CHAPTER 2.3.9.

SPRING VIRAEMIA OF CARP

1. Scope[1]

Spring viraemia of carp (SVC) is a rhabdovirus infection capable of inducing an acute haemorrhagic and contagious viraemia in several carp species and of some other cyprinid and ictalurid fish species. For the purpose of this chapter, SVC is considered to be infection with spring viraemia of carp virus (SVCV). Comprehensive references can be found in reviews by Wolf, 1988, Ahne et al., 2002and Dixon, 2008.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

The aetiological agent of SVC is Spring viraemia of carp virus (SVCV), a species in the genus *Vesiculovirus* in the virus family *Rhabdoviridae* (Carsten, 2010). The virus genome is a non-segmented, negative-sense, single strand of RNA. The genome contains 11,019 nucleotides encoding five proteins in the following order: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G) and an RNA-dependent, RNA polymerase (L). The genome does not contain a non-virion (NV) gene between the G and L genes as is found in fish rhabdoviruses of the genus *Novirhabdovirus* (Ahne et al., 2002). The type strain of SVCV is available from the American Type Culture Collection (ATCC VR-1390). Two complete genome sequences of the type strain have been submitted to Genbank (Genbank accession U18101 by Björklund et al., 1996and Genbank accession AJ318079 by Hoffmann et al., 2002). The complete genome sequence of isolates from China (People's Rep. of) has also been deposited in Genbank (Genbank accession DQ097384 by Teng et al., 2007and Genbank accession EU177782 by Zhang et al., 2009).

Stone et al., 2003used sequence analysis of a 550 nucleotide region of the G-gene to compare 36 isolates from different fish species and geographical locations previously identified as SVCV or pike fry rhabdovirus (PFRV) by serology. The analysis showed that the isolates could be separated into four distinct genogroups and that all of the SVCV isolates could be assigned to genogroup I, sharing <61% nucleotide identity with viruses in the other three genogroups. Genogroup II comprised a single isolate from grass carp, previously identified by serology as PFRV, genogroup III comprised the reference PFRV isolate, and genogroup IV comprised a large number of unassigned isolates and isolates previously identified as PFRV. The latter genogroup was called the tench rhabdovirus (TenRV) group after the species from which the earliest member was isolated. Further analysis also showed that SVCV genogroup I could be further subdivided into at least four subgenogroups. Ahne et al., 1998showed that the two viruses could also be differentiated by a ribonuclease protection assay using a G-gene probe, suggesting that genetic differences exist between the two viruses.

Antibodies directed against SVCV cross-react to various degrees with members of the other three genogroups, indicating that the viruses possess common antigens, whilst being genetically distinct. The viruses have been shown to share common antigenic determinants on the G, N and M proteins, but can be differentiated by neutralisation assays (Jørgensen et al., 1989).

2.1.2. Survival outside the host

The virus has been shown to remain viable outside the host for 5 weeks in river water at 10°C, for more than 6 weeks in pond mud at 4°C reducing to 4 days in pond mud at 10°C (Ahne, 1976).

2.1.3. Stability of the agent

The virus is inactivated at 56°C for 30 minutes, at pH 12 for 10 minutes and pH 3 for 2 hours (Ahne, 1986). Oxidising agents, sodium dodecyl sulphate, non-ionic detergents and lipid solvents are all effective for

1 NB: Version adopted by the World Assembly of Delegates of the OIE in May 2012.

inactivation of SVCV. The following disinfectants are also effective for inactivation: 3% formalin for 5 minutes, 2% sodium hydroxide for 10 minutes, 540 mg litre^{-1} chlorine for 20 minutes, 200–250 ppm (parts per million) iodine compounds for 30 minutes, 100 ppm benzalkonium chloride for 20 minutes, 350 ppm alkyltoluene for 20 minutes, 100 ppm chlorhexidine gluconate for 20 minutes and 200 ppm cresol for 20 minutes (Ahne, 1982; Ahne & Held, 1980; Kiryu et al., 2007). The virus can be stored for several months when frozen in medium containing 2–5% serum. The virus is most stable at lower temperatures, with little loss of titre for when stored for 1 month at –20°C, or for 6 months at –30 or –74°C (Ahne, 1976; Kinkelin & Le Berre, 1974). The virus is stable over four freeze(–30°C)–thaw cycles in medium containing 2% serum (Kinkelin & Le Berre, 1974).

2.1.4. Life cycle

The virus appears to enter the host via the gill. A viraemia follows and the virus rapidly spreads to the liver, kidney, spleen and alimentary tract. The virus can be detected in faeces and is also shed into the water via faeces and urine.

2.2. Host factors

2.2.1. Susceptible host species

Naturally occurring SVC infections have been recorded from the following cyprinid species: common carp (*Cyprinus carpio carpio*) and koi carp (*Cyprinus carpio koi*), crucian carp (*Carassius carassius*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), grass carp (white amur) (*Ctenopharyngodon idella*), goldfish (*Carassius auratus*), orfe (*Leuciscus idus*), and tench (*Tinca tinca*) and bream (*Abramis brama*) (Basic et al., 2009; Dixon, 2008). Three Indian carp species, merigal (*Cirrhinus merigala* [= *C.cirrhosus*]), rohu (*Labeo rohita*) and catla (*Catla catla* [= *Gebelion catla*]) have been reported to be hosts of SVCV (Haghighi Khiabanian Asl et al., 2008a), but the nucleotide sequence data from a confirmatory reverse-transcription polymerase chain reaction (RT-PCR) deposited at Genbank does not align with known SVCV nucleotide sequence data (D.M Stone, pers. comm.). In addition, the deduced amino acid sequence shares only limited similarity with SVCV, and therefore further work is required to determine whether this virus is SVCV in origin. The virus has also been isolated from the non-cyprinid sheatfish (also known as European catfish or wels) (*Silurus glanis*) and from pike (*Esox lucius*); the viral nucleic acid has also been detected in pike by combined RT-PCR and nested PCR (Koutná et al., 2003).

SVCV has also been reported to have been isolated from Nile tilapia (*Sarotherodon niloticus*) (Soliman et al., 2008) and rainbow trout (*Oncorhynchus mykiss*) (Jeremic et al., 2004; Haghighi Khiabanian Asl et al., 2008b). Immunohistochemistry constituted the sole basis of identification of SVCV from Nile tilapia; electron microscopy purported to show virus in the nucleus, which is not a feature of SVCV infection. Haghighi Khiabanian Asl et al., 2008bused the same RT-PCR to identify the virus in rainbow trout that produced equivocal results when used to type the virus in Indian carp described above, and so the identity of that virus in rainbow trout awaits confirmation. The virus isolated from rainbow trout by Jeremic et al., 2004was subsequently confirmed to be SVCV by nucleotide sequence analysis, but attempts to infect rainbow trout with the virus by intraperitoneal injection were unsuccessful, although the virus was virulent for common carp (P.F. Dixon, J. Munro & D.M. Stone, unpublished data). Hence, the status of rainbow trout and tilapia as hosts for SVCV remains unresolved, and awaits further confirmatory data. Some serological tests do not distinguish SVCV from members of the other genogroups described by Stone et al., 2003, and it is imperative that sequence data are used to confirm the identity of putative SVCV isolates from new hosts.

Other cyprinid species have been shown to be susceptible to SVCV by experimental bath infection, including roach (*Rutilus rutilus*) (Haenen & Davidse, 1993) whilst zebra fish (*Danio rerio*) and the golden shiner (*Notemigonus crysoleucas*) have been infected with SVCV by intraperitoneal injection (see Dixon, 2008). It is reasonable to assume that other cyprinid species in temperate waters may be susceptible to infection. Other species can also be infected experimentally, e.g. guppy (*Lebistes reticulatus*). The pumpkinseed (*Lepomis gibbosus*) has been reported to have been experimentally infected with SVCV, but there are no supporting data.

The nucleotide sequence of the G gene of a rhabdovirus isolated from the Pacific white shrimp, *Litopenaeus (Penaeus) vannamei*, in Hawaii is over 99% identical to that of SVCV (Johnson et al., 1999), and is serologically related to SVCV. The virus caused mortality in juvenile Pacific blue shrimp, *L. stylirostris*, fed food pellets soaked in the virus (Lu & Loh, 1994).

2.2.2. Susceptible stages of the host

Generally, young fish up to 1 year old are most susceptible to clinical disease, but all age groups can be affected. Moreover, there is a high variability in the degree of susceptibility to SVC among individuals of the

same fish species. Apart from the physiological state of the fish, the role of which is poorly understood, age or the age-related status of innate immunity appears to be extremely important: the younger the fish, the higher the susceptibility to overt disease, although even adult broodfish can be susceptible to infection.

2.2.3. Species or subpopulation predilection (probability of detection)

Common carp varieties are the principal hosts for SVCV and are considered to be most susceptible to SVCV infection followed, in order of susceptibility, by other carp species (including hybrids), other susceptible cyprinid species and finally susceptible non-cyprinid fish species. When sampling during surveillance programmes for SVC, common carp or strains such as koi or ghost (koi × common) carp are preferentially selected, followed by carp hybrids (e.g. common carp × crucian carp), then other carp species such as crucian carp, goldfish, grass carp, bighead carp and silver carp. Should these species not be available then other known susceptible species should be sampled in the following preferential order: tench, orfe, wels catfish and, finally, any other cyprinid species present. For disease surveillance purposes all cyprinid species should be considered as potential covert carriers of SVCV. Cyprinid species are commonly mixed together in polyculture systems and the risk of transmission of SVCV between species during disease outbreaks is high.

2.2.4. Target organs and infected tissue

High titres of virus occur in the liver and kidney of infected fish, but much lower titres occur in the spleen, gills and brain (Dixon, 2008).

2.2.5. Persistent infection with lifelong carriers

The reservoirs of SVCV are clinically infected fish and covert virus carriers among cultured, feral or wild fish. Factors affecting persistence and duration of the carrier state have not been studied.

2.2.6. Vectors

Among animate vectors, the parasitic invertebrates *Argulus foliaceus* (Crustacea, Branchiura) and *Piscicola geometra* (Annelida, Hirudinea) transferred SVCV from diseased to healthy fish under experimental conditions and the virus has been isolated from *A. foliaceus* removed from infected carp (Ahne et al., 2002; Dixon, 2008). Herons (*Ardea cinerea*) were fed SVCV-infected carp and made to regurgitate the fish at intervals post-feeding. Virus was isolated from fish regurgitated 120 minutes after feeding.

2.2.7. Known or suspected wild aquatic animal carriers

Most reports of SVC have been from cultured fish, but the virus has been isolated from both diseased and apparently healthy feral carp in lakes.

It has been suggested that a possible mode of transmission of the virus is by the movement of baitfish, but there are no data to show that this has occurred (Goodwin et al., 2004). The main mode of transmission of the virus from one area to another is by movements of infected fish. The virus is often found in ornamental fish such as goldfish and koi carp, which are regularly transported around the world.

2.3. Disease pattern

2.3.1. Transmission mechanisms

The mode of transmission for SVCV is horizontal, but ‘egg-associated’ transmission (usually called 'vertical' transmission) cannot be ruled out following one report of isolation of SVCV from carp ovarian fluid although there have been no further such reports. Horizontal transmission may be direct or vectorial, water being the major abiotic vector (Fijan, 1988). Animate vectors (Section 2.2.6) and fomites may also be involved in transmission of SVCV (Fijan, 1988). Once SVCV is established in pond stock or pond farm stock, it may be very difficult to eradicate without destroying all types of life at the fish production site.

2.3.2. Prevalence

There are very few data on the prevalence of SVC, although there have been a small number of surveys of prevalence of antibody against the virus. In one such survey, carp in 19 of 20 hatcheries surveyed were positive for antibody against the virus. Data collected over the 10-year period 1992–2002 from Serbia showed that the virus had been isolated from carp at 12 of 38 hatcheries. The virus can occur sporadically in different ponds on one site, and sporadically from year to year at different sites.

2.3.3. Geographical distribution

For a long time, the geographical range of SVC was limited to countries of the European continent that experience low water temperatures during winter. Consequently, the disease has been recorded from most European countries and from certain of the western Independent States of the former Soviet Union (Belarus, Georgia, Lithuania, Moldova, Russia and the Ukraine) (see Dixon, 2008for references to these and the following locations). However, in 1998, the disease was recorded in goldfish in a lake in Brazil, in 2002 it was reported for the first time in two separate sites in the USA, and it was reported from Canada in 2006. Detection of the virus in carp in China (People's Rep. of) was confirmed in 2004. Confirmation of the isolation of SVCV from rainbow trout and Indian carp in Iran, and from Nile tilapia in Egypt awaits more data, and so the addition of those countries to the geographical range of the virus, also awaits those data.

2.3.4. Mortality and morbidity

Disease patterns are influenced by water temperature, age and condition of the fish, population density and stress factors. The immune status of the fish is also an important factor with both nonspecific (e.g. interferon) and specific immunity (serum antibodies, cellular immunity) having important roles. Poor physiological condition of over-wintered fish may be a contributory factor to disease susceptibility. In European aquaculture, losses can be up to 70% in young carp (Ahne et al., 2002), but are usually from 1 to 40%. Approximately 20% of the carp population in a lake in the USA died from SVC during a disease outbreak.

2.3.5. Environmental factors

Disease outbreaks in carp generally occur between 11 and 17°C. They rarely occur below 10°C, and mortalities, particularly in older fish, decline as the temperature exceeds 22°C (Fijan, 1988). Secondary and concomitant bacterial and/or parasitic infections can affect the mortality rate and display of signs. In carp, the disease is often observed in springtime (hence the common name for the disease), particularly in countries having cold winters. It is believed that the poor condition of the over-wintered fish may be a contributory factor in disease occurrence. The disease can occur in fish in quarantine following the stress of transportation, even though there has been no evidence of virus in the fish prior to transportation. The virus was isolated from apparently healthy fish from a lake in Canada that had been sampled over a 13-day period during which the water temperature varied between 27.3°C and 24.2°C.

2.4. Control and prevention

Methods to control SVC disease mainly rely on avoiding exposure to the virus coupled with good hygiene practices. This is feasible on small farms supplied by spring or borehole water and a secure system to prevent fish entering the farm via the discharge water. Hygiene measures should include disinfection of eggs by iodophor treatment (Ahne & Held, 1980), until it has been confirmed unequivocally that vertical transmission does not occur, regular disinfection of ponds, chemical disinfection of farm equipment, careful handling of fish to avoid stress and safe disposal of dead fish. Reducing fish stocking density during winter and early spring will reduce the spread of the virus. In rearing facilities with a controlled environment, elevation of water temperature above 19–20°C may stop or prevent SVC outbreaks. A safe and effective vaccine is not currently available. However, a number of experimental inactivated preparations, live attenuated vaccines and DNA vaccines have given encouraging results (Dixon, 2008).

2.4.1. Vaccination

A number of studies have reported the efficacy of vaccination, and vaccination trials in the field have been reported from the former Yugoslavia, Austria and the former Czechoslovakia (Fijan, 1988); a vaccine was once marketed in the latter country, but is no longer available. Laboratory trials have shown that DNA vaccination can protect fish (Dixon, 2008; Emmenegger & Kurath, 2008), but further developmental work is required.

2.4.2. Chemotherapy

Methisoprinol inhibits the replication of SVCV *in vitro*, but has not been tested under carp culture conditions.

2.4.3. Immunostimulation

Injection into carp of single-stranded and double-stranded RNA (which is an interferon inducer) protected carp for longer than 3 weeks, but the treatment is not effective by bath administration.

2.4.4. Resistance breeding

The “Krasnodar” strain of common carp has been bred for increased resistance to SVCV.

2.4.5. Restocking with resistant species

Not reported. The wide host range of the virus means that rigorous selection procedures would have to be applied to prospective alternative species.

2.4.6. Blocking agents

Not identified.

2.4.7. Disinfection of eggs and larvae

The virus is not considered to be transmitted via the egg, but if thought necessary, eggs could be disinfected by iodophor treatment (Ahne & Held, 1980).

2.4.8. General husbandry practices

Ponds should be disinfected regularly and effective disease biosecurity practices should be used. Equipment, particularly nets, should not be used in different ponds unless first disinfected. Practices that might cause stress should be minimised, and high stocking densities should be avoided.

3. Sampling

3.1. Selection of individual specimens

3.1.1. Diseased fish

Moribund fish or fish exhibiting clinical signs of the disease should be collected; fish should be alive when collected. However, there are no pathognomonic gross lesions and there may be no clinical signs in cases of sudden mortality (see Section 4.1.1). An identification label that includes information on the place, time, date, species, number of samples collected, dead/moribund state on collection, and the name and contact information of the individual collecting the sample(s) should be attached to the sample(s). A general approach to surveillance and sampling is provided in Chapter 1.4. of the *Aquatic Animal Health Code*. See also the OIE *Guide for Aquatic Animal Health Surveillance* (2009).

3.1.2. Fish appear to be clinically normal

Fish collection should encompass a statistically significant number of specimens, but it is obvious that failure to detect certain pathogens from the sample does not guarantee the absence of these agents in the specimen examined or in the stock. This is particularly true of free-ranging or feral stocks from which it is difficult to collect a representative and random sample. However, the risk of a pathogen escaping the surveillance system is reduced in fish farms whose fish stocks have been inspected and checked for pathogens for several years (at least two), insofar as they are not exposed to possible recontamination by feral fish.

Samples should comprise all susceptible species on the site with each lot of a species being represented in the sample group. A lot is defined as a group of the same fish species that shares a common water supply and that originates from the same broodfish or spawning population.

Any moribund fish present in the fish population to be sampled should be selected first for sample collection and the remainder of the sample should comprise randomly selected live fish from all rearing units that represent the lot being examined.

A general approach to surveillance and sampling is provided in Chapter 1.4. of the *Aquatic Animal Health Code*. See also the OIE *Guide for Aquatic Animal Health Surveillance* (2009).

3.2. Preservation of samples for submission

Samples for virus isolation should be transported to the laboratory at 4°C using refrigerated containers or on ice, preferably in virus transport medium (Chapter 2.3.0, Section A.2.2.1.), and tested within 24 hours or, in exceptional circumstances, 48 hours. The shipment of organ samples is preferred, but live or whole dead fish can be submitted

to the testing laboratory if necessary. If this is not possible, samples can be frozen, but there may be loss of virus viability on thawing the samples. Repeated freeze–thawing of the sample must be avoided. Samples for RT-PCR can be preserved in commercially available RNA preservation solutions according to the manufacturers' recommendations, or, alternatively, samples can be fixed in ethanol.

3.3. Pooling of samples

Samples from up to five fish can be pooled.

3.4. Best organs or tissues

Subclinically infected fish (apparently healthy fish): kidney, spleen, gill and encephalon (any size fish).

Clinically affected fish: whole alevin (body length ≤ 4 cm), entire viscera including kidney and encephalon (> 4 cm body length ≤ 6 cm) or, for larger sized fish, liver, kidney, spleen and encephalon.

3.5. Samples/tissues that are not suitable

It can be difficult to isolate virus from subclinically infected carrier fish and, in particular, from fish surviving a disease outbreak with increasing time after the disease occurrence. Likewise, isolation of virus from such fish at temperatures outside the clinical range for the disease is problematic. It may be possible to detect antibody against the virus in such fish (Dixon, 2008), but see the caveat in Section 4below. Virus isolation may not be possible from decomposed clinical samples, so the presence of signs of SVC disease and a positive indirect immunofluorescent antibody test (IFAT) or enzyme-linked immunosorbent assay (ELISA) may be considered sufficient to initiate control measures. A number of studies in which attempts were made to isolate virus from reproductive fluids were unsuccessful, although the virus has been isolated at low frequency from ovarian, but not seminal, fluids.

4. Diagnostic methods

Diagnosis of SVC in clinically affected fish may be achieved by virus isolation or, more rapidly, by IFAT or ELISA on infected tissues. Ideally, direct diagnosis by IFAT or ELISA should be confirmed by virus isolation followed by a virus neutralisation (VN) test or RT-PCR and sequence analysis.

The detection of fish antibodies to viruses has not thus far been accepted as a routine screening method for assessing the viral status of fish populations because of insufficient knowledge of the serological responses of fish to virus infections. However, the validation of some serological techniques for certain fish virus infections could arise in the near future, rendering the use of fish serology more widely acceptable for health screening purposes. As SVCV cannot be detected at all times of the year, or with confidence from all carrier fish, there are occasions when detection of fish antibody may provide useful information for epidemiological studies or risk assessments. However, it must be borne in mind that the presence of specific antibody only indicates previous exposure to the virus, and is not an indicator of the current presence of virus in a fish. Antibody surveys are best used at the population level, rather than the individual level as an indicator of previous exposure to the virus.

4.1. Field diagnostic methods

4.1.1. Clinical signs

During an outbreak of SVC there will be a noticeable increase in mortality in the population. Diseased fish usually appear darker in colour. Typical clinical signs include exophthalmia, pale gills, haemorrhages on the skin, base of the fins and the vent, abdominal distension or dropsy and a protruding vent (anus), often with trailing mucoid faecal casts. All these clinical signs may not be present in individual fish, and they may not all be present in the affected population. Some of these signs may be present in diseases caused by other pathogens. There may be no clinical signs in cases with a sudden onset of mortality.

4.1.2. Behavioural changes

Generally, young fish up to 1 year are most susceptible to clinical disease, but all age groups can be affected. Fish become lethargic, separate from the shoal and gather at the water inlet or sides of a pond and some may experience loss of equilibrium.

4.2. Clinical methods

4.2.1. Gross pathology

There are no pathognomonic gross lesions. Final diagnosis must await direct detection of viral antigen or nucleic acid in tissues or virus isolation and identification. Lesions may be absent in cases of sudden mortality. Gross pathologies are mainly documented for common carp and may include excess ascitic fluid in the abdominal cavity, usually containing blood, degeneration of the gill lamellae and inflammation of the intestine, which contains mucus instead of food. Oedema and haemorrhage of the visceral organs is commonly observed. Focal haemorrhages may be seen in the muscle and fat tissue, as well as in the swim bladder.

4.2.2. Clinical chemistry

In the absence of large-scale studies, clinical chemistry is an unreliable means of indicating SVC disease. The data presented below are only indicative of nonspecific disease processes.

Some groups of sheatfish experimentally infected with the virus exhibited lowered haematocrit values, but in other groups the values remained unchanged. Transaminase activity increased in all groups.

During 3 months following an outbreak of SVC in carp in ponds there was an increase in neutrophils, monocytes, eosinophils, and basophils. The numbers of lymphocytes declined then rose back to the starting levels. Over the same period, fish with signs of SVC had an increase in plasma levels of Ca^{2+}, inorganic phosphate levels, total bilirubin, alanine aminotransferase activity, lactic acid dehydrogenase activity and α-hydroxybutyryl dehydrogenase activity. Levels of total protein, cholesterol and alkaline phosphatase activity decreased.

4.2.3. Microscopic pathology

Histopathological changes can be observed in all major organs. In the liver, blood vessels show oedematous perivasculitis progressing to necrosis. Liver parenchyma shows hyperaemia with multiple focal necroses and degeneration. The heart shows pericarditis and infiltration of the myocardium progressing to focal degeneration and necrosis. The spleen shows hyperaemia with hyperplasia of the reticuloendothelium and enlarged melanomacrophage centres, and the pancreas is inflamed with multifocal necrosis. In the kidney, damage is seen to excretory and haematopoietic tissue. Renal tubules are clogged with casts and the cells undergo hyaline degeneration and vacuolation. The intestine shows perivascular inflammation, desquamation of the epithelium and atrophy of the villi. The peritoneum is inflamed and lymph vessels are filled with detritus and macrophages. In the swim bladder, the epithelial lamina changes from a monolayer to a discontinuous multi-layer and vessels in the submucosa are dilated with nearby lymphocyte infiltration.

4.2.4. Wet mounts

Not relevant.

4.2.5. Smears

Only of value if an immunohistological procedure such as the IFAT (see Section 4.3.1.2.2.1) or immunoperoxidase procedure is used but see caveats in Section 4.3.1.2

4.2.6. Fixed sections

See Section 4.2.3Fixed sections can also be used for immunohistochemical procedures as in 4.2.5, but see caveats in Section 4.3.1.2

4.2.7. Electron microscopy/cytopathology

The virus has the typical bullet shape of a rhabdovirus and is approximately 60–90 nm wide by 90–180 nm long, following negative staining. The virus comprises a nucleocapsid surrounded by an envelope.

4.3. Agent detection and identification methods

See the following sections in Chapter 2.3.0.:

- Section A.2.2.1. for further details of transportation.
- Section A.2.2.2. for virus extraction and obtaining organ homogenates.

4.3.1. Direct detection methods

The virus can be observed directly by electron microscopy, but this will only indicate the presence of a rhabdovirus and further identification will be needed. Virus antigen and nucleic acid can potentially be identified in extracts of tissues from clinically infected fish, and virus can usually be isolated from those fish. However, it is much less likely that virus antigen or nucleic acid will be detected directly from tissues from subclinically infected carrier fish. Virus isolation is the preferred method for detecting such fish, but is not 100% effective.

4.3.1.1. Microscopic methods

Microscopic methods by themselves are not recommended for diagnosis of SVC as the histopathological picture is not specific for the disease. They may, however, provide supporting evidence, particularly, when immunohistological methods are used, but see caveats in Section 4.3.1.2

4.3.1.1.1. Wet mounts

Not relevant.

4.3.1.1.2. Smears/tissue imprints

Only of value if an immunohistological procedure such as the IFAT (see Section 4.3.1.2.2.1) or immunoperoxidase procedure is used but see caveats in Section 4.3.1.2

4.3.1.1.3. Fixed sections

Only of value if an immunohistological procedure such as the IFAT (see Section 4.3.1.2.1.2) or immunoperoxidase procedure is used but see caveats in Section 4.3.1.2See Chapter 2.3.0., Section B.3.3.1. for details of fixation of specimens.

4.3.1.2. Agent isolation and identification

Following isolation, the virus must be identified and this can be achieved by antigen detection methods, virus neutralisation or nucleic acid identification methods. The former two methods must be regarded as presumptive unless fully validated monoclonal or polyclonal antibodies are used, as cross reactions with other viruses occur (Section 2.1.1and Section 5). Commercially available kits using polyclonal antibodies may lack specificity, and those using monoclonal antibodies may not detect all subgenogroups of SVCV (Dixon & Longshaw, 2005). Nucleic acid detection methods must always be followed up by sequencing or use of a method such as reverse hybridisation (Sheppard et al., 2007) to confirm the identity of the virus.

4.3.1.2.1. Cell culture/artificial media

4.3.1.2.1.1. Cell line to be used

EPC or FHM (Chapter 2.3.0., Section B.1.1.).

4.3.1.2.1.2. Virus extraction

Use the procedure described in Chapter 2.3.0, Section A.2.2.2.

4.3.1.2.1.3. Inoculation of cell monolayers

Make two serial tenfold dilutions of the 1/10 organ homogenate supernatants in cell culture medium (i.e. the homogenate supernatants will be 1/100 and 1/1000 dilutions of the original organ material) and transfer an appropriate volume of each of these two dilutions on to 24-hour-old cell monolayers drained of their culture medium. Alternatively, make a single tenfold dilution of the 1/10 organ homogenate (i.e. a 1/100 dilution of the original organ material) and add an appropriate volume of both the 1/10 and 1/100

dilutions directly to undrained 24-hour-old cell monolayers, to effect 1/100 and 1/1000 final dilutions of the organ homogenate. Should toxicity of the sample be a problem, make two serial tenfold dilutions of the 1/10 organ homogenate supernatants in cell culture medium as described above and inoculate at least 2 cm^2 of drained cell monolayer with 100 µl of each dilution. Allow to adsorb for 0.5–1 hour at 10–15°C, withdraw the inoculum and add cell culture medium buffered at pH 7.6 and supplemented with 2% fetal calf serum (FCS) (1 ml/well for 24-well cell culture plates). Incubate at 20°C.

4.3.1.2.1.4. Monitoring incubation

Follow the course of infection in positive controls and other inoculated cell cultures by microscopic examination at ×40–100 magnification for 7 days. The use of a phase-contrast microscope is recommended.

Maintain the pH of the cell culture medium at between 7.3 and 7.6 during incubation. This can be achieved by the addition to the inoculated medium of sterile bicarbonate buffer (for tightly closed cell culture flasks) or HEPES-buffered medium (HEPES = N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid) or 2 M Tris (Tris [hydroxymethy]) aminomethane)/HCl buffer solution (for cell culture plates).

If a cytopathic effect (CPE) appears in those cell cultures inoculated with the dilutions of the tested homogenate supernatants, identification procedures must be undertaken immediately (see Sections 4.3.1.2.1.1, 4.3.1.2.1.2, 4.3.1.2.1.3and 4.3.1.2.3.1below).

If no CPE develops in the inoculated cultures (despite normal progression of CPE in the virus controls), the inoculated cultures should be subcultured for a further 7 days. Should the virus control fail to develop CPE, the process should be repeated with fresh susceptible cells and new batches of samples.

4.3.1.2.1.5. Subcultivation procedures

Using a pipette, try to dislodge cells from the cell culture vessels and collect aliquots of cell culture medium plus cells from all inoculated monolayers, keeping different groups separate. The aliquots of the 1/100 and 1/000 dilutions are pooled and inoculated on to fresh 24-hour-old cell cultures to effect 1/10 and 1/100 final dilutions of the pooled aliquots. Incubate and monitor as described above. If no CPE occurs, the test may be declared negative.

4.3.1.2.1.6. Confirmation of virus identity by neutralisation

i) Collect the culture medium of the cell monolayers exhibiting CPE and centrifuge at 2000 ***g*** for 15 minutes at 4°C, or filter through a 0.45 µm pore membrane to remove cell debris.

ii) Dilute the virus-containing medium from 10^{-2} to 10^{-4}.

iii) Mix aliquots of each dilution with equal volumes of an antibody solution against SVCV, and similarly treat aliquots of each virus dilution with cell culture medium. The neutralising antibody (NAb) solution must have a 50% plaque reduction titre of at least 2000 based on neutralisation of 50–100 plaque-forming units (PFU) of SVCV.

iv) In parallel, other neutralisation tests must be performed against:

- a homologous virus strain (positive neutralisation test),
- a heterologous virus strain (negative neutralisation test).

v) Incubate all the mixtures at 20°C for 1 hour.

vi) Transfer aliquots of each of the above mixtures on to cell monolayers (inoculate two cell cultures per dilution) and allow adsorption to occur for 0.5–1 hour at 15–20°C; 24- or 12-well cell culture plates are suitable for this purpose, using a 50 µl inoculum.

vii) When adsorption is completed, add cell culture medium, supplemented with 2% FCS and buffered at pH 7.4–7.6, to each well and incubate at 20°C.

viii) Check the cell cultures for the onset of CPE and read the results as soon as it occurs in non-neutralised controls (cell monolayers being protected in positive neutralisation controls). Results are recorded either after a simple microscopic examination (phase-contrast preferable) or after discarding the cell culture medium and staining the cell monolayers with a solution of 1% crystal violet in 20% ethanol.

ix) The tested virus is identified as SVCV when CPE is prevented or noticeably delayed in the cell cultures that received the virus suspension treated with the SVCV-specific antibody, whereas CPE is evident in all other cell cultures.

NOTE: Presumptive SVCV isolates identified by the ELISA or the IFAT may not be neutralised by NAb to SVCV. Also, some SVCV subgenogroups may not be completely neutralised by NAb prepared against an isolate from a different subgenogroup. Where neutralisation by NAb to SVCV is absent or incomplete, confirmation by the RT-PCR and nucleotide sequence analysis of RT-PCR products is recommended to confirm the presence of SVCV.

4.3.1.2.1.7. Confirmation of virus identity by the indirect fluorescent antibody test (IFAT)

i) Prepare monolayers of cells in 2 cm^2 wells of plastic cell culture plates, flasks or on cover-slips or glass slides in order to reach approximately 80% confluency within 24 hours of incubation at 25°C (seed six cell monolayers per virus isolate to be identified, plus two for positive and two for negative controls). The FCS content of the cell culture medium can be reduced to 2–4%. If numerous virus isolates have to be identified, the use of Terasaki plates is strongly recommended.

ii) When the cell monolayers are ready for infection, i.e. on the same day or on the day after seeding, inoculate the virus suspensions to be identified by making tenfold dilution steps directly in the cell culture wells or flasks. For tests using cells cultured on glass cover-slips or slides, the dilutions are made in sterile containers and then used to inoculate the cells.

iii) Dilute the control virus suspension of SVCV in a similar way, in order to obtain a virus titre of about 5000–10,000 PFU ml^{-1} in the cell culture medium.

iv) Incubate at 20°C for 24 hours.

v) Remove the cell culture medium, rinse once with 0.01 M phosphate-buffered saline (PBS), pH 7.2, then three times briefly with cold acetone (stored at –20°C) for slides or cover-slips or 80% acetone in water or 30% acetone in ethanol, also at –20°C, for cells on plastic substrates. Let the fixative act for 15 minutes. A volume of 0.5 ml is adequate for 2 cm^2 of cell monolayer.

vi) Allow the cell monolayers to air-dry for at least 30 minutes and process immediately or freeze at –20°C.

vii) Rehydrate the dried cell monolayers, if they have been stored frozen, by four rinsing steps with PBS containing 0.05% Tween 20 (PBST) and remove this buffer completely after the last rinse. Block with 5% skim milk or 1% bovine serum albumin, in PBST for 30 minutes at 37°C.

viii) Rinse four times with PBST, 5 minutes for each rinse. The slides or plastic culture plates can be gently agitated during the rinses.

ix) Prepare a solution of purified antibody or serum to SVCV in PBST, at the appropriate dilution (which has been established previously or as given by the reagent supplier).

x) Incubate the cell monolayers with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur.

xi) Rinse four times with PBST.

xii) Incubate the cell monolayers with a solution of fluorescein isothiocyanate (FITC)-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These FITC antibodies are most often rabbit or goat antibodies.

xiii) Rinse four times with PBST.

xiv) View the treated cell monolayers on plastic substrates immediately, or mount the slides or cover-slips using glycerol saline at pH 8.5, or a commercially available mountant.

xv) Examine under incident ultraviolet (UV) light using a microscope with ×10 eye pieces and ×20 or ×40 objective lenses having numerical apertures of >0.65 and >1.3, respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

4.3.1.2.1.8. Confirmation of virus identity by enzyme-linked immunosorbent assay (ELISA)

i) Coat the wells of microplates designed for ELISAs with appropriate dilutions of purified immunoglobulins (Ig) specific for SVCV, in 0.02 M carbonate buffer, pH 9.5 (200 µl/well). Ig may be polyclonal or monoclonal Ig originating most often from rabbit or mouse, respectively. For the identification of SVCV, monoclonal antibodies (MAbs) specific for certain domains of the nucleocapsid (N) protein are suitable.

ii) Incubate overnight at 4°C.

iii) Rinse four times with PBST.

iv) Block with skim milk (5% in carbonate buffer) or other blocking solution for 1 hour at 37°C (300 µl/well).

v) Rinse four times with PBST.

vi) Add 2% non-ionic detergent (Triton X-100 or Nonidet P-40) to the virus suspension to be identified.

vii) Dispense 100 µl/well of two- or four-step dilutions of the virus to be identified, and of the non-infected cell culture harvest (negative control). Also include SVCV positive control virus. Incubate for 1 hour at 37°C.

viii) Rinse four times with PBST.

ix) Add to the wells, 200 µl of horseradish peroxidase (HRPO)-conjugated MAb or polyclonal antibody to SVCV; or polyclonal IgG to SVCV. A MAb to N protein specific for a domain different from the one of the coating MAb and previously conjugated with biotin can also be used. Incubate for 1 hour at 37°C.

x) Rinse four times with PBST.

xi) If HRPO-conjugated antibody has been used, go to step xiii. Otherwise, add 200 µl of HRPO-conjugated streptavidin or ExtrAvidin (Sigma) to those wells that have received the biotin-conjugated antibody and incubate for 1 hour at 37°C.

xii) Rinse four times with PBST.

xiii) Add 200 µl of a suitable substrate and chromogen, such as tetramethylbenzidine dihydrochloride. Stop the course of the test when positive controls react, and read the results.

4.3.1.2.2. Antibody-based antigen detection methods directly on fish tissues

4.3.1.2.2.1. Indirect fluorescent antibody test

i) Bleed the fish thoroughly.

ii) Make kidney imprints on cleaned glass slides or at the bottom of the wells of a plastic cell culture plate.

iii) Store and transport the kidney pieces as indicated in Chapter 2.3.0., Section A. 2.2.1.) together with the other organs required for virus isolation.

iv) Allow the imprint to air-dry for 20 minutes.

v) Fix with cold acetone (stored at –20°C) for glass slides or 80% acetone in water or 30% acetone in ethanol, also at –20°C, for plastic wells. Let the fixative act for 15 minutes. Allow the imprints to air-dry for at least 30 minutes and process immediately or freeze at –20°C.

vi) Rehydrate the imprints if they have been stored frozen by four rinsing steps with PBST, and remove this buffer completely after the last rinse. Block with 5% skim milk or 1% bovine serum albumin, in PBST for 30 minutes at 37°C.

vii) Rinse four times with PBST, 5 minutes for each rinse. The slides or plastic culture plates can be gently agitated during the rinses.

viii) Prepare a solution of purified antibody or serum to SVCV in PBST, at the appropriate dilution (which has been established previously or as given by the reagent supplier).

ix) Incubate the imprints with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur.

x) Rinse four times with PBST.

xi) Incubate the imprints with a solution of FITC-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These FITC antibodies are most often rabbit or goat antibodies.

xii) Rinse four times with PBST.

xiii) View the treated imprints on plastic plates immediately, or mount the slides with cover-slips using glycerol saline at pH 8.5, or a commercially-available mountant.

xiv) Examine under incident ultraviolet (UV) light using a microscope with ×10 eye pieces and ×20 or ×40 objective lenses having numerical aperture of >0.65 and >1.3, respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

4.3.1.2.2.2. Enzyme-linked immunosorbent assay (ELISA)

See Chapter 2.3.0., Section A.2.2.2. for obtaining organ homogenates.

i) Coat the wells of microplates designed for ELISAs with appropriate dilutions of purified immunoglobulins (Ig) specific for SVCV, in 0.02 M carbonate buffer, pH 9.5 (200 µl/well). Ig may be polyclonal or monoclonal Ig originating most often from rabbit or mouse, respectively. For the identification of SVCV, monoclonal antibodies (MAbs) specific for certain domains of the nucleocapsid (N) protein are suitable.

ii) Incubate overnight at 4°C.

iii) Rinse four times with PBST.

iv) Block with skim milk (5% in carbonate buffer) or other blocking solution for 1 hour at 37°C (300 µl/well).

v) Rinse four times with PBST.

vi) Store a 1/4 aliquot of each homogenate at 4°C, in case the test is negative and virus isolation in cell culture is required.

vii) Treat the remaining part of the homogenate with 2% Triton X-100 or Nonidet P-40 and 2 mM of phenyl methyl sulphonide fluoride; mix gently.

viii) Dispense 100 µl/well of two- or four-step dilutions of the sample to be identified, and of negative control tissues. Also include an SVCV positive control virus. Incubate for 1 hour at 37°C.

ix) Rinse four times with PBST.

x) Add to the wells, 200 µl of horseradish peroxidase (HRPO)-conjugated MAb or polyclonal antibody to SVCV; or polyclonal IgG to SVCV. A MAb to N protein specific for a domain different from the one of the coating MAb and previously conjugated with biotin can also be used. Incubate for 1 hour at 37°C.

xi) Rinse four times with PBST.

xii) If HRPO-conjugated antibody has been used, go to step xiv. Otherwise, add 200 µl of HRPO-conjugated streptavidin or ExtrAvidin (Sigma) to those wells that have received the biotin-conjugated antibody and incubate for 1 hour at 37°C.

xiii) Rinse four times with PBST.

xiv) Add 200 µl of a suitable substrate and chromogen, such as tetramethylbenzidine dihydrochloride. Stop the course of the test when positive controls react, and read the results.

xv) If the test is negative, process the organ samples stored at 4°C, for virus isolation in cell culture as described in Section 4.3.1.2.1

4.3.1.2.3. Molecular techniques

4.3.1.2.3.1. Reverse-transcription polymerase chain reaction (RT-PCR) (confirmation of virus identity or directly from fish tissue extracts)

The genome of SVCV consists of a single strand of RNA of approximately 11 kb, with negative polarity. Amplification of a 714 bp fragment of SVCV cDNA is performed using primers derived from sequences of the region coding for the glycoprotein gene: 5'-TCT-TGG-AGC-CAA-ATA-GCT-CAR*-R*TC-3' (SVCV F1) and 5'-AGA-TGG-TAT-GGA-CCC-CAA-TAC-ATH*-ACN*-CAY*-3' SVCV R2), using a modification of the method of Stone et al., 2003.

i) Total RNA is extracted from 100 µl of supernatant from cell cultures exhibiting CPE or 100 µl of fish tissue extract and dissolved in 40 µl molecular biology grade DNase- and RNase-free water.

A number of total RNA extraction kits are available commercially that will produce high quality RNA suitable for RT-PCR. Examples are Trizol ReagentT (RL, Life Technologies, Paisley, UK), SV Total RNA isolation system (Promega) and Nucleospin® RNA AB gene).

ii) For cDNA synthesis, a reverse transcription reaction is performed at 37°C for 1 hour in a 20 µl volume consisting of 1 × M-MLV RT reaction buffer (50 mM Tris, pH 8.3, 75 mM KCl, 10 mM DTT, 3 mM $MgCl_2$) containing 1 mM dNTP, 100 pmol SVCV R2 primer, 20 units M-MLV reverse transcriptase (Promega, Southampton, UK) or equivalent reverse transcriptase and 1/10 of the total RNA extracted above.

iii) PCR is performed in a 50 µl reaction volume 1 × PCR buffer (50 mM KCl, 10 mM Tris/HCl, pH 9.0, and 0.1% Triton X-100) containing 2.5 mM $MgCl_2$, 200 µM dNTPs, 50 pmol each of the SVCV R2 and SVCV F1 primers, 1.25 units of Taq DNA polymerase, and 2.5 µl reverse transcription reaction mix. The reaction mix is overlaid with mineral oil and subjected to 35 temperature cycles of: 1 minute at 95°C, 1 minute at 55°C and 1 minute at 72°C followed by a final extension step of 10 minutes at 72°C. Amplified DNA (714 bp) is analysed by agarose gel electrophoresis.

iv) If the CPE in culture is not extensive it is possible that a product will not be generated using a single round of amplification. To avoid such problems, use the semi-nested assay using primers: 5'-TCT-TGG-AGC-CAA-ATA-GCT-CAR*-R*TC-3' (SVCV F1) and 5'-CTG-GGG-TTT-CCN*-CCT-CAA-AGY*-TGY*-3' (SVC R4) according to Stone et al., 2003.

v) The second round of PCR is performed in a 50 µl reaction volume 1 × PCR buffer (50 mM KCl, 10 mM Tris/HCl, pH 9.0, and 0.1% Triton X-100) containing 2.5 mM $MgCl_2$, 200 µM dNTPs, 50 pmol each of the SVCV R4 and SVCV F1 primers, 1.25 units Taq DNA polymerase, and 2.5 µl of the first round product. The reaction mix is overlaid with mineral oil and subjected to 35 temperature cycles of: 1 minute at 95°C, 1 minute at 55°C and 1 minute at 72°C followed by a final extension step of 10 minutes at 72°C. Amplified DNA (606 bp) is analysed by agarose gel electrophoresis.

vi) All amplified products are confirmed as SVCV in origin by sequencing, and the SVCV subtype (Ia-Id) is identified using a BLAST search (http://www.ebi.ac.uk/Tools/sss/ncbiblast/) or by phylogenetic analysis using the SVCV sequences available in public sequence databases. Phylogenetic analysis is undertaken using a 426 bp region corresponding to nucleotides 429–855 of the glycoprotein gene.

vii) In some cases where the CPE is extensive and the virus replicates to a high titre, sufficient PCR amplicon will be available for direct sequencing. Where the amplified product is weak it is recommended that the product be inserted into an appropriate sequencing vector (e.g. pGEM-T, pCR® 4- TOPO®) prior to undertaking the sequencing. At least two independent amplification and sequencing events should be undertaken to eliminate potential sequence errors introduced by the Taq polymerase.

NOTE: SVCV primer-annealing sites were identified by the alignment of the published amino acid sequences for the glycoprotein of SVCV (Björklund et al., 1996; Genbank accession U18101), and the vesicular stomatitis virus (VSV) New Jersey (Gallione & Rose, 1983; Genbank accession V01214), and Piry strains (Genbank accession D26175). Primers were then designed to anneal to the regions encoding the conserved amino acids using the published sequence for SVCV (Björklund et al., 1996) as a skeleton, and introducing degenerate bases at the 3' termini to allow for potential differences in codon usage. The appropriate IUB codes have been used where appropriate and are indicated by an asterisk (*).

4.3.1.2.4. Agent purification

The virus can be purified as described by Hill et al., 1975.

i) Harvest medium from infected cell cultures.

ii) Clarify by centrifugation at 2000 ***g*** for 15 minutes at 4°C. Remove the supernatant.

iii) Sediment the virus by centrifuging the supernatant at 40,000 ***g*** for 1 hour.

iv) Remove and discard the supernatant. Resuspend the pellet in a small volume of PBS or TNE (0.01 M Tris, 0.1 M NaCl, 1 mM ethylenediaminetetra-acetic acid, pH 7.2). The volume will depend on the original amount of cell culture medium and the size of tube used to make the gradient. If the supernatant fluids have been centrifuged in several tubes, combine the resuspended pellets.

v) Prepare a 15–45% sucrose gradient made up in the buffer used in step iv.

vi) Gently overlay the resuspended pellets and centrifuge at 40,000 ***g*** for 2 hours at 4°C.

vii) An opalescent band of virus should be visible in the gradient. Harvest the band and dilute at least tenfold with the buffer in use.

viii) Centrifuge at 40,000 ***g*** for 2 hours at 4°C.

ix) Resuspend the pellet in the buffer in use.

4.3.2. Serological methods

Fish produce an immune response following infection with SVCV, and this has been studied mainly by following antibody development. The antibody response is influenced by water temperature. Following SVCV infection at low temperatures such as 10°C, antibody may not be detected, or may be present at low titre and may take several weeks to develop, whereas at 20°C, antibody develops sooner (after 1 week), and high titres can be present (Dixon, 2008). The duration of the antibody response is not known, but antibody has been detected 1 year after a natural infection in a fishery, and after over 2 years following experimental infection (unpublished observations). Detection of neutralising antibody has been used in many surveys for the virus, but ELISA methods are more sensitive. Dixon et al., 1994developed a competitive ELISA, which is applicable to detection of antibody in a wide range of hosts.

The suitability of antibody detection has been discussed in Sections 3.5and 4

5. Rating of tests against purpose of use

The methods currently available for targeted surveillance and diagnosis of SVC are listed in Table 5.1.The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category ‘a’ or ‘b’ have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. *Methods for targeted surveillance and diagnosis*

Method	Targeted surveillance		Presumptive diagnosis	Confirmatory diagnosis
	Juveniles	Adults		
Gross signs	d	d	b	d
Histopathology	d	d	b	c
Transmission EM	d	d	d	d
Isolation in cell culture	a	a	a	a
Test for virus antigen	d	d	a	c
Test for fish antibody against the virus	c	c	c	d
RT-PCR	c	c	a	a
Sequence	na	na	a	a

EM = electron microscopy; RT-PCR = reverse-transcription polymerase chain reaction; na = not applicable.

NOTE: Isolation in cell culture can only be regarded as presumptive until the identity of the isolated virus is confirmed by a suitable method.

Four genogroups of piscine rhabdoviruses have been described (Stone et al., 2003): genogroup I (SVCV), genogroup II (grass carp rhabdovirus), genogroup III (pike fry rhabdovirus) and genogroup IV (tench rhabdovirus). Further analysis also showed that the SVCV genogroup could be further subdivided into at least four subgenogroups. Antibodies directed against SVCV cross-react to various degrees with all of the rhabdoviruses in the other three genogroups. The ability to confirm SVCV based on results from serological tests, such as ELISA, IFAT and serum neutralisation, relies on the specificity of the antibodies used in the tests. Results from those serological tests can only be accepted as confirming the presence of SVCV if the antisera used have been validated as detecting viruses in all four subgenogroups of genogroup I and do not cross-react with isolates from the other three genogroups.

Many diagnostic laboratories have encountered difficulties in obtaining antibodies against SVCV that are suitable for use in serological tests and have turned to commercially available test kits. Two commercial test kits are available for identification of SVCV, the TestLine ELISA kit (TestLine, Brno, Czech Republic) and the Bio-X IFAT kit (Bio-X Diagnostics, Jemelle, Belgium). Recently the tests have been assessed for their specificity against virus isolates from genogroups I, II, III and IV by Dixon & Longshaw, 2005who found that the TestLine ELISA, which uses a polyclonal rabbit antibody, was nonspecific and could not distinguish SVCV from viruses in the other three genogroups. Conversely, the Bio-X IFAT, which uses a monoclonal mouse antibody, was too specific and could only detect SVCV isolates from one of the four SVCV subgenogroups. These commercial test kits can be applied for presumptive diagnosis of SVC, but the problems of specificity severely limit their application for confirmatory diagnosis.

It is recommended that RT-PCR and nucleotide sequence analysis of the PCR products are used for confirmatory identification of SVCV.

6. Test(s) recommended for targeted surveillance to declare freedom from spring viraemia of carp

The method for surveillance of susceptible fish populations for declaration of freedom from SVC is inoculation of cell culture with tissue extracts (as described in Section Cell culture/artificial mediaabove) to demonstrate absence of the virus.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

SVC should be considered as a cause of disease when rapid mortalities and significant numbers of mortalities occur in a population of susceptible fish species, particularly if accompanied by clinical signs of SVC.

A suspect case of SVC disease is defined as the presence of typical clinical signs of the disease in a population of susceptible fish OR presentation of typical histopathology in tissue sections OR typical CPE in cell cultures without identification of the causative agent OR a single positive result from one of the diagnostic assays described above.

7.2. Definition of confirmed case

The first case of the disease in a new area, or in an area where SVC has occurred before but has not been identified over a 2-year surveillance period is described as the index case. A confirmed index case is defined as a suspect case that has produced a typical CPE in cell culture with subsequent identification of the causative agent by one of the serological tests using validated antisera or RT-PCR plus sequencing described above OR a second positive result from a separate and different diagnostic assay described above. If a serological test is used, the antisera must be “fit for purpose” as indicated in Section 5. If RT-PCR is used, the product obtained must be sequenced in order to confirm SVCV; if not the case is suspect SVCV.

During follow-up investigations after a confirmed index case, a case can be confirmed on the basis of RT-PCR plus sequencing alone.

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NB: There are OIE Reference Laboratories for Spring viraemia of carp
(see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list:
http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on Spring viraemia of carp