Intervet Aquatic Animal Health
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In this issue ……..

VIBRIOSIS IN ATLANTIC COD – A CHALLENGE IN INTENSIVE CULTURE

By Solveig Nygaard, Fish Health and Environment Inc.
Veterinarian Specializing in Fish

Vibriosis, as caused by the bacteria *Listonella (Vibrio) anguillarum*, was the first of the known bacterial fish diseases. The disease was described (in Italy) as early as 1718 (1). *Listonella anguillarum* is divided into several serotypes, where 0_1 is dominant in salmonids while 0_2 is usual found in Atlantic cod (1). Despite the long history of the disease, vibriosis still causes problems in cod farming, thereby increasing the usage of antibacterial remedies in coldwater aquaculture.

Characteristics of vibriosis in Atlantic cod (*Gadus morhua*)

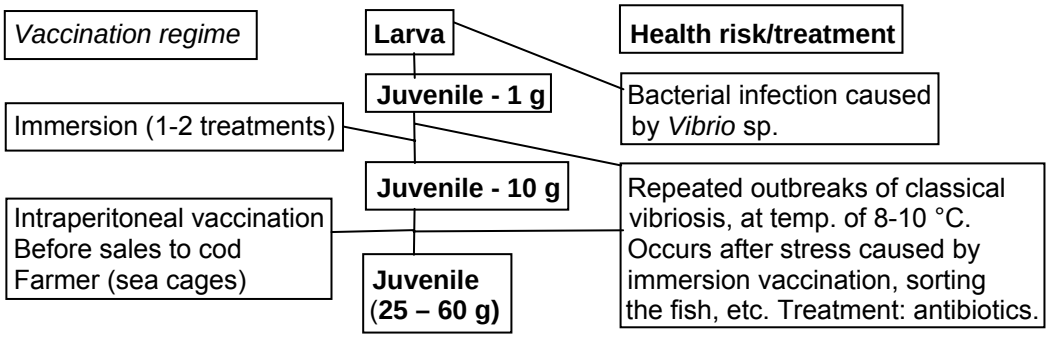
Distribution	In the marine environment
Species affected	Cod, saith, salmonids, pike, eel, turbot, halibut, etc.
Symptoms on cod	Haemorrhages on the jaw and exophthalmia ("pop eye") in small fish; haemorrhages and wounds on the skin and fins (also enlarged) in larger fish
Mortality	High in juveniles, ≥ 50%; lower in larger fish
Contributing factors	High water temperature, stress
Prevention	Vaccination (as of now, two water-based vaccines are available for immersion and injection vaccination in Norway)
Treatment	Antibiotics - good effect from (for example) oxolinic acid as long as the bacteria have not developed resistance

Haemorrhages on the jaw of a juvenile cod with vibriosis
Photo: Solveig Nygaard

Experiences from farming

Vibriosis outbreaks are often prevalent at temperatures > 14 °C. When cod juveniles are produced in lagoons, vibriosis has a tendency to occur shortly after dip vaccination, while catching the fish or in other situations which cause stress, especially when combined with high water temperature. Prevention of vibriosis in large cod has been possible by moving the pens to locations which are deeper and have stronger currents (personal experience).

Current production of juveniles in intensive cod farming



Tail wounds on a cod (approx. 10 g) with vibriosis
Photo: Solveig Nygaard

Status:
- The juveniles are produced in tanks with higher densities now than in the earlier days of cod farming

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- More repeated outbreaks of vibriosis at lower temperatures than experienced earlier
- The juveniles are infected by vibriosis before, immediately after and some months after vaccination
- Repeated treatment with antibiotics is beginning to result in resistance (1)


Atlantic cod (*Gadus morhua*)

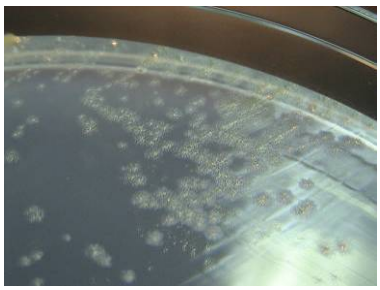
The juvenile producers have spent a lot of money both in the building of production facilities and in the large-scale production of juveniles. They vaccinate according to the instructions from the vaccine producers but still experience problems caused by repeated outbreaks of vibriosis, both before and after vaccination.
Note: The effect of the vaccine will be dramatically reduced if fish already suffering from disease (including an outbreak of vibriosis) are vaccinated. It is important that the fish have gained the necessary degree days in order to achieve good protection. If not, this may be an explanation for why the fish repeatedly experience outbreaks of vibriosis after immersion with vibriosis vaccine.

What to do:
- Improve vaccination conditions in order to reduce stress
- Improve water quality in hatcheries – this may be the causal agent in repeated outbreaks
- Improve production and documentation of effects and side-effects for water-based vaccines
- Test oil-based vaccines with vibriosis components on cod fish. Is it possible to reduce the vaccine quantity so that the IP injection may be given to the base of the pectoral fin? Injection site at the pectoral fin will not leave vaccine residues in liver and most likely avoid migration of vaccine to the fillet.

Production of cod juveniles is still in its infancy and problems caused by vibriosis will surely be eliminated through increased improvements by both the juvenile producers and the vaccine producers.

(1) Fish Health and Fish Diseases, Universitetsforlaget 1999, Chapter on Vibrio bacteria; Jens Lauritz Larsen and Karl Pedersen

COLUMNARIS IN TILAPIA


Flavobacterium columnare on agar plate

Introduction
Continuing the theme of diseases in tilapia we have highlighted in the previous two issues (Intervet Aquatic Animal Health Newsletter no. 11 and 12), another major bacterial disease known to have a devastating effect on survival rate in tilapia farms throughout the world is discussed here. Columnaris caused by *Flavobacterium columnare* (previously called *Flexibacter Columnaris, Cytophaga columnare* or *Myxobacterium columnare*) is one of the most common diseases in tilapia culture. The disease is highly contagious, especially at the fry and fingerling stages. Infected fish often display external lesions such as skin and gill erosion, and necrosis. In acute cases, these lesions may spread quickly and lead to high mortality within a matter of hours.


Mouth necrosis

Causative agent
F. columnare is a Gram negative, rod-shaped bacterium forming typical “hay stacks” or “columns” in wet-mount preparations (hence the name). These bacteria have a characteristic rhizoid pattern of growth on a low nutrient agar medium. Outbreaks are known to occur as a result of both temperature and environmental stress.

Clinical signs
External
Most columnaris infections are external manifested and present first as brown to yellowish-brown lesions on the gills, skin and fins. The lesions may first be seen only as a paler area


Tail rot caused by columnaris


Saddleback lesion


Gill necrosis


Grow-out tilapia tank

that lacks the normal shiny appearance. These sores are usually surrounded by a zone with a distinct reddish tinge. Early signs of infection also include fin erosion.
Lesions on the back often extend down the sides, giving the appearance of a 'saddle', typical of columnaris disease. On the mouth, the lesions may look moldy or cottony, and the mouth can be severely affected. The gill lesions are typically necrotic and the filaments disintegrate as the bacteria invade them. Damage to the gills causes the fish to begin breathing rapidly and 'gasping' at the surface due to lack of oxygen.

Internal
Less commonly, the infection will be observed internally. During acute outbreaks, bacteria sometimes reach the blood system, resulting in a systemic infection.

Epidemiology
Fish are susceptible to columnaris following some degree of stress. Abrupt variations in water temperature are likely to induce and accelerate the progression of this disease. Poor water quality, inadequate diet, handling and overcrowding are also stress factors likely to induce an outbreak. Columnaris occurs frequently in fry production units (hatcheries) but also in cages and closed recirculation systems (grow-out facilities).
Once established, the disease is highly contagious and may spread horizontally from fish to fish, causing high mortality rates. Infections may also stem from the environment, through contaminated nets, specimen containers and even food. The presence of columnaris may also lead to secondary infection or other diseases; for example, winter saprolegniosis is often preceded by columnaris.

Diagnostic methods
A presumptive diagnosis is obtained by observation of typical clinical signs such as saddleback lesions, or necrotic gills and mouth. Additionally, the bacteria can be observed in a wet mount of infected tissues observed under light microscopy. Using phase contrast at 400x magnification, the bacteria show a slow gliding movement, gathering into characteristic column-like masses or "haystacks".
A definitive diagnosis requires bacterial isolation on a low nutrient medium (such as cytophaga agar) and identification in the laboratory.

Control and treatment
The ideal way to eliminate the occurrence of columnaris is to alleviate stress in the cultured fish population. The bacteria thrive on organic wastes and these can be controlled by regular water changes. Proper diet, maintaining good water quality and avoidance of excessive handling will keep the fish from being stressed. To avoid spreading the bacteria, it is important to disinfect all equipment after each use and to use separate equipment at each rearing facility. But, this can be difficult in practice; however, stress should be minimized as much as possible. Salt (5-10 ppt) can be used to control the disease in hatchery tanks and to reduce the chance of infection during transportation.
In many cases, farmers are only able to partially control outbreaks by using antibiotics. However, this is not a sustainable practice. Best results are obtained if affected fish are treated as soon as the disease is detected. Infected fish display a reduced appetite and, consequently, antibiotics applied orally are generally ineffective. This may lead to selection of resistant bacteria leading to enhanced problems in the future. In general, antibiotics only prevent the infection from developing further. Farmers' reports indicate that, once the infection is over, subsequent infections are less likely, indicating that an immune response can be elicited after infection.
A live-attenuated immersion vaccine, Aquavac-COL™, against columnaris in channel catfish and manufactured by Intervet Inc. is on the market in the USA. In the future, we hope that a vaccine against *F. columnare* in tilapia and oher species will also be available as the key preventative technique to combat this devastating disease.

INTERVET ATTENDS AUSTRALASIAN AQUACULTURE CONFERENCE

Matt Bransden (Skretting Tasmania), Kate Woodward (Intervet Australia) and Carol Cox (Marine Harvest Scotland) during a Skretting-sponsored workshop and tour on Fish Health Management for the Tasmanian salmonid industry in Nov. 2005. Kate discussed the role of Intervet in the production of vaccines for the agricultural industry and its development of vaccines for aquaculture. Kate also highlighted the technical and regulatory processes required to produce vaccines to meet future health challenges in the local industry.

The Australasian Aquaculture conference is a biennial event and was held this year in Adelaide, Australia during 27-30th August. The theme was "Innovation in Aquaculture" and the conference attracted over 1,300 delegates from the Asia-Pacific region and elsewhere. Delegates represented all levels of industry and included farmers, processors, scientists, educators, government representatives, business operators and equipment suppliers.
The programme was broad, encompassing all types of aquaculture, including finfish, oysters, shellfish and crustaceans. Topics covering the entire production process were presented, from selective breeding and practical aspects of farming right through to harvest, processing and marketing the final product. The opening plenary sessions were particularly interesting and relevant to all members of the industry; Viggo Halseth from Skretting ARC discussed the key issues and primary drivers of the industry globally. Rosemary Stanton, a well-known independent Australian dietician, promoted the health benefits of fish and seafood in our diets.
Regarding aquatic animal health, one session was dedicated to vaccines and new technologies. Cedric Komar (and co-authors) presented a paper on vaccination as a disease management tool for barramundi (Asian sea bass), an extremely relevant topic as many Australian barramundi producers are starting to consider and implement vaccination against *Streptococcus iniae* into their production systems. Other papers presented in this session included a paper on protective effects of multivalent vaccines (Paul Midtyling), serotypic variation in *S. iniae* isolates (Andrew Barnes), RNA interference in the control of fish viral diseases (Serge Corbeil) and immune responses in invertebrates (David Raftos). Other health and disease management issues were presented within various other sessions, including risk management and surveillance, where there was an update on the recent herpes-like viral disease outbreak in farmed abalone in Australia.
Intervet Australia supported Kally Gross from the fish health unit at Tasmania's DPIWE to attend the conference and present a poster on outcomes of some of the work conducted with Anguillvac C, a vaccine against *Vibrio anguillarum* manufactured in Australia by Intervet.
Further information about the conference, including the abstracts, can be found on the National Aquaculture Council website at www.australian-aquacultureportal.com

HIGHLIGHTS OF THE 5th INTERNATIONAL SYMPOSIUM OF AQUATIC ANIMAL HEALTH

The symposium was held at the San Francisco Marriott on Sept. 2-6 and was attended by approximately 275 people. The programme consisted of several plenary lectures followed by lectures on selected topics. In addition, a poster session was organized. A brief overview of some of the key notes follows.

Fish health
A. Mitchell gave an historical overview of finfish health in the US from 1797 to 1920, mentioning the health pioneers, their investigations and their (sometimes) unusual treatments. *F. Berthe* reviewed the management of aquatic animal health from a global perspective. Proper health management practices are of primary importance in preventing, monitoring, controlling and even eradication of infectious diseases. Proper diagnostic tools, communication, reporting and establishment of quarantine methods should lead to emergency planning and actions. Fish providers should preferably be certified. Legislation, enforcement of risk analysis and a list of potentially important pathogens should allow farmers to manage disease and reduce their risks. Examples such as TSV in shrimp and VHS in Canada were highlighted. *A. Goodwin* dealt with the biology and politics of listed fish pathogens and why some are on the OIE list while others are not.

Immunity

C. Secombes gave a presentation on the cytokine network in fish; some vertebrate cytokines are known in fish while others have still not been found. The question remains as to whether fish have a Th1/Th2 pathway or not? Some part of this pathway may be present but another part not. In addition, some fish-specific cytokines have been identified, not occurring in vertebrates. Eventually, the fish cytokine network might be as complex as in vertebrates. *K. Söderhall* explained the innate defence system of crustaceans based on the proPO system and a variety of factors produced: penaeidins, crustins, hemocyanins, callinectines. New molecular methods have revealed new categories of genes which seem to be up or down regulated upon infection. The potential use of this knowledge in disease prevention has still to be demonstrated.

L. Brown used genomic and proteomic tools to investigate the pathogenesis of *A. salmonicida* infection. Information was generated on the importance of superoxide dismutase, pili and flagellar proteins as virulence factors. In addition, the host response was studied in salmon at different times after infection. Combination of the information generated in vitro and in vivo could lead to possible new vaccine antigen candidates. *R.Thune* studied single-tagged mutagenesis of *Edwardsiella ictaluri* strains which were tested in an *in vitro* invasion model in fish cells highlighting the role of bacterial urease.

Emerging diseases

P. Dixon described the occurrence of viral diseases in cyprinids as rhabdoviruses, KHV, CyHV-2, Koi sleepy disease and aqua reoviruses. Placing hosts in new environments might lead to new viral infections. Better diagnosis and access to better diagnostic tools would also lead to a greater awareness amongst farmers. Global warming and changed management practices (such as overcrowding) might also play a part.

B. Nowak reviewed the status of amoebic gill disease which is a serious problem in Tasmania, affecting cultivated salmon but not wild fish. Several other amoebic pathogens were identified in other fish species and sometimes taxonomic relationships could be identified. However, still no effective treatment (except fresh water treatment) is available for amoebic gill disease in salmon.

INTERVET AT CAA2

The 2nd International Symposium on Cage Aquaculture in Asia (CAA2) was held in Hangzhou, China July 4 – 6, 2006. The symposium was organized by the Asian Fisheries Society with support from Zhejiang University and several other organizations. Intervet was one of the supporting organizations together with NACA, AIT and the Asian-Pacific chapter of WAS. Approximately 300 people (including 150 from overseas) attended the event. There were about 150 papers presented and 23 of them (about 15%) were related to fish health.

A strong team from Intervet was present, participating in the scientific session, expert panel and exhibition. The team members were Alistair Brown (Intervet International bv), Dr. Brian Sheehan and Dr. Zilong Tan (Intervet Norbio Singapore), and Zhao Shuang (Intervet Hong Kong) (see photo). Intervet and collaborators presented five scientific papers, including one as keynote presentation (see abstract below; for the full paper, contact info.aquaINS@intervet.com). Zilong and Brian also chaired the disease session.

Dr. Leong Tak Seng (co-authored with **Intervet**) from Malaysia presented a paper entitled “Impact of infection with capsalid monogeneans in marine fish cultured in Asia”. He pointed out that many capsalid monogeneans, mainly *Benedenia epinepheli*, *B. lutjani* and *Neobenedenia girellae* (*N. melleni*), infected marine fish all year round. Among them, *N. girellae* appeared to cause the greatest economic losses, at least in the S.E. Asian region. The high density of the monogenean population in the cage culture ecosystem is the direct cause of the parasitic disease outbreaks. Improved farming practices, and prevention and control measures must be used to tackle the problem. Reduction in stocking density, frequent cleaning of cage nets, application of antifouling paint and use of pelleted feed can

decrease the incidence of parasitic infection. More research needs to be done on developing products for prevention and/or treatment.

A study that monitored the health status of marine fish in Asia was conducted by Dr. An-xing Li and colleagues from Sun Yat-sen University in Guangzhou, and **Intervet** Singapore. This epidemiological survey on a variety of farmed fish studied the prevalence of viral, bacterial and parasitic diseases during the July 2003 to April 2006 period, and found that 84% of fish (mostly clinically-abnormal) were infected with one or more pathogens. Several species were co-infected with multiple pathogens. Bacterial pathogens identified included *Streptococcus iniae, S. dysgalactiae, Pasteurella damsela* subsp. *piscicida, Nocardia seriolae* and *Tenacibaculum maritimum*. Parasites were monogenean trematodes and *Cryptocaryon irritans*. The authors called for improvements in the farming environment and health management practices.

Ms. Xiaoli Yu from Guangxi Institute of Fisheries, in collaboration with **Intervet**, gave a presentation entitled: "Trial vaccination of channel catfish *Ictalurus punctatus* with a live attenuated vaccine against enteric septicemia of catfish in China". Confirming data from the USA where the vaccine, Aquavac®-ESC, has been already marketed for several years, the results indicated that the vaccine was safe and ELISA data showed that it could stimulate the immune system of channel catfish to produce specific antibodies, even in the fry stage. The feed conversion rate of the vaccinated group was 16.0% better than that of the control group. It suggests that proper application of Aquavac®-ESC would provide economic benefits to the farmers.

In his presentation on the global success of the salmon industry in Norway, Alistair Brown (co-authored with Dr. William Enright, both from **Intervet** International) said that, with the use of vaccination programmes, 5.6 million tonnes of salmon were produced during the period from 1986 to 2005. This figure would be only about 3.6 million tonnes without vaccines. Translated into value, the extra 2 million tonnes equaled USD 8 billion at current salmon prices. He also emphasized the reductions in the use of antibiotics as a result of vaccination and the overall improvement of health management practices. Although it was emphasized that vaccination had a major role in the salmon success story, concurrent developments with feeds, culture operations, marketing, etc. also contributed.

At the CAA2 Intervet booth. From left: Zhao Shuang, Alistair Brown, Dr. Brian Sheehan and Dr. Zilong Tan

Health management practices for cage aquaculture in Asia - a key component for sustainability

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ABSTRACT

The intensification of aquaculture and globalization of the seafood trade have led to remarkable developments in the aquaculture industry. Nevertheless, the industry, particularly Asian aquaculture (> 90% of world production), is paying a price for this unprecedented growth in terms of deterioration in environmental and health conditions. The industry has been plagued with disease problems caused by viral, bacterial, fungal and parasitic pathogens. In recent years, disease outbreaks are becoming more frequent in the region and the associated mortality and morbidity have caused substantial economic losses.

Asian aquaculture is characterized by an enormous diversity of species, with several dozen marine species being farmed. Consequently, more resources are needed to understand the basic epidemiology of diseases in the various species. In Asia, some disease-causing agents have been described but comparative studies between isolates from different geographical locations and fish species are generally not available. Epidemiology data are scarce, as are basic data on the immune systems of Asian fish species. This hampers development of effective strategies for disease control. Also, most farming is operated on a small scale and technical support, including disease diagnosis and training, is often lacking at farm level.

Increased trade of live aquatic animals and the introduction of new species for farming, without proper quarantine and risk analysis in place, result in the further spread of diseases. In Asia, most individual fish farms produce several species of fish. Trash fish are widely used as feed. Fry are often wild caught or derived from wild-caught broodstock. Furthermore, legislation for and implementation of farming licenses and zoning policies are not in place in most Asian countries. Coupled with a 'gold rush' mentality, this often results in too many fish and too many farms in a concentrated area, which in turn promotes disease transmission. The combination of all these factors, together with the diversity of organisms in tropical waters, leads to a truly challenging disease situation.

At present, many farmers still focus more on treatment than prevention. Irresponsible use of antibiotics and chemicals in aquaculture can lead to residue problems, an increasing consumer concern, and to the development of drug resistance among the bacterial pathogens. In Asia, with the exception of Japan, few fish vaccines are yet commercially available. The major advantages of prophylactic vaccination over therapeutic treatment are that vaccines provide long-lasting protection and leave no problematic residues in the product or environment.

Asian aquaculture will continue to grow at a fast pace due to both area expansion and production intensification. Under these conditions, the prevalence and spread of infectious diseases will unavoidably increase as a result of higher infection pressure, deterioration of environmental conditions and movement of aquatic animals. Accordingly, the effective control of infectious diseases has become more and more important in the cultivation of aquatic animals. Good health management is the "silver bullet" for disease control. Collectively, this includes the use of healthy fry, quarantine measures, optimized feeding, good husbandry techniques, disease monitoring (surveillance and reporting), sanitation and vaccination, and proper control and biosecurity measures when diseases do occur. Overall, the emphasis must be on prevention rather than treatment. Remember, 'one gram of prevention is better than a kilogram of cure'.

INTERVET SUPPORTS AwF

Intervet International bv (www.intervet.com) is dedicating its 2006 Christmas donation (in lieu of sending out Christmas and New Year cards) to four non-governmental organizations (NGO's). Four initiatives from different parts of the world have been selected. The first initiative, Aquaculture without Frontiers (AwF), is an independent non-profit organization that promotes and supports responsible and sustainable aquaculture and alleviation of poverty by improving livelihoods in developing countries. AwF does not seek to promote aquaculture in isolation, but as a component of integrated rural and coastal development plans, and of strategies to alleviate poverty (see www.aquaculturewithoutfrontiers.org for more details).
The other three NGO's aim to support disadvantaged communities in Africa or to taking care of orphaned animals. A South African-based non-profit NGO initiative, Animal Aid for Africa (AAA), aims to uplift disadvantaged communities across sub-Saharan Africa. Focus is on delivering sustainable solutions to malnourishment and the spread of zoonotic diseases, by exchanging skills and knowledge between veterinarians.
(www.animalaidafrica.co.za).
The two other organizations are Sophianum explores South Africa, supported by the Dutch Ministry of foreign affairs (www.ZA.DEBIOLOOG.nl and www.africanorphanage.com), and Global Vets (www.ovc.uoguelph.ca/associations/globalvets), a programme at the Ontario Veterinary College that offers students a unique opportunity to investigate animal health care in developing countries. Global Vets was formed to promote international collaboration on animal health and welfare, agricultural development and ecosystem health.

NEWSLETTER EDITOR ENDS 6-YEAR TERM

William with two other new colleagues in Intervet Ireland, Ciara Feeney and Lesley Pim

Dr. William Enright, International Marketing & Pharmaceutical Manager for the Aquatic Animal Health (AAH) Division, has decided to return to his native country of Ireland. After nearly 11 years with Intervet International bv in Boxmeer, The Netherlands, William has accepted the position of Marketing Manager for the Companion Animal & Equine Business Unit at Intervet Ireland. It is a great opportunity for William to return to Ireland and yet stay within the Intervet family. His new position is within a newly restructured team in Ireland and the change in species focus and the more commercial environment will offer William new and exciting challenges and learning opportunities.

One of the more obvious initiatives that William was responsible for is this series of Intervet AAH Newsletters, this issue 13 being the last that William will edit. The first issue was published in October 2000 and has appeared at 6-month-intervals ever since. We believe that the usefulness and high quality of this publication has been due to a large extent to the efforts of William. The future Editor will be mentioned in issue 14.

We are obviously sorry to see William leave AAH after seven years (he joined the AAH Division on August 1, 1999) but understand his desire to return to his roots. We thank William for his contribution not only to the AAH newsletters but indeed to our AAH programme in general, and we wish him well for his continued success within Intervet.

Alistair Brown, Director, Intervet AAH Division

EDITOR'S NOTE: I would like to take this opportunity to send all the newsletter readers my very best wishes for their future activities related to the broad field of aquaculture, one of the great socio-economic areas of the future. And a big thank you to all those who have contributed in any way to the thirteen issues of this newsletter – it could not have been possible without you! Best regards, William

NEW TEAM MEMBER AT INTERVET NORBIO SINGAPORE

Neil Wendover joined the Veterinary Services Department of Intervet Norbio Singapore in September 2006 in a new position as Technical Officer. He comes most recently from GenoMar, a world-renowned tilapia selective-breeding company. He has extensive experience in tilapia production on both the Asian and African continents. He hopes to become a key team member and will, amongst other things, play an active role in supporting the vaccine business being developed by Intervet Norbio Singapore.
Neil is a keen adventurer, having explored the wilds of Africa, from the Zambezi river right up to the peaks of Kilimanjaro and, most recently, investigating China where he met his Spanish girlfriend. Water sports are also his passion and Neil will kite surf and scuba dive wherever possible. He hopes sports like field hockey, running and racket games will become part of the norm as he settles in to his new life in Singapore.

INTERVET PARTICIPATE AT ISTA 7 IN MEXICO

The seventh International Symposium for Tilapia Aquaculture (ISTA 7) was held in Veracruz, Mexico from the 6th to the 9th of September, 2006. Three Intervet staff (Cedric Komar of Intervet Norbio Singapore, and Oscar Parra and Marcos Godoy of Intervet Chile) attended the symposium. This series of symposia was initiated in Israel in 1983 and was followed by symposia in Thailand, Ivory Coast, USA, Brazil and, most recently, the Philippines in 2004. The proceedings of these meetings bring together the most up to date information for farmers, scientists and processors and are a very important vehicle for bringing together the various sectors of this very diverse and rapidly expanding industry. Delegates from over 20 different countries were at the conference. Given the increasing importance of infectious diseases in tilapia farming, one entire session was devoted to *Streptococci* and other pathologies. In this session, chaired by Dr. Phil Klesius, Cedric Komar from Intervet Norbio Singapore presented an overview of the emerging diseases in tilapia. Due to the economical impact of infectious diseases in tilapia farming during recent years, there is an increasing awareness among farmers that prevention is better than cure and that health management and vaccination strategies should become a critical factor in the success of this industry. Three new tilapia books have just been published and were launched at the conference, one by Professor Abdel-Fattah M. El-Sayad from Alexandria University (Egypt), titled “Tilapia Culture”, and the other two by Kevin Fitzsimmons, titled “Tilapia Biology Culture and Nutrition” and “Atlas of Tilapia Histology”.

NACA: 5th ASIA REGIONAL ADVISORY GROUP MEETING ON AAH

The fifth Asia Regional Advisory Group Meeting on Aquatic Animal Health (AGM-5) was held at the Headquarters of NACA in Bangkok, Thailand. The advisory group was established by the Governing Council of the Network of Aquaculture Centres (NACA) to provide advice to NACA members in the Asia-Pacific region on aquatic animal health management through review and evaluation of quarterly regional aquatic animal disease reporting, and through review, evaluation and revision of guidelines, procedures and diagnostic guides. Members of the advisory group included invited aquatic animal disease experts, the World Animal Health Organization (OIE), the Food and Agricultural Organization of the United Nations (FAO) and collaborating regional organizations. This year, AGM-5 was held from November 22 to 24.

Staff of Intervet Norbio Singapore (INS) have been members of the Advisory Group since the inception of the programme in 2002 and, as such, represent the private sector. Dr. Lauke Labrie from INS attended AGM-5 and gave a presentation on emerging bacterial diseases in finfish. Data from epidemiological investigations in the region were presented as well as data on emerging bacterial diseases caused by pathogens such as *E. tarda, S. dysgalactiae, Francisella* sp. and Big Belly (due to a new *Vibrio* species). Unknown diseases described as visceral toxicosis in catfish (VTC) and loss of mucus and septicemia in eel (LMSS) were brought on the agenda. However, emphasis was equally placed on diseases which are and remain of important economic concern in the region, such as *S. iniae, T. maritimum, N. seriolae, F. columnare* and *S. agalactiae*. An official report will soon be issued by NACA.

Members of the fifth Asia Regional Advisory Group Meeting on Aquatic Animal Health (AGM-5) was held at the Headquarters of NACA in Bangkok, Thailand. Dr. Lauke Labrie is 5th from the left.

ERRATUM IN NEWSLETTER 12

In the article titled "Streptococciosis in Tilapia" in the previous Intervet AAH Newsletter (Issue 12), the sentence "*Streptococococcus* spp. are Gram positive, non-acid fast, non-motile, **oxidase-positive**, catalase-negative cocci" should have read "*Streptococococcus* spp. are Gram positive, non-acid fast, non-motile, **oxidase-negative**, catalase-negative cocci". We apologise for any inconvenience caused.

NEWSFLASH!!

Coming in January 2007, the new Intervet website dedicated to aquatic animal health.

http://AQUA.INTERVET.COM

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SCIENTIFIC SUMMARIES

Viral and bacterial diseases of Atlantic cod *Gadus morhua*, their prophylaxis and treatment: a review
Dis Aquat Organ 71:239-254, 2006
Samuelsen OB, Nerland AH, Jorgensen T, Schroder MB, Svasand T, Bergh O (Norway)
This review summarises the state of knowledge of both viral and bacterial diseases of Atlantic cod *Gadus morhua*, and their diagnosis, prophylaxis and treatment. The most important losses have been at the larval and juvenile stages, and vibriosis has long been the most important bacterial disease in cod, with *Listonella (Vibrio) anguillarum* dominant among pathogenic isolates. Vaccination of cod against pathogens such as L. anguillarum and *Aeromonas salmonicida* clearly demonstrates that the cod immune system possesses an effective memory and appropriate mechanisms sufficient for protection, at least against some diseases. Well-known viruses such as the nodavirus that causes viral encephalopathy and retinopathy (VER), infectious pancreatic necrosis virus (IPNV) and viral haemorrhagic septicaemia virus (VHSV) have been isolated from Atlantic cod and can be a potential problem under intensive rearing conditions. No commercial vaccines against nodavirus are currently available, whereas vaccines against IPNV infections based upon inactivated virus as well as IPNV recombinant antigens are available. A number of investigations of the pharmacokinetic properties of antibacterial agents in cod and their efficacy in treating bacterial infections have been reviewed.

Immunostimulation of larvae and juveniles of cod, *Gadus morhua* L.
Magnadottir B, Gudmundsdottir BK, Lange S, Steinarsson A, Oddgeirsson M, Bowden T, Bricknell I, Dalmo RA, Gudmundsdottir S (Iceland)
J Fish Dis 29:147-155, 2006
Cod larval culture is currently hampered by high mortalities in the first 2-3 weeks after hatching, often due to infectious diseases. The immune system of cod is not fully competent until 2-3 months after hatching. Conventional vaccination is, therefore, not of value before this time, and the larvae are wholly reliant on non-specific parameters for their defence against infection. A range of substances, generally derived from bacterial, fungal or plant origin, can activate these non-specific parameters. During three hatching seasons, 2001-2003, at the Marine Institute's Experimental Station, Stadur, Grindavik, Iceland, the effects of several immunostimulants on survival and disease resistance of cod larvae and juveniles were examined. Both bathing treatments and administration in the feed were used. One of these substances, lipopolysaccharide (LPS), isolated from the bacterium Aeromonas salmonicida (ssp. salmonicida or achromogenes), appeared in some instances to improve survival and have a beneficial effect on disease resistance. Other substances tested had limited effects. The results emphasize the need for further work in this field.

Infectious pancreatic necrosis virus establishes an asymptomatic carrier state in kidney leucocytes of juvenile Atlantic cod, *Gadus morhua* L.
Garcia J, Urquhart K, Ellis AE (UK)
J Fish Dis 29:409-413, 2006
Juvenile Atlantic cod (10 g) were infected with infectious pancreatic necrosis virus (IPNV) by intraperitoneal injection and cohabitation. Fish showed no signs of disease but IPNV could be re-isolated from kidney tissue for up to 12 weeks. On weeks 2, 5, 8, 10, 11 and 12 following infection, kidney leucocytes were fractionated on Percoll gradients, and cells separated into plastic adherent and non-adherent cell populations after overnight incubation. IPNV was detectable in lysates of both cell populations and in supernatants by culture in CHSE-214 cells. Wells containing 10(5)-10(6) macrophages had an IPNV TCID(50) of about 10(3)/well and in serially diluted macrophages the minimum number of cells required to detect virus ranged from 10(1) to 10(4). These data indicate that about

one in 10(4) macrophages were infected and the mean number of virus/infected cell was about 10. Replication of IPNV in the macrophages was low as the titre of the virus in macrophage lysates did not increase between days 1 and 3 of culturing the macrophages, but virus was released into the supernatant over this time.

Francisella sp. (Family _Francisellaceae_) causing mortality in Norwegian cod (_Gadus morhua_) farming

Nylund A, Ottem KF, Watanabe K, Karlsbakk E, Krossoy B (Norway)
Arch Microbiol 185:383-392, 2006

In 2004, a new disease was detected in cod (*Gadus morhua*) in western Norway. Affected cod had white granulomas in the visceral organs and skin. A species of *Francisella* was isolated on blood agar plates from moribund cod. The bacterium could be grown at temperatures ranging from 6 to 22 degrees C, but did not grow at 37 degrees C. Challenge experiments showed that *Francisella* sp. was the cause for the new disease. The 16S rDNA gene sequence from *Francisella* sp. showed 99.17% similarity to *F. philomiragia*, and the 16S-23S ribosomal RNA intergenic spacer (249 nt), shows a similarity with that from *Francisella* isolated from tilapia and *F. tularensis* of 96.8 and 35.9%, respectively. The 23S sequence is more similar to *F. tularensis*, 97.7% (2,862 nt), compared to the tilapia isolate 96.8% (2,131 nt). The partial putative outer membrane protein (FopA) sequence (781 nt) from *Francisella* sp. shows a similarity with that from *F. tularensis* and *F. philomiragia* of 77.3 and 98.2%, respectively. Based on sequence data, culturing temperatures and pathogenicity for cod, it is suggested that this Francisella sp. from cod could be a new species of *Francisella*, Family *Francisellaceae.*

Immunization of eyed channel catfish, _Ictalurus punctatus_, eggs with monovalent _Flavobacterium columnare_ vaccine and bivalent _F. columnare_ and _Edwardsiella ictaluri_ vaccine

Shoemaker CA, Klesius PH, Evans JJ (USA)
Vaccine Oct 2, 2006 E-publication ahead of print version

The efficacy of a modified live monovalent *Flavobacterium columnare* vaccine and bivalent *F. columnare* and *Edwardsiella ictaluri* vaccines were evaluated following immersion vaccination of eyed channel catfish (*Ictalurus punctatus*) eggs. The modified live *F. columnare* vaccine was grown in modified Shieh broth and administered at 1.35 x 10(7) CFU/ml for 15 min exposure (1 L water). Booster immunization was conducted at day 34 with 2.17 x 10(7) CFU/ml for 15 min. Bivalent vaccines consisted of a 1:1 ratio of the modified live *F. columnare* and AQUAVAC-ESC® vaccine for the 15 min exposure (1 L immersion bath). Non-vaccinated controls were held in 1 L water without vaccine for 15 min. Fish were challenged with *F. columnare* (ALG-00-530) by immersion at days 109, 116, and 137 post-primary immunization or E. ictaluri (AL-93-75) by immersion at day 116 (bivalent vaccine group). Efficacy of monovalent modified live *F. columnare* vaccine administered singly or with a booster vaccination was shown to be protective with relative percent survival (RPS) values ranging from 50.0 to 76.8. Some variation was seen in RPS values following bivalent immunization, ranging from 33.0 to 59.7 in the fish challenged with *F. columnare* and 44.5 to 66.7 in fish challenged with *E. ictaluri*. However, the RPS values were not statistically different. The results suggest that administration of live bivalent vaccine at the eyed-egg stage is safe and elicits protection upon single pathogen challenge.

Molecular diversity and growth features of _Flavobacterium columnare_ strains isolated in Finland

Suomalainen LR, Kunttu H, Valtonen ET, Hirvela-Koski V, Tiirola M (Finland)
Dis Aquat Org 70:55-61, 2006

Columnaris disease caused by *Flavobacterium columnare* is a problem in fish farming worldwide. During the last 15 yr, outbreaks have started to emerge in Finland*. Flavobacterium columnare Type Strain NCIMB 2248T and 30 Finnish* F. columnare

isolates were studied using analysis of 16S rDNA by restriction-fragment length polymorphism (16S RFLP), length heterogeneity analysis of polymerase chain reaction (LH-PCR) products, automated ribosomal intergenic spacer analysis (ARISA), and 16S rDNA sequence analysis. All isolates fell into RFLP Genomovar I and had the same length in the LH-PCR analysis. Based on ARISA, 8 genetically different strains were selected for further analyses. The growth of these strains under different temperatures, NaCl concentrations, and pH values was tested. The Finnish *F. columnare* strains did not grow at NaCl concentrations >0.1% or at pH values < or = 6.5, and they were susceptible to several antimicrobial agents, but not to Polymyxin B or neomycin. These findings may aid in development of methods for disease management at fish farms.

Live vaccine trials against nocardiosis in yellowtail *Seriola quinqueradiata*
Itano T, Kawakami H, Kono T, Sakai M (Japan)
Aquaculture 261:1175-1180, 2006
In an attempt to develop a vaccine against *Nocardia seriolae*, related species of live bacteria *N. soli*, *N. fluminea*, and *N. uniformis* were injected into yellowtail *Seriola quinqueradiata*. In addition, fish were challenged with a low virulence strain of *N. seriolae* to model the concept of use of a live vaccine. The fish injected with live *N. soli* and *N. fluminea* cells showed slight resistance against an artificial challenge with *N. seriolae*. On the other hand, the fish that survived the *N. seriolae* infection showed complete resistance to the *N. seriolae* challenge. These results suggest that protective immune responses against *N. seriolae* are induced in yellowtails.

Aquatic *Francisella*-like bacterium associated with mortality of intensively cultured hybrid striped bass *Morone chrysops* × *M. saxatilis*
Ostland VE, Stannard JA, Creek JJ, Hedrick RP, Ferguson HW, Carlberg JM, Westerman ME (USA, UK)
Dis Aqua Org 72:135-146, 2006
The present study identifies an emerging disease associated with an aquatic *Francisella*-like bacterium that can cause mortality in hybrid striped bass *Morone chrysops* × *M. saxatilis* reared intensively in freshwater. Clinically affected fish were lethargic, had scattered haemorrhagic cutaneous lesions and diffuse gill pallor. The head kidney and spleen were markedly swollen and contained numerous interstitial granulomas; histological examination revealed small, pleomorphic Gram-negative coccobacilli within vacuolated cells. The bacterium could not be cultured from head kidney homogenates either with standard or enriched microbiological media or following inoculation of a Chinook salmon embryo (CHSE)-214 cell line. No amplification product was obtained from head kidney DNA by polymerase chain reaction (PCR) assay using *Piscirickettsia salmonis*-specific primers. PCR analysis of infected head kidney homogenate with primers designed for the eubacterial 16S rRNA produced a single amplicon. Phylogenetic analysis of this DNA sequence demonstrated that the sequence aligned most closely with members of the genus *Francisella*, identified from tilapia *Oreochromis* spp. in Taiwan and an aquatic *Francisella* species that was recently isolated from the three-line grunt *Parapristipoma* trilineatum in Japan. This *Francisella*-like disease was transmitted to naïve hybrid striped bass fingerlings by intraperitoneal injection of tissue homogenates prepared from a natural outbreak. All fish developed gross and histological lesions identical to those from natural outbreaks. Intracellular Gram-negative bacteria were observed within the cytoplasm of cells (presumably macrophages) within the granulomas, but bacteria were not recovered. The 16S DNA sequence of the bacterium obtained from tissues of experimentally infected fish was identical to that obtained from the fish used as infected donor tissue.

Use of hydrogen peroxide against the fish pathogen *Tenacibaculum maritimum* and its effect on infected turbot (*Scophthalmus maximus*)
Avendaño-Herrera R, Magariños B, Irgang R, Toranzo AE (Spain)
Aquaculture 257:104-110, 2006
Hydrogen peroxide (H2O2) has recently received attention for its effective control of numerous external pathogens to fish. This study examines whether this chemical disinfectant at concentrations ranging from 30 to 240 ppm has the capacity to kill *Tenacibaculum maritimum* in in vitro assays. Moreover, in order to evaluate the effectiveness of H2O2 in infection conditions, marine tenacibaculosis was induced in turbot (8 to 10 g), *Scophthalmus maximus*, by bath exposure of 106 T. maritimum cells ml− 1 and treated with 30 or 240 ppm H2O2. Under the in vitro conditions, all concentrations tested were efficacious at killing a high proportion of *T. maritimum* in the seawater after 30 min of exposure. In the case of treated skin mucus, the effect of 30 ppm H2O2 on the bacterium was mitigated by the presence of mucus and treatment with higher concentration of H2O2 (240 ppm) is needed to kill the pathogen. Although these concentrations were not toxic for the fish, they were not effective for the treatment of experimentally infected turbot due to the increasing levels of stress, which did not lead to death, but accelerated the tenacibaculosis outbreak. Based on our results, we recommend the use of H2O2 at a concentration of 240 ppm only as a general disinfection preventive method for treating water culture and surface of tanks before the introduction of fish.

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