ATYPICAL SPRING VIRAEMIA OF CARP VIRUS (SVCV) ISOLATED FROM CARP IMPORTED INTO THE UK : EVIDENCE FROM PHYLOGENETIC ANALYSIS OF PARTIAL GENE SEQUENCES FOR A NUMBER OF DISTINCT SUB-GROUPS OF SVCV

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Introduction

Spring viraemia of carp virus (SVCV) is a rhabdovirus, tentatively classified in the genus *Vesiculovirus*, that is recognised as an important pathogen of freshwater fish associated with major disease epizootics in cultured carp populations. The geographic range of SVC disease is generally considered to be restricted to the European continent, including European Russia and some of the states of the former Soviet Union such as the Ukraine, Moldova and Georgia. However, in 1998, during routine virus screening of coldwater ornamental fish, SVCV was isolated from koi carp and goldfish imported into the United Kingdom (UK) from Asia. All of these isolates exhibited growth characteristics that were different from that of typical SVCV and were difficult to identify using some immunodiagnostic tests. Further "atypical" isolates were obtained in 2001 and 2002 from sites connected with imports of fish from Asia and also from carp imported illegally into the UK from Europe.

Materials and Methods

Virus isolation and identification

Fish underwent post mortem and tissue samples were transported, on ice, in viral transport medium (Glasgow Minimal Essential Medium (GMEM) + 10% Newborn calf serum + 10x antibiotic / antimycotic solution). Virological examination followed procedures recommended for the isolation and identification of SVCV in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000) and tissue homogenates, diluted in Tris buffered GMEM + 5% Foetal calf serum, were inoculated onto EPC cells and incubated at 20°C.

Preliminary identification of viral cpe was by an amplified sandwich-ELISA following OIE recommended procedures. Identification was confirmed by serum neutralisation assay using polyclonal rabbit antisera against SVCV and PFRV. A list of the virus isolates included in the study is given in table 1.

Genetic analysis

The reverse transcription-polymerase chain reaction (RT-PCR) method was applied to the detection of a 426 nucleotide fragment of the glycoprotein (G) gene of putative SVCV isolates. The nucleotide sequence of the RT-PCR amplicons was then determined by inserting the amplicon into the pGEM-T vector and sequencing both strands of the inserted DNA using the M13 universal sequencing primers and ABI Prism cycle sequencing system. Sequencing reactions were analysed on an ABI 310 genetic analyser. Amplification and sequence reactions were performed in duplicate and additional cloning and sequencing performed where necessary to produce concensus sequence data for each isolate. Phylogenetic trees were generated by neighbour joining and maximum parsimony analysis.

Table 1: SVCV isolates included in the studies. Initial identification was by ELISA or serum neutralisation and genogroups were assigned as part of this study

Isolate	Country and year of isolation	Host name	Genogroup
S30	Yugoslavia (1971)	Common carp (*Cyprinus carpio carpio*)	1d
770346	UK (1977)	Common carp	1d
P4	Russia (1983)	Common carp	1b
M2-78	Moldova (1983)	Silver carp (*Hypophthalmichthys molitrix*)	1d
860115	UK (1986)	Common carp	1d
N1-5	Ukraine (1986)	Bighead carp (*Aristichthys nobilis*)	1c
N3-14	Ukraine (1986)	Grass carp (*Ctenopharyngodon idella*)	1d
88062; 88085; 880100; 880110; 880124; 880163	UK (1988)	Common carp	1d
RHV	Ukraine (1989)	Rainbow trout (*Oncorhynchus mykiss*)	1c
2-90	Moldova (1990)	Common carp	1b
910109	UK (1991)	Common carp	1d
940626	UK (1994)	Ghost carp (*Cyprinus carpio carpio x koi*)	1d
940648; 940768	UK (1994)	Common carp	1d
950115; 950255; 950418	UK (1995)	Common carp	1d
960650	UK (1996)	Common carp	1d
970469	UK (1997)	Common carp	1a
980451; 980528	UK (1998) [Asian origin]	Koi carp (*Cyprinus carpio koi*)	1a
980548	UK (1998)	Tench (*Tinca tinca*)	1a
980619	UK (1998) [Asian origin]	Goldfish (*Carassius auratus*)	1a
D120; D148	UK (2001) [Asian origin]	Koi carp	1a
E134	UK (2002) [Illegal import]	Common carp	1c
E208; E232	UK (2002) [Asian origin]	Koi carp	1a
US02-46; US-6K	USA (2002)	Koi carp	1a
PB02-131	USA (2002)	Common carp	1a

Results

Atypical characteristics

- When grown in EPC cells at 20°C, atypical SVCV isolates produce a distinctive cytopathic effect (cpe). The cpe appears as discrete foci enlarging to form plaques (see fig. 1) rather than spreading rapidly to produce a more generalised destruction of the cell monolayer as is seen with typical European SVCV isolates.
- Atypical SVCV isolates also reduce the pH of Tris buffered cell culture medium during growth (see fig. 2). Typical SVCV isolates reduce the pH by 0.1 to 0.2 of a pH unit after 3 days growth in EPC cells but during growth of atypical isolates the pH was seen to drop by as much as 0.6 of a pH unit after 3 days.
- Not neutralised to the same extent as other SVCV isolates by polyclonal rabbit antiserum raised against the S30 reference SVCV isolate (see Table 2).
- In immunoperoxidase staining tests on infected cell cultures some monoclonal antibodies specific for SVCV did not recognise atypical SVCV antigens (data not shown).

History of isolations

- 1998: First isolations of SVCV showing atypical growth characteristics. Three isolations were made from asymptomatic koi carp and goldfish during routine CEFAS Fish Health Inspectorate checks on ornamental fish imports from the Republic of China. A fourth isolation was made from tench held at a site that had recently received fish imported from China.
- 1999 & 2000: Import checks were increased and SE Asian imports targeted but no further isolations.
- 2001: More atypical SVCV isolations made from a koi carp mortality in a hobbyists pond and further isolations at the supplier. The supplier had received a recent consignment containing ornamental fish imported from south-east Asia.

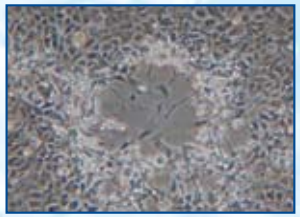

Figure 1: Cytopathic effect (cpe) produced by an atypical SVCV isolate after 3 days growth in EPC cells

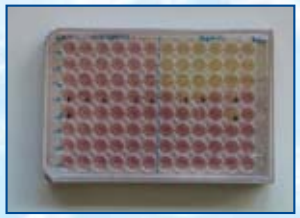

Figure 2: Microtitration of a typical European SVCV isolate (left side) and an atypical Asian SVCV isolate (right side) showing the reduction in pH of the tris buffered GMEM culture medium in the wells where the Asian isolate is showing cpe

- 2002: Two more isolations, again from a koi carp mortality in a hobbyists pond and from a retail site. The hobbyists supplier and the retail site were found to have a wholesaler in common who imported fish from Asia.
- 2002: Isolation of atypical SVCV from an illegal import of carp from continental Europe.
- 2002: First isolations of SVCV in the USA from disease outbreaks in koi carp at a farm in North Carolina (Goodwin 2002) and in feral common carp in Cedar Lake, Wisconsin. Both isolates showed atypical growth characteristics.

Growth characterisation and serological studies were carried out on other SVCV isolates in the virus collection at CEFAS Weymouth.

- This revealed a further atypical SVCV isolated from carp in 1997 from a site in the UK that had received a number of consignments of ornamental fish from the Republic of China.

Isolates from Russia and former Soviet states

- The majority of these were isolated during SVC outbreaks, between 1983 and 1994, in common carp but also from big-head, silver and grass carp (Shchelkunov & Shchelkunova, 1989). Many of these isolates were found to be "atypical".

Phylogenetic analysis

Phylogenetic analysis produced trees in which the isolates were assigned to four distinct sub-groups that were supported by bootstrap values of >985 (see figures 3 and 4).

Isolates from Western Europe formed one sub-group (designated 1d) and isolates from Russia and the former Soviet states were divided between two sub-groups (1c and 1b). Two isolates from the former USSR (M2-78 & N3-14) were also assigned to sub-group 1d. The isolates of Asian origin formed a fourth distinct group along with three isolates associated with SVC outbreaks in the USA during 2002. The SVCV isolated from carp imported illegally into the UK (UK E134) was assigned to sub-group 1c.

Table 2: Neutralisation titres of five SVCV isolates and Tench rhabdovirus and Pike fry rhabdovirus with polyclonal rabbit antiserum raised against SVCV isolate S30

Virus isolate	Microneutralisation titre *
SVCV Ger. 10/3	10,240
SVCV UK 88:62	5,120
SVCV Ukr. N3-14	2,560
SVCV Ukr. RHV	640
SVCV UK980528 (Asian import)	320
TRV UK950237	80
PFRV F4	<40

* titre = reciprocal of last antiserum dilution giving 100% inhibition of virus growth (virus diluted to 100 TCID50/ml)

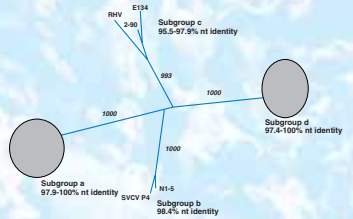

Figure 3: Re-analysis of the SVCV genogroup using the Neighbour joining method on a 426bp region of the G-gene identified 4 sub-groups supported by bootstrap values of >985

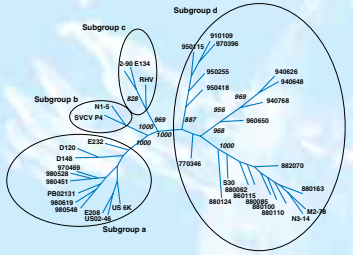

Figure 4: Subgroups of the SVCV genogroup identified using Maximum Parsimony analysis

Discussion and further studies

- Only 2 of 9 isolates of Asian origin have been associated with outbreaks of SVC disease in the UK. Preliminary virus challenge studies on two atypical SVCV isolates have shown them to be of low virulence in carp compared to some typical European SVCV isolates.
- Atypical SVCV isolates are difficult to identify with some immunodiagnostic tests. The atypical isolates probably represent a separate serotype of SVCV.
- The phylogenetic analysis has highlighted the genetic diversity that exists among SVCV isolates. Analysis of this region of the SVCV G gene is a valuable epidemiological tool to identify the source of an SVCV isolate. Analysis assigned the illegal import SVCV isolate to sub-group 1c and indicates an Eastern European origin.
- A reverse hybridisation assay using oligonucleotide probes specific for the SVCV sub-groups has been developed at CEFAS Weymouth (Sheppard *et al.* 2003). It is hoped that this can be used as a rapid method for genotyping SVCV isolates.
- Studies are currently in progress at CEFAS Weymouth on the development of an RT-PCR method for detecting SVCV RNA in archive paraffin-embedded tissue sections. The amplicons generated can be sequenced and genotyped.

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Acknowledgements

This work was funded by Defra contract F1136. The authors would like to acknowledge Andy Goodwin, Nikolai Fijan and Igor Shchelkunov who kindly donated SVCV isolates.

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