



**HAL**  
open science

## Detection of spring viremia of carp virus by hybridization with biotinylated DNA probes

Sf Oreshkova, Nv Tikunova, Is Shchelkunov, Aa Ilyichev

► **To cite this version:**

Sf Oreshkova, Nv Tikunova, Is Shchelkunov, Aa Ilyichev. Detection of spring viremia of carp virus by hybridization with biotinylated DNA probes. *Veterinary Research*, 1995, 26 (5-6), pp.533-537. hal-00902387

**HAL Id: hal-00902387**

**<https://hal.science/hal-00902387>**

Submitted on 11 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Detection of spring viremia of carp virus by hybridization with biotinylated DNA probes

SF Oreshkova<sup>1</sup>, NV Tikunova<sup>1</sup>, IS Shchelkunov<sup>2</sup>, AA Ilyichev<sup>1</sup>

<sup>1</sup> Institute of Biotechnology, State Research Center 'Vector' Koltsovo,  
Novosibirsk Province, 633159;

<sup>2</sup> All-Russian Research Institute of Pond Fisheries, Rybnoe, Moscow Province, 141821 Russia

Spring viremia of carp virus (SVCV) is a fish rhabdovirus that causes a disease which remains largely untreatable. It is responsible for heavy economic losses in the pond fisheries of Russia. Typically, the detection of viral diseases in fish is based on the isolation of the virus in a sensitive cell culture and confirmation of its presence by serum neutralization (Amos, 1985). Since these techniques are rather time-consuming and expensive, an important priority in SVCV research has been the development of more rapid and sensitive diagnostic methods. In order to obtain a specific and sensitive hybridization test for SVCV we constructed DNA probes using the technique of reverse transcription, amplification by PCR and cDNA cloning in plasmid and phage vectors.

The sequence of the cDNA coding for the matrix protein of SVCV is known (Kiuchi and Roy, 1984) and it was shown that this virus may be related (according to the structure of the 3' end of the viral RNA) to the vesiculovirus (Roy *et al*, 1984). The cDNA to genome RNA of SVCV was synthesized with the aid of both random and primer 1.

Primer 1 (5'CATGTCTACTCTAAGAAAGC) and primer 2 (5'ACCGAATCGATGAGGCACAT) were used for PCR.

A DNA fragment of the *M* gene was inserted into the single-stranded phage vectors M13mp8 and M13mp9. This enabled us to construct DNA probes to the genome RNA and to the mRNA of SVCV. The single-stranded DNA probes were biotinylated by the 2-step method for labelling DNA. The method is based on the reaction of the cytosine residues of DNA with 4-aminooxybutylamine followed by further modification with biotin.

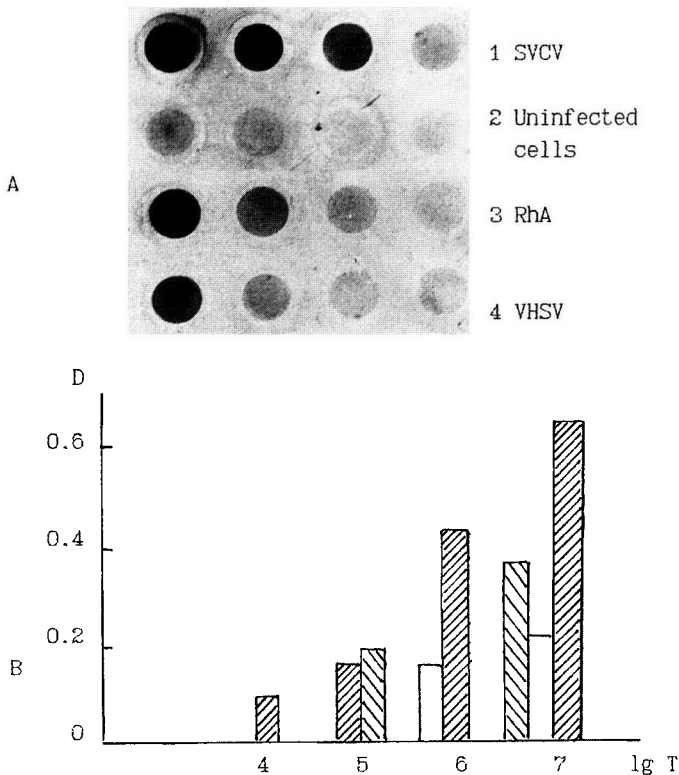
Studies were carried out to establish the sensitivity and specificity of viral RNA detection using non-radioactive probes in an infected cell culture and in the pathogenic material taken from fish. The total RNA was isolated from the infected cells using proteinase and then further processed with phenol extraction. We simultaneously analysed the cultures of cells infected by other fish rhabdoviruses: hemorrhagic septicemia virus of salmonid (VHSV) and the rhabdovirus of *Anguilla* (RhA). Comparative analysis

\* Correspondence and reprints

revealed that the biotinylated DNA probe containing a fragment of the *M* gene to the genome RNA of SVCV detects SVCV with a sensitivity of  $10^4$  viral particles per dot. It could also be hybridized with the other 2 fish rhabdoviruses but this gave a lower efficiency (fig 1). This indicates a certain degree of homology between the *M* gene sequences of these viruses, and the hybridization data presented further proof for the fact that both SVCV and RhA (Wolf,

1988) belong to the *Vesiculovirus* genus while VHSV belongs to the *Lyssavirus* genus. It is important to mention that the DNA probe based on the *M* gene of SVCV could not be hybridized with either the RNA of hepatitis A virus or with the DNA of bovine herpesvirus type 1, which were used as negative controls.

The presence of SVCV was tested in samples taken from different organs of the



**Fig 1.** Detection of fish rhabdoviruses in infected FHM (fathead minnow) cells by hybridization with the DNA probe for the *M* gene of SVCV. **A:** Dot hybridization of RNA, extracted from cells infected with fish rhabdoviruses. For the colour development of a biotinylated probe, the membranes were incubated with streptavidin/alkaline phosphatase conjugate followed by processing with a solution containing nitro-blue tetrazolium and 5-bromo-3-indolyl phosphate. The volume of the culture samples for analysis was 0.1 ml. There were 10-fold dilutions of the viral RNAs on the membrane. **B:** Sensitivity diagram of the DNA probe. T: virus titer in TCID<sub>50</sub>/0.1 ml; D: density of the colour dots, measured by a densitometer in relative units. SVCV ▨, VHSV | and RhA ▩.

**Table I.** Detection of SVCV viral RNA in clinical samples <sup>a</sup> by hybridization with a biotinylated DNA probe.

Sample	Hybridization	Virus titre <sup>b</sup>
Fish N1		
Gills	—	10 <sup>4.4</sup>
Kidney + spleen + liver	—	10 <sup>6.8</sup>
Fish N2		
Kidney	—	10 <sup>6.0</sup>
Spleen	—	10 <sup>6.0</sup>
Liver	—	10 <sup>6.0</sup>
Gills	+	10 <sup>6.0</sup>
Fish N3		
Brain	+	ND
Gills	+	ND
Kidney + spleen + liver	—	ND
Fish N4		
Brain	+	ND
Gills	+	ND
Kidney + spleen + liver	—	ND
Uninfected fish		
Gills	—	
Kidney + spleen + liver	—	

<sup>a</sup> Tissue samples from infected fish were homogenized and the viral RNA was extracted for further dot-hybridization. Replicated samples were titred. <sup>b</sup> Titre in TCID per ml. ND: not detected.

infected fish with all the clinical signs of acute disease (swelling of the peritoneum, bristled up scales, and anemia of the gills). In table I, the data of the virus detection by the DNA probe indicate that the genome RNA of SVCV is more often found in the brain and gills of the fish and less frequently in the organs of the abdominal cavity. The detection of SVCV mRNA in fish organs by another probe containing a complementary fragment to the *M* gene demonstrate lower sensitivity than the probe to the genome RNA of SVCV.

## REFERENCES

- Amos KH (1985) *Fish Health Section*. American Fisheries Society, 3rd ed, Corvallis, OR, USA
- Kiuchi A, Roy P (1984) Comparison of the primary sequence of spring viremia of carp virus M protein with that of vesicular stomatitis virus. *Virology* 134, 238-243
- Roy P, Gupta KC, Kiuchi A (1984) Characterization of spring viremia of carp virus mRNA species and the 3'-sequence of the viral RNA. *Virus Res* 1, 189-202
- Wolf K (1988) *Fish Viruses and Fish Viral Diseases*. Comstock Publishing Associates, Ithaca, NY, USA