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Risk Analysis on Spring Viraemia of Carp from Imported Cyprinidae

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Abstract

Spring Viraemia of Carp (SVC), also known as catfish infectious ascites, is an acute, hemorrhagic infectious septicaemia. The disease can harm salmon, catfish, *Carassius auratus*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, etc., and is popular in both Europe and Asia. SVC is the first type of quarantine target for fish port quarantine. The World Organization for Animal Health (OIE) lists SVC as a disease that needs to be declared, and China's Ministry of Agriculture defines it as a class of animal disease. In order to avoid the risk of introduction of IHN due to the introduction of fingerlings, and provide decision-making departments with scientific decision-making data, this paper conducts a systematic risk analysis of SVC from risk assessment, risk management, and risk communication.

Keywords

Spring Viraemia of Carp (SVC), Carp Fish, Risk Analysis

1. Hazard Determination

1.1. Pathogens

Spring viraemia of carp virus (SVCV), also known as rheumatoid virus, is tentatively listed by the International Viral Classification Commission in its seventh report in 2000 as a provisional membership of Rhabdoviridae and Vesiculovirus genus [1]. The Rhabdoviridae family is divided into 3 genera: Lyssavirus, vesicular virus genus, and plant rhabdovirus group. There are currently five rhabdoviruses that cause important fish diseases. The RNA and protein composition are basically similar to mammalian rhabdoviruses. Among them, protein profiles of members of Hunchun virus and pikefry rhabdovirus (PFRV) and vesicular stomatitis virus are similar. It is classified as a tentative member of the vesicular

stomatitis virus genus; viral hemorrhagic septicemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV) and salmon rhabdovirus. Under electron microscope, SVCV exhibits a typical elastic shape of vertebrate rhabdoviruses. The virus particles are approximately 80 - 180 nm in length, approximately 60 - 90 nm in diameter, and have a spiral symmetric core shell with a diameter of approximately 50 nm body. The genome of the virus is a linear, single-stranded, negative-stranded RNA with no segments. The buoyant density of SVCV in lanthanum chloride is 1.195 to 1.200 g/mL. At pH 3 and 12, fat solvent and heat (56˚C) can destroy the infectivity of virus particles, 3% formalin, 0.01% organic iodine, 2% NaOH, and UV (254 nm) can inactivate virus. Repeated freeze-thaw can destroy the partial activity of the virus. Adding 2% to 10% of bovine serum during lyophilization can protect its activity. The SVCV viral genome is a linear single-stranded negative-stranded RNA with a sedimentation coefficient of 38 to 40 s in a sucrose gradient of 5% to 20%. The SVCV genomic RNA is about 11,019 bp in length and contains five open reading frames (ORFs) encoding the five major structural proteins of SVCV. From the 3' to 5' ends of the genomic RNA, they are nucleoproteins in turn: (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA polymerase (L). In 1984, Kiuchi and some other people first studied the characteristics of the SVCV viral genome and published the M protein gene sequence and a base sequence that is located at the 3' end of the genomic RNA and is approximately 70 bp in length. From 1984 to 1994, the study of the SVCV genome was almost in a stagnant state. From 1995 to 1996, Bjorklund *et al.* published the partial sequence of the L protein gene, the full sequence of the G protein gene, and the sequence at the intergenic junction site. In 2001, Bjorklund *et al.* and Hoffman *et al.* published the complete genome sequence of the SVCV reference strain VR-1390, respectively. The sequence numbers in GenBank were U18101 and AJ318079, respectively [1].

1.2. Epidemiology

1.2.1. Historical Geographical Distribution and Current Status

The geographic scope of SVC has only been confined to the high latitude of European continent where water temperature is low in winter. By and by, SVC infection has been reported in most European countries and several member states of the former Soviet Union close to Europe. Now SVC has also been reported in more than 30 countries including Austria, Bulgaria, Croatia, the Czech Republic, France, Germany, the United Kingdom, Hungary, Italy, Israel, Poland, Romania, Slovakia, Spain, and Russia. In 1998, a goldfish in a lake in Brazil was reported to be infected with SVC. In April 2002, an outbreak of 135,000 Koi was reported in a farm in North Carolina, USA, with an incidence rate of 10%. In June of the same year, wild salmon in Wisconsin of the United States was also heavily affected. In October 2002, the United States confirmed that both epidemic pathogens were SVCV. In 2006, there were reports of SVC infection in Canada. The earliest reports of SVC in China were in 1998. At that time, the SVC virus was discovered in Britain from goldfish and Koi that were exported from Beijing.

According to the requirements of OIE's operation manual, the investigation team took samples from fisheries that had recently purchased goldfish for export. They brought 582 goldfish samples back to the laboratory for SVC virus isolation, and collected blood from the heart of the larger 70 fishes and extracted serum detection antibody according to conventional methods. Results: No CPE was produced after all goldfish samples were inoculated into FHM cells; no anti-SVC neutralizing antibodies were found in all serum samples; the positive and negative controls in the experiment were normal, indicating that the test results were reliable. The British side was satisfied with the investigation report and considerer it credible. After investigation, the Beijing area is designated as a "monitoring area" and fisheries that have been monitored for more than two years are allowed to export ornamental fish. In 2001, after two consecutive years of monitoring, the British side agreed to withdraw the "monitoring area." Usually, people think that it is an endemic in cool region, since the internationally known SVC epidemic areas are distributed in the area north of 35 degrees north latitude (equivalent to north of Zhengzhou, China), but it appears that the disease has spread southwards in China's isolation and monitoring of the virus trend [2].

1.2.2. Susceptible Animals

Carp-like carpio (Cyprinus carpio Carpio), carpio Koi, Carassius carassius, Hypophthalmichthysmolitrix, Aristichthys Nobilis, white amur, goldfish (Carassius auratus), Leuciscus idus, and Tinca tinca can be infected by SVC under natural conditions. In addition to carps, SVCV also infects non-carpodidae such as silurus glanis, rainbow trout (Oncorhynchus mykiss), and white pekin (Esox lucius). In laboratory infection conditions, there are other carp species that are sensitive to SVCV, including Rutilusrutilus and Danio rerio, and it can be concluded that other species of carp are also susceptible to SVCV. Some species are susceptible to experimental conditions. For example, Lebistes reticulatus, Lepomis gibbosus [3]. However, hybrids are not sensitive to SVCV. SVCV can infect salmon of all ages, younger fish being more susceptible.

1.2.3. Modes of Transmission and Sources of Infection

Diseased fish, dead fish, contaminated water, and nets are the main sources of horizontal transmission. In the artificial infection test, 100% of the infections were dead and only 20% were soaked. This shows that trauma is an important route of transmission. The mortality rate is not so high under natural conditions. The incubation period of the disease is about 20 days. The virus can invade the fish from the gill or digestive tract. It can also spread through parasite invertebrate warts, ticks or eggs, spread through contaminants, and finally pass through the feces, and urinary excretion. The fish remaining after SVCV infection will have a strong immune protection and circulating antibodies will become asymptomatic carriers of the virus. The incubation period after SVCV infection depends not only on the water temperature but also on the status of the carp it-

self. Onset salmon, post-mortem rehabilitation salmon, wild or farmed salmon can all become SVCV storage hosts. Fish gills, otters, fish-eating birds (such as herons) and aquatic arthropods can all become biological media for SVCV transmission [4]. SVCV-infected salmon can excrete viruses via feces and urine, and viruses that are excreted in vitro can maintain infection activity in water for more than 4 weeks, and can maintain infection activity in mud at 4˚C to 10˚C for more than 6 weeks. In addition, equipment contaminated with SVCV can also be a source of infection. In summary, SVCV is mainly horizontal and vertical transmission is not the main route of SVCV infection.

1.3. Clinical Symptoms and Latency, Latent Infection

SVCV infection is lethal. It destroys the balance of water and salt in fish. It is clinically manifested as edema and hemorrhage. When the diseased fish has a dominant infection, the kidney, spleen, sputum, and brain contain large amounts of virus. Water temperature is a key environmental factor for SVCV infection. SVCV mainly infects carp species at a water temperature of 10˚C to 15˚C, among which juvenile fish species are susceptible. Scarlet fish naturally infected with SVCV initially showed diseased fish tending to flow. The dead fish had dark body color, slow breathing, abdominal distension, side-swimming and swimming dyskinesia, spotting of blood spots in the skin, ankles and eyes, inflammation of the anus, and edema. The crotch is pale. The common symptoms of necropsy are: hemorrhoids, edema of internal organs, ascites, and catarrhal enteritis. Subsequent bacterial or parasitic infections increase the mortality of diseased salmon. Few fry and adult fish rarely develop dominant infections above 17˚C. In addition to the direct relationship between water temperature and the outbreak of SVC, it is related to the age, stocking density, and environmental conditions of carp. After catching SVCV, the carp tends to accumulate at the water inlet. The parade and the response to sensory stimulation are slow, and the respiratory intensity is weakened. Before the death, the symptoms are depression, rest and body tilt.

1.4. Diagnosis

1.4.1. Initial Diagnosis

The dying squid infected with SVCV has obvious clinical symptoms, such as skin bleeding, pale sputum and ascites. Changes in necropsy mainly include enteritis, peritonitis, edema, and bleeding from fish gills, muscles, and other internal organs. A preliminary diagnosis can be made by the clinical symptoms and necropsy changes, and final diagnosis requires virological examination after sampling.

1.4.2. Virus Isolation and Identification

Currently, there are two cell lines recommended by OIE for the isolation of SVCV: salmon epithelial neoplasia (EPC) cells and fat mantle cells (FHM). The cytopathogenic effect (CPE) produced after cell inoculation with SVCV is cha-

racterized by cell rounding, lysis and marginalization of nuclear chromatin. The time of appearance of CPE depends on the temperature at which the virus is infected and the cell line used. When CPE is observed in sensitive cells, virus identification should be performed immediately. Identification methods include neutralization test, IFAT, ELISA or RT-PCR.

1.4.3. Foreign Standards

The diagnostic procedures recommended by the OIE's Diagnostic Manual for Aquatic Animals include immunohistochemistry, fluorescent antibody technology, cell culture, ELISA and PCR techniques.

1.4.4. Domestic Standard

SN/T 1155-2002 Industrial Standard for Entry-Exit Inspection and Quarantine of the People's Republic of China, "Hypovirus Virusemia Virus (SVCV) Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Detection Method", released on November, 25, 2002. SN/T 1155-2011 The Industrial Standard for Entry-Exit Inspection and Quarantine of the People's Republic of China "Qinchun Viralemia Quarantine Technical Specifications" was issued on May 1, 2011 and implemented on July 1. GB/T 15805.5-2008 People's Republic of China National Standard" Fish Quarantine Methods Part 5: Hirsch Viralemia Virus (SVCV)" was officially released on July 31, 2008.

1.5. Prevention and Control (Immunization, Monitoring, Disinfection, Restriction, Destruction, Medication, etc.)

When the water temperature is above 20˚C, the squid body can produce higher levels of interferon and antibodies to withstand the SVCV attack. Therefore, to date, there have been no reports of SVC outbreaks in the tropics and subtropics. Common comprehensive preventive measures include the following: Prevent water pollution, disinfect ponds and fingerlings before fish release, cultivate disease-resistant fish species, ensure the quality of feed, reasonably feed baits, and control water temperature and dissolved oxygen, pH and other physical and chemical factors. Small salmon farms can avoid the outbreak of SVC by controlling the water temperature, while large-scale farms are difficult to control due to the surrounding environmental conditions, and general control measures are difficult to achieve. At present, seedlings with SVCV resistance have not yet been cultivated by selection, hybridization and genetics techniques. Inactivated vaccines and attenuated vaccines can produce certain protection after transvaginal or intraperitoneal injection, but there are still many deficiencies, such as high cost, poor protection, and low safety. The SVCV inactivated vaccine was launched in Europe in the early 1980s and the SVCV attenuated vaccine was successfully developed. Regardless of the type of vaccine, fish should be immunized when the water temperature is above 19˚C - 20˚C. Emerging DNA vaccines have many advantages over traditional vaccines. They are relatively inexpensive and easy to produce and store. Multivalent vaccines can be produced by

mixing multiple plasmids together or inserting multiple genes encoding antigens into one Plasmid were constructed on vectors. Moreover, the DNA vaccine can stimulate the body to produce strong and lasting humeral and cellular immune responses without the need for booster immunization. In the aquaculture industry, DNA vaccines against VHSV and IHNV have shown promising applications, but no SVCV nucleic acid vaccine has been reported to date. Ahne and some people believe that more studies are needed in the future to establish models and standards for the development of aqua vaccines [5]. The future development criteria for SVCV vaccines (including live, inactivated, subunit, and nucleic acid vaccines) should depend on the vaccine's effectiveness, safety, availability of local regulations and compliance with local epidemiological conditions.

2. Risk Assessment

Assessment of the possibility of the introduction, colonization and transmission of pathogens with imported animals or animal products The Hunchun virus mainly infects carp species. Once the outbreak mortality rate is high, it can cause huge losses. China is a large country of ornamental fish breeding, export, and import. International trade is more and more frequent and trade volume is large. Each year, China's ornamental fish trade volume is about 200 million U.S. dollars. Hunchun virus is mainly spread horizontally. The fish that are infected and survive will become asymptomatic carriers of the virus. The possibility of the virus being introduced, colonized, and spread with imported fish is very high.

Consequence Assessment. The key issue now is that the SVC has a high mortality rate when it is popular in foreign countries, but there has not been a large-scale outbreak in China. Preliminary studies have shown that it is not fully adapted between the virus and the Chinese salmon strain. According to monitoring data in recent years, SVCV may be mutating and become more and more adaptable to Chinese cultured species, making it an SVC susceptibility species. If we do not take effective measures now, let SVC's epidemic go unchecked and the outbreak of the outbreak will only happen sooner or later. The consequences will be unthinkable. According to the statistics of the Ministry of Agriculture, the production of carp aquaculture in China accounts for more than 20% of the freshwater yield, and the carp species accounts for more than 50% of the freshwater aquaculture production. Once a large-scale outbreak happens, it is likely to have a devastating effect on the nation's salmon farming industry, much worse than the impact of white spot disease on shrimp.

Carangid fish is a traditional and popular cultured species in many provinces and cities in China. It has large aquaculture production and is a delicious food on people's table. Ornamental goldfish is a characteristic breeding breed in China and is the main force of recreational fishery. It not only beautifies people's lives, but also inherits Chinese culture. However, with the expansion of the scale of farming, various infectious diseases continue to occur and spread, often causing huge economic losses. Hunchun virus is one of the most dangerous infec-

tious diseases of carp. The disease has an acute morbidity, high mortality rate, juvenile fish and adult fishes may be infected, causing great harm. Even the infected fish survived, they can became chronically infected and showed a latent infection. Its host range is wide, vertically and horizontally, and pathogens are easy to spread. At present, rapid and accurate diagnosis of this epidemic can be conducted at home and abroad, but there is no effective treatment. Therefore, we must attach great importance to the prevention and supervision of this epidemic.

3. Risk Management

3.1. Overview of Current Laws and Regulations in China

The new version of the "Category of Category I, II and III Animal Diseases" issued by the Ministry of Agriculture at the end of 2008 classified SVC as a category I animal disease. Article 31 of these regulations specifies the control and extinguishing measures that should be taken when a type 1 animal epidemic occurs.

3.2. Regulations of the OIE "International Animal Health Code"

The international organizations that control the occurrence and spread of SVC are mainly the World Organization for Animal Health (OIE) and the World Food and Agriculture Organization's Asia Pacific Aquatic Diseases Network Center (NACA). The OIE stipulates that the SVC is one of the fish diseases that must be declared, but according to the OIE's Aquatic Animal Health Code, when the SVC epidemic occurs, its control, disinfection, and destruction are required to be treated in accordance with Class A disease.

3.3. Risk Management Measures

3.3.1. National Conditions

In light of the inevitability and seriousness of the future outbreak of SVC, the Ministry of Agriculture began a large-scale investigation of SVC across the country in 2003 and increased monitoring efforts year by year, which has achieved a phased result. However, there are still many problems that have not been solved. For example, it takes a long time to detect viruses in the prior art; lack of early-warning programs; monitoring coverage is limited; no effective measures to prevent and control the disease in a specific area. The best virus identification method recommended by the OIE standard is an indirect fluorescent antibody test and an ELISA assay based on monoclonal antibodies. However, the detection of SVCV in China can only be confirmed by cytotoxicity and PCR. Although molecular biology techniques including DNA probes and PCR have been developed for the identification of many aquatic animal diseases, the advantages of high sensitivity compared to other diagnostic techniques are often judged by problems or techniques. The sensitivity of the aspects is offset. Moreover, the PCR identification test is quite dependent on the conditions, including

the operation conditions and whether the laboratory was contaminated by the earlier PCR product, and it will lead to false positive results. The methods established on the basis of direct culture and serology are relatively precise and strict. Chinese scholars have been actively conducting research on various aspects of SVC.

3.3.2. Hygienic Requirements for Commodities

SVC can be spread through the cross-border transport of sprout, and can also be transmitted vertically through parent fish spawning. Every year, a large number of parent fish and roe are imported from abroad, and the risk of pathogen introduction through these sprouts is extremely high. Therefore, when imported related sprouts, they must have a full certificate of origin, health certificate and other certificates.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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