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Prosjektopplysninger

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Veileder:	
Prosjekttittel:	Studies of factors related to susceptibility or resistance to IPN virus infection in Atlantic salmon throughout the production cycle- with focus on virus virulence

C Faglig rapport

Oppnådde faglige resultater

ACTIVITIES BY WORK PROGRAM (WP)

WP1: COLLECTION OF IPN VIRUS FROM CLINICAL OUTBREAKS OF DISEASE IN DIFFERENT CATEGORIES OF ATLANTIC SALMON

Groups of Atlantic salmon reared under two different categories of infectious pancreatic necrosis (IPN) disease epizootiological situations, *i.e.* one with low risk (few IPN problems in previous years) and another with high risk (recurrent problems) were monitored for infection with IPNV from roe to grow-out stages. About 900 samples, including eggs, milt, fry, kidney/livers of brood stock fish, parr and smolts were collected from 2002 to 2004. Figures 1 and 2 outline the distribution of production material from brood stations to sea water sites. ST stamfisk and recipients of production materials from this brood station were categorised as low IPN risk while Erfjord stamfisk and corresponding fresh and sea water sites were considered as high risk.

As expected, subclinical IPN virus infections were mostly observed in the low risk category. On the other hand, clinical cases and mortalities due to IPN were most predominant in the high risk category.

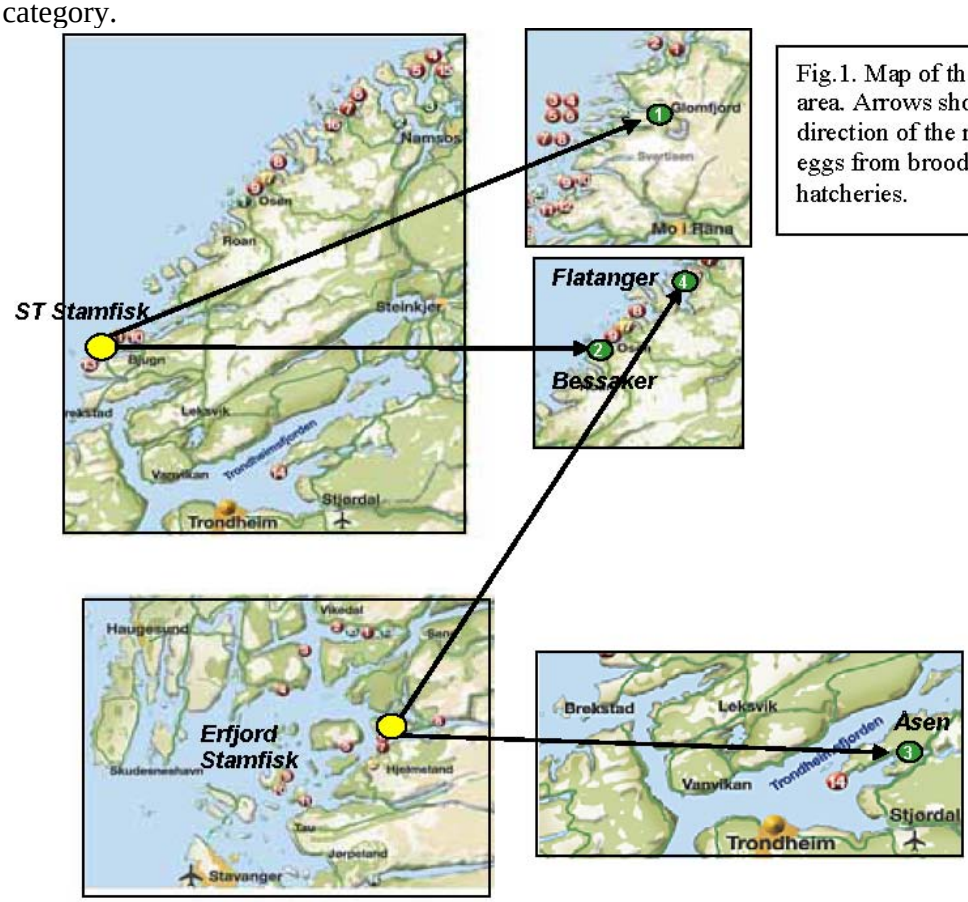

Fig.1. Map of the project area. Arrows show the direction of the movement of eggs from brood stations to hatcheries.

A. CATEGORY WITH LOW IPN RISK

A summary of mortalities due to IPN at all the sites examined in this study is shown in Fig. 2. At broodstock stage, there was neither IPN disease outbreak nor viruses isolated from ST stamfisk (broodfish station). This suggests that vertical transmission was not important in this production cycle.

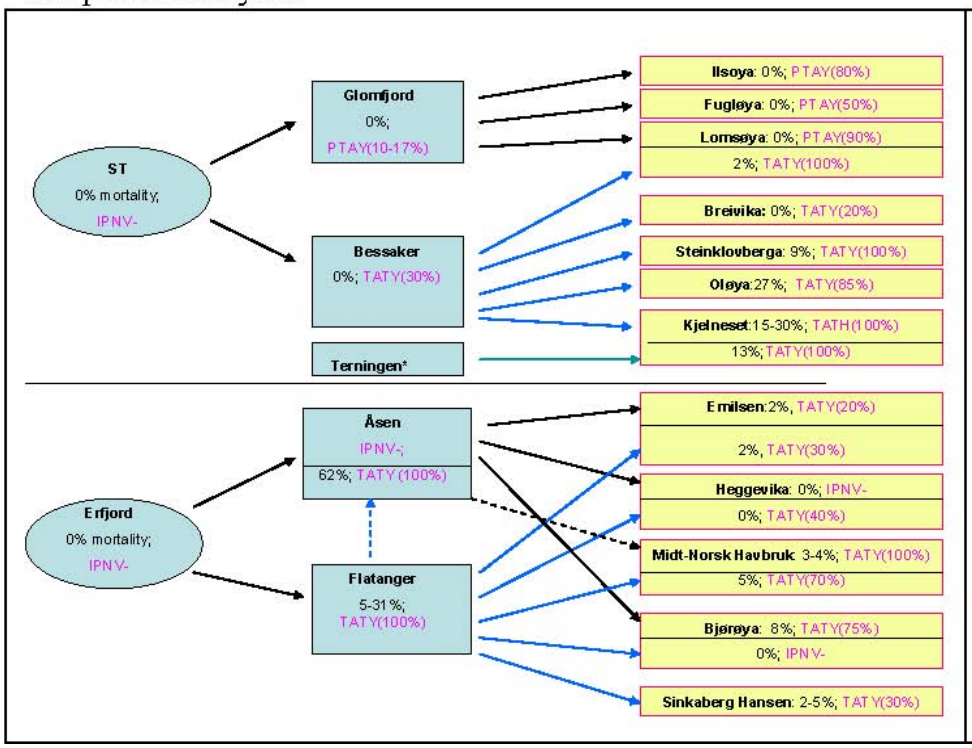

Fig.2. Mortalities due to infectious pancreatic necrosis virus (IPNV) infections in Atlantic salmon at different stages of production and sites. Percentages (black ink) represent mortalities; percentages (coloured ink) in brackets = % of fish found infected with IPNV; IPNV- means no IPNV isolated; PTAY or TATY = virus virulence motif corresponding to amino acid posistions 217, 221, 247 and 500 of VP2. *additional external hatchery; dotted arrows represent one group of fry that resulted in an outbreak at Åsen, and also at the corresponding sea water site.

During the fresh water stage, no outbreak was recorded at any of the hatcheries in this category but the virus was isolated from kidney/livers of parr, demonstrating the presence of subclinical infections. The estimated infectivity (no. of test positives divided by total sampled) per site is given in Fig. 2 in percentages.

At the sea water stage, no IPN disease broke out in any of the fish groups transferred from Glomfjord although the infectivity in fish increased from fresh water to sea water stage (Fig. 2), suggesting that this virus strain at these locations was avirulent. This is concurrent with previous studies carried out in our group on virulence mechanisms of IPNV. In contrast, clinical IPN was observed at sea water sites that received smolts from Bessaker. One of these sea water sites, Lomsøya, also received smolts from Glomfjord. Interestingly, mortalities at this site were only observed in fish from Bessaker and not those from Glomfjord. Interestingly, the genotype of the virus in the two groups, corresponded to what was found in the freshwater stage, *i.e.* PTAY for Glomfjord and TATY for Bessaker.

B. CATEGORY WITH HIGH IPN RISK

At Erfjord stamfisk, neither clinical nor subclinical IPN was recorded, suggesting that vertical transmission in this production cycle also was not important.

During the freshwater stage, clinical cases and mortalities were observed at Flatanger hatchery. Here, all fish groups contracted clinical disease with mortalities ranging from 531% and

infectivity approaching 100%. In contrast, neither IPNV nor disease outbreaks in the fish at Åsen were recorded except for one group of fry that had been transferred from Flatanger. In this group, an outbreak occurred with virus infectivity of 100% and a mortality of 62% (Fig 2).

Unlike in category A where rearing of smolts from more than one hatchery at sea water sites was minimized, this practice was common in this category (Fig. 2) and outbreaks in both exposed and apparently "clean" fish were often observed (e.g. at Emilsen and Midt-Norsk havbruk; Fig. 2.). However, exceptional cases were also observed (e.g. Heggevika) whereby smolts that originated from Åsen were reared together with fish from Flatanger but mortalities were only observed in the latter.

All smolts from Flatanger (except the groups at Bjørøya and Heggevika) developed clinical IPN during the sea water stage. Incidentally, no IPNV was isolated from fish transferred from Flatanger to Bjørøya, but an infectivity of 40% was found in a similar group at Heggevika (Fig. 2.). These results suggest that either environmental factors play a role in the maintenance of IPNV in fish or sea water sites are also important sources of infection.

The only group of parr from Åsen in which an outbreak occurred during the fry stage, also had another outbreak at sea water stage (Midt-Norsk Havbruk; Fig. 2.), indicating that infection during the fresh water stage does not preclude sea water outbreaks in a population.

WP2: CHALLENGE EXPERIMENTS WITH ISOLATES OF IPN VIRUS IN FRY OF ATLANTIC SALMON

Two challenge experiments were conducted at the NVH/VI shared Wetlab. All the experiments were done using virus isolated from fish collected from field outbreaks of IPN outside the NVH/ Marine Harvest (MH) collaboration, prior to the start of this project. Some of the isolates used were from fish farms in Chile. These isolates were used instead of isolates from different stages of hatchery operations and grow-out stages in order to overcome the limitation associated with the seasonal availability of fry for challenge experiments. The results of these challenge experiments are however relevant to the evaluation of IPNV from hatcheries/farms as shown below.

Cumulative mortalities of fry challenged in the different experiments are given below (Fig. 3&4). The results show that different IPNV isolates cause different levels of mortalities and that in general, the virulence of these isolates can be linked to specific motifs in the VP2 gene. More details are discussed under WP3.

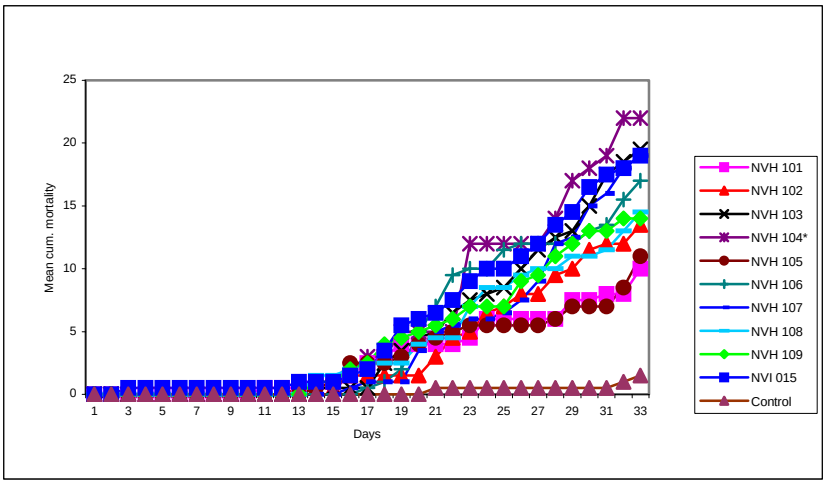

Fig. 3. Cumulative mortalities of Atlantic salmon fry following experimental challenge with infectious pancreatic necrosis virus. The positive control (NVI-015) was found to have high virulence in a previous experiment.

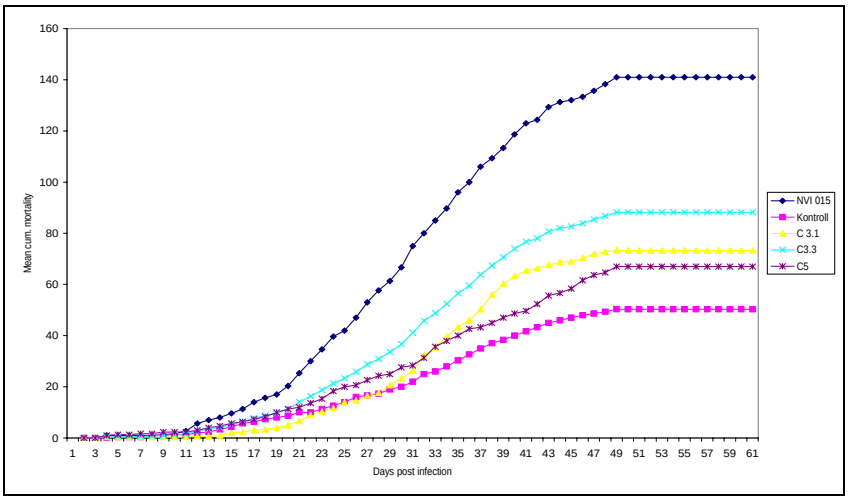

Fig. 4. Cumulative mortalities in Atlantic salmon fry following fry challenge. NVI 015 – a known virulent isolate from Norway; C3.1, C3.3 and C5 – Chilean isolates.

WP3: Sequence data from different isolates of IPNV both from field isolates and isolates tested in challenge experiments

Virus isolates used in challenge experiments were genotyped before and after the challenge. Of the field isolates collected, at least three from each site were genotyped. The results are summarised below.

Challenge experiments

The sequence results of the VP2 segment of the virus isolates used in the challenge experiments in this study are provided in the table below (Table 1). All virus isolates used in the first challenge experiment (Fig. 3.) were highly virulent, with amino acid residues Threonine (T), Alanine (A), Threonine and Tyrosine (Y) or Histidine (H) at position number 217, 221, 247 and 500 respectively (Santi et al., 2004; Song et al., 2005). In the second experiment (Fig. 4.) 2 of the 3 Chilean isolates used had Proline (P), A, A and Histidine (H) in these positions. This motif (PAAH) is consistent with low virulent isolates (Song et al., 2005). One isolate (C5) however had a motif similar to the high virulent Norwegian isolate (NVI-015) although in this experiment the two caused contrasting mortalities. The difficulty in controlling environmental factors such as the flow of oxygen in individual buckets during the challenge experiment is one of the reasons that have been suggested for this anomaly.

Strain	217	221	247	500
NVI-015	T	A	T	Y
NVH-101	T	A	T	H
NVH-102	T	T	T	H
NVH-103	T	A	T	H
NVH-104	T	A	T	H
NVH-105	T	A	T	H
NVH-106	T	A	T	H
NVH-107	T	A	T	Y
NVH-108	T	A	T	Y
NVH-109	T	A	T	Y
Chile 3.1	P	A	A	H
Chile 3.3	P	A	A	H
Chile 5	T	A	T	H

Table 1. Putative virulence motifs of virus isolates used in fry challenge experiments. 217, 221, 247 and 500 represent amino acid residue positions in the VP 2 gene.

Field isolates

The putative virulence motifs of different isolates collected from different fresh and sea water sites is given in Fig. 2. These motifs are in two groups, PTAY and TATY. This grouping is consistent with subclinical versus clinical grouping respectively, and also with the main clustering in the phylogenetic tree (Fig.5.). These results are in agreement with the findings of challenge experiments and also with what has been previously observed regarding virus isolates with different motifs (Santi et al., 2004; Song et al., 2005). Avirulent isolates (PTAY motifs) were not among the isolates tested in the challenge experiment probably because of their dominance in subclinical infections hence less likely to be picked up in clinical outbreaks.

As has already been mentioned, comparison of sequences (Fig. 5) shows that the virus isolates can be divided into two main clusters. Isolates from Bessaker, Åsen, Flatanger and corresponding sea water sites as well as the virulent isolates used in the challenge experiments on one side and those from Glomfjord and corresponding sea water sites on the other. Most of the isolates from Chile form a group of their own with isolates from Øyerhamn (a site which was not initially included as study). In addition, the smaller clusters within these groups show that there is a high level of similarity between isolates from fresh and corresponding sea water sites, suggesting that sea water infections are a recurrence of fresh water infections.

Conclusions

- The prevalence of subclinical and clinical IPN virus infections has been defined. Subclinical infections have a low IPNV infectivity in fry or parr while clinical IPN is associated with high virus prevalence. In smolts, the infectivity of isolates causing subclinical infections can be very high but without causing clinical symptoms.
- The presence of virus isolates in fish at fresh water stage and yet absent at sea water stage suggests lack of persistence in some virulent isolates. It must be noted however that sampling in this study was purposive during outbreaks to maximise the chances of isolating the virus. Because of this, virus infectivity in fish populations must be interpreted with caution.
- The similarities between sequences of fresh and sea water isolates of related fish groups suggest that sea water outbreaks are a recurrence of fresh water infections. Each hatchery seems to have an “in-house” strain. The fish pick up the virus during the fresh water stage and are transferred to sea with it probably through persistent infections.
- Infection with IPNV during the fresh water stage does not necessarily lead to resistance against the disease in the sea water as outbreaks may re-occur.
- Introduction of fish groups from more than one fresh water site encourages intermixing of different virus isolates and is a recipe for disease outbreaks. However, it is also interesting to note that isolates with PTAY motifs (Glomfjord isolate) seem to confer protection against virulent isolates. This finding requires further investigation.
- The contribution of vertical transmission towards the perpetuation of IPNV in the production cycles examined in this study is of limited importance since no virus was isolated from the brood stock, eggs or milt. Our findings however suggest that hatcheries on the other hand are an important source of infection. Nevertheless, brood stock testing is still recommended as a means of keeping vertical transmission of the virus in check.
- Finally, the differences in mortalities of fish from the same group when transferred to different sea water sites suggests that the environment plays a role in the epidemiology of IPN. Factors such as pH, superoxygenation etc are well known to play a role in predisposing fish to IPN disease outbreaks and must be studied in more detail.

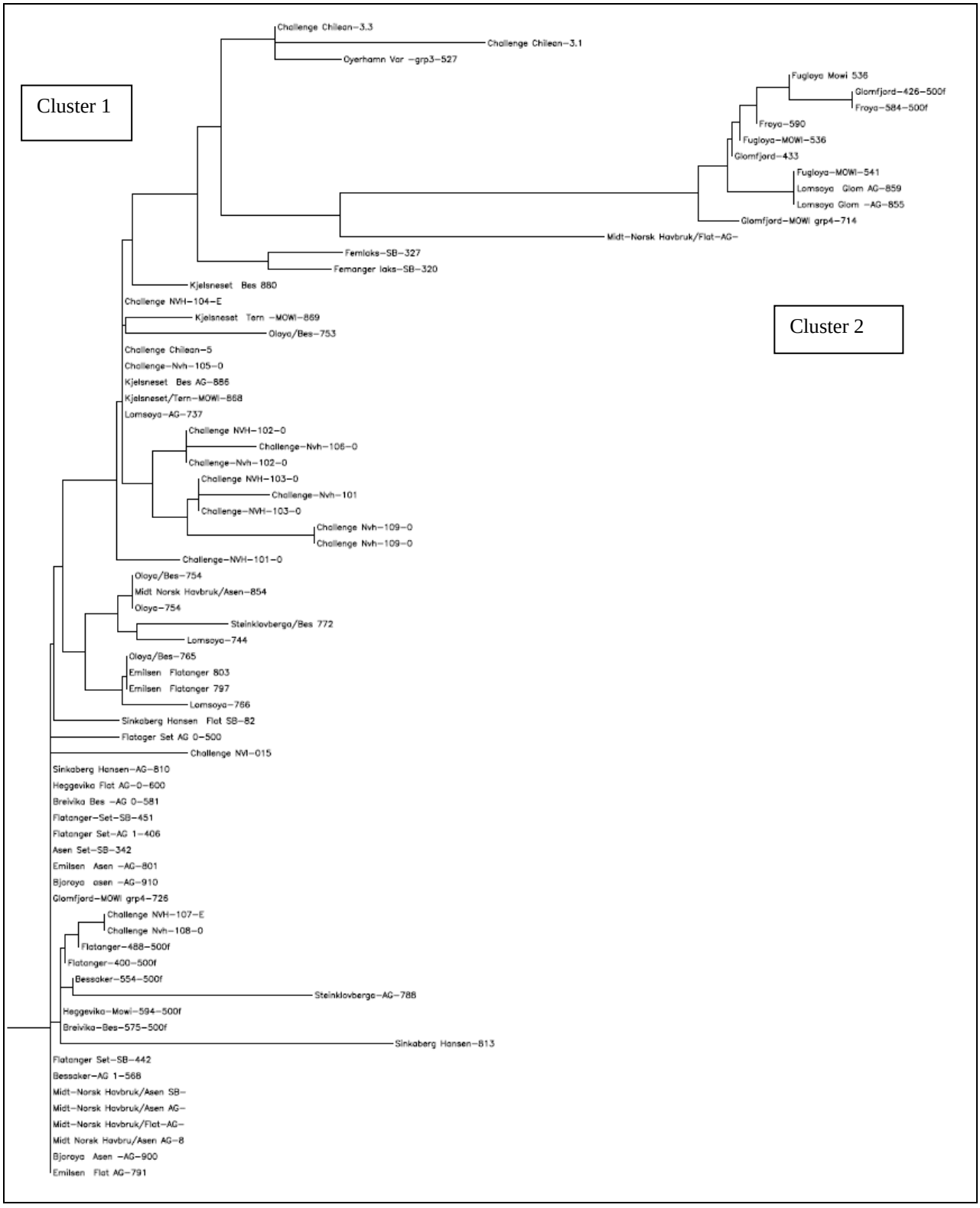

Fig. 5. Clustal W dendrogram of amino acid sequences of infectious pancreatic necrosis virus isolated from Atlantic salmon in the field as well as those used in fry challenge experiments. Labels of isolates used in challenge experiments start with “Challenge.”