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Can we get the upper hand on viral diseases in aquaculture of Atlantic salmon?

Børre Robertsen

Norwegian College of Fishery Science, University of Tromsø, Tromsø, Norway

Correspondence: B Robertsen, Norwegian College of Fishery Science, University of Tromsø, 9037 Tromsø, Norway. E-mail: borre.robertsen@uit.no

Abstract

Viral diseases are a major problem in Atlantic salmon aquaculture. The development of more effective viral vaccines is crucial for a sustained control of viral diseases in salmon farming because the present viral vaccines are much less effective than the bacterial vaccines. On the other hand, research and lessons from Norwegian aquaculture suggest that major progress in the control of viral diseases can still be made through classical combat principles. Improved and enforced strategies for avoidance and control of viral pathogens by breaking horizontal transmission have yielded encouraging results both for the infectious salmon anaemia virus (ISAV) and for the pancreas disease virus (PDV). More emphasis should also be placed on the possible vertical transmission of viral pathogens. Interestingly, salmon appears to have a relatively strong innate immunity against viruses as it possesses a very well-developed interferon system. Interferons protect salmon cells very well against the infectious pancreatic necrosis (IPN) and PDV and salmon show high resistance against these viruses in challenge experiments. In contrast, these viruses cause high mortality in salmon farming. This suggests that the avoidance of production conditions that result in the suppression of the immune system is of primary importance for the prevention of IPN and PD in the aquaculture industry. Interferons protect salmon cells against the ISAV less effectively, which confirms that ISA must be combated by avoidance and vaccination. Breeding of virus-resistant salmon shows promising results, especially with respect to IPN. Finally, to effectuate the above combat principles, it is important to educate highly competent aquamedicine personnel within the aquaculture industry. In Norway, this is accomplished through a 5-year integrated MSc programme in Aquamedicine.

Keywords: Atlantic salmon, virus, horizontal transmission, vertical transmission, innate immunity, interferon, antiviral, vaccine, aquamedicine biologist

Introduction

Farming fish in dense populations in the open sea inevitably leads to outbreaks of infectious diseases. Weak individuals within the farm population will pick up pathogens from the external environment and transmit the agent to healthy individuals. This article discusses how to deal with viral problems in the farming of Atlantic salmon, which is one of the major aquaculture species in the world. Without vaccines, Atlantic salmon farming would have been impossible due to bacterial diseases such as vibriosis, cold water vibriosis and furunculosis. Today, viruses represent the main challenge. The threat posed by viral pathogens is highlighted by the recent devastating effect of the infectious salmon anaemic virus (ISAV) infection in the Chilean aquaculture of Atlantic salmon.

The Norwegian Atlantic salmon aquaculture industry has experienced outbreaks of the following viral diseases: infectious pancreatic necrosis (IPN), ISA and pancreas disease (PD). In 1999, a new viral disease, heart skeletal muscle inflammation (HSMI), was discovered (Kongtorp, Kjerstad, Taksdal, Guttvik & Falk 2004). During the last 10 years, ISA outbreaks have remained at a relatively low level of about 13 outbreaks per year, while there have been on average

180 annual IPN outbreaks (Fig. 1). However, the actual losses due to IPN appear to have declined due to a combination of several factors as mentioned below. On the other hand, PD has increased alarmingly in the south-western part of Norway. Various actions are now being implemented to hopefully reduce this problem. Finally, HSMI has also increased quite drastically in incidence, but no procedures have been carried out as yet to control this disease.

In Norwegian aquaculture, viral diseases are mainly controlled by efforts to stop the transmission of viruses from initial outbreak areas. Commercial vaccines are being used against IPN and PD, but the efficacy of the vaccines in the field is still uncertain. Breeding of salmon for increased resistance against viruses is also underway. More attention should, however, be paid to the possibility that viral outbreaks may occur due to suppression of the fish immune system as a result of unfavourable environmental conditions.

Atlantic salmon has a relatively strong innate immunity against viruses

The non-vaccinated fish is dependent on the innate immune system. The problems associated with viral diseases may leave the impression that Atlantic salmon, by nature, are quite susceptible to viruses. However, both the history of Norwegian salmon farming in relation to its exceptional output in biomass and the recent immunological research demonstrate that, under optimal husbandry conditions, salmon have a high level of innate immunity against most viruses. Firstly, until recently, farmed salmon have managed very well without viral vaccines. Secondly, salmon demonstrate high resistance against IPN virus (IPNV) and PD virus (PDV) in laboratory challenge experiments, where it has been very difficult to obtain high mortality in such experiments. And thirdly, recent research has revealed that Atlantic salmon, in common with mammals, have a very well-developed interferon system, which is the most important component in the first line of defence against viruses.

Interferons (IFNs) are alarm proteins that are produced upon viral infection. The host cell recognizes viral RNA by various receptors, starting a signal process that leads to the synthesis and secretion of IFN. Most cells in the body have receptors for IFNs. Binding of IFN to the IFN receptor initiates another signalling process, which leads to the synthesis of multiple antiviral proteins and the host cells become protected against further viral infection. Recent research has shown that fish possess an IFN system that is very similar in function to that of mammals (Robertsen 2006). Fish have all the receptors for viral RNA found in mammals including RIG-I/MDA5 in the cytoplasma and TLR3, TLR7, TLR8 and TLR9, which are imbedded in endosomal membranes (Meijer, Gabby Krens, Medina Rodriguez, He, Bitter, Ewa Snaar-Jagalska & Spaink 2004; Rodriguez, Wiens, Purcell & Palti 2005; Sarkar, Desalle & Fisher 2008). Moreover, my group has shown that salmon has a remarkable arsenal of IFNs, which is comparable to that of mammals in complexity (Robertsen, Bergan, Røkenes, Larsen & Albuquerque 2003; Robertsen

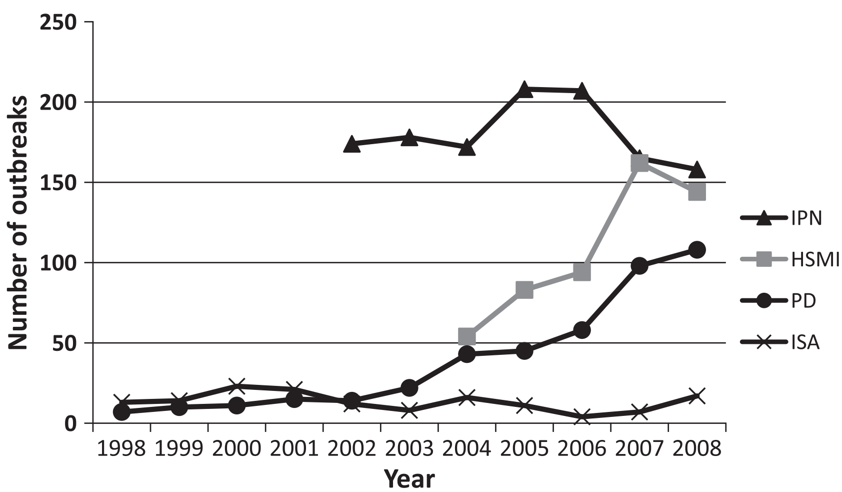

Figure 1. Number of annual viral outbreaks in Norwegian salmon aquaculture from 1998 to 2008. Source: National Veterinary Institute, Oslo, Norway (http://vetinst.com).

2006; Sun, Robertsen, Wang & Liu 2009). Notably, salmon possess a cluster of at least 11 IFN genes linked to the growth hormone 1 gene (Sun *et al.* 2009). The genes encode three different IFN subtypes, IFNa, IFNb and IFNc, which are quite different in sequence and are regulated differently. At present, we know that Atlantic salmon possess at least 14 IFN genes. Recombinant IFNa, IFNb and IFNc have been prepared and they all show potent antiviral activity against IPNV in cell assays (Robertsen *et al.* 2003, T. Svingerud & B. Robertsen, unpubl. obs.). IFNa1 also protects cells very well against the PDV. Salmon thus seem to be well equipped to fight viral infections. It might be possible to stimulate the innate immune system with IFNs or IFN-stimulating compounds, although the effect is likely to be short-lived compared with the effect of vaccines. One possibility is to stimulate the IFN system shortly before the release of smolts into the sea as a relief to this very stressful event.

Some virulent virus strains are able to break through the innate immune barriers of the host by encoding IFN antagonistic proteins. This is apparently the case for the ISA virus (ISAV). Neither recombinant IFNa1 nor the IFN-inducing compound poly I:C was able to protect salmon cells against ISAV even though the stimulated cells contained high levels of the antiviral proteins Mx and ISG15 (Jensen & Robertsen 2002; Kileng, Brundtland & Robertsen 2007). Recent data also suggest that ISAV encodes proteins that inhibit the induction of IFNa1 in cells (Garcia-Rosado, Markussen, Kileng, Baekkevold, Robertsen, Mjaaland & Rimstad 2008). Finally, ISAV also causes high mortality in salmon in cohabitant challenge experiments. These observations underline the importance of developing effective vaccines against ISAV. Fortunately, ISA outbreaks are still relatively rare along the Norwegian coast and can be controlled by the prevention of horizontal transmission as detailed below.

Avoidance of immunosuppressive production conditions

As described above, salmon appear to have a relatively strong innate immunity against IPNV and PDV. Outbreaks of these diseases may thus be due to the appearance of high-virulent viral strains that are able to break through the innate immune barriers of the host or to conditions in the farm environment that suppress the IFN system of salmon. While little is known about the effect of IPNV and PDV on the IFN system, it is well known that various kinds of stress have a suppressive effect on the innate immunity of fish against pathogens (Maule, Tripp, Kaattari & Schreck 1989; Schreck 1996). Handling stress can result in the reoccurrence of covert IPNV (Taksdal, Ramstad, Stangeland & Dannevig 1998) and hyperoxygenation in the fresh water phase increases the susceptibility to IPNV challenge following seawater transfer (Fridell, Gadan, Sundh, Taranger, Glette, Olsen, Sundell & Evensen 2007). The stress resulting from high stocking densities of large salmon in net pens is probably of importance. Infection with sea lice and lice treatment may also lead to immunosuppression (Mustafa, MacWilliams, Fernandez, Matchett, Conboy & Burka 2000). Furthermore, under field conditions, a substantial fraction of smolts released into the sea may be immunosuppressed because they have not achieved sufficient seawater tolerance. It is obviously highly important to avoid such an immunosuppressive condition to minimize the risk of a viral disease outbreak.

Vaccination against viruses

Vaccines stimulate the adaptive immune system by initiating antibody production and T-cell-mediated immune responses. Fish have an adaptive immune system that is remarkably similar to that of mammals, although fish possess fewer immunoglobulin classes and lack class switching mechanisms (Secombes & Pilstrom 2000; Solem & Stenvik 2006). The success with bacterial vaccines shows that Atlantic salmon has an effective adaptive immune system against most bacterial pathogens. Salmonids can also be immunized against viral pathogens, but the protective effects of most viral vaccines are not comparable to that of bacterial vaccines (Biering, Villoing, Sommerset & Christie 2005). Commercial vaccines are available against IPNV, based on both killed virus particles and recombinant VP2 capsid protein expressed in bacteria. Good protective effect of the IPN vaccine based on recombinant VP2 has been obtained in laboratory and field experiments (Ramstad, Romstad, Knappskog & Midtlyng 2007). The beneficial effect of IPN vaccines in salmon aquaculture has been questioned due to the number of IPN outbreaks remaining at a very high level (Fig. 1). A commercial vaccine against PD based on killed virus is also available, but the effect is uncertain due to the lack of satisfactory challenge models for the PDV. Killed ISAV also elicits a protective immune response in salmon, but vaccine development is still at a primitive stage (Jones, Mackinnon & Salonius 1999). More recently, DNA vaccines against rhabdoviruses have shown excellent protective effects in the laboratory both with Atlantic salmon (IHNV) and rainbow trout (IHNV and VHSV), illustrating the potential that lies in this new vaccine technology (Kurath 2005). A DNA vaccine against IHNV has been licensed for use in aquaculture in British Columbia, Canada (Salonius, Simard, Harland & Ulmer 2007), but DNA vaccines are not yet permitted in Europe.

The general scientific opinion appears to be that the present viral vaccines are not effective enough in the field and may not exert sufficient long-lasting protective effects. Vaccines with low efficacy may result in fish that are carriers of viruses and allow viruses to adapt more easily to the antibodies generated by vaccination. No doubt, much more effort has to be invested into fish viral vaccine research. With the exception of DNA vaccines, surprisingly little advances have occurred in fish vaccine research during the last 20 years. One of the problems is to achieve sufficiently high levels of viral antigens in the vaccines. This may be achieved by increasing virus yields in cell cultures or by producing recombinant viral proteins. Both approaches are demanding. A major problem with recombinant viral proteins is to achieve correct folding. Live-attenuated viral vaccines generated by reverse genetics also have a potential, but may not be permitted due to environmental safety concerns. Finally, adjuvants optimized for viral vaccines need to be developed and vaccination strategies have to be improved with respect to delivery methods and repeated immunization.

Breeding of virus-resistant fish

Different salmon families have been shown to vary substantially in susceptibility, especially to IPNV, but also to ISAV (Kjoglum, Larsen, Bakke & Grimholt 2006; Storset, Strand, Wetten, Kjøglum & Ramstad 2007). This may be related to the differences in innate immunity, but could also be due to other factors such as differences in the host proteins involved in viral invasion and replication. These observations have motivated breeding companies to select fish with increased resistance against IPNV and offer these selected breeding lines to the farmers. Although it is likely that these efforts have contributed to a reduction in losses due to IPN during the last few years, the number of outbreaks is still at a very high level. Aggressive breeding for resistance against specific viruses may not be beneficial for the industry in the future because this may lead to one-gene resistance that can be overcome by emerging virulent strains of the virus. Furthermore, concerns have been raised that fish bred for resistance against one disease may become more susceptible to another.

The importance of stopping horizontal transmission

It is now well accepted that horizontal transmission is the main route of the spread of viral diseases in salmon farming. In fact, stopping further horizontal viral transmission is and has been a major success factor in Norwegian salmon farming.

Viral outbreaks originate either from infected wild fish or from infected smolts released into the net pens. In the latter case, well boats are regarded as the main problem because they often transport both smolts and fish ready for slaughter (Murray, Smith & Stagg 2002). The virus may spread to neighbouring farms either by the water current, shared equipment or by escaped fish. Without effective vaccines, breaking the horizontal transmission pathways is perhaps the most important measure to combat viral disease in salmon farming. This is obvious when it comes to the eradication of exotic viruses such as IHNV and VHSV, but is also important for the control of viral diseases such as ISA and PD. The combat principles include maintaining appropriate distances between fish farms, practicing separation of generations by the 'all-in-all-out' principle, non-use or UV treatment of sea water in the fresh water phase, a ban on transportation of fish to and from infected sites, stamping out of infected stocks, allow several months of fallowing between outbreaks, disinfection of well boats, screening of smolts for viruses, avoiding fish escapes and making the disease notifiable to veterinary authorities. Many of the procedures and principles listed above have been implemented as legal measures in Norway and the EU. The importance of these efforts in stopping the spread of viral disease in Norwegian salmon farming was first demonstrated by the successful handling of ISAV outbreaks (Håstein, Hill & Winton 1999). In 1990, ISA outbreaks were alarmingly high, with approximately 80 new outbreaks and the virus spread rapidly along the whole Norwegian coast. Several legal measures were then implemented to stop horizontal transmission, which resulted in an astonishingly rapid decline in ISA outbreaks. In 1994, only one outbreak of ISAV was recorded. Annual ISA outbreaks have since increased and have varied between 4 and 24. However, the recent ISA outbreaks in Chile illustrate the devastating effect of not paying enough attention to the horizontal spread of ISAV.

Unfortunately, Norway did not learn enough from the ISA problem and PD has been allowed to become out of control in the south-western part of Norway. Fortunately, PD is not yet endemic in Norway and outbreaks in northern Norway are most probably due to the transport of infected smolts from southern Norway. In this case, the industry itself has taken action in collaboration with the veterinary authorities and have launched the 'Stop PD' campaign (http://pdfri.no). The main goals are to stop the spread of PDV outside the south-western counties and to reduce losses within the problem areas. This campaign includes many of the principles and procedures mentioned above, but also involves a much more coordinated action between farmers in the same area. Also, released smolts are vaccinated against salmonid alphavirus 3 (SAV3), the causal agent of PD in Norway. Furthermore, transport of smolts from the problem region to non-affected areas is avoided. These efforts will hopefully bring PD under control in the near future.

Vertical transmission of viruses

Vertical transmission is the transfer of virus from one generation to its offspring. The recent outbreaks of ISA in Chile have increased worries about the transmission of viruses via fertilized eggs. Although the survival of viruses within salmon eggs has yet to be proven, this cannot be excluded (Dorson 1985). Transmission of viruses via reproductive products is well proven (Dorson 1985; Bootland & Leong 1999) and viruses are known to attach to sperm and fertilized eggs from salmonids (Mulcahy & Pascho 1984; Ahne & Negele 1985). Viruses may thus spread with fertilized eggs that have not been successfully disinfected or possibly within eggs. Both events are likely to be rare in modern aquaculture, but the transfer of salmonid viruses between continents demonstrates that egg-associated transmission has occurred. Sequence analyses of genes from Chilean ISAV isolates obtained in 2007 and 2008 suggest that these ISAV strains have a European origin and therefore must have been introduced to Chile via embryos (Godoy, Aedo, Kibenge, Groman, Yason, Grothusen, Lisperguer, Calbucura, Avendano, Imilan, Jarpa & Kibenge 2008; Vike, Nylund & Nylund 2009). Whether this has happened in connection with aquaculture is, however, uncertain. The transfer of another salmonid virus, IHNV, between continents is well known and must also have occurred via fertilized eggs (Bootland & Leong 1999). Vertical transmission of IPNV in Atlantic salmon is suspected, but has not been proven. On the other hand, the Norwegian strain of PDV, SAV3, has apparently not yet appeared in Ireland or Scotland despite frequent transport of fertilized salmon eggs from Norway (Hodneland, Bratland, Christie, Endresen & Nylund 2005; Karlsen, Hodneland, Endresen & Nylund 2006; Fringuelli, Rowley, Wilson, Hunter, Rodger & Graham 2008).

To be on the safe side, precautions against vertical transmission need to be taken. Disinfection of fertilized eggs is now a routine practice, but screening of juveniles for virus may also have to be considered. Virus-free broodfish stocks should be cultured and the parental fish must also be examined for the presence of virus or viral genes before the eggs are released onto the market.

The importance of education in fighting disease

Finally, I want to point out how important it is for a sustainable aquaculture industry to maintain education of highly qualified professionals in aquamedicine. Veterinarians have played a major role in the development of the legislation and surveillance related to fish diseases in Norway. However, with the advent of Norwegian aquaculture, the need for specialized fish health professionals became apparent. A 5-year integrated MSc programme in aquamedicine was therefore established at the University of Bergen and the University of Tromsø in 1989. Courses with a special emphasis on fish are included in the programme, among which are the following: aquaculture, physiology, immunology, diseases, pathology, pathogens, parasitology and pharmacology. Graduates obtain the professional title 'Aquamedicine biologist' and are licensed to work on fish health in aquaculture on an equal footing with veterinarians. They hold important jobs within fish farm companies, as well as within vaccine companies and research and governmental administration related to aquaculture. It cannot be doubted that aquamedicine biologists and veterinarians have made a strong positive impact on the health of farmed salmon in Norway.

Conclusion

Viral diseases will remain a continuous problem in aquaculture and efforts to stop horizontal and vertical spread of viruses therefore need to be at the forefront of disease control strategies. Both legislation and voluntary involvement are important for a successful control of horizontal transmission of viral pathogens. Potential immunosuppressive conditions need to be avoided and much more research has to be carried out in the field of fish viral vaccines. Finally, education of specialized aquamedicine professionals must have a high priority to maintain a sustainable aquaculture industry.

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