

Newsletter

May 2005

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2005 – A MILESTONE YEAR FOR INTERVET AQUATIC ANIMAL HEALTH

- 🚧 20 years experience in AAH
- 🚧 10 salmon vaccines
- 🚧 5 years of R&D operations in Singapore
- 🚧 5 years/10 issues of Intervet AAH Newsletter
- 🚧 2nd fish vaccine in USA
- 🚧 1st fish vaccine for Japan
- 🚧 1st warmwater fish vaccine for Asia

TWENTY YEARS OF AAH EXPERIENCE IN INTERVET

In 1993, Intervet International made a key decision – to enter the aquatic animal healthcare market via the acquisition of Norbio, a small Norwegian company devoted to providing vaccines to the salmon industry since 1985.



Location of Intervet Norbio, Bergen, Norway

Now, the Intervet Aquatic Animal Health (AAH) Division is an integral part of Intervet International. A pioneer in state-of-the-art aquatic animal health products, the AAH division has consistently been at the forefront of technology in vaccines and disease prevention, and has grown hand in hand with the rapidly developing aquaculture industry. Dedicated exclusively to the research of fish and shrimp diseases, and the development, production and marketing of a complete range of effective aquatic animal health products and disease prevention strategies, Intervet has established two specialised Aquatic Animal Health Research & Development Centres, each

located in a major aquaculture production area. Each centre brings together expertise in disciplines like biotechnology, microbiology, immunology and aquatic veterinary science. All this makes Intervet ideally placed to act when a new pathogen is discovered or when vaccine development and tests are required for any aquaculture species. Intervet is fully committed to the development of new preventive measures to tackle the most devastating diseases, in ways that are safe for the environment. Furthermore, Intervet organises and/or supports conferences, workshops and exhibitions.

Economic Losses

Most economic losses related to diseases are caused by mortality and reduced growth. Although comprehensive data do not generally exist in the industry, the scale of losses can be estimated from the few existing examples. Estimates from various organisations have indicated that approximately 50 % of all fish and shrimp put into cages and ponds are lost due to disease before they reach market size. The actual economic losses in the aquaculture industry world-wide are estimated to be between 7 and 9 billion US\$ per year, which is roughly 16% of the world's farmed fish and shellfish value.

Prevention better than cure

While under certain circumstances antibiotics can provide a useful means of helping to treat some bacterial diseases, there are many problems associated with their use. An important side effect of the use of antibacterial drugs in aquaculture, apart from residue concerns, is the development of drug resistance among the bacterial pathogens. Furthermore, there is no effective chemotherapy for the control of viral diseases. It is against this background that vaccination, based on effective vaccines and vaccination strategies, does and will continue to play a most important and increasing role in aquaculture. Indeed, the growth in salmonid production (> 5 % per annum) over the last five years would not have been possible without the use of effective vaccines.

Highlights in Intervet's AAH vaccine history

- 1988: First water-based vibriosis vaccine (Norvax[®] Vibriose).
- 1990: First water-based vibriosis/ furunculosis combination vaccine (Norvax[®] Triple).
- 1993: First non-mineral oil-based triple vaccine (Norvax[®] Protect).

- 1995: World's first pentavalent fish vaccine including a viral component (IPN) based on recombinant DNA technology (Norvax[®] Protect-IPN).
- 1997: Vibriosis vaccine for sea bass and seabream in the Mediterranean (Norvax[®] Vibriose).
- 1999: New Norvax[®] Compact and Norvax[®] Minova fish vaccine ranges. New oil-based, hexavalent combination vaccine against all major diseases in Atlantic salmon in Norway (Norvax[®] Compact 6).
- 2000: Opening of a new state-of-the-art AAH R&D Centre in Asia (Singapore).
- 2001: First live-attenuated vaccine against ESC in channel catfish, USA (AQUAVAC-ESC[®]). First salmon vaccine in Ireland (Norvax[®] Compact 4). First Intervet salmon vaccine in Chile (Compact[™] IPN).
- 2003: Vibriosis vaccine for Atlantic cod in Norway (Norvax Vibriose Marine).
- 2004: First vaccine against salmon pancreas disease, Ireland (Norvax[®] Compact PD). First Intervet combination vaccines in Chile (Compact[™] Fur-IPN; Compact[™] Vib-IPN).
- 2005: First Intervet vaccine in Japan, against vibriosis in yellowtail (Norvax[®] Vibrio mono). First Intervet vaccine in Asia (outside Japan), against *Streptococcus iniae* in warmwater fish (Norvax[®] Strep Si). First live-attenuated vaccine against columnaris in channel catfish, USA (AQUAVAC-COL[™]). First Intervet salmon vaccine in Canada (Norvax[®] Compact 4).

FIVE FRUITFUL YEARS OF OPERATIONS AT INTERVET NORBIO SINGAPORE

Intervet decided to set up Asia-Pacific's first and only private aquatic animal health (AAH) R&D centre, Intervet Norbio Singapore Pte. Ltd. (INS), which was completed in January 2000 (see also Intervet AAH Newsletter no. 1). The U-shaped laboratory complex of nearly 900 M² consists of offices, laboratories and aquarium facilities. The centre focuses on the warm water species found in the Asia-Pacific region, such as grouper, Asian sea bass, yellowtail,

amberjack, tilapia and shrimp. In addition to disease epidemiology and vaccine development, investigations are underway to develop new, high quality and safe pharmaceuticals for responsible use as part of an overall health care management plan for farms. The Singapore centre faces a more challenging task than most R&D centres because it must tackle a wide range of aquatic animal species cultured in Asia Pacific, ranging from temperate to warmwater species.



Intervet Norbio Singapore R&D Centre

After several years of extensive epidemiology investigations and close contact with the industry in many Asian countries, a good understanding of the most important diseases, and their aetiology and prevalence, has now been achieved. Indeed, through this work, some new very pathogenic bacteria of important fish species have been identified. With this knowledge, it has been possible to develop vaccines and vaccination strategies for several important Asian diseases. In addition, staff from INS have given many seminars and technical training sessions to scientists and farmers in many Asian countries.

Based largely on the success of INS and the fact that a series of fish vaccines are going to enter the Asian market in the next few years, Intervet AAH decided to have a strong presence at the nearby World Aquaculture Society 2005 congress and exhibition in Bali in May. Intervet will have a booth, will present three scientific papers during the congress and will put on a 2-hour satellite seminar "Fish Vaccination in the Asia-Pacific Region" in order to introduce a new concept, vaccination as a key element of good health management, to the Asian aquaculture industry.

In addition, INS is now recognised as a centre of excellence in AAH. Evidence of this is the

fact that Dr. Luc Grisez (R&D Director) and Dr. Zilong Tan (Tech. Services Director) have both served two-year terms as committee members of the Asia Regional Advisory Group on AAH in the Network of Aquaculture Centres in Asia-Pacific organisation. Furthermore, INS receives clinical samples from throughout the region on a regular basis for diagnosis and epidemiology purposes. As an example, INS was a key participant in the 2002 Indonesia Koi Herpes Virus investigation (see Intervet AAH Newsletter no. 6). Also, Dr. Grisez, Dr. Tan and INS were all profiled in Asian Aquaculture magazine in 2003. In addition, INS staff get frequent requests to speak at congresses and meetings throughout the region.

Since Asia produced about 90% of world aquaculture production in recent years, the region will continue to dominate global aquaculture for decades to come. Just like for the salmon industry, vaccination will become a critical factor for economically viable and sustainable aquaculture in Asia, but it is only the combination of vaccination with several other factors, such as good nutrition, using fingerlings of high quality, and good management and husbandry practices, that will guarantee the highest possible survival rate and the best profit margins. Thus, the success of Intervet's laboratory in Singapore in developing vaccines and other health products for aquatic animals is very significant and it will help lift the aquaculture industry in Asia Pacific to the same level as that observed for terrestrial farm animals and salmon, where vaccination is routinely practiced as the way to prevent diseases. The extensive knowledge accumulated at INS over the past five years means that Intervet will be able to help the Asian aquaculture industry remain strong and viable, and we look forward to continued commercial success due to the work and dedication of the "fish" team in Singapore.



The Intervet Norbio Singapore team

INTERVET'S FIRST FISH VACCINE FOR JAPAN

This year will mark the launch of Intervet's first fish vaccine in Japan, Norvax® Vibrio mono, an immersion vaccine against *Vibrio anguillarum* infections of Japanese yellowtail. Several more complex Intervet fish vaccines are expected to be registered in Japan over the next few years.



INTERVET'S FIRST WARMWATER FISH VACCINE

This year marks the launch of Intervet's first south-east Asian fish vaccine, Norvax® Strep Si, an immersion and injection vaccine against *Streptococcus iniae* infections in warmwater fish (see Intervet AAH Newsletter no. 7). Field trials have been completed in Indonesia and Singapore, and a special licence has been granted by the Indonesian government, with full registration expected in both countries later this year. This pathogen affects many marine and freshwater species throughout Asia and registration in several other countries is envisaged over the next few years. A brochure about the product is available on request: mail to info.aquaINS@intervet.com



NOCARDIA SERIOLAE – A CHRONIC PROBLEM

Mark Sheppard, B.Sc., DVM
Sakana Veterinary Services Ltd., Canada

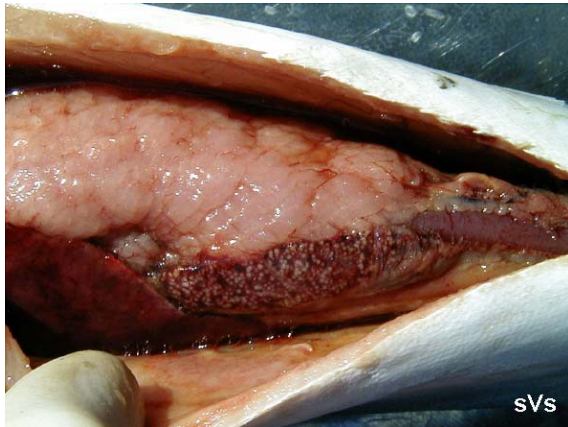
Marine nocardiosis is a long-term and problematic bacterial infection of warm-water fish. It eventually presents itself as an underlying debilitating factor in many types of fish. Affected fish often have other concurrent or secondary infections. Nocardiosis begins as a “silent infection”, developing undetected for months in fry or juvenile fish. The duration of the infection is a long-term (chronic) phenomenon. *Nocardia* bacteria multiply slowly within fish tissues before any visual symptoms arise, and certainly before lethargy and death rates increase. The typical outcomes within affected fish populations are: poorly performing yearling and pre-harvest fish, elevated feed conversion rates, and emaciation and rising mortality rates near the end of the summer.

Causative Agent

Many *Nocardia* species are found in the terrestrial and marine environments, but *Nocardia seriolae* (previously, *N. kampachi*) is considered the most likely pathogen of *Seriola* fish. The bacteria do not stimulate a septicemic reaction or an acute immune response. Rather, *Nocardia* is thought to progressively invade (and perhaps dwell and multiply inside) various types of fish host cells, including white blood cells. Relatively limited information about the microbiology, chemistry and patho-physiology related to *Nocardia* is published. This may be due to the inherent problems of researching slow-growing microorganisms.

Transmission and Epidemiology

The initial exposure of fry to *Nocardia* is the likely result of the fry consuming uncooked fish tissues (live, raw or frozen) or by the horizontal transmission of *Nocardia* from sick fish. Amberjack and yellowtail juveniles fed raw fish or moist pellets are likely the first to be infected, so the use of raw, low quality, trash fish should be avoided when rearing fish of any type. The infection develops silently as the bacteria multiply slowly over months within major organs, such as the spleen, kidney and liver.



Spleen with hundreds of 1- to 2-mm white-yellow spots.

Cohabitation with infected or diseased fish is also a contributing factor of this disease. Research indicates that yellowtail sharing tank space with sick juveniles (previously injected with live *Nocardia*) eventually exhibit internal pathology (white spots in their spleens) after 3 months of cohabitation, yet no external visual symptoms are evident. On the other hand, the injected cohort fish began dying within two weeks of their intraperitoneal injections. Various shellfish populations have also been shown (by RT-PCR) to contain genetic material indicative of *Nocardia* and *Mycobacterium*. However, the question remains whether the shellfish should be considered an environmental and contributing source of these pathogens, or simply accumulators of bacteria from affected finfish populations.

In marine finfish culture, nocardial infections appear to progress more quickly during the summer months when water temperatures reach 24°C or more, but the mortality due to *Nocardia* is more commonly experienced in the autumn and early winter months, perhaps as the fish becomes overwhelmed and its immune system wanes.

Clinical signs and Gross Pathology

The visual symptoms of this disease vary somewhat. The typical external lesions are: thin fish, skin nodules (focal, multifocal or coalescing), skin ulceration, opercular erosion and irregularly-shaped fleshy white masses at the base of the gill filaments. The internal pathology of nocardiosis is easily confused with other 'white-spot-forming' diseases, such as mycobacteriosis ('fish tuberculosis') and photobacteriosis (formerly *Pasteurella* or 'pseudo-tuberculosis'), especially if mixed infections exist. The white-yellow granulomata are usually 1-2 mm in size. The spots are most obvious in the spleen, kidney and liver but can be found in any tissue. Fish that mount a significant immune reaction to the disease eventually 'heal' somewhat and exhibit hard black spots (melano-macrophage accumulations) in place of the white spots in the liver and adipose tissues. Brown-black crusty plaques often develop on the dorsal inner surface of the swim bladder.



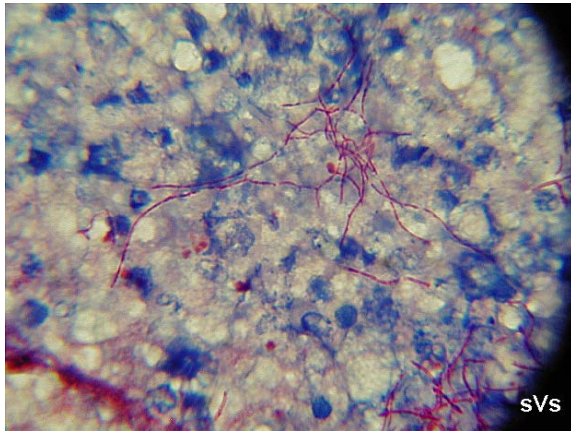
A classic nocardial lesion: whitish-yellow irregularly-shaped masses at the base of the gill filaments.



A coalescing cluster of nocardial skin abscesses. These dry abscesses protrude individually or in groups, each containing massive numbers of *Nocardia* bacteria. Many burst leaving a 'dry' yellow skin ulcer.

Microbiology

The bacterium is thread-like, beaded and branching. It is variable-staining when using Gram's stain and the bacteria are acid-fast positive (pink). The culture and isolation of *Nocardia* is relatively easy, yet somewhat tedious. Several types of agar and broth media will support nocardial growth, but these media also support the growth of other faster-growing species of bacteria. The use of selective antibiotic agar-tube media, such as Lowenstein-Jensen, is most efficient to isolate *Nocardia* directly. The incubation time may range from 4-10 days depending on incubation temperatures of 25°C to 35°C. The colonies appear dry and 'stacked'. The results of *in vitro* antibiotic sensitivity testing tend to be ambiguous and mis-leading. In general, *Nocardia* appears to be inherently resistant to most commercially available antibiotics when challenged *in vitro* and *in vivo*.



A spleen imprint or 'stamp' (x1000, acid-fast stain) showing bright pink, thread-like branching and beaded *Nocardia seriolae*.

Diagnosis and Primary on-site tests

A thorough visual examination of the fish is always the best way to begin an assessment. Feel the skin and body wall for lumps and ulcers. Upon cutting through the firm skin nodules, one will find a dry, grey-yellow, inspissated abscess. Lift the operculum to look for pale gills and irregular whitish lumps at the base of the filaments.



Colonies of *Nocardia seriolae* isolated on Lowenstein-Jensen selective (antibiotic) agar.

Gill, kidney or spleen imprints or 'stamps' are easily collected (in duplicate), dried and stained using Gram's or an acid-fast stain. Five-mm sections of the same tissues are helpful for a histological diagnosis when preserved in 10% buffered formalin.

Management and Control

The best prevention and control of this disease would be through vaccination; however, *Nocardia* vaccine development remains experimental. To date, I am unaware of the development of a commercially available, efficacious antigen-adjuvant combination that will prevent nocardial infections in fish. Therefore, the early detection of silent infections amongst juvenile live fish is the goal. However, the efficacy and practicality of detecting sub-clinical nocardiosis remains questionable in that the surveillance for infection may involve expensive experimental tests, such as: mucus testing by polymerase chain reaction (PCR) and antibody serology.

The use of drugs to control *Nocardia* is controversial. Environmentally, consumers are not in favour of drug treatments. From a fish production viewpoint, it is very difficult to ensure that fish will consume sufficient volumes of

medicated feed to achieve a therapeutic daily dose. In making the attempt, the fish may reduce their daily food intake and slow their weight gain, thus creating another cost to the farmer. Overall, the cost-effectiveness of antibiotic therapies to control nocardiosis in finfish is debatable. Antibiotic doses need to be high and the duration of treatment must be extended to the point that the use of antibiotics is largely impractical. That said, it is speculated that the application of two prophylactic treatments applied to asymptomatic juvenile fish may be useful. Using specific antibiotics that can penetrate fish cells (when in high serum concentration) for an extended period of time (i.e., 10 - 14 days) may interfere with *in vivo* nocardial development.

Control and prevention through husbandry and good management practices is the best approach for nocardial infections. Avoid the use of uncooked fish feeds (live, raw or frozen) when rearing fish of any age or type. Feed only dry cooked feed. Reduce the shellfish fouling (i.e., barnacles) on floats and ropes near your finfish cages whenever possible. Disinfect hands and marine equipment, practice strict diving hygiene between pens, farm sites and rearing areas, and minimize fish stress as much as possible.

Key References

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- Itano, T. and H. Kawakami (2002): Drug susceptibility of recent isolates of *Nocardia seriolae* from cultured fish. *Fish Pathol.*, 37, 152-153. (in Japanese with English abstract)
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- Sako, H. (1988): Survival of fish pathogenic bacteria in seawater. *Bull. Natl. Res. Inst. Aquaculture*, 13, 45-53. (in Japanese with English abstract)
- Kusuda, R. and A. Nakagawa (1978): Nocardial infection of cultured yellowtail. *Fish Pathol.*, 13, 25-31. (in Japanese with English abstract)

INTERVET STAFF ATTEND 7th ASIAN FISHERIES FORUM IN PENANG, MALAYSIA

Mr. Alistair Brown, Director of Intervet AAH, and Dr. Zilong Tan, Director of Technical Services at Intervet Norbio Singapore, attended the 7th Asian Fisheries Forum in Penang, Malaysia, Nov. 30 – Dec 3, 2004. Mr. Brown presented a talk entitled: "Salmon farming, a global success story – with focus on the impact of vaccines on the Norwegian industry". Dr. Tan (co-authored with Dr. Luc Grisez) gave a presentation on: "Health management practices in Asian mariculture – current status and challenges. Links to presentations are on the NACA website:

<http://www.enaca.org/Grouper/AFF-2004/Alistair-Brown.pdf>

<http://www.enaca.org/Grouper/AFF-2004/Zilong-Tan.pdf>

INTERVET IS PROUD SPONSOR OF DAA VI

Intervet is a proud sponsor of the 6th Symposium on Diseases in Asian Aquaculture (DAA VI). The Fish Health Section (FHS) of the Asian Fisheries Society is organizing "The 6th Symposium on Diseases in Asian Aquaculture (DAA VI)" with the theme "Aquatic Animal Health - Facing New Challenges" to be held on 25-28 October 2005 in Colombo, Sri Lanka. For detail information, you can visit <http://www.daasix.org/>

LARGE INTERVET AAH PRESENCE AT WORLD AQUACULTURE 2005 IN BALI

Intervet will have a booth (no. 209 - 210) during the World Aquaculture 2005 conference and trade show in Bali in May.

In addition, three presentations will be made by Intervet Norbio Singapore staff during the conference:

- Demonstration and confirmation of etiology of a new facultative intracellular bacterium causing mass mortality in Asian sea bass *Lates calcarifer*. (Luc Grisez et al.).
- *Tenacibaculum maritimum*, an

underestimated fish pathogen in Asian marine fish culture. (Lauke Labrie et al.).

- Diseases and vaccination strategies in Asian sea bass (*Lates calcarifer*). (Cedric Komar et al.).

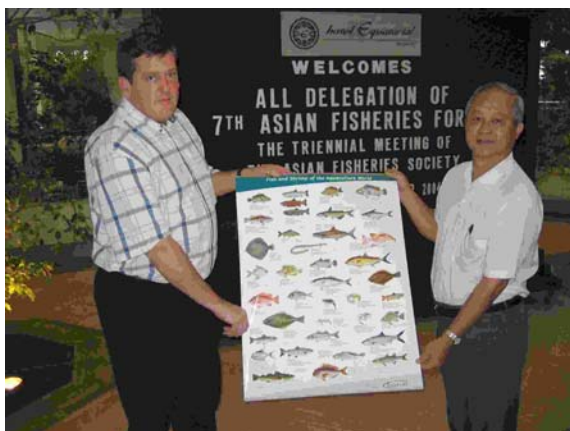
Intervet is also organising a satellite seminar entitled: "Fish Vaccination in the Asia-Pacific Region" on Wednesday May 11th. The programme is as follows:

- Asian aquaculture – challenges for sustainability
(Dr. Rohana Subasinghe, FAO)
- Fish immunology and vaccinology
(Dr. Sylvie Quiniou, USDA)
- Vaccination in the salmon industry
(Dr. William Enright, Intervet International)
- Aquatic animal diseases in Asia with a focus on the role of vaccination
(Dr. Luc Grisez, Intervet Norbio Singapore)

A report about the seminar will appear in the next newsletter.

INTERVET POSTER – FISH AND SHRIMP OF THE AQUACULTURE WORLD

Intervet recently produced a wall poster titled "Fish and Shrimp of the Aquaculture World". It consists of 36 representative fish and shrimp species which are being cultured in various areas of the world. The species are named in Latin, English, Spanish, Chinese and Japanese. We thank Scandinavian Fishing Year Book and the artists involved for the very nice professional work. Like Intervet vaccines, the fish drawings are made to a very high quality.



Mr. Alistair Brown, Director of Intervet Aquatic Animal Health Division, proudly

presented the first print to Dr. Leong Tak Seng, a prominent fish health specialist in Asia, during the 7th Asian Fisheries Forum in Malaysia.

INTERVET AAH NEWSLETTER – OUR 10th ISSUE AND STILL GOING STRONG

This is the 10th issue of the Intervet AAH Newsletter and marks five years of dissemination of useful information on aquatic animal health to thousands of people worldwide.

Topics have included profiles on the following important fish and shrimp diseases:

- Vibriosis in Shrimp (Issue 1)
- Winter Disease Syndrome (Issue 1)
- Infectious Pancreatic Necrosis (Issue 2)
- Pasteurellosis (Issue 3)
- Enteric Septicemia of Catfish (Issue 3)
- Pancreas Disease of Salmon (Issue 4)
- Furunculosis and Vibriosis in Salmonids (Issue 5)
- Coldwater Vibriosis in Salmonids (Issue 6)
- Streptococciosis in Asian Fish (Issue 7)
- Atypical Vibriosis in Chile (Issue 8)
- Salmon Rickettsial Syndrome (Issue 9)
- Proliferative Kidney Disease (Issue 9)
- Nocardia Disease (Issue 10)

Thanks again to all the internal and external contributors over the last five years. If someone wishes to contribute an interesting feature article (e.g., on a disease), please contact info.aqua@intervet.com

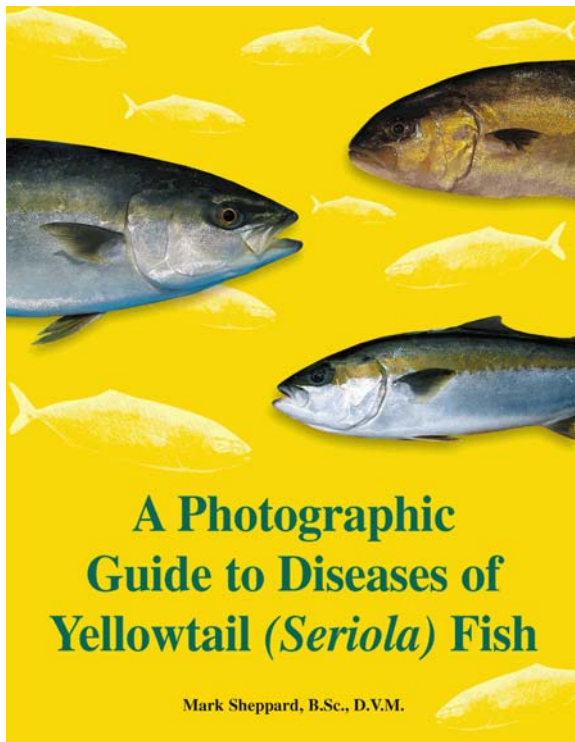
Copies of all Newsletters can be downloaded from www.intervet.com/AAH



Dr. William Enright, Marketing Manager, Intervet Aquatic Animal Health Division and Newsletter Editor holding all ten issues.

A COLOUR ATLAS OF DISEASES OF YELLOWTAIL (SERIOLA) FISH

A new 2005 Japanese publication is available: ***A Colour Atlas of Diseases of Yellowtail (Seriola) Fish*** (ISBN 0-920225-15-2), written by Canadian veterinarian, Dr. Mark Sheppard. The 64-page guidebook (originally published in English, ISBN 0-920225-14-4) has been translated by Dr. Hiroshi Yokoyama. The book is comprised of 30 pages of high resolution, detailed pathology photographs depicting 29 of the most typical diseases and related topics found in hamachi, kampachi, hiramasa and other fish. Each topic includes one page of easy-to-read information, descriptions and diagnostic collection recommendations. The topics range from farm-hygiene to bacterial, viral, parasitic and complex disease syndromes. The book is designed as a basic, hands-on, "what am I looking at now" diagnostic field guide for fish farmers, fish health professionals, laboratory technicians and students. To preview the book, visit <http://oberon.ark.com/~svs>



SUMMARIES OF SCIENTIFIC PUBLICATIONS

Vaccines for fish in aquaculture

Expert Rev Vaccines. 4:89-101, 2005
Sommerset I, Krossoy B, Biering E, Frost P (Intervet Norbio, Bergen, Norway)
Vaccination plays an important role in large-scale commercial fish farming and has been a key reason for the success of salmon cultivation. In addition to salmon and trout, commercial vaccines are available for channel catfish, European seabass and seabream, Japanese amberjack and yellowtail, tilapia and Atlantic cod. In general, empirically developed vaccines based on inactivated bacterial pathogens have proven to be very efficacious in fish. Fewer commercially available viral vaccines and no parasite vaccines exist. Substantial efficacy data are available for new fish vaccines and advanced technology has been implemented. However, before such vaccines can be successfully commercialized, several hurdles have to be overcome regarding the production of cheap but effective antigens and adjuvants, while bearing in mind environmental and associated regulatory concerns (e.g., those that limit the use of live vaccines). Pharmaceutical companies have performed a considerable amount of research on fish vaccines; however, limited information is available in scientific publications. In addition, salmonids dominate both the literature and commercial focus, despite their relatively small contribution to the total volume of farmed fish in the world. This review provides an overview of the fish vaccines that are currently commercially available and some viewpoints on how the field is likely to evolve in the near future.

A review of the main bacterial fish diseases in mariculture systems

Aquaculture 246:37-61, 2005
Toranzo AE, Magariños B, Romalde JL (Spain)
The aim of this review is to compile some dispersed literature published about different aspects of the most threatening bacterial diseases occurring in fish cultured in marine waters worldwide such as vibriosis, "winter ulcer", photobacteriosis, furunculosis, flexibacteriosis, "winter disease", streptococcosis, lactococcosis, BKD, mycobacteriosis and piscirickettsiosis. Therefore, the geographic distribution of each disease and the main host species affected, together with the biochemical and antigenic diversity existing in the aetiological agents are

described. In addition, the genetic studies that have been performed to determine the possible existence of intraspecific heterogeneity or clonal lineages within each pathogen are included. We review also in brief the classical methods to isolate the microorganisms from their hosts as well as the serological and/or genetic tools for a rapid diagnosis of the diseases. Finally, the current status in the development of vaccination strategies to prevent these bacterial diseases is also addressed.

A framework for understanding the potential for emerging diseases in aquaculture

Preventive Vet Med. 67:223-235, 2004

Murray AG, Peeler EJ (U.K.)

Numerous diseases have emerged as serious economic or ecological problems in aquaculture species. The combination of factors behind the emergence of each disease is unique, but various common factors are apparent. We combine risk-analysis methods and virulence theory with historical examples (mainly from salmonid production) to identify key disease-emergence risk factors. Diseases have emerged through pathogen exchange with wild populations, evolution from non-pathogenic micro-organisms and anthropogenic transfer of stocks. Aquacultural practices frequently result in high population densities and other stresses (such as intercurrent disease) which increase the risk of infection establishment and spread. As aquaculture expands and new species are farmed, diseases will continue to emerge and affect both wild and farmed fish adversely. The rate and extent of emergence can be reduced by the application of biosecurity programmes designed to mitigate the risk factors for disease emergence.

Mycobacterial infection in farmed turbot *Scophthalmus maximus*

Dis Aquat Organ. 7:87-91, 2002

dos Santos NM, do Vale A, Sousa MJ, Silva MT (Portugal)

Mycobacteriosis (piscine tuberculosis) has been reported to affect a wide range of freshwater and marine fish species; however, this is the first report describing mycobacterial infections in turbot *Scophthalmus maximus*. High numbers of granulomas were initially observed in the organs of moribund farmed turbot. Bacteriological analysis of organs with granulomas led to the isolation of *Mycobacterium marinum*. Further analysis, to determine the prevalence of the infection in the farm and to identify its source, showed the

occurrence of a dual infection by *M. marinum* and *M. chelonae*. The presence of *Nocardia* sp. in some of the fish infected with mycobacteria was also detected. The presence of granulomas in internal organs of apparently healthy fish indicated a high prevalence of the disease, a conclusion that was supported by isolating mycobacteria from all fish with or without granulomas. The infection was probably responsible for the mortality observed (approximately 2% mo(-1)), as most of the recently dead fish presented high numbers of granulomas and isolation of mycobacteria was possible from all of the fish. The isolation of *M. marinum* from the inlet water suggested this as the most plausible source for the infection occurring in the farm.

Side effects in sea bass (*Dicentrarchus labrax* L.) due to intraperitoneal vaccination against vibriosis and pasteurellosis

Fish Shellfish Immunol. 19:1-16, 2005

Afonso A, Gomes S, da Silva J, Marques F, Henrique M (Portugal)

Sea bass (*Dicentrarchus labrax* L.) were injected intraperitoneally with monovalent (*Photobacterium damsela* subsp. piscicida or *Vibrio anguillarum*) and divalent (*Ph. damsela* subsp. piscicida and *V. anguillarum*) vaccine formulations, with or without adjuvants (mineral oil, liposome or alginate), to evaluate the short and long-term pathological effects. Eight animals from each group were sampled one, two, four and 11 months after intraperitoneal injection. The acute peritoneal response and the progression to a chronic status were evaluated by analysing peritoneal leucocytes collected during the first days post-injection. To evaluate the chronic response, the late peritoneal leucocyte response was analysed and the peritoneal cavity was examined and the intra-abdominal lesion level scored based on a pre-defined scale. Correlation between leucocyte exudative response, tissue inflammatory response and the development of granuloma were sought. The acute leucocyte response was characterized by an early (24-48 h) mobilization of neutrophils and macrophages, with phagocyte numbers dependent on the formulation, but no significant variations were observed in lymphocytes/small cells and EGCs. Later on, a steady increase occurred in lymphocytes/small cells and EGCs and a high concentration in neutrophils and macrophages was maintained up to 30-60 days in groups i.p. injected with oil adjuvanted formulations with antigen. All the lesions observed were

moderate, indicating that in sea bass, the pathological effects due to intraperitoneally injected vaccines are less severe than in other fish species. The divalent oil adjuvanted vaccine induced the most severe side effects, with macroscopic granulomas consistently present up to 11 months.

Effects of gill abrasion and experimental infection with *Tenacibaculum maritimum* on the respiratory physiology of Atlantic salmon *Salmo salar* affected by amoebic gill disease

Dis Aquat Organ. 63:169-174, 2005

Powell MD, Harris JO, Carson J, Hill JV (Australia) The effects of gill abrasion and experimental infection with *Tenacibaculum maritimum* were assessed in Atlantic salmon *Salmo salar* with underlying amoebic gill disease. The respiratory and acid-base parameters arterial oxygen tension (P(a)O₂), arterial whole blood oxygen content (C(a)O₂), arterial pH (pHa), haematocrit and haemoglobin concentrations were measured at intervals over a 48 h recovery period following surgical cannulation of the dorsal aorta. Mortality rates over the recovery period were variable, with gill abrasion and inoculation with *T. maritimum* causing the highest initial mortality rate and unabraded, uninoculated controls showing the lowest overall mortality rate. Fish with abraded gills tended to show reduced P(a)O₂ and lower C(a)O₂ compared with unabraded fish. Infection with *T. maritimum* had no effect on P(a)O₂ or C(a)O₂. All fish showed an initial alkalosis at 24 h post-surgery/inoculation which was more pronounced in fish inoculated with *T. maritimum*. There were no significant effects of gill abrasion or infection upon the ratio of oxygen specifically bound to haemoglobin or mean cellular haemoglobin concentration. Histologically, 48 h following surgery, abraded gills showed multifocal hyperplastic lesions with pronounced branchial congestion and telangiectasis, and those inoculated with *T. maritimum* exhibited focal areas of branchial necrosis and erosion associated with filamentous bacterial mats. All fish examined showed signs of amoebic gill disease with multifocal hyperplastic and spongy lesions with parasome-containing amoeba associated with the gill epithelium. The results suggest that respiratory compromise occurred as a consequence of gill abrasion rather than infection with *T. maritimum*.

Spatial and temporal variations in sea lice (Copepoda: Caligidae) infestations of three salmonid species farmed in net pens in southern Chile

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Zagmutt-Vergara FJ, Carpenter TE, Farver TB, Hedrick RP (USA)

Sea lice infestations have become a major health problem for farmed salmonids throughout the world including Chile. In southern Chile, 6 geographical areas, divided into 22 geographical zones with a total of 127 salmon farming centers and 1519 sea pens, were regularly sampled from December 1999 to April 2002. A linear mixed-effects model (LME) approach was used to describe the infestations of adult forms of sea lice on 3 salmonid species farmed in southern Chile. The variables fish species, water temperature, water salinity, fish weight, juvenile parasite count, pen shape, treatment status in previous month and the interaction of previous and current month treatments were found to be statistically significant fixed effects for the population sampled. The most susceptible species to sea lice infestation was rainbow trout *Oncorhynchus mykiss*, while the least susceptible species was coho salmon *O. kisutch*. Fishes in pens treated in the previous month with avermectins were associated with the smallest sea lice count compared to fishes in pens not treated or treated with other products. The variability in sea lice infestations in areas and zones within areas was not statistically significant when controlling for the previously mentioned fixed variables. The variability between centers, the within-pen variability, and the interaction between within-pen effect and the date of measurement were statistically significant and not explained by the fixed effects. Potential sources for this variability are discussed. We conclude that the epidemiology of sea lice infestations in farmed salmonids in southern Chile is complex and in need of further study.

Lancefield group C *Streptococcus dysgalactiae* infection responsible for fish mortalities in Japan

J Fish Dis. 27:679-686, 2004

Nomoto R, Munasinghe LI, Jin DH, Shimahara Y, Yasuda H, Nakamura A, Misawa N, Itami T, Yoshida T (Japan)

A Lancefield serological group C *Streptococcus* sp. was isolated from cultured amberjack, *Seriola dumerili* Risso, and yellowtail, *Seriola quinqueradiata* Temminck and Schlegel, immunized with *Lactococcus garvieae*

commercial vaccines in Japan. The isolated bacteria were Gram-positive cocci, auto-aggregating in saline, morphologically long chains in growth medium, catalase negative and alpha-haemolytic on blood agar. An almost complete gene sequence of the 16S rDNA of two isolates was determined and compared with that of bacterial strains in the database. The isolates were identified as *Streptococcus dysgalactiae* based on the results of the 16S rDNA sequence, the bacteriological properties and the Lancefield serological grouping. Oligonucleotide primers specifically designed for the 16S-23S rDNA intergenic spacer region of *S. dysgalactiae* amplified a gene from all the fish isolates, as well as the type strains alpha-haemolytic *S. dysgalactiae* subsp. *dysgalactiae* ATCC430738 and beta-haemolytic *S. dysgalactiae* subsp. *equisimilis* ATCC35666, but not those of *S. equi* ATCC33398, *Lactococcus garvieae* ATCC43921 and *L. garvieae* KG9408. The severe necrotic lesions of the caudal peduncle seen in experimentally infected fish were similar to those seen in naturally infected fish.



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