

## The First Report of SVC from Indian Carp Species by PCR and Histopathologic Methods in Iran

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**Abstract:** In this research, we confirmed the positive SVC in three Indian carps spp. (1) Rohu (*Labeo rohita*), (2) Merigal (*Cirrhinus merigala*) and (3) Catla (*Catla catla*) with typical histopathologic signs and PCR sequencing. Nested-PCR used to amplify a fragment of viral glycoprotein gene in Shahid Beheshti University M.C. and PCR product was purified and submitted to sequencing and deposited to GenBank at accession No. FJ711168. Two sites in this research that, were placed in Khuzestan Province (Ahvaz City, South of Iran) and Gilan Province (Astaneh Ashrafiyeh City, North of Iran) aquaculture farms. Samples were prepared for PCR method in both sites (30 pieces from Khuzestan and 30 pieces from Gilan Province), that all of the (100%) samples were positive in Nested-PCR. In addition, other 60 samples for histopathologic studies in both sites (30 pieces from Khuzestan and 30 pieces from Gilan Province), (according to 10% prevalence). All of the (100%) typically samples were with major histopathologic signs. The results of this study indicate that SVC infection can be found in some *Indian carp* in Iran.

**Key words:** SVC, Indian carp, PCR, histopathology

### INTRODUCTION

Spring Viremia of Carp (SVC) is an important disease affecting cyprinids (Hoole and Bucke, 2001). Mainly Carps are common carp (*Cyprinus carpio*) and Indian carp's sp. (1) Rohu (*Labeo rohita*), (2) Merigal (*Cirrhinus merigala*) and (3) Catla (*Catla catla*). Natural infections have been recognized in common carp, koi carp, grass carp, silver carp, big head carp, crucian carp and goldfish. Designated a notifiable disease by the Office International des Epizooties (OIE), SVC is caused by a rhabdovirus, Spring Viremia of Carp Virus (SVCV). This virus is a member of the family Rhabdoviridae (Haghghi *et al.*, 2008b) and has been tentatively placed in the genus *Vesiculovirus*. Affected fish show destruction of tissues in the kidney, spleen and liver, leading to hemorrhage, loss of water salt balance and impairment of immune response. High mortality occurs at water temperatures of 10 to 17°C typically in spring. The virus is shed mostly with the feces and urine of clinically infected fish and by carriers (Fijan, 1972). Waterborne

transmission is believed to be the primary route of infection, but bloodsucking parasites like leeches and the carp louse may serve as mechanical vectors of SVCV (Ahne *et al.*, 2002). The genome of SVCV is composed of a single molecule of linear, negative-sense, single-stranded RNA containing five gene in the order 3'-NPMGL-5' coding for the viral nucleoprotein, phosphoprotein, matrix protein, glycoprotein. Although, fish of any age can become ill, disease is most common in young fish up to a year of age. The morbidity and mortality rates vary with stress factors and population density, as well as the species, age and condition of the fish. Water temperature affects the development of disease. Virulent virus is shed via feces, urine, gill and skin mucus and exudate of skin blisters or oedematous scale pockets. However, liver, kidney, spleen, gill and encephalon are the organs in which SVCV is most abundant during the course of over infection (Faisal and Ahne, 1984). The mode of transmission for SVCV is horizontal, but an 'egg-associated' transmission (vertical) cannot be ruled out. Horizontal transmission may be direct

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or vectorial, water being the major abiotic vector. Incubation periods from 7 to 15 days have been reported in experimental infections (Ahne, 1985). For additional information about SVC disease in carp in other countries, data shown by Haghghi *et al.* (2008a). The aim of this study was detection of SVC virus in Indian carp's farm in Iran.

**Clinical signs:** Indian Carp sp. can carry SVCV with or without symptoms. The clinical signs are abdominal distension, exophthalmia, inflammation or edema of the vent (often with trailing mucoid fecal casts) and petechial hemorrhages of the skin, gills and eyes. The body is often darkened with pale gills. Diseased fish tend together at the water inlet or sides of the pond, swim and breathe more slowly than normal (Faisal and Ahne, 1984).

Loss of equilibrium, with resting and leaning, are seen in the late stages. The body is often darkened with pale gills and petechial hemorrhages may be seen in the skin, gills or eyes. The abdominal cavity typically contains serous fluid, which may be mixed with blood or necrotic material. The muscles and fat may contain petechial or focal hemorrhages. Similar hemorrhages are also common on the internal organs, particularly on the walls of the swim bladder. The intestines are often severely inflamed and dilated and may contain necrotic material. The spleen is frequently swollen, with a coarse surface texture. Other lesions may include degeneration of the gill lamellae, edema of other internal organs, hepatic necrosis, jaundice, cardiac inflammation and pericarditis. In fish that die suddenly, gross lesions may be absent (Fijan, 1999).

## MATERIALS AND METHODS

**Sampling:** The samples were selected among Indian carp with the symptoms of SVC (kidney swallowing, exophthalmia, general petechiae, bronchi mottling, mouth inflammation, ascites, spotting bleeding on adipose tissues and preocular bleeding) under perishing between May 2006 and September 2007. PCR samples were stored in ethanol 70% and transmitted to the Biotechnology Laboratory.

**RNA extraction:** Viral RNA extraction was done by RNX<sup>plus</sup> buffer (CinnaGen, Iran) as described previously by Kazemi *et al.* (2004). Briefly, about 1 mm<sup>3</sup> of fish tissue was transferred to 1.5 mL micro tube, then 200 µL RNX plus buffer was added. The mixture was incubated for 5 min at room temperature and then 50 µL of chloroform was added and centrifuged at 12000 rpm for 15 min at 4°C.

Total tissue RNA (include viral RNA) was precipitated by ethanol and then dissolved in 10 µL of diethyl pyrocarbonate treated water.

**Reverse transcription reaction:** Reverse Transcription (RT) was performed as previously described by Pfeffer (1998). Briefly, template RNA (1 µg) was incubated in a 20 µL reaction mixture containing: 40 pico mole of specific antisense external primer (SVC R 5' - CAC ACT CAT GCT GTA CAG TCT C - 3'), 100 unit of reverse transcriptase enzyme (RT) (Fermentas, Lithuania), 20 unit RNAsin (Fermentas, Lithuania), 1X RT buffer, 0.2 mM dNTP, for 1 h at 42°C.

**PCR reaction:** A nested-PCR was used to amplify a fragment of viral glycoprotein gene. First PCR reaction mixture was contained 10 µL of synthesized cDNA, 1.5 mM MgCl<sub>2</sub>, 0.1 mM dNTP, 1X PCR buffer, 40 pico mole each forward and reverse primers (Nest I primers: SVC F 5' - CCT ACC AAA CAC GAT GGG TTT G - 3' and SVC R 5' - CAC ACT CAT GCT GTA CAG TCT C - 3, were amplified 445 bp of viral glycoprotein gene) and 1.25 unit of Taq DNA polymerase (CinnaGen Iran) and was carried out within 30 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec and extension at 72°C for 40 sec. One micro liter of PCR product was used as template DNA for second PCR. Second PCR reaction also was done like first PCR within 30 cycles (Nest II primers: SVC2 F 5' - TGC CAC CAA ATT GAC AAT AAA - 3' and SVC2 R 5' - GTG ATC CAA ATA GAG TGA TTC - 3', were amplified 398 bp of viral glycoprotein gene) (Mc Pherson *et al.*, 2000).

**Detection of PCR product:** PCR product was electrophoresed on 2% agarose gel, stained by ethidium bromide and UV light under UV Transilluminator observed DNA band (Boffy, 1984).

**Sequence analysis:** PCR-product was purified and submitted to sequencing.

**Histopathology:** In histopathology method, tissues from live or moribund (but not dead), fish or targeted tissue should be fixed in formalin salin 10%. The fixative ought to be change in another new formalin salin 10% after 24 h and samples prepared in processing set in almost 20 h, then transfer to be processed embedding and sectioning at 5 µm thickness that prepared to tissue staining standard method on the slides with haematoxylin and eosine (H and E) (Fig. 1) or (PAS) (Fig. 2) (Roberts, 2001).

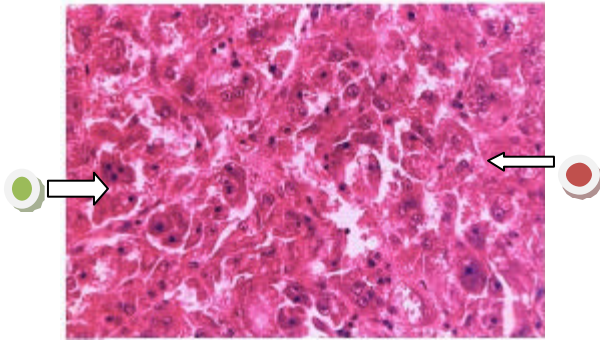


Fig. 1: Liver, many degenerative changes and necrotic fossa with karyorrhexis and chromatolysis in nucleus of hepatocytes are present among atrophic hepatocytes H and E X320



Fig. 2: Intestine of Indian carp (Ruho sp. (*Labeo rohita*), Villi have acute necrotizing zones (arrowed) with severely inflammation and loss of villar mucosa, pyknosis and degeneration of mucosal tissue in the crypts. PAS X120

### RESULTS

We collect 60 samples of Indian carps sp. (1) Rohu (*Labeo rohita*) (2) Merigal (*Cirrhinus merigala*) and (3) Catla (*Catla catla*) from Khuzestan Province (Ahvaz City, South of Iran) and Gilan Province (Astaneh Ashrafieh City, North of Iran) aquaculture farms in Iran. The SVC virus was detected by using PCR and histopathological methods. The PCR product was purified and submitted to sequencing and deposited to GenBank at accession No. FJ711168.

In 60 samples for histopathologic studies in both sites (30 pieces from Khuzestan and 30 pieces from Gilan Province). According to 10% prevalence, all of the 100%, typical samples (Table 1) with major signs as the muscles and fat contain petechial or focal hemorrhages. Similar hemorrhages are also common on the internal

Table 1: Sampling of Indian carp sp. farms in two provinces and frequency of SVC virus positive by Nested-PCR and histopathological methods

Provinces	Sample size (Prevalence 10%)	Positive SVC			
		Histopathology		Nested-PCR	
		Pieces	Percent	Pieces	Percent
Khuzestan	60	30	100	30	100
Gilan	60	30	100	30	100
Total	120	60	100	60	100

organs, particularly on the walls of the swim bladder. The intestines are often severely inflammation and may contain necrotic material. The spleen is frequently swollen, with a coarse surface texture. Other lesions may include degeneration of the gill lamellae, edema of other internal organs, hepatic necrosis.

### DISCUSSION

The results of this study indicate that SVC infection can be found in some Indian carps in Iran. To study the pathogenicity and to obtain isolates of SVC virus the establishment of a laboratory with virus culture equipment is necessary (Haghighi *et al.*, 2008a). The severity of disease was dependant to the period of disease, body immunological resistance of fish, stress and environmental factors related to season, temperature and pH changes (Ahne, 1985).

The respect for on-site hygiene rules, control of disease in fish propagation and breeding centers, isolation and quarantining of infected Indian carp or fish with abnormal behavior have a major role for the prevention of disease (Fijan, 1999). Often, one of the most important causes of little incidence of disease is also the weakness of virus carriers (Haghighi *et al.*, 2007). Therefore, the innovation of sensitive and specific techniques for the diagnosis of disease such as PCR and histopathology are necessary (Faisal and Ahne, 1984). Other techniques such as gene sequencing method. However, because of the cross reaction with other viruses, there is the probability of error. The advantage of this study is detection of SVC virus in Indian carp in Iran for first time.

In brief, the PCR and gene sequencing are useful for the diagnosis of SVC disease and these methods have almost perfect agreement.

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