpio* from carp in Hungary, *Magyar Allatoovosk Lapja*, 6, 369-71.
6. Bucke D, Finlay J, 1979, Identification of spring viremia in carp (*Cyprinus carpio* L.) in Great Britain, *Vet Record*, 104, 4, 69-71.
7. Fijan N, 1972, Infectious dropsy of carp - a diseases complex, Book of Proceedings, Symp Zool Soc, London, 30, 35-51.
8. Goodwin EA, 2002, First Report of Spring Viremia of Carp Virus (SVCV) in North America, *J Aquat An Health*, 14, 161-4.
9. Hofmann B, Shutze H, Mettenleiter A, Thomas C, 2002, Determination of the complete genomic sequence and analysis of the gene products of the virus of Spring Viremia of Carp, a fish rhabdovirus, *Vir Res*, 84, 1-2, 89-100.
10. Köbl O, 1975, Neue Erkenntnissue über die Bauchwassersucht bei Karpfen, *Österreich Fischerei*, 28, 5-6, 69-72.
11. Macura B, Dedek L, Tesarčik J, 1973, The positive cytopathic effect from a case of spring viremia of carp, *Bull VUHR Vodnany*, 9, 7-14.
12. Marcotegui MA, Estepa A, Frias D, Cooi JM, 1972, First report of a Rhabdovirus affecting carp in Spain, *Bull Eur Ass Fisch Pathol*, 12, 2, 50.
13. Roberts JR, 2001, Fish Pathology, Third edition, Harcourt Publishers Limited, WB Saunders, 386-90.
14. Rudikov NJ, Griščenko L, Lobuncov KA, 1975, Весенняя вирусная болезнь рыб, *Bull Vses Ord Lenina Inst Exp Vet*, 20, 16-9.
15. Tesarčik J, Macura B, Dedek L, Valiček L, Šmid B, 1977, Isolation and electron microscopy of a Rhabdovirus from the acute form of infectious dropsy of carp (spring viraemia of carp), *Zbl Vet Med B*, 24, 340-3.
16. European Community Reference Laboratory for Fish Diseases (ECRL), (2005): SVC tutorial, Danish Institute for Food & Veterinary Research, Århus, Denmark.
17. Stone D, 2004, Project FC 1152 – Studies on the pathogenicity, tissue tropism and transmission of VHS In: Trout News, 38, 23-4.
18. Sulimanović Đ, Bambir S, Sabočanec R, Čuljak K, Miyazaki, 1986, Spring viremia of carp: Microscopic pathology: International symposium of ichthyopathology in aquaculture, Dubrovnik, october 21-24, 1986.
19. Wolf K, 1988, Fish Viruses and Fish Viral Diseases. Cornell University Press, Ithaca, NY.
20. Wunner WH, Peters D, 1991, The Rhabdoviridae, *Arch Virol*, Suppl 2, 250-62.

**RHABDOVIRUS CARPIO KAO UZROČNIK OBOLJENJA KALIFORNIJSKE
PASTRMKE (*Oncorhynchus mykiss* – Walbaum)**

JEREMIĆ SVETLANA, IVETIĆ V i RADOSAVLJEVIĆ V

SADRŽAJ

Rhabdovirus carpio je uzročnik prolećne viremije šarana koji je najvažnija ugrožena vrsta u svim starosnim kategorijama, iako oboljevaju i druge ciprinidne vrste riba. Do nedavno nije bilo dokazano da može oboleti i kalifornijska pastrmka. U radu je opisano kliničko oboljenje jednogodišnje mlađi kalifornijske pastrmke, sa visokim mortalitetom, koje je ukazivalo na tipičnu akutnu septikemiju. Za laboratorijska ispitivanja uzeto je 39 uzoraka obolele jednogodišnje mlađi kalifornijske pastrmke, sa sledećim kliničkim sptomima: uvećanje abdomena, tamna pigmentisanost, egzoftalmus, krvarenje u prednjoj očnoj komori, i 10 uzoraka riba bez kliničkih simptoma bolesti.

Deo promenjenih organa (parenhimatozni organi i škrge) je pripremljen za virusološka ispitivanja. Za izolaciju virusa su korišćene RTG-2 i EPC ćelijske linije, a identifikacija je vršena serum neutralizacionim testom, ELISA testom i imuno-fluorescencijom (IFAT). Za histološko ispitivanje, deo promenjenih organa (parenhimatozni organi, škrge, creva) je fiksiran u 10% formalinu, uklopljen u parafin, napravljeni tkivni isečci debljine 6 μm , i obojeni hematoksilin-eozinom.

Najčešće patohistološke promene utvrđene su u jetri, bubregu i crevima u vidu zapaljenskih i nekrotičnih promena. Veštačka infekcija mlađi šarana je vršena i/p inokulacijom 0,2 ml 10^7 c.f.u./ml izolata *Rhabdovirus carpio*, izolovanog iz kalifornijske pastrmke. *Rhabdovirus carpio* je izolovan iz svih uginulih i moribundnih šarana. Šta više, patogen je reizolovan iz parenhimatoznih organa preživelih riba na kraju ogleđa.

Ovo je prvi objavljeni slučaj pojave oboljenja izazvanog sa *Rhabdovirus carpio* kod kalifornijske pastrmke, i prva izolacija *Rhabdovirus carpio* iz kalifornijske pastrmke u Srbiji. Takođe, u radu je opisana i prva veštačka infekcija šarana sa *Rhabdovirus carpio* izolovanim iz kalifornijske pastrmke.