SURVEILLANCE OF INFECTIOUS DISEASES IN ANIMALS AND HUMANS IN SWEDEN 2020

Chapter excerpt -

Infectious diseases in fish, crustaceans and molluscs

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Cover: Juvenile mink in hand. Photo: Elina Kähkönen

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, produced by authors, 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 using pandoc and R to the LaTeX typesetting language. Most figures and maps are produced using the R software for statistical computing and the LaTeX library pgfplots. Development for 2020 has further improved the importing of content from Excel files to automatically build figures in the pgfplots LaTeX library. The tool is available as an R-package on GitHub (https://github.com/SVA-SE/mill/). The report generation R-package and process was designed by Thomas Rosendal, Wiktor Gustafsson and Stefan Widgren. In 2020, final typsetting was done primarily by Wiktor Gustafsson with contributions from the report authors.

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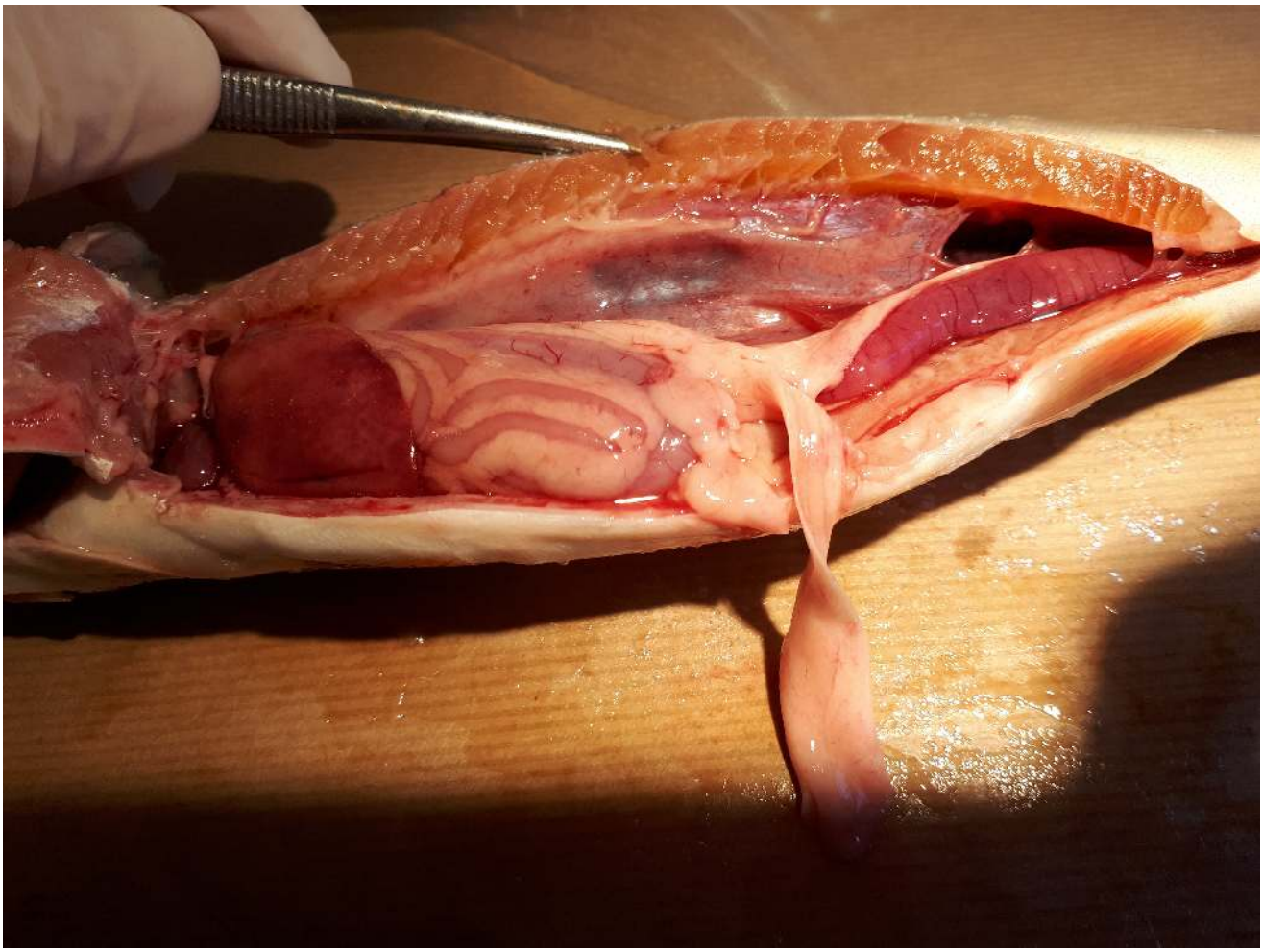

Necropsy of an Arctic char (*Salvelinus alpinus*) with furunculosis. There are multiple petechial haemorrhages in the swim bladder wall. Photo: Charlotte Axén.

BACKGROUND

All registered aquaculture farms are obligated to participate in the Official Health Control Programme, regulated in accordance with SJVFS 2014:4 and by Council Directive 2006/88/EG. Sweden has a very 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 accordance with Commission Implementing Decision (EU) 2015/1554) and Council Directive 2006/88/EC. Sweden has an approved disease-free zone status (2002/308/EC) for Viral haemorrhagic septicaemia (VHS) and Infectious haematopoietic necrosis (IHN) (2008/427/ EC). Additional guarantees are in place for the whole country for Spring Viraemia of Carp (SVC), and for the inland zone for Infectious Pancreatic Necrosis (IPN) (2010/221/EU). The inland zone has an eradication programme for Renibacteriosis/bacterial kidney disease (BKD) and the coastal zone for IPN (2010/221/EU). These diseases are included in the Swedish legislation on notifiable diseases (SJVFS 2013:23). Further, IHN, VHS, IPN (other than genogroup 2) and SVC are included in the Swedish Act on epizootic diseases (SFS 1999:657 with amendments). In addition, testing is routinely done for Koi herpes virus (KHV) in imported, quarantined koi, and for Crayfish plague in crayfish. These diseases are also regulated by the Swedish legislation on notifiable

diseases (SJVFS 2013:23). Other notifiable diseases such as furunculosis (*Aeromonas salmonicida salmonicida*/ASS), yersiniosis/Enteric redmouth disease (ERM), Marteiliosis and Bonamiosis (shellfish) and Whitespot disease (crayfish) 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. Susceptible species present in Sweden are rainbow trout, European/redfin perch, Northern pike and pikeperch. Fish is 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. 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. IHN was found in two Bothnian bay farms in Finland in 2017, but 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 are the most common internal disease signs. IPN appears sporadically in Swedish east coast farms.

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 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 of up to 40% mortality can occur. Infected fish may have reduced growth 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. 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 fungus (*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 fungus 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. 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 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 unicellular parasite (*Marteilia refringens* in oysters and *M. pararefringens* in blue mussels). 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 on the Swedish west coast.

Bonamiosis

Bonamiosis is a disease in oysters caused by the protistan parasite *Bonamia ostreae*. 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.

SURVEILLANCE

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 (I), medium (II) or low risk (III)) after a risk analysis, based on the risk for the farm becoming infected, the risk that the farm will further spread the pathogen and the impact of the pathogen. The risk categorisation is performed by the Swedish Board of Agriculture. Farms within risk categories I and II are tested every year and every second year, respectively, whereas farms within risk category III are only tested upon suspicion of disease. The aim of the Official Health Control Programme is to document freedom from disease and to contribute to the maintenance of this status.

There is also active surveillance in imported quarantined fish (eel - IPN and koi/carp - KHV). Active surveillance is also done when potential 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.

Crayfish plague is monitored by passive surveillance and testing is done based on suspicion of disease outbreaks.

DIAGNOSTIC PROCEDURES

All diagnostic virus analyses are performed according to recommendations by the EU (EU 2015/1554) or the OIE aquatic manual at the Swedish reference laboratory for fish diseases at the National Veterinary Institute. Pooled organ material (for EHN, VHS, IHN and IPN spleen, kidney, heart/brain are tested, for SVC spleen, kidney, brain and gill are tested) by cell culturing. 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 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 and WSSV are detected with real-time PCR. The number of sampled animals varies from case to case.

RESULTS

Official health control programme for fish farms and crustacean surveillance

The number of samples analysed during 2020 and results are shown in Table 35. In summary, the active surveillance detected two cases of BKD (one case = one outbreak). The BKD cases were reinfections of recently sanitised farms.

Voluntary health control programme for fish farms

There were seven recorded outbreaks of “other” notifiable diseases in fish during 2020. Furunculosis (ASS) was detected in six cases. One farm had recurrent disease and concurrent BKD infection. In 2019 another production site within the same company also got infected with ASS and in 2020 the disease was spread to two more production sites. Another farm also had recurrent disease and one case was detected in wild spawning salmon with saprolegniosis. Yersiniosis was detected in one restocking farm.

Few cases (n=6) of flavobacteriosis due to *Flavobacterium psychrophilum,* usually the predominant production disease, were detected compared to previous years (15–30 cases in the last 10 years). Instead, *Aeromonas* bacteria other than *A. salmonicida salmonicida* or *A. salmonicida* atypically dominated with 16 cases. The cause for this shift is unknown. *Flavobacterium columnare* was detected in five disease cases during the summer.

OUTBREAKS IN WILD FISH, CRUSTACEANS AND MOLLUSCS

During 2020, suspicion of crayfish plague was investigated in ten outbreaks of mortality in noble crayfish. Crayfish plague was detected in six cases. Mortality cases that could not be attributed to crayfish plague were investigated for presence of WSSV but the virus was not identified. Further analyses will be performed during 2021 as a PCR for *Thelohania contijeani* (causing porcelain disease) is currently being evaluated.

DISCUSSION

The number of farms that were sampled during 2020 are listed in Table 35. Swedish aquaculture has a good health status, where all severe diseases of EU/OIE importance are absent. This is confirmed by the surveillance results from 2020.

The most problematic disease to control is renibacteriosis/BKD, due to its vertical transmission and variable clinical presentation. In 2020 only two cases were detected, one recurring in an on-growth farm and one in wild broodstock. 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 five 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 be improved by modified sampling and improved methodology, from today's post mortem sampling to an *in vivo* method. Also, rapid slaughter to avoid manifestation of the bacterium in wild fish is imperative to avoid reinfection in farms and secondary bacterial diseases that require antibiotic treatment. Additional resources must be invested in risk-based analysis of individual aquaculture farms to get a more reliable assessment for health surveillance.

Table 35: Samples taken in the Swedish surveillance programmes for notifiable diseases in fish, crustaceans and molluscs during 2020. One case = one outbreak.

Disease	No. of sampled production sites	No. of infected production sites	No. of tested individuals	No. of tested pools	No. of infected individuals/pools
Fish					
VHS	68	0	-	509	-/0
IHN	68	0	-	509	-/0
IPN	68	0	-	509	-/0
SVC	2	0	5[A]	3	0/0
KHV	3[B]	0	7	0	0/0
BKD	64	2	3194	-	4/-
Crustaceans					
Aphanomyces astaci	10[C]	6[C]	17	-	9/-
WSSv	0	0	8	-	0/-
Molluscs					
Bonamia ostreae[D]	4	0	150	0	0/-
Marteilia refringens[D]	4	0	150	0	0/-

[A] One koi import company tests individual fish
[B] One koi import company, two private fish owners (diseased koi).
[C] A total of 10 locations were sampled, representing 10 separate waterways with wild crayfish. Six waterways were positive.
[D] This sampling was performed as part of a project within the European Sea and Fisheries Fund.

Abbreviations:

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