Epidémiol. santé anim., 1997, 31-32

A PROSPECTIVE STUDY OF INFECTIOUS PANCREATIC NECROSIS IN FARMED ATLANTIC SALMON POST-SMOLT

Jarp J. [1] Melby H.[1]

*La nécrose pancréatique infectieuse (NPI) est responsable de pertes sérieuses dans les élevages norvégiens de salmonidés. Une analyse prospective de terrain a été planifiée pour rechercher des associations entre présence du virus de la NPI (IPNV), exposition à différents facteurs, et risque d'apparition de NPI clinique après smoltification chez les jeunes saumons atlantiques (*Salmo salar *L.)*

Les premiers enregistrements de données quantitatives ont porté sur 99 groupes de smolts répartis dans 42 élevages d'eau douce. Après transfert en mer 111 groupes distribués sur 70 sites marins d'élevage ont été suivis. L'observation s'est prolongée depuis le moment du transfert jusqu'au 1er octobre de la même année, représentant en moyenne 156 jours. De plus, dans 40 des groupes suivis dans les établissements d'eau douce, 25 sujets ont fait l'objet juste avant transfert de prélèvements de tissu rénal destinés à rechercher le virus de la NPI.

La prévalence de l'infection par IPNV avant transfert en mer était en moyenne de 27,5 %. A 1 an et 2 ans les proportions d'animaux infectés étaient respectivement de 21 et 67 %.

L'incidence cumulée de NPI clinique durant le séjour en mer s'est élevée à 18,9 %. En moyenne les épisodes cliniques étaient observés 35,9 jours après le transfert. Plus la période écoulée entre le transfert et l'apparition des troubles cliniques était brève, plus la mortalité était élevée. Par contre, le risque de développer la maladie clinique ne différait pas entre groupes NPI-positifs ou NPI-négatifs. L'analyse du risque proportionnel par le modèle de Cox a révélé de plus grandes chances de maladie pour des saumons transférés en fin de printemps que pour ceux transférés plus tôt, le risque d'apparition de NPI clinique augmentant de 3,4 % pour chaque jour de délai supplémentaire.

Infectious pancreatic necrosis virus (IPNV) is a world wide distributed virus in various marine and freshwater animal species. The prevalence of IPNV has not been systematically investigated in different Norwegian fish populations. It has, however, been detected in fish from nearly all commercial salmonid sea farms (Melby et al. 1991). The aetiology of clinical infectious pancreatic necrosis (IPN) is complex, but the presence of IPNV is necessary but not sufficient to cause disease, as latent virus carriers are common. Clinical IPN has for several years been a major disease problem in various age groups of the Norwegian farmed salmonid population. In 1991 the cumulative site-level incidence of clinical IPN in post-smolt was estimated to 39.5 % during a time period of approximately four months (Jarp et al. 1995). As outbreaks of IPN in post-smolt are related to fish mortality, it is of importance to clarify the causal mechanisms of the disease outbreaks

The objective of a prospective field study was to analyse the association between presence of IPNVprior to seawater transfer, various exposure factors in the freshwater hatcheries and the seawater sites, and the risk of clinical IPN in farmed Atlantic salmon (*Salmo salar* L.) post-smolt.

MATERIAL AND METHODS

Initially, 99 smolt groups from 42 freshwater hatcheries were included and various fish and farm-level factors were recorded. After seawater transfer, the material comprised 111 post-smolt groups located in 70 sea sites. Prior to seawater transfer the local veterinarians recorded site and fish data at the freshwater hatchery and collected tissue samples for IPNV isolation. After seawater transfer in the spring the fish were observed for the occurrence of specific diseases and total cumulative mortality until the 1st October the same year. The mean length of the observation period was 156 days. Clinical IPN was diagnosed as described in Jarp et al. 1996. Various exposure factors in the sea sites were recorded by the veterinarians on a questionnaire at the end of the observation period.

Virus analyses

Kidney tissue from 25 fish were collected from 40 groups raised in 22 commercial freshwater hatcheries in order to detect IPNV in cell culture. Samples of five fish were pooled, homogenised in Glasgow modification minimum essential medium (GMEM) supplemented with gentamicin (50 µg/ml) and centrifuged. The clarified homogenate was added to CHSE-214 cells (Lannan et al. 1984) , grown in GMEM with 2% foetal bovine serum, to a final tissue dilution of 1:200 and 1:2000. The plates were incubated at 15 °C for a maximum of 10 days. If cytopathogenic effect was observed, the virus was identified by indirect immunoflourescence using monoclonal antibodies against IPNV (Taksdal et al. 1997). GMEM was used as negative control. A sample was classified as

[1] National Veterinary Institute, PO-box 8156 Dep., N-0033 Oslo, Norway

negative if no cytopathogen effect was observed in any of the two dilutions after 10 days incubation. The group was classified as IPNV-positive if one of five samples were positive.

Statistical analyses

The observation unit in the study was a smolt group. The statistical analyses were performed in the SAS-PC System® Version 6.10 for Windows (SAS Institute Inc., Cary, NC, USA, 1989). The relationships between the various exposure factors and the risk of clinical IPN were analysed in a Cox proportional hazard model. The relative time interval from seawater transfer until the first outbreak of IPN or until the end of the observation period was used as the survival time. The observations were censored if IPN occurred. Since several groups were represented from each freshwater site the data were analysed in a stratified model with the freshwater site as the stratifying variable. The assumption of proportional hazard for the independent variables was evaluated by the log-log survival plot of stratified continuous variables or the various categories of qualitative variables.
For the part of the study associated with IPNV exact confidence intervals (CI) for the various estimates were calculated.

RESULTS

Prior to seawater transfer IPNV was detected in 11 of 40 smolt groups leading to an overall prevalence of 0.28 (95% CI: 0.15-0.44). The mean within-group IPNV-prevalence in the positive groups was 0.59. The proportions of IPNV-positive groups in one-year-old and two-year-old smolt were 21% and 67%, respectively, leading to an estimated relative risk (RR) of 0.31 (95% CI: 0.13-0.74). The within-group prevalence of IPNV was also significantly higher in two-year-old smolt groups (0.43) than in corresponding one-year old (0.11)(Student T-test: $p<0.05$). IPNV could not be demonstrated in any of the groups which had been affected by clinical IPN earlier during the freshwater stage. The incidence of clinical IPN during the observation period in sea water was 0.20 in IPNV-positive smolt and 0.24 in IPNV-negative smolt, leading to an estimated RR of 0.84 (95%CI: 0.2-3.3). The time interval lasting from seawater release to the first outbreak of IPN was quite similar for IPNV-positive and IPNV-negative groups as the median values were 35 days and 44 days, respectively.

During the observation period in sea water, the overall cumulative incidence of clinical IPN was 18.9 %. The estimated mean mortality rate per group day at risk was 0.03 %, being 0.02% and 0.10% in IPN-negative and IPN-positive groups, respectively. On average, the first outbreak was observed 35.9 days after seawater transfer. If the relative time period from seawater transfer to the first outbreak of IPN was short the crude fish mortality was higher compared to the mortality in groups experiencing outbreaks later in the observation period (r^2=-0.35, $p<0.20$, $n=19$).

In an unstratified model various exposure factors both in freshwater and seawater stage were found to influence the risk of an outbreak of IPN. The model stratified by freshwater site had to be restricted because many of the independent variables on freshwater site were singular. The final model included the date of seawater transfer (2 Log Likelihood ratio=5.379, 1df, $p=0.021$). Smolt transferred to sea water late in the season were at a higher risk of experiencing IPN during the post-smolt period than the smolt released early. For each day the seawater transfer was postponed the risk of IPN increased by 4.1%. The date of seawater transfer was positively correlated to the temperature in freshwater prior to transfer (r^2=0.43, $p<0.001$) and the temperature in sea water at the date of release (r^2=0.55, $p<0.001$), and negatively correlated to the weight of smolt at release (r^2=-0.38, $p<0.001$).

In an unstratified model two other exposure variables recorded in the freshwater site were connected to the hazard of IPN; the length of the time period for the eggs in the hatching cylinder and the type of vaccine used at the latest vaccination of the smolt prior to seawater release. In the stratified model these were absorbed in the freshwater site.

Table I
The hazard ratios for the variable in the final Cox proportional hazard model of IPN in post-smolt.

	Hazard ratio with 95% CI	Wald's p-value of parameter	Percent change in hazard per one-unit change in covariate
Date for seawater transfer of the smolt	1.041 (0.99-1.085)	0.0577	4.1

DISCUSSION

The relationship between carrying IPNV and the subsequent risk of clinical IPN is debated. In the current study no difference in the risk of clinical IPN during the post-smolt stage could be demonstrated between groups with or without carriers of IPNV prior to seawater transfer. In an earlier study it was shown that the risk of clinical IPN in a seawater site during the corresponding time period was higher when the smolt were purchased from several freshwater hatcheries, and that the cumulative mortality in post-smolt was related to outbreaks of furunculosis, IPN or vibriosis, transport stress in the fish and the age of the smolt at sea water release. In experimental trials it has been difficult to mediate outbreaks of clinical IPN in Atlantic salmon after challenge with IPNV only (Swanson

& Gillespie 1979, Rimstad et al. 1991). Recently clinical IPN has been induced in Atlantic salmon fry after challenge with IPNV (Taksdal et al. 1996) and in post-smolt after challenge and stressing (Stangeland et al. 1996). The findings in the epidemiological and experimental studies support the practical experiences that in addition to IPNV, stress is a critical factor for the induction of IPN.

On the other hand it is not easy to verify whether outbreaks of IPN are caused by a new infection or reactivation of carried IPNV. It has been suggested that stress-mediated reactivation of IPNV might play an important role of the high incidence of clinical IPN during the post-smolt stage. In an experimental trial Melby & Falk (1995) found no activation of IPNV infection when Atlantic salmon parr were challenged with tissue homogenate infected with Infectious salmon anaemia. No difference was detected between isolates of the Sp strain of IPNV from clinical versus symptom-less carriers (Melby et al. 1994). The impact of carrying IPNV may be of minor importance for disease outbreaks compared to host and environmental factors.

Smolt transferred to sea early in the season were at lower risk of experiencing IPN in the post-smolt period than smolt transferred later. It has been shown that two-year-old smolt is best adapted to sea water in April and May when comparing to June (Staurnes et al. 1993). As the largest smolt in the present study were transferred to sea water earlier than smaller smolt it might be explained by the fact that larger smolt is better adapted to sea water conditions. However, in a prior smaller study no relation could be shown between the smoltification status as measured by the plasma chloride values, of the smolt and the risk of IPN during the post-smolt period (Jarp et al. 1996). Later sea water transfer was connected to higher temperature. Higher temperature could be associated with an increased immune response and to an increase in the IPNV-titre. It has been shown that specific antibodies against IPNV more often were present in smolt groups kept at higher temperature than in groups at lower temperatures, and that the specific immunity protected against outbreaks of IPN (Jarp et al. 1996).

REFERENCES

Jarp J., Gjevre A.G., Olsen A.B., Bruheim T., 1995. Risk factors for furunculosis, infectious pancreatic necrosis, and mortality in post-smolt of Atlantic salmon, *Salmo salar* L. Journal of Fish Disease 18, 67-78.

Jarp J., Taksdal T., Tørud B., 1996. Infectious pancreatic necrosis in Atlantic salmon *Salmo salar* in relation to specific antibodies, smoltification, and infection with erythrocytic inclusion body syndrome (EIBS). Diseases of Aquatic Organisms 27, 81-88.

Lannan C.N., Winton J. R., Fryer J.L, 1984. Fish cell lines: establishment and characterization of nine cell lines from salmonids. In Vitro 20, 671-676.

Melby H.P., Krogsrud J., Håstein T. Stenwig H., 1991. All commercial Atlantic salmon seawater farms in Norway harbour carriers of infectious pancreatic necrosis virus (IPNV). In *Proceedings from 2nd International Symposium on Viruses of Lower Vertebrates* (ed. by J.L. Fryer), Oregon State University, Corvallis, Oregon, USA, pp. 211-217.

Melby H.P., Caswell-Reno P., Falk K., 1994. Antigenic analysis of Norwegian Aquatic birnavirus isolates using monoclonal antibodies - with special reference to fish species, age and health status. Journal of Fish Disease 17, 86-91.

Melby H.P., Falk K., 1995. Study of the interaction between a persistent infectious pancreatic necrosis virus (IPNV) infection and experimental infectious salmon anaemia (ISA) in Atlantic salmon, *Salmo salar* L Journal of Fish Disease 18, 579-586.

Rimstad E., Poppe T., Evensen Ø., Hyllseth B., 1991. Inoculation of Infectious pancreatic necrosis virus Serotype Sp did not cause Pancreas disease in Atlantic salmon (*Salmo salar* L.) Acta Veterinaria Scandinavica 32, 503-510.

Stangeland K., Høie S., Taksdal T., 1996. Experimental induction of infectious pancreatic necrosis in Atlantic salmon, *Salmo salar* L., post-smolts. Journal of Fish Disease 19, 323-327.

Staurnes M., Lysfjord G., Hansen L.P., Heggberget T.G., 1993. Recapture rates of hatchery-reared Atlantic salmon (Salmo salar) related to smolt development and time of release. Aquaculture 118, 327-337.

Swanson R.N., Gillespie J.H., 1979. Pathogenesis of infectious pancreatic necrosis in Atlantic salmon *Salmo salar.* Journal of Fish research Board Canadian 36, 587-591.

Taksdal T., Stangeland K., Dannevig B.H., 1997. Induction of infectious pancreatic necrosis (IPN) in Atlantic salmon *Salmo salar* and brook trout *Salvelinus fontinalis* by bath challenge of fry with of infectious pancreatic necrosis virus Serotype Sp. Diseases of Aquatic Organisms 28, 39-44.

ACKNOWLEDGEMENTS

This study was funded by the Norwegian Research Council and the Norwegian Ministry of Agriculture. The authors acknowledge the assistance from fish farmers and field veterinarians.