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Validation of a model for ranking aquaculture facilities for risk-based disease surveillance

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1 Abstract

A semi-quantitative model for risk ranking of aquaculture facilities in Switzerland with regard to the introduction and spread of Viral Haemorrhagic Septicaemia (VHS) and Infectious Haematopoietic Necrosis (IHN) was developed in a previous study (Diserens et al., 2013). The objective of the present study was to validate this model using data collected during field visits on aquaculture sites in four Swiss cantons compared to data collected through a questionnaire in the previous study. A discrepancy between the values obtained with the two different methods was found in 32.8% of the parameters, resulting in a significant difference ($p<0.001$) in the risk classification of the facilities. As data gathered exclusively by means of a questionnaire are not of sufficient quality to perform a risk-based surveillance of aquaculture facilities a combination of questionnaires and farm inspections is proposed. A web-based reporting system could be advantageous for the factors which were identified as being more likely to vary over time, in particular for factors considering fish movements, which showed a marginally significant difference in their risk scores ($p\geq0.1$) within a six- month period. Nevertheless, the model proved to be stable over the considered period of time as no substantial fluctuations in the risk categorisation were observed (Kappa agreement of 0.77).Finally, the model proved to be suitable to deliver a reliable risk ranking of Swiss aquaculture facilities according to their risk of getting infected with or spreading of VHS and IHN, as the five facilities that tested positive for these diseases in the last ten years were ranked as medium or high risk. Moreover, because the seven fish farms that were infected with Infectious Pancreatic Necrosis (IPN) during the same period also belonged to the risk categories medium and high, the classification appeared to correlate with the occurrence of this third viral fish disease.

Key words: fish, disease, risk ranking, risk-based surveillance, Viral Haemorrhagic Septicaemia, Infectious Haematopoietic Necrosis, Infectious Pancreatic Necrosis

2 Introduction

Risk-based disease surveillance aims to identify surveillance needs to protect the health of livestock and consumers, thus setting priorities and allocating resources effectively and efficiently (Stärk et al., 2006). Over recent years there have been considerable methodological developments in the field of risk-based disease surveillance in terrestrial animals, and these have demonstrated the substantial potential for cost savings. A similar benefit is also expected for aquatic animals (Oidtmann et al., 2013), though such risk-based surveillance approaches were, until recently, largely inexistent for aquatic animal diseases. On the first of August 2008, the Council Directive 2006/88/EC on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals (Anon., 2006) became operative in all countries of the European Union (EU). This directive demands a risk-based surveillance of aquaculture facilities in relation to their risk of contracting and spreading notifiable diseases.

Consequent to the entering into force of the Council Directive 2006/88/EC, several models for risk ranking of aquaculture facilities have either been proposed in the scientific literature (Oidtmann et al., 2011; Diserens et al., 2013), or were presented in the non-peer-reviewed literature (Kleingeld, 2010). While most authors focused on Viral Haemorrhagic Septicaemia (VHS), an economically important viral fish disease, particularly in the northern hemisphere (Jensen et al., 2014), other model proposals also included Infectious Salmon Anaemia (ISA) (Tavornpanich et al., 2013) or Infectious Haematopoietic Necrosis (IHN) (Diserens et al., 2013).

The main constraint to the application of risk analysis in aquatic animal health is lack of data (Peeler et al., 2007). In particular, lack of published data on risk factors, diagnostic test performance, or the expected prevalence in infected populations pose a challenge to the design of aquatic risk-based surveillance strategies (Oidtmann et al., 2013). To overcome this problem, expert opinion can be integrated within the epidemiologic assessment (Gustafson et al., 2013). This procedure is often required to develop a model for risk-based surveillance of aquaculture facilities (Oidtmann et al., 2014a). However, while expert panels can offer timely decision support in the absence of empirical data (Gustafson et al., 2014), a validation of such expert-based models using questionnaire or field collected data is often necessary. These data allow to verify that those fish farms with a high risk classification are also the ones which in reality experience an epizootic outbreak or spread pathogens. Furthermore, to implement the benefits from a risk-based approach, farm data collection must be cost-effective (Oidtmann et al., 2014b), but also of sufficient quality to ensure a correct risk classification of the farms. Moreover, the parameters assessed in the risk classification should be stable over time, as seasonal fluctuations in these parameters would result in repeated changes of the risk classification several times a year.

We previously presented a semi-quantitative model for risk ranking aquaculture facilities in Switzerland (Diserens et al., 2013). This model was developed specifically for the surveillance of VHS and IHN, given their importance and the obligation to notify these diseases in both Switzerland and the EU. Infectious Pancreatic Necrosis (IPN) was not considered during the development of the model since, in contrast to Switzerland, the disease is no longer notifiable in the EU. Viral Haemorrhagic Septicaemia is a disease affecting primarily rainbow trout (*Oncorhynchus mykiss*). Besides the acute infection with rapid onset of mortality, the infection can also be chronic where affected fish do not generally exhibit external signs (World Organisation for Animal Health (OIE), 2015a). Infectious Haematopoietic Necrosis is a viral disease which affects most species of salmonid fish. The main clinical and economic consequences of IHN occur on farms rearing rainbow trout, where acute outbreaks can result in very high mortality among younger fish, whereas older fish are typically more resistant to clinical disease (OIE, 2015b). Infectious Pancreatic Necrosis is a major cause of mortality in freshwater salmonids, especially in brook trout

(*Salvelinus fontinalis*) and rainbow trout, whereby only young fish become clinically ill and die, but all fish can become infected and remain chronic carriers of the virus (Noga, 2010). The primary objective of this study was to validate the risk ranking model developed within the context of a previous study (Diserens et al. 2013) using data collected during field visits on the aquaculture sites, and to identify those risk factors that are more likely to vary over time. A secondary objective was to assess how the model performs in classifying fish farms for the risk of IPN introduction or spread. The rationale was to assess the model's suitability for performing risk-based surveillance of aquaculture facilities in Switzerland. Additionally, the challenges in applying a risk-based method for fish disease surveillance are described, together with the advantages and disadvantages of the different approaches currently available to obtain the data necessary to perform a risk assessment.

3 Materials and Methods

3.1 The model

In this study we apply the model of Diserens et al. (2013), which was developed to perform a risk-based classification of aquaculture facilities with regards to the specific situation of fish farming in Switzerland. This model considered six risk factors for the introduction of VHS and IHN (i.e. (i) susceptibility of the fish species kept, (ii) type of water supply, (iii) overall purchase [based on the frequency of purchases and number of suppliers], (iv) number and distance of fish farms in the vicinity, (v) processing activities and (vi) biosecurity measures). For the spread of the two diseases the model comprised seven factors (i.e. (i) susceptibility of the fish species kept, (ii) type of water runoff, (iii) type of fish sales, (iv) number and distance of fish farms in the vicinity, (v) risk of flooding, (vi) soil type and (vii) biosecurity measures). In the previous study, data were collected through a voluntary questionnaire that was sent to all known Swiss fish farms. Farmers who did not return the questionnaire on time were contacted by phone. Thereby a 95% return rate was achieved. All the main aquaculture facilities took part in the survey, and the 17 farmers who did not respond were mainly hobby farmers or only ran a fish farm as a side-line activity. These questionnaire data were then used to categorize each individual risk factor using a scale from 0 to 4 (where 0, 1, 2 and 4 corresponded to a null, low, medium or high risk). The scores for the risk of introduction and spread were then calculated by summing up the individual scores and dividing the total by six and seven, respectively. The overall risk categorization score was then obtained by summing up the scores for the risk of disease introduction and the risk of disease spread. This resulted in risk scores between 0.5 and 6.3 depending on the farm. In order to perform a risk-based surveillance of aquaculture facilities, the obtained risk scores were converted into risk categories (low, medium, high). For this purpose, two theoretical trout farms, supplied solely or partly with surface water and possessing a water runoff into surface waters (the typical fish farm type in Switzerland), were defined as the upper limit for low-risk farms and the upper limit for medium-risk farms respectively. These two farms were characterized as follows: as flooding is quite a rare incident, a null risk for this factor was attributed to each of the two farms, and because processing of fish from other farms applies to only a few plants in Switzerland, a null risk was attributed for this factor as well. For all other factors, a low risk was set for the model farm corresponding to the upper limit of a low risk, and a medium risk was set for the model farm corresponding to the upper limit of a medium risk.

In the present study, a few modifications were made to some of the risk factors considered. In the original model, “risk of flooding” was based on whether the farm had experienced flooding in the past. Therefore, when no flooding had occurred, the risk was categorized as "null", whereas if the farm had experienced flooding the risk "low" was attributed. However, since a new owner of an aquaculture facility may not be aware of previous flooding and as structural measures may largely influence this risk, the factor was re-categorized by taking into consideration the flooding hazard maps of each Swiss canton (Anon., 2016). These maps classify geographic areas into the following categories: no or negligible hazard, residual hazard, minor hazard, medium hazard and considerable hazard. Therefore, if the flooding hazard for the area of the fish farms was depicted as negligible, residual or minor, the fish farms were classified as having "null risk of flooding", whereas if the flooding hazard was depicted as medium or considerable, the fish farms were classified as having a "low risk of flooding".

Since the data for the risk factor "biosecurity", which consisted of eight individual sub-factors [presence of foot bath, disinfection equipment, disinfection of vehicles, fencing, access control, bird control, presence of gate and quarantine strategies], could not be collected by questionnaire during the previous study, the maximal risk value for this factor was given to all aquaculture facilities. However, in the present study such biosecurity measures could be assessed during the field visits and individual risk scores were therefore assigned.

3.2 Data collection

Four pilot cantons with a representative composition of Swiss aquaculture facilities were chosen for the validation of the model: Bern (48 fish farms), Vaud (38), Valais (26) and Zürich (17) (Figure 1), in total 129 farms. These cantons were selected because they represent approximatively 40% of all known Swiss aquaculture facilities and include both types of fish farms: food fish production (including aquaculture production businesses, small hobby facilities, recirculation systems and angling ponds) and production of fish for stocking of rivers and lakes (including cantonal fish farms and aquaculture farms owned by fisheries societies). These 129 fish farms were visited once either during autumn 2012 or spring 2013 to collect the necessary data (including data related to the factor “biosecurity”). All visits within the framework of the project were performed by the same fish veterinarian, and the data were collected with a form specifically developed for this purpose.

To verify whether the parameters being considered to determine the farm risk categorization are stable over time, 30 of the 129 fish farms were inspected twice, once in autumn 2012 and once again in spring 2013. To avoid that a canton was over- or under-represented, the proportion of facility numbers per canton within the 30 farms that were inspected twice reflected the overall farm numbers of the respective cantons. Furthermore, the facilities were classified in the following categories: aquaculture production businesses, small hobby facilities, recirculation systems, angling ponds, cantonal fish farms and aquaculture farms of fisheries societies. The facilities were then selected such that all fish farm types were proportionally represented. Lastly, the selection of the individual farms within all farms of a farm type was performed randomly in an Excel spreadsheet (Microsoft Office Excel©, 2010) where a number was randomly assigned to all facilities and the smallest number was selected each time.

3.3 Sampling

To investigate the relationship between the risk classification based on the model and effective epizootic outbreaks, the VHS- and IHN-outbreaks of the 129 inspected farms since 2004 were considered. Furthermore, fish samples of species susceptible for these diseases were taken during the inspection of those farms that met one or more of the following criteria: i) suspicion of an epizootic outbreak based on visual inspection of fish observed during a walk-through inspection of the farm (no farm met this criterion); ii) farm had experienced a VHS- or IHN-outbreak from 2000 onwards (five aquaculture facilities); and iii) farm was sampled in 2000/2001 within the context of a study to estimate the prevalence of viral fish diseases in Switzerland (Knuesel et al., 2003) (all sampled farms complied with this criterion). A sample comprised 4 pools per farm, each consisting of 5-10 fish. A total of 25 facilities (19.4% of the facilities visited during the study) were sampled during spring 2013. If weak, abnormally behaving or freshly dead (not decomposed) fish were present, such fish were selected for the sampling. Furthermore, if the tanks were connected serially, fish from the last tank of the series were sampled. Finally, if rainbow trout were present in the facility, fish of this species were preferentially sampled because of their increased susceptibility to both VHS and IHN.

The virological examinations were performed according to recommendations made by the World Organisation for Animal Health (OIE, 2015a; OIE, 2015b). Specifically, tissues from the spleen, heart, head kidney and pyloric caeca including pancreas, of 5-10 fish were pooled in one sample, while the brain tissues of the 5-10 fish were pooled in a second sample. The tissue samples were conditioned and then incubated on bluegill fry (BF-2) and epithelioma papulosum cyprini (EPC) cells for 2 passages at 15°C, each of 7 days. If a cytopathic effect occurred in the cell culture, an indirect immunofluorescence test for VHS, IHN and IPN was performed.

3.4 Data management and Statistical Analysis

All data entry and data cleaning were carried out in an Excel Spreadsheet (Microsoft Office Excel©, 2007), while the statistical analyses were carried out in Stata 12.1® (StataCorp LP, Texas, USA).

Descriptive statistics were performed, and risk factors were checked for missing values and variability in the observations. Risk categories with few observations were pooled to allow for further statistical analysis.

As questionnaire data from the previous study were available for 125 of the 129 aquaculture facilities visited in this study, a Kappa agreement test was performed to determine the agreement between the risk categorisation of the nine different risk factors based on the questionnaire and those based on farm inspection. Specifically, the risk factors included: fish species present on farm, water supply, overall purchase [based on the sub-factors frequency and number of suppliers], processing activities, water runoff, type of sales and soil type. Since the “risk of flooding” was assessed differently in this study, and “biosecurity” data were not available from the previous study, these two factors were not included in the analysis. Furthermore, the risk factor “other fish farms in the vicinity” (which was characterised through the distance along the waterway to the next farm situated upstream or downstream and the number of farms in a radius of 5km) was also not included in the comparison as its assessment was based on an identical computer calculation in both studies.

If more than two risk categories were possible for a certain risk factor, a weighted Kappa agreement was calculated, as this better accounts for partial agreement between scores compared to the non-weighted Kappa (Dohoo et al., 2009). Kappa values were then interpreted using the scale described by Dohoo et al. (2009), where values ≤0.0, or between 0.01-0.20, 0.21-0.40, 0.41-0.60, 0.61-0.80 and 0.81-1.00, were considered indicative of poor, slight, fair, moderate, substantial and almost perfect agreement, respectively.

The risk classification for each farm was then calculated using the data collected on site. To evaluate the effect of the risk factors based on field-data collection on the overall risk score, a first score was calculated where a high risk was attributed to all parameters characterising the factor “biosecurity” (to keep it comparable with the previous risk categorizations based on questionnaire data). In a second step, the risk classification was calculated including the real values of the factor “biosecurity”, to allow for the evaluation of this factor on the overall risk.

A Wilcoxon-signed rank test was then performed to determine whether there was a statistically significant ($p \leq 0.05$) difference between each farm’s overall risk score based on the old classification (i.e. using questionnaire data), and the new classification (i.e. using data collected during farm inspection), using both a constant high risk score for biosecurity and the real values obtained through field visits.

Lastly, to determine possible differences in the risk categorisations between the two visits for the 30 farms visited twice, a Wilcoxon-signed rank test was performed for the 18 individual risk factors as well as for the overall risk score. Risk factors with a p-value <0.20 and ≤0.05 were considered to represent a marginally and statistically significant difference, respectively.

4 Results

4.1 Agreement between the questionnaire and the farm inspection data

A Kappa agreement test was computed for the categorisation of 9 risk factors on 125 fish farms based on information collected through a questionnaire and information collected during the field visits (Table 1). Of these nine factors, one factor (soil type) showed almost perfect agreement, three factors showed substantial agreement (fish species kept, water supply and type of fish sales), while the remaining five factors showed fair agreement (overall purchase, frequency of purchases, number of suppliers, processing activities and water runoff).

As the change of a single parameter does not necessarily result in a change of the risk categorisation (e.g. if the frequency of purchase of a fish farm would be seven, the risk factor “frequency of purchase” would be categorised as high, but if the parameter frequency of purchase would change to nine, the categorisation of this risk factors would still be categorised as high), more parameter changes (n=369 or 32.8%) were observed than risk category changes of the individual factors (n=227 or 20.2%). Of the 227 changes in risk category, 184 (81.1%) resulted in a categorisation of the risk factors in a higher risk class after the correction of the parameters according to the findings from farm inspections.

4.2 Effect of using field-collected data on the overall risk classification

The overall risk score of the 125 fish farms differed significantly between the old classification and the new classification using both a constant high risk score for biosecurity ($p<0.001$), and using the real values obtained through field visits ($p<0.001$). In the old classification (i.e. based on questionnaire data), 3.4% of the aquaculture facilities were classified in the low risk category, 39.5% were classified in the medium risk category and 57.1% were classified in the high risk category (Figure 2). Using the new classification (i.e. based on field visit data) while keeping the risk factor “biosecurity” constantly high, 76.3% of the plants kept their original risk categorisation, while 20.3% were classified in a higher risk category and 3.4% in a lower risk category. Thus, 0.9% of the farms were classified as low risk, 27.7% as medium risk and 71.4% as high risk (Figure 3). Finally, when the true values for the factor “biosecurity” were included in the new classification, the following distribution was observed: 16.0% of the farms were classified in the low risk category, 63.0% in the medium risk, and 21.0% in the high risk category. In comparison with the old categorisation, 45.8% of the farms kept their original risk categorisation, 3.4% were classified within a higher risk category, while 50.8% were classified in a lower risk category (Figure 3).

4.3 Agreement between data from the autumn and the spring visit for aquaculture facilities that were visited twice

Thirty fish farms were visited twice (once in autumn 2012 and once again in spring 2013) to collect information on the same 18 risk factors. For 9 of these 18 factors, the risk categorization was identical on both visits and the p-value could therefore not be computed (Table 2). The other nine factors where the risk score changed concerned the factors “type of fish sales”, “overall purchase” and “biosecurity” and their sub-factors. However, none of these factors showed statistically significant differences in the risk score assigned on the autumn and spring visit, though three factors showed a marginally significant difference in the risk scores. These included: “frequency of purchases” (p=0.10), “overall purchases” (p=0.17), and “bird control” (p=0.10). Interestingly, five of the six risk score changes observed in the factor “bird control” during the spring inspection resulted in a lower risk categorization. In contrast, the majority of the changes observed in the risk scoring of “overall purchases” and “frequency of purchases” during the spring visit resulted in a higher risk categorisation.

Between the autumn and spring inspection, the overall risk score changed for 13 of the 30 aquaculture facilities. However, as a change in the overall risk score does not necessarily result in a change in the overall risk categorisation, the overall risk categorisation only changed for six farms. The Kappa agreement between the risk categorization score based on the autumn and spring visit was 0.77.

4.4 Relationship between risk classification and effective epizootic outbreaks

The model was run using the data collected on site in autumn 2012 and spring 2013. The categorization of fish farms which were tested positive for VHS, IHN and/or IPN at any time since 2004 was compared with the one of the farms which were not tested positive for these three diseases (Figure 4).

Since 2004, within the four cantons, three still existing fish farms were tested positive for VHS once. All three cases were diagnosed in rainbow trout during the year 2006. Based on the proposed model, all three aquaculture facilities were classified as having a medium risk of disease introduction and spread.

Two IHN outbreaks were registered since 2004 within the four cantons (one in 2012 and one in 2013), both in rainbow trout. Of the two corresponding farms, one was categorized as having a high risk, and the other as having a medium risk of disease introduction and spread.

Since 2004, IPN was diagnosed 12 times within the 4 cantons: six of the cases were in six different aquaculture facilities (one in 2010, one in 2012 and four in 2013) while the other six were always in the same facility (in 2005, 2006, 2007, 2008, 2010 and 2013). All cases were found in rainbow trout. Five of these fish farms were classified as having a medium risk and two as having a high risk of disease introduction and spread. The facility with the multiple outbreaks was classified in the upper range of the medium risk category.

As mentioned above, samples from 25 fish farms were collected. As it was not always possible to get 4 pools of 5-10 fish from every farm, a mean of 3.56 pools consisting of 7.6 fish were taken (41 times rainbow trout, 34 times brown trout *Salmo trutta* and 14 times char *Salvelinus* sp.). Of these 25 fish farms, 3 were tested positive for a notifiable viral disease: 1 for IHN (this aquaculture facility was classified in the medium risk category) and 2 for IPN (one was categorized as having a high risk and the other as having a medium risk). All three samples were taken from rainbow trout which did not show any signs of disease. No fish tested positive for VHS.

5 Discussion

5.1 Data collection for risk-based surveillance of aquaculture facilities

Data collection using questionnaires represents a convenient and cheap variant for gathering information (Cummings et al., 2007) required for risk-based surveillance of aquaculture facilities. However, the agreement between the data collected through questionnaires in a previous study and that collected during field visits on the same farms in this study showed that the quality of data collected by questionnaire may be equivocal. Based on the field visit data, almost one third (32.8%) of the parameters had to be modified. As the model is semi-quantitative, it has some degree of buffering capacity, whereby not every parameter correction resulted in a change in the score of the corresponding risk factor. In fact, the modification of 32.8% of parameters resulted in a score change in 20.2% of the single risk factors. Nevertheless, the overall risk categorizations of the 125 fish farms based on the old and new classification (either keeping “biosecurity” constantly high, or including the real values collected during field visits) were significantly different. This underlines the importance of having data related to all risk factors, as missing data (as it was the case for the “biosecurity” risk factor) results in a bias of the risk scores.

A high discrepancy was observed in the degree of agreement between the different factors. However, the fair agreement shown for the factors “processing activities” and “water runoff” should be put into perspective as the observed absolute agreement for both factors was good. A change in the risk factor score was only observed on 6 (for processing activities) and 9 (for water runoff) of the 125 farms. Therefore, the low Kappa value observed in this case is likely due to the low variability in both data sets (i.e. for these two risk factors, the majority of the facilities was classified in only one category). Some risk factors (e.g. frequency of purchase) were more likely to change over time, compared with others (e.g. water supply). These differences in agreement can be due to different reasons. Firstly, actual changes may have occurred on the facilities included in this study. Two years elapsed between the first and second study, and it is thus reasonable to assume that some parameters might have changed during this time. Changes in the supply and demand, or the loss of fish due to diseases, strongly influence purchasing behaviour. Therefore, risk factors concerning the “overall purchase” are very likely to change over time. This can explain why factors related to fish movements have a low agreement. The fact that changes concerning these risk factors were also observed between the two inspections performed in autumn 2012 and spring 2013 on 30 fish farms further confirms this assumption. Another plausible explanation for the differences observed between the data collected via questionnaire and that collected during the field visits is a possible misinterpretation of some of the questions in the questionnaire. Some of the risk factors, such as “water supply”, “water runoff” and “soil type” are rather stable, so that a score change is not very likely for them. The risk factor “processing activities” (i.e. processing of fish from other farms) is also not very likely to change over time, as the construction and certification of the necessary infrastructure is rather expensive and time consuming. Thus, a variation over time of one of these four risk factors should be an exception. The good agreement found for these factors shows that this is indeed true in this study. Nevertheless, it was observed during the field visits that some of the responses given in the questionnaire concerning these four factors were erroneous because the fish farmer had misinterpreted the question. For example, the definition of “spring water” was not clear to all participants, and this might have led to different interpretations. To minimize such misclassification errors, a detailed guideline providing definitions and additional information could be adjoined with the questionnaire. However, the additional effort required by the fish farmers may decrease their willingness to complete the questionnaire.

Lastly, intentional provision of wrong data may also have been responsible for some of the observed differences between the two studies. Many of the changes made in the risk factor scores following the farm inspection led to an increase in the risk score. It is thus possible that some of the data provided by the fish farm owners in the questionnaires were intentionally brightened. Escher and Büsser (2005), who conducted the last official compilation of the Swiss fish production, reported that some of the fish farmers were reluctant to divulge operational data. Therefore, compared to questionnaires, farm inspections provide two advantages in this regard: firstly, the data can be directly recorded and controlled and, secondly, through personal contact with the fish farmer a relationship of trust can be established from which future information exchanges stand to benefit (Andaleeb 1996).

This study showed that a questionnaire does not suffice to gather farm data of a sufficient quality for performing a risk-based surveillance of aquaculture facilities. However, depending on the resources available, it might not be possible for the authorities to visit every single farm on a regular basis for the collection of data necessary for a risk analysis (Thrush et al. 2016). Moreover, the cost-benefit ratio of collecting field data versus relying solely on questionnaire data needs to be considered. A possible alternative might be a combination of questionnaires and farm inspections, whereby aquaculture facilities are visited alternately while data are also collected regularly via questionnaires (Oidtmann et al., 2014b). A web-based self-reporting system, as proposed by Oidtmann et al. (2013), would also be advantageous to such an approach, particularly for those factors that are more likely to vary over time, such as “fish movements”.

5.2 Suitability of the model for ranking aquaculture facilities for risk-based disease surveillance

Regarding the individual risk factors and the overall risk categorization score for the 30 farms that were visited twice, no statistically significant difference between the two visits was found. This suggests that the risk classification performed with the model was stable over the considered period of time (i.e. six months), and no substantial fluctuations due to natural and seasonal circumstances could be detected. Risk factors characterising the “fish movements” and “biosecurity” were the only ones where some variation could be observed, and this resulted in a new risk score for six fish farms. As discussed above, changes in the “fish movement” factors were mostly due to natural yearly fluctuations in purchasing behaviour. With regards to the variations observed for the risk factors concerning “biosecurity”, these might have been either due to an improvement in the biosecurity measures after the first visit or due to seasonal changes. Regarding the latter, this could be observed for the factor “bird control”, whereby some fish farmers remove the nets, usually in place over the tanks to prevent bird access, during the autumn and winter months. This is done because the weight of fallen leaves and/or snow occurring during these months might cause the nets to fall in the water, thus posing a threat to the fish. However, the removal of these nets may also result in increased bird predation, particularly since the grey herons (*Ardea cinerea*), the main piscivorous bird in Swiss fish farms, eat more farmed fish in winter (Lekuona, 2001). This is relevant not only from a fish welfare perspective, but also from an epidemiological point of view, as it has been shown that predatory birds can act as vectors of viral fish diseases (Peters and Neukirch, 1986). Therefore, when collecting data for the risk classification, inspectors need to verify whether the biosecurity measures are effective all year round.

As Swiss aquaculture facilities are only subject to passive disease surveillance and are not categorized according to the health status concept dictated by the Council Directive 2006/88/EC, no Swiss fish farm can be considered free of VHS, IHN and/or IPN. The exact health status of the facilities which were not tested positive for these diseases is therefore unknown. In case of epizootic detections, the concerned facilities are subject to an eradication program. Nevertheless, the health status of these farms after the eradication procedures has again to be considered as unknown. Accordingly, the aquaculture facilities which were tested negative for these epizootic agents within the framework of the project cannot be considered as disease free. As they were only sampled at a single point in time, infections with low prevalence or subclinical infections could have been difficult to detect and might thus have been missed (Lyngstad et al. 2016). For these reasons, the validation of the model cannot be supported with the ranking of the farms which were tested negative for the diseases. Only the ranking of the facilities tested positive should be considered.

The relationship between risk classification of the individual aquaculture facilities and the history of epizootic outbreaks was satisfactory, though better results were expected. Unlike for the model that has been used for this study, in models developed by other countries (Kleingeld, 2010; Oidtmann et al., 2011) the individual risk factors assessed were weighted. Specifically, the risk factors assessing fish movements, such as “purchase”, got the highest weighting (e.g.in the model presented by Oidtmann et al. (2013) the factor “fish movements” contributed to 63% of the overall risk score of a farm). Nevertheless, a comparison between the overall risk score and the risk score for the factors “overall purchases” for those farms that tested positive for IHN and VHS since 2004 showed that, in our model, a higher weighting of this factor would not have offered an advantage for calculating the overall risk score. In fact, the risk ranking of four of these five fish farms was equal or higher than the ranking of the factor “overall purchase” (Table 3).

Live fish movement is considered as having the most important role in the transmission of infectious diseases (Yatabe et al. 2015) and we are aware that the risk factor “overall purchase” probably represents the weak point of the model. The hazard to get infected with an epizootic increases with an increase in the number of purchases and suppliers (Oidtmann et al., 2011). Yet, as discussed above, these factors are also subject to variation within a short period of time. Furthermore, the health status of the supplied fish is also important, as a single introduction of fish from an infected plant represents a much greater risk, compared to several deliveries from a disease-free aquaculture facility. As to date the health status concept dictated by the Council Directive 2006/88/EC (Anon., 2006) is not applied in Switzerland (i.e. all Swiss fish farms are considered to have an unknown health status), an adjustment of the model considering the status of delivering farms could not be performed.

This project also highlights some of the difficulties encountered during the validation of a model for ranking aquaculture facilities for risk-based surveillance. One of the major difficulties was the small number of aquaculture facilities that were tested positive for VHS or IHN. With only 5 cases reported on the 129 fish farms inspected in the last 10 years, the number of positive cases is too low to allow for a reliable calculation of the correlation between risk classification and effective epizootic outbreaks. Gustafson et al. (2014) were confronted with a similar problem during the field validation of risk factors predictive for Infectious Salmon Anaemia (ISA) in Chile. Their factors were assessed using expert estimates. Nevertheless, despite few differences, they could find similarities in direction and magnitude between expert predictions and field findings.

Moreover, the time lapse between the occurrence of the epizootic outbreaks and the data collection might also hinder such model validation exercises. While the two IHN cases occurred during the study period, all VHS outbreaks were recorded in 2006. It can therefore be reasonably expected that the situation on the fish farms and, consequently, of the risk factors assessed, may have partly changed since these outbreaks. As an example, the biosecurity measures are usually improved after such severe events. Thus, the overall risk score calculated for these farms during the study period may differ from the one that would have been obtained at the time of the outbreaks.

Furthermore, IHN and, to a lesser extent, VHS infection can occur without any signs of disease (OIE, 2015a; OIE, 2015b). Subclinical infections could pose a problem for detection (Lyngstad et al. 2016). As the fish disease surveillance in Switzerland up until 2016 was based on disease notification and not on active inspection of farms, it can be assumed that some plants might hold chronic carrier fish for a long period of time, possibly even years. Therefore, the time of detection of VHS and IHN virus in fish may not correspond with the time of infection. This may apply for the IHN case found within the scope of the sampling performed during this project. It is therefore possible that the overall risk score of the farm at the time of introduction of the pathogen was different from the one calculated during this study.

Finally, although the model was developed for VHS and IHN, a correlation between the fish farm risk classification and the occurrence of IPN outbreaks since 2004, could be seen. As transmission routes for IPN are similar to those for IHN and VHS, the model seems to be valid for IPN too.

To conclude, this study showed that the model is stable over short time periods as no substantial fluctuations in the risk categorisation due to natural and seasonal circumstances were observed during the project period. Furthermore, when all the correct risk factor parameters were included, the model proved to be able to deliver a reliable risk ranking of Swiss aquaculture facilities according to their risk of getting infected with, or of spreading, VHS and IHN. One weak point that has been identified relates to the risk factor “purchase”. However, an adjustment of the model based on the health status of the suppliers is not possible at present as, unlike the EU, Switzerland does not currently perform a health status categorization of fish farms. Finally, this study showed that the model may also be adapted for a risk-based surveillance concerning IPN, a third viral disease which is notifiable in Switzerland.

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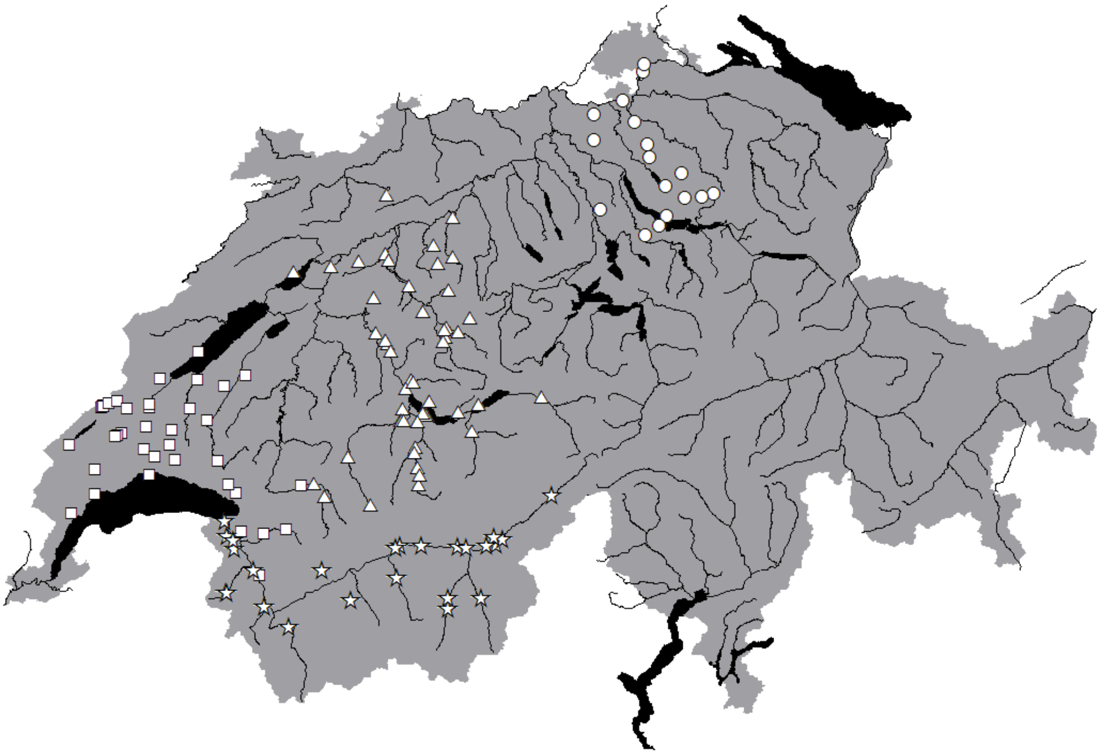

Figure 1: Distribution of 129 aquaculture facilities in the Swiss cantons Bern (triangle), Vaud (square), Valais (star) and Zürich (circle) that were inspected and ranked based on their risk for disease introduction and spread.

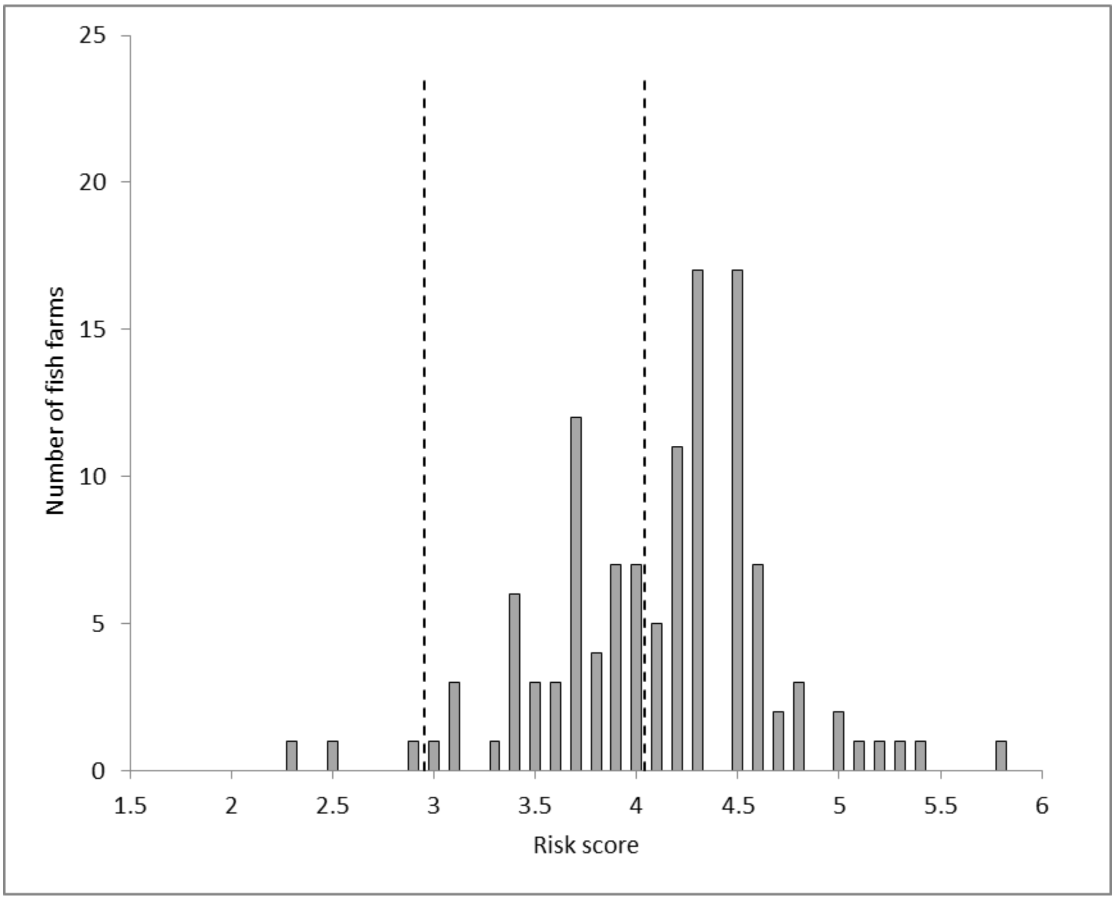

Figure 2: Distribution of the risk scores of the 125 fish farms in the 4 selected Swiss cantons based on data collected via questionnaire (Diserens et al., 2013). The first dashed vertical line represents the upper limit of the low risk category, and the second dashed line the upper limit of the medium risk category.

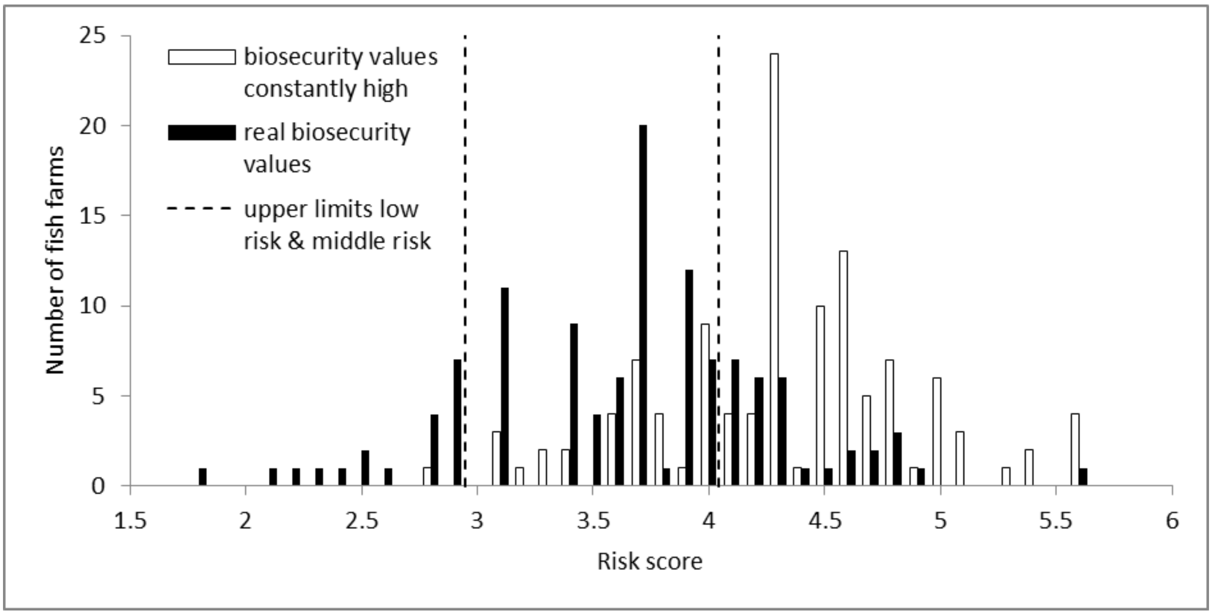

Figure 3: Distribution of the risk scores of the 125 fish farms in the 4 selected Swiss cantons based on data collected during the farm inspection, while keeping the risk factor "biosecurity" constantly high (white bars) and with the real biosecurity values (black bars). The first dashed vertical line represents the upper limit of the low risk category and the second dashed line the upper limit of the medium risk category.

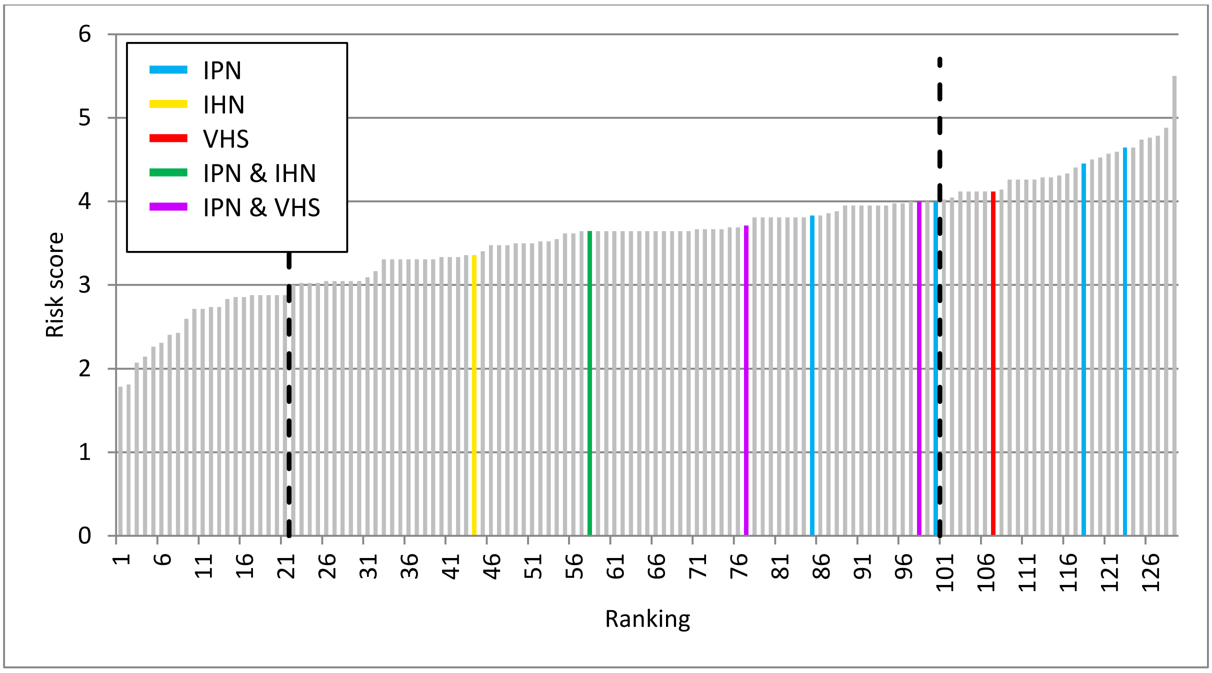

Figure 4: Risk classification of the aquaculture facilities tested positive for VHS, IHN and IPN since 2004 in the four selected Swiss cantons. The first vertical dashed line characterises the upper limit of the low risk category and the second dashed line the upper limit of the medium risk category.

Table 1: Kappa agreement between the categorisation of 9 risk factors based on questionnaire data and farm visit data collected on 125 fish farms in the 4 selected Swiss cantons.

Risk factors	**Data changes**	**Score changes (a; b)[1]**	**Kappa value**	**Level of agreement**
Fish species kept	36	3 (1; 2)	0.79	Substantial
Water supply	21	13 (5;8)	0.79	Substantial
Overall purchase	52	52 (8; 44)	0.31	Fair
Frequency of purchases	82	52 (8; 44)	0.34	Fair
No. of purchase suppliers	63	58 (9; 49)	0.25	Fair
Processing activities	6	6 (2; 4)	0.27	Fair
Water runoff	17	9 (0; 9)	0.31	Fair
Type of fish sales	56	23 (4; 19)	0.70	Substantial
Soil type	36	11 (6; 5)	0.81	Almost perfect
Total	369	227		

[1] a: number of farms with a higher risk on the basis of the data collected by questionnaire; b: number of farms with a higher risk on the basis of the data collected by farm inspection

Table 2: Risk factors and corresponding p-value obtained from a Wilcoxon signed-rank test to determine whether there was a statistically significant difference in the score assigned to each factor during two consecutive visits on 30 fish farms in the four selected Swiss cantons.

Risk factor	**Data changes**	**Score changes (a; b)**[1]	**p-value**
Fish species kept	2	0	*
Water supply	0	0	*
Overall purchase	5	5 (1; 4)	0.17
Frequency of purchases	9	6 (1; 5)	0.10
No. of purchase suppliers	10	7 (3; 4)	0.69
Processing activities	0	0	*
Biosecurity	9	9 (5; 4)	0.74
Foot bath	3	3 (2; 1)	0.56
Disinfection equipment	6	6 (3; 3)	1.00
Disinfection vehicles	0	0	*
Fencing	0	0	*
Access control	0	0	*
Bird control	6	6 (5; 1)	0.10
Presence of gate	0	0	*
Quarantine strategies	4	4 (2; 2)	1.00
Water runoff	0	0	*
Type of fish sales	5	3 (1; 2)	0.55
Soil type	0	0	*

*p-value could not be computed as the mean difference between each pair of observations was 0.

[1] a: number of plants with a higher risk when based on the first inspection; b: number of farms with a higher risk when based on the second inspection

Table 3: Comparison of the risk categorisation, and their categorisation for the risk factor „overall purchase“, of the five fish farms that tested positive for VHS and IHN since 2004 (using field collected data and the real values for biosecurity).

	Disease	**Year of outbreak of epizootic**	**Risk categorisation of the fish farm**	**Categorisation of the risk factor “overall purchase”**
Fish farm 1	VHS	2006	Middle	Middle
Fish farm 2	VHS	2006	Middle	Middle
Fish farm 3	VHS	2006	Middle	High
Fish farm 4	IHN	2012	High	Middle
Fish farm 5	IHN	2013	Middle	Low