

Newsletter

March 2003

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AQUATIC ANIMAL HEALTH IN INTERVET IRELAND

Intervet Ireland Ltd. is a local company representative of Intervet International B.V. The Aquatic Animal Health Division of Intervet is headquartered in Boxmeer, The Netherlands and has R&D sites in Bergen, Norway (salmon and other coldwater species; **see Newsletter no. 2**), Boxmeer (temperate species; **see Newsletter no. 4**) and Singapore (Asian and other warmwater species; **see Newsletter no. 1**). Intervet Ireland Ltd. markets products for use in a wide range of farm and companion animal species, now including fish. Ireland, as an annual producer of more than 22,000 tonnes (worth €69 million) of Atlantic salmon, is an important market for Intervet. In 1997, trials began on two prototype vaccines. Results from these trials, among others carried out in Norway and the U.K., led to further research and development of combination products and the use of lower volume (0.1 ml) dose vaccines.



Dag Knappskog, Dr. Marian McLoughlin and Bosco Cowley on a typical Irish sunny day at Fanad, Co. Donegal, an important location for Irish aquaculture

Trials began on these “Compact” vaccines in 1998 and continued through 2000 with two vaccines: Norvax® Compact 4 (**see page 3**) and Norvax® Mono PD. Local reactions to vaccines became an area of concern to fish farmers just prior to this period and these clinical trials demonstrated the new Compact vaccines to have a much improved safety profile compared with older vaccines. The trials also led to the approval of Norvax Compact 4 for licensing under the Animal Remedies Regulations 1996 legislation on an AR16 License. This vaccine has been found to be very successful both in terms of safety and efficacy. Norvax Mono PD is a mono-valent inactivated vaccine for Pancreas Disease (**see Newsletter no. 4**), a condition that is gaining recognition among veterinarians and fish farmers as a primary cause of production losses. Intervet, with the key assistance of Dr. Marian McLoughlin MVB, a leading expert on PD, has produced Norvax Compact Mono PD as a result of extensive research into the disease and its prevention through vaccination. This vaccine, once formally approved (expected mid 2003), will be of great

assistance to farmers trying to deal with this economically devastating disease.

Intervet Ireland estimates that they had a market share of about 50% in 2002. The people responsible for this success include Dr. Frank Hughes, Director, who has spearheaded the guidance of the Norvax vaccines through the process of approval, while additional support is provided by Technical Services Veterinarian, Bosco Cowley MBV, and Intensive Livestock Representative, Michael Roe. The company also relies on the expert guidance of Dr. McLoughlin, in terms of market development as well as technical support. Additional support is provided by Dag Knappskog, Intervet Norbio Bergen (Technical) and Dr. William Enright, Intervet International (Marketing).

FIELD TRIP FOR INTERVET MANAGEMENT.....

The Koyo farm in Singapore is a recent hot spot for Intervet visitors! Within a 2-week period, two groups of people from Intervet's headquarters in The Netherlands visited the farm to have a better "feel" for Asian aquaculture. The Koyo Aquaculture Center is famous for reproduction of various marine species, such as grouper, snapper, sea bass and threadfin. The company also produces shrimp for the local market.



Mr. Yeo (far right), Director of the Koyo Aquaculture Center, explaining shrimp farming (shrimp pond in the background) to the visitors. From left to right: Dr. Eric Rijke (Aquatic Animal Health R&D Coordinator), Dr. Dieter Lütticken (Vice President R&D), Dr. Grisez (Research Manager, Intervet Norbio Singapore), Dr. Frank Sterner (Director International R&D Biologicals) and Cedric Komar DVM (Technical Officer, Intervet Norbio Singapore).



Accompanied by Dr. Zilong Tan (centre; Technical Manager, Intervet Norbio Singapore), Dr. John Battison (right; Asia-Pacific Area Director) visited the Koyo broodstock farm. Mr. Yeo (left) was showing the very small 1-day-old Asian sea bass fry at the farm.

NEW ARRIVALS IN SINGAPORE....

Cedric Komar DVM (right) and Lauke Labrie DVM (left) recently took up important new positions in Intervet Norbio Singapore. Cedric, from France originally, is a new Technical Officer working with Dr. Zilong Tan, Veterinary Services Manager.



Lauke is from The Netherlands and is a Project Leader in Bacteriology working with Dr. Luc Grisez, Research Manager. They both received a degree in veterinary medicine in Toulouse (France) and a M.Sc. in Aquatic Veterinary Studies from the University of Stirling (Scotland). They met during their first year in Toulouse and shared a mutual interest for aquatic animal health (AAH). During their vet studies, they focused on fish pathology and have always tried to combine international travelling with work. So far, they worked on fish disease-related subjects in Israel, Malta,

Scotland, Mexico and, most recently, in the USA. There, they spent one year as AAH interns at the Mississippi Veterinary College in Stoneville, providing fish disease diagnostics to the farmers and doing applied fish disease research for the catfish industry.

For them, working in Asia for Intervet is an exciting and challenging prospect, due to the tremendous variety of fish species and diseases in the region. They are both happy and proud to have become part of the “Intervet family” in Singapore and hope to get to know “family members” in other countries soon.

NORVAX® COMPACT 4

Norvax® Compact 4 is a vaccine containing *Aeromonas salmonicida* subsp. *salmonicida*, *Vibrio salmonicida*, *Vibrio anguillarum* serotype O1 and *Vibrio anguillarum* serotype O2a. The vaccine induces protective immunity in Atlantic salmon against furunculosis (see Newsletter 5) caused by *Aeromonas salmonicida* subsp. *salmonicida*, coldwater vibriosis (see following article) caused by *Vibrio salmonicida*, vibriosis (see Newsletter 5) caused by *Vibrio anguillarum* serovar O1 and/or *Vibrio anguillarum* serovar O2a. Inactivated bacterial cultures are incorporated in a water-in-oil emulsion in order to enhance and prolong the immunity.

The onset of immunity is documented from 500 degree-days. Duration of immunity has been proven for 33 weeks by challenge studies and for 47 weeks by antibody studies under laboratory conditions. No outbreaks have been seen in field trials.

Efficacy and Duration of Immunity Study

The efficacy and duration of immunity following vaccination of Atlantic salmon with one dose of Norvax® Compact 4 was investigated by means of challenge experiments and measurements of specific antibodies. The trial was carried out at VESO Vikan AkvaVet in Norway.

A total of 715 Atlantic salmon (average weight 26 grams) were vaccinated with Norvax® Compact 4 while untreated fish were used as controls. The groups were tagged and kept separate until challenge.

At 6 and 33 weeks post vaccination, fish from each of the experimental groups were transferred to challenge units. The fish were challenged by i.p. injection. After each

challenge, the mortality among vaccinated fish and controls were registered daily for three weeks.

Lower mortality ($P < 0.001$) was obtained in vaccinated fish compared with controls in all challenge experiments. The RPS values are presented as RPS₆₀ in Table 1. [RPS = $(1 - M/60) \times 100$ where M = mortality among vaccinated fish at 60% control mortality].

Table 1. RPS₆₀ values after bacterial challenges at 6 and 33 weeks post vaccination.

Challenge strain	6 weeks	33 weeks
<i>V. ang</i> O1	100%	97%
<i>V. ang</i> O2a	100%	96%
<i>V. salm.</i>	100%	100%
<i>A. salm.</i>	90%	96%

The serological response to the vaccination with Norvax® Compact 4 was monitored with ELISA tests. The antibody response against all antigens increased from 6 to 33 weeks post vaccination.

Conclusion

The results of the challenge experiments at both 6 and 33 weeks post vaccination in addition to the high and increasing levels of specific antibodies against all four bacterial components demonstrate a duration of immunity against *Aeromonas salmonicida* subsp. *salmonicida*, *Vibrio salmonicida*, *Vibrio anguillarum* serovar O1 and *Vibrio anguillarum* serovar O2a for at least 33 weeks.

COLDWATER VIBRIOSIS IN SALMONIDS

Coldwater vibriosis, also called “Hitra” disease, is a problem in farmed salmon. It has caused heavy losses in Norway since the 1970’s. The disease is caused by the bacterium *Vibrio salmonicida* (*V. salm*). Clinical signs are inappetence and erratic swimming. Pathology varies with stage of the disease process, but common external signs are pallor of the gills, haemorrhage on the fin base, redness and swelling. Internally, the fish is pale due to anaemia. *V. salm* is transmitted through the water from fish to fish.

Causative agent

Vibrio salmonicida is a Gram-negative, peritrichously flagellated, non-spore-forming, curved, rod shaped bacterium. The usual size is 1.5-1.8 µm by 1.0-3 µm. *V. salm* is a facultative, anaerobic, catalase and oxidase positive bacterium fermenting glucose with production of acid.

The surface layer antigen of *V. salm* is called the VS-P1 antigen. This cell surface product is a single polypeptide, the monomeric form of which has an apparent molecular weight of 40 kD. There are also oligomeric forms with molecular weights in the range 300-700 kD. The antigen contains 6% carbohydrate and several isoelectric forms can be distinguished. Whether VS-P1 is related to the pathogenesis of *V. salm* is not known, but it is hypothesised that VS-P1 is released from the bacteria and binds specific antibodies, thus saving the bacteria from complement-mediated killing and phagocytosis. Whether plasmid or chromosomal genes encode the virulence of *V. salm* or a combination of the two is not known. Some experiments indicate, however, that the virulence is completely or partly plasmid mediated.

Prevention and Control

General methods

Control of the described disease is best achieved by maintenance of water quality, good husbandry and low stocking densities. However, this is not always possible and, where outbreaks occur, treatment with antibiotics is the only option. In areas where a disease is not endemic, it is possible to exclude the causative agents by a legislative policy such as (1) restrictions on importation/movement of live fish/eggs and (2) slaughter and disinfection in infected fish farms.

Antibiotics

Treatment of established infections with antimicrobial compounds has been and will be extensively used for the control of many infectious diseases. However, its value is limited since clinically affected fish do not eat and therefore cannot be well treated. A successful treatment is dependent on a rapid diagnosis and immediate treatment.

Vaccination

Vaccination has proven to be an efficacious method in preventing many bacterial diseases,

including furunculosis, vibriosis and coldwater vibriosis. Vaccines are preparations of inactivated antigens derived from pathogenic organisms, which will stimulate the immune system to increase the resistance to disease from subsequent infection by a pathogen.

The method of choice for prevention of the disease is therefore vaccination with inactivated whole cells or subunits and an appropriate adjuvant for enhancing the immune response (see above regarding Norvax[®] Compact 4).

KOI CARP DISEASE IN INDONESIA – INTERVET HELPS OUT!

Intervet Participation in an Investigation of a Serious Disease Outbreak of Koi and Common Carp in Indonesia

A serious disease outbreak in Koi carp (*Cyprinus carpio koi*, an ornamental fish) and common carp (*Cyprinus carpio carpio*, a food fish) occurred early this year in Indonesia. The disease started in the area of Blitar in East Java in April 2002, then spread rapidly throughout Java Island, causing very high mortality (80-90%) in both common and Koi carp. The loss is estimated to be 50 to 80 billion Rupiahs (approximately 5 to 8 million USD).



Koi carp can be worth up to several thousand US dollars each

Clinical signs in infected fish include severe gill necrosis, increased mucus production, superficial haemorrhages, fin rot and

enlargement of the kidney and liver with haemorrhages and discolouration. Indonesia has not experienced a disease of this nature in the past. It was suspected that the disease was introduced through importation of koi carp from another country.

At the request of the government of Indonesia, the Network of Aquaculture Centers in Asia-Pacific (NACA) organized an Emergency Disease Control Task Force Team (Task Force) to assist the local fish health authority to identify the problem and to find control measures for the disease. The Task Force visited outbreak farms and took clinical specimens for laboratory testing.

In response to the call from NACA, Intervet participated in the investigation. With assistance from our local company, Intervet Indonesia, we received a number of samples of both koi and common carp from the Task Force in mid-July. In the weeks that followed, Intervet Singapore and colleagues in pathology and virology at Intervet International worked very hard on the diagnosis testing. Various methods were employed to search for the causative agent: polymerase chain reaction (PCR), *in vivo* passage through koi carp, virus isolation in cell lines and electron microscope examination. The Intervet team, led by Dr. Ellen Ho in Singapore, worked closely with scientists from other institutes and universities, such as the Aquatic Animal Health Research Institute in Thailand, the University of California at Davis, and the Institute of Aquaculture at the University of Stirling. Based on the detection of koi herpes virus (KHV) by PCR, KHV might have played a role in the disease outbreak. Other agents such as parasites and bacteria might be also involved. The Task Force has classified the outbreak as "Mass mortality of koi and common carp" until a clear association with KHV or any other specific disease can be established.

Intervet's active involvement in this joint investigation has been highly appreciated. A letter from Mr. Pedro Bueno, Director General of NACA, stated "NACA is pleased to acknowledge officially and express gratitude to Intervet for its participation and contribution to the Emergency Disease Control Task Force on a Serious Disease of Koi and Common Carp in Indonesia..... We appreciate very much the valuable expert assistance, and your personal and keen interest, to this work and look forward to more cooperation in the future."

Authors note:

Koi herpes virus is a herpes virus that is distinctly different from Herpes cyprini, the most commonly known herpes virus in cyprinid fish. KHV has only been identified as a pathogen of Koi and common carp since 1998. It was found in the UK, continental Europe, the USA and Israel. Most mortalities occur at water temperatures of 22-27°C, being very much reduced below this and there being virtually no occurrence at 30°C or above. Mortalities are very rapid and severe. In many cases, 80-100% mortality occurs within 10 days of disease outbreak. Diagnosis is typically based on clinical observation, PCR, virus isolation, histopathology and electron microscope examination. While biosecurity measures will help, there is no effective treatment for KHV at present.

Sources of information:

NACA Newsletter. April-June 2002.
NACA and ACIAR, December 2002. Report of the Emergency Disease Control Task Force on a Serious Disease of Koi and Common Carps in Indonesia, June 2002
OIE, 28 June 2002. Disease Information. Vol. 15, No. 26.
Ornamental Aquatic Trade Association. Koi Herpes Virus (KHV). December 2001.

CHLORASOL™ – NEW DISINFECTANT FOR AQUACULTURE

Intervet's broad-spectrum chloramine-T disinfectant for use in the aquaculture industry will become available during 2003.

Disinfection is defined as: "to free from infection, especially by destroying harmful micro-organisms". Disinfection is a crucial pre-requisite for the prevention of infectious diseases and successful production of quality fish and shrimp. Disinfection must be practiced throughout the production cycle. Applications include rearing houses, tanks, equipment, water, footbaths, broodstock, eggs, nauplii and live food such as *Artemia*.

Globally, a variety of disinfectants are used in fish and shrimp production facilities. One of the most effective and frequently used active molecules is chloramine-T (the active in Chlorasol™). Regardless of what disinfectant

you use, care should be given to the concentration of the active ingredient in each commercial product. While Chlorasol is nearly a pure substance (> 98% chloramine-T), many other chlorine-based and other disinfectants are available at various and lower strengths (concentrations) in the market. Also, particular care should be given to the strength of the product when following recommendations given in “ppm” (parts per million). The calculation should always be based on the actual concentration of the active ingredient and not on the bulk product. Note that a 1 “ppm” solution means 1 part of active ingredient per 1 million parts of liquid. For example, 100 ppm in an aqueous solution = 100 gram active per 1,000,000 gram (1,000 litres or 1 tonne) water.

Indication and Mechanism of Action

Chlorasol™ is a multi-purpose disinfectant effective against a wide range of bacteria (Gram - and +), fungi, viruses and parasites. It is used to disinfect fish and shrimp pond and tank water, equipment, housing and other facilities, aquatic animals (e.g., fish and shrimp) and their development stages (e.g., eggs and larvae), and live food (e.g., *Artemia* cysts). It acts as a biocidal agent due to its ability to produce hypochlorous acid when dissolved in water, which in turn releases active chlorine and oxygen. The chlorine, and particularly the chloramine-T ion itself, kills micro-organisms by oxidation of and/or irreversibly binding to cellular material like proteins and nucleic acids. Also, because of this special mode of action, and because it truly acts as a disinfectant and not as an antibiotic, it will not induce resistance.

Benefits of Using Chlorasol™

- Known efficacy against a range of bacteria, viruses, fungi and parasites, including the most important pathogens in shrimp and finfish aquaculture.
- Due to its unique mode of action, it remains “dormant” until required – the presence of micro-organisms “turn it on”! Furthermore, bacterial resistance cannot develop.
- Biodegradable and environmentally friendly, leaving no harmful “footprint” in the water, sediment, or plant and animal life.
- Very stable as a powder and in solution. When you require a disinfectant to be available for several days, Chlorasol is ideal.
- The active component, chloramine-T, has a long history of safe and effective use in the food, agriculture and aquaculture industries.

Chloramine-T has Annex II status in the European Union (i.e., no MRL needed; no tissue residue concerns).

Known efficacy against a range of pathogens important in aquaculture

Chloramine-T has been shown to be effective against bacterial, viral and parasitic pathogens important in aquaculture including:

Bacteria	<i>Vibrio harveyi</i> , <i>V. anguillarum</i> , <i>V. parahaemolyticus</i> , <i>V. harveyi</i> , <i>V. alginolyticus</i> , <i>V. salmonicida</i> , <i>Photobacterium damsela</i> ssp. <i>Piscicida</i> , <i>Pseudomonas fluorescens</i> , <i>Streptococcus iniae</i> , <i>Lactococcus garvieae</i> , <i>Aeromonas</i> spp., <i>Edwardsiella tarda</i> , <i>Aeromonas salmonicida</i> and <i>Yersinia ruckerii</i>
Viruses	Infectious pancreatic necrosis virus (IPNV), Infectious salmon anaemia virus (ISAV), White spot syndrome virus (WSSV)
Fungi	<i>Aspergillus</i> sp
Parasites	<i>Gyrodactylus</i> (skin flukes), <i>Dactylogyrus</i> (gill flukes), <i>Costia</i> , <i>Epistylis</i> , <i>Ichthyobodo</i>

The actual dose required is dependant on individual and practical conditions at your hatchery or farm, such as contact time, and the prevailing organic matter and temperature; thus, a conservative approach should be taken until sufficient on-site experience with the product is gained.

METHODOLOGY

Fish and shellfish vaccination VI. Strategy for Reduction of Side Effects with Injection Vaccination - 3

This is the third 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).

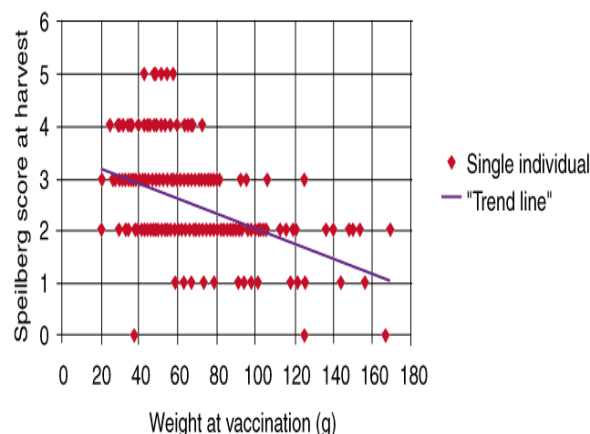
Size at vaccination

A population with a normal fish size (weight) distribution was vaccinated, and each specimen was marked so that each fish could be identified throughout the trial period (see table). In this manner, observations at harvest could be related to size at the time of vaccination.

Objective of trial	Investigate the importance of size at vaccination with respect to the development of side effects
Time of vaccination	November 1998
Time of sea transfer	May 1999
Weight (g) at vaccination	20-168; average 58
Evaluation of side effects	Up to and including 88 weeks after vaccination

The findings indicated a clear correlation between size at vaccination and the degree of side effects at harvest. The smallest fish at vaccination had the most severe side effects. This indicates that the variation in side effects found at harvest may be caused by vaccination of populations with large differences in size.

The most interesting size parameter is the minimum size for vaccination. In the trial in question it was difficult to determine a minimum size because the population had a normal size distribution and, consequently, had few individuals in the smallest (and largest) size intervals. For this reason, future research plans call for similar trials in order to obtain more data in this area.



Side effects at harvest related to size at vaccination.

Summary

Vaccination of a population with identified individual fish indicates a clear correlation between size at vaccination and the development of side effects. Increasing size leads to fewer side effects at harvest.

How is the development of side effects in a population of S1 smolts?

The development of side effects following vaccination is a dynamic process. In the first phase after vaccination (the first 1-3 months), adhesions tend to be rather weak and diffusely distributed in the abdominal cavity. In the middle phase (3-12 months after vaccination), the adhesions appear stronger and more concentrated in spatial areas of the abdominal cavity. During this phase the side effects will be at their highest level. In the last phase (more than 12 months after vaccination), a healing process takes place and the degree of side effects is reduced. Seasonal variations, caused by variations in temperature, will also show up as cycles in the level of side effects in the course of the production period. The severity of side effects observed in the latter part of the middle phase, will give a picture of what to expect at harvest.

Light after vaccination

Three groups of fish were subjected to different light (photoperiod) regimes for a period of 16 weeks after vaccination (see table). The groups were initially given 24 hours of light per day for 10, 6 and 2 weeks after vaccination, respectively. They were then given 12 hours of light per day for 6, 10 and 14 weeks, respectively.

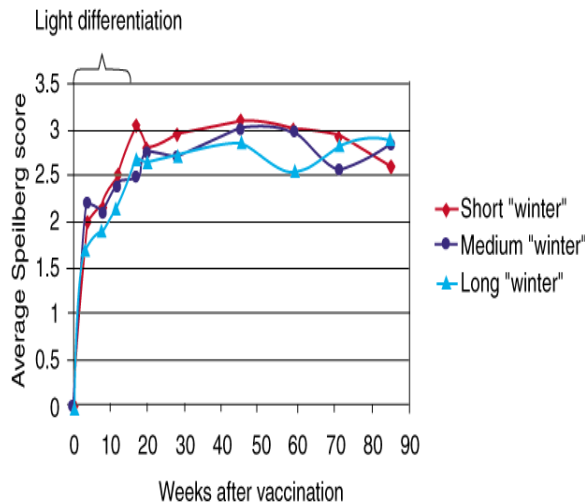
Objective of trial	Investigate the importance of light after vaccination with respect to the development of side effects
Time of vaccination	October 1997
Time of sea transfer	April 1998
Weight (g) at vaccination	58
Time of light differentiation	0-16 weeks after vaccination

Light groups	Short "winter": 24 hr light/day for 10 wk; 12 hr for 6 wk Medium "winter": 24 hr light/day for 6 wk; 12 hr for 10 wk Long "winter": 24 hr light/day for 2 wk; 12 hr for 14 wk
Evaluation of side effects	Up to and including 85 wk after vaccination

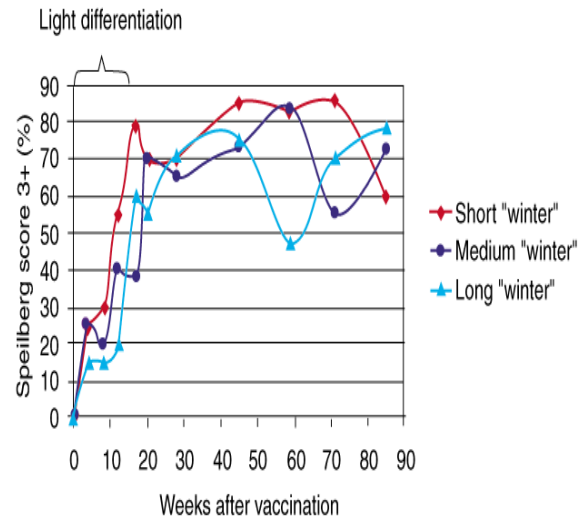
The group receiving 12 hours of light per day for 6 weeks (short "winter") developed the highest degree of side effects, while the group receiving 12 hours of light per day for 14 weeks (long "winter") had the least side effects. The group receiving 12 hours of light per day for 10 weeks (medium "winter") had a side effect profile between the two other groups. The differences between the groups were largest in the freshwater phase (during the period of light differentiation). After that, the differences between the groups grew smaller, but it must still be concluded that light has a certain influence on the development of side effects.

Summary

The different light regimes in the period 0-16 weeks after vaccination resulted in a weak correlation between light and development of side effects. Long "winter" gave a low rate of side effects, while short "winter" gave a higher rate of side effects.



Development of side effects in relation to light after vaccination.



Frequency of severe side effects (Spielberg score of ≥ 3) in relation to light.

SUMMARIES OF SCIENTIFIC PUBLICATIONS

Initial characteristics of koi herpesvirus and development of a polymerase chain reaction assay to detect the virus in koi, *Cyprinus carpio koi*

Dis Aquatic Organisms 11:101-108, 2002

Gilad O, Yun S, Andree KB, Adkison MA, Zlotkin A, Bercovier H, Eldar A, Hedrick RP (USA)

Since 1998, episodes of mass mortality have occurred in populations of common carp *Cyprinus carpio* in Israel and in populations of koi *Cyprinus carpio koi* in Israel and the USA. A herpesvirus isolated from infected fish has been shown in experimental studies to induce disease and mortality similar to those observed in outbreaks at infected farms. Initial characteristics of the virus show that it is clearly different from Herpesvirus cyprini (CHV), the most commonly known herpesvirus from cyprinid fish. The koi herpesvirus (KHV) has 31 virion polypeptides. Twelve of the virion polypeptides of KHV have similar molecular weights to those of CHV and 10 are similar to those of channel catfish virus (CCV). Both virion polypeptide and restriction fragment length polymorphism analyses of genomic DNA showed that the first KHV isolates from Israel and the USA were identical. In contrast, the genomic DNA restriction fragments clearly distinguish KHV from CHV and CCV. A polymerase chain reaction (PCR) assay to detect the virus in koi tissues was developed with sequences obtained from 1

restriction fragment of KHV DNA. The PCR assay effectively detected a 484 base pair sequence from KHV but did not amplify genomic DNA from either CHV or CCV. The PCR assay detected as little as 1 pg of KHV DNA mixed with 100 ng of host DNA. Viral sequences were amplified from koi obtained from field collections and from koi that were experimentally exposed to 10(2) TCID₅₀ ml(-1) of KHV via the waterborne route. All KHV exposed fish dying of infection between 8 and 10 d post exposure or surviving to 14 d post exposure were found to be positive by PCR, while unexposed control koi were all negative. The assay also showed the presence of KHV DNA in tissues of koi obtained from farms in Israel. The PCR assay should assist virus isolation procedures and histologic and electron microscopic analyses now commonly used to detect KHV infection. Current studies are examining the possibility of using the PCR to detect KHV DNA in live fish and the relative sensitivity and specificity of the KHV PCR assay compared with other diagnostic tests.

Comparison of two aquatic alphaviruses, salmon pancreas disease virus and sleeping disease virus, by using genome sequence analysis, monoclonal reactivity, and cross-infection

J Virol 76:6155-6163, 2002

Weston J, Villoing S, Bremont M, Castric J, Pfeffer M, Jewhurst V, McLoughlin M, Rodseth O, Christie KE, Koumans J, Todd D. (UK, Norway, The Netherlands)

Cell culture isolates of salmon pancreas disease virus (SPDV) of farmed Atlantic salmon and sleeping disease virus (SDV) of rainbow trout were compared. Excluding the poly(A) tracts, the genomic nucleotide sequences of SPDV and SDV RNAs include 11,919 and 11,900 nucleotides, respectively. Phylogenetic analysis places SPDV and SDV between the New World viruses of Venezuelan equine encephalitis virus and Eastern equine encephalitis virus and the Old World viruses of Aura virus and Sindbis virus. When compared to each other, SPDV and SDV show 91.1% nucleotide sequence identity over their complete genomes, with 95 and 93.6% amino acid identities over their nonstructural and structural proteins, respectively. Notable differences between the two viruses include a 24-nucleotide insertion in the C terminus of nsP3 protein of SPDV and amino acid sequence variation at the C termini of the capsid and E1 proteins. Experimental infections

of Atlantic salmon and rainbow trout with SPDV and SDV confirmed that the disease lesions induced by SPDV and SDV were similar in nature. Although infections with SPDV and SDV produced similar levels of histopathology in rainbow trout, SDV induced significantly less severe lesions in salmon than did SPDV. Virus neutralization tests performed with sera from experimentally infected salmon indicated that SPDV and SDV belonged to the same serotype; however, antigenic variation was detected among SDV and geographically different SPDV isolates by using monoclonal antibodies. Although SPDV and SDV exhibit minor biological differences, we conclude on the basis of the close genetic similarity that SPDV and SDV are closely related isolates of the same virus species for which the name Salmonid alphavirus is proposed.

Characterisation of a pathogenic virus isolated from marine threadfin fish (*Eleutheronema tetradactylus*) during a disease outbreak

Aquaculture 214:1-18, 2002

Seng K, Fang Q, Chang SF, Ngoh GH, Qin QW, Lam TJ, Sin YM (Singapore, China)

An unknown virus was isolated from massive mortality of cultured threadfin (*Eleutheronema tetradactylus*) fingerlings. The virus replicated in BF-2 fish cell line and produced a plaque-like cytopathic effect. Electron micrographs revealed non-enveloped, icosahedral particles approximately 70-80 nm in diameter composed of a double capsid layer. Viroplasm and subviral particles approximately 30 nm in diameter and complete particles of 70 nm in diameter were also observed in the infected BF-2 tissue culture cells. The virus was resistant upon pH 3 to 11 and ether treatment. It is also stable to heat treatment (3 h at 56 °C). Replication was not inhibited by 5-iododeoxyuridine (5-IUdR). Acridine orange stain revealed typical reovirus-like cytoplasmic inclusion bodies. Electrophoresis of purified virus revealed 11 segments of double-stranded RNA and five major structural polypeptides of approximately 136, 132, 71, 41 and 33 kDa. Based on these findings, the virus isolated was identified to belong to the genus *Aquareovirus* and was designated as threadfin reovirus. This virus differed from a majority of other aquareovirus by its increase in virus infectivity upon exposure to various treatments such as high and low pH, heat (56 °C), ether and 5-IUdR. The RNA and virion protein banding pattern of the threadfin reovirus was shown to

differ from another Asian isolate, the grass carp hemorrhage reovirus (GCV). Artificial injection of the threadfin reovirus into threadfin fingerlings resulted in complete mortality, whereas sea bass (*Lates calcarifer*) fingerlings infected via bath route showed severe mortality within a week after exposure. These results indicate that the threadfin virus is another pathogenic Asian aquareovirus isolate that could cross-infect into another marine fish, the sea bass.

Nodavirus Infection in Hatchery-reared Orange-spotted Grouper *Epinephelus coioides*: First Record of Viral Nervous Necrosis in the Philippines

Fish Pathology 37:87-89, 2002

Maeno Y, de la Pena LD, Cruz-Lacierda ER (Japan)

Mass mortality occurred in 34-day old larval orange-spotted grouper *Epinephelus coioides* reared at a hatchery in the Philippines with clinical signs such as anorexia and abnormal swimming behavior. Histopathology of moribund fish demonstrated marked vacuolation of the brain, spinal cord and retina. Cytopathic effects were observed in SSN-1 cells inoculated with the tissue filtrate of affected grouper. Electron microscopy revealed non-enveloped virus particles measuring 20 to 25 nm in diameter in the cytoplasm of degenerated SSN-1 cells. Piscine nodavirus (betanodavirus), the causative agent of viral nervous necrosis (VNN), was detected in the affected tissues and SSN-1 cells inoculated with the tissue filtrate of affected fish by RT-PCR. This is the first record of VNN in the Philippines.

Chemicals and biological products used in south-east Asian shrimp farming, and their potential impact on the environment - a review

Sci Total Environ 280:93-131, 2001

Graslund S, Bengtsson BE (Sweden)

A wide variety of chemicals and biological products are used to treat the water and sediment of ponds in semi-intensive and intensive south-east Asian shrimp farming. These products are also often used in shrimp hatcheries and to disinfect equipment for shrimp pond management. In spite of the size and importance of the shrimp farming industry in several south-east Asian countries, documentation of the quality and quantity of chemicals and biological products used during farming is scarce. This paper is a compilation of the literature available on substances used in

shrimp farming, and the possible environmental effects of these products are analysed to the extent allowed by the limited information. The role of shrimp farm managers, the chemical industry, governments, inter-governmental organisations and scientists in the development of a sustainable practice is discussed. It is concluded that shrimp farmers should reduce the use of chemicals and biological products because of the risks to the environment, human health and to production, and also, because many chemicals and biological products used in pond management have not been scientifically shown to have a positive effect on production. Clearly, the use of some chemicals, i.e. certain antibiotics, poses a risk of danger towards human health. Some chemicals used in shrimp farming, such as organotin compounds, copper compounds, and other compounds with a high affinity to sediments leave persistent, toxic residues, and are likely to have a negative impact on the environment. However, to assess the reality of these risks, substantial new information about the quantity of chemicals used in marine south-east Asian shrimp farming is needed.

A simple analytical procedure to replace HPLC for monitoring treatment concentrations of chloramine-T on fish culture facilities

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Dawson VK, Meinertz JR, Schmidt LJ, Gingerich WH (USA)

Concentrations of chloramine-T must be monitored during experimental treatments of fish when studying the effectiveness of the drug for controlling bacterial gill disease. A surrogate analytical method for analysis of chloramine-T to replace the existing high-performance liquid chromatography (HPLC) method is described. A surrogate method was needed because the existing HPLC method is expensive, requires a specialist to use, and is not generally available at fish hatcheries. Criteria for selection of a replacement method included ease of use, analysis time, cost, safety, sensitivity, accuracy, and precision. The most promising approach was to use the determination of chlorine concentrations as an indicator of chloramine-T. Of the currently available methods for analysis of chlorine, the DPD (*N,N*-diethyl-*p*-phenylenediamine) colorimetric method best fit the established criteria. The surrogate method was evaluated under a variety of water quality conditions. Regression analysis of all DPD colorimetric analyses with the HPLC values

produced a linear model ($Y=0.9602 X+0.1259$) with an r^2 value of 0.9960. The average accuracy (percent recovery) of the DPD method relative to the HPLC method for the combined set of water quality data was 101.5%. The surrogate method was also evaluated with chloramine-T solutions that contained various concentrations of fish feed or selected densities of rainbow trout. When samples were analyzed within 2 h, the results of the surrogate method were consistent with those of the HPLC method. When samples with high concentrations of organic material were allowed to age more than 2 h before being analyzed, the DPD method seemed to be susceptible to interference, possibly from the development of other chloramine compounds. However, even after aging samples 6 h, the accuracy of the surrogate DPD method relative to the HPLC method was within the range of 80–120%. Based on the data comparing the two methods, the U.S. Food and Drug Administration has concluded that the DPD colorimetric method is appropriate to use to measure chloramine-T in water during pivotal efficacy trials designed to support the approval of chloramine-T for use in fish culture.

Interactions between fish larvae and bacteria in marine aquaculture

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Jan A. Olafsen (Norway)

Modern aquaculture provides effective means for intensive seafood production under "controllable" conditions. This rapidly growing industry, however, has experienced relatively severe disease problems owing to lack of control of the microbiota in rearing systems. Disease control is an inherent part of any intensive animal production system; however, in the aquatic environment, the intimate relationship between bacteria and their host and the frequent use of open production systems adds to this challenge. The use of antibiotics in aquatic ecosystems is presently kept to a minimum, and fortunately, vaccines and other health control means have so far kept most diseases under relative control. Various organisms, however, may not respond to vaccines, and new diseases or variants are a constant challenge to the industry. In aquaculture, eggs are kept in incubators with a microflora that differs considerably from that in the sea, and become heavily overgrown with bacteria within hours after fertilisation. Fish larvae ingest bacteria by drinking and are, thus, primed with antigens before active feeding

commences. This may result in the formation of an indigenous larval microflora; however, at present, we know little about this process. The microflora of marine invertebrates may harbour bacteria that are pathogenic to other organisms and, thus, invertebrate co-inhabitants or food organisms in aquaculture may serve as vectors for transfection of fish pathogens. In intensive egg production and larviculture, the numbers of bacteria are kept low by various forms of water treatment and disinfection. These approaches, however, may disturb the balance between microbial communities, or favour proliferation of opportunistic bacteria or unpredictable development of bacterial communities. Thus, there is a need for better microbial control during intensive larval production. The use of probiotics has proven advantageous in domestic animal production, and microbial management may also have a potential in aquaculture. Better control of host–microbe interactions is a prerequisite for stable production of marine larvae in intensive systems.

Local company contact details:

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Co-ordination, final copy editor and contributor: Dr. William Enright

Other contributors to this issue:

Dr. Odd Magne Rødseth and Lone Holst, Intervet Norbio, Bergen, Norway; Dr. Zilong Tan, Dr. Ellen Ho and Cedric Komar, Intervet Singapore; Bosco Cowley, Intervet Ireland