# SURVEILLANCE FOR VIRAL HAEMORRHAGIC SEPTICAEMIA

## 1. Purposes of aquatic animal health surveillance

Surveillance is the systematic on-going collection, collation and analysis of information related to animal health and the timely dissemination of information for policy and control actions. Although more details for developing surveillance systems are provided in the OIE *Guide for Aquatic Animal Health Surveillance* and in Chapter 1.4 *Aquatic animal health surveillance* of the *Aquatic Animal Health Code*, this document applies these concepts to a specific viral disease in finfish: viral haemorrhagic septicaemia (VHS).

Surveillance for VHS virus (VHSV) infection can be used to provide data on which claims of disease freedom can be based. It can also be used to describe disease occurrence, and for early detection of emergence or of an outbreak in a population of cultured or wild fishes.

## 2. VHSV transmission in the aquatic environment

### 2.1. Transmission mechanisms

Transmission primarily occurs horizontally through water, with excretion of virus in the urine (Neukirch, 1985). Studies using bioluminescence imaging of live trout infected with recombinant virulent Infectious haematopoietic necrosis virus (IHNV), a virus very similar to VHSV, carrying a reporter gene, elegantly illustrated that virus uptake and multiplication on the base of fish fins was very high (Harmache *et al.,* 2006). This remains to be examined for VHS-infected fish. Smail (1999) found that it was likely that direct excretion of virus from the skin is a source of virus spread from sea fish bearing ulcers. Although only the presence of VHSV and not excretion was shown (Mortensen *et al.,* 1999), studies of wild marine fish showed that VHSV could be isolated from both skin and internal organs of asymptomatic carriers also implicating skin as a tissue for virus excretion. Furthermore, VHSV has been isolated from ulcers from wild sea fish, although the VHSV was not necessarily responsible for the ulcers (reviewed in Skall *et al.,* 2005b).

True vertical transmission is unlikely to occur (Bovo *et al.,* 2005a). Infected spawners can excrete large amounts of virus in ovarian fluid, but the eggs will tend to be free of virus after a few days in clean, flow-through water (Jørgensen, 1970). All life stages of infected fish can excrete virus.

Although no intermediate hosts have been confirmed, insects have been shown to carry IHNV when migrating upstream, and IHNV has been isolated from freshwater leeches and copepods (Mulcahy *et al.,* 1990). Recently, it was also demonstrated that leeches may play an important role in disease transmission of VHSV type IVb as the virus could be isolated from more than 50% of leeches collected from fish in an endemically infected area (Faisal & Schulz, 2009). In view of the large range of susceptible species, no vector fish species has been identified. The virus is expected to be able to multiply in all teleosts. However, clinical signs have never been observed in infected individuals of many fish species.

The reservoirs of VHSV are primarily clinically infected fish as well as covert carriers among cultured, feral or wild fish. Several factors influence susceptibility to VHS disease. In rainbow trout, there is genetic variability for susceptibility (Henryon *et al.,* 2002a; 2002b), and the age of the fish appears to be of some importance, younger fish being more susceptible. In general, older fish experiencing high VHS mortality have not had previous exposure. Pathogenicity varies from minor pathological changes and few mortalities to very severe and high proportional mortality according to the virus isolate and host (Skall *et al.,* 2005b).

### 2.2. Pathogen transmission in aquaculture

In rainbow trout, virus excretion increases exponentially a short time before and peak at the onset of the first clinical signs, i.e. under experimental conditions at 10–12°C virus excretion begins 3–5 days post-infection with a peak 7–8 days post-infection.

High titres of virus can be found in outlet water from infected farms with high mortality (1000 plague-forming units [PFU] $ml^{-1}$ river water), and farms situated downstream using nondisinfected inlet water should be considered as exposed and potentially infected. Outbreaks will appear quite soon in nearby farms but can take up to 1 year when the distance between sites is 40 km or more (Olesen, pers. obs.). In open marine conditions, the virus will spread readily within 1 km. Infection from one farm to another has been observed at a distance of

7 km when the farms are situated in an area with significant stream activity. The VHS eradication programme in Denmark showed that after downstream infection, movement of fish in the nonsymptomatic incubation phase was the most important route of pathogen spread.

Piscivorous birds, such as herons, have been shown to contribute to the spread of virus between farms (Olesen & Vestergård Jørgensen, 1982; Peters & Neukirch, 1986). Spillage of water from vehicles transporting infected fish to slaughter was shown to pose a serious risk for spread. Finally, placement of a fish farm in proximity to a processing plant will increase its risk of contracting VHS (Olesen, pers. obs.; Stone *et al.,* 2008).

### 2.3. Transmission between farmed and wild aquatic animals

The presence of various genotypes of VHSV in various wild fish species poses a constant threat to rainbow trout farming in some regions (Skall *et al.,* 2005b). At least four reported cases of disease outbreaks in fish farms were attributed to wild fish reservoirs, two in turbot in Scotland and Ireland, respectively (Munro, 1996; Ross *et al.,* 1994), one in Sweden with recurrent outbreaks with VHSV genotype Ib isolates (Skall *et al.,* 2005b), and one in Norway with a genotype III isolate (Dale *et al.,* 2009). The first disease outbreaks in Finland in 2000 (Raja-Halli *et al.,* 2006) and Denmark in the 1960s were suspected to have originated from wild fish sources, possibly through feeding raw marine fish (Dixon 1999; Skall *et al.,* 2005b; Stone *et al.,* 1997). Lastly, it is possible that the VHS outbreak in Scottish turbot may have originated from raw minced fish, mainly haddock, used as feed, though virus was not isolated from the remaining feed (Munro, 1996).

In Europe, very few clinical VHS outbreaks have been reported in feral fish; clinical disease is primarily linked to farmed fish. In North America, VHS outbreaks are primarily observed in wild fish in the Great Lakes area (Elsayed *et al.,* 2006; Groocock *et al.,* 2007; Lumsden *et al.,* 2007); outbreaks of genotype IVb are also reported from East Coast marine areas (Gagné *et al.,* 2007). Genotype IVa occurs on the West coast of North America, distinct from East Coast genotypes (reviewed in Meyers & Winton, 1995 and Skall *et al.,* 2005b). In Asia, the virus (mainly genotype IVa) has been observed primarily in Japanese flounder (*Paralichthys olivaceus*) both under wild and farming conditions (Isshiki *et al.,* 2001; Kim *et al.,* 2003; Takano *et al.,* 2000; Watanabe *et al.,* 2002).

VHS (IVa)-infected Pacific herring are believed to transmit the virus to farmed Atlantic salmon on the West Coast of North America, causing outbreaks with low mortalities, when they migrate in close proximity to farms (Amos & Thomas, 2002; Traxler, pers. comm. in Skall *et al.,* 2005b).

### 2.4. Other transmission considerations

Water-borne exposure is the prime cause of disease spread, and all susceptible populations in direct water contact with infected populations should be considered as potentially infected.

Survival of VHS in water and sediments highly depends on temperature and water properties (Bovo *et al.,* 2005b). As an enveloped virus, VHSV is quite susceptible to high temperatures and both chemical and enzymatic degradation. Survival in pond water is short (<7 days). Thus, exposure to other populations through direct contamination of water or transport containers is limited by time. Trace-back of exposed groups can usually rely on more immediate past exposures, however, temperature should always be taken into consideration as virus survival can be prolonged to more than 3 months at temperatures below 5°C.

Wild or feral susceptible fish are considered very important factors for disease transmission. Escaped VHS-infected mature rainbow trout have been shown to spread VHS far upstream during their attempts to reach spawning grounds upriver (Olesen, unpublished obs.). When controlling VHS in fish farms, eliminating or reducing exposure to feral and wild susceptible fish surrounding the farm(s) should be considered.

## 3. Populations

### 3.1. The concept of populations applied to VHSV surveillance

The population structure should consider the following life stages:

i) Fertilised eggs, which can carry the VHS virus externally without being affected: transmission risk can be mitigated with proper disinfection;

ii) Fry, which usually are held in closed units with clean well water, but if infected will reach mortalities of almost 100%;

iii) Fingerlings, which are also highly susceptible and especially vulnerable if transferred to infected waters;

iv) Adults, which are less susceptible.

Experience regarding life-stage susceptibility is primarily derived from rainbow trout farming as observations of young stages of wild fish is lacking and high mortality is rarely detected.

All life stages appear to be susceptible to infection, but covert carriers can be found among larger fish (i.e. older life stages) in endemically infected areas. If no weak or moribund fish with clinical signs are apparent in susceptible ages, larger life stages can be selected for virological examination.

It is uncommon for VHS to occur without clinical signs (i.e. mortalities) in susceptible life stages at a rainbow trout hatchery. Mortalities are obvious and occur very soon after exposure. Hence, describing subsets of the population with common attributes for exposure and disease is not very useful for designing surveillance programmes.

Diseased fish display clinical signs in the early stages of infection, with rapid onset of mortality (which can reach up to 100% in fry), lethargy, darkening of the skin, exophthalmia, anaemia (pale gills), haemorrhages at the base of the fins, gills, eyes and skin, and a distended abdomen due to oedema in the peritoneal cavity. In the chronic state of infection, affected fish do not generally exhibit external signs. VHS can also occur in a nervous form, characterised by severe abnormal swimming behaviour, such as constant flashing and/or spiralling.

Clinical signs are very similar to signs exhibited by other haemorrhagic diseases such as enteric redmouth disease (*Yersinia ruckerii*), furunculosis (*Aeromonas salmonicida*), vibriosis (*Listonella anguillarum*), and infectious salmon anaemia. However, distinct petechial haemorrhages in dorsal muscles are quite characteristic, and the posterior gut is often pale and atonic in contrast to infectious septicaemic infections caused by bacterial infections. Dark, lethargic fish that do not tend to escape while catching them at the outlet or on the side of ponds should also led to suspicion of VHS. When selecting fish for the detection of infections, fish with these clinical signs should be included in samples for diagnostic testing.

### 3.1.1. Susceptible species

It is likely that VHSV is endemic in fish populations in large areas of the temperate Northern Hemisphere. So far, it has been isolated from more than 82 different fish species in North America, Asia and Europe (European Food Safety Authority, 2008). A further 11 fish species have been shown to be susceptible to clinical disease caused by VHSV under experimental conditions. The number of host species is increasing with increasing monitoring efforts. The fish species most susceptible to genotype Ia is rainbow trout, although this VHS genotype was also reported in one case to cause mortality in farmed turbot (*Psetta maxima*) (Schlotfeldt *et al.,* 1991) and in brown trout (*Salmo trutta fario*) (de Kinkelin & Le Berre, 1977). VHSV Ia has also been isolated from pike (*Esox lucius*) causing high mortalities in pike fry (Meier & Jørgensen, 1979), grayling (*Thymallus thymallus*) (Wizigmann *et al.,* 1980) and white fish (*Coregonus lavaretus*) (Meier *et al.,* 1986; Skall *et al.,* 2004).

Severe mortality has been observed in recent years in the Great Lakes region of the USA and Canada involving at least 28 freshwater fish species. All VHSV isolated from these outbreaks belonged to genotype IVb (Thompson *et al.*, 2011).

The most susceptible fish species for a particular genotype should be sampled as the first priority. For example, sampling priority would be rainbow trout in Europe and muskellunge (*Esox masquinongy*) in the Great lakes. In a fish species such as white fish (*Coregonus lavaretus*), which are able to carry the virus without developing disease (Skall *et al.,* 2004), susceptibility is less than for rainbow trout. However, experimental infection has shown that the number of asymptomatic carriers among white fish may be higher than in rainbow trout 3 weeks after infection. In the absence of rainbow trout to sample, whitefish should be considered a suitable alternative.

In Scotland, Ireland and Norway, all Atlantic salmon farms have been monitored routinely for the presence of VHS (and IHN) since 1995 to maintain the status as VHS (and IHN) free areas. As thousands of samples have been tested during these years without a single detection of VHSV in Atlantic salmon, it may be appropriate that the sampling strategy focus on rainbow trout farming and passive surveillance for Atlantic salmon farming in these areas.

Turbot (*Scophthalmus maximus*) have shown to be highly susceptible to VHSV genotype III isolates and should be selected for surveillance in farmed non-salmonid marine fish species, when available. In wild marine fish, fish species such as herring (*Clupea harengus* and *C. pallasii*), Norway pout (*Trisopterus esmarkii*), pilchard (*Sardinops sagax*) and sprat (*Sprattus sprattus*) are likely to have the

highest probability of infection, and these should be included in samples for detection (Mortensen *et al.,* 1999). A highly migratory fish species such as the European eel (*Anguilla anguilla*) has previously been considered as a nonsusceptible species (Jørgensen *et al.,* 1994), with no mortality observed by experimental VHSV infections. However, VHSV has been isolated from eels in both France and Germany (Castric *et al.,* 1992), indicating that this species might carry the virus and contribute to upstream transmission during its migration.

If sentinel species are considered, juvenile rainbow trout should be selected in Europe as the most susceptible age and species for VHSV type I. Sentinel fish were used to assess if continuous rainbow trout production was possible after the first VHS outbreak in a marine fish farm in Sweden in 1999. In 2001, the disease reoccurred in these sentinel fish and thus management changes, such as stocking with fish larger than fingerlings and avoiding wintering of rainbow trout in sea water, were necessary for rainbow trout production.

#### 3.1.2. Disease exposure history

High virus titres can be found in outlet water from infected farms with high mortality, therefore farms situated downstream not using disinfected inlet water should be considered as infected. Outbreaks will appear quite soon in nearby farms. Surveillance activities should consider all fish populations sharing exposure histories, with particular attention to the movement of fish that has occurred during the nonsymptomatic incubation phase.

Piscivorous birds can contribute to the spread of virus between farms (Olesen & Vestergård Jørgensen, 1982; Peters & Neukirch, 1986). Contamination from vehicles transporting infected fish poses a serious risk for spreading the virus. Fish farms in close proximity to processing plants have increased risk of contracting VHS (Stone *et al.,* 2008).

#### 3.1.3. Higher probability samples

Risk-based surveillance should target components of the population with a higher probability of being infected or being infected early after introduction. In fish farms, experience suggests that the first appearance of VHS occurs in:

i) Populations a few days after stocking (if fish were purchased in the VHS incubation phase);

ii) Ponds in the most remote locations where birds can enter without being disturbed; or

iii) Channels just before the effluent outlet (fish in these channels may have been in contact with water from many ponds in the farms).

When designing surveillance for VHS, considerations should account for the fact that older, larger fish can have covert infections with the virus and these infections can transmit to naïve groups of other larger fish, thus increasing the prevalence, or to naïve smaller fish resulting in clinical mortality. This exposure to new groups should be taken into consideration when attempting to identify shared risk among groups.

Weak or moribund fish should be sampled when applying risk-based surveillance. Experience has shown that only one or two fish in an entire farm population may demonstrate clinical signs of disease, and that only these fish were positive for VHS, while the 30 fish randomly collected at the same time were negative (Henrik Korsholm, pers. comm.). Directed sampling of moribund fish is much more efficient at VHS detection than random sampling of apparently healthy fish.

### 3.2. Host factors affecting population definitions

#### 3.2.1. Carrier states

Fish with spiralling and abnormal swimming behaviours, including lack of escape responses when being netted, are more likely to be positive for VHSV compared with clinically healthy fish. Following clinical infection, larger rainbow trout remain infective VHSV carriers capable of shedding virus for extended periods. Virus can often be recovered from these asymptomatic carriers, and a negative test may be falsely negative. Past clinical episodes or positive diagnostic tests at the population level should be considered positive from that time forward, and any trout populations exposed to this positive group should also be considered positive. Serological examination and detection of anti-VHSV antibodies in fish have been used as indicators of previous VHS infection (Schyth *et al.,* 2012).

#### 3.2.2. Factors affecting disease expression

Low environmental temperatures (i.e. less than 5°C) appear to result in prolonged, lower mortality and most disease expression occurs as water temperatures increase or fluctuate widely.

Disease expression is often low at water temperatures greater than16°C and the disease can remain undetected when occurring during summer or in fish produced at higher temperatures (Schyth *et al.,* 2012).

Age and previous exposure affects disease expression. All ages of naïve susceptible species may express clinical disease, but generally younger fish have the highest mortality.

#### 3.2.3. Group-level susceptibility

High fish density may lead to more rapid spread of disease and a higher proportion of mortalities. Post-exposure carriers occur in rainbow trout.

#### 3.2.4. Vaccination

Although vaccination against VHSV is still not commercially available, DNA-based vaccines have shown significant promise for future control strategies.

### 3.3. Epidemiological units

#### 3.3.1. Sharing risk of exposure

Fish from the same farm site will have a similar level of risk of exposure. Risk of exposure within a geographical area or watershed will depend on the distance and potential to share water or wild reservoir species. Proximity to susceptible farming units will influence risk exposure.

Within a farm, fish subgroups (e.g. tanks) that share water through multiple use or recirculation will share exposure risk. It may be possible to define epidemiological units based on water sharing pathways within a farm or series of farms, but in practise, most farms should be considered epidemiological units as biosecurity measures are difficult to maintain at a level that will prevent internal spreading of the disease.

### 3.4. Zones and compartments

Zoning can be applied to VHSV surveillance, particularly in freshwater as it is possible to determine the movement of contaminated water or wild fish reservoirs between farm locations. An example may be exposure below, versus above, a barrier (e.g. dam) where fish above the barrier will not be exposed to water or wild species but fish below may be exposed to water from above. Zoning in marine areas is more problematic due to the migration of wild species and the wide range of hosts susceptible to VHSV.

In areas where VHSV may potentially be present, compartmentalisation is possible for hatcheries and freshwater farms that can be isolated from possible exposure to surface water, wild fish, or birds. Water treatment (e.g. ground water with sterilisation for viral agents) and high biosecurity would be necessary to reduce the likelihood of incursion into the compartment.

#### 3.4.1. Geographical barriers

Although poorly understood, it would appear that certain natural geographical barriers have a strong influence on the localisation of VHSV genotypes. This phenomenon is probably caused by a combination of factors including lack of natural movement of wild host species beyond the extremes of the current VHSV genotype ranges and water current patterns. For example, the Great Lakes watershed is very large, but current patterns appear to localise the genotype IVb to wild populations sharing this geographical location. Therefore, it seems probable that the geographical patterns contributing to this localisation of genotype are sufficient to support zones based on genotype.

As genotypes and subtypes of VHSV are closely related to their geographical location and host, it is crucial not to introduce genotypes to new areas even if the area is not officially recognised as VHS free. For example, the highly rainbow trout pathogenic VHSV genotype Ia would have devastating impact on rainbow trout farming if introduced into the Americas, while the American genotype IVb would severely affect European or Asian fish populations if introduced.

#### 3.4.1. Prevalence zones

A high prevalence of infection will occur in some susceptible populations of carrier fish, such as rainbow trout, if they have previously experienced an outbreak. Naïve fish usually experience high mortalities once exposed. Therefore, the prevalence is expected to be extremely low in populations that have very recent introductions of the pathogen. It is difficult to estimate the prevalence in wild populations in which very few of the clinically affected individuals are observable. For practical purposes, it should be considered extremely low to account for the lack of access to the higher probability individuals (i.e. clinically sick or dead animals).

In farming situations, the prevalence in older populations that are apparently healthy but have been previously exposed is expected to be reasonably high.

# 4. General design considerations

## 4.1. Types of surveillance system

#### 4.1.1. Passive surveillance

Passive surveillance is conducted by fish farmers, veterinarians and consultants (such as feed companies). As the conditions and disease patterns in rainbow trout farming are conducive to clinical disease, passive but vigilant surveillance has proven to be an efficient tool for the detection of VHSV. When the sampling and diagnostic procedures are followed, VHS can be diagnosed within a few days.

Passive surveillance is only applicable in approved disease free or in known infected areas. In free-living fish, passive surveillance can be done by diagnosing and reporting unexplained mortalities.

#### 4.1.2. Active surveillance

Active surveillance comprises programmed health inspections at regular intervals, where all stocks are inspected. Collection of fish for laboratory examination occurs if there is suspicion of a notifiable disease, while targeted surveillance comprises the prerequisite sampling of fish for laboratory examination. Assuming that there are credible barriers to introduction of pathogens to an area, historical test results provide valuable evidence, especially over many years, contributing to the declaration of freedom. This evidence can be as useful as on-going sampling strategies employed over a short period and thus reduce the required sample sizes.

Both active and targeted surveillance have been used for surveillance of the disease in approved VHS-free areas in European fish farming for decades, with credible results. The direct impact of targeted surveillance is however more difficult to judge, as almost all new outbreaks in populations with naïve fish were based on virological examination of fish with clinical signs. During 50 years of VHS surveillance in Denmark, VHSV was only isolated in a couple of cases from clinically healthy fish with no initial suspicion of VHS. Early detection based on targeted surveillance should not be underestimated as VHS is often misinterpreted as other diseases with similar presentations, such as enteric redmouth disease, proliferative kidney disease or bacterial kidney disease. These masked VHS cases would not have been detected if the sampling of fish was not compulsory as it is for targeted surveillance (Olesen, pers. obs.).

## 4.2. Sampling from markets or processing plants

Sampling from processed marketed product for routine disease surveillance is usually not an option, as the origin of fish is difficult to trace and as VHSV will degrade quite rapidly in dead fish because of enzymatic processes, lowering the test sensitivity. However, sampling from processing plants might be an option in cases where on-site surveillance of farms is not possible or difficult to conduct. To increase prevalence, weak or moribund fish should be selected at on-site inspections for laboratory testing. As such fish are seldom identifiable in the processing plant, the sample size should be increased to obtain the same sensitivity and probability of detecting the virus. Recent studies showed, however, that meat and filets from infected fish may contain a high amount of virus (Oidtmann *et al.,* 2011) and it would be likely that virus can be isolated from skin and muscles as well as internal organs.

## 4.3. Questionnaire-based surveillance

Active surveillance using gross clinical signs and questionnaire-based surveys is an option when inspections are performed by trained personnel. Few clinical signs are pathognomic for VHS, increasing the probability of missed detections by non-trained personnel. However, VHS usually causes mortality to some level in some life

stages of susceptible species (e.g. fry stages of rainbow trout), so focusing samples during these mortality episodes will increase the probability of detection.

## 4.4. Case definition

According to the OIE *Aquatic Manual*, the presence of VHSV shall be suspected if at least one of the following criteria is met:

i) The presence of post-mortem findings consistent with VHS, with or without clinical signs of disease.

ii) Cytopathic effect (CPE) in cell cultures before confirmation

iii) Where an investigation reveals epidemiological links to VHS suspected or confirmed farms: detection of antibodies to VHSV in fish

Suspicion of VHS can be ruled out if continued investigations reveal no further evidence of the presence of the pathogen, while the presence of VHSV is considered to be confirmed if, in addition to the criteria in the suspect cases, VHSV isolation is carried out in cell culture followed by virus identification by either an antibody-based test and/or PCR followed by the sequencing of the amplicon.

The tissue material for virological examination may, in some cases, be accompanied by supplementary material for bacteriological, parasitological, histological or other examination to allow for a differential diagnosis.

Four major genotypes of VHSV have been identified, based on sequencing of full-length and/or truncated genes from the N-gene (Einer-Jensen *et al.,* 2004; Snow *et al.,* 1999; 2004), G-gene (Einer-Jensen *et al.,* 2004; 2005) and NV-gene (Einer-Jensen *et al.,* 2005):

Genotype I: Several sublineages (Ia–Ie) containing European freshwater VHSV isolates, isolates from the Black Sea area and a group of marine isolates from the Baltic Sea, Kattegat, Skagerrak, the North Sea and the English Channel

Genotype II: A group of marine isolates from the Baltic Sea

Genotype III: Isolates from the North Atlantic Sea (from the Flemish Cap (López-Vázquez *et al.,* 2006) to the Norwegian coast (Dale *et al.,* 2009), the North Sea, Skagerrak and Kattegat.

Genotype IV: North American and Japanese/Korean isolates (two sublineages IVa and IVb [Elsayed *et al.,* 2006]).

Genotype I is divided into several sublineages, where the marine isolates from wild fish fall into sublineage Ib. The best resolution of genotype I sublineages is obtained when analysing the full-length G-gene (Einer-Jensen *et al.,* 2005).

As genotype I comprises VHSV isolated from wild marine fish, as well as isolates causing mortality in rainbow trout from continental Europe, a relationship between freshwater and marine types was suggested (Skall *et al.,* 2005b).

All the Japanese and other Asian isolates but one falls into the North American IVa genotype. The remaining isolate falls into the traditional European genotype Ib (Nishizawa *et al.,* 2002). This isolate is considered to have been introduced from outside Japan.

In North America, at least two sublineages are found: genotype IVa on the Pacific coast and genotype IVb in the Great Lakes. Isolates found on the Atlantic coast of North America are most related to the IVb group but have tentatively been suggested placed in a subgroup IVc.

Case definition information for VHS positive in rainbow trout:

i) Increased mortality during fry stages, usually greater than 1% per day;

ii) Mortality (with no clinical signs) or signs of septicaemia present in some animals;

iii) Positive tests on at least two fish by two tests, or on at least one fish by viral cell culture.

## 4.5. Surveillance and denominator-based information

VHSV reservoirs are clinically infected fish as well as covert carriers among cultured, feral or wild fish. Several factors influence susceptibility to VHS disease. Genetic variability for susceptibility exists in rainbow trout

(Henryon *et al.,* 2002a; 2002b), and the age of the fish appears to be of some importance: the younger the fish the higher the susceptibility. In general, older fish experiencing high mortality caused by VHS have never been in contact with the disease before. The population at risk, especially in naïve fish, would thus be all sizes and life stages except eggs, as fertilised eggs are not affected by VHSV. Whole farm stocks of susceptible species or population of wild susceptible fish should therefore be considered at risk.

## 5. Diagnostic tests

Information is provided on the characteristics of available diagnostic tests, as they have been assessed in published and unpublished studies.

***Table 5.1.*** *Comparative properties of four methods for VHS diagnosis*

| | **Cell cultivation followed by virus identification** | **RT-PCR (Snow *et al.,* 2004)** | **Real time RT-PCR (Jonstrup *et al.,* 2012)** | **Immunohistochemistry (Evensen *et al.,* 1994)** |
|---|---|---|---|---|
| Diagnostic Se | 0.86 | Approximately the same level as real-time PCR | 0.90 | No data but indicated to be lower than cell cultivation and PCR |
| Diagnostic Sp | 1.0 | No data, false positive reactions observed | 1.0 | No data, false positive staining patterns observed |
| Analytical Se | Approx. 125 $TCID_{50}$ $g^{-1}$ tissue | | Same as cell culture | Higher than histology |
| Analytical Sp | 1 | | 1 | |
| Repeatability | Not relevant | | 1 | |
| Reproducibility | Median $TCID_{50}$ and 50% fractals from inter-laboratory proficiency tests including 25 labs: 1 $log_{10}$ variation in titres (Ariel *et al.,* 2009) | | 1 (1–2 Ct variation) from proficiency test including four laboratories | |
| Advantages | Robust method with high Se and Sp. Virus in hand for further studies and typing | Sensitive method, no need for specialised laboratories. Direct genotyping possible by sequencing amplicon | Fast, robust and reliable with Se and Sp comparable to cell culture. Semi quantitative method | Histopathological changes corroborate staining patterns |
| Disadvantages | Need of highly specialised cell culture facilities. Long time from sampling to test | Specificity problematic due to false positive bands from cell lines. Controls are crucial. No quantitative measures | Need of equipment for real time PCR. No virus in hand. No use of amplicon for genotyping | Low sensitivity – only for clinical cases. No virus in hand for typing |

Ct: threshold cycle; PCR: polymerase chain reaction; Se: sensitivity; Sp: specificity; TCID: tissue culture infective dose.

The standard diagnostic test for surveillance for VHS has been based for decades on direct methods for virus identification, i.e. the isolation of VHSV in cell culture followed by identification using antibody-based methods such as indirect fluorescent antibody test (IFAT) and the enzyme-linked immunosorbent assays (ELISA) or nucleic acid-based methods as reverse transcription (RT)-PCR. This system has proven to be highly sensitive, specific, and suitable for purpose. The direct immunological demonstration of VHSV antigen in infected fish tissues has low sensitivity, and can only be used when VHS infection is suspected (based on clinical signs, epidemiological data and histopathology). Two recently published validated real-time RT-PCRs for direct identification of the VHSV N-genome in fish tissue was found to have sensitivity and specificity values very similar to cell culture methods followed by identification (Garver *et al.,* 2011; Jonstrup *et al.,* 2012) and will, if accepted by OIE Member Countries, be used as an alternative to cell culture in surveillance programmes for achieving VHS-free status.

Detection of VHS-specific antibodies in farmed rainbow trout has been proposed as an alternative or supplement to the currently approved direct methods, as surveys based on serological tests have several advantages, especially in cases where water temperatures are too high for virus isolation and in endemically infected populations where there are no clinical signs of disease and the virus cannot be isolated in cell culture. A disadvantage of serological tests is that antibody cannot be detected earlier than 2 weeks to 3 months after infection, depending on the water temperature of the pond where the fish are cultured, with antibody developing faster at higher temperatures (Schyth *et al.,* 2012).

## 6. Sampling considerations for surveillance

### 6.1. General considerations

Sampling of aquatic animals is more relevant to active surveillance, but can be used in passive surveillance to facilitate outbreak investigations or other instances of increased disease occurrence.

Sampling design depends on the objectives and scope of the study and on the structure of the examined populations. A major distinction is made between studies that aim to estimate occurrence of infection in a population and studies that aim to detect infection or claim that the population is free from infection. In the case of estimating infection occurrence, a representative sample is sought, while in the case of infection detection, sampling may be biased toward detection of the infection or disease (i.e. by selecting animals that are moribund or show clinical signs). Variations in the probability of disease presentation, such as younger fish compared with older fish, should be considered when designing surveys. For example, as infection prevalence is higher in young fish, surveys should be directed at different life stages. If detection of infection is the objective, then the sensitivity of the detection system can be increased by conducting the survey using juvenile life stages in which infection prevalence peaks.

Surveillance of VHS will be based on the presence or absence of the pathogen in a population, farm, compartment or zone. Estimating disease frequency has not been relevant under farming conditions as the disease management strategy, e.g. stamping-out procedures, has been independent of disease frequency. However, in endemic areas, disease frequency and impact studies are relevant to the consideration of such issues as vaccination strategy or use of less susceptible fish species. In wild and feral fish, infection frequency is relevant for investigating clinical outbreaks or for estimating risk of transfer to farms.

Inspection of farms should consist of analysis of records taken on the fish farm, with particular attention to mortality records, to enable an assessment to be made of the health status history of the farm, including all epidemiological units present on the site. If available, a representative selection of recently dead or moribund fish should be examined clinically, both externally and internally, for major pathological changes suggesting VHS. If the outcome or other factors lead to any suspicion of the presence of VHS, fish should be subjected to laboratory examination.

### 6.2. Sample sizes

The structure of the population needs to be considered in the design of the survey and the calculation of sample sizes. For example, the survey may concern only one farm or site, or it may be targeted at multiple sites or an entire region or country. In the latter cases, sampling should be conducted separately at each stage. For example, a sample of regions could be taken from all the regions in the country (sampling unit: the region), next a sample of farms within each selected region (sampling unit: the farm), next a sample of cages, tanks or raceways within each farm (sampling unit: the tank) and finally a sample of animals from each selected tank (sampling unit: the tank). Special attention should be given to the definition of design parameters (e.g. design prevalence, test sensitivity and specificity) at each sampling level. For example, the region-level prevalence would be the proportion of regions in the country that are infected, while the farm-level prevalence would be the proportion of infected farms in a region etc. For more details, examples and calculations, see chapter 1.4 of the *Aquatic Animal Health Code* and the *Guide for Aquatic Animal Health Surveillance*. In the case of VHSV, a collection of units (i.e. the farm) will be classified as infected if at least one of its units is found to be infected. Collection-level test sensitivity and specificity should be defined accordingly.

Optimal sampling size should accommodate special considerations for VHSV infection, such as the fact that VHSV may be detected in only one or two fish in a large populations (e.g. more than 100,000 fish). Random sampling of apparently healthy fish can be extremely inefficient, making efforts to demonstrate freedom difficult in situations for which no prior knowledge of infection prevalence exists. A careful clinical inspection of all ponds and areas of a farm should be conducted and sampling directed at diseased fish. Priority should focus on fish with clinical signs most resembling VHS infection. If other fish species are present, the most susceptible for the area must be chosen (e.g. rainbow trout, turbot, or round goby). If all fish on the farm appear healthy, sample sizes of 30–150 fish (depending on objectives and diagnostic tests chosen) should be collected representing all sizes and areas of the farm, but higher probability samples (e.g. early life stages) should

dominate the investigation. When clinically affected fish are selected, sample sizes of 35 fish are generally sufficient for surveillance. Past history of surveillance, with repeatedly negative tests, is very important to certainty for declarations of freedom. Experience with VHS control and eradication in Denmark has demonstrated that repeated inspections for a prolonged period of time (2–4 years) using selective, risk-based sampling is far more efficient than single inspections with large sample sizes using random selection.

Assuming 0.001 design prevalence (i.e. 1 fish infected per 1000) and sensitivity of 0.9 (RT-PCR estimate of Se), a sample of more than 3300 fish would need to be collected to declare 95% confidence of freedom from infection. As a single survey, this is highly inefficient and underestimates the sample size if the infection is truly as low as 1 fish per 50–100,000. A more efficient method would be to establish multiple sampling events to identify clinically sick fish, which will increase the prevalence (i.e. in the component of the population that is tested) to at least 0.1 and reduce the sample size to 32 clinically sick fish using the same test characteristics. Multiple years of absence of clinical signs of VHSV coupled with testing samples of sick fish with or without VHS signs will provide much greater certainty of infection freedom. Specifics of the situation, such as biosecurity level, are important considerations as past history of pathogen freedom is much more predictive of future pathogen freedom if there is maximal biosecurity preventing new introductions. When the opportunity for pathogen introduction is constantly high, past diagnostic history is virtually worthless for predicting future pathogen status.

Some indicative calculations are included in the following table, using two different diagnostic tests. For prevalence estimation, sample sizes are provided for different levels of required precision (half of the width of the confidence interval) and assumed true population prevalence (0.02, 0.10). Calculations presented are for an infinite population and confidence level of 0.95. For these calculations the online EpiTools epidemiological calculators were used (http://epitools.ausvet.com.au/content.php?page=home). For surveys for demonstrating disease freedom, three different design prevalences were used (0.001 for recent introduction and apparently healthy fish, 0.10 for post-exposure older fish, and 0.80 for diseased fish with clinical signs compatible with VHS) and the probability of type I and type II error were set at 0.05 and a population size of 100,000. No cut-point number of reactors is presented as both tests are assumed to have a specificity of 1, hence no false positives are possible. Calculations were done using the FreeCalc software (Survey Tool Box: AusVet Animal Health Services. Includes FreeCalc – Cameron AR Software for the calculation of sample size and analysis of surveys to demonstrate freedom from disease. Available for free download at: http://www.ausvet.com.au/content.php?page=software).

***Table 6.2.*** *Calculation of fish numbers to collect randomly according to estimates of prevalences in fish populations*

| Objective | Required precision | Assumed true population prevalence | Virus isolation (Se: 0.86; Sp: 1) | RT-PCR (Se: 0.9; Sp: 1) |
|---|---|---|---|---|
| Prevalence estimation | 0.05 | 0.02 | 36 | 34 |
| | 0.02 | | 220 | 210 |
| | 0.01 | | 878 | 839 |
| | 0.05 | 0.05 | 86 | 82 |
| | 0.02 | | 535 | 510 |
| | 0.01 | | 2138 | 2039 |
| | 0.05 | 0.10 | 164 | 156 |
| | 0.02 | | 1021 | 972 |
| | 0.01 | | 4083 | 3885 |
| Disease freedom demonstration | | 0.001* | 3482 | 3328 |
| | | 0.10* | 34 | 32 |
| | | 0.80* | 3 | 3 |

* design prevalence

## 6.3. Sampling units

### 6.3.1. Individual to aggregate

Sampling units should be defined in the surveillance programme design based on characteristics of shared disease exposure potential. They can range from fish to tank to groups of tanks (e.g. shared water through recirculation) to entire sites (e.g. with shared history or management) to watershed/region (e.g. shared water source) to nation or geographical area.

## 6.4. Sampling strategies

### 6.4.1. Sampling in disease situations

Infected fish display clinical signs in the early stages of infection, with rapid onset of mortality (which can reach up to 100% in fry), lethargy, darkening of the skin, exophthalmia, anaemia (pale gills), haemorrhages at the base of the fins, gills, eyes and skin, and a distended abdomen caused by oedema in the peritoneal cavity. In the chronic state of infection, affected fish do not, in general, exhibit external signs. Moribund fish are common in clinical cases, particularly in early life stages. When the objective of the survey is detection of infection, moribund fish should be sampled preferentially, as they are more likely to be VHSV positive. By focused sampling of these fish, probability of detection is expected to increase if the pathogen is present.

Although clinical signs are similar to other haemorrhagic diseases, distinct petechial haemorrhages in dorsal muscles are quite characteristic. In addition, posterior gut is often pale and atonic in contrast to infectious, septicaemic infections. When selecting fish for the detection of infections, fish with these clinical signs should be included in the samples.

Non-probability sampling for VHS is advisable in many situations in the sense that sampling should focus on samples from clinically diseased fish and fish should not be sampled at random or by convenience. However, such sampling requires trained personnel who are sampling fish for laboratory examination. Uniform international application of judgement sampling is difficult to describe and implement, and the risk of inappropriate sampling may occur with inexperienced investigators. Probability sampling with no emphasis on clinical disease signs is recognised as inefficient. It must be noted that when sampling dead fish, it is important to take only fresh fish as the degradation process will quickly inactivate the virus.

### 6.4.2. Convenience and purposive samples

Physically crowding fish for sampling is stressful and may not be an advisable management strategy, except possibly when sampling is associated with other production-related fish handling events. Using feed to attract and then net fish in tanks or cages is likely to select for more aggressive fish with good appetites. However, such convenience sampling is often the only alternative possible for collection of healthy fish. This type of sample is likely to bias away from detection as VHSV-positive fish are more likely to be excluded from the component of the population attracted to feed. Although convenience sampling of moribund or diseased fish could create a bias toward higher prevalence estimates useful for pathogen detection, it would not be useful for estimates of prevalence in populations that are not free.

A number of surveys have been conducted to detect the presence or estimate prevalence of VHSV in wild and feral fish around the world (Bain *et al.,* 2010; Brudeseth & Evensen 2002; Dopazo *et al.,* 2002; King *et al.,* 2001; Meyers & Winton 1995; Mortensen *et al.,* 1999; Skall *et al.,* 2005a;). The major bias in these surveys has been the lack of juvenile life stages in the netted samples. Also, most vessel access only provides opportunity for sampling open water fish species, missing fish samples from shallow water or reefs that are inaccessible. Continuously monitoring of disease situations is rarely possible for wild fish because of the discrete time points involved in disease surveys.

## 6.5. Probability sampling

Effective probability sampling requires the existence of good records of the holding units in which the sampled populations are organised. Information on location, size and other characteristics of the holding units must be available. Sampling frames (lists of all units) are generally needed but other approaches that do not require the existence of a sampling frame (e.g. systematic sampling) can be applied at certain levels of organisation of the population. Probability (usually systematic random) samples can be obtained at specific times during the farm production cycle, such as collecting eggs by random location in a tray or by selecting larger fish at slaughter.

Within a farm, it is possible to produce a sampling frame of tanks, but not of individual animals. Probability sampling requires maps of fish farms and maps indicating the distribution of tanks within sites. It also requires

more planning and foreground knowledge and may be more complicated than non-probability sampling, but it is the recommended method for the generation of unbiased prevalence estimates. Non-probability sampling is recommended for pathogen detection when the objective is to gather evidence for declaration of freedom (i.e. requiring strategies to bias toward higher prevalence populations).

### 6.5.1. Collection of true probability samples of fish

Collecting a true probability sample is possible but not usually practical. Different methodologies can be used in different situations. The following is offered as an example of a probability sampling strategy.

- Country>farms>tanks>fish

Probability sampling can be done with simple random sampling of farms and tanks (or cages) and then systematic sampling of individuals if they are to be handled for another reason. Once tanks or cages have been selected, then individual fish can be sampled using systematic samples based on presentation or timing of removal. For example, carcasses can be selected in systematic fashion as they move past a reference point when entering the processing plant. When each fish is handled for vaccination, a systematic random sample could be collected during this procedure.

### 6.5.2. Potential biases introduced with systematic random sampling

Biased samples can be obtained if characteristics exist that may influence the risk of infection and that have a cyclical distribution and if the frequency of systematic sampling is the same as the frequency of this cyclical distribution. For example, if one of every three tanks is sampled and tanks are arranged in lines of three then the sample will include only tanks from one specific side of the farm. If conditions affecting infection on that side of the farm are different or there are different risk factors for the infection or the disease compared with other areas of the farm, then samples obtained will probably be biased.

### 6.5.3. Probability sampling at different life/production stages

It is more difficult to obtain a probability sample when targeting early life stages.

### 6.5.4. Possibility of non-probability sampling for detection of VHSV

If the objective is to assess disease freedom then sampling should be biased towards pathogen detection. In this case, higher-risk groups (for example, moribund individuals) should be sampled preferentially. However, if the objective of the system is to estimate prevalence or describe disease occurrence in an endemic population, then non-probability sampling should be avoided.

### 6.5.5. Convenience and purposive samples

Fish collected for other health surveillance purposes can also be used as convenience samples. In such cases, however, the representativeness of the samples will need to be addressed. Unknown biases may be introduced and hamper the interpretation, limiting the usefulness of the test results.

### 6.5.6. Potential biases associated with using convenience sampling

As the distribution of infection in a population in a given area may not be homogeneous, considerable biases can be introduced using convenience sampling. The direction of the bias will depend on the criteria that lead to selection of specific animals and will be influenced by the specific characteristics of the animals that are included in the sample in relation to those that are not. Examples of additional biases include sampling high mortality groups (e.g. tanks), which could induce biases in the prevalence estimates obtained. In fact, underestimation may result from sampling after the peak of mortality, while overestimation may occur if the prevalence is very high in the sampled stock and not representative of the target population. Similar biases may be produced when sampling is not done throughout the production cycle and when the age structure, and thus the infection prevalence, is not considered. Finally, sampling only harvested animals can induce an overestimation of the prevalence as marketed animals are more likely to be carriers than younger animals. On the other hand, this strategy may produce an underestimation of the prevalence for naïve components of the population.

### 6.5.7. Potential attributes that can be considered when adopting purposive sampling

Purposive sampling (i.e. directing units included for testing based on chosen attributes) can be based on clinical signs, the stage of production, time of the year, morbidity, or species.

#### 6.5.8. Most common haphazard sampling method and its associated biases

The most common haphazard sampling method is to net fish when feed is used to attract them within a convenient range. As described previously, this is likely to give samples from the healthier part of the population and will bias away from VHSV detection.

### 6.6. Selection bias

#### 6.6.1. Independence between individuals

Individuals held in the same holding units and having the same history will have a similar probability of exposure to infection compared with individuals from other units. Within ponds or tanks, infected weak fish will tend to cluster near outlets or sides of the enclosure. At the farm level, outbreaks are usually first observed in one pond or tank, and disease then spreads quickly within the farm. Clustering will thus be difficult to predict, but attention should focus on fish from the latest delivery or ponds with greater access to bird predators. Other factors that may affect infection and severity of disease include the age and size of animals, population density, origin (e.g. hatchery or wild) of stocked animals. Other factors such as farming practices (e.g. recirculation versus flow-through hatcheries), contact with wild species or piscivorous birds can have an impact on the occurrence of the infection.

### 6.7. Multi-stage sampling

#### 6.7.1. Groupings of characteristics or disease patterns

For pathogen detection and multistage sampling, risk factors must be taken into consideration by selecting farms and areas with the highest risk of contracting VHS, such as areas or farms in geographical proximity to previous VHS detections, or farms with many sites exchanging stocks of fish. (Some guidelines for the purpose of planning risk-based aquatic animal health surveillance schemes are given in European Commission, 2008.)

## 7. Flow of information and tools/methods

### 7.1. Collecting health data and information

The primary stakeholders of the surveillance system will be farm owners and workers, farmer associations, veterinary services and their inspectors, consultants and health specialists (veterinary practitioners), and the diagnostic laboratories involved.

**Table 7.1.** *Stakeholders involved in surveillance systems, their required capacity and gross observations*

| Stakeholders | Required capacity | Gross observations |
|---|---|---|
| Farm owners and workers | Regular training including knowledge of the most characteristic signs of VHS. Record keeping on movements of fish, daily mortalities, feeding including feed conversion rates etc. | Increased mortality<br>Darkening of skin and exophthalmia, haemorrhages especially at fin basis and ventral part of the eye, lethargy, cerebral disturbances. Knowledge of epidemiological evidence of contact with infected fish |
| Farmer associations | Ability to support and to participate in the development and the organisation of disease surveillance programmes. Training courses for fish farmers | |
| Veterinary Services and their inspectors | Training especially on inspection, sampling, and disease signs, record keeping of authorisations and health status of individual farms. Updated legislation and taking responsibility that legislation is followed. Issuing trade documents and certificates. Sampling and correct shipment of samples to diagnostic laboratories | Same gross pathology + pale, anaemic gills and internal organs, petechial bleedings especially in dorsal muscles. Pale atonic gut. Ascites. Knowledge on the epidemiological status of VHS in the area |
| Consultants and health specialists (veterinary practitioners) | Advanced knowledge of VHS and related diseases, sampling and reporting obligations. Being able to provide consultancy in connection with suspect and confirmed cases | Gross pathology and pale, anaemic gills and internal organs, petecchiation especially in dorsal muscles. Pale atonic gut. Ascites |

| Stakeholders | Required capacity | Gross observations |
|---|---|---|
| Diagnostic laboratories | Being able to prove capacity and performance, e.g. inter-laboratory proficiency testing. Providing quick and reliable identifications and consultancy in relation to the findings | Same as above if whole fish are received. Histopathology: focal necrosis and degeneration in kidney, spleen and heart, cytoplasmic vacuoles, pyknosis, karyolysis, and lymphocytic invasion |

Competent Authorities must immediately be notified at the first suspicion of a VHS outbreak or when unexplained increased mortality is observed by farmers, health specialists or consultants. Likewise laboratories involved in diagnosis must notify the authorities immediately when there is suspicion of the presence of VHSV in samples received. The notification is usually done by a telephone call, explaining the signs and cause of the suspicion, followed by an electronic notification.

### 7.2. Reporting from laboratories

Performance of diagnostic laboratories, test methods, and registration of received samples must be in accordance with the general principles of laboratory quality assurance (e.g. the ISO 17025 standards). Full traceability, documented use of validated methods and standardised procedures for registration and response to customers are required. Diagnostic laboratories shall be approved officially to conduct tests as either national or regional reference laboratories for VHS and must comply with requirements for such laboratories, such as obligatory participation in inter-laboratory proficiency testing.

## 8. Data management

All collected data should be kept in a structured format; usually the Competent Authorities have the responsibility for managing epidemiological data while the respective laboratories have the responsibility for keeping data in relation to diagnosis.

## 9. Statistical aspects

### 9.1. Quantifying uncertainty

In general it is not possible to test all the animals (or units) in a population as all standardised tests for VHSV detection are lethal. However, when testing brood stocks, which are killed after stripping (Atlantic salmon), it is possible to test all individuals.

The cost of taking large samples is very significant; cell culture methods and real-time RT-PCR are also especially costly. Cost can be reduced by pooling samples. It has been shown that pooling of samples from fish with low virus levels (carrier fish) causes a reduction in sensitivity for VHS detection. As the test cost is usually the limiting factor in a survey, however pooling samples will provide more positives than testing fish individually, i.e. 150 fish in 5-fish pools will provide more positives than 30 fish examined as single fish (Helle Frank Skall, pers. comm.). When clinically infected fish are included, the high virus titre in a single fish will easily overcome the dilution effect of pooling tissues from more fish.

### 9.2. The power of the statistical hypothesis test and sample size

The disease-specific power and confidence level achievable in a surveillance system for VHS freedom under farm conditions depends largely on the target of the sampling, on diagnostic sensitivity, on the biosecurity measures implemented, and on the time period through which the surveillance system is conducted. Sample size and numbers play a small role compared with these factors. Under non-farming conditions, however, sample size should be considered more carefully, e.g. testing all highly susceptible fish individually when caught by electrofishing when sample size is not excessive or stratify sampling on research vessels trawling and catching very large numbers of fish and fish species.

### 9.3. Statistical estimation

#### 9.3.1. Precision of the estimate

Precision and the confidence level of estimates of disease occurrence are primarily calculated on the basis of historical data. In approved VHS free areas, it would appear that the OIE provisions and equivalent national or international procedures used to date have been sufficient to prevent

introduction, giving high precision to these estimates, whereas they have not been able to reduce spread and disease outbreaks in endemic areas.

## 9.4. Design prevalence

### 9.4.1. Specification of design prevalence values

As VHS surveillance in fish farming has been based on the presence or absence of the virus, prevalence studies within populations have not been performed.

In wildlife, however, Skall *et al.* (2005a) attempted to carry out prevalence studies for wild fish in the Baltic Sea, Kattegat, Skagerrak and the North Sea. They concluded that the highest prevalences were found in herring and sprat, and in an area around Bornholm, an island in the Baltic Sea. The study showed that prevalence can be estimated even when samples are pooled.

Prevalences vary from 1 to 15% indicating that a high number of fish has to be tested to obtain reliable results. This study included testing of more than 27,000 fish. The study was biased as it only included fish that were caught using officially accepted fishing gear.

## 9.5. Sensitivity and power of the surveillance system

The sensitivity of the VHS surveillance system shall be very close to 1 where the surveillance aims at proving freedom from disease. For prevalence studies in endemic areas, high sensitivity is not as crucial when the sensitivity is known and used in the prevalence estimation. A number of tools can be used for modifying the sensitivity of surveillance, such as sample size, time span of the surveillance, fish species, size, season of inspection, reporting, etc.

### 9.5.1. Power of the surveillance system

The power of a VHS surveillance system is the probability that no positive cases are reported if the disease is truly not there. As a false positive case can have a very serious economic impact for affected aquaculture and regions, the power of a VHS surveillance should be as high as possible without a negative influence on sensitivity, a power estimate of around 90% would be acceptable.

# 10. Responsibilities and resources

See the general comments applicable to all disease chapters.

# 12. Monitoring and evaluation

## 12.1. Flexibility of surveillance

In suspect cases, surveillance intensity and frequency of response should be modified according to a prescribed plan given in the contingency plan for VHS. The response should include immediate inspection and diagnostic sampling, re-testing and molecular characterisation of VHSV. Communication with the farmers involved and other stakeholders must be thorough and documented.

## 12.2. Risk-based surveillance

Disease surveillance must use resources optimally to detect early emergence of VHS. Risk-based surveillance can increase confidence in surveillance programmes by focusing efforts on defined high risk points, e.g. focus on risk for contracting the disease together with risk for spreading the disease. A comprehensive description on how to design a risk-based surveillance programme for VHS is given in a European Commission Decision (2008), which provides guidelines on risk-based surveillance.

### 12.2.1. Improved technologies

Detection technologies recently developed for VHS primarily use real-time RT-PCR assays that are highly sensitive, specific, robust and applicable for testing of large sample sizes. Validation of real-time RT-PCR has occurred for diagnosing and surveying for freedom of VHS (Garver *et al.,* 2011; Jonstrup *et al.,* 2012). The methods were adopted and included in the OIE *Aquatic Manual*.

Serological methods for detection of antibodies against VHS have been developed and validated in recent years and may supplement VHS surveillance in future (Fregeneda-Grandes *et al.,* 2007; 2009, Schyth *et al.,* 2012).

## 12.3. Sensitivity and specificity of surveillance programmes

### 12.3.1. Surveillance programme evaluation process

Once established, surveillance programmes must undergo regular evaluation, especially in cases where the surveillance has failed to detect or detection of new outbreaks is delayed. Independent assessment of weaknesses and gaps or inefficiencies is warranted on a regular basis.

## 12.4. Component testing

### 12.4.1. Passive surveillance

Passive surveillance for VHS may produce delays in detection of the pathogen emerging during a period of declared freedom, but the severity of disease in susceptible populations means that complete failure to detect the presence of VHS is unlikely when appropriate notifications of mortality events occur.

### 12.4.2. Performance on field samples

Diagnostic test evaluations for VHSV based on a long history of testing field samples on cell cultures have provided strong evidence of the strengths and weaknesses of these methods. Implementation of direct real-time RT-PCR or serological methods in surveillance requires appropriate evaluation as methods are more recently established.

## 12.5. Timeliness

The capacity of the surveillance programme for detecting and investigating suspect cases as they arise is crucial to success. As diagnostic tests and viral characterisation usually take 2–4 weeks for confirmation (positive or negative) of suspect cases, biosecurity measures and intensified surveillance plans must be in place. Conducting exercises (following contingency plans evolved for VHS) with simulated disease outbreaks are very useful for identifying weaknesses prior to experiencing a VHS outbreak.

# 13. Special design considerations for surveillance of wild, ornamental and sessile aquatic organisms

## 13.1. Wild populations

The highest prevalence of VHS has been observed in schooling fish, but virus has also been isolated from predators of schooling fish as well as in benthic (bottom dwelling) fish species. If prevalence in wild fish is the objective, sampling should focus on schooling fish. No studies have been performed on the importance of water depth for VHS prevalence, but there is no indication that water depth is an important consideration.

In fresh water, especially in streams or rivers, electro-fishing is an efficient method for collecting fish for laboratory testing. Fishing gear placed closed to fish farms or traditional trawling for pelagic fish is the method of choice. However, bias in all methods excludes juvenile stages of wild fish species.

Collecting samples from commercial fishers for VHS surveillance in marine waters is possible. However, rapid inactivation in wild fish harbouring naturally low levels of virus (i.e. just above detection levels) will interfere with interpretation of test results. A study conducted during a marine VHS survey in waters around Denmark showed that freezing fish for later virological examination gave a significant decrease in virus recovery, i.e. 2 × 15 pools of 10 sprat (*Sprattus sprattus*) were collected. Fifteen pools were processed on board while 15 pools were frozen at –30°C and processed at the laboratory. All pools (100%) processed on board were positive, whereas only six pools (40%) were positive when frozen and processed at the laboratory (Helle Frank Skall, pers. comm.). Collecting fish from commercial fishers is expected to result in under reporting.

## 13.2. Ornamental aquatic animals

Generally, VHS does not affect ornamental species and ornamental fish are not considered as hazards for VHS spread.

With the implementation of real-time RT-PCR and serology in VHS surveillance non-lethal sampling has become more feasible in valuable individuals. However, molecular techniques do not appear to have same high sensitivity for non-lethal samples compared to testing internal organs.

## Literature cited


AMOS K. & THOMAS J. (2002). Disease interactions between wild and cultured fish: Observations and lessons learned in the Pacific Northwest. *Bull. Eur. Assoc. Fish Pathol.*, **22** (2), 95–102.

ARIEL E., NICOLAJSEN N., SKALL H.F., ANDERSEN J.S., MADSEN S. & OLESEN N.J. (2009). Proficiency testing of national reference laboratories for fish diseases. *Aquaculture*, **294** (3-4), 153–158.

BAIN M.B., CORNWELL E.R., HOPE K.M., ECKERLIN G.E., CASEY R.N., GROOCOCK G.H., RODMAN G.G., BOWXER P.R., WINTON J.R., BATTS W.N., CANGELOSI A. & CASEY J.W. (2010). Distribution of an invasive aquatic pathogen (viral hemorrhagic septicemia virus) in the Great Lakes and its relationship to shipping. *PLoS ONE*, **5**, e10156-doi:10.1371/journal.pone.0010156.

BOVO G., HÅSTEIN T., HILL B., LAPATRA S., MICHEL C., OLESEN N.J., SHCHELKUNOV I., STORSET A., WOLLFROM T. & MIDTLYING P.J. (2005a). QLK2-CT-2002-01546: Fish Egg Trade Work package 1 report: Hazard identification for vertical transfer of fish disease agents, 1–35. VESO, P.O. Box 8109, Dep., N-0032 Oslo, Norway.
Available at http://www.crl-fish.eu/upload/sites/eurl-fish/reports/links/fisheggtrade%20wp_1.pdf

BOVO G., HILL B., HUSBY A., HÅSTEIN T., MICHEL C., OLESEN N.J., STORSET A. & MIDTLYNG P. (2005b). Fish Egg Trade Work package 3 report: Pathogen survival outside the host, and susceptibility to disinfection, 1–53. VESO, P.O. Box 8109 Dep., N-0032 Oslo, Norway.
Available at: http://www.crl-fish.eu/upload/sites/eurl-fish/links/fisheggtrade%20wp_3.pdf

BRUDESETH B.E. & EVENSEN O. (2002). Occurrence of viral haemorrhagic septicaemia virus (VHSV) in wild marine fish species in the coastal regions of Norway. *Dis. Aquat. Org.*, **52**, 21–28.

CASTRIC J., JEFFROY J., BEARZOTTI M. & DE KINKELIN P. (1992). Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild elvers *Anguilla anguilla*. *Bull. Eur. Assoc. Fish Pathol.*, **12**, 21–23.

DALE O.B., ØRPETVEIT I., LYNGSTAD T.M., KAHNS S., SKALL H.F., OLESEN N.J. & DANNEVIG B.H. (2009). Outbreak of viral haemorrhagic septicaemia (VHS) in seawater-farmed rainbow trout in Norway caused by VHS virus Genotype III. *Dis. Aquat. Org.,* **85**, 93–103.

DE KINKELIN P. & LE BERRE M. (1977). Isolement d'un Rhabdovirus pathogéne de la Truite Fario (*Salmo trutta* L., 1766). *C.R. Acad. Sci. Paris*, **284**, 101–104.

DIXON P.F. (1999). VHSV came from the marine environment: Clues from the literature, or just red herrings*? Bull. Eur. Assoc. Fish Pathol.*, **19**, 60–65.

DOPAZO C.P., BANDÍN I., LÓPEZ-VAZQUEZ C., LAMAS J., NOYA M. & BARJA J.L. (2002). Isolation of viral hemorrhagic septicemia virus from Greenland halibut *Reinhardtius hippoglossoides* caught at the Flemish Cap. *Dis. Aquat. Org.*, **50** (3), 171–179.

EINER-JENSEN K., AHRENS P., FORSBERG R. & LORENZEN N. (2004). Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. *J. Gen. Virol.,* **85**, 1167–1179.

EINER-JENSEN K., AHRENS P. & LORENZEN N. (2005). Parallel phylogenetic analyses using the N, G or Nv gene from a fixed group of VHSV isolates reveal the same overall genetic typing. *Dis. Aquat. Org.,* **67**, 39–45.

ELSAYED E., FAISAL M., THOMAS M., WHELAN G., BATTS W. & WINTON J. (2006). Isolation of viral haemorrhagic septicaemia virus from muskellunge, *Esox masquinongy* (Mitchill), in Lake St Clair, Michigan, USA reveals a new sublineage of the North American genotype. *J. Fish Dis.,* **29**, 611–619.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA) (2008). Scientific Opinion of the panel on AHAW on a request from the European Commission on aquatic animal species susceptible to diseases listed in the Council Directive 2006/88/EC. *The EFSA Journal,* **808**, 1–144.

EUROPEAN COMMISSION (2008). 2008/896/EC: Commission Decision of 20 November 2008 on guidelines for the purpose of the risk-based animal health surveillance schemes provided for in Council Directive 2006/88/EC (notified under document number C(2008) 6787) (Text with EEA relevance). *Official J. European Communities,* **L 322**, 30–38.

EVENSEN Ø., MEIER W., WAHLI T., OLESEN N.J., JØRGENSEN P.E.V. & HÅSTEIN T. (1994). Comparison of immunohistochemistry and virus cultivation for detection of viral haemorrhagic septicaemia virus in experimentally infected rainbow trout *Oncorhynchus mykiss*. *Dis. Aquat. Org.,* **20**, 101–109.

FAISAL M. & SCHULZ C.A. (2009). Detection of viral hemorrhagic septicemia virus (VHSV) from the leech *Myzobdella lugubris* Leidy, 1851. *Parasit. Vectors,* **2**, 45.

FREGENEDA-GRANDES J.M. & OLESEN N.J. (2007). Detection of rainbow trout antibodies against viral haemorrhagic septicaemia virus (VHSV) by neutralisation test is highly dependent on the virus isolate used. *Dis. Aquat. Org.,* 74, 151–158.

FREGENEDA-GRANDES, J.M., H.F. SKALL & N.J. OLESEN (2009). Antibody response of rainbow trout with single or double infections involving viral haemorrhagic septicaemia virus and infectious haematopoietic necrosis virus. *Dis. Aquat. Org., 83* (1), 23–29.

GAGNÉ N., MACKINNON A.-M., BOSTON L., SOUTER B., COOK-VERSLOOT M., GRIFFITHS S. & OLIVIER G. (2007). Isolation of viral haemorrhagic septicaemia virus from mummichog, stickleback, striped bass and brown trout in eastern Canada. *J. Fish Dis.,* **30**, 213–223.

GARVER K.A., HAWLEY L.M., MCCLURE C.A., SCHROEDER T., ALDOUS S., DOIG F., SNOW M., EDES S., BAYNES C. & RICHARD J. (2011). Development and validation of a reverse transcription quantitative PCR for universal detection of viral hemorrhagic septicemia virus. *Dis. Aquat. Org.,* **95**, 97–112.

GROOCOCK G.H., GETCHELL R.G., WOOSTER G.A., BRITT K.L., BATTS W.N., WINTON J.R., CASEY R.N., CASEY J.W. & BOWSER P.R. (2007). Detection of viral hemorrhagic septicemia in round gobies in New York State (USA) waters of Lake Ontario and the St. Lawrence River. *Dis. Aquat. Org.,* **76**, 187–192.

HARMACHE A., LEBERRE M., DROINEAU S., GIOVANNINI M. & BREMONT M. (2006). Bioluminescence imaging of live infected salmonids reveals that the fin bases are the major portal of entry for Novirhabdovirus. *J. Virol.,* **80**, 3655–3659.

HENRYON M., JOKUMSEN A., BERG P., LUND I., PEDERSEN P.B., OLESEN N.J. & SLIERENDRECHT W.J. (2002a). Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. *Aquaculture,* **209**, 59–76.

HENRYON M., JOKUMSEN A., BERG P., LUND I., PEDERSEN P.B., OLESEN N.J. & SLIERENDRECHT W.J. (2002b). Erratum to "Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout" (*Aquaculture*, 2002, **209**, 59–76). *Aquaculture,* **216**, 389–390.

ISSHIKI T., NISHIZAWA T., KOBAYASHI T., NAGANO T. & MIYAZAKI T. (2001). An outbreak of VHSV (viral hemorrhagic septicemia virus) infection in farmed Japanese flounder *Paralichthys olivaceus* in Japan. *Dis. Aquat. Org.,* **47**, 87–99.

JONSTRUP S.P, KAHNS S., SKALL H.F, BOUTRUP T.S. & OLESEN N.J. (2012). Development and validation of a novel Taqman based real time RT-PCR assay suitable for demonstrating freedom from viral haemorrhagic septicaemia virus. *J. Fish Dis*., in press.

JØRGENSEN P.E.V., CASTRIC J., HILL B., LJUNGBERG O. & DE KINKELIN P. (1994). The occurrence of virus infections in elvers and eels (*Anguilla anguilla*) in Europe with particular reference to VHSV and IHNV. *Aquaculture*, **123**, 11–19.

JØRGENSEN P.E.V. (1970). The survival of viral haemorrhagic septicaemia (VHS) virus associated with trout eggs. *Riv. It. Piscic.,* **1**, 13–15.

KIM S.-M., LEE J.-I., HONG M.-J., PARK H.-S. & PARK S.-I. (2003). Genetic relationship of the VHSV (viral hemorrhagic septicemia virus) isolated from cultured olive flounder, *Paralichthys olivaceus* in Korea. *J. Fish Pathol*., **16**, 1–12.

KING J.A., SNOW M., SMAIL D.A. & RAYNARD R.S. (2001). Distribution of viral haemorrhagic septicaemia virus in wild fish species of the North Sea, north east Atlantic Ocean and Irish Sea. *Dis. Aquat. Org.,* **47** (2), 81–86.

LÓPEZ-VÁZQUEZ C., RAYNARD R.S., BAIN N., SNOW M., BANDÍN I. & DOPAZO C.P. (2006). Genotyping of marine viral haemorrhagic septicaemia virus isolated from the Flemish Cap by nucleotide sequence analysis and restriction fragment length polymorphism patterns. *Dis. Aquat. Org.* **73**, 23–31.

LUMSDEN J.S., MORRISON B., YASON C., RUSSELL S., YOUNG K., YAZDANPANAH A., HUBER P., AL-HUSSINEE L., STONE D. & WAY K. (2007). Mortality event in freshwater drum *Aplodinotus grunniens* from Lake Ontario, Canada, associated with viral haemorrhagic septicemia virus, type IV. *Dis. Aquat. Org.,* **76**, 99–111.

MEIER W., AHNE W. & JØRGENSEN P.E.V. (1986). Fish viruses: Viral haemorrhagic septicaemia in white fish (*Coregonus* sp.). *J. Appl. Ichthyol.,* **2**, 181–186.

MEIER W. & JØRGENSEN P.E.V. (1979). Egtved virus: Characteristics of a virus strain isolated from pike fry (*Esox lucius* L.). *Nord. Vet. Med.,* **31**, 484–485.

MEYERS T.R. & WINTON J.R. (1995). Viral hemorrhagic septicemia virus in North America. *Ann. Rev. Fish Dis.,* **5**, 3–24.

MORTENSEN H.F., HEUER O.E., LORENZEN N., OTTE L. & OLESEN N.J. (1999). Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea, Kattegat, Skagerrak and the North Sea. *Virus Res.,* **63**, 95–106.

MULCAHY D., KLAYBOR D. & BATTS W.N. (1990). Isolation of infectious hematopoietic necrosis virus from a leech (*Piscicola salmositica*) and a copepod (*Salmincola* sp.), ectoparasites of sockeye salmon *Oncorhynchus nerka*. *Dis. Aquat. Org*., **8**, 29–34.

MUNRO A.L.S. (1996). Report of the first recorded outbreak of viral haemorrhagic septicaemia (VHS) in GB and subsequent actions to contain, eradicate and investigate the origins of the infection. Scottish Aquaculture Research Report 3. The Scottish Office Agriculture, Environment and Fisheries Department, 1–12.

NEUKIRCH M. (1985). Uptake, multiplication, and excretion of viral haemorrhagic septicaemia virus in rainbow trout (*Salmo gairdneri*). *In:* Fish and Shellfish Pathology, Ellis A.E., ed. Academic Press, Inc., USA, 295–300.

NISHIZAWA T., IIDA H., TAKANO R., ISSHIKI T., NAKAJIMA K. & MUROGA K. (2002). Genetic relatedness among Japanese, American and European isolates of viral haemorrhagic septicaemia virus (VHSV) based on partial G and P genes. *Dis. Aquat. Org.,* **48**, 143–148.

OIDTMANN B., JOINER C., STONE D., DODGE M., REESE R.A. & DIXON P. (2011). Viral load of various tissues of rainbow trout challenged with viral haemorrhagic septicaemia virus at various stages of disease. *Dis. Aquat. Org*., **93**, 93–104.

OLESEN N.J. & VESTERGÅRD JØRGENSEN P.E. (1982). Can and do herons serve as vectors for Egtved virus? *Bull. Eur. Assoc. Fish Pathol.,* **2**, 48.

PETERS F. & NEUKIRCH M. (1986). Transmission of some fish pathogenic viruses by the heron, *Ardea cinerea*. *J. Fish Dis.,* **9**, 539–544.

RAJA-HALLI M., VEHMAS T.K., RIMAILA-PARNANEN E., SAINMAA S., SKALL H.F., OLESEN N.J. & TAPIOVAARA H. (2006). Viral haemorrhagic septicaemia (VHS) outbreaks in Finnish rainbow trout farms. *Dis. Aquat. Org*., **72**, 201–211.

ROSS K., MCCARTHY U., HUNTLY P.J., WOOD B.P., STUART D., ROUGH E.I., SMAIL D.A. & BRUNO D.W. (1994). An outbreak of viral haemorrhagic septicaemia (VHS) in turbot (*Scophthalmus maximus*) in Scotland. *Bull. Eur. Assoc. Fish Pathol.,* **14**, 213–214.

SCHLOTFELDT H.-J., AHNE W., JØRGENSEN P.E.V. & GLENDE W. (1991). Occurrence of viral haemorrhagic septicaemia in turbot (*Scophthalmus maximus*) – a natural outbreak. *Bull. Eur. Assoc. Fish Pathol.,* **11**, 105–107.

SCHYTH B.D., ARIEL E, KORSHOLM H & OLESEN N.J. (2012). Diagnostic capacity for viral haemorrhagic septicaemia virus (VHSV) infection in rainbow trout (*Oncorhynchus mykiss*) is greatly increased by combining viral isolation with specific antibody detection. *Fish Shellfish Immunol.,* **32**, 593–597.

SKALL H.F., KJÆR T.E. & OLESEN N.J. (2004). Investigation of wild caught whitefish, Coregonus lavaretus (L.), for infection with viral haemorrhagic septicaemia virus (VHSV) and experimental challenge of whitefish with VHSV. *J. Fish Dis.,* **27**, 401–408.

SKALL H.F., OLESEN N.J. & MELLERGAARD S. (2005a). Prevalence of viral haemorrhagic septicaemia virus in Danish marine fishes and its occurrence in new host species. *Dis. Aquat. Org*., **66**, 145–151.

SKALL H.F., OLESEN N.J. & MELLERGAARD S. (2005b). Viral haemorrhagic septicaemia virus in marine fish and its implications for fish farming – a review. *J. Fish Dis.,* **28**, 509–529.

SMAIL D.A. (1999). Viral haemorrhagic septicaemia. *In:* Fish Diseases and Disorders, Volume 3: Viral, Bacterial and Fungal Infections, Woo P.T.K. & Bruno D.W., eds. New York: CABI Publishing, 123–147.

SNOW M., BAIN N., BLACK J., TAUPIN V., CUNNINGHAM C.O., KING J.A., SKALL H.F. & RAYNARD R.S. (2004). Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). *Dis. Aquat. Org.,* **61**, 11–21.

SNOW M., CUNNINGHAM C.O., MELVIN W.T. & KURATH G. (1999). Analysis of the nucleoprotein gene identifies distinct lineages of viral haemorrhagic septicaemia virus within the European marine environment. *Virus Res.,* **63**, 35–44.

STONE D.M., FERGUSON H.W., TYSON P.A., SAVAGE J., WOOD G., DODGE M.J., WOOLFORD G., DIXON P.F., FEIST S.W. & WAY K. (2008). The first report of viral haemorrhagic septicaemia in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), in the United Kingdom. *J. Fish Dis.,* **31**, 775–784

STONE D.M., WAY K. & DIXON P.F. (1997). Nucleotide sequence of the glycoprotein gene of viral haemorrhagic septicaemia (VHS) viruses from different geographical areas: a link between VHS in farmed fish species and viruses isolated from North Sea cod (*Gadus morhua* L.). *J. Gen. Virol.,* **78**, 1319–1326.

TAKANO R., NISHIZAWA T., ARIMOTO M. & MUROGA K. (2000). Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild Japanese flounder, *Paralichthys olivaceus*. *Bull. Eur. Assoc. Fish Pathol.,* **20**, 186–192.

WATANABE L., PAKINGKING R.J., IIDA H., NISHIZAWA T., IIDA Y., ARIMOTO M. & MUROGA K. (2002). Isolation of aquabirnavirus and viral hemorrhagic septicemia virus (VHSV) from wild marine fishes. *Gyobyo Kenkyu* (= Fish Pathology), **37**, 189–191.

WIZIGMANN G., BAATH CH. & HOFFMANN R. (1980). Isolierung des Virus der viralen hamorrhagischen Septikamie (VHS) aus Regenbogenforellen-, Hecht- und Aschenbrut. *Zbl. Vet. Med*., **27B**, 79–81.

*
* *

**NB:** There is an OIE Reference Laboratory for Viral haemorrhagic septicaemia
(see Table at the end of the *Aquatic Manual* or consult the OIE Web site for the most up-to-date list:
http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/ ).
Please contact the OIE Reference Laboratories for any further information on
diagnostic tests, etc., for viral haemorrhagic septicaemia