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Evaluation of the surveillance and control programme for viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN)

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Norwegian Food Safety Authority (Mattilsynet)

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Contents

Mandate and working group

The Norwegian Food Safety Authority (NFSA) has asked the National Veterinary Institute (NVI) for proposals for future surveillance program for viral haemorrhagic septicemia (VHS) and infectious haematopoietic necrosis (IHN) (Norwegian Food Safety Authority ref. 2009/23133, National Veterinary Institute ref. 09/01169)

The NFSA wants to ensure that Norway maintains a surveillance programme that facilitates early detection of signs of VHS and IHN so that appropriate measures to prevent further spread of infection can be implemented, and that the approval of Norway as free of VHS and IHN will not be threatened. Furthermore, the NFSA asks for cost considerations in relation to different alternative surveillance programmes. The NFSA also asks if VHS and IHN should be considered separately.

The work has been organized by a working group at the NVI supported by a group of people with experience and expertise on VHS and aquaculture (expert group).

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Summary

The National Veterinary Institute has evaluated the surveillance and control programme for viral haemorrhagic septicemia (VHS) and infectious haematopoietic necrosis (IHN). The methodology used is a quantitative analysis of multiple complex data sources, and is based on scenario tree analysis and stochastic simulation. By evaluating the surveillance programme according to this method, we will have quantitative estimates for the probability of detecting disease (the system surveillance component sensitivity, *SSCSe*) for the various surveillance strategies. The most cost-effective surveillance strategy is the strategy that yields at least 95% *SSCSe* with lowest cost.

The present model shows that if surveillance is risk-based we have a high probability of detecting disease. However, the surveillance system component sensitivity is dependent on the number of samples taken within farms, and the design prevalence (i.e. the hypothetical prevalence of disease that the surveillance program is assessed against).

If the surveillance is targeted towards farms with rainbow trout, a minimum of 20 samples per farm from fish with disease signs will be needed for the detection of VHS given the farm is infected with a within-farm prevalence of 5 %. Furthermore, a number of 487 farms (a total of 9740 samples) will be needed to achieve a 95 % certainty that the programme will detect a VHS-infected farms assuming that there is at least 2 infected farms in the whole salmon farm population (design prevalence of 0.2 %). Because there are not that many farms with rainbow trout, 20% of the salmon farms have to be added in the strategy to achieve at least 95% sensitivity.

Cost considerations indicate that running PCR for VHS virus on few, large batches of samples will be more cost effective than the present programme using cell culture. However, rapid reply is not possible if samples are collected over a period of time, so the need for early detection must be considered in relation to response time.

The model presented is targeted towards VHS. Historically, IHN has been included as part of the same sampling regime as VHS. The results and conclusions in this model may therefore be relevant also for IHN but needs to be validated.

Sammendrag

Veterinærinstituttet har evaluert overvåkings- og kontrollprogrammet for viral hemoragisk septikemi (VHS) og infeksiøs hematopoetisk nekrose (IHN). Vi har brukt en metode som er basert på stokastisk analyse av scenariotrær. Analyse av ulike overvåkingsprogram etter denne metoden gir kvantitative estimater av sannsynligheten for at sykdom blir oppdaget (*SSCSe*). Den mest kostnadseffektive strategien er den som gir minst 95 % *SSCSe* med lave kostnader.

Modellen viser at sannsynligheten for å oppdage sykdom (*SSCSe*) er avhengig av antall prøver som tas ut fra hver lokalitet, antall lokaliteter som er inkludert i overvåkningen og design prevalensen, dvs. den hypotetiske prevalensen av sykdom som overvåkingsprogrammet er vurdert i forhold til.

Hvis overvåkingsstrategien målrettes mot regnbueørret, og minst 20 fisk med sykdomstegn blir prøvetatt, vil VHS kunne påvises på en hypotetisk smittet lokalitet. I tillegg må minst 487 lokaliteter prøvetas (totalt 9740 prøver) for å kunne påvise VHS dersom design prevalensen er 0,2 %. I og med at vi har relativt få lokaliteter med regnbueørret i Norge, må 20 % av oppdrettsanleggene med laks inkluderes i overvåkingsprogrammet for å kunne oppnå minst 95 % sannsynlighet for at sykdom oppdages.

Kostnads vurderinger tilsier at analyse av få og store grupper av prøver med PCR vil være mer kostnadseffektivt sammenlignet med dagens program hvor prøvene analyseres ved hjelp av cellekultur. Behovet for et raskt analysesvar må imidlertid vurderes opp mot en forsinket responstid en får dersom en ønsker å samle prøver over noe tid for å kjøre større prøveserier.

Modellen som er presentert i denne rapporten er rettet mot VHS. Historisk sett har IHN vært inkludert i det samme prøvetakingsregime som har vært benyttet for VHS. Resultatene og konklusjonen fra denne modellen kan derfor også være gyldig for IHN, men dette gjenstår å validere.

Introduction

International legislation requires that in order to trade fish and fish products on the international market, a country must prove freedom of certain diseases. This is usually done by maintaining surveillance systems targeting the specific diseases. In order to consider the effectiveness of different surveillance strategies, an evaluation of the surveillance system in place needs to be carried out. A new methodology for quantitative analysis of multiple complex data sources to support claims of freedom from diseases has recently been suggested (Martin et al 2007), and is applied in this report to evaluate the surveillance and control program for viral haemorrhagic septicaemia (VHS).

Viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) are two important viral diseases in salmonid fish listed as non-exotic diseases according to EU legislation (Council directive 2006/88/EC). The aetiological agents for VHS and IHN are the rhabdoviruses; VHS virus or Egtved virus, and IHN virus (Anonymous 2009, OIE). VHS occurs both in freshwater and seawater, and the disease has been reported in several species, both farmed and wild. The disease has most frequently been observed in farmed rainbow trout, and young stages are generally more susceptible to the disease. Disease generally occurs at temperatures between 4º C and 14º C. VHS was present in Norway between 1964 and 1974, but was successfully eradicated and has not been detected until the outbreak in Storfjorden in 2007 (Dale et al 2009). IHN has been reported in both freshwater and seawater, and the disease occurs in several salmonid species, both farmed and wild. The main impact of the disease has been in fry and juveniles of farmed rainbow trout. Clinical disease is most often observed at temperatures between 8º C and 15º C. IHN is present in Europe but has never been diagnosed in Norway.

Norway was approved free from VHS and IHN in 1994 (EFTA decision 71/94/COL), and has operated a surveillance programme in accordance with Council directive 91/67/EEC from 1994 until 2008 (Surveillance and control programmes - annual reports). When VHS virus was detected in Norway in 2007, the approved status was temporarily suspended. Since May 2008, Norway, with the exemption of the coastal zones in the affected area, has again been recognized as an approved zone (EFTA decision 302/08/COL).

The requirements regarding maintenance of disease free status for VHS and IHN were amended in 2008, when Council directive 91/67/EEC was replaced by the new Council directive 2006/88/EEC. It follows from Article 52 in the new directive that a member state declared free from VHS or IHN may discontinue targeted surveillance and maintain its disease-free status provided that the conditions conducive to clinical expression of the disease in question exist, and that the directive 2006/88/EEC is followed.

Occurrence of either VHS or IHN in Norway is expected to result in obvious disease signs in the infected fish. As host species, age and temperature ranges show great overlap between VHS and IHN, we presuppose that these two diseases may be covered by similar surveillance systems (as has previously been done). The model presented in this report is targeted towards VHS, but the main conclusions are considered to be relevant also for IHN.

Ongoing surveillance activity where VHS may be detected

Risk-based health control has to be conducted on all fish farms according to Council directive 2006/88/EEC. In Norway, health control on fish farms is carried out on a routine basis by authorized/certified personnel in private or industry owned fish health services (veterinarian or fish health biologist). According to the present legislation, each site must have an operation journal which is audited at each visit. Autopsy and relevant investigations will be carried out on a representative sample of recently dead animals and/or animals showing abnormal behaviour to determine the cause of death or disease. For any increase (abnormal) in mortality a cause has to be identified either by the farmer or by the health service. In the case of unexplained mortality or suspicion of notifiable infectious diseases there is an obligation to notify the Norwegian Food Safety Authority.

At least one health control has to be carried out by the fish health service when a site is stocked and before any transport of fish out of the farm is allowed.

Norway has operated a surveillance and control program for VHS and IHN in accordance with EU regulations since 1994. The aim of the program has been to document absence of VHS virus and IHN virus

in fish farms within the approved zone to maintain Norway's status as free of VHS and IHN. The programme consisted of an inspection and a sampling regime. The Norwegian surveillance programme was amended in 2009 and sampling is limited to rainbow trout farms only. The history and design of the Norwegian surveillance programme is described in the annual reports for surveillance and control programmes for terrestrial and aquatic animals in Norway (Surveillance and control programmes - annual reports).

Materials and methods

Principle

The methodology used to evaluate the surveillance programme is a quantitative analysis of multiple complex data sources (Martin et al 2007), and is based on scenario tree analysis and stochastic simulation. The analysis provides an assessment of the ability of the programme to prove freedom of disease by calculating the surveillance system component sensitivity (*SSCSe*) (Martin et al 2007). *SSCSe* is an overall measurement of how effective a given surveillance system is at detecting disease among farms and fish included in the system. By evaluating various alternatives of surveillance strategies by this method we get a quantitative measure of the effect of these strategies and thus gain better support for choosing an optimal strategy for claiming freedom from disease.

Description of the scenario tree

A scenario tree is a tool that may be used to assist in the calculation of the sensitivity of a component of a surveillance system. In contrast to the analysis of representative samples, the scenario tree takes into account the fact that not all animals in the population have the same probability of being infected and/or detected (Cameron 2009).

On the basis of current knowledge on VHS and discussions in the expert group, a scenario tree for VHS – surveillance was created (Fig. 1). Through the nodes and branches of the scenario tree, the Norwegian salmonid fish population is gradually divided into smaller (more homogeneous) groups. Within each of the smaller groups, each fish is assumed to have equal probability of being detected as VHS-infected given VHS infection is present within the population. Risk category nodes divide the population into groups with respect to their relative risk (RR) of being infected. Detection category nodes divide the population into groups with respect to their probability of being detected as infected. Farm (including sea sites and hatcheries) and fish are categorized as Infection nodes which are defined by their respective design prevalence, P^*_{farm} and P^*_{fish}. Design prevalence (P*) represents the assumed or expected level of infection in the population of fish farms.

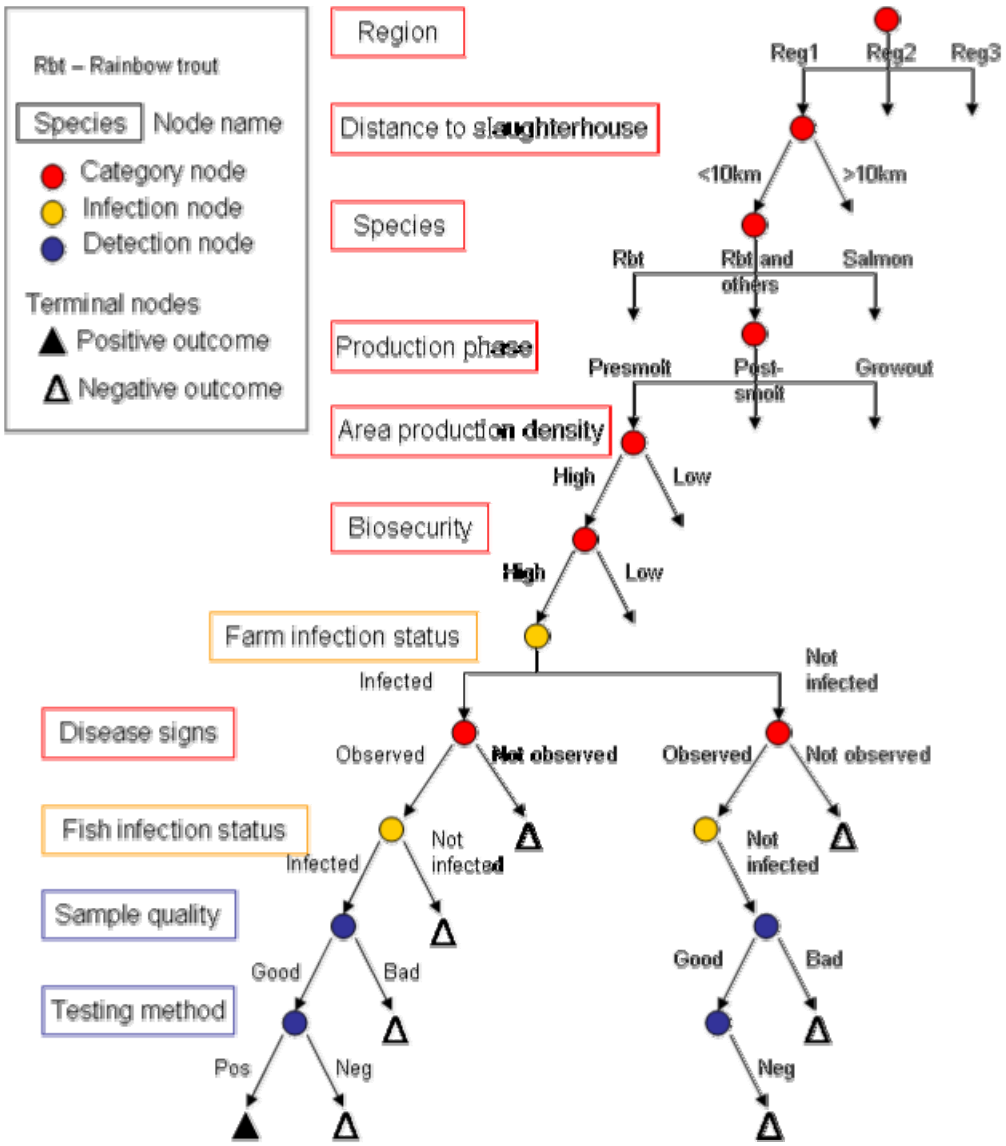

Figure 1. Scenario tree describing the model. Only one of the possible pathways is followed to the stage of farm infection status (the other main branches are identical in structure). The branches illustrate risk-based surveillance (sampling of fish with disease signs). The chance of getting a false positive test result is ignored (we assume 100% specificity) See Appendix I for description of the different nodes.

Risk category nodes at farm level:

- Region: All commercial fish farms are grouped into 3 regional groups based on proximity to areas where VHS has been reported either in commercial fish farms or in wildlife population: 1) Fish farms in the counties from south of Norway up to Møre and Romsdal, 2) Fish farms in the county of Trøndelag, and 3) Fish farms in the counties of Nordland, Troms and Finnmark.
- Distance to slaughter and processing plant: All commercial fish farms were grouped into two groups, where one group consisted of farms located within a seaway distance of 10 km from fish slaughter- and processing plant and the other group farms located in farther distance. Data on seaway distance to the nearest slaughterhouse was obtained from Kristoffersen et al. 2008. Distance to slaughter and processing plant was included due to discussions in the expert group. Moreover, the increased risk of infection due to a closed distance to slaughter plants was presented in a study of Infectious Salmon Anemia (ISA) in sea sites of farmed Atlantic salmons (Jarp and Karlsen, 1997).
- Species: VHS outbreaks have been reported mainly in farmed rainbow trout, but the virus has also affected other species including farmed Atlantic salmon. All commercial salmon fish farms were therefore grouped into three groups: 1) farms with rainbow trout only, 2) farms with both rainbow trout and Atlantic salmon, 3) farms with Atlantic salmon only. For the mixed populations, the relative species proportion was not taken into account.
- Production stage: Fish farms are grouped based on stage of production: pre-smolt, post-smolt, and on-growing. Pre-smolt is the freshwater production phase. Post-smolt is the seawater production phase during the first 3 months after seawater transfer. On-growing is the seawater phase after 3 months.
- Area production density: ¼ of salmon fish farms are placed in areas where the average fish density is 6925 kg/km^2 or more. We define these farms as being placed in a high area production density. Monthly estimates of area production densities for each of the sea locations was obtained by using a kernel density function (using the ArcView extension Spatial Analyst) to smooth out the biomass for all active sea farms on the Norwegian coast, and then extracting the biomass density of farmed salmonids surrounding each farm.
- Biosecurity level: Fish farms are grouped in two groups, high and low level of biosecurity due to discussions in the expert group. Although the effect of biosecurity for aquaculture facilities is not easily defined this node is kept in the model in order to try to test the effect of improved biosecurity level on the *SSCSE* of surveillance system..

Risk category nodes at within-farm level:

- Disease signs: The fish are grouped into two groups; fish with and without disease signs. The fish with disease signs is a combine of VHS-infected fish that develop observable disease signs and VHS-not infected fish that are sick and develop VHS-compatible disease signs. The proportion of VHS-infected fish that develop observable disease signs was estimated for the two groups (with and without observable clinical signs).

Infection nodes:

- Farm infection status: Farms are divided into two groups, one being VHS-infected the other not. We use design prevalence of 0.1, 0.2 and 0.3 % as an estimate of VHS prevalence on fish farm level (P^*_{farm}).
- Fish infection status: Fish on a farm is divided into two groups; one being VHS-infected the other not. We use design prevalence of 5 % as an estimate of VHS prevalence within a VHS infected farm (P^*_{fish}). This in accordance with the OIE Aquatic Animal Health Code (Anonymous 2009).

Detection nodes:

- Sample quality: The probability that representative fish and relevant organs are sampled, samples properly treated and transported to laboratory for relevant diagnostic purpose was estimated (combination of samples are correctly collected and sent).
- Test sensitivity for the detection of infection using histopathology, PCR and cell culture was estimated. Test specificity was assumed to be 100%.

Data on the population (the reference population) were obtained from industry statistics (see Appendix II). For data on the sea locations we used demographic information from the Norwegian salmonid fish production in 2009 (data compiled as described in Kristoffersen et al. 2008), and for land locations (hatcheries) we used data from the aquaculture license register of the Directory of Fisheries.

As input to the simulation model, the risk of having the infection in one group of farms compared to another group of farms (Relative Risk) was estimated from the expert opinions (see the expert opinion section) and included in the model as described by Martin et al. 2007 (Appendices I and III). At each risk

node we obtained adjusted risk (AR) by adjusting the relative risks of the specific risk categories so that the branch proportions add to 1 at the population proportions (ensures that the average risk for the reference population is 1).

Each of all the possible pathways/branches in the scenario tree (Fig. 1) ends with a positive or negative outcome. For each of these branches, the overall pathway probability is the product of all the conditional probabilities along the path. In the calculations we have assumed that there are no false positive outcomes due to imperfect specificity of the diagnostic tests (100% specificity).

For the whole tree, the probability to get a positive outcome, Pr(positive outcome), is then the sum of all the individual branch probabilities with positive outcomes. This is the probability that a chosen unit from the population will give a positive outcome (will be detected), given that the Norwegian fish production is infected, and is referred to as the Unit Sensitivity (*USe*) of the surveillance program tested.

With disease present at the assigned design prevalence, the surveillance system component sensitivity (*SSCSe*) is the probability that at least one fish in the in the surveillance system does not give a negative result, defined by

$$SSCSe = 1 - (1- USe)^n$$

where n is the number of samples tested during the chosen time period of one year.

Expert opinion

As data on relative risk of being VHS infected and being detected as VHS infected given VHS infection do not exist in Norwegian salmonid fish population, it is necessary to elicit expert opinion. A group of experts were asked to answer questions necessary to estimate relative risk of VHS among sub-populations (if hypothetically present in Norway), effectiveness of sample collection procedures, and accuracy of VHS diagnostic tests, and to provide their own level of expertise related to each question asked (See Appendix I, Sources for risk estimates). Information about sub-populations of each risk category node in the scenario tree and the difference in risk between them are needed for calculation of relative risk (RR). The RR was defined as the risk of having VHS in one sub-population compared with the risk of having VHS in the sub-population that has the lowest risk (usually denoted as the baseline group).

The experts were also asked to provide their opinion about VHS prevalence within an infected salmonid farm (if hypothetically present in Norway). Due to the high uncertainty and variability in the estimate, the design prevalence of 5% for within-farm prevalence was used to replace the experts estimate in the model.

The model is stochastic as it takes into account the uncertainty and variability of the model parameters. Uncertainty in the expert opinion was taken into account by asking them to provide the "minimum", "most likely" and "maximum" values for each question asked. We used a PERT distribution to describe the estimates from the experts. The estimates from each expert were then combined and weighted based on their provided level of expertise. Monte Carlo simulation using 1000 iterations was run to create a distribution for each input value. Our model results showed that the majority of expert opinion distributions are widely spread and non-symmetric (figures not shown here).

The tables in Appendix III present the expert opinion on each parameter needed in the model. We present the median, minimum, mean, and maximum values of the distributions and use the median as the representative value.

Results

Sensitivity for detection of VHS within an infected farm

Different surveillance strategies were evaluated in order to determine a strategy that provides at least 95% sensitivity of VHS detection with low cost. Factors influencing VHS detection within a farm were taken into consideration. This includes within-farm design prevalence, number of sampled fish per farm, choice of diagnostic test, and difference in risk of having VHS between groups of fish with and without disease signs.

Appendix VI Table A.1 shows that at 5% within-farm prevalence, targeted sampling of fish with disease signs (risk-based surveillance) yielded a considerable higher sensitivity than random sampling from all fish. PCR and histopathological examination yielded comparable results, and sampling 5 or 10 fish per farm were not sufficient to detect VHS with 95% sensitivity.

Appendix VI Table A.2 shows the increase in the sensitivity of VHS detection as sample size increased. The result shows that a minimum of 20 samples per farm yields a sensitivity of at least 95%, assuming that the farm is infected at 5% within-farm prevalence, targeted sampling of fish with disease signs is used, all samples are properly collected and sent, and PCR is the testing method.

System surveillance component sensitivity (*SSCSe*)

The surveillance system component sensitivity (*SSCSe*) was estimated for different surveillance strategies to determine the optimal cost-effective surveillance strategy for detecting a VHS-infected farm, if hypothetically present in Norway. The optimal cost-effective surveillance strategy should yield at least 95% SSCSe with low cost.

Appendix VI Table B.1 shows the results of *SSCSe*. The first strategy includes all farms into the surveillance system, and for each farm a minimum of 20 samples from fish with disease signs are properly collected, sent to laboratories, and tested with PCR. The second strategy follows the same farm selection process, except that random sampling is used instead of targeting fish showing clinical signs of disease. Given that the same number of samples is tested for both strategies, the first strategy has a higher *SSCSe*.

Appendix VI Table B.2 shows the comparison between surveillance strategies focusing on risk of VHS associated with species. Farms rearing rainbow trout only or farms rearing mixed species have a higher risk of having VHS than farms with only salmon.

If we assume that there is one -1- VHS infected farm in Norway, the *SSCSe* of a surveillance strategy targeting mainly on rainbow trout and mixed species farms shows that this strategy is not sufficient to provide the 95% SSCSe (SSCSe of 80%-85%). This is likely due to the small number of rainbow trout and mixed species farms in the system resulting in an inadequate number of samples tested (less than 10000 samples per year).

Appendix VI Table B.3 and table B.4 show the *SSCSe* estimates based on the assumption that there is two -2- and three -3- infected farms in the population, respectively. The results show at least 97% *SSCSe* when 20 samples per farm were used, and 99% *SSCSe* when 30 samples per farm were used. This surveillance strategy focuses on targeting species with high risk and targeting fish with disease signs, as well as assuming that all samples are properly collected, sent, and tested with PCR. A smaller number of samples is needed for this surveillance strategy (9740 samples per year) compared with sampling from all farms (20480 samples per year).

Discussion

The main results from evaluation of the surveillance programme for VHS in the Norwegian salmonid farming industry show that risk-based surveillance targeting sampling of fish with disease signs gives a high probability of detecting the disease. The surveillance system component sensitivity, *SSCSe,* is dependent on both the number of samples tested per farm, the number of farms included in the surveillance programme and the design prevalence (i.e., the hypothetically prevalence of disease that the surveillance program is assessed against).

The number of samples investigated within an infected farm is important. Risk-based sampling targeted towards fish showing clinical signs compatible with the disease gives a higher probability of detecting VHS than if the sampling is based on random selection of individuals. At least 20 samples per farm annually are needed in order to detect VHS with an appropriate probability. Investigating only 5 or 10 samples is not sufficient to detect VHS with 95% probability. In the present model the time period of analysis is one year, and it is assumed that the risk of obtaining VHS is the same throughout the year (assuming that sampling is carried out when the water temperature is below 14^{o}C). A production cycle could however be more appropriate and will be tested in future work. Furthermore, the probability of detecting disease within an infected farm is almost the same whether we use PCR or histopathology, and the sensitivity will be

improved if we assume that the samples are properly collected and sent to the laboratory. Cell culture, being the test method considered as the gold standard for VHS, takes a long turnaround time and has a slightly lower sensitivity than PCR. Cell culture therefore yielded a lower *SSCSE* (results not shown here).

Early detection is emphasized in the model by the choice of a low within-farm design prevalence of 5%. By using this low level of prevalence we allowed the new surveillance programme to detect the infection at its early spread ($\geq$5% within-herd prevalence) within an infected farm.

When all farms are included and the risk-based surveillance is targeted at within-farm level only, a large number of samples (20480 samples per year) are needed in order to obtain an appropriate high probability of detecting VHS, if present. This strategy was compared with a sampling strategy where risk-based sampling was also targeted at farm level. We show that if the surveillance is limited to sea sites with rainbow trout and mixed species, the *SSCSe* is only 80-81% assuming there is at least 1 infected farm in the salmon farming population (a design prevalence of 0.1%). This is likely due to the small number of rainbow trout and mixed species farms in the system resulting in inadequate number of samples tested (less than 10000 samples per year). If 10% and 20 % of the salmon farms are added to the surveillance programme the *SSCSe* is slightly improved to 81% and 85%, respectively (Appendix IV, Table B.2). In this model, the design prevalence of 0.1% VHS-infected farms was used. However, if we instead allow the design prevalence to be 0.2% (assuming there are at least 2 infected farms in the salmon farming population), we obtain a *SSCSe* of at least 95% and a considerable smaller number of samples are required to be investigated (Appendix IV, Table B.3).

Risk-based surveillance is effective to increase the efficiency of a surveillance system, provided the disease under consideration is less common in the general population than in the targeted group and specific risk factors are known. In our model, the large uncertainty in the relative risk estimates of the risk groups illustrates that the risk factors for VHS are not well established in Norwegian salmonid industry. The wide uncertainty in the relative risk estimates indicates that by removing the risk categories not currently used in the model we might reduce the large uncertainty and simplify the scenario tree. This is because, the more variables (with associated uncertainty distributions) that are included, the more uncertain the model output will be. Moreover, this uncertainty maybe due to the experts not being so familiar with VHS in Norwegian salmonid farming environment and that knowledge on risk factors associated to VHS genotype 3 in rainbow trout is scarce.

In the future, we can test a risk-based surveillance strategy targeting farms with increased mortality in addition to targeting fish with disease signs. This may closer reflect the on-going risk-based health control program currently implemented, where a larger proportion of samples are likely to be taken from farms with increased mortality level.

The model presented here can also be used in the risk-ranking of farms that is required by the new directive 2006/88/EEC, which requires that all farms be ranked according to their risk of obtaining a specific disease. The expert group has thus identified important risk factors that can be evaluated for each farm when grouping them into the five different risk-categories.

The present surveillance programme is based on isolation of VHS virus in cell culture followed by virus identification using antibody-based methods (IFAT, ELISA) or nucleic acid based methods (e.g. reverse-transcription polymerase chain reaction [RT-PCR]). According to the OIE 2010 Manual of Diagnostic tests for Aquatic Animals, "PCR-based technology using direct identification of the VHS virus genome in fish tissue has yet to be validated for use in direct surveillance programmes for obtaining approved VHS-free status".

In 2009, the programme included rainbow trout only and involved testing of 200 pooled samples from approximately 50 farms by cell culture. Cost per sample are NOK 3831 (including 25% VAT). Prior to 2009, the programme included other susceptible species as Atlantic salmon. In 2008, 1398 pooled samples (= 13 980 individual fish) from 444 farm were tested at a total cost of more than NOK 1,880,000 (25% VAT included).

Cell culture takes time and is labour intensive. In addition, samples for cell culture must arrive at the laboratory within 72 hours, a factor which severely restricts days available for field sampling. Sampling for PCR will be easier to plan as samples can be preserved in RNA stabilizing liquids, such as RNAlater®. If samples for VHS and IHN surveillance can be collected during episodes of increased mortality (in addition to diagnostic material), stored and analysed in large batches the cost per individual sample will be approximately 1/10 of cell culture of a pooled sample (Appendix V).

The cost of surveillance mainly depends on the methods used and the time available. Running PCR for VHS virus on a few, large batches of individual samples will be better cost effective than the present programme using cell culture of pooled samples. Using PCR on large batches; however, does not allow for a rapid reply as samples must be collected over time. On the other hand, PCR analysis of small batches of samples will provide quick replies, but not improve cost-efficiency.

Risk-based surveillance as shown for VHS in the present model is also applicable for IHN given that farms with Atlantic salmon are included in the sampling scheme.

Conclusion

We have compared different surveillance and sampling regimes to determine a cost-effective surveillance strategy for the detection of VHS in the Norwegian salmonid farming population. In general, a cost-effective strategy should yield at least the same probability of detecting the disease as the current strategy does, at a lower cost. In our model we aimed to find a strategy that yielded at least 95% probability of detecting VHS-positive fish given the infection is present in the Norwegian farmed fish population.

The present model shows that if surveillance is risk based, we have a high probability of detecting disease. However, the surveillance system component sensitivity is dependent on the number of samples within farms and the design prevalence (i.e., the hypothetically prevalence of disease that the surveillance program is assessed against). If the surveillance strategy is targeted towards rainbow trout farms, a minimum of 20 samples per farm from fish with disease signs will be needed for detection of VHS-infected farm (design prevalence of 5 % within-farm prevalence). Furthermore, a minimum number of 487 farms will be needed for detection of VHS if there are at least 2 infected farms in the salmonid farming population (design prevalence of 0.2 % farm prevalence). Because there are too few farms with rainbow trout, 20% of salmon farms have to be added in the strategy to achieve at least 95% sensitivity.

Cost considerations indicate that running PCR for VHS virus on a few, large batches of samples will be better cost-effective than the present programme using cell culture.

The model presented is targeted towards VHS. Historically, IHN has been included as part of the same sampling regime as VHS. The results and conclusions in this model may therefore be relevant also for IHN but this need to be validated.

Although we believe that this model will give a good breakdown structure of the various components of the surveillance of VHS, further evaluation of the values given and the effect of possible simplification of the tree needs to be investigated. A refinement of the present model will therefore be carried out as part of an ongoing research project (NFR project no 190245).

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References

Anonymous (2009) OIE Aquatic Animal Health Code 2009. http://www.oie.int/eng/normes/fcode/en_sommaire.htm, accessed 30.08.2010

Anonymous (2009) OIE Manual of Diagnostic Tests for Aquatic Animals 2010 http://www.oie.int/eng/normes/fmanual/A_summry.htm?e1d11, accessed 01.09.2010

Cameron AR. Risk-based diseases surveillance: a manual for veterinarians. The Food and Agriculture Organization of the United Nations (FAO). 2009

Commission Decision 2001/183/EC of 22 February 2001 laying down the sampling plans and diagnostic methods for the detection and confirmation of certain fish diseases

Council Directive 2006/88/EC of 24 October 2006 on animal health requirements for aquaculture animals and the products thereof, and on the prevention and control of certain diseases in aquatic animals

Dale OB, Ørpetveit I, Lyngstad TM, Kahns S, Skall HF, Olesen NJ and Dannevig BH. Outbreak of viral haemorrhagic septicaemia (VHS) in sea-water farmed rainbow trout in Norway caused by VHS virus genotype III. Dis Aquat Organ. 2009 Jun 10; 85(2):93-103

EFTA Surveillance Authority Decision No. 302/08/COL of May 2008

EFTA Surveillance Authority Decision No. 71/94/COL of June 1994

Jarp J. and Karlsen E. Infectious salmon anaemia (ISA) risk factors in sea-cultured Atlantic salmon *Salmo salar*.Dis Aquat Organ, 1997; 28: 79-86

Kristoffersen, A. B., H. Viljugrein, R. T. Kongtorp, E. Brun, and P. A. Jansen. 2009. Risk factors for pancreas disease (PD) outbreaks in farmed Atlantic salmon and rainbow trout in Norway during 2003-2007. Prev. Vet. Med. 90:127-136

Martin PAJ, Cameron AR, Greiner M. Demonstrating freedom using multiple complex data sources 1: A new methodology based on scenario trees. Prev Vet Med. 2007; 79; 71-97

Skall, H. F., N. J. Olesen, and S. Mellergaard. 2005. Viral haemorrhagic septicaemia virus in marine fish and its implications for fish farming--a review. J. Fish. Dis. 28:509-529

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Appendix I

Table of nodes, node type, node name, branch name, sources and dependency

Node	Node type	Node name	Branch name	Sources for proportions	Sources for risk estimates
1	Risk category	Region	Region 1 Region 2 Region 3	Industry statistics*	Expert opinion
2	Risk category	Distance to slaughterhouses	< 10 km > 10 km	Industry statistics*	Expert opinion
3	Risk category	Species	Rainbow trout Mixed Salmon	Industry statistics*	Expert opinion
4	Risk category	Production stage	Pre-smolt Post-smolt On-growing	Industry statistics*	Expert opinion
5	Risk category	Area production density	High density Low density	Industry statistics*	Expert opinion
6	Risk category	Biosecurity level	High biosecurity Low biosecurity		No data
7	Infection	Farm status	Infected Not infected	Design prevalence	
8	Infection	Fish status	Infected Not infected	Expert opinion Expert opinion	
9	Detection	Disease signs	Disease signs No disease signs	Expert opinion -	
10	Detection	Sample quality	Good sample Bad sample	Expert opinion Expert opinion	
11	Detection	Histopathology	Positive Negative	Expert opinion Expert opinion	
12	Detection	PCR	Positive Negative	Expert opinion Expert opinion	
13	Detection	Culture	Positive Negative	Expert opinion Expert opinion	

* Demographic information from the Norwegian salmon fish production in 2009 (data compiled as described in Kristoffersen et al. 2008).

Appendix II

Table showing the population proportion of fish farms by risk category

Risk category	Branch name		Number of fish farms*	Population proportion**
Region	Region 1		440	0.43
	Region 2		200	0.20
	Region 3		384	0.38
Species	Region 1	Rainbow trout	32	0.03
		Atlantic salmon	255	0.25
		Mixed	153	0.15
	Region 2	Rainbow trout	8	0.01
		Atlantic salmon	131	0.13
		Mixed	61	0.06
	Region 3	Rainbow trout	7	0.01
		Atlantic salmon	286	0.28
		Mixed	91	0.09
Production stage	Region 1	Pre-smolt	146	0.14
		Post-smolt	65	0.06
		On-growing	229	0.22
	Region 2	Pre-smolt	56	0.05
		Post-smolt	32	0.03
		On-growing	112	0.11
	Region 3	Pre-smolt	82	0.08
		Post-smolt	66	0.06
		On-growing	236	0.23
Distance to slaughterhouses	Region 1	Within 10km	94	0.13
		Outside 10km	191	0.27
	Region 2	Within 10km	35	0.05
		Outside 10km	99	0.14
	Region 3	Within 10km	60	0.08
		Outside 10km	230	0.32

* All hatcheries registered in the aquaculture licence register of the Directory of Fisheries were assumed to be active.

** For the risk categories “Distance to slaughterhouses” and “Area production density” the proportion of fish farms is based on sea locations only (hatcheries excluded).

Cont. Appendix II

Proportion of sites by risk category	Branch name		Number of fish farms	Population proportion*
Area production density	Region 1	High	64	0.09
		Low	228	0.31
	Region 2	High	40	0.05
		Low	104	0.14
	Region 3	High	66	0.09
		Low	236	0.32

* For the risk categories "Distance to slaughterhouses" and "Area production density" the proportion of fish farms is based on sea locations only (hatcheries excluded).

Appendix III

The tables below present the expert opinion on each input needed in the model. The majority of expert opinion distributions are widely spread and non-symmetric (figures not shown here). We, therefore, present the median, minimum, mean, and maximum values of the distribution, and use median as the representative value.

Table 1: The relative risk (RR) of VHS among different regions. Farms located in region 3 has the lowest risk of having VHS. Farms in Region 1 have 2 times higher risk of having VHS than Farms in region 3. Farms in Region 2 have 1.3 times higher risk of having VHS than Farms in region 3.

Region	Median	Minimum	Mean	Maximum
Region 1	2	1	3	16
Region 2	1.3	1	1.5	4
Region 3	1	1	1	1

Table 2: The relative risk (RR) of farms located within a seaway distance of 10 km from fish slaughter- and processing plant and the other group farms located in farther distance (Outside 10 km). Farms located within a seaway distance of 10 km have 2.4 times higher risk of having VHS than farms located in farther distance.

Distance to slaughter and processing plant	Median	Minimum	Mean	Maximum
Within 10km	2.4	1	2.7	8.6
Outside 10km	1	1	1	1

Table 3: The relative risk (RR) of VHS among farms rearing rainbow trout only, mixed species including rainbow trout and salmon, and salmon only. Farms with salmon only have the lowest risk. Farms with mixed species have 14 times higher risk of having VHS than farms with salmon only.

Species	Median	Minimum	Mean	Maximum
Rainbow trout	13	1	13	42
Mix	14	1	16	67
Salmon	1	1	1	1

Table 4: The relative risk (RR) of VHS related to production stages; pre-smolt, post-smolt and on-growing farms. There is a slight difference in the risk of having VHS among farms among different production stage. The pre-smolt and post-smolt farms showed protective effect compared with on-growing farm.

Production stage	Median	Minimum	Mean	Maximum
Pre-smolt	1	0.02	1	3
Post-smolt	1.3	0.25	1.6	5
On-growing	1	1	1	1

Table 5: The relative risk (RR) of VHS related to area production density; high and low (baseline group). Farms in high production density have 2.5 times higher risk than farms in low production density area.

Area production density	Median	Minimum	Mean	Maximum
High	2.5	1	3	9
Low	1	1	1	1

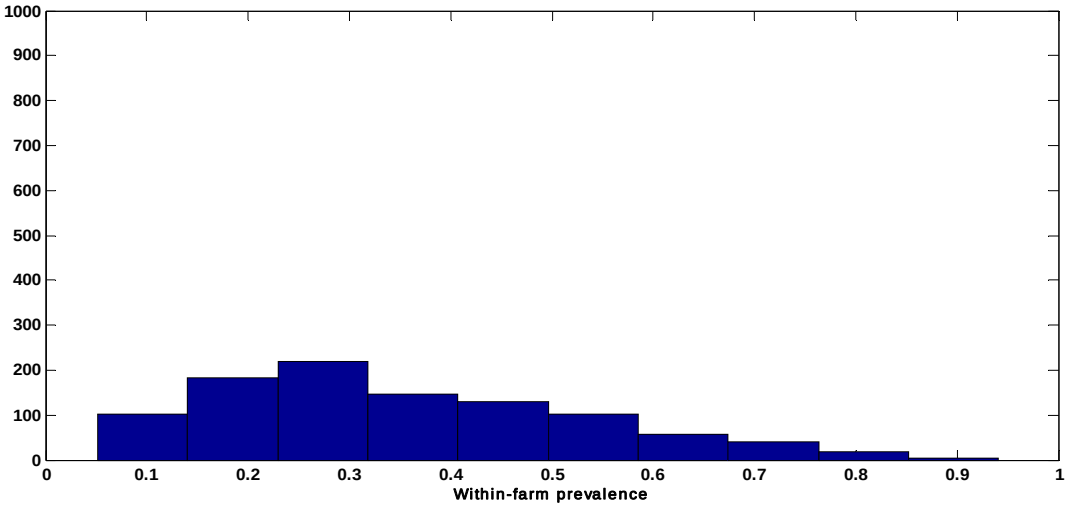
Table 6: The relative risk (RR) of VHS related to the biosecurity level; low and high (baseline group). The model assumes that farms with low level of bio-security have 2 times higher risk than farms with high level of bio-security.

Biosecurity	Median	Minimum	Mean	Maximum
Low	2	2	2	2
High	1	1	1	1

Table 7: The relative risk (RR) of fish having disease signs. This is the risk category node at fish level. Within an infected farm, fish are categorized into 2 groups: fish with disease signs; and fish without disease signs. The probability to observe disease signs is assumed to be dependent on the production stage. In post-smolt and on-growing farms, fish with disease signs have 11 times higher risk of having VHS than fish without disease signs.

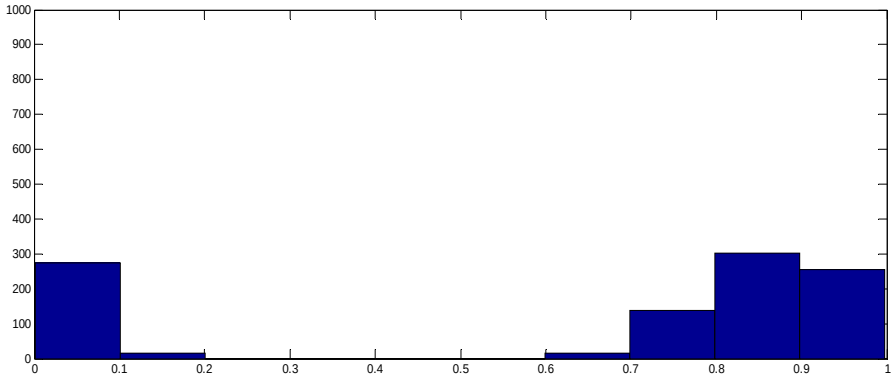
Production stage	Relative risk (RR)	Median	Minimum	Mean	Maximum
Pre-smolt	Disease signs	13	1	19	145
	No disease signs	1	1	1	1
Post-smolt	Disease signs	11	1	12	30
	No disease signs	1	1	1	1
On-growing	Disease signs	11	1	12	85
	No disease signs	1	1	1	1

Table 8: The figure shows the expert opinion on prevalence of VHS within an infected farm. The estimate ranges from 5% to 94%. Due to the high uncertainty of the expert opinion, the design prevalence of 5% replaced the expert opinion for the within-farm prevalence.



	Minimum	Median	Maximum
Prevalence of VSH-infected fish within a farm	0.05	0.31	0.94

Table 9: The figure shows the expert opinion on the proportion of samples that are properly collected and sent to laboratories (good sample). The result shows 2 separate opinions: high and low proportions of good samples.



	Median	Minimum	Mean	Maximum
Good sample	0.82	0.0015	0.62	0.997

Table 10: The expert opinion on the test sensitivity (se) of PCR, histopathology and cell culture. Overall, PCR has the highest sensitivity with a wider distribution ranging from 0.32 to 1.

Sensitivity (se)	Minimum	Median	Maximum
PCR	0.32	0.95	1
Histopathology	0.58	0.85	0.997
Cell culture	0.55	0.95	0.998

Table 11: The expert opinion on the test specificity (sp) of PCR, histopathology and cell culture. Both PCR and cell culture have very high test specificity (at least 0.97) compared, with histopathology with sp of 0.89.

Specificity (sp)	Minimum	Median	Maximum
PCR	0.82	0.97	1
Histopathology	0.63	0.89	1
Cell culture	0.69	0.98	1

Appendix IV

Results of the surveillance sensitivity for detection of VHS within an infected farm and the surveillance system component sensitivity (*SSCSe*) are presented in this section.

A. The sensitivity for detection of VHS within an infected farm

Table A.1. Sensitivity (%) for detection of VHS within an infected farm (the design prevalence is 5%, and the time period is one year)

Stage	Number of samples per farm and year	Test	Sensitivity (%) based on targeting fish with disease signs	Sensitivity (%) based on random sampling
Pre-smolt	5	PCR	40	26
	5	HP	41	25
	10	PCR	64	45
	10	HP	65	44
	30	PCR	95	83
	30	HP	96	82
Post-smolt	5	PCR	37	26
	5	HP	35	24
	10	PCR	61	46
	10	HP	57	43
	30	PCR	94	84
	30	HP	92	81
On-growing	5	PCR	35	34
	5	HP	35	23
	10	PCR	57	42
	10	HP	58	41
	30	PCR	92	80
	30	HP	93	80

Table A.2. Sensitivity (%) for detection of VHS within an infected farm assuming that all samples are properly collected and sent, within-farm design prevalence of 5%, targeting on fish with disease signs, and testing using PCR.

Production stage	Number samples per farm	sensitivity (%)
Pre-smolt	5	57
	10	82
	20	97
	30	99
Post-smolt	5	58
	10	82
	20	97
	30	99
On-growing	5	58
	10	82
	20	96
	30	99

B. The surveillance system component sensitivity (SSCSe) based on different surveillance strategies

Table B.1 Comparison of *SSCSe* results between targeting of fish with disease signs and random sampling, assuming there is 1 infected farm in the population, within-farm prevalence of 5%, samples properly collected, sent, and tested with PCR

Surveillance strategy	Total no. of samples per year	*SSCSe* (%)
1) Include all farms and test fish with disease signs within the farms	20480 (20 samples x 1024 farms)	96
2) Include all farms and test random fish within the farms	20480 (20 samples x 1024 farms)	85

Table B.2 Comparison of *SSCSe* results between 4 different surveillance strategies targeting on risk associated with species, assuming there is 1 infected farm in the population, within-farm prevalence of 5%, targeted sampling of fish with disease signs, samples properly collected, sent, and tested with PCR

Surveillance strategy	Total no. of samples per year	*SSCSe* (%)
Include all farms	20480 (20 samples x 1024 farms)	96
Include all rainbow trout and mixed species farms and exclude all salmon farms	7040 (20 samples x 352 rainbow trout and mixed species farms)	80
Include all rainbow trout and mixed species farms and include 10% of salmon farms	7800 (20 samples x 352 rainbow trout and mixed species farms, and 20 samples x 68 salmon farms)	81
Include all rainbow trout and mixed species farms and include 20% of salmon farms	9740 (20 samples x 352 rainbow trout and mixed species farms, and 20 samples x 135 salmon farms)	85

Table B.3 Estimation of *SSCSe* for surveillance strategies assuming that there is 2 infected farms in the population, within-farm prevalence of 5%, targeted sampling of fish with disease signs, samples properly collected, sent, and tested with PCR

Surveillance strategy	Total no. of samples per year	*SSCSe* (%)
Include all rainbow trout and mixed species farms and include 20% of salmon farms	9740 (20 samples x 352 rainbow trout and mixed species farms. and 20samples x 135 salmon farms)	97
Same as above	14610 (30 samples x 352 rainbow trout and mixed species farms, and 30 samples x 135 salmon farms)	99

Table B.4 Estimation of *SSCSe* for surveillance strategies assuming that there is 3 infected farms in the population, within-farm prevalence of 5%, targeted sampling of fish with disease signs, samples properly collected, sent, and tested with PCR

Surveillance strategy	Total no. of samples per year	*SSCSe* (%)
Include all rainbow trout and mixed species farms and include 20% of salmon farms	9740 (20 samples x 352 rainbow trout and mixed species farms, and 20 samples x 135 salmon farms)	98
Same as above	14610 (30 samples x 352 rainbow trout and mixed species farms, and 30 samples x 135 salmon farms)	99

Appendix VI

Table showing calculated cost of analysis for different methods

Surveillance strategy	Total no. of samples per year	Cost NOK per sample	Cost NOK per fish
Random by cell culture 2008	1398*	1345*	135
Random by cell culture 2009	200*	3831*	383
Risk based by PCR	10000** (9740)	300***	300***

*Pooled samples 10 by 10 fish
** Individual samples
***Price for PCR presumes analysing large batches

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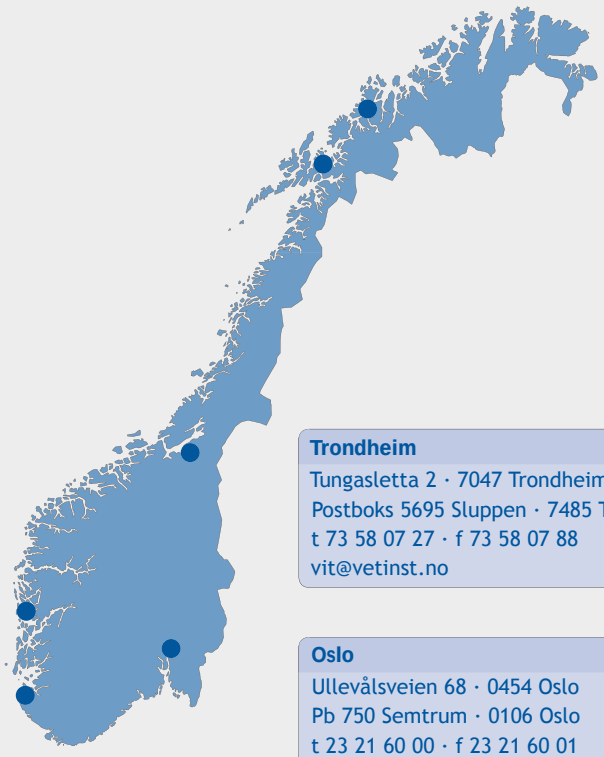

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