Antiviral immunity in fish – functional analysis using DNA vaccination as a tool

In fish, DNA vaccines encoding the glycoproteins (G proteins) of the salmonid rhabdoviruses VHSV and IHNV have proved very efficient under experimental conditions. Nano-gram amounts of plasmid DNA can induce long-lasting protective immunity when delivered by intramuscular injection in rainbow trout fingerlings. Vaccination of fish at an early stage appears advantageous, since larger fish require higher doses of vaccine to be protected. Even in fish with an average size of 0.5 g at the time of vaccination, good protection can be obtained. Interestingly, immunity is established already a few days after vaccination and cross-challenge experiments have revealed that protection in the early phase is non-specific. Later on, protection becomes very specific in terms of virus species. The protection in the early non-specific phase is related to interferon induced defence mechanisms whereas specific antibodies and cellular components both play a role in the long lasting protection. The similarity of the functional immune response profile to that induced by a natural virus infection is striking and is most likely one of the major reasons for the efficacy of the rhabdovirus DNA vaccines. Although other elements like CpG motifs in the plasmid backbone sequence might play a role, the viral G protein appears to have an inherent ability to stimulate innate immune mechanisms by receptors and pathways that still remain to be characterized in detail. Immunity to VHS in rainbow trout can be induced by DNA vaccination across a temperature range of at least 5-15°C. Interestingly, the initial non-specific phase is significantly prolonged at lower temperatures, hereby ensuring protection despite a slow activation of adaptive mechanisms. Expression of the rhabdovirus G protein on the surface of transfected muscle cells induces a histologically visible local inflammatory reaction with higher doses of VHSV G DNA vaccine. Cell surface expression may be important for a proper activation of the fish immune system, since blocking of the intracellular trafficking of the expressed glycoprotein G-gene interferes with protection. It may be anticipated that the viral G protein acts like a PAMP (pathogen associated molecular pattern), but it remains to be determined which PRRs (pattern recognition receptors) that may be involved in the recognition of the G protein. Recent data from DNA vaccination trials with variant forms of the G protein gene suggest that the structural requirements for antigenicity are different from the requirements for immunogenicity.
DNA vaccination in fish promotes an early chemokine-related recruitment of B cells to the muscle

In fish, intramuscular injection of plasmid DNA encoding viral proteins has proved as the most effective vaccination strategy against many viral pathogens. The efficacy of DNA vaccination in teleost fish is based on a high level of viral antigen expression in muscle cells inducing a strong and long-lasting protection. However, the mechanisms through which this protection is conferred in fish are still not understood. Moreover, similarities to mammalian models can not be established since DNA vaccination in mammals induces much lower responses. In this work, we have focused on the characterization of immune cells that infiltrate the muscle at the site of DNA delivery in vaccinated fish and the chemokines that may be involved in their infiltration. It was observed that B lymphocytes, both IgM+ and IgT+, represent a major infiltrating cell type in fish vaccinated with a viral hemorrhagic septicemia virus (VHSV) DNA vaccine, whereas in control fish injected with an oil adjuvant mainly granulocytes were attracted. While IgM+ cells were the major B cell population at early time points post vaccination, IgT+ cells represented the predominant cell type later on. Among twelve chemokine genes studied in the injected muscle tissues, only CXCL10, CK5B and CK6 were more strongly transcribed in DNA vaccinated fish compared to control fish injected with the corresponding vector backbone. In vitro tests performed with recombinant trout CK5B and CK6 revealed that these chemokines have chemotactic capacities which might explain the recruitment of immune cells to the site of DNA injection. Our results suggest that B cells are involved in the initial phase of the immune response to intramuscular DNA vaccination against VHSV. This appears to be a major difference to what we know from mammalian models where T cells play a major role.

General information

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Authors: Castro, R. (Ekstern), Martínez-Alonso, S. (Ekstern), Fischer, U. (Ekstern), Álvarez de Haro, N. (Ekstern), Soto-Lampe, V. (Ekstern), Lorenzen, N. (Intern), Lorenzen, E. (Intern), Wang, T. (Ekstern), Secombes, C. J. (Ekstern), Tafalla, C. (Ekstern)
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Use of DNA vaccination for determination of onset of adaptive immunity in rainbow trout fry

Vaccine producers often recommend a minimum size of 5g for vaccination of rainbow trout, but implementation of prophylactic vaccination in smaller sized fish would be an advantage for several infectious diseases. To implement a cost efficient vaccination strategy, it is important to know the duration and nature of the protective immunity induced by the vaccines in the fish. The present work aimed at determination of the smallest size at which specific immunity could be induced in rainbow trout fry by DNA vaccination against viral haemorrhagic septicaemia (VHS). Earlier experiments revealed that intramuscular injection of the DNA vaccine encoding the viral glycoprotein G induced protective immunity to VHS in rainbow trout fry of 0.5g. However, the vaccine is known to induce both innate and adaptive protection. The present work therefore aimed at determination of which type of protection the DNA vaccine induced in such early life stages of rainbow trout. Vaccination trials were performed with fry at average sizes of 0.25 g and 0.5 g respectively and included both the homologous VHSV G-gene vaccine and a heterologous DNA vaccine encoding the G-protein of infectious haematopoietic necrosis virus (IHNV). The fish were challenged by immersion at different times post vaccination. Protective immunity was induced in both sizes of fish, but whereas clear-cut specific protection was evident in the fish vaccinated at 0.5g, the results suggested that the protection in the fish vaccinated at 0.25 g was mainly due to innate cross-reactive antiviral mechanisms of shorter duration. The critical size for induction of an adaptive immune response in rainbow trout to this type of vaccination thus appears to be between 0.25 and 0.5g. This work was supported by the “DAFINET” grant from the Danish Council for Strategic Research.
DNA vaccination of small rainbow trout fry against VHSV

Small rainbow trout fry were DNA vaccinated by intramuscular injection at 0.25g and other fish later at 0.5g. Vaccine groups included pcDNA3-vhsG, heterologous vaccine (pcDNA3-ihnG), empty vector (pcDNA3) and unhandled fish. Fry vaccinated at 0.25g were challenged with VHSV by immersion at 3wpv, 11wpv and 21wpv. The challenge at 3wpv was started 1wpv, however as no mortality was observed, the fish were re-challenged 3wpv using a modified setup. Fry vaccinated at 0.5g were challenged with VHSV by immersion at 11wpv.

By early challenge (3wpv) of fish vaccinated at 0.25g both homologous and heterologous vaccines induced unspecific protection (10 % mortality for both). Challenge 11wpv showed waning unspecific protection (60 % mortality) but also a poor specific protection (30 % mortality). By challenge 21wpv, hardly no specific (75 % mortality) or unspecific (81 % mortality) protection was observed. In contrast, fish vaccinated at 0.5 g and challenged at 11wpv showed good specific protection.

The results indicate that DNA vaccination of very small fry (0.25g) can induce an early innate response. However a late adaptive immune response is apparently not established. Vaccination of fry at 0.5g induces an adaptive response like in larger fish.

The experiment was repeated with same vaccination groups. Rainbow trout fry were vaccinated at 0.25g followed by challenge with homologous or heterologous virus at 13 dpv, 11 wpv and 21 wpv. At 13 dpv unspecific protection was induced with both homologous and heterologous challenge (5% mortality). At 11 wpv an unspecific protection with 30 % mortality was observed. At 21 wpv protection against VHSV had dropped further (50 % mortality). Protection against IHNV was better (10 % mortality) but equal for both homologous and heterologous vaccines confirming previous results, that vaccination of fry at 0.25g induces unspecific protection but no adaptive response.

Improved Protection of Rainbow Trout Against Furunculosis by an Autologous Vaccine Under Experimental Conditions

Despite vaccination with oil-adjuvanted vaccines against vibriosis and furunculosis, sea reared rainbow trout in Denmark often encountered outbreaks of furunculosis during warm summer periods. To address this issue under experimental conditions, two groups of rainbow trout were vaccinated by i.p. injection with two different oil-adjuvanted vaccines: (1) a commercial vaccine comprising Vibrio anguillarum serotype O1 and O2, and Aeromonas salmonicida subspecies salmonicida bacterins, with all bacteria originating from Atlantic salmon, and (2) an experimental vaccine based on cultures of the same bacterial species originating from rainbow trout reared in Danish sea farms. The experiment also included a third group of non-vaccinated controls. All fish were individually chip-tagged to allow mixing of all groups in
three replicate aquaria. After 770 dg (day degrees) or 77 days at 10°C, half of the fish in each group were challenged by i.p. injection of 1x10^5 cells of the A. salmonicida isolate used in the experimental vaccine. The other half was tagged by cutting off the adipose fin (non-injected cohabitants). While the non-vaccinated, i.p.-injected fish all died within 2 weeks, a certain level of protection was evident among the vaccinated groups although high mortality also occurred here. No mortality/clinical disease was evident among the non-injected cohabitants. However, when the water temperature was gradually risen to 15-17-20°C, the cohabitants started to die. Some variability was evident between replicate tanks, but the experimental vaccine tended to provide better protection than the commercial counterpart. The results indicate that tailor-making of a vaccine against furunculosis for sea reared rainbow trout in DK is an important approach for optimal protection.

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Authors: Lorenzen, E. (Intern), Kjær, T. E. (Intern), Henriksen, N. H. (Intern), Dalsgaard, I. (Intern), Holten-Andersen, L. (Intern), Madsen, S. B. (Ekstern), Krossøy, B. (Ekstern), Buchmann, K. (Intern), Lorenzen, N. (Intern)
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General and family-specific gene expression responses to viral hemorrhagic septicaemia virus infection in rainbow trout (Oncorhynchus mykiss)
The ability of rainbow trout (Oncorhynchus mykiss) to respond successfully to infection by viral hemorrhagic septicemia virus (VHSV) is expected to involve a large number of biochemical processes. We hypothesized that this would be reflected at the gene expression level in infected fish, and we tested it by examining gene expression levels in the head kidney of trout at a genome-wide scale with a 16K cDNA microarray for salmonids. Expression levels were recorded during 16 days following bath challenge. The challenge experiment included a relatively low susceptibility (32% survival following challenge) and a relatively high susceptibility (18% survival following challenge) trout family that were both split into a group exposed to virus and a non-exposed control group. In total, 939 genes were differentially expressed between infected and non-infected fish (FDR p = 0.05). Five groups of Gene Ontology categories were involved in immune-related processes and over-represented in infected fish: (i) stress and defense response, (ii) NFkappaB signal transduction, (iii) response to non-self, (iv) antigen processing and presentation, and (v) proteasome complexes. The first four categories were also over-represented among the 642 differentially expressed genes in the low-susceptibility trout family but not among the 556 differentially expressed genes in the high-susceptibility trout family. Expression profiles for most immune genes discussed showed increased transcription from day 3 post-challenge. The results suggest that the innate immune system may play an important role in the successful response to VHSV in rainbow trout. In addition, the results indicate that a superior regulation of the transcription of several key innate immune-related genes contribute to the increased survival in resistant fish. (C) 2011 Elsevier Ltd. All rights reserved.

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http://www.sciencedirect.com.globalproxy.cvt.dk/science?_ob=ArticleURL&_udi=B6T9R-527FRRR-1&_user=641802&_coverDate=04%2F30%2F2011&_rdoc=1&_fmt=high&_orig=gateway&_origin=gateway&_sort=d&_docanchor=&view=c&_acct=C000034418&_version=1&_urlVersion=0&_userid=641802&md5=5877feab16ecf1c57f03a8ca3bfb1e6&searchtype=a
Temperature influences the expression profiling of immune response genes in rainbow trout following DNA vaccination and VHS virus infection

A DNA vaccine encoding the glycoprotein (G) genes of the salmonid rhabdovirus viral haemorrhagic septicaemia virus (VHSV) has proven highly efficient against the disease caused by this virus in rainbow trout (Oncorhynchus mykiss). Several studies have demonstrated that this vaccine induces both an early unspecific antiviral response as well as a long-lasting specific protection. However, temperature appears to influence immune response with respect to the nature and duration of the protective mechanisms. In this study, groups of fish were temperature acclimated, vaccinated and challenged at three different temperatures (5, 10 and 15°C). Tissue and organ samples were collected at numerous time points post vaccination (pv) and post viral challenge (pch). Then, gene expression levels of a two immune genes (Vig-1 and Mx3) involved in unspecific antiviral response mechanisms were determined by Q-PCR. The expression profiles appeared similar for the two genes in terms of temperature dependency with a faster induction and shorter duration at the higher temperature. In order to analyze the temperature effect on the relative expression profiles across a larger set of immune genes time points displaying similar Mx3 expression levels post vaccination were identified: 4 days pv (15°C); 7 days pv (10°C); 23 days pv (5°C), respectively. A targeted trout immune gene array including probes for VHSV genes was used for analysis of vaccinated vs control fish per temperature (4-5 replicates). Temperature altered the transcriptome response to the viral challenge, and the number of responsive genes increased at higher temperature: 51 (5°C), 64 (10°C) and 72 (15°C), respectively, indicating that fish kept at higher temperature may have an enhanced immune response. Additional correlation analysis allowed identification of genes that were significantly up- or down regulated synchronous with expression of the vaccine G gene. These findings encompass that for example in fish kept at 5°C, putative CD3, CD4, CD9, CD28, CD53, CD63, CD83, CD84 were up regulated, while CD59, CD83 were down regulated, potentially indicating balancing mechanism of the immune system. An experimental VHSV challenge was performed 7 weeks pv. Similar protection levels of approximately 10% mortality were found for the vaccinated fish, regardless of temperature during immunisation and challenge, whereas the course and level of mortality among the controls were temperature dependent. At day 5 post infection, the number of differentially regulated genes in the livers of vaccinated versus control fish was highest in fish kept at 10°C and lowest in fish at 5°C. This difference presumably reflects the temperature dependent progression of the disease in the controls. Further analysis of the obtained data with respect to gene regulation pathways as a result of DNA vaccination and/or viral infection will be presented.

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**Authors:** Einer-Jensen, K. (Intern), Gautier, L. (Intern), Rasmussen, J. S. (Intern), Lorenzen, E. (Intern), Christensen, M. B. (Intern), Villanueva, S. A. (Ekstern), Martin, S. (Ekstern), Evensen, Ø. (Ekstern), Schyth, B. D. (Intern), Lorenzen, N. (Intern)

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**The Protective Mechanisms Induced by a DNA Vaccine in Fish Depend on Temperature**

In veterinary vaccinology, DNA-vaccines encoding the viral glycoproteins of viral haemorrhagic septicaemia virus (VHSV) and infectious haematopoietic necrosis virus (IHNV) have proved highly efficient in fish under experimental conditions. In the early phase following vaccination, innate cross-protective mechanisms are dominating but the protection becomes highly specific within 3–4 weeks at 12–15 C. Temperature is known as an important external parameter affecting the immune response in fish and the present study aimed at characterizing temperature effects on the immune response to a VHS DNA vaccine. Rainbow trout fingerlings acclimated at 5, 10 or 15 C, were given an intramuscular injection of 1 lg purified plasmid DNA and challenged with virulent VHSV 9 or 36–40 days later. The vaccine protected the fish well at all three temperatures, however the non-specific mechanisms lasted for a longer period of time at lower temperatures, hereby apparently compensating for a delayed adaptive response. At 36 dpv fish kept at 5 C thus had no detectable response of neutralising antibodies while 67% of the fish kept at 15 C had seroconverted. Immunohistochemical time-course studies of the injection site of vaccinated fish also showed a clear effect of temperature; in fish maintained at 15 C the vhsG-protein occurred earlier on the surface of transfected myocytes than in fish kept at the lower temperatures and the inflammatory response clearing away these myocytes similarly arose earlier at high temperature. Long persistence of transfected myocytes expressing the vaccine antigen might explain the prolonged stimulation of innate protective mechanisms at low
temperature. Quantitative gene expression profiles suggested interferon related mechanisms as the explanation for the early protection and also supported their temperature dependent kinetics.

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Experimental vaccination of small turbot against bacterial and viral pathogens

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Immersion exposure of rainbow trout (Oncorhynchus mykiss) fry to wildtype Flavobacterium psychrophilum induces no mortality, but protects against later intraperitoneal challenge
Flavobacterium psychrophilum, the causative agent of RTFS or rainbow trout fry syndrome, causes high mortality among hatchery reared rainbow trout (Oncorhynchus mykiss) fry in Europe and the USA. Despite several attempts, no efficient vaccines have yet been developed, the main obstacle being that the fry have to be vaccinated very early, i.e. around 0.2–0.5 g, where RTFS usually starts to give problems in the fish farms. Consequently, only oral or bath vaccines are relevant. Immersion of fry in inactivated or attenuated bacteria has resulted in RPS values of less than 50%. However, the results are biased by the fact that the fish have been challenged by intraperitoneal (ip) or subcutaneous (sc) injection against which an immersion/oral vaccine may not protect. Therefore, the present study was undertaken in order to investigate whether the presumably most potent immersion immunization, i.e. bathing in high titres of non-attenuated isolates of F. psychrophilum, was able to induce immunity to a subsequent ip challenge. Immersion in live bacteria for 30 or 50 min caused no mortality and protected a major fraction of the fry against challenges 26 and 47 days later with RPS values of 88.2 and 60.3%, respectively. Increased specific antibody titres suggested that adaptive immune mechanisms were involved in the protection.
N-Linked Glycans on the Viral Glycoprotein are not Required for Induction of Protective Immunity to VHSV when Delivered as a DNA Vaccine

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Protection Against Viral Haemorrhagic Septicemia Virus (VHSV) in Rainbow Trout Using a DNA Vaccine with MX1 Promotor Controlled Expression of the Viral G Protein

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Response to Viral Infection differs between families of Rainbow Trout

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**Temperature effects on vaccine induced immunity to viruses in fish**

Abstract In poikilothemic vertebrates such as teleost fishes, temperature affects all physiological processes including host-pathogen interactions like immune response and propagation of infection. Whether an infection with a pathogenic virus in fish results in development of clinical disease often depends on the balance between virus multiplication and anti-viral immune reactions in the host. Water temperature is one of the most important factors influencing the balance between the fish and its environment. Usually, an optimal immune response of a particular fish species is obtained at its normal summer temperature whereas low temperatures may be immunosuppressive. Although innate and adaptive immune response mechanisms should be considered as integrated parts of the immunedefence, low temperatures appears to affect (inhibit) adaptive mechanisms more than innate mechanisms. This might represent a problem in terms of inducing a protective immune response by vaccination in aquaculture, since it is often desirable to vaccinate fish during autumn, winter, or spring. In experimental vaccination trials with rainbow trout (Oncorhynchus mykiss) using a DNA-vaccine encoding the viral glycoprotein of viral haemorrhagic septicaemia virus (VHSV), non-specific as well as specific immune mechanisms seemed to be delayed at low temperature. At five weeks post vaccination fish kept at 5°C had no detectable response of neutralising antibodies while two thirds of the fish kept at 15°C had sero-converted. While protective immunity was still established at both temperatures, specificity analysis suggested that protection at the lower temperature was mainly due to non-specific innate antiviral mechanisms, which appeared to last longer at low temperature. This was presumably related to a prolonged persistence of the vaccine. In DNA vaccination trials with spring viremia of carp (SVC) in common carp (Cyprinus carpio), protection at low temperature (10°C) appeared to require considerable longer time to develop compared to at 19°C, stressing that determination of optimal vaccination strategies in terms of temperature related effects need to be based on experimental evidence with the actual host and pathogen species rather on general principles.

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Main Research Area: Technical/natural sciences

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**DNA Vaccines against Viral Diseases: Basic Immunological Aspects and Applied Perspectives**

**General information**

State: Published

Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute

Authors: Lorenzen, N. (Intern), Lorenzen, E. (Intern), Rasmussen, J. S. (Intern), Einer-Jensen, K. (Intern)

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Publication information
Dual DNA vaccination of rainbow trout (Oncorhynchus mykiss) against two different rhabdoviruses, VHSV and IHNV, induces specific divalent protection

DNA vaccines encoding the glycoprotein genes of the salmonid rhabdoviruses VHSV and IHNV are very efficient in eliciting protective immune responses against their respective diseases in rainbow trout (Oncorhynchus mykiss). The early anti-viral response (EAVR) provides protection by 4 days post vaccination and is non-specific and transient while the specific anti-viral response (SAVR) is long lasting and highly specific. Since both VHSV and IHNV are endemic in rainbow trout in several geographical regions of Europe and Atlantic salmon (Salmo salar) on the Pacific coast of North America, co-vaccination against the two diseases would be a preferable option. In the present study we demonstrated that a single injection of mixed DNA vaccines induced long-lasting protection against both individual and a simultaneous virus challenge 80 days post vaccination. Transfected muscle cells at the injection site expressed both G proteins. This study confirms the applied potential of using a combined DNA vaccination for protection of fish against two different rhabdoviral diseases.
Functional demonstration of adaptive immunity in zebrafish using DNA vaccination.

Due to the well characterized genome, overall highly syntenic with the human genome and its suitability for functional genomics studies, the zebrafish is considered to be an ideal animal model for basic studies of mechanisms of diseases and immunity in vertebrates including humans. While several studies have documented existence of a classical innate immune response, there is mainly indirect evidence of functional adaptive immunity. To address this aspect, groups of zebrafish were vaccinated with DNA-vaccines against the rhabdoviruses VHSV, IHNV and SVCV. Seven weeks later, the fish were challenged with SVCV by immersion. Despite some variability between replicate aquaria, there was a protective effect of the homologous vaccine and no effect of the heterologous vaccines. The results therefore confirm the existence of not only a well developed but also a fully functional adaptive immune system in zebrafish.

Interference of an ERM-vaccine with a VHS-DNA vaccine in rainbow trout

Simultaneous vaccination of fish against several diseases is often desirable in order to minimise cost and handling of the fish. Intramuscular DNA-vaccination of rainbow trout against viral haemorrhagic septicaemia virus (VHSV) has proved to provide very good protection. However, preliminary results showed that intraperitoneal injection of a commercial vaccine against Enteric Redmouth Disease (ERM) based on formalin-killed bacteria in oil adjuvant immediately followed by intramuscular injection of an experimental DNA-vaccine against VHSV, decreased the protective effect of the DNA-vaccine against challenge with VHSV 11 weeks post vaccination (pv). This experiment was performed with rainbow trout of 30 g injected with 0.5 g VHS-DNA vaccine. The experiment was later repeated with smaller fish (2.5 g) and using two different doses of DNA-vaccine, 1 g and 0.05 g. Both doses provided good protection in the control groups not given the ERM vaccine. But among fish given both vaccines, those vaccinated with the lower DNA dose had significantly higher mortality when challenged with SVCV 9 weeks pv. When challenged with VHSV 8 days pv, not even the 1 µg DNA dose protected such fish. A plasmid dose of 0.05 g VHSV DNA vaccine would normally induce good protection in small fish (2-3 g). To ensure complete protection in larger fish, higher doses are needed. This could explain the negative effect of ERM vaccination observed in the 30 g fish described above. It thus appears, that if the fish are vaccinated with a VHS-DNA vaccine dose according to their size, a simultaneous intraperitoneal vaccination against ERM can compromise the protective effect of the DNA-vaccine. The negative effect appears to be strongest in the early phase following vaccination. The immune mechanisms behind this interference will be discussed.
Studies on herd-immunity and primary versus secondary infection of VHSV in challenge and vaccination trials with rainbow trout

Abstract for Scofda meeting 4-5.11.09 Studies on herd-immunity effect and primary versus secondary infection of VHSV by Ellen Lorenzen, Torben Eigil Kjær & Niels Lorenzen, National Veterinary Laboratory, Århus The phenomenon of "herd-immunity" is one of the basal principles behind vaccination as well as selective breeding, i.e. the more non-susceptible individuals in a population, the lower the risk of disease among susceptible individuals. Thus as part of a recent field trial with a VHS-DNA-vaccine vaccinated as well as naïve fish from a Danish fish farm were brought to the laboratory at a size of 24g to be subjected to an experimental challenge with VHSV. The setup included 7 aquaria with 100 fish in each: 2 aquaria with 100 vaccinated fish (+VHSV-challenge), 2 aquaria with 100 naïve fish (+ VHSV-challenge), 2 aquaria with 50 vaccinated + 50 naïve fish (+VHSV-challenge), and 1 aquarium with non-challenged control fish (vaccinated + naïve). Mortality in the aquaria with only vaccinated fish was 2-3 %. Mortality in the aquaria with only naïve fish was 60-70 %. However, mortality among naïve fish in the mixed aquaria was only 6-18 %, the mortality among vaccinated fish being 0-6 %, and we interpreted this as an effect of herd-immunity, where the vaccinated fish protected the naïve fish, probably by secreting less virus compared to the naïve fish. We tried to demonstrate this phenomenon in 3 later experiments, but without success, probably due to a too high challenge load in relation to the susceptibility of the fish included in these studies, i.e. it was shown that the challenged vaccinated fish secreted large amounts of virus, although still less than challenged naïve fish. However, these results led to the question if the fish die due to the challenge virus or due to the virus secreted from fish in the same aquarium that become diseased at an early time point. This question was addressed in 3 subsequent parallel challenge experiments (3 different virus doses) including only one fish in 24 aquaria and 24 fish in 3 aquaria. The study showed, that at high challenge doses, mortality was comparable in the single-fish group (24 aquaria) and the group with 24 fish in each of 3 aquaria. At lower challenge doses, however, the survival rate increased in the single fish group the lower the virus titer during challenge. i.e. at lower challenge doses, secondary infections seem more pronounced. These results will be presented and discussed.

The protective mechanisms induced by a fish rhabdovirus DNA vaccine depend on temperature

DNA vaccines encoding the viral glycoproteins of viral haemorrhagic septicaemia virus (VHSV) and infectious haematopoietic necrosis Virus (IHNV) have proved highly efficient in rainbow trout (Oncorhynchus mykiss) under experimental conditions. Non-specific as well as specific immune mechanisms seem to be activated. Temperature is an important external parameter affecting the immune response in fish. The present study aimed at determining the effectiveness of a DNA vaccine against VHS at different temperatures. Rainbow trout fingerlings acclimated at 5 degrees C, 10 degrees C or 15 degrees C, were given an intramuscular injection of 1 mu g purified plasmid DNA and challenged with virulent VHSV 8 or 36-40 days later. The vaccine protected the fish well at all three temperatures, but the involvement of innate and adaptive mechanisms differed: at low temperature, non-specific protection lasted longer and at 36 dpv fish kept at 5 degrees C had no detectable response of neutralizing antibodies while 67% of the fish kept at 15 degrees C had seroconverted. Induction of Mx as measured in liver samples was delayed at 5 degrees C with no detectable response 7 dpv whereas fish maintained at 10 C had significantly elevated levels of Mx3-transcripts at that time point. Immunohistochemical studies of the injection site of vaccinated fish also showed a clear effect of temperature: in fish maintained at 15 degrees C the vhsG-protein appeared earlier on the surface of transfected myocytes and the inflammatory response clearing away these myocytes arose earlier Compared to fish kept at the lower temperatures of 5
and 10 degrees C.

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Authors: Lorenzen, E. (Intern), Einer-Jensen, K. (Intern), Rasmussen, J. S. (Intern), Kjær, T. E. (Intern), Collet, B. (Ekstern), Secombes, C. (Ekstern), Lorenzen, N. (Intern)
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Web of Science (2007): Indexed yes
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Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.194 SNIP 1.065
Web of Science (2005): Indexed yes
THE PROTECTIVE MECHANISMS INDUCED BY A FISH RHABDOVIRUS DNA-VACCINE DEPENDS ON TEMPERATURE

DNA-vaccines encoding the viral glycoproteins of viral haemorrhagic septicaemia virus (VHSV) and infectious haematopoietic necrosis virus (IHNV) have proved highly efficient in rainbow trout (Oncorhynchus mykiss) under experimental conditions. In the early phase following vaccination, innate cross-protective mechanisms are dominating but the protection becomes highly specific within 3-4 weeks at 12-15°C. Temperature is known as an important external parameter affecting the immune response in fish and the present study aimed at characterizing temperature effects on the immune response to a VHS DNA vaccine. Rainbow trout fingerlings acclimated at 5°C, 10°C or 15°C, were given an intramuscular injection of 1 g purified plasmid DNA and challenged with virulent VHSV 9 or 36-40 days later. The vaccine protected the fish well at all three temperatures, however the non-specific mechanisms lasted for a longer period of time at lower temperatures, hereby apparently compensating for a delayed adaptive response. At 36 dpv fish kept at 5°C thus had no detectable response of neutralising antibodies while 67% of the fish kept at 15°C had seroconverted.

Immunohistochemical time-course studies of the injection site of vaccinated fish also showed a clear effect of temperature: in fish maintained at 15°C the vhsG-protein occurred earlier on the surface of transfected myocytes than in fish kept at the lower temperatures and the inflammatory response clearing away these myocytes similarly arose earlier at high temperature. Long persistence of transfected myocytes expressing the vaccine antigen might explain the prolonged stimulation of innate protective mechanisms at low temperature. From a practical point of view the results suggest that DNA vaccination against rhabdoviruses might be applied as a prophylactic measure within a broad temperature range.

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Authors: Lorenzen, N. (Intern), Lorenzen, E. (Intern), Einer-Jensen, K. (Intern), Rasmussen, J. S. (Intern), Kjær, T. E. (Intern), Collet, B. (Ekstern), Secombes, C. J. (Ekstern)
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Time course study of in situ expression of antigens following DNA-vaccination against VHS in rainbow trout (Oncorhynchus mykiss Walbaum) fry

The present study was performed as a time course study of fish vaccinated with 20 μg plasmid DNA vaccine encoding either the VHSV G-protein or the VHSV N-protein. Samples of the injection site were collected sequentially over a 7-week period. The study revealed an intense positive staining by immunohistochemistry for the viral G-protein mainly in the membrane of intact myocytes, most prominent by days 10-27, and with concomitant infiltration of inflammatory cells by days 13-38 that subsequently lead to a marked reduction in the number of myocytes expressing the G-protein. By immunofluorescence, infiltrating cells positive for MHC II, IgM, and C3 were demonstrated. By contrast, in fish vaccinated with the VHSV-N construct, fewer, diffusely positive myocytes were found, most prominent by days 13-38, these having a positive reaction for the N-protein mainly in the cytoplasm and variably in the membrane. N-protein positive myocytes did
not attract infiltrating cells to the same degree. Positive reaction for the N-protein almost ceased by day 48 post-vaccination.

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Authors: Lorenzen, E. (Intern), Lorenzen, N. (Intern), Einer-Jensen, K. (Intern), Brudeseth, B. (Ekstern), Evensen, O. (Ekstern)  
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BFI (2013): BFI-level 1  
Scopus rating (2013): SJR 1.001 SNIP 1.149 CiteScore 3.11  
ISI indexed (2013): ISI indexed yes  
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Scopus rating (2012): SJR 1.151 SNIP 1.174 CiteScore 3.02  
ISI indexed (2012): ISI indexed yes  
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BFI (2011): BFI-level 1  
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ISI indexed (2011): ISI indexed yes  
Web of Science (2011): Indexed yes  
BFI (2010): BFI-level 1  
Scopus rating (2010): SJR 1.131 SNIP 1.056  
Web of Science (2010): Indexed yes  
BFI (2009): BFI-level 1  
Scopus rating (2009): SJR 0.96 SNIP 1.101  
Web of Science (2009): Indexed yes  
BFI (2008): BFI-level 2  
Scopus rating (2008): SJR 0.952 SNIP 1.062  
Scopus rating (2007): SJR 0.842 SNIP 1.378  
Web of Science (2007): Indexed yes  
Scopus rating (2006): SJR 0.954 SNIP 1.298  
Web of Science (2006): Indexed yes  
Scopus rating (2005): SJR 0.789 SNIP 0.861  
Web of Science (2005): Indexed yes
A DNA vaccine directed against a rainbow trout rhabdovirus induces early protection against a nodavirus challenge in turbot

A DNA vaccine encoding the envelope glycoprotein from a fish rhabdovirus, viral hemorrhagic septicemia virus (VHSV), has previously been shown to induce both early and long time protection against the virus in rainbow trout. Challenge experiments have revealed that the immunity established shortly after vaccination is cross-protective against heterologous fish rhabdoviruses. In this study, we show that the DNA vaccine encoding the VHSV glycoprotein also induces early protection against a non-enveloped, positive-sense RNA virus belonging to the Nodavirus family, the Atlantic halibut nodavirus (AHNV). In a vaccine efficacy test using juvenile turbot as model fish, the fish injected with the VHSV vaccine were completely protected against a nodavirus challenge performed 8 days post vaccination, while the cumulative mortality in the control group reached 54%. A DNA vaccine carrying the gene encoding the capsid protein of AHNV revealed no protective properties against the nodavirus challenge. Histological examination of muscle tissue sections from the vaccine injection site showed that the DNA vaccine against VHSV triggered a pronounced inflammatory response in turbot similar to what has earlier been observed in rainbow trout.

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Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute
Authors: Sommerset, I. (Ekstern), Lorenzen, E. (Intern), Lorenzen, N. (Intern), Bleie, H. (Ekstern), Nerland, A. (Ekstern)
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Immunity induced shortly after DNA vaccination of rainbow trout against rhabdoviruses protects against heterologous virus but not against bacterial pathogens

It was recently reported that DNA vaccination of rainbow trout fingerlings against viral hemorrhagic septicemia virus (VHSV) induced protection within 8 days after intramuscular injection of plasmid DNA. In order to analyse the specificity of this early immunity, fish were vaccinated with plasmid DNA encoding the VHSV or the infectious haematopoietic necrosis virus (IHNV) glycoprotein genes and later challenged with homologous or heterologous pathogens. Challenge experiments revealed that immunity established shortly after vaccination was cross-protective between the two viral pathogens whereas no increased survival was found upon challenge with bacterial pathogens. Within two months after vaccination, the cross-protection disappeared while the specific immunity to homologous virus remained high. The early immunity induced by the DNA vaccines thus appeared to involve short-lived non-specific anti-viral defence mechanisms.
Immunity to viral haemorrhagic septicaemia (VHS) following DNA vaccination of rainbow trout at an early life-stage

Rainbow trout fry of average weight 0.5 g were vaccinated against viral haemorrhagic septicaemia (VHS) by intramuscular injection of 1 µg of plasmid DNA encoding the VHS virus glycoprotein gene. Challenge with a lethal dose of virus at two different time points, 9 and 71 days post-vaccination respectively, revealed that a highly protective and lasting immunity was established shortly after vaccination, in accordance with earlier experiments with larger fish. The defence mechanisms activated by the DNA vaccine are thus functional at an early life-stage in rainbow trout.

General information
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Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute
Authors: Lorenzen, N. (Intern), Lorenzen, E. (Intern), Einer-Jensen, K. (Intern)
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Scopus rating (2014): SJR 1.138 SNIP 1.089 CiteScore 2.92
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.001 SNIP 1.149 CiteScore 3.11
ISI indexed (2013): ISI indexed yes
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ISI indexed (2011): ISI indexed yes
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BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.131 SNIP 1.056
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.96 SNIP 1.101
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DNA vaccination of rainbow trout against viral hemorrhagic septicemia virus: A dose-response and time-course study
Viral hemorrhagic septicemia (VHS) in rainbow trout Oncorhynchus mykiss is caused by VHS virus (VHSV), which belongs to the rhabdovirus family. Among the different strategies for immunizing fish with a recombinant vaccine, genetic immunization has recently proven to be highly effective. To further investigate the potential for protecting fish against VHS by DNA vaccination, experiments were conducted to determine the amount of plasmid DNA needed for induction of protective immunity. The time to onset of immunity and the duration of protection following administration of a protective vaccine dose were also analyzed. The dose-response analysis revealed that significant protection of rainbow trout fingerlings was obtained following intramuscular injection of only 0.01 μg of plasmid DNA encoding the VHSV glycoprotein gene. In addition, higher doses of DNA induced immunity to a virus isolate serologically different from the isolate used for vaccine development. Following administration of 1 μg of a DNA vaccine, significant protection against VHS was observed in the fish as early as 8 d postvaccination. At 168 d postvaccination, the fish had increased in size by a factor of 10 and protection against a lethal dose of VHSV was still evident. The results confirm the great potential for DNA vaccination in inducing efficient immunoprophylaxis against viral diseases in aquacultured fish.

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Authors: Lorenzen, E. (Intern), Einer-Jensen, K. (Intern), Martinussen, T. (Ekstern), LaPatra, S. (Ekstern), Lorenzen, N. (Intern)
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Immunoprophylaxis in fish by injection of mouse antibody genes

Antibodies are a crucial part of the body’s specific defense against infectious diseases and have considerable potential as therapeutic and prophylactic agents in humans and animals. The development of recombinant single-chain antibodies allows a genetic application strategy for prevention of infectious diseases. To test this in a fish model, a gene construct encoding a neutralizing single-chain antibody to the fish-pathogenic rhabdovirus VHSV (viral hemorrhagic septicemia virus) was administered to rainbow trout by intramuscular injection of plasmid DNA. Circulating recombinant antibodies could later be detected in the fish, and protective immunity to the viral disease was established.

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Inter-laboratory comparison of cell lines for susceptibility to three viruses: VHSV, IHNV and IPNV

Eleven European National Reference Laboratories participated in an inter-laboratory comparison of the susceptibility of 5 selected cell lines to 3 fish pathogenic viruses. The test included viral hemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis Virus (IPNV), and the cell lines derived from bluegill fry (BF-2), chinook salmon embryo (CHSE-214), epithelioma papulosum cyprini (EPC), fathead minnow (FHM) and rainbow trout gonad (RTG-2). The results showed that for isolation of VHSV, BF-2 and RTG-2 cells performed equally well and had higher sensitivity compared to the other cell lines. For IHNV, EPC and FHM cells gave the best results, and for IPNV it was BF-2 and CHSE-214 cells. FHM cells showed the largest variability among laboratories, whereas EPC was the cell line showing the smallest variability.
Protective immunity to VHS in rainbow trout (Oncorhynchus mykiss, Walbaum) following DNA vaccination

Rainbow trout fingerlings were immunized by intramuscular injection of a plasmid DNA vector encoding the viral haemorrhagic septicaemia virus (VHSV) glycoprotein (G) or nucleocapsid protein (N) genes under the control of a cytomegalovirus promoter. Challenge with VHSV 52 days later demonstrated that both viral genes, and the G gene in particular, were able to induce protective immunity against VHS. In contrast to sera taken from fish injected with the N gene, neutralizing antibody activity could be detected both before and after challenge in the sera of a major proportion of the fish injected with the G gene.

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Characterization of isolates of Flavobacterium psychrophilum associated with coldwater disease or rainbow trout fry syndrome II: serological studies

The possibility of serological differentiation between isolates of Flavobacterium psychrophilum was analyzed by ELISA and slide agglutination. Twenty-five Danish isolates and 20 isolates from other European countries were studied using polyclonal rabbit antisera and whole-cell preparations. Unabsorbed as well as reciprocally absorbed antisera and purified Ig preparations derived from the antisera were included. Most of the isolates originated from clinical outbreaks of rainbow trout fry syndrome (RTFS) or coldwater disease (CWD), but some were isolated from asymptomatic fish or from other fish species with different disease signs. The ELISA showed the existence of different serotypes most distinctly, but slide agglutination supported the ELISA results. Three serotypes were found among the isolates studied: 1 major serotype (serotype Th) represented most of the Danish isolates and isolates from other European countries; 2 minor serotypes (Serotypes Ed and Fp(T)) also occurred. Serotype Th could be further divided into a major subtype, Th-1, and a minor subtype, Th-2. Serotype Fp(T) was defined by the type strain F. psychrophilum NCIMB 1947(T), and seemed to include mostly isolates from asymptomatic fish or from fish species other than rainbow trout.

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Web of Science (2015): Indexed yes
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Scopus rating (2014): SJR 0.889 SNIP 0.881 CiteScore 1.86
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.812 SNIP 0.918 CiteScore 1.77
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.912 SNIP 1.092 CiteScore 2.04
Characterization of isolates of Flavobacterium psychrophilum associated with coldwater disease or rainbow trout fry syndrome I: phenotypic and genomic studies

Isolates of Flavobacterium psychrophilum (formerly Cytophaga psychrophila and Flexibacter psychrophilus) mainly originating from clinical outbreaks of either coldwater disease (CWD) or rainbow trout fry syndrome (RTFS) were studied for selected biochemical, physiological, morphological and genomic characteristics, and compared with previously characterized French and American strains. DNA hybridization studies showed that the Danish isolates were highly related to the type strain, F. psychrophilum NCIMB 1947(T). Plasmid profiling of Danish isolates and those from other European countries revealed differences, which might be related to differences in pathogenicity. European isolates originating from clinical outbreaks of either RTFS or CND usually harboured one plasmid of 3.2 kb, whereas isolates originating from fish with different or no disease signs had other profiles. Phenotypically, the Danish isolates appeared very homogeneous and shared most characteristics with the type strain, and with French and American strains studied by other authors. Further studies on the importance of the plasmids and the proteolytic activities of the bacterium might help in elucidating possible virulence factors.

General information

State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute, Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Lorenzen, E. (Intern), Dalsgaard, I. (Intern), Bernardet, J. (Ekstern)
Pages: 197-208
First demonstration of *Renibacterium salmoninarum* / BKD in Denmark

**General information**
State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute, National Food Institute
Authors: Lorenzen, E. (Intern), Olesen, N. J. (Intern), Korsholm, H. (Ekstern), Heuer, O. E. (Intern), Evensen, Ø. (Ekstern)
Pages: 140-144
Publication date: 1997
Main Research Area: Technical/natural sciences

**Publication information**
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Volume: 17
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Web of Science (2018): Indexed yes
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Web of Science (2017): Indexed Yes
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Scopus rating (2016): CiteScore 0.49 SJR 0.234 SNIP 0.421
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.27 SNIP 0.496 CiteScore 0.64
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.32 SNIP 0.414 CiteScore 0.68
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.365 SNIP 0.431 CiteScore 0.62
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.257 SNIP 0.49 CiteScore 0.47
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.236 SNIP 0.364 CiteScore 0.41
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.34 SNIP 0.469
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.292 SNIP 0.459
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.325 SNIP 0.373
Scopus rating (2007): SJR 0.31 SNIP 0.386
Scopus rating (2006): SJR 0.436 SNIP 0.592
Simultaneous demonstration of infectious pancreatic necrosis virus (IPNV) and Flavobacterium psychrophilum in paraffin-embedded specimens of rainbow trout Oncorhynchus mykiss fry by use of paired immunohistochemistry

The Gram-negative bacterium Flavobacterium psychrophilum, which is the causative agent of rainbow trout fry syndrome (RTFS), and infectious pancreatic necrosis virus (IPNV), the causative agent of infectious pancreatic necrosis (IPN), are both highly pathogenic for rainbow trout fry. Several ‘persistent’ cases of RTFS have been observed concomitant with IPNV. Cultivation alone might not be sufficient for evaluation of the disease situation as both pathogens can be cultivated from fish that do not show any clinical signs of disease. In such cases it may be difficult to decide which pathogen should be considered the primary cause of the mortality observed. Further, it may be difficult to cultivate the bacterium in later stages of the disease or from dead fish that have been transported without cooling. In the case of (suspected) double infections it is therefore suggested that immunohistochemistry be included as a supplementary diagnostic tool, allowing correlation of the presence of either pathogen with pathological lesions. In the present study, fry representing different stages of RTFS from 3 clinical outbreaks were shown to suffer from ongoing double infections as demonstrated by immunohistochemistry and supported by cultivation of the 2 pathogens. The general finding was that single cells of the exocrine pancreas were positive for the virus, whereas bacteria were mainly demonstrated in the interstitial tissue surrounding the pancreatic islets. In some endothelial cells of the head kidney, both pathogens were detected in the same cell. These findings as well as various protocols in relation to the methodology are discussed.

General information
State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute, Technical University of Denmark
Flavobacterium psychrophilum, paired immunohistochemistry, IPNV, rainbow trout fry syndrome, infectious pancreatic necrosis

An immunohistochemical study of Flexibacter psychrophilus infection in experimentally and naturally infected rainbow trout (Oncorhynchus mykiss) fry

An immunohistochemical method is described for the detection of Flexibacter psychrophilus in formalin-fixed, paraffin-wax-embedded fry of rainbow trout. Rabbit antiserum as well as rainbow trout hyperimmune serum were used in the study. The distribution and tissue localization of the bacterium was compared in naturally and experimentally (intraperitoneal injections) infected fry by use of immunohistochemistry. This study showed that F. psychrophilus could be detected in paraffin-wax-embedded tissue of rainbow trout fry by immunohistochemistry. The principal immunohistochemical findings in naturally and experimentally infected fry showed that there was a localization of bacteria in the monocyte-macrophage system, in skin lesions, and in the retina and the choroid gland of the eye. The dermal changes included superficial or deep ulcers extending to the subcutaneous tissue or the musculature accompanied by inflammatory cell infiltrates in which polymorphonuclear inflammatory cells were shown to contain the bacterium in the cytoplasm by immunostaining. The eye changes were likewise a common finding in chronic cases with severe inflammatory changes in the retina and with numerous bacteria in inflammatory (mainly polymorphonuclear) cells. F. psychrophilus infection in rainbow trout fry involves the monocyte-macrophage system extensively, and the concurrent localization of bacteria in the skin ulcers and retinal inflammation points to the probable involvement of the bacterium in the development of the lesions which are typically found during the chronic stage of the disease.

Vaccination of rainbow trout against VHS using live attenuated vaccines: Danish field trials from 1978 to 1983.
Sammendrag af undersøgelser vedr. yngeldødelighedssyndromet. Afsnit 1

General information
State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute
Authors: Lorenzen, E. (Intern), Olesen, N. J. (Intern)
Pages: 35-39
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Original language: Danish
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Publication: Research › Journal article – Annual report year: 1996

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General information
State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute
Authors: Lorenzen, E. (Intern), Olesen, N. J. (Intern)
Pages: 52-56
Publication date: 1996
Main Research Area: Technical/natural sciences

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Issue number: 3
ISSN (Print): 0015-0223
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Original language: Danish
Source: orbit
Source-ID: 241554
Publication: Research › Journal article – Annual report year: 1996

Differentiation of VHS virus isolates by use of monoclonal antibodies

General information
State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute
Authors: Olesen, N. J. (Intern), Lorenzen, E. (Intern), Lorenzen, N. (Intern)
Publication date: 1995
Event: Abstract from 7th International Conference on Diseases of Fish and Shellfish, Palma de Mallorca, Spain.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 241659
Outbreaks of IPN in reared fry of Atlantic cod Gadus morhua

General information
State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute
Authors: Lorenzen, E. (Intern), Olesen, N. J. (Intern), Evensen, Ø. (Ekstern), Strum, A. (Ekstern)
Publication date: 1995
Event: Abstract from 7th International Conference on Diseases of Fish and Shellfish, Palma de Mallorca, Spain.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 241660
Publication: Research › Conference abstract for conference – Annual report year: 1995

Serological studies on Danish isolates of Flexibacter psychrophilus associated with coldwater disease or rainbow trout fry syndrome

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State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute
Authors: Lorenzen, E. (Intern), Olesen, N. J. (Intern)
Publication date: 1993
Event: Abstract from International Conference of the European Association of Fish Pathologists, Brest, France, .
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 241589
Publication: Research › Conference abstract for conference – Annual report year: 1993

Vaccination trials on rainbow trout fry using formaline killed cells of F.psychrophilus

General information
State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute
Authors: Lorenzen, E. (Intern), Olesen, N. J. (Intern)
Publication date: 1993
Event: Abstract from The Nordic Symposium on Fish Immunology, Lysekil, Sweden, .
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 241585
Publication: Research › Conference abstract for conference – Annual report year: 1993

Projects:

Improved vaccination strategies in marine aquaculture

Section of Fish Diseases
Division of Poultry, Fish and Fur Animals
National Veterinary Institute
National Institute of Aquatic Resources
University of Copenhagen
Danish Aquaculture Association
Aller Aqua A/S
Fishlab
AquaSearch Vet
Schering-Plough A/S
Period: 01/04/2008 → 30/09/2012
Number of participants: 15
Project ID: 22452

Project participant:
- Rasmussen, Jesper Skou (Intern)
- Lorenzen, Ellen (Intern)
- Olesen, Niels Jørgen (Intern)
- Buchmann, Kurt (Ekstern)
- Madsen, Simon B. (Ekstern)
- Melingen, Geir Olav (Ekstern)

Project Manager, organisational:
- Lorenzen, Niels (Intern)
- Dalsgaard, Inger (Intern)
- Pedersen, Karl (Ekstern)
- Hansen, Per Juel (Ekstern)
- Henriksen, Niels Henrik (Ekstern)
- Hørlück, Viggo (Ekstern)
- Sørensen, Kirsten Engell (Ekstern)
- Nielsen, Torben (Ekstern)
- Nylén, Jørgen (Ekstern)

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