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Spring Viremia of Carp Virus (SVCV): Global Status of Outbreaks, Diagnosis, Surveillance, and Research

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Abstract

The spring viremia of carp virus (SVCV) is an OIE-listed rhabodovirus historically responsible for losses of cultured fish in Europe. Acute disease is associated with high mortality, especially in common carp *Cyprinus carpio* during their first spring season when water temperatures are 10-15°C. Mortality has been reported in other cyprinids and in the Wels catfish *Siluris glanis*. The disease is characterized by hemorrhages on the skin and bloody mucus in the intestine, clinical signs shared by other diseases including bacterial infections. In 2002, SVCV was detected on a large koi farm in the USA. The USA isolate was 98% identical to isolates associated with koi and goldfish imported from China, but distantly related to European strains. In spring 2002, a major SVCV kill of common carp occurred in Cedar Lake, Wisconsin. This isolate was also of the Asian type, as were subsequent isolates from wild and cultured fish in several states. In the USA, all infected farmed populations were destroyed and no additional isolates have been detected since 2004. One of the most critical aspects of SVCV diagnosis is to differentiate the disease from the koi herpesvirus (KHV). The most obvious difference is that KHV generally occurs in temperatures of 20-28°C while SVCV disease occurs below 18°C and commonly at 10-15°C.

Introduction

There are excellent papers covering the molecular epidemiology of spring viremia of carp virus (SVCV; Stone et al., 2003; Miller et al., 2007; Sheppard et al., 2007; Warg et al., 2007), the emergence of SVCV in Asia (Liu et al., 2004; Teng et al., 2007), the impact on fish populations in North America (Goodwin, 2002; Dikkeboom et al., 2004; Garver et al., 2007), and descriptions of the disease (Fijan, 1999; Ahne et al., 2002). In this review, I will emphasize those aspects of SVCV history, taxonomy, and diagnosis most likely to provide insights into the future of SVCV in Asia.

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The Virus

SVCV was first isolated and characterized by Nicola Fijan as *Rhabdovirus carpio* (Fijan et al., 1971). Descriptions of carp losses in older literature indicate that the virus was endemic in Europe long before that time. Initial work on the virus involved a great deal of taxonomic confusion caused by the morphologic and antigenic similarities between SVCV and other members of the rhabdovirus family. Some case reports describing infectious dropsy of carp were probably SVC, as were cases of "infectious ascites, hemorrhagic septcemia, red contagious disease, and carp rubella" (Fijan, 1999). More recently, it appears that there were cases of confusion between the pike fry rhabdovirus (PFRV) and SVC virus. Fortunately, modern methods that rely on sequence data and analyses of genetic similarities have brought order to this field and retrospective studies have cleared up much of the confusion in the older literature.

Using viral gene sequence data, consistent phylogenetic trees genogrouping SVCV and related viruses have been produced (Miller et al., 2007; Sheppard et al., 2007; Warg et al., 2007). In many cases, similarities between genogroups are so high that serological methods (including those suggested in the OIE Manual) often fail to correctly assign isolates to the correct genogroup (Sheppard et al., 2007). The four genogroups of closely-related SVCV-like viruses include Genogroup I - classic SVCV isolates, Genogroup II - a single isolate of a grass carp rhabdovirus, Genogroup III - the pike fry rhabdovirus group (PFRV), and Genogroup IV - the tench rhabdovirus group.

According to the International Committee on the Taxonomy of Viruses (ICTVdB Management, 2006), the SVCV Genogroup I viruses are in the Rhabdoviridae family, in the order Mononegavirales. The genus is *Vesiculovirus* and the species carries the formally tentative name "spring viremia of carp virus". The Genogroup II grass carp rhabdovirus is listed as a synonym of the Group III pike fry rhabdovirus, and the Group IV tench rhabdovirus has not yet been recognized. The SVCV Genogroup I viruses are divided into Subgroup a - SVCV of Asia, Subgroup b - SVCV of eastern Europe, Subgroup c - SVCV of eastern Europe, and Subgroup d - SVCV of western Europe.

The first isolations of subgroup Ia were made in 1997 in the UK during routine import inspections of fish arriving directly from China (Liu et al., 2004; Teng et al., 2007). Subsequent isolations were made in farmed koi in the USA and in feral common carp in the USA and Canada. Surveillance programs identified this subgroup on farms in northern China. Subtyping and epidemiological studies of Subgroup Ia isolates from North America and Europe indicate a link with ornamental fish imports of Chinese origin (Miller et al., 2007).

Few studies demonstrate biological differences between the subgroups. One study showed major differences in virulence between two subgroup Ia isolates (Warg et al., 2007). It may also be noteworthy that most reports of SVC in non-cyprinid species appear to involve subgroups b and c.

The Disease

Clinical signs and lesions. The clinical signs of SVC are not very different from those associated with bacterial septicemias and other systemic infections of carp. Infected fish typically have hemorrhages of the skin, protruding eyes (exophthalmia), abdominal distension (ascites), and a discharge of bloody mucoid material from the vent. Internal signs include petechial hemorrhages of the swim bladder and muscle, ascites, fluid accumulation in the kidney, liver, and spleen, and enteritis characterized by hemorrhage and the accumulation of viscous mucus. Outbreaks of type Ia that occurred in North America did not produce the swim bladder petechia that are commonly found in outbreaks of Genogroup Id in Europe (Fijan, 1999; Goodwin, 2002; Dikkeboom et al., 2004).

Temperatures and seasons. In typical outbreaks, SVCV spreads horizontally during the winter when water temperatures are low and host immune systems are less active. When spring

approaches and temperatures rise toward 10°C, fish develop clinical signs of SVC and begin to die. The peak mortality for SVC occurs at 15-17°C. Above 20°C, most fish are able to develop immunity to the virus and mortality ceases. Fish also develop SVC in the fall if naive populations are exposed at temperatures in the right range for virus transmission and disease. The virus has not been isolated from infected fish populations following periods in water temperatures of 25-30°C. In experimental infections, immunosuppression triggered by cold temperatures is critical to the development of SVC. The temperature required to produce immunosuppression is apparently related to the thermal optimum of the infected fish species. It is not known if stressing coolwater fish with temperatures above the optimal for the fish but below the maximum for virus replication in culture induces clinical SVC (Fijan, 1999; Ahne et al., 2002; Goodwin and Winton, 2004).

Most reported losses from SVC have occurred in farmed carp during their first winter and spring. Little mortality has been reported in older fish in Europe, but this may be due to immunity in older fish previously exposed to the virus. The first outbreak reported in Wisconsin occurred in large mature carp, perhaps indicating their naive status. Reports of losses of wild fish from Genogroup Id are rare in Europe, however, at least two major kills of common carp in the USA are attributed to SVC. One in Cedar Lake Wisconsin involved more than 10,000 kg of fish (Ahne et al., 2002; Dikkeboom et al., 2004).

Transmission. While SVCV has been isolated from fish lice (*Argulus* sp.) and leeches (*Pisicola* sp.) feeding on infected fish, there is no evidence that these mechanical vectors are important in the spread of SVCV (Ahne, 1985; Ahne et al., 2002). It has not been shown that vertical transmission is a significant mechanism for the spread of SVCV. However, there is one report of the virus being found in ovarian fluid from a population of common carp broodfish tested on a farm in Europe with a history of SVC (Bekesi and Csontos, 1985). Cell culture during the heat of the summer, testing thousands of fish of a koi population that experienced acute spring mortality from SVC, did not detect the virus. The virus may be present during the summer in the ovaries of SVCV survivors but at a level and prevalence so low that detection is unlikely.

Many publications describe vaccines for SVCV that work well in laboratory settings, including DNA vaccines (Kanellos et al., 2006). Killed SVCV vaccines have historically been used in cultured carp in eastern Europe (Ellis, 1988). However, vaccination of farmed fish for SVCV is not a common practice (Fijan, 1999).

Hosts. Natural infections have occurred in bighead carp, common carp, crucian carp, goldfish, koi, pike, silver carp, tench, and the wells catfish (sheatfish). Experimental infections produced disease or demonstrated viral replication in golden shiners, grass carp, guppies, pike, pumpkinseeds, roach, zebra danios, and fruit flies (OIE, 2006). The outbreak in Wels catfish occurred in very young fish crowded in a hatchery setting. Early confusion regarding rhabdovirus taxonomy may have led to some confusion between SVCV and PFRV infections in pike, but more recent studies show that pike are a host for SVCV. There is a single report of SVCV isolation from Penaeid shrimp (Johnson et al., 1999) and one from trout (Jeremic et al., 2006). Summarizing what is known about host specificity, it is clear that mortality is most likely to occur in *Cyprinus carpio* and that Asian carps may also develop the disease. However, under stressful environmental conditions or impaired immune status, a very taxonomically diverse group of fish species may replicate the virus and even die of the disease. An example of this is a report showing mortality in zebrafish (*Danio rerio*) exposed to SVCV at the lower range of the fish's temperature tolerance (Sanders et al., 2003).

Diagnosis

In both the OIE Manual and the American Fisheries Society Fish Health Blue Book, the method of choice for detecting SVCV is cell culture. The SVC virus grows best in fat head minnow (FHM) and epithelioma papulosum cyprini (EPC) cells inoculated with homogenates of spleen, kidney, and encephalon. Cultures are incubated at 20°C and cytopathic effects may be evident in as little as

48 h in cases of acute disease. A blind passage is required if a fish population is to be officially proven negative. The largest difficulty with SVCV is selection of an appropriate confirmatory test.

Published papers and the OIE Manual describe a number of confirmatory tests for identifying SVCV isolated in cell culture. However, the taxonomic diversity within Genogroup I can lead to false negatives and the similarities between Genogroup I and the other rhabodiviris genogroups has led to false positives. Monoclonal antibodies raised against the European Genogroup Id often fail to react to the Asian Genogroup Ia. Likewise, polyclonal antibodies raised against Genogroup Id often cross-react with Genogroup III (Ahne et al., 2002; Dixon and Longshaw, 2005). Due to these constraints, the current method of choice is polymerase chain reaction (PCR), but this approach must also deal with these same challenges. While specific primers for Genogroups Ia and Id have been developed, the OIE approach is to use degenerate primers that amplify all of Genogroup I and then confirm the identity of the isolate by sequencing the PCR product and identifying its appropriate placement among the rhabdovirus genogroups. Obviously, this requires access to PCR and DNA sequencing technology, a database of rhabdovirus sequences, and appropriate software and expertise for the construction and interpretation of taxonomic trees. Given that SVCV is an OIE reportable disease, it is recommended that all suspect isolates involving new hosts, geographic ranges, or previously negative zones or compartments be confirmed by the OIE SVCV Reference Laboratory (CEFAS, Weymouth, UK) or other national reference laboratory with specific expertise in the identification of SVCV.

Differential diagnosis of SVCV vs KHV. When the first outbreak of SVC in North America occurred (Goodwin, 2002), it triggered a great deal of concern among koi hobbyists and fish diagnosticians. Given the non-specific nature of SVC clinical signs, diseases caused by other pathogens, parasites, and water quality problems are often confused with SVCV. Of these, the most problematic are infections by koi herpesvirus (KHV). The following factors are helpful in distinguishing suspect SVC cases from KHV and other fish health problems.

SVCV vs KHV, temperature – Check the water temperature that prevailed when the first losses occurred. Disease from KHV typically occurs when water temperatures are in the range of 21-26°C and peaks are about 23°C. SVC occurs at significantly lower temperatures. Deaths from SVC usually occur at 5-18°C with the peak at about 16°C (Fijan, 1999). Fall koi losses that occur when water temperatures drop into the mid-twenties are much more likely to be KHV. Spring losses that occur as water temperatures warm to 10-18°C might be SVC.

SVCV vs KHV, gross clinical signs – SVC produces clinical signs typical of those seen with many septicemias. These include exophthalmia, petechial hemorrhage of the skin (especially ventral), abdominal distention, and bloody mucus trailing from the vent or easily expressed by slight pressure (Fig. 1). KHV produces few grossly visible clinical signs other than focally necrotic gill lesions that bear some resemblance to columnaris disease (Hedrick et al., 2000; Fig. 2).

SVCV vs KHV, gross internal signs – European strains of SVCV produce petechial hemorrhages of the muscle, swim bladder, and peritoneum (Fijan, 1999). These signs do not seem to be as common in Asian-related strains isolated in the USA. Both European and Asian-related strains produce ascites, edema of the internal organs (especially the posterior kidney), and bloody catarrhal inflammation of the intestine. Fish with KHV have few grossly visible internal signs.

SVC vs bacterial infections – Bacterial septicemias (especially motile aeromonads) produce clinical signs similar to those seen with SVC. However, most koi bacterial infections (other than the atypical *Aeromonas salmonicida* associated with ulcer disease) are unlikely to occur at the low temperature favored by SVC. If it looks like septicemia, but water temperatures have been in the 5-15°C range, suspect SVC. In addition, if the koi owner already unsuccessfully treated the fish with appropriate doses of powerful systemic antibiotics (a very common occurrence), a diagnosis of bacterial septicemia is less likely.

KHV vs bacterial infections – The bacterial infection that most resembles KHV is focal columnaris infection of the gills. This can easily be ruled out by looking for characteristic flexing, long,

Fig. 1. Exophthalmia and petecchial hemorrhages on a common carp experimentally infected with spring viremia of carp virus (SVCV).

Fig. 2. Gill lesions in koi and common carp experimentally infected with koi herpesvirus (KHV).

rod-shaped bacteria in haystacks or floating against the cover glass. The absence of these bacteria rules out columnaris disease. The presence of these bacteria does not rule out KHV because they may be secondary invaders of KHV gill lesions.

SVCV vs parasites – Common protozoan infections of koi may cause damage to the skin and gills with many of the same external signs as SVC. A notable exception is that the parasites are unlikely to cause bloody catarrhal inflammation of the intestine that occurs in a high percentage of SVCV-infected fish. Of course, external parasites can easily be ruled out by microscopic examination, but non-professionals with substandard microscopes may not be able to detect *Ichthyobodo.*

Water quality vs virus – Water quality problems can quickly and easily be ruled out with suitable kits. Acute outbreaks of KHV may produce patterns of mortality resembling those usually associated with water quality problems, however, gill damage from water quality problems is much more diffuse than typical KHV lesions. Poor water quality may predispose fish to bacterial infections that resemble SVCV (see SVC vs bacterial infections, above)

Fish size and age factors – These are not useful indications of SVC or KHV infection. In European fish farms, most outbreaks occur in naive young of the year carp. However, the outbreak of SVCV that occurred in feral carp in Wisconsin, USA, killed large mature fish, demonstrating that naive populations are vulnerable at any age. KHV kills all ages of koi with very high mortality.

The Future of SVCV

The SVCV Genogroup Id has a long history in Europe where it clearly causes high mortality and has the ability to effectively spread to new areas with movements of common carp and other susceptible species. The only significant fish kills attributed to Genotype Id occurred on fish farms or in water bodies adjacent to farms with acute SVC outbreaks (personal communications with Drs. Nikola Fijan, Igor Shchelkunov, and Barry Hill). The geographic boundaries of SVC disease are clearly limited by temperature with all cases occurring in temperate climates, most cool enough to support populations of salmonids in nearby streams.

The picture with Genogroup Ia (Asian) is much less clear. While no kills attributable to SVCV have been reported in China, two large kills of common carp have occurred in the USA. Common carp are exotic to the USA and these large epizootics may be occurring where there are dense populations of feral carp. However, these kills in wild populations may indicate a significant difference between the Ia and Id genogroups. The virus seems to be established in the upper Mississippi River and Great Lakes, but there has been no further evidence of the virus in wild populations surrounding the more southern isolations in Missouri and North Carolina.

Genogroup Ia outbreaks in cultured fishes have occurred in regions of the USA that are much farther south than those in which Genogroup Id outbreaks have occurred, but the outbreak in North Carolina occurred on a farm adjacent to mountain streams that sustain trout and the outbreak in Missouri occurred during the winter immediately following the introduction of fish from the northern USA. Thus, neither case seems to contradict our understanding of the temperature limitations of SVCV.

Because there is little potential for the translocation of Genogroup Id to Asia, the biggest SVCV threat to Asian aquaculture is Genogroup Ia. This pathogen is present in Asia and clearly has the potential to produce significant mortality in cultured common carp and probably other cyprinid species including the economically important bighead and silver carps (Shchelkunov and Shchelkunova, 1989). There are three scenarios of major concern, (a) the establishment of SVCV in regions of Asia with temperate climates, (b) sporadic losses of fish during cool seasons in sub-tropical climates, and (c) regulatory impacts from any detection of SVCV, even in tropical climates where the pathogen is unlikely to cause disease. Based on our experience in Europe and North America, the biggest threat for the movement of this pathogen is koi coming from colder climates where the pathogen may be permanently established.

References

Ahne W., 1985. *Argulus foliaceus* l. and *Philometra geometra* l. as mechanical vectors of spring viraemia of carp virus (SVCV). *J. Fish Dis.*, 8:241-242.

Ahne W., Bjorklund H.V., Essbauer S., Fijan N., Kurath G. and J.R. Winton, 2002. Spring viremia of carp (SVC). *Dis. Aquat. Org.*, 52:261-272.

Bekesi L. and L. Csontos, 1985. Isolation of spring viraemia of carp virus from asymptomatic broodstock carp, *Cyprinus carpio* L. *J. Fish Dis.*, 8:471-472.

Dikkeboom A.L., Radi C., Toohey-Kurth K., Marcquenski S., Engel M., Goodwin A.E., Way K., Stone D.M. and C. Longshaw, 2004. First report of spring viremia of carp virus (SVCV) in wild common carp in North America. *J. Aquat. Anim. Health*, 16(4):169-178

Dixon P.F. and C.B. Longshaw, 2005. Assessment of commercial test kits for identification of spring viraemia of carp virus. *Dis. Aquat. Org.*, 67:25-29.

Ellis A.E., 1988. Current aspects of fish vaccination. *Dis. Aquat. Org.*, 4:159-164.

Fijan N., 1999. Spring viraemia of carp and other viral diseases and agents of warmwater fish. pp. 177-244. In: P.T.K. Woo, D.W. Bruno (eds.). *Fish Diseases and Disorders, Vol. 3: Viral, Bacterial and Fungal Infections.* CAB Int., London.

Fijan N., Petrinec Z., Sulimanovic D. and L.O. Zwillenberg, 1971. Isolation of the viral causative agent from the acute form of infectious dropsy of carp. *Veterinarski Archiv,* 41:125-138.

Garver K.A., Dwilow A.G., Richard J., Booth T.F., Beniac D.R. and B.W. Souter, 2007. First detection and confirmation of spring viraemia of carp virus in common carp, *Cyprinus carpio* L., from Hamilton Harbour, Lake Ontario, Canada. *J. Fish Dis.,* 30:665-671.

Goodwin A.E., 2002. First report of spring viremia of carp virus (SVCV) in North America. *J. Aquat. Anim. Health*, 14:161-164.

Goodwin A.E. and J.R. Winton, 2004. Spring viremia of carp. In: *FHS Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens*, 2007 ed. Am. Fish. Soc. - Fish Health Section (AFS-FHS), Bethesda, Maryland.

Hedrick R.P., Gilad O., Yun S., Spangenberg J.V., Marty G.D., Nordhausen R.W., Kebus M.J., Bercovier H. and A. Eldar, 2000. A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. *J. Aquat. Anim. Health*, 12(1):44-57.

ICTVdB Management, 2006. 01. Mononegavirales. In: C. Buchen-Osmond (ed.). *ICTVdB - The Universal Virus Database, version 3.* Columbia Univ., New York.

Jeremic S., Ivetic V. and V. Radosavljevic, 2006. *Rhabdovirus carpio* as a causative agent of disease in rainbow trout (*Oncorhynchus mykiss* - Walbaum). *Acta Vet.(Beograd)*, 56:553-558.

Johnson M.C., Maxwell J.M., Loh P.C. and J.S. Leong, 1999. Molecular characterization of the glycoproteins from two warm water rhabdoviruses: snakehead rhabdovirus (SHRV) and rhabdovirus of penaeid shrimp (RPS)/spring viremia of carp virus (SVCV). *Virus Res.*, 64:95-106.

Kanellos T., Sylvester I.D., Mello D.F., Howard C., Colin R., Mackie A., Dixon P.F., Chang K.C., Ramstad A., Anne W., Midtlyng P.J. and P.H Russell, 2006. DNA vaccination can protect *Cyprinus carpio* against spring viraemia of carp virus. *Vaccine*, 24(23):4927-4933.

Liu H., Gao L., Shi X., Gu T., Jiang Y. and H. Chen, 2004. Isolation of spring viraemia of carp virus (SVCV) from cultured koi (*Cyprinus carpio koi*) and common carp (*Cyprinus carpio carpio*) in P.R. China. *Bull. Eur. Assoc. Fish Pathol.*, 24:194-202.

Miller O., Fuller F.J., Gebreyes W.A., Lewbart G.A., Shchelkunov A.S., Shivappa R.B., Joiner C., Woolford G., Stone D.M., Dixon P.F., Raley M.E. and J.F. Levine, 2007. Phylogenetic analysis of spring viremia of carp virus reveals distinct subgroups with common origins for recent isolates in North America and the UK. *Dis. Aquat. Org.,* 76:193-204.

OIE, 2006. Spring viremia of carp. In: *Diagnostic Manual for Aquatic Animal Diseases,* 5th ed. World Organization for Animal Health, Paris. http://www.oie.int/eng/normes/fmanual/A_summ-ry.htm.

Sanders G.E., Batts W.N. and J.R. Winton, 2003. Susceptibility of zebrafish (*Danio rerio*) to a model pathogen, spring viremia of carp virus. *Comp. Med.*, 53:14-21.

Shchelkunov I.S. and T.I. Shchelkunova, 1989. *Rhabdovirus carpio* in herbivorous fishes: isolation, pathology and comparative susceptibility of fishes. pp. 333-348. In: W. Ahne and E. Kurstak (eds.). *Viruses of Lower Vertebrates.* Springer-Verlag, New York.

Sheppard A.M., Le Duff R.M., Martin P.D., Woolford G., Way K. and D.M. Stone, 2007.

Genotyping spring viraemia of carp virus and other piscine vesiculo-like viruses using reverse hybridization. *Dis. Aquat. Org.*, 76:163-168.

Stone D.M., Ahne W., Denham K.L., Dixon P.F., Liu C.T.Y., Sheppard A.M., Taylor G.R. and K. Way, 2003. Nucleotide sequence analysis of the glycoprotein gene of putative-spring viraemia of carp virus and pike fry rhabdovirus isolates reveals four genogroups. *Dis. Aquat. Org.*, 53:203-210.

Teng Y., Liu H., Lv J.Q., Fan W.H., Zhang Q.Y. and Q.W. Qin, 2007. Characterization of complete genome sequence of the spring viremia of carp virus isolated from common carp (*Cyprinus carpio*) in China. *Arch. Virol.,* 152:1457-1465.

Warg J.V., Dikkeboom A.L., Goodwin A.E., Snekvik K. and J. Whitney, 2007. Comparison of multiple genes of spring viremia of carp viruses isolated in the United States. *Virus Genes,* 53:87-89.