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Comparison of multiple genes of spring viremia of carp viruses isolated in the United States

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Abstract Five spring viremia of carp viruses (SVCV), *Rhabdovirus carpio*, were isolated in the United States (US) between 2002 and 2004. Single tube reverse transcription-polymerase chain reaction (RT-PCR) was used to generate overlapping cDNA fragments from the US isolates of SVCV. Multiple pairs of specific primers were designed to amplify a portion of the phosphoprotein gene, the matrix gene, and the glycoprotein gene of SVCV genogroup Id (corresponding to nucleotides 2174–4942 of GenBank accession NC_002803). Sequences were proofread and aligned to generate a consensus sequence for each isolate. Phylogenetic analysis of the 2705 nucleotide consensus sequence revealed that all five US isolates belong to SVCV genogroup Ia, Asian origin isolates, and a PCR primer binding site unique to SVCV genogroup Ia was identified.

Keywords carp · koi · Rhabdovirus · SVCV · US

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Introduction

Spring viremia of carp virus (SVCV), is a member of the family *Rhabdoviridae* in the genus *Vesiculovirus* [1] and causes an acute contagious disease of the common carp (*Cyprinus carpio*) and koi (*Cyprinus carpio koi*) [2]. Natural occurrences of disease have also been documented in other members of the cyprinid family including bighead carp (*Aristicthys nobilis*) [3], crucian carp (*Carassius carassius*) [4], grass carp (*Ctenopharyngodon idella*) [5], silver carp (*Hypophthalamicthys molitrix*), and in a non-cyprinid species the sheatfish (*Silurus glanis*) [6]. Fish species susceptible in experimental studies include guppies (*Lebistes reticulates*) [7], northern pike (*Esox lucius*) [8], pumpkinseed (*Lepomis gibbosus*) [9], roach (*Rutilus rutilus*) [10], and zebrafish (*Danio rerio*) [11].

The genome of SVCV consists of a single-stranded negative sense RNA of approximately 11 kb in length [12, 13]. The RNA genome encodes five structural proteins: the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G), and a RNA-dependent polymerase (L) [12–16]. The P and N proteins are associated with the viral genome and play an important role in viral transcription and replication

[17]. The G protein forms trimeric peplomers on the virus surface that function by binding to a cellular receptor which triggers viral endocytosis [18]. The G protein carries neutralizing epitopes and is a potential target for DNA vaccines [19, 20]. Phylogenetic analysis of the nucleotide sequence of the G protein has been used to differentiate SVCV isolates into four sub genogroups: genogroup Ia contains viruses originating from Asia; genogroups Ib and Ic contain viruses originating from Moldova, Ukraine, and Russia; and genogroup Id contains viruses primarily originating from the UK with the exception of a few viruses from the former USSR [21].

Outbreaks of SVC disease have historically occurred throughout Europe. These epizootics have been associated with high mortality, substantial economic loss, and a significant risk for international movement of the virus, resulting in SVC being classified as a listed disease by the Office International des Epizooties (OIE), World Organization for Animal Health. Diseases listed by the OIE are reportable diseases that may affect international trade including restrictions on the movement of susceptible animal species, or their products. Discovery of a reportable disease often leads to expensive eradication or control efforts and suspect populations may be quarantined until a definitive diagnosis can be made. Unfortunately, genetic diversity in the fish *Rhabdoviridae* family and serological similarities with other rhabdoviruses [22], in particular pike fry rhabdovirus (not listed by OIE) can make the identification of SVCV a time consuming process.

The first reports of SVCV in the US occurred in the spring of 2002. In April and May, clinical disease was observed at a private koi farm in North Carolina [23], and in wild common carp at Cedar Lake [24] in northwestern Wisconsin. In 2004, a third US case of SVCV occurred at a backyard koi hobbyist's pond in Washington state, and a fourth case occurred in koi at a commercial fish hatchery in Missouri. In all four of these clinical cases, SVCV was isolated in cell culture. A fifth isolate of SVCV was made from a pool of tissues collected from healthy-looking common carp that were harvested during a 2003 invasive fish passive net survey conducted in the Calumet Sag Channel in Illinois. Multiple occurrences of this reportable disease in a short time span in such geographically diverse areas resulted in an interest in the genetic relatedness of the five US isolates.

The purpose of this study was to determine the nucleotide sequence of a portion of the P protein, the M protein, and G protein genes of the five US isolates, and to compare them to each other and to representative isolates of the four sub genogroups that represent the geographic and genetic diversity of SVCV. The data revealed that all five US isolates belong to the SVCV genogroup Ia, Asian origin isolates.

Materials and methods

Cells and viruses

Fathead minnow (FHM) cells were used for propagating the viruses. The cells were grown in monolayer cultures in Earles minimum essential medium supplemented with 10% fetal bovine serum (FBS), 0.5% edamin type S, 1 mM sodium pyruvate, 2.4 mM L-glutamine, 0.02 M sodium bicarbonate, 25 μg of gentamicin sulfate per ml, and 250 μg amphotericin B per ml. Reference viruses (Table 1) used in this study included SVCV S30 (genogroup Id) obtained from the Centre for Environment, Fisheries and Aquaculture Science, Weymouth, UK; and isolates 970469 (genogroup Ia), RHV (genogroup Ib), pike fry rhabdovirus (PFRV) V76 (genogroup II) and PFRV 950237 (genogroup IV) obtained from Dr J. Winton, Western Fisheries Research Center, Seattle Washington. The five US isolates used in this study were as follows: Wisconsin (common carp) isolate [24], North Carolina (koi) isolate [23], Illinois (common carp) isolate, Washington (koi) isolate, and Missouri (koi) isolate. The viral isolates were received from the originating diagnostic laboratories and passaged on FHM cell cultures 1–2 times. Fish cells were infected with SVCV isolates and incubated at 20°C until the cytopathic effect was complete (2–5 days). Total RNA was extracted from 300 μl of cell culture supernatants with TRIzol® LS reagent (Gibco, Invitrogen, Carlsbad, California, USA). Purified RNA was suspended in diethylpyrocarbonate (DEPC)-treated water and stored at –70°C until used.

Single tube reverse transcription-polymerase chain reaction (RT-PCR) and sequencing

The rTth RNA PCR kit (Applied Biosystems, Foster City, CA, USA) was used to generate overlapping cDNA fragments. Primers (Table 2) were designed from GenBank accession number NC_002803. Reaction mixtures (50 μl) consisted of 2 μl of the extracted RNA, 1.0 μM of each primer, 200 μM of each dNTP, 1× reaction buffer, 2.5 mM manganese acetate, 5 U of rTth enzyme, and RNase-free water up to the reaction volume. The reaction conditions consisted of reverse transcription for 30 min at 60°C, and 1 min at 94°C, followed by 40 amplification cycles of 20 s at 94°C and

Table 1 SVCV isolates compared in this study

GenBank accession	Isolate identifier	Country of origin	Source of isolate	Genogroup	Year
DQ097384	741	China		Ia	
AY842486	772	China	*Carassius auratus*	Ia	
AY842487	926	China	*Cyprinus carpio*	Ia	
AY842484	461	China	*Cyprinus carpio*	Ia	
AY842488	978	China	*Cyprinus carpio*	Ia	
AY842489	992	China	*Cyprinus carpio*	Ia	2003
AJ538067	970469	United Kingdom[a]	*Cyprinus carpio*	Ia	1997
DQ227500	Wisconsin	United States of America	*Cyprinus carpio*	Ia	2002
DQ227502	Illinois	United States of America	*Cyprinus carpio*	Ia	2003
AJ538066	980528	Asian import to United Kingdom	*Cyprinus carpio koi*	Ia	1998
AJ538065	980451	Asian import to United Kingdom	*Cyprinus carpio koi*	Ia	1998
AY842485	464	China	*Cyprinus carpio koi*	Ia	
DQ227501	North Carolina[b]	United States of America	*Cyprinus carpio koi*	Ia	2002
DQ227503	Washington	United States of America	*Cyprinus carpio koi*	Ia	2004
DQ227504	Missouri	United States of America	*Cyprinus carpio koi*	Ia	2004
AJ538060	2/90	Moldova	*Cyprinus carpio*	Ib	1990
AJ538075	RHV	Ukraine	*Oncorhynchus mykiss*	Ib	1989
AJ538064	N1-5	Ukraine	*Aristichthys nobilis*	Ic	1986
AJ538074	P4	Russia	*Cyprinus carpio*	Ic	1983
AJ538062	N3-14	Ukraine	*Ctenopharyngodon idella*	Id	1986
AJ538079	880062	United Kingdom	*Cyprinus carpio*	Id	1988
AJ538080	860115	United Kingdom	*Cyprinus carpio*	Id	1986
AJ538078	880124	United Kingdom	*Cyprinus carpio*	Id	1988
AJ538076	970395	United Kingdom	*Cyprinus carpio*	Id	1997
AJ538077	770346	United Kingdom	*Cyprinus carpio*	Id	1977
NC_002803	Fijan Reference Strain[c]	Yugoslavia	*Cyprinus carpio*	Id	1971
AJ318079	Fijan Reference Strain[c]	Yugoslavia	*Cyprinus carpio*	Id	1971
AJ538061	Fijan Reference Strain[c]	Yugoslavia	*Cyprinus carpio*	Id	1971
AJ538063	M2-78	Moldova	*Hypophthamichthys molitrix*	Id	1983
AJ538068	V76	Germany	*Ctenopharyngodon idella*	II	1982
AJ538082	950237	United Kingdom	*Tinca tinca*	IV	1995

[a] Isolated from fish that was held on site with fish from China

[b] Accession AY527273 is sequence for the North Carolina isolate generated by other investigators

[c] Multiple sequences for same isolate in GenBank. Only sequences with nucleotide differences utilized. Accession U18101 is identical to NC_002803

40 s at 59°C, with a final cycle of 59°C for 7 min. Products were visualized with UV light following electrophoresis and ethidium bromide staining. The PCR fragments were purified in Microcon^{TM} 100 (Amicon, Pullman, Washington, USA) filters and sequenced in both directions using the corresponding primers. Sequencing services were provided by Iowa State University, Ames, Iowa, USA. Sequences were proofread, primer binding sites' sequences removed, and assembled into a contig to form a consensus sequence for each viral isolate using the Sequencher program from Gene Codes Corporation. Alignment, analysis for open reading frames (ORF), translation of sequences, and analysis of protein products of the five consensus sequences along with reference SVCV sequences obtained from GenBank Data libraries were performed using Align Plus 5, sci-ed software and MEGALIGN, Lasergene, DNAStar, Inc. Phylogenetic analyses were conducted using Mega version 3.1 [25]. The sequence data has been deposited in the GenBank Data libraries under accession numbers DQ227500, DQ227501, DQ227502, DQ227503, and DQ227504.

Results

Ten overlapping cDNAs (Table 2) were used to obtain a 2705 nucleotide consensus sequence for a portion of the P protein, the M protein, the G protein, and the start of the L protein genes for each of the five US isolates of SVCV. The cDNA fragments were sequenced a minimum of three times, two times in one direction and once in the opposite direction, using the PCR primers that were used to generate the cDNA. The consensus sequences and the deduced amino acids for each isolate were compared with SVCV reference

Table 2 PCR primers used to generate cDNA fragments

Primer set	Primer IDS[a]	Sequence	Amplification area
1	2174	5′-AGA AGA TGA GCC GCC ACT AA	End of phosphoprotein gene and start of matrix gene
	2581	5′-TTC AAG AGT CCG AGA AGG TC	
2	2448	5′-GCG ACT GCG CCA GTG TTA AT	Portion of the matrix gene
	3015	5′-GCA CCA CGT CTA CTG GCA TA	
3	2557	5′-TGT CGG ATC TTC TCG GAC TC	Portion of the matrix gene
	2975	5′-CCA TTC TCC TGT GGC TGA AC	
4	2645	5′-CCG TTG TGC GTT AGA ACT CA	End of the matrix gene and start of glycoprotein gene
	3336	5′-TTC AGC TGC ATG GCA GAT CC	
5	3180	5′-ATC ATG GCA GCC TGT GAT TC	Portion of the glycoprotein gene
	3573	5′-ATG TCC TCC GTA TGG CTC TA	
6[b]	R2 (3358)	5′-AGA TGG TAT GGA CCC CAA TAC ATH ACN CA	Portion of the glycoprotein gene
	F1 (4071)	5′-TCT TGG AGC CAA ATA GCT CAR RTC	
7	3392	5′-TTC ATC CGA TCA GTC CTA CC	Portion of the glycoprotein gene
	3930	5′-ACC GGA GAC CAA CGT TCC AT	
8	3863EU	5′-CAG CCG GAA CAT TAA CAA CG	Portion of the glycoprotein gene
	3863US	5′-CAG CTG AAA CAT TGA CGA A	
	4559	5′-GTG CAA GCA ACA CAG CAT CT	
9	4215	5′-CTG GGA CGA CTG GGA GTT AG	End of the glycoprotein gene and start of the RNA-dependent polymerase gene
	4733	5′-CTC ATC CGG CAA TCC AGA CG	
10	4450	5′-GCA GTG GTA GAA TGG GTT AG	End of the glycoprotein gene and start of the RNA-dependent polymerase gene
	4942	5′-GTC TTA ATG ATG CCG ACT CC	

[a] Corresponds to nucleotide position in GenBank accession NC_002803

[b] Previously published primers [21]

virus; the G protein nucleotide sequences were utilized for phylogenetic analysis along with other isolates' sequences deposited in GenBank.

In general, the five US isolates of SVCV had nucleotide identities between 98.4 and 99.9% with each other and identities between 92.7 and 93.0% with the SVCV reference virus (GenBank NC_002803). Nucleotide sequence differences were observed throughout the region sequenced, but occurred more frequently in the G protein gene. The majority of nucleotide differences between the SVCV reference virus and the five US isolates occurred in all five US isolates except for nucleotide changes that were observed in either the three koi isolates or the two common carp isolates. Single nucleotide changes unique to an individual isolate were observed. All five sequences from US isolates contained an insertion of 10 nucleotides between nucleotides 4636 and 4637 of the SVCV reference virus. The gene junctions were conserved in all five US isolates as evidenced by all US isolates having a conserved TATG(A)7 transcription stop signal/polyadenylation signal, an untranscribed dinucleotide (CT) region between the P/M and M/G genes, and a tetranucleotide (CTAT) between the G/L genes.

At the amino acid level, G protein amino acid identities (Fig. 1) among the five US isolates were between 98.0 and 99.8%, and between 94.3 and 95.1% with the SVCV reference virus. Fewer amino acid changes were observed in the deduced amino acid sequences for the M protein of the US isolates. Among the five US isolates, M protein amino acid identities were between 99.6 and 100%. Identities between the US isolates and the SVCV reference virus were between 97.3 and 97.8%.

A phylogenetic tree was constructed using the 550 nucleotide region of G gene that has been used to separate SVCV isolates into their respective genogroups [21]. This tree revealed that all five US isolates belong in the SVCV Ia cluster (Fig. 2). Closer examination of the nucleotides in this region revealed a potential PCR primer binding site that would differentiate SVCV Ia genogroups from SVCV Id genogroups (Fig. 3). Two separate RT-PCR reactions were performed utilizing either primer 3863EU or primer 3863US with primer 4559 (Fig. 4). Primer pair 3863US/4559 amplified all five of the US SVCV isolates and reference isolate 970469 (SVCV genogroup Ia), but failed to amplify SVCV reference virus S30 (genogroup Id), RHV (genogroup Ib), PFRV V76 (genogroup II), or PFRV 950237 (genogroup IV). Primer pair 3863EU/4559 amplified SVCV reference viruses S30 and RHV, but failed to amplify any of the US isolates, reference virus 970469 (genogroup Ia), PFRV V76 (genogroup II), or PFRV 950237 (genogroup IV).

Subsequent to this study, the complete genome for a Chinese SVCV isolate 741 was released in GenBank (accession number DQ097384). Phylogenetic analysis

PANEL A

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DQ097384          1 MSIISYIAFLLLIDSTLGIPIFVPSGQNISWQPVIQPFDYQCPIHGNLPNTMGLSATKLTIKSPSVFSTDKVSGWICHAAEWKTTCDYRWYGPQYITHSI
DQ227500          1 ....................................................................................................
DQ227502          1 ....................................................................................................
DQ227501          1 .................F..................................................................................
DQ227503          1 ....................................................................................................
DQ227504          1 ....................................................................................................
NC_002802         1 ................N..........R.........................................................................

DQ097384        101 HPISPTIDECKRIISRIASGTDEDLGFPPQSCGWASVTTVSNTNYKVVPHSVHLEPYGGHWIDHDFNGGECREKVCEMKGNHSIWITDETVQHECEKHIE
DQ227500        101 ..........................................................E.....................................
DQ227502        101 ..................T.....................................................E.....................................
DQ227501        101 ..........................................................E.....................................
DQ227503        101 ....................................................................................................
DQ227504        101 ..........................................................E.....................................
NC_002802       101 ..........R...Q..............................R..................E.......................E.......A....

DQ097384        201 EVEGIMYGNAPRGDAIYINNFIIDKHHRVYRFGGSCRMKFCNKDGIKFTRGDWVEKTAGTLTNIYENIPECADGTLVSGHRPGLDLIDTVFNLENVVEYT
DQ227500        201 ................L.........................D.................E...K..A.T.............................
DQ227502        201 ................L.........................D.................E...K.................................
DQ227501        201 ..............................................................E......A.............................
DQ227503        201 ..........................................................A.....................................
DQ227504        201 ..........................................................A.....................................
NC_002802       201 .........V....VM.A......R...........Q...........A.............T.HD.V.K.V.........................

DQ097384        301 LCEGTKRKINKQEKLTSVDLSYLAPRIGGFGSVFRVRNGTLERGSTTYIRIEVEGPVVDSLNGIDPRTNASRVFWDDWELDGNIYQGFNGVYKGKDGKIH
DQ227500        301 ..........E.....................................................I....T.T.....................................
DQ227502        301 ..........E.....................................................I....T.T.....................................
DQ227501        301 ..........N.....................................K......I......T.....................................
DQ227503        301 ..........N.....................................K......I......T.....................................
DQ227504        301 ..........N.....................................K......I......T.....................................
NC_002802       301 ..............................................K......I......T.....................................

DQ097384        401 IPLNMIESGIIDDELQHAFQADIIPHPHYDDDEIREDDIFFDNTGENGNPVDAVVEWVSGWGTSLKFFGMTLVALILIFLLIRCCVACTYLMKKSKRPAT
DQ227500        401 ..........................................................................T.....................
DQ227502        401 ..........................................................................T.....................
DQ227501        401 ..........................................................................T.....................
DQ227503        401 ..........................................................................T.....................
DQ227504        401 ..........................................................................T.....................
NC_002802       401 ..................................................................................R..L...

DQ097384        501 ESHEMRSLV
DQ227500        501 .........
DQ227502        501 .........
DQ227501        501 .......F.
DQ227503        501 .......F.
DQ227504        501 .......F.
NC_002802       501 .........
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PANEL B

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DQ097384          1 MSTLRKLFGIKKSKGTPPTYEETLATAPVLMDTHDTHSHSLQWMRYHVELDIQLDTPLKTMSDLLGLLKNWDVDYKGSRNKRRFYRLIMFRCALELKHVS
DQ227500          1 ....................................................................................................
DQ227502          1 ....................................................................................................
DQ227501          1 ....................................................................................................
DQ227503          1 ....................................................................................................
DQ227504          1 ....................................................................................................
NC_002802         1 .........T.....................................VK.................................................

DQ097384        101 GTYSVDGSALYSNKVQGSCYVPHRFGQMPPFKREIEVFRYPVHQHGYNGVVDLRMSICDLNGEKTGLNLLKECQVAHPNHFQKYLEEVGLEAACSATGEW
DQ227500        101 ..................................................................................................V....
DQ227502        101 ....................................................................................................
DQ227501        101 ....................................................................................................
DQ227503        101 ....................................................................................................
DQ227504        101 ....................................................................................................
NC_002802       101 ..............................................M.............I.....................................

DQ097384        201 ILDWTFPMPVDVVPRVPSLFMGD
DQ227500        201 ........................
DQ227502        201 ........................
DQ227501        201 ........................
DQ227503        201 ........................
DQ227504        201 ........................
NC_002802       201 ........................
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Fig. 1 Comparison of the amino acid sequences of the US isolates of SVCV with reference virus NC_002803 (genogroup Id) and isolate DQ097384 (genogroup Ia). The dots (·) indicate where amino acids are identical to those of DQ097384

of the 550 nucleotide region used to genotype SVCV isolates clusters isolate 741 in the Ia genogroup. Comparison of the deduced M protein amino acids identities of Chinese isolate 741 with the US SVCV isolates were between 99.6 and 100%, and 97.8% between isolate 741 and SVCV reference virus (NC_002803). The deduced G protein amino acids identities between Chinese isolate 741 and the US isolates were between 97.6 and 98.6%, and 94.9% between isolate 741 and SVCV reference virus (NC_002803). Alignment of the nucleotide sequences of Chinese isolate 741 with the consensus sequences of the five US isolates and NC_002803 revealed an insertion of 85 nucleotides (4642–4726 in DQ097384)

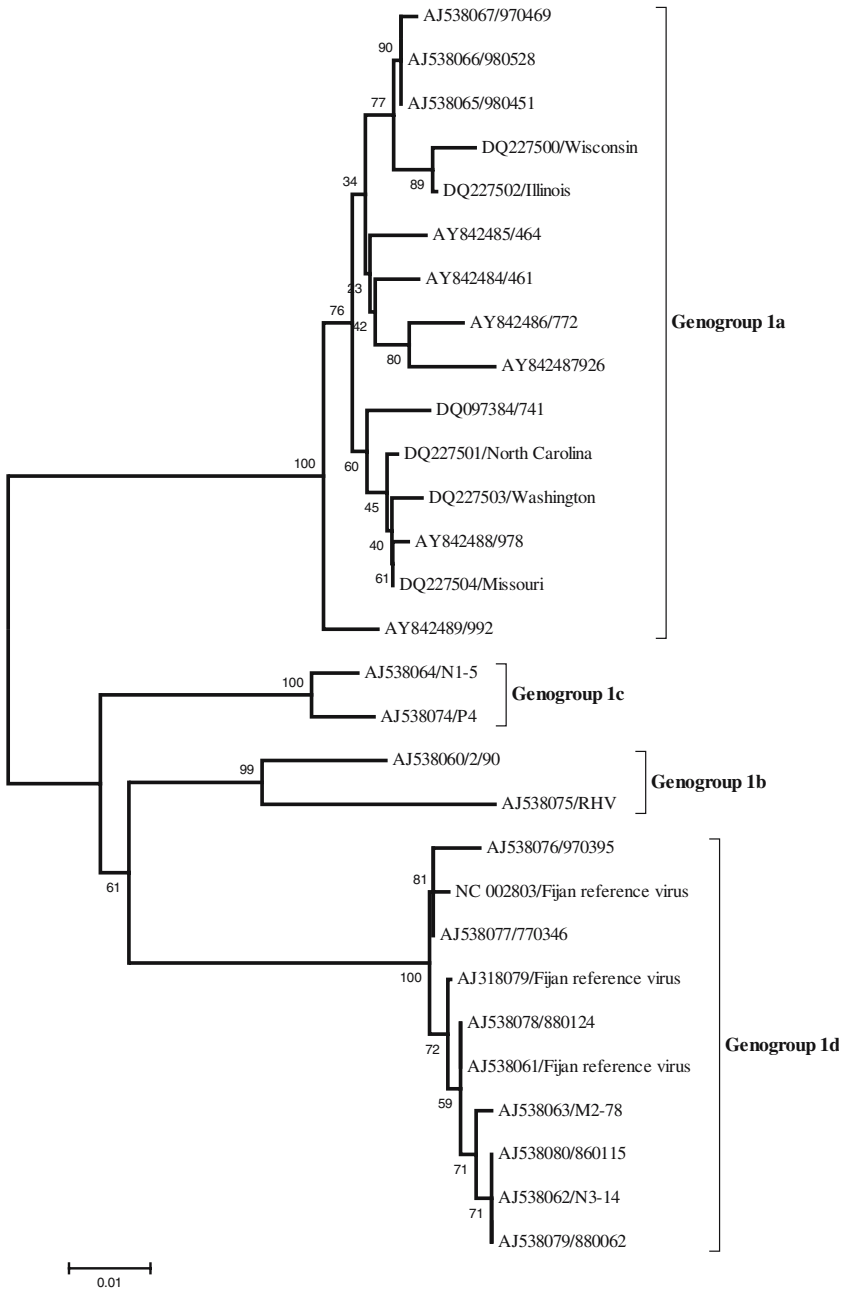
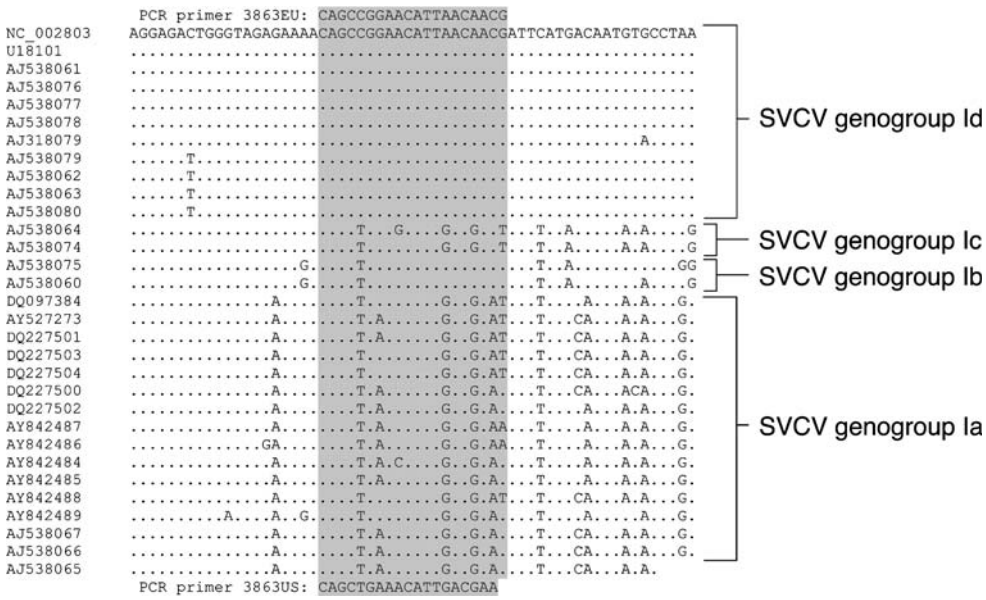


Fig. 2 Phylogenetic trees of US SVCV isolates and representatives of the SVCV genogroups based on the 550 nucleotides of the G gene used to differentiate SVCV into genogroups. This is a Neighbor-Joining tree using the *p*-distance substitution model with bootstrap test of 1,000 replicates and a cut-off value of 70%

in the non-coding region of the G gene of isolate 741 (Fig. 5). This 85 nucleotide insertion includes the 10 nucleotide insertion (nucleotides 4717–4726 of DQ097384) identify in the five US isolates that is absent in SVCV reference virus NC_002283.

Discussions

The sequence analysis and phylogenetic studies on the US SVCV isolates and published data on historical isolates [21] suggest that US isolates are most closely related to Asian strains of SVCV. The nucleotide and deduced amino acid sequences data for a portion of the P gene, the complete M and G genes, and a portion of the L gene showed that all five US isolates cluster within SVCV genogroup Ia. The gene junctions of all five US isolates contained the conserved TATG (A) 7 transcription stop signal/polyadenylation signal, an untranscribed dinucleotide (CT) region between the P/M and M/G genes, and a tetranucleotide (CTAT) between the G/L genes [12]. Alignment of the nucleotide sequences and deduced amino acid of the five US isolates showed a high degree of sequence homology,



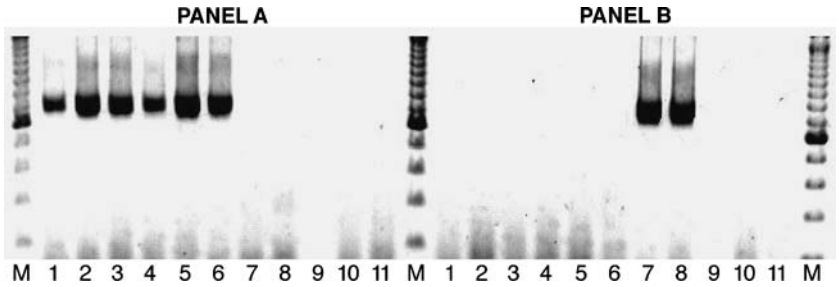


Fig. 4 PCR amplicons produced by RT-PCR with primers that distinguish SVCV genogroup Ia. Panel **A** represents amplification using primers 3863US and 4559. Panel **B** represents amplification using primers 3863EU and 4559. M: 100 bp marker. Lane 1: Wisconsin isolate. Lane 2: North Carolina isolate. Lane 3: Illinois isolate. Lane 4: Washington isolate. Lane 5: Missouri isolate. Lane 6: Isolate 970469 (SVCV genogroup Ia). Lane 7: Isolate RHV (SVCV genogroup Ib). Lane 8: S30 (SVCV genogroup Id). Lane 9: water (no RNA). Lane 10: Isolate PFRV V76 (genogroup II). Lane 11: Isolate PFRV 950237 (genogroup IV)

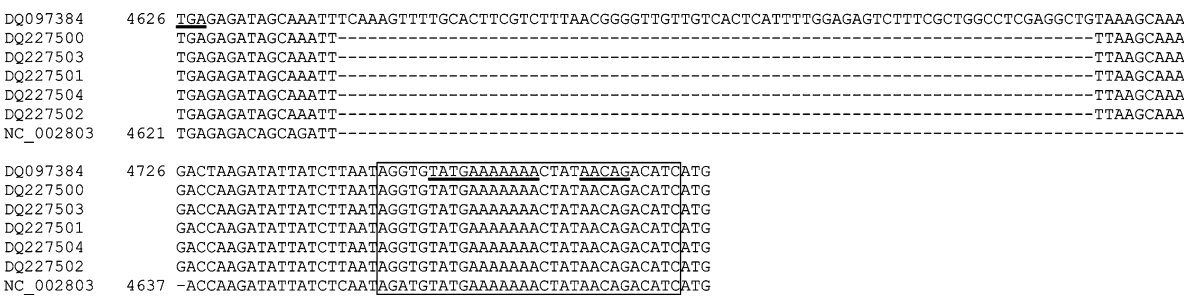


Fig. 5 Comparison of the alignment of the nucleotide sequences in the G/L gene junction of the Chinese SVCV isolate 741 (DQ097384) with US isolates and reference virus NC_00283. The dots (·) indicate where nucleotides are identical to those of isolate 741. The dashes (–) indicate nucleotides not present. The transcription termination/polyadenylation sequences and the potential initiation signals are underlined

with the greatest variation occurring in the G gene/protein. The two isolates from common carp were more similar to each other than to the three koi isolates. The three koi isolates were more similar to each other than to the common carp isolates. It is not known if these similarities represent divergence of two strains of SVCV as a result of adaptation to different hosts; koi and common carp are both of the same species

(*Cyprinus carpio*), but a long history of selective breeding has resulted in separate varieties. Cell culture passage levels could influence nucleotide differences between the US isolates as GenBank sequences for Fijan reference virus do show some sequence variation (NC_002803, AJ318079, and AJ538061). This is not likely, since US isolates were received from the originating laboratories at passage 2 or 3, and only passaged one or two times, so that all isolates were evaluated at roughly the same culture passage level. Alternatively, these similarities could represent separate events of virus introduction into US wild fish and hobby koi.

It is interesting that the majority of the amino acid variation (Fig. 2) was detected in the G protein, since rhabdovirus G proteins stimulate an antibody response and are responsible for both virus attachment and for membrane fusion after endocytosis [18, 26]. A recent comparison of SVCV diagnostic kits that utilize either a polyclonal rabbit serum or a monoclonal antibody suggest there are antigenic differences between the different SVCV sub genogroups [27], which is supported by our comparison of the deduced amino acid sequences. In general, G proteins of rhabdoviruses share low amino acid identity [21, 28], but are predicted to all share the same ectodomain folding structure [28] with four distinct domains (post fusion confirmation). Analysis of the deduced amino acids for the US SVCV isolates G protein reveal single amino acid changes throughout the protein, with a clustering of differences in domain III. Whether these amino acid substitutions play a role in virus attachment and endocytosis is not known.

Sequencing of multiple isolates of the Asian strain of SVCV has also provided data that will enable improved diagnosis and contribute baseline data for future epidemiological studies of SVCV. Current diagnostic tests for SVCV include ELISA for the detection of antigen or antibodies, and virus isolation with virus identification by immunofluorescence, ELISA, virus neutralization or RT-PCR [27, 29–40]. None of these methods distinguish between the SVCV genogroups. This study identified a PCR primer binding site that appeared to be unique for SVCV genogroup Ia isolates, and used it in an RT-PCR assay that differentiated genogroup Ia (Asian) isolate representative (970469) from genogroups Ib an Id (UK, Moldavia, Russia) representatives, RHV and S30, and from genogroups II and IV representatives V76 and 950237. A representative isolate from the Ic genogroup was not available for use during this study, but review of nucleotide sequences for isolates in this genogroup suggests that the 3863US/4559 primer pair will not amplify genogroup Ic isolates. Although not tested in this study, sequence analysis of Chinese isolate 741 predicts that the 3863US/4559 primer pair would amplify this isolate. When fully validated, this assay will be an important tool providing rapid information useful in future epidemiological investigations of SVCV outbreaks.

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