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Intervet Aquatic Animal Health

Newsletter

October 2003

In this issue ……..

LABORATORIOS INTERVET IN SPAIN

Laboratorios Intervet S.A is located in Salamanca, a city with plenty of monuments and where one of the oldest universities in the world was set up back in the 13th century. UNESCO recently declared it a World Heritage City. Salamanca has a population of 170,000 people, a high percentage of them university students, and is situated in the northwest corner of the Iberian Peninsula, some 200 km from Madrid.

Intervet began to operate in Spain in 1974, after buying a small local laboratory with an area of 3,000 m^2, 1,200 m^2 of which were buildings. This was subsequently renamed Laboratorios Intervet S.A. Back then the company had a workforce of just 30 people employed in administration, production and commercialisation of pharmaceutical specialities for veterinary use in the domestic market. However, the development of Laboratorios Intervet S.A. has run parallel to the growth of Intervet globally, and nowadays it employs 250 people across various departments, which are now situated on a 28,000-m^2 site, 20,000 m^2 of which are buildings. The production activities are an integral part of Intervet's global production and products made in Spain are sold all over the world.

The Production Department, which has 175 employees, has grown rapidly during the last ten years, being today regarded as a cornerstone in Intervet's global production strategy. It has state-of-the-art production facilities for vaccines, including equipment to produce live and inactivated vaccines for poultry, mammals and fish. The antigens used to produce the various viral and bacterial vaccines are obtained from fertile chicken eggs, from cell cultures and by means of bioreactors. Its biological production capacity is one of the biggest of its kind in the world.

The rapid expansion of aquaculture in recent years in many parts of the world resulted in Intervet's decision to develop fish vaccines to help the industry. With this in mind, much of Intervet's aquatic animal vaccine production has now been transferred to Laboratorios Intervet S.A.'s T.C. production facilities.

The reasons behind Laboratorios Intervet S.A.'s continuous growth are the outstanding results obtained over the years in quality, productivity, costs and flexibility. All these achievements have been attained by always complying strictly with GMP, GLP and internal quality systems.

STREPTOCOCCUS INIAE INFECTIONS IN ASIAN AQUACULTURE

Streptococcal disease caused by *Streptococcus iniae* is without doubt one of the major bacterial diseases in fish. It has been reported to cause significant mortality in more than 12 different aquaculture species. Its distribution is worldwide in both freshwater and marine environments. The annual impact to aquaculture has been estimated to be over US$100 million. However, in the past, very few reports have described its presence in Asia. Over the last 3 years, Intervet Norbio Singapore has gathered a substantial amount of information on the severity and frequency of *Streptococcus* outbreaks in cultured fish of the entire Asian-Pacific region.

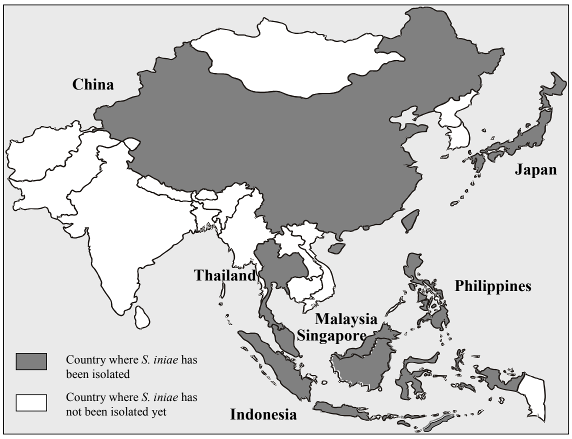
Aetiology

Streptococci are Gram-positive bacteria. Streptococcal disease in fish is mainly caused by three bacteria: *S. iniae, S. difficile* and *S. agalactiae*. *S. iniae* is the most common and pathogenic one in the marine environment.

Host range, geographic distribution

S. iniae infection is a major problem of warmwater aquaculture, but has very few limitations in regard to geographic boundaries or host ranges. The affected species reported include rainbow trout (*Oncorhynchus mykiss*), tilapia *(Oreochromis* spp.*)*, yellowtail *(Seriola quinqueradiata)*, European seabass (*Dicentrarchus labrax*), European seabream (*Sparus aurata*), red drum (*Sciaenops ocellatus*), bastard halibut (*Paralichthys olivaceus)* and Asian seabass (*Lates calcarifer*).

The following map shows the countries of Asia where Intervet has isolated *S. iniae* from cultured fish to date.

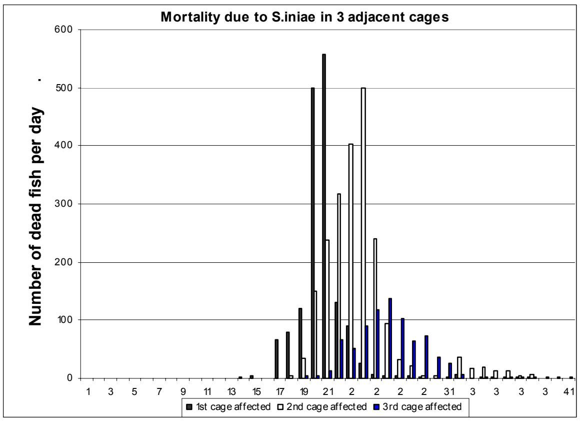


In these countries, *S. iniae* has been isolated in a variety of species as illustrated in the following table.

Fish species	Country of isolation
Asian seabass/Barramundi (*Lates calcarifer)*	Malaysia, Singapore, Taiwan, Indonesia, Thailand
Four-finger threadfin (*Eleutheronema tetradactylum*)	Malaysia
Grouper (*Epinephelus* spp.)	Malaysia, China
Pomfret (*Trachinotus* spp.)	Malaysia, China
Seabream (*Rhabdosargus* spp., *Sparus* spp.; *Plectorhynchus* spp.)	China
Snapper (*Lutjanus* spp.)	Malaysia, China
Tilapia (*Oreochromis* spp.)	Philippines, Indonesia, China
Yellow croaker (*Larimichthys polyactis)*	China

Epidemiology

The transmission is horizontal with infection coming from direct contact with infected fish, contaminated fish food or a contaminated environment. As *Streptococci* bacteria can survive for several months in frozen fish, feeding trash fish considerably increases the chances of infection. Transmission from wild fish to cultured fish has also been reported.
Both acute and chronic mortality occurs. Acute outbreaks often occur during the warmer months of the year or when fish are subject to increased stress, with cumulative mortality reaching 80% within a 10-day-period. In a marine cage-farming situation, the peak mortality usually starts suddenly from one cage and spreads progressively to the neighbouring cages as illustrated in the following graph.



After an acute outbreak, a low-level chronic mortality can carry on for weeks or months with a small number of fish dying every day. Any size of fish can be affected by *S. iniae*. But most outbreaks will take place on fish of at least 10 g.

Clinical signs and gross pathology

Usually, fish infected with *S. iniae* become lethargic and refuse to feed. As these bacteria target the brain and nervous system, erratic swimming, disorientation and swirling behaviour are commonly observed. Very often, fish show unilateral or bilateral exophthalmia with opacification of the cornea. Petechial haemorrhage can be present at the base of the fins, or around the mouth, operculum or anus. Darkening of the skin is another common external sign.
Internally, the symptoms are typical of a systemic bacterial infection with presence of ascites, splenomegaly, enlarged kidney, pallor and haemorrhages of the liver.

Affected black seabream (Spondyliosoma cantharus) with opacification of the cornea

Diagnosis

Clinical signs and impression smear. The presence of typical clinical signs and demonstration of Gram-positive cocci from internal organs by Gram-stained impression smears constitute a presumptive diagnosis.
Bacteriology. Samples from brain, liver, spleen and kidney tissues plated on standard, non-selective TSA or BHIA media supplemented with salt if appropriate, incubated at 26ºC for 24 to 48 hours show small (0.5-1.0 mm diameter), whitish, translucent, rounded and slightly raised colonies. *Streptococci* are Gram-positive, non-acid fast, non-motile, oxydase-positive, catalase negative, cocci. *S. iniae* are ß-haemolytic. The identification to the species level is done on the basis of biochemical and phenotypical profile.
Histopathology. Invasion of a large number of Gram-positive cocci can be observed in most organs. Proliferative inflammation with infiltration of macrophages engulfing bacteria and multifocal areas of necrosis are particularly notable in the central nervous system, eye, heart, spleen, kidney and ateral muscles. No notable changes are observable in the gills.

Control

Considering the acute nature of the development of the disease, most fish will not be treatable through antibiotic treatment. Presently there is no real effective cure.

Prevention

Avoidance. Screening and quarantine of incoming fingerlings and avoiding the feeding of trash fish diet are the two major means of avoidance relevant to Asia.
Good husbandry practices. Reducing overfeeding, overcrowding and unnecessary stress (such as handling or transportation) can reduce the risk of outbreak. The collection and sanitary disposal of moribund or dead fish

should be observed on a daily basis. These measures may prevent outbreaks or at least reduce their severity.
Vaccination. A safe and effective vaccine against *S. iniae* outbreak in fish has been developed by Intervet Norbio Singapore and will soon be available in South East Asia as a major means to control this disease.

DISSEMINATION OF INTERVET SINGAPORE RESEARCH

[Reprinted from Asian Aquaculture magazine, Nov/Dec 2003]
Infectious disease is considered to be the single most important cause of mass mortality and economic loss in the aquaculture industry. A range of infectious organisms, including bacteria, viruses, fungi and parasites, causes diseases. At the recent Asian Pacific Aquaculture 2003 conference held in September in Bangkok, Dr Cedric Komar and Dr Zilong Tan, from Intervet Norbio Singapore presented information on two important bacterial pathogens that are threatening the fish farming industry in Asia.

Bacterial diseases in farmed fish in Asia

Dr. Komar's presentation was entitled "The prevalence of *Streptococcus iniae* infection in cultured fish of South East Asia". *S. iniae* is a fish pathogen affecting a wide range of aquaculture species of both marine and freshwater environments (see previous article).

Over the last few years, Intervet has investigated the importance of this disease in the South East Asia region. A large number of samples were analysed and *S. iniae* was identified based on phenotypical, biochemical and serological tests, and histopathological examination. *S. iniae* was found in a wide range of species, including Asian seabass (*Lates calcarifer*), four-finger threadfin (*Eleutheronema tetradactylum*), pomfret (*Trachinotus* spp), grouper (*Epinephelus* spp), snapper (*Lutjanus* spp) and tilapia (*Oreochromis* spp), originating from various countries, such as Singapore, Indonesia, Malaysia, the Philippines and China.

Tilapia and Asian seabass appear to be the most frequently affected species; the pathogen was isolated in fish as small as 10 g but was also often found to cause mortality in market-size animals. Observed mortality associated with the pathogen could reach 30-80% over a period as short as 10 days.

Control of Streptococcal Diseases

- Due to sudden and acute mortality, no real effective treatment.
- Preventative measures
  - Choice of fingerlings
  - Quarantine of incoming fish
  - Regular cleaning and disinfection
  - Disposal of dead fish
  - Reduce stress on fish
  - Good nutrition
  - Good feeding management
  - Vaccination

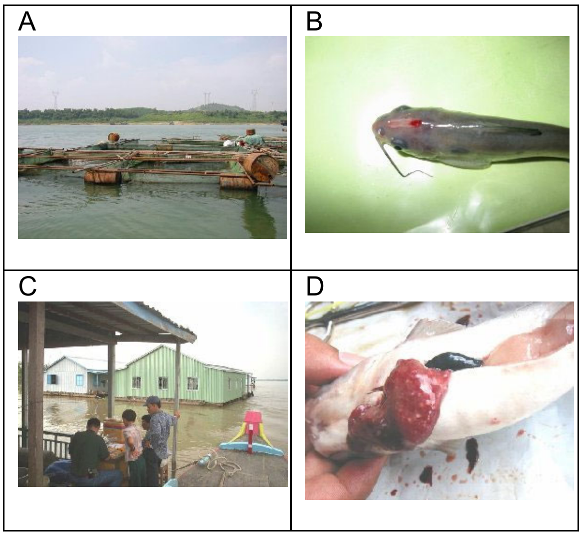
Frequently observed clinical signs included darkening of the fish, loss of equilibrium, whirling swimming behaviour, pop eyes and pinpoint skin haemorrhages.

Prevention is better than cure. Vaccination will be a valuable tool for the control of streptococciosis and other infectious diseases.

Another disease situation, *Edwardsiella ictaluri* infection, was described by Dr. Tan, specifically *E. ictaluri* isolated from catfish in China and Vietnam. *E. ictaluri* is the etiological agent of enteric septicaemia of catfish (ESC), already the major disease of channel catfish (*Ictalurus punctatus*) in the USA. Until now, the disease has not been well recognised in Asia except for a few reported cases in walking catfish (*Clarias batrachus*) in Thailand, Japanese eel (*Anguilla japonica*) in Taiwan and tra catfish (*Pangasius hypophthalmus*) in Vietnam.

In the investigations jointly conducted by Intervet and local institutions and organizations in China and Vietnam, *E. ictaluri* was isolated from several natural outbreaks in channel catfish and tra catfish. In channel catfish, typical ESC clinical signs were observed with approximately 30% mortality in 10- to 30-g fish:

head-up swimming behaviour, a bump at the cranial foramen, haemorrhage or inflammation in the skin under the jaw, and haemorrhage in the liver. Histopathologically, severe systemic infection (often with degeneration and necrosis) was observed in the major organs and tissues. The clinical findings in tra catfish were different from those in channel catfish. Findings in the latter were characterised by skin discolouration (pale) and multifocal, small white lesions on the liver and kidney. Severe necrosis was common in the liver with a lot of bacteria present.



***Edwardsiella ictaluri* infections in catfish: a) channel catfish cultured in river cages in Southern China; b) lesion on the head of a channel catfish infected with *E. ictaluri*; c) culture of tra catfish in house cages in Vietnam; and d) white lesions on liver caused by *E. ictaluri*.**

These findings indicate that *S. iniae* and *E. ictaluri* are prevalent in South East Asia and cause major economic losses to the aquaculture industry. Up to now, it has been common for some of the farmers in this region to use antibiotics for disease control. However, apart from the issues of residue problems and drug resistance, antibiotics are often not effective in combating these diseases. In the case of a streptococcal outbreak, onset of the disease is sudden and usually results in acute mortality. Therefore, it is often too late for therapeutic treatments. In addition, sick fish do not eat well so antibiotics cannot be effectively delivered. In the USA, oxytetracycline (OTC) and an ormetoprim-potentiated sulfa drug are the only approved antibiotics for *E. ictaluri* infection. However, we have found that some of the *Edwardsiella* isolates are resistant to antibiotics belonging to the same drug families as OTC and sulfa.

Effective control of infectious diseases must rely on prevention rather than cure. Thus, good husbandry practices and health management must be emphasized. As part of the health management measures, vaccination has been proven to be a very effective approach for disease control. In relation to these two specific diseases, an efficacious live attenuated *E. ictaluri* vaccine is already available in the USA for catfish and an inactivated *S. iniae* vaccine is being developed and will be available in the near future.

Dr. Zilong Tan (middle) and Dr. Cedric Komar (on right) examining diseased fish during an on-farm investigation in Vietnam.

KHV ISOLATED AND TO BE RENAMED

[See Intervet AAH Newsletter no. 6 for related article.] According to research findings in a forthcoming article in the quality journal Vaccine, the virus that has been the cause of high mortalities in Koi populations around the globe has been isolated. The article, "Efficient vaccine against the virus causing a lethal disease in cultured *Cyprinus carpio*", by Ariel Ronen et al. of the Department of Pathology, The Hebrew University-Hadassah Medical School, Jerusalem, announces that the virus previously known as Koi Herpes Virus (KHV) is not a member of the Herpes virus family, and that renaming it is in order. The new name proposed is "carp nephritis and gill necrosis virus" (CNGV). It may not be a mouthful for the Koi, but it is for us! For further details, see http://www.koi-news.com/CNGV.html

GONAZON™, FIRST REGISTERED GnRH SPAWNING AID IN EU/NORWAY

In salmonid aquaculture, the normal spawning season extends over many weeks (typically 6 to 8), requiring repeated anaesthesia and handling of a large number of fish. As a consequence, it is difficult to optimise the use of labour and hatchery facilities. The season is even longer and ovulation efficiency is lower in Atlantic salmon when broodstock fish are kept in sea- or brackish water for sanitary and/or technical reasons. This means repeated handling of broodstock fish resulting in additional diseases and mortality, as well as increased labour. Thus, reducing both the duration of the spawning season and the number of fish manipulations will maximise egg production, limit broodstock mortality and optimise production and selection. It will also allow for better use of labour and facilities, and give an element of control to management that will optimise marketing efforts for egg suppliers.

Ovulation is a complex physiological process wherein oocytes that are ready to be fertilised are shed. For fish farming purposes, the control of ovulation may be achieved by several hormonal means, such as pituitary extracts, human chorionic gonadotrophin (hCG; like Chorulon® **see Intervet AAH Newsletter no. 4**) and gonadotrophin-releasing hormone (GnRH, LHRH) analogues (GnRHa). Intervet has developed a GnRHa product, Gonazon™, to induce and synchronise ovulation of female salmonids and other finfish species. Due to the demand for full transparency in the food chain, a pre-requisite for the use of such products in aquaculture is the obtainment of a marketing authorisation (i.e., full registration). This was obtained for Gonazon in 2003 in the EMEA countries (EU/Norway) and the product will be available in certain markets early next year.

Azagly-nafarelin, a long-acting GnRH agonist

The active ingredient of Gonazon is azagly-nafarelin, a synthetic GnRH analogue composed of 10 amino acids (pGlu-His-Trp-Ser-Tyr-[D-Nal(2)]-Leu-Arg-Pro-[aza-Gly]). The half-life of azagly-nafarelin in trout was prolonged (about 5 hr) compared with other GnRH analogues and other animals (typically minutes). This is presumably the result of its lower susceptibility to peptidases (that metabolise GnRH into smaller peptides and amino acids).

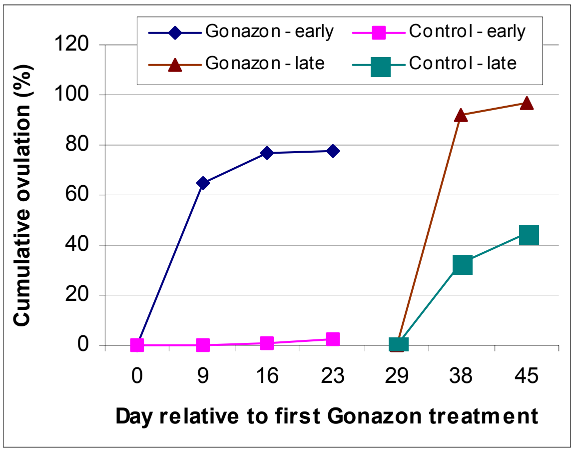
The clinical trials with female broodstock salmonids were performed in commercial fish farms in France, Norway and Scotland between 1997 and 2000 and the studies involved about 900 rainbow trout, 500 Atlantic salmon and 360 Arctic charr. This is the first time that such a large number of females were used to evaluate the efficiency of a specific GnRH in fish. All females were anaesthetised before manipulation to minimise stress. Gonazon was injected once intraperitoneally at the clinical dose of 32 µg/kg body weight (0.2-0.5 ml/kg body weight depending on the fish weight).

Gonazon™, an effective inducer of ovulation

One application of Gonazon is sufficient to advance and/or synchronise ovulation early in the spawning season in salmonids. This will decrease the cost of labour in the hatcheries and the number of anaesthesia sessions needed to strip females (currently, this is often 1-2 times per week for 6-10 weeks). In the trials, unless otherwise stated, treatment with Gonazon was performed when females had just begun their spawning season (0.1-6 % ovulation in the population). A tight synchronisation was observed with 90 to 95 % of ovulations being obtained in a narrow window of time (9-19 days depending on species and water temperature). Gonazon typically advanced mean spawning time by 2-3 weeks.

A second very practical application of Gonazon is to decrease the cost of production and labour in the hatcheries in the last 3-4 weeks of the spawning season. At that time, fewer eggs are being produced in the broodstock facility due to the declining number of fish yet to spawn. Gonazon has been administered to rainbow trout and Atlantic salmon (see Figure below) when up to 70% of the total population had already spawned. In these trials, 94.2 and 91.7% of these late-spawning fish ovulated by 9 days after treatment (vs. 27.6 and 33.3% in the controls, respectively).

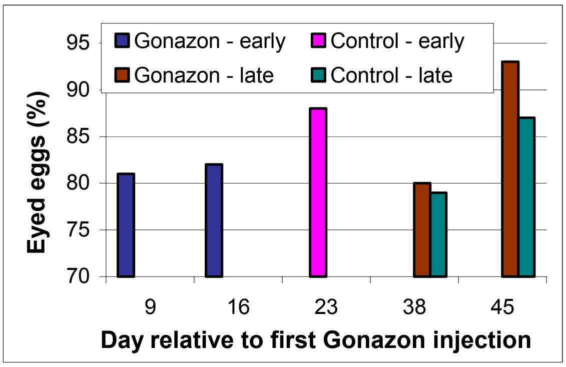
A third important application for Gonazon is to induce ovulation in sub-optimal environmental conditions when ovulation is delayed or blocked. This is the case in Atlantic salmon held in sea- or brackish water. Approx. 65% of Atlantic salmon held in brackish water (25-30 g NaCl/l) and receiving Gonazon were successfully stripped 9 days later (vs. 0 % in the controls); this had increased to 77% by day 16 (vs. 0.7% in the controls).



On day 0, 120 females were injected with Gonazon and compared with 152 controls. On day 29, 60 of the non-ovulated control females were treated with Gonazon and compared with 60 contemporary controls.

Gonazon™ does not significantly affect egg production and quality

During all trials, there was no mortality, behavioural changes or changes in body weight associated with treatment. In all trials, there were also no statistical differences between fish treated with the recommended clinical dose (32 µg/kg) and control fish in terms of relative fecundity, egg weight, ovarian fluid production and egg development to the eyed stage (see Figure below). Furthermore, no differences in hatchability, survival during yolk-sac resorption, mean weight of swimming fry, and survival and growth until 2 months after the first feeding were observed between treated and control rainbow trout.



Percentage eyed eggs from Atlantic salmon held in brackish water and treated with Gonazon (see earlier Figure for corresponding ovulation rates). Day 38- and 45-treated fish were non-ovulated controls from the earlier injection phase that had received Gonazon on day 29; thus, the eyed egg rates for these fish were for eggs stripped at 9 and 16 days, respectively, following the later injection.

In one trout trial, a lower rate of development was observed but this can be easily explained by the fact that treatment was given 2 weeks before the beginning of the spawning season in rainbow trout. This is in keeping with the literature. It is concluded that the possible benefits of using Gonazon several weeks ahead of the initiation of the natural spawning season (to have eyed eggs earlier for sale and/or for juveniles to gain weight earlier) have to be balanced against the cost of the (limited) loss of embryos and larvae.

Importance of using approved treatments in aquaculture

Before Intervet obtained a marketing authorisation for Gonazon within the EU/Norway, the use of spawning inducers in fish was not legally allowed in these countries. Production of healthy food is the goal of all fish producers. Concurrently, consumers and national authorities are more and more focussed on the safety of the food consumed. The EU/Norway approval of Gonazon provides breeders with an efficient product that respects EU and national regulations for animal and human safety, and that is produced to a high pharmaceutical quality (according to Good Manufacturing Practices). In addition, Gonazon will be prescribed by veterinarians in these countries. This will ensure better traceability and will support the development of labelling, certification and HACCP procedures, more and more required by end-users (supermarket, consumers, etc.).

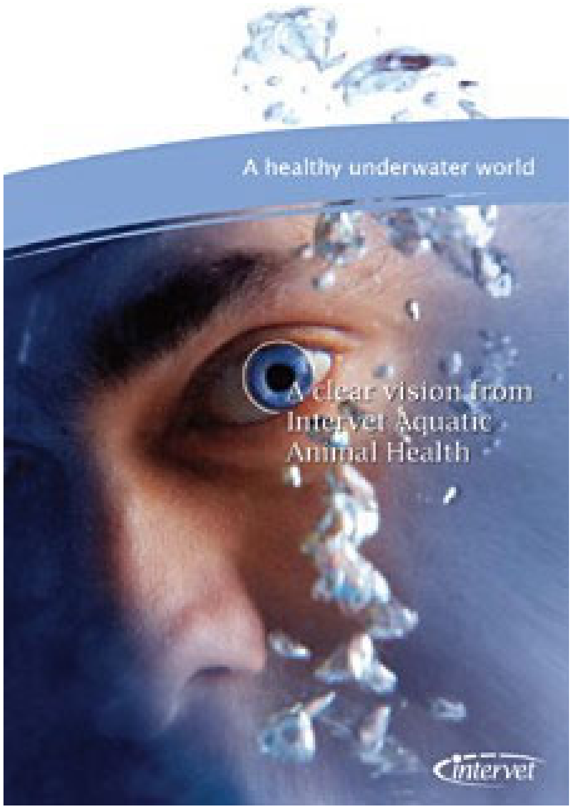
Gonazon comprises a kit containing two sterile vials, one a concentrated solution of azagly-nafarelin (2 ml) and the other an aqueous diluent (100 ml). These should be mixed in the sterile bottle(s) to give a concentration of active product to provide for a salmonid dose of 32 µg/kg body weight. This could be 320 µg/ml for use in large Atlantic salmon at 0.1 ml/kg body weight or could be 32 µg/ml for use in small Arctic charr at 1 ml/kg body weight, thus supplying lots of flexibility.

Gonazon can be stored for 3 years at 4-8 °C and, when opened, for 4 weeks in the fridge. Once concentrate and diluent are mixed, the product should be injected immediately. The recommended delay between the injection and stripping is around 50-100 degree-days depending on water temperature and local farming conditions. When the water temperature is higher, a reduction in the delay between injection and stripping (to 50 degree days) is recommended as egg quality quickly decreases with increasing temperatures.

Conclusion
All field studies have underlined the ability of Gonazon to induce ovulation in salmonids and have clearly demonstrated that such a product can help farmers to optimise their production capacity, especially by simplifying the management of egg production. Synchronisation of ovulation, even under sub-optimal environmental conditions, is also observed. Shortening of the late phase of the spawning season is another very useful application of this product. Gonazon could also advance the spawning season when administered around 2 weeks before its initiation. This may be, however, associated with a limited increase in mortality up to the fry stage (2-3 g).

Other species
Several Polish trials with Gonazon in common carp, even without the use of anti-dopaminergic compounds, have also proven the efficacy of azagly-nafarelin in this species (Mikolajczyk et al., 2003 [see 6th abstract below]). Gonazon has also been successfully used in Channel catfish. Trials to evaluate the specific effect of Gonazon on other species have not yet been performed, although it can be assumed that Gonazon will be at least as potent (on a µg to µg basis) as any GnRH product currently being used in aquaculture. The use of a single GnRHa injection regime has already been shown to be successful in species such as European sea bass, sea bream, turbot, various catfish species, etc. For many species that are synchronous or asynchronous multiple-batch spawners (flatfish, Sparids, etc.) or that present reproductive dysfunctions (e.g., sturgeons), control of ovulation by a single GnRHa injection may be a little more difficult but multiple injections should provide no problems. Further trials are also required to explore the ability of Gonazon to stimulate spermiation in male fish (both quality and quantity of milt).

A HEALTHY UNDERWATER WORLD



A clear vision from Intervet Aquatic Animal Health

- We see the challenges ahead
- Setting our sights on a healthy future together
- If your view of the way ahead is becoming clouded ...
- ... our foresight will open up new horizons
- Need a global outlook but with the right local solutions?
- Our ongoing work is clear for all to see

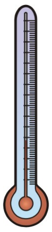
We are proud to herald the arrival of Intervet's new 8-page Aquatic Animal Health brochure. It is available from your local Intervet office. The text and some photos can also be viewed at www.intervet.com/AAH

METHODOLOGY

Fish and shellfish vaccination VI. Strategy for Reduction of Side Effects with Injection Vaccination - 4

This is the last of four sections in an article on the importance of water temperature, fish size and light (photoperiod) on the development of side effects after intraperitoneal injection vaccination of Atlantic salmon. These are the results of a collaborative research project involving Matre Aquaculture Research Station and Intervet Norbio, both located in Bergen, Norway. The key people involved at Matre Aquaculture Research Station were Arne Berg (Researcher), Tom Hansen (Manager) and Eva-Kristine Hansen (Master's student, University of Bergen). *Sections 1 – 3 of this article have appeared in Newsletters 4 – 6, respectively.*

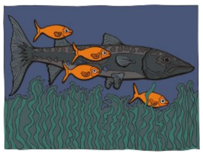
Recommended vaccination strategy *(more details can be seen in the three previous Newsletters)*



Temperature

Water temperature is a factor with significant impact on the severity of side effects after vaccination. The fish will perceive the vaccine as a foreign element, and will attempt to render it harmless. The immune system induces an inflammatory reaction as an important part of building immunity, but the same reaction may also lead to side effects in its efforts to eliminate/encapsulate the vaccine. At high temperatures, the initial inflammatory reaction will be strong and quick. This quickly builds immunity, but also increases the risk that the inflammatory reaction manifests itself as unacceptable side effects.

High temperature in the first phase after vaccination has a stronger impact on the level of side effects than high temperature later in the production period. Based on the findings observed in our research, as well as in other trials, vaccination at temperatures exceeding 15 °C should certainly be avoided. Furthermore, vaccination should ideally take place at low (≤ 9 °C), stable temperatures and without large fluctuations in the period immediately following. After some time with stable temperatures, however, the temperature may be gradually increased.



Size

Fish size (weight) at the time of vaccination is the second factor with significant impact on the level of side effects. In the trials carried out so far, we used a vaccine that previous experience had shown to carry a high risk of side effects. Based on the results from the trials, the majority of Speilberg scores 3, 4 and 5 may be eliminated by only vaccinating fish weighing over 70 grams. The findings also showed that it was important to grade the fish well before vaccinating. In an non-graded population with a satisfactory mean weight, there is still the risk that a considerable portion of the fish are too small.

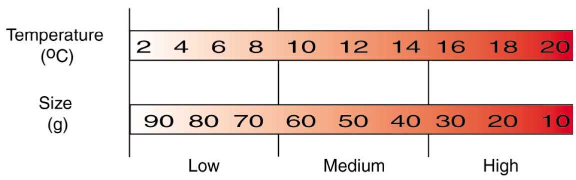
The connection between size at vaccination and the development of side effects is viewed as a general property of products based on water in oil emulsions. However, in determining the recommended minimum size at vaccination, the properties of each product must be considered. Fish weighing less than 70 g may quite possibly be vaccinated with oil-based vaccines available today, without any significant risk of adverse side effects. This is being further documented through follow-up trials at Matre Aquaculture Research Station.

Light

Compared with temperature and size, light (photoperiod) is of relatively less importance to the development of side effects after vaccination. Different light regimes influence the endocrinological process. The light will, amongst other things, hasten the smoltification process and increase the rate of growth. However, the research done so far is insufficient to provide firm evidence on the importance of light.

Thus, in summary, it is recommended that vaccination of S1 smolts should be done:

- at low, stable water temperatures
- on a well-graded population with the highest possible mean weight



Risk of developing side effects from vaccination

Asia: Intervet Norbio Singapore · Phone: +65 6397 1121 · E-mail: info.aquaINS@intervet.com
Salmonid countries: Intervet Norbio · Phone: +47 555 43750 · E-mail: info.norbio@intervet.com
Elsewhere: Intervet International · P.O. Box 31 · 5830 AA Boxmeer · The Netherlands
Phone: +31 485 587600 · E-Mail: info.aqua@intervet.com · URL: www.intervet.com/AAH

Vaccination within the indicated areas of low risk and, to some extent, medium risk, will be a good insurance against severe side effects. There will be an additive/synergistic negative effect when moving the vaccination time in the direction of high-risk areas. The referenced intervals are valid for a vaccine with a known high risk of side effects. For other vaccines, the risk areas must be adjusted specifically.

SUMMARIES OF SCIENTIFIC PUBLICATIONS

Innate defence: Evidence for memory in invertebrate immunity
Nature 425:37-38, 2003
Kurtz J, Franz K (Germany)
[First paragraph of paper]
Acquired immunity in vertebrates is characterized by immunological memory and specificity, whereas the innate defence systems of invertebrates are assumed to have no specific memory. Here we use a model system of a copepod, which is a minute crustacean, and a parasitic tapeworm to show that the success of reinfection depends on the antigenic resemblance between the consecutively encountered parasites. This finding indicates that an invertebrate defence system may be capable of specific memory.

Vaccination trials with *Penaeus japonicus* to induce resistance to white spot syndrome virus
Aquaculture In press. Available online, June 2003.
Namikoshi A, Wu JL, Yamashita T, Nishizawa T, Nishioka T, Arimoto M, Muroga K (Japan)
Crustaceans do not possess an adaptive immune response with immunoglobulins; however, recently, "quasi-immune response" has been reported by which kuruma shrimp (*Penaeus japonicus*) surviving from natural or experimental white spot syndrome virus (WSSV) infections possess a resistance against challenge with WSSV. In this study, efficacy of vaccines made of inactivated WSSV with or without immunostimulants ß-1,3-glucan or killed *Vibrio penaeicida*) and of recombinant proteins of WSSV (rVP26, rVP28) were tested by intramuscular vaccination followed by intramuscular challenge of kuruma shrimp with WSSV. The shrimp vaccinated with formalin-inactivated WSSV showed a resistance to the virus on 10th day post-vaccination (dpv) but not on 30th dpv. Heat-inactivated WSSV did not induce a resistance in the shrimp even on 10th dpv. Additional injections with glucan or *V. penaeicida* enhanced the efficacy of formalin-inactivated WSSV vaccine; however, the relative percent survival (RPS) values did not exceed 60% even when shrimp were vaccinated three times. On the other hand, two injections with rVP26 or rVP28 induced a higher resistance, with RPS values 60% and 95%, respectively, in the shrimp on 30th dpv. These results indicate the possibility of vaccination of kuruma shrimp with recombinant proteins against WSSV.

Topically acquired bacterial zoonoses from fish: a review
Med J Aust 173:256-259, 2000
Lehane L, Rawlin GT (Australia)
The main pathogens acquired topically from fish (through spine puncture or open wounds) are *Aeromonas hydrophila*, *Edwardsiella tarda*, *Erysipelothrix rhusiopathiae*, *Mycobacterium marinum*, *Streptococcus iniae*, *Vibrio vulnificus* and *Vibrio damsela*. S. iniae has recently emerged as a public health hazard associated with aquaculture, and M. marinum often infects home aquarium hobbyists. With the expansion of aquaculture and popularity of recreational fishing in Australia, medical practitioners can expect to see more infections of this nature. Diagnosis and treatment may be difficult, especially in view of emerging antibiotic resistance in fish pathogens.

Infection of barramundi *Lates calcarifer* with *Streptococcus iniae*: effects of different routes of exposure
Dis Aquat Organ 52:199-205, 2002
Bromage ES, Owens L (Australia)
The use of various challenge techniques has allowed the formation of a hypothesis for the mode of infection of *Streptococcus iniae* in barramundi. A bacterial dose of 1 x 10(3) colony forming units (cfu), corresponding to the LD50, delivered orally to barramundi could initiate the sub-acute form of the disease observed at the farms. The acute form of the disease could be initiated through bath exposure to the pathogen. S. iniae was equally as infective in freshwater, saltwater or when fish were subject to skin trauma prior to exposure, with LD50 values of 3.2 x 10(4), 2.0 x 10(4), 3.2 x 10(4) cfu, respectively, when observed over a 10 d period. It is suggested that sub-acute infection occurs orally, with mass mortalities occurring through the increased presence of the bacterium in the environment.

Streptococcus iniae, a bacterial infection in barramundi _Lates calcarifer_
Dis Aquat Organ 36:177-81, 1999
Bromage ES, Thomas A, Owens L (Australia)
The cause of ongoing mortality in barramundi *Lates calcarifer* (Bloch) in seawater culture was identified as *Streptococcus iniae* by biochemical and physiological tests. This is the first published record of this bacterial species in Australia and the first confirmed report of S. iniae causing mortality in barramundi. The bacterium was highly pathogenic for barramundi when challenged by bath exposure. The pathogen was found to have a LD50 of 2.5 x 10(5) and 3.2 x 10(4) colony-forming units at 48 h and 10 d respectively. Experimental challenge of barramundi resulted in high levels of mortality (> 40%) within a 48 h period. Ten days after the challenge, S. iniae could not be isolated from kidney, spleen, liver or eye of surviving fish. However, the organism was easily isolated from the brain of both moribund and healthy fish, indicating that barramundi can carry the bacterium asymptomatically.

Attempts to induce an LH surge and ovulation in common carp (_Cyprinus carpio_ L.) by differential application of a potent GnRH analogue, azagly-nafarelin, under laboratory, commercial hatchery, and natural conditions
Aquaculture 223:141–157, 2003
Mikolajczyk T, Chyb J, Sokolowska-Mikolajczyk M, Enright WJ, Epler P, Filipiak M, Breton B (Poland, The Netherlands, France)
The study was performed in order to try to provoke the gonadotropin wave and ovulation in common carp using a potent GnRH analogue, azagly-nafarelin (AZAGLY), without the use of dopamine (DA) antagonists. Different doses, routes of delivery, and injection protocols were applied, as well as different experimental conditions (laboratory, hatchery, and natural). It has been shown that the best and the simplest way of AZAGLY administration was by intraperitoneal injection. Despite dose (40 or 80 Ag/kg body weight) and number of injections (one or two), maximal plasma carp-luteinizing hormone (cLH) concentrations never exceeded 40–50 ng/ml. There was no priming effect on final plasma cLH concentrations. Using different treatment protocols, it was possible to obtain 40–60% ovulated females in laboratory as well as hatchery conditions. However, time of ovulation after AZAGLY treatment in some cases was less compressed in comparison with Ovopel-treated (a local commercial product, containing a GnRH analogue plus the dopamine antagonist, metoclopramide) fish. Egg quality was not altered by AZAGLY treatment. With natural spawning, in unfavourable thermal conditions, AZAGLY treatment induced spawning in a limited number of females. It is postulated that, if antidopaminergic drugs are ever banned from use in aquaculture, AZAGLY treatment alone can be considered as an alternative method of inducing ovulation in cyprinid fish for artificial spawning.

Assessment of ovulation of common carp (_Cyprinus carpio_) females selected for induced spawning on the basis of external morphological characteristics
Acta Vet Hung. 48:1-8, 2000
Szabo T, Szabo R, Urbanyi B, Horvath L (Hungary)
Secondary sexual characteristics such as softening and rounding of the abdomen as well as reddening and protrusion of the anal papilla and vent can be of help to breeders in selecting common carp (Cyprinus carpio) females prepared for propagation. To assess the reliability of this method, long-term data obtained on induced spawning of common carp at a large-scale fish hatchery were evaluated. The average spawning ratio of 2,620 females receiving hormonal injections was 79.8%. The average pseudogonadosomatic index (PGSI) calculated from data on the egg production of 2,086 females was 16.3 +/- 5.87% (mean +/- SD) for the same period. There was a correlation between fish weight and the time of induction determined by the breeder on the basis of external morphological characteristics. The similarity of the responses of females, including both spawning ratio and PGSI, among the different weight categories proved the reliability of this method for identification.

The effects of reserpine and LHRH or salmon GnRH analogues on gonadotropin release, ovulation and spermiation in common carp (_Cyprinus carpio_ L.)
Reprod Nutr Dev. 28:889-897, 1988
Sokolowska M, Mikolajczyk T, Epler P, Peter RE, Piotrowski W, Bieniarz K. (Poland)
The effects of reserpine (catecholamine depletor) and LHRH analogues on gonadotropin secretion, spermiation and ovulation of common carp were investigated. Injections of reserpine alone at a dose of 1 or 7 mg/kg of body weight stimulated spermiation, and reserpine at a dose of 1 mg/kg of body weight in combination with (D-Arg6, Trp7, Leu8, Pro9, NEt)-LHRH (s-

GnRH-A) or with (D-Ala6, Pro9)-LHRH (LHRH-A) at a dose of 50 micrograms/kg of body weight caused an increase of plasma gonadotropin levels, spermiation and ovulation in 80-90% of the females. Simultaneous injection of reserpine and LHRH analogues was as effective as injection of reserpine followed by injection of LHRH analogues 6 h later.

Multiple spawning and egg quality of individual European sea bass (*Dicentrarchus labrax*) females after repeated injections of GnRHa
Aquaculture 221:605–620, 2003
Mylonas CC, Sigelaki I, Divanach P, Mananõs E, Carrillo M, Afonso-Polyviou A (Greece, Spain, Cyprus)
Using multiple injections of gonadotropin-releasing hormone agonist (GnRHa) (10 Ag kg^{-1} bw) spaced 7–14 days apart, we investigated the multiple-batch group-synchronous spawning kinetics of individual European sea bass females (n = 6) and examined batch fecundity and egg quality parameters. Spawning was obtained 3–4 days after each GnRHa injection. A mean of 2.8 spawns $female^{-1}$ was obtained, with a minimum of 2 and maximum of 4. Mean relative fecundity of the first spawn was 320,211 eggs kg^{-1} bw and decreased continuously in subsequent spawns, reaching 52,381 eggs kg^{-1}. Total mean relative fecundity for all spawns was 637,900 eggs kg^{-1}. Mean fertilization success did not vary significantly among sequential spawns and ranged between 67% and 91%. On the contrary, there were significant differences among the four spawns in embryo survival 1 day after egg collection, hatching percentage and larval survival 4 days after egg collection, but there was no consistent trend during the study. Significant positive correlations existed between 4-day larval survival and hatching percentage (P= 0.0002), and between hatching percentage and embryo survival 1 day after egg collection (P= 0.03), indicating that 1-day embryo survival can be a potential biomarker for egg quality in commercial hatcheries. The results of the study demonstrate that European sea bass can produce up to four sequential spawns of high quality eggs in response to repeated acute treatments of GnRHa, and suggest that a pulsatile treatment of GnRHa may be necessary to induce the appropriate cycles of hormone changes for the recruitment, maturation and ovulation of multiple batches of viable eggs.

INTERVET GLOBAL AQUATIC ANIMAL HEALTH MEETING, THE NETHERLANDS, MAY 2003

Back row (L to R): D. Knappskog, R. Busch, F. Sterner, A. Brown, O. Parra. Middle row (L to R): E. Hendricks, K. Schuster, D. Lütticken. O.-M. Rødseth, S. Koumans, M. McLoughlin, A. Tanaeno. Seated (L to R): Y. Wada, A. Bolland, L. Grisez, W.J. Enright, Z. Tan.

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