SURVEILLANCE OF INFECTIOUS DISEASES

IN ANIMALS AND HUMANS IN SWEDEN 2022

Chapter excerpt:
Infectious diseases in fish, crustaceans and molluscs

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Cover: A cultivation of *Salmonella* at the Public Health Agency of Sweden.
Photo: Nicklas Thegerström/DN/TT. Cover design by Rodrigo Ferrada Stoehrel.

Reporting guidelines: Reporting guidelines were introduced in 2018 for those chapters related to purely animal pathogens. The guidelines build on experiences from several EU projects, and have been validated by a team of international experts in animal health surveillance. The aim is to develop these guidelines further in collaboration within the global surveillance community and they have therefore been made available in the form of a wiki on the collaborative platform GitHub (https://github.com/SVA-SE/AHSURED/wiki). Feel free to contribute!

Layout: The production of this report continues to be accomplished using a primarily open-source toolset. The method allows the source text to be edited independently of the template for the layout which can be modified and reused for future reports. Specifically, the chapter texts, tables and captions are authored in Microsoft Word and then converted to the LaTeX typesetting language using a custom package written in the R software for statistical computing. The package uses the pandoc document conversion software with a filter written in the lua language. Most figures and maps are produced using R and the LaTeX library pgfplots. Development for 2022 has focused on generalising the R package to accommodate conversion into formats other than LaTeX and PDF, with a focus on markdown files which can be published as HTML websites using the Quarto publishing system. The report generation R package and process was designed by Thomas Rosendal, Wiktor Gustafsson and Stefan Widgren.

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Infectious diseases in fish, crustaceans and molluscs

Figure 68: Wild arctic char infected with bacterial kidney disease. Photo: Victor Felix.

BACKGROUND

All registered aquaculture farms are obligated to participate in the Official Health Control Programme, regulated in accordance with the Animal Health Law, (EU) 2016/429 and its implementing regulation (EU) 2018/1882. Sweden has a good health status in aquaculture as well as in wild populations of fish and shellfish in the aspect that none of the serious viral diseases that occur in other European countries are prevalent in Sweden. A restrictive approach to imports of live fish for restocking/farming, an early introduction of health control in farms and the presence of hydroelectric power dams in most Swedish rivers (acting as migration barriers for feral fish from the coastal zone) all contribute to maintaining this health status. The presence of dams also results in a different health status at the coast compared to the more disease-free continental zone. To maintain this situation, all transport of live fish from the coast to the inland zone is forbidden and Sweden has a national restocking programme for salmonids to maintain populations that cannot migrate and spawn in nature.

LEGISLATION AND DISEASES

All Swedish fish farms have participated in surveillance for the diseases mentioned below since the late 1980s. In 2021, when the new Animal Health Law (EU 2016/429) was implemented, legislation for disease surveillance and methodology was also changed. Previously the Commission Implementing Decision (EU) 2015/1554 was followed regarding both surveillance and diagnostics. Currently, surveillance is regulated by the Commission Implementing Regulation (EU) 2018/1882 and diagnostic manuals have been removed and are now the responsibility of the European Union Reference Laboratory for Fish and Crustacean diseases, Denmark and the European Union Reference Laboratory for Molluscs diseases (Ifremer), France. Sweden is officially free from Viral haemorrhagic septicaemia (VHS) and Infectious haematopoietic necrosis (IHN) in accordance with (EU) 2021/620. Sweden is considered free from SVC and has approved national measures in accordance with (EU) 2021/620. Sweden is considered free from Infectious Pancreatic Necrosis (IPN) in the inland zone and has approved

national measures in accordance with (EU) 2021/260. The inland zone has an eradication programme for renibacteriosis/bacterial kidney disease (BKD) and the coastal zone for IPN (EU) 2021/620. These diseases are notifiable in accordance with SJVFS 2021:10. Further, IHN, VHS, IPN (other than genogroup 2/serotype ab) and SVC are included in the Swedish Act of epizootic diseases (SFS 1999:657 with amendments). In addition, testing is routinely done for Koi herpes virus (KHV) in imported, quarantined koi. Marteiliosis and bonamiosis (shellfish) and crayfish plague and white spot syndrome (crayfish) are actively tested for within the wild crustacean and mollusc surveillance programmes. These diseases are also regulated by the Swedish legislation on notifiable diseases (SJVFS 2021:10). Other notifiable diseases such as furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*, ASS) and yersiniosis/enteric redmouth disease (ERM), are not actively tested for within surveillance programmes.

Epizootic haematopoietic necrosis (EHN)

EHN is caused by a ranavirus. The disease is considered exotic to EU and is a listed disease (category A, D and E) in the Animal Health Law, (EU) 2016/429. Susceptible species present in Sweden are rainbow trout, European/redfin perch, Northern pike and pike-perch. Fish are susceptible at all ages. Farm outbreaks have occurred at 11–20°C with a rapid onset of high mortality rates and there is no evidence of a carrier state.

Infectious haematopoietic necrosis (IHN) and viral haemorrhagic septicaemia (VHS)

Both diseases are caused by rhabdoviruses and occur frequently in Europe. VHS and IHN are listed diseases (category C, D and E) in the Animal Health Law, (EU) 2016/429. They are transferred horizontally, but vertical transmission cannot be completely ruled out for IHN. Both diseases have greatest impact in freshwater rainbow trout (*Oncorhynchus mykiss*) aquaculture but have also been detected in several other species. Infected fish exhibit behavioural changes, lethargy and abnormal swimming (whirling). The fish are anaemic with varying degrees of haemorrhage in multiple organs. VHS also exists in a marine form, and a low prevalence in wild populations of sensitive species cannot be excluded in the Swedish coastal zone since the virus has been identified in wild fish from Skagerrak and the Bornholm basin. A large outbreak of IHN occurred in Danish rainbow trout farms and associated put and take lakes in the spring and summer of 2021. The disease was spread to Åland (Finland archipelago) rainbow trout farms by sale of live fish. Consecutive outbreaks occurred in both Denmark and Åland in 2022 and eradication is still ongoing. Extra surveillance of wild salmon returning to Swedish river systems has been done during the summers because they pass Åland en route, but the virus has not been detected in our wild salmon populations. The virus has not yet been identified in Sweden.

Infectious pancreatic necrosis (IPN)

IPN is caused by an Aquabirnavirus which is highly infectious to juvenile salmonids. Susceptibility declines with increasing age. Fish that survive infection become subclinical carriers. In addition to salmonids, the virus has been detected in several other species. The virus is transmitted both horizontally and vertically.

There are seven genogroups with varying virulence. Some genogroups cause up to 90% mortality in fry, and IPN is considered one of the costliest fish diseases in several European countries. Symptoms include darkening, abdominal distension and corkscrew swimming. Petechial haemorrhage in abdominal fat and internal organs or severe anaemia are the most common internal disease signs. IPN appears sporadically in Swedish east coast fattening farms as well as in salmon and sea trout brood stock on both the east and west coast.

Renibacteriosis (BKD)

BKD is caused by a gram-positive bacterium, *Renibacterium salmoninarum*. The infection can be transmitted both horizontally and vertically. The disease favours low water temperatures, and outbreaks mainly occur at temperatures between 7 and 15°C.

Salmon and Arctic char are the native species most susceptible to BKD and mortality can reach 80%. In rainbow trout, the disease is chronic with a continuous low mortality of about 5–10%, however outbreaks with up to 40% mortality can occur. Infected fish may have reduced growth and secondary bacterial infections such as ASS, which demands antimicrobial treatments, and disease can result in a deterioration of the meat quality. BKD is present in a few farms in the Swedish inland zone.

Spring viraemia of carp (SVC)

SVC is caused by a rhabdovirus. The disease occurs in Asia and several European countries. SVC is not present in Sweden. Several species within the cyprinid family are susceptible to infection and the virus is transmitted horizontally. Clinical signs are usually general, such as darkening, exophthalmia and slow breathing. The fish swim lazily with sporadic periods of hyperactivity. Other common findings are pale gills, ascites and skin and gill haemorrhage. Internally, haemorrhage is found in various organs including muscle, swim bladder and the brain.

Koi herpes virus (KHV) infection

KHV is a herpesvirus and affects common carp (*Cyprinus carpio*) and variants thereof, including koi. KHV is a listed disease (category E) in the Animal Health Law, (EU) 2016/429. The virus was first detected in 1998 and has since then been reported from all continents except Australia. Transmission is horizontal. KHV can cause severe problems and is associated with high mortality. Infected fish usually swim at the surface and have an increased breathing frequency. Disease signs include enophthalmia, gill necrosis and secondary bacterial or parasitic infections on gills and skin. Surviving carps can become subclinical carriers.

The prevalence in Sweden is unknown. Koi is frequently imported, but only a few farms use quarantine and sampling. Two outbreaks in koi, with 90–100% mortality, occurred in 2018.

Crayfish plague

Crayfish plague is caused by an aquatic oomycete (*Aphanomyces astaci*) that spread with live crayfish from the United States to Europe in the late 1800s. The disease occurs throughout Europe and North America. The oomycete reproduces by spores spread in the water. When the spores infect crayfish, they grow through the skin and attack the underlying tissues.

The signal crayfish becomes subclinically infected and may exhibit black (melanised) areas in the shell adjacent to the presence of the fungus in the skin. The spots disappear when the shell is shed but may gradually reappear.

When noble crayfish are infected, the first sign is high mortality in affected populations. Disease in the individual is characterised by behavioural changes such as movement during daytime and, reduced coordination and balance.

Crayfish plague is spread in the southern parts of Sweden.

White spot syndrome (WSS)

WSS is caused by White spot syndrome virus (WSSv), a *Whispovirus* that can infect a wide range of aquatic crustaceans, including marine, brackish and freshwater shrimps, crabs, crayfish and lobsters. WSS is a listed disease (category A, D and E) in the Animal Health Law, (EU) 2016/429. Outbreaks with high mortality occur at water temperatures of 18–30°C. The most common clinical sign in penaeid/giant shrimps is white spots in the exoskeleton. In species with a thicker exoskeleton the disease can occur without obvious external signs.

The virus is transmitted both horizontally and vertically and has a long survival time outside the host animal. Viable virus can be present in imported frozen raw giant shrimps. There is a non-negligible risk that the virus will be introduced to the aquatic environment by anglers using these shrimps for bait. The consequences are difficult to predict but if introduced, the virus may have a negative impact on Swedish crustacean populations. WSSv has never been detected in Sweden.

Marteiliosis

Marteiliosis, a disease in oysters and blue mussels, is caused by a protist parasite (*Marteilia refringens* in oysters and *M. pararefringens* in blue mussels). Marteiliosis is a listed disease (category C, D and E) in the Animal Health Law, (EU) 2016/429. The parasite needs a crustacean (*Paracartia grani*) as an intermediate host. The disease causes reduced fitness, impaired growth and resorption of the gonads and hence reduced reproductive capacity. *M. pararefringens* is present in the Swedish west coast waters.

Bonamiosis

Bonamiosis is a disease in oysters caused by the protistan parasite *Bonamia ostreae*. Bonamiosis is a listed disease (category C, D and E) in the Animal Health Law, (EU) 2016/429. The parasite invades and destroys the haemocytes. Usually, the only sign of disease is increased mortality in the infected oyster population. *B. ostreae* is found along the European Atlantic coast as far up as Denmark, where it has been found in Limfjorden.

Perkinsosis

Perkinsosis is a disease in bivalves caused by protists of the genus *Perkinsus*. Perkinsosis is a listed disease (category A, D and E) in the Animal Health Law, (EU) 2016/429. The most relevant species under Swedish conditions is *P. marinus* that infects *Magallana gigas* among other species. *M. gigas* usually do not show any signs of disease. The disease has never been found in Sweden or neighbouring countries.

Mikrocytosis

Mikrocytosis is a disease in oysters that is caused by the protist *Mikrocytos mackini*. Mikrocytosis is a listed disease (category A, D and E) in the Animal Health Law, (EU) 2016/429.

The parasite usually infects connective tissue and muscles. Infected animals often die but subclinical infections occur. The disease has never been found in Sweden or neighbouring countries.

SURVEILLANCE

The aim of the Official Health Control Programme is to document freedom from disease and to contribute to the maintenance of this status.

Within the Official Health Control Programme, there is active surveillance for the viruses causing EHN, IHN, VHS, IPN and SVC, and for renibacteriosis/BKD. Sampling frequency is based on classification of each farm into one of three categories (high, medium or low risk) after a risk analysis, based on the risk for the farm becoming infected and the risk that the farm will further spread an introduced pathogen. The risk categorisation is performed by the Swedish Board of Agriculture. Sampling is performed annually to every third year based on risk class and pathogen. There is also active surveillance in imported quarantined fish (eel - IPN and koi/carp - KHV). Active surveillance is also done when invasive alien species - like the marble crayfish - are discovered.

Passive disease surveillance has been done through diagnostics related to disease outbreaks in farms and wild fish.

Since 2020 there is also an active health surveillance of wild fish, crustaceans and molluscs. Within this surveillance samples for different infectious diseases are taken.

Crayfish plague, WSS and porcelain disease (the latter not notifiable) are monitored by passive surveillance and testing is done based on suspicion of disease outbreaks. Infectious diseases in shellfish are monitored through sampling of 150 blue mussels and 150 European oysters per year.

Table 35: Samples taken in the Swedish surveillance programmes for notifiable diseases in fish, crustaceans and molluscs during 2022. One case = one outbreak.

Disease	No. of sampled production sites[A]	No. of infected production sites[A]	No. of tested individuals[A]	No. of tested pools[A]	No. of infected individuals/pools
Fish					
VHS	70/20	0	0/9	434/49	-/0
IHN	70/20	0	0/9	434/49	-/0
IPN	70/20	1/0	0/9	434/49	-/1
ISA	4/1	0	114/4	-	0/-
SVC	2	0	7[B]	3	0/0
KHV	1[C]	1	7	-	1/0
BKD	62/7[D]	6/2[D]	2961/214	-	not relevant/3
Crustaceans					
Aphanomyces astaci[E]	15[F]	4[F]	107	-	not relevant
WSSv[E]	12	0	81	-	0/-
Molluscs					
Bonamia sp.[E]	5	0	150	0	0/-
Marteilia (para)refringens[E]	10	0	300	0	0/-
Perkinsus sp.[E]	5	0	150	0	0/-
Mikrocytos mackini[E]	5	0	150	0	0/-

[A] Farms/wild fish sampling points.
[B] One koi import company tests individual quarantined fish.
[C] One koi import company that tests quarantined fish.
[D] Two lakes with connected rivers.
[E] This sampling was performed as part of the wild fish, crustaceans and shellfish health monitoring by mission from the Agency for Marine and Water Management.
[F] A total of 8 locations were sampled, representing 7 separate waterways with wild crayfish. Three waterways were positive.

Abbreviations:

EHN	Epizootic haematopoietic necrosis
VHS	Viral haemorrhagic septicemia
IHN	Infectious haematopoietic necrosis
IPN	Infectious pancreatic necrosis
ISA	Infectious salmon anaemia
SVC	Spring viraemia of carp
KHV	Koi herpesvirus
BKD	Bacterial kidney disease

Within the wild fish programmes, samples are taken based upon suspicion of infectious disease or through short-term prevalence estimating projects.

For details about the different programmes included in the wild fish, crustaceans and molluscs surveillance, please see chapter "Wild fish surveillance programme" (page 144).

DIAGNOSTIC PROCEDURES

All diagnostic virus analyses are performed at the Swedish reference laboratory for fish diseases at the National Veterinary Institute in accordance with e manuals published by the EU reference laboratories or the WOAH aquatic manual. Pooled organ material (for EHN, VHS, IHN and IPN spleen, kidney, heart/brain, for SVC spleen, kidney, brain and gill) are tested by inoculation on cell cultures. A pool consists of organs from up to ten fish (up to five fish for SVC). A cell culture is defined as virus-positive if a cytopathogenic effect is detected within two weeks, after which the virus is identified by ELISA and confirmed by real-time PCR, or in some cases by serum neutralisation (SN) test. Thirty fish are sampled in regular fish farms, and in restocking farms all females are sampled after stripping of roe. In eel quarantine, 120 glass eels are sampled at arrival, and after two months 120 cohabitated rainbow trout are sampled for detection of virus. In the case of carp/koi, only a few fish may be sampled. KHV is tested on individual fish (pooled gill and kidney) by real-time PCR.

BKD is tested on kidney tissue from individual fish and demonstrated by an ELISA method. Verification is done by real-time PCR. Thirty fish are sampled in regular farms, and in restocking farms all females are sampled after stripping of roe.

A. astaci, WSSv and *Thelohania contejeani* are detected with real-time PCR. The number of sampled animals varies from case to case.

Bonamia sp. is detected by real-time PCR. *Marteilia refringens*, *Perkinsus* sp. and *Mikrocytos mackini* are preliminary detected by histology and confirmed by real-time PCR.

RESULTS

Official health control programme for fish farms and broodstock sampling

The number of samples analysed during 2022 and results are shown in Table 35. In summary, the active surveillance detected two cases of BKD (one case = one outbreak). In addition, subsequent sampling of contact farms to farms that were found to be BKD infected in the autumn 2021 identified another four cases of BKD. All cases were detected in previously uninfected farms. In one of the subsequently sampled farms it is hard to interpret if the disease was moved to the lake from the contact farm, since other farming sites

within the same lake as the receiving farm have BKD. *Renibacterium salmoninarum* does not have variable DNA, and thus molecular epidemiology to trace the source of infection is not possible.

One pool from salmon brood stock females from river Lagan on the west coast tested positive for infectious pancreatic necrosis virus (IPNV) genogroup 5. The pool contained organs from five females, but it is unlikely that more than one of the females were infected.

Voluntary health control programme for fish farms

KHV was detected in one imported koi carp. Sequencing showed a genotype that is considered to have low pathogenicity. No symptoms were seen in the batch although an attempt to trigger disease was done. Further sampling revealed no more infected fish in the batch. There were three recorded outbreaks of "other" notifiable diseases in fish during 2022. One concerned furunculosis (ASS) and two yersiniosis. Seven cases of flavobacteriosis due to *Flavobacterium psychrophilum* were detected. This is similar to the previous three years with 5–11 cases respectively, but fewer than the regular 20–30 cases per year during the past decades until 2018. *Flavobacterium columnare* was detected in five disease cases during summer and autumn. Infection with *Aeromonas* bacteria other than *A. salmonicida* subsp. *salmonicida* was identified in a few cases.

OUTBREAKS IN WILD FISH, CRUSTACEANS AND MOLLUSCS

During 2022, mass mortality of crayfish, caused by crayfish plague, in river Skellefteälven lead to a massive sampling, trying to detect the source of the outbreak. The river Ångermanälven was also struck by a mass mortality in crayfish, but once this was realised, there were no crayfish carcasses left to sample. The mass mortality was most likely caused by crayfish plague.

Crayfish plague was detected in four cases. Most cases were also investigated for presence of WSSV and *Thelohania contejeani* (causing porcelain disease). *Thelohania contejeani* was detected in two individuals with crayfish plague and in one individual without other identified pathogens

Esocid herpesvirus-1 was detected in a blue-spotted pike that was caught for surveillance of pike lymphosarcoma. This is the first time this benign virus is identified in Sweden.

DISCUSSION

The number of farms that were sampled during 2022 are listed in Table 35. Swedish aquaculture has a good health status, where all severe diseases of EU/WOAH importance are absent. This is confirmed by the surveillance results from 2022.

The most problematic disease to control is renibacteriosis/BKD, due to its vertical transmission and variable clinical presentation. In 2022 two preliminary cases and four secondary cases were detected. More farms are currently known to be infected and thus not sampled. Prolonged time from diagnosis to slaughter can lead to secondary health issues and increased antibiotic use, as well as decreased welfare. As an example: in the last seven years, ASS has been causing problems in one BKD infected farm and there is an apparent lack of treatment effect. The reason is probably the underlying BKD infection, facilitating the ASS infection and itself being accelerated by the concurrent ASS infection. The farm has also managed to spread both BKD and ASS between production sites. Control of BKD could potentially be improved by adding a modified sampling method to allow *in vivo* sampling of brood stock in commercial farms. Also, rapid slaughter in infected net pen farms to avoid manifestation of the bacterium in wild fish is imperative to avoid reinfection at re-stocking and secondary bacterial diseases that require antibiotic treatment. To get a more reliable assessment for health surveillance additional resources invested in risk-based analysis of individual aquaculture farms would be needed.