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Intervet Aquatic Animal Health

Newsletter

May 2004

In this issue ……..

Intervet Canada enters market with Norvax® Compact 4

This year Intervet Canada Ltd. (ICL) celebrates its 20th anniversary. From a very modest beginning with the introduction of some poultry vaccines, ICL has become a major animal health company in Canada.

"We now sell vaccines and pharmaceuticals for all species groups: swine, cattle, poultry and companion animals. Sales between the species are almost even, although sales in the swine segment are the largest, mainly because of a new vaccine we introduced last year," says Dr. Jorgensen. As a result of the rapid expansion, ICL built a brand new headquarters building (including warehouse) in Whitby, Ontario just 3 years ago, right beside the shore of Lake Ontario. It was an immense leap for the employees, both physically as well as mentally, to move into new quarters - although about half of the 50 staff works from other locations in Canada.

"And now we are truly excited to be shortly entering the Canadian aquatic animal health segment with Norvax® Compact 4 as Canada represents the fourth largest salmonid farming area in the world, and is right on the heels of the UK who are currently in third place." says Dr. Jorgensen. "Canadian fish farming has the potential for tremendous growth and we would like to be a part of that growth by helping to find cost-effective, environmentally-friendly solutions to emerging diseases. Intervet is looking forward to the opportunity to offer an increasing range of fish vaccines to both our national and global salmonid customers."

Norvax® Compact 4 induces long-lasting protective immunity in Atlantic salmon against furunculosis caused by *Aeromonas salmonicida* subsp. *salmonicida*, cold-water vibriosis caused by *Vibrio salmonicida*, and vibriosis caused by *Vibrio anguillarum* serovar O1 and/or *Vibrio anguillarum* serovar O2a (see **Intervet AAH Newsletters no. 5 and 6** for

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more information on these diseases and the vaccine).

The company in Canada is in the process of employing experienced technical representatives, on both major salmon farming coastlines, to support the customers. “ICL has a good reputation regarding technical support already; we believe that that is what makes a difference, along with innovative products based on sound R&D. We could not have come so far so quickly in Canada without that”, Dr. Jorgensen adds. In Canada, mail info.canada@intervet.com or telephone 800.268.4257 for further information.

Norvax® Compact 4

- Tetravalent injection vaccine against furunculosis, vibriosis and coldwater vibriosis
- Proven high and long-lasting protection against all targeted pathogens
- Safe and efficacious across a range of vaccination weights
- Proven in the field since 1999
- Benefits sustainable and environmentally friendly aquaculture

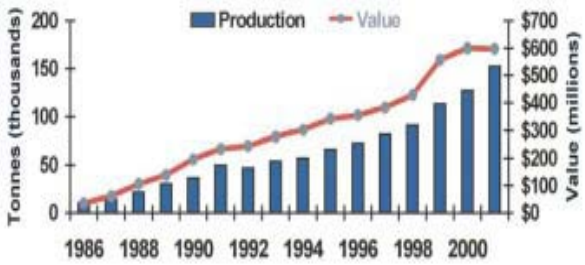
The following is an excerpt from:

Recommendations for Change, Report of the Commissioner for Aquaculture Development to the Minister of Fisheries and Oceans Canada, 2004.

The full report can be found at:
http://ocad-bcda.gc.ca/egreetings.html

In 1986, Canadian aquaculture production amounted to only 10,488 tonnes, valued at $35 million. Growing at an average annual rate in excess of 19 percent, in 2001, the Canadian aquaculture industry produced 152,523 tonnes of fish and seafood valued at $597 million representing roughly 24 percent of the value of total fish landings (see Figure). This extraordinary growth, however, has not kept pace with other leading fish-farming nations. In fact, today, Canada ranks twenty-second among aquaculture producing nations and accounts for less than one-third of 1 percent of global farmed fish and seafood production. This level of production falls far below Canada's capacity and capability. Canada has the natural resource base that can enable it to be in the top three global competitors in aquaculture production.

Aquaculture production and value for the period 1986 - 2001



Production is dominated by five main categories: salmon 69.0 percent, blue mussels 14.2 percent, oysters 7.0 percent, trout 4.3 percent and steelhead 3.1 percent. Finfish represents 77 percent of the tonnage and 90 percent of the value of Canadian aquaculture production. From 2000 to 2001, farmed salmon production increased 21 percent to almost 95,000 tonnes. In 2001, shellfish production in Canada surpassed 34,000 tonnes, worth more than $58 million. Blue mussel is the most important shellfish species grown in Canada, followed by oysters and clams. Today, a total of 72 cold-water species are licensed for rearing in the country. They include 50 species of finfish, 18 species of marine shellfish, two species of amphibians and two species of marine plants. Aquaculture is carried out in all provinces and in one territory.

With a bountiful biophysical resource base and proximity to two of the world's largest, growing seafood markets (the US and Pacific Rim), Canada is well-positioned to be a more significant producer of aquaculture products. However, in Canada's drive to become a leading supplier of fish and seafood, we are also aware of the broader objectives of Canadian society to bring economic development to our coastal and rural regions in harmony with the social fabric of these communities, while preserving environmental integrity and the culture and traditions of Canada's Aboriginal Peoples. Building upon a solid foundation established by individual entrepreneurs, small and medium-sized businesses and multi-national organizations, the sector has a tremendous capacity to expand and diversify.

Within the next 15 years, it is projected that the Canadian aquaculture sector, growing at 10 to 15 percent annually, could generate in excess

of $2.8 billion annually in farm-gate revenues and provide sustainable, year-round employment to more than 47,000 people living in coastal, rural and Aboriginal communities. Value added processing and revenues generated in the related supplies and services sector could push the total economic value of the Canadian aquaculture sector beyond $6.6 billion.

Intervet AAH Director visits fish and shrimp farms in Asia

In view of the importance of Asian aquaculture, Alistair Brown, Director of Intervet's Aquatic Animal Health Division, recently spent two weeks in Asia. During his trip, Alistair visited a number of farms in five Asian countries covering fish and shrimp, freshwater and marine, and warmwater and coldwater species. He also met people from various organizations, research institutes and nutrition companies. It was indeed a busy and productive trip. Fourteen flights were taken in 14 days, not to mention the car and boat rides. "The scale and diversity of Asian aquaculture is fascinating," said Alistair, "It represents a great number of challenges to and opportunities for Intervet."

Above: ***A tilapia broodstock farm outside Bangkok, Thailand. Workers are collecting eggs from brooders. In recent years, tilapia has become one of the most consumed fish in the world. Thailand produced 100,000 metric tones tilapia in 2002, 10% of which were exported to the USA.***

Below left: ***Alistair Brown with Mr. Dai Quen Chai, President of Long Diann Aquatic Products Farming Technology, in his marine fish hatchery in southern Taiwan. Taiwan is the best known fish reproduction country in the world and Long Diann has been a pioneer of reproduction and commercialization of several important marine species.***

Above: ***An eel farm in Fujian Province, China. Fujian is the biggest eel production area in the world with an annual output of 60,000 metric tonnes, accounting for over a quarter of world production.***

Above: ***A turbot farm in Northern China. The turbot industry is growing fast. China produced approximately 8,000 metric tonnes last year. The restaurant price is about US$40 per kg due to its delicious taste and high market demand.***

Above: ***One of the biggest shrimp hatcheries in the world, located in Lampung, Indonesia. Over 4 billion post larvae are produced annually, mostly Litopenaeus vannamei with some Penaeus monodon.***

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Chlorasol™ MIC summary data

A recent study performed at the Intervet aquatic animal health R&D centre in Singapore demonstrated the efficacy of Chlorasol™ against a range of important aquatic bacterial pathogens. Minimum inhibitory concentrations (MIC) were calculated for, amongst others, *Aeromonas hydrophila, Vibrio anguillarum, Vibrio harveyi, Yersinia ruckerii, Lactococcus garvieae, Streptococcus agalactiae, Streptococcus iniae, Streptococcus difficile, Photobacterium damsela subsp. Piscicida, Edwardsiella tarda, Edwardsiella ictaluri, Tenacibaculum maritinum, Flavobacterium columnarae, Nocardia asteroides.*

Some of the conclusions of the report are:

1. There was no clear relationship between efficacy and the Gram reaction.
2. The carbonate hardness of the water does affect efficacy – when all else is equal, the higher the carbonate, the higher the MIC value. However, the presence of metal ions (like calcium and magnesium) in the water can reduce the negative effect of carbonates on Chlorasol efficacy.
3. The longer the exposure, the lower the MIC value. However, after a certain minimal duration, the MIC does not decline further.
4. Although there is variation between bacterial species tested, 40 ppm is always effective in seawater with 2 minutes of exposure while this drops to 10 ppm with 60 minutes of exposure.
5. In freshwater, it is advisable to measure the free carbonate hardness – for example, 20 ppm is always effective with 60 minutes of exposure in very soft water but as high as 200 ppm may be needed in very hard water.

Safety Precautions for Animal Treatment

Chlorasol may be effectively used in water volumes containing live fish and shrimp. However, as for all products, a small number of animals should be first exposed to the disinfection solution at the recommended concentration and exposure time, as water hardness (soft), temperature (high) and pH (low) may make animals more sensitive. In addition, the water needs to be well aerated during treatment if live animals are present. As a rule of thumb, consider the following maximum dose recommendations.

- Warmwater marine fish: 15-30 ppm for 30-60 minutes.
- Coldwater freshwater fish: 10-20 ppm for 30-60 minutes.
- Warmwater shrimp: 3-15 ppm for 24-72 hours.
- Ornamental fish: 3-20 ppm for 4-24 hours.

Intervet well represented at AquaSur 2004

The third AquaSur aquaculture international fair was held in March in the city of Puerto Montt, Chile. This year the exhibition involved the participation of 32 countries, deployed in nearly 266 stands distributed across an area of almost 8.000 M^2. There were more than 10,000 visitors during the three days.

For the third exhibition running, Intervet Chile's Aquatic Animal Health Division, also located in Puerto Montt, was present at this important exhibition. This reaffirms the commitment existing to each one of Intervet's clients and it was welcoming to see that the distinguished Intervet stand was continuously visited by a great number of clients and friends that, as always, showed a great interest in the products, research and service activities carried out by Intervet both in Chile and around the world.

(L to R): Dr. Sergio Vasquez, Maria Elena Bartsch (hostess), Dr. Dag Knappskog, Valeria Gallardo, Alistair Brown, Cynthia Ferrada (hostess) at AquaSur 2004.

In addition to the whole Intervet Chile team (Andrés Engelbreit, Oscar Parra, Sergio Vásquez, Juan Pablo López and Valeria Gallardo), several senior Intervet staff were in Chile for AquaSur and other meetings: Alistair Brown, Director, Intervet AAH Division, The

Netherlands; Dr. Eric Rijke, Director of R&D, Intervet AAH, The Netherlands; Dr. Dag Knappskog, Research Manager, Intervet Norbio, Norway; Dr. Luc Grisez, Research Manager, Intervet Singapore; and Jorgen Jorgensen DVM, General Manager, Intervet Canada.

Dr. Eric Rijke (left) and Dr. Luc Grisez taking some tissue samples in Chile.

The presence of these multinational AAH specialists allowed for some excellent external and internal meetings, the primary objective being toward the development innovative products for the aquaculture market in Chile. Intervet realizes the current importance of the Chilean industry; added to its dynamic growth, it is also the recent appearance of several important diseases like (atypical) vibriosis (see related article in this newsletter), streptococcosis and furunculosis. However, rickettsiosis in salmon continues to be the most economically-important disease where there is still no effective tool for prevention.

Salmonid production in Chile in recent years

Product	Quantity (net; 1,000 MT)		Average value (USD /kg FOB)	
	2002	2003	2002	2003
Salmon	257	218	3.0	4.2
Trout	74	68	2.6	3.6
Total	331	286	2.9	4.0

Atypical Vibriosis in Chile

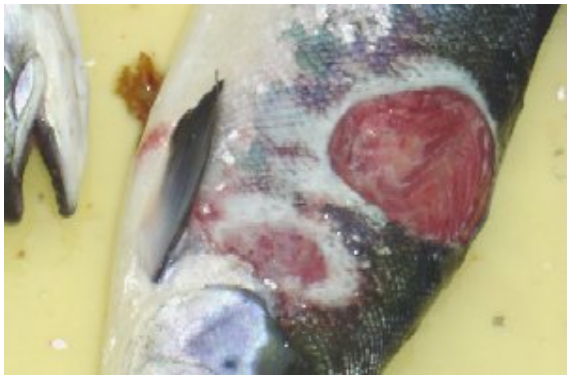
During the winter and spring of 2003, Atlantic salmon (*Salmo salar*) reared in sea and brackish water net pen sites on the coast of Southern Chile were affected by several unusual disease outbreaks. The first clinical cases were reported by a private Chilean fish health lab, ADL Diagnostic Chile Ltda., who first described the clinical signs of the disease and the bacterial genus. The external clinical symptoms were characterized by the presence of lesions in skin, haemorrhaging ulcers and mortality of the affected fish, while the internal signs were pericarditis, peritonitis and multiple necrotic foci in the liver, and signs of systemic septicaemia.

The mortality level of fish at different farms ranged from 2 to 22% before treatment with antibiotics was instigated. In all clinical cases, bacteria were isolated from the lesions and internal organs of the affected fish and, by using conventional bacteriological identification assays, a *Vibrio* sp. was suspected to be the agent of the disease. *Vibrio* isolates have been described in Chile prior to these new clinical cases, but they have so far not been related to disease outbreaks of farmed fish. At present, the disease seems to cause mortality in Atlantic salmon in hatcheries that use seawater to optimize smolting and in Atlantic salmon during the period from sea transfer until a size of 1 kg; however, the problem will most likely become bigger in the future as bacterial diseases typically affect fish of all sizes. Indeed, there are already reports of the bacterium being found in 4- to 5-kg Atlantic salmon, but without causing mortality. At present, sick fish are treated with antibiotics; a good response is observed if it is applied in the early stages of the disease outbreak. The bacteria seem to be sensitive to the various antibiotics registered for fish in Chile.

In order to obtain the final identification of the agent, Intervet initiated a co-operation programme with ADL, which provided Intervet's R&D Centre in Bergen (Norway), Intervet Norbio, with the bacterial isolates collected from

the field outbreaks. Two researchers from ADL spent a week working with the bacteriological team of Intervet Norbio in order to get familiarized with the laboratory methods employed. The final identification of the bacterial isolates was made after amplification and characterization of the 16S rRNA of the isolates using polymerase chain reaction (PCR), followed by comparison of the nucleotide sequence with previously submitted bacterial 16S rRNA sequences in a gene bank. The results from these analysis showed that it was possible to identify the bacterium as belonging to the group *Vibrio ordalii*.

The dissimilarities discovered by using the morphological, physiological, biochemical, enzymatic and antigenic assays, indicated important differences from the reference strains of *V. ordalii*; thus, a new sub species of *V. ordalii* may well have been discovered. The name for this bacterium was proposed to be Atypical *V. ordalii*. In both laboratories (ADL and Intervet Norbio), Koch's Postulates were applied and the strong virulence of the causative agent was observed as the disease could cause total losses of the inoculated and cohabitated challenged fish.



A second objective of this work was to initiate a feasibility study using prototype vaccines that hopefully would protect the vaccinated fish from an atypical *Vibrio ordalii* challenge. Vaccines have been produced and several vaccination studies have been completed. The results show that the vaccine is safe and highly efficacious in protecting the vaccinated fish after a challenge 6 weeks post vaccination. It is envisaged that the vaccine will soon be available for field testing in the Chilean market.

Intervet Vietnam launches Aquaculture e-Newsletter

Intervet Vietnam has recently launched a regular (several times per month) electronic newsletter with aquaculture news items relevant to Vietnam for the benefit of Intervet customers and contacts. The brainchild of Nguyen Vo Hoang, there are both Vietnamese and English versions.

For example, the May 4th, 2004 issue contained the following news items:

- Viet Nam, Laos cooperate in aquaculture
- Taiwan buys all the catfish oil going
- Workshop - Vibrios of Aquatic Animals.
- Jiangsu successfully breeds jellyfish plants
- Cheap prawn imports force NT farmer to stop production
- Soy Complex Higher On Crush Margins
- CP eyes Madagascar as gateway to European shrimp market

To subscribe to the newsletter, please send a mail to vanquoc@hcm.vnn.vn

International workshop on KHV

In February 2004, an international workshop on koi herpesvirus (and related) disease took place in the U.K. The objectives were: to assess the current status of the disease regarding its impact on the ornamental fish sector; discuss the practical management of the disease throughout the ornamental fish supply chain; and discuss the trans-national research priorities for a better comprehension, diagnostic and management of the disease. The major conclusions were: that the industry should develop a self-regulatory process; that standardised diagnostic tests be developed and implemented globally; that it is imperative to clearly establish where and in what species the disease is present; and that research priorities (including a better understanding of the immune system response to KHV, and vaccine development) be established and acted on through collaboration between scientists in all affected countries. The bottom line is that international cooperation will be essential to

combat this increasingly important disease.in the long term.

The full report and many of the presentations can be downloaded from:
http://www.frmltd.com/Workshop_KHV.htm

See also pieces on KHV in **Intervet AAH Newsletters 6 and 7**, and the last two abstracts in the Summaries of Scientific Publications section below.

SUMMARIES OF SCIENTIFIC PUBLICATIONS

Recent Advances in Our Knowledge of the Myxozoa
J. Eukaryot. Microbiol., 48:395–413, 2001
Kent MJ, Andree KB, Bartholomew JL, El-Matbouli M, Desser SS, Devlin RH, Feist SW, Hedrick RP, Hoffmann RW, Khattra J, Hallett SL, Lester RJG, Longshaw M, Palenzeula M, Siddall ME, Xia C (USA, Germany, Canada, UK, Australia, Spain)
In the last few years two factors have helped to significantly advance our understanding of the Myxozoa. First, the phenomenal increase in fin fish aquaculture in the 1990s has lead to the increased importance of these parasites; in turn this has lead to intensified research efforts, which have increased knowledge of the development, diagnosis, and pathogenesis of myxozoans. The hallmark discovery in the 1980s that the life cycle of *Myxobolus cerebralis* requires development of an actinosporean stage in the oligochaete, *Tubifex tubifex,* led to the elucidation of the life cycles of several other myxozoans. Also, the life cycle and taxonomy of the enigmatic PKX myxozoan has been resolved: it is the alternate stage of the unusual myxozoan, *Tetracapsula bryosalmonae,* from bryozoans. The 18S rDNA gene of many species has been sequenced, and here we add 22 new sequences to the data set. Phylogenetic analyses using all these sequences indicate that:1) the Myxozoa are closely related to Cnidaria (also supported by morphological data); 2) marine taxa at the genus level branch separately from genera that usually infect freshwater fishes; 3) taxa cluster more by development and tissue location than by spore morphology; 4) the tetracapsulids branched off early in myxozoan evolution, perhaps reflected by their having bryozoan, rather than annelid hosts; 5) the morphology of actinosporeans offers little information for determining their myxosporean counterparts (assuming that they exist); and 6) the marine actinosporeans from Australia appear to form a clade within the platysporinid myxosporeans. Ribosomal DNA sequences have also enabled development of diagnostic tests for myxozoans. PCR and in situ hybridisation tests based on rDNA sequences have been developed for *Myxobolus cerebralis, Ceratomyxa shasta, Kudoa* spp., and *Tetracapsula bryosalmonae* (PKX). Lectin-based and antibody tests have also been developed for certain myxozoans, such as PKX and *C. shasta.* We also review important diseases caused by myxozoans, which are emerging or re-emerging. Epizootics of whirling disease in wild rainbow trout (*Oncorhynchus mykiss*) have recently been reported throughout the Rocky Mountain states of the USA. With a dramatic increase in aquaculture of fishes using marine netpens, several marine myxozoans have been recognized or elevated in status as pathological agents. *Kudoa thyrsites* infections have caused severe post-harvest myoliquefaction in pen-reared Atlantic salmon (*Salmo salar*), and *Ceratomyxa* spp., *Sphaerospora* spp., and *Myxidium leei* cause disease in pen-reared sea bass (*Dicentrarchus labrax*) and sea bream species (family Sparidae) in Mediterranean countries.

Pathogenesis of liver lesions caused by experimental infection with *Piscirickettsia salmonis* in juvenile Atlantic salmon, Salmo salar L.
J. Vet. Diagn. Invest. 12:552-557, 2000
Almendras FE, Fuentealba IC, Frederick Markham RF, Speare DJ (Canada)
Piscirickettsia salmonis, the etiologic agent of salmonid rickettsial septicemia (SRS), or piscirickettsiosis, causes substantial economic losses to the salmon industry. The pathogenesis of the disease has not been fully characterized. The aim of this study is to describe the hepatic lesions associated with experimental *P. salmonis* infection in Atlantic salmon juveniles. Fish were maintained in fresh water and inoculated intraperitoneally (IP), orally, or on the gill surface with P. salmonis. A group of uninfected fish was kept as control. Liver samples from 5 fish in each inoculated group and 3 controls were collected weekly and processed for histological and immuno-histochemical examination. Thickening of the liver capsule by inflammatory cells was a characteristic histologic feature of IP inoculated

fish. Three weeks post-IP inoculation, 8 fish had died and 2 fish were sampled. Histological changes at this time consisted of vasculitis, presence of fibrin thrombi, vacuolated hepatocytes and focal areas of necrosis. Leukocytes containing intracytoplasmic basophilic microorganisms were seen within hepatic sinusoids. Vasculitis and intra-cytoplasmic vacuoles were prominent features in fish inoculated orally and on the gill surface. The presence of P. salmonis within hepatocellular vacuoles, endothelial cells, and leucocytes was confirmed by immunohistochemistry. The intracellular location of *P. salmonis* and the vascular damage seen in infected fish are characteristic of rickettsial infections. Histological lesions induced by experimental infection with P. salmonis using the oral and gill surface routes were similar to those observed in natural outbreaks of piscirickettsiosis. The tropism of P. salmonis for endothelial cells explains the vascular lesions observed in SRS, whereas hepatic lesions are due to ischemic necrosis and direct injury by intracytoplasmic organisms.

Characterization of a novel envelope protein (VP281) of shrimp white spot syndrome virus by mass spectrometry

Proteins 55:229-235, 2004

Huang C, Zhang X, Lin Q, Xu X, Hew CL (Singapore)

The primary structure of a novel envelope protein from shrimp white spot syndrome virus (WSSV) was characterized using a combination of SDS-PAGE and mass spectrometry. The resulting amino acid sequence matched an open reading frame (ORF), ORF1050, of the WSSV genome ORF database. ORF1050 contained 843 nt, encoding 281 aa, and was termed the vp281 gene. Computer-assisted analysis showed that both the vp281 gene and its product shared no significant homology with other known viruses. However, they shared striking identity/similarity with another WSSV structural protein, VP292, at both the nucleotide and amino acid sequence level, suggesting that vp281 and vp292 might have evolved by gene duplication from a common ancestral gene. WSSV VP281 cDNA was cloned into a pET32a(+) expression vector containing a T7 RNA polymerase promoter to produce (His)(6)-tagged fusion proteins in Escherichia coli strain BL21. Specific mouse antibodies were raised using the purified fusion protein (His)(6)-VP281. Western blot analysis showed that the mouse anti-(His)(6)-VP281 antibodies bound specifically to VP281 of WSSV, without cross-reactivity with VP292. The transmission electron microscope immunogold-labelling method was used to localize VP281 in the WSSV virion as an envelope protein. The cell attachment 'Arg-Gly-Asp' motif in VP281 indicated that this protein might play an important role in mediating WSSV infectivity.

Propagation of yellow grouper nervous necrosis virus (YGNNV) in a new nodavirus-susceptible cell line from yellow grouper, *Epinephelus awoara* (Temminck & Schlegel), brain tissue

J. Fish Diseases 24:299, 2001

Lai Y-S, Murali S, Chiu H-C, Ju H-Y, Lin Y-S, Chen S-C, Guo I-C, Fang K, Chang C-Y (Taiwan)

A nodavirus was isolated from diseased yellow grouper, *Epinephelus awoara*, larvae cultured in southern Taiwan. The histopathology and RT-PCR results confirmed that it was a fish nodavirus; its coat protein gene sequence was similar to that of red spotted grouper nervous necrosis virus (RGNNV) and it is named yellow grouper nervous necrosis virus (YGNNV). A new nodavirus-susceptible cell line, grouper brain (GB) was established and characterized from the brain tissue of yellow grouper. The GB cells multiplied well in Leibovitz's L-15 medium supplemented with 10% foetal bovine serum at temperatures between 24 and 32 °C, and have been subcultured more than 80 times, becoming a continuous cell line. The GB cell line consists of fibroblast-like cells and some epithelioid cells. The cell line yielded titres of YGNNV up to $10^{8.5}$ $TCID_{50}$ mL^{-1}. The GB cells effectively replicated the virus at 28 °C, which could be purified to homogeneity by caesium chloride gradient centrifugation. Electron microscopy studies showed that purified virus particles were 25-30 nm in diameter. The cytoplasm of infected cells was filled with aggregates of virus particles. These results indicate that the GB cell line is a significant tool for the study of fish nodaviruses.

Susceptibility of cultured juveniles of several marine fish to the sevenband grouper nervous necrosis virus

J. Fish Diseases 26:109, 2003

Tanaka S, Kuriyama I, Nakai T, Miyazaki, T (Japan)

Piscine nodaviruses (betanodaviruses) have been tentatively divided into four genotypes (SJNNV, RGNNV, TPNNV and BFNNV) and it is suggested that host specificity is different among these genotypes. In the present study, a

betanodavirus [sevenband grouper nervous necrosis virus (SGNNV)] belonging to the redspotted grouper nervous necrosis virus (RGNNV) genotype, to which most betanodaviruses from warm water fish are identified, was evaluated for its pathogenicity to hatchery-reared juveniles of several marine fish species. When challenged with the virus by a bath method ($10^{5.1}$ $TCID_{50}$ mL^{-1}, sevenband grouper, *Epinephelus septemfasciatus*, Japanese flounder, *Paralichthys olivaceus*, and tiger puffer, *Takifugu rubripes*, displayed behavioural abnormalities and mortalities with distinct histopathological signs of viral nervous necrosis and heavily immunostained cells were observed in the central nervous tissues and retina. Bath-challenged rock fish, *Sebastiscus marmoratus*, and a hybrid of sevenband grouper and kelp grouper, *E. moara*, did not display any behavioural abnormality or mortality during the experimental period, although many fish showed slight signs of viral infection in nerve cells. Kelp grouper and red sea bream, *Pagrus major*, showed no behavioural abnormality, mortality or immunohistopathological changes after the virus challenge. These results are, in part, consistent with the natural host range of RGNNV, indicating the complexity in the host specificity of betanodaviruses.

Mycobacteriosis in Atlantic salmon farmed in British Columbia
Can Vet J. 44:486-489, 2003
Brocklebank J, Raverty S, Robinson J (Canada)
Mycobacterium chelonae was identified as the cause of incidental mortality in Atlantic salmon smolts following introduction to seacages. Source of infection was not confirmed. Polymerase chain reaction was a useful method of detecting and speciating the genus *Mycobacterium* in infected stocks. Clinical management and public health implications of infection are discussed.

Restriction fragment length polymorphism of the pMJ101-like plasmid and ribotyping in the fish pathogen *Vibrio ordalii*
Epidemiol Infect. 117:385-391, 1996
Pedersen K, Koblavi S, Tiainen T, Grimont PA (Denmark)
A total of 32 *Vibrio ordalii* strains were studied for their plasmid content and shown to carry a plasmid of approximately 32 kb. This plasmid was subsequently subjected to restriction fragment length polymorphism (RFLP) studies. Using Hind III, three different restriction patterns were identified while BamH I cleaved the plasmid into a single linear fragment. The results suggest that the 32 kb plasmid is highly conserved but that some variation in restriction pattern occurs. The same set of strains was subjected to ribotyping. Using Mlu I, six different restriction patterns were demonstrated. Strains from the USA and Canada shared profiles with strains from Australia and Japan. Strains from Australia generated a single pattern whereas strains from North America were subdivided into three patterns, and the Japanese strains fell into five patterns. The results suggest that ribotyping in combination with RFLP studies of the pMJ101-like plasmid may be useful in epidemiological studies of *V. ordalii*.

Isolation and characterization of pathogenic Vibrio alginolyticus from diseased cobia *Rachycentron canadum*
J. Basic Microbiol. 44:23-28, 2004-05-05
Liu PC, Lin JY, Hsiao PT, Lee KK (Taiwan)
Outbreaks of serious mortality among cultured juvenile cobia *Rachycentron canadum* L. (weighing 8-10 g) characterized by lethargy, dark skin and ascites in the peritoneal cavity while some fish possessing damaged eyes occurred in July and August of 2001 in Taiwan. Fifteen motile bacterial strains were isolated from head kidney and/or the ascites on tryptic soy agar supplemented with 1% NaCl (TSA1) and/or thiosulphate citrate bile salt (TCBS) sucrose agar plates during the two outbreaks. All the isolates were characterized and identified as *Vibrio alginolyticus* on the basis of biochemical characteristics, and comparisons with those of the reference strain V. alginolyticus ATCC 17749. The strain C3c01 (a representative of the 15 similar field isolates), was virulent to the cobia with an LD50 value of 3.28 x 10(4) colony forming units/g fish body weight. All the moribund/dead fish exhibited lethargy, dark skin and ascites in the peritoneal cavity as that observed in natural outbreaks. The same bacteria could be reisolated from kidney and the ascites of fish after bacterial challenge using TSA1 and TCBS plates. The results reveal that *V. alginolyticus* is an infectious agent of vibriosis in the cobia.

Global distribution of KHV with particular reference to Europe
International Workshop on Koi Herpesvirus, 12-13 February 2004, U.K.
Haenen OLM, Engelsma MY (The Netherlands)
The KHV problems are global, especially now that Japan is also positive for KHV since May

2003. In this paper we present the current global situation, as recorded via e-mails, our network and the literature. Also the impact of KHV on a global scale will be discussed.

EUROPE:

• Belgium: The disease is present since 1999 in koi, with mortalities up to 90%. Sometimes carp showed symptoms.

• Denmark: Since July 2002 KHV positive: in carp <10 cm from a wholesaler pond KHV diagnosed by PCR on gill tissue (also SVCV was isolated). Summer 2003: two importers with Japanese koi (13-15 cm) were KHV positive from the same source. KHV diagnosis was done by PCR on gill tissue.

• England: 36 outbreaks in 2002 in a wide range of carp sizes, all detected by PCR, 9 also by virus isolation at KF-1 cells; outbreaks continued in 2003.

• Finland: no outbreaks so far, virus never isolated from koi.

• France: KHV outbreak in 2003 in carps/koi (?) from Israel (Munich KHV meeting, 2 Dec 2003, organized by R. Hoffmann). Virus isolation and PCR are both available for diagnosis.

• Germany:

Riems: 21 KHV cases in 2002 by PCR, virus isolation, immunofluorescence, E.M., June 2003 67 cases, since June 123 cases (4-6 cases in carp) in all sizes of koi/carp, but mostly in larger fish; mortalities 50-100% (koi) and 80-100% (carps); 2003 KHV in imported 2-3 years old carp from a non-EU European country to carp farms. Mortalities in affected farms 30-100%. Diagnosis by PCR, but no culturable KHV at KF-1 or CCB cells. Asymptomatic fish were KHV negative by PCR.

Munich: regular outbreaks: up to 60 in 2002 in all sizes of koi, partly in common carp; mortalities up to 100%. In so called “survivors” or “immunized“ carp only a few deaths in combination with transport stress; method: PCR and histology.

• Austria: first outbreak summer 2003 in koi in a private pond, tested by PCR (Munich)

• Switzerland: KHV outbreak in 2003 (Munich meeting, Dec 2003).

• Poland: exported common carp were KHV positive (Riems).

• Hungary: no outbreaks so far, and no suspicions.

• Luxemburg: KHV positive (Munich meeting, Dec 2003).

• Italy: 1 serious suspicion in juvenile koi by E.M. and histology, no CPE after freezing at –80°C; Italy is KHV positive (Munich meeting, Dec 2003).

• Spain: no diagnosis yet and no suspicions of KHV.

• Scotland: no outbreaks so far.

• Ireland: no outbreaks so far.

• Sweden: no outbreaks so far, prevention by quarantine with virus isolation at KF-1 cells.

• The Netherlands: 2002: 6 outbreaks, by PCR, histology, of which only 1 in by virus isolation; 2003: 27 positives (koi) out of 68 samples (61 koi and 5 common carp) as detected by KHV PCR. Additionally, histopathology of gills and internal organs was done: in case of clinical outbreaks, some positive PCR results were confirmed. So far it occurred only in koi, and not in carp, mostly in larger fish. Generally the water temperature was between 20 and 27°C. At 30°C mortality was halted. KHV is considered to be endemic in The Netherlands, in import sites, pet shops, garden centres and private ponds.

• Russia: no suspicions, no outbreaks, but also no diagnosis.

ASIA:

• Indonesia since April 2002 (NACA)(confirmed by PCR : Java since May 2002, 30% mortality in carp; Sumatra since Nov 2002, 80% mortality in carp), outbreaks continued.

• Thailand: no KHV yet? It actively does surveillance for KHV.

• Singapore: no KHV yet? It actively does surveillance for KHV.

• Taiwan (January 2003: many outbreaks, >80% mortality in koi, no confirmation yet?)

• Philippines: KHV positive?

• Malaysia: KHV positive?

• Japan (pers. comm. M. Sano): KHV PCR tests are done since 2001. In May-June 2003 the first outbreak occurred in common carp and koi of 1-3 kg in a river in Okayama Prefecture, Western Japan. Oct 2003 acute mortalities occurred in Ibaraki Prefecture, Lake Kasumigaura (660 tons) and Lake Kitaura (200 tons), Eastern Japan. The water temp was 17°C. Phytoplankton bloom may have been a stress factor. In the mean time 22 metric tons were distributed from Lake Kasumigaura to 21 prefectures (Fukuda, pers. comm.). Mid Nov already 4 rivers were infected (Yoshimizu, pers. comm.). By end Nov 2003 mortalities reached 1200 tons in total. Clinical signs included severe gill necrosis and sunken eyes. There was new legislation in Japan since July 2003 (requirement of an infection free certificate). Ibaraki Prefecture has officially prohibited movements of common carp from the affected areas to other areas. All koi shows were cancelled for Nov 2003. By January 2004 23 of

the 47 Prefectures of Japan were infected with KHV (Asahi Shimbun, 2004) and killed thousands of tons of fish. It threatens the USD 75 million ornamental carp industry of Japan further (Australian, 2003).

• China: handpicked koi from China were KHV positive (CEFAS). In Hong Kong, a KHV outbreak in 2001 killed many koi in 2 weeks (pers. comm. G. Chu).

• Other NACA (Network of Aquaculture Centres in Asia-Pacific) countries: Bangladesh, Cambodia, Hong Kong SAR, India, Korea (DPR), Myanmar, Nepal, Pakistan, Sri Lanka, Thailand, Vietnam. Other participating (non-member) governments include Iran, Rep. of Korea, Lao PDR: no outbreaks yet?

MIDDLE EAST:

• Israel: May 1998 KHV was first diagnosed (Tinman & Bejerano, 1999) after imports of koi from Europe. Since than it spread further. Israel uses active immunisation schemes and developed an attenuated vaccine.

AFRICA:

• South Africa: There was an outbreak autumn 2003, in which KHV was proven (Munich meeting, Dec 2003) (details not known).

UNITED STATES:

The disease is present since 1990; KHV was isolated from diseased adult koi, showing irregularly coloured gills (Hedrick et al., 2000).

SOUTH AMERICA:

• Chile: no outbreaks of KHV so far.

• No further data available.

AUSTRALIA: No outbreaks so far.

ELSEWHERE: Furthermore no data on KHV in other countries were available to the authors.

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Outbreak of disease causing mass mortality in koi and common carp (*Cyprinus carpio*) in Indonesia

International Workshop on Koi Herpesvirus, 12-13 February 2004, U.K.

Sunarto A, Rukyani A (Indonesia)

Since March 2002, Indonesian carp culture has been faced with a serious epizootic of mass mortality caused severe economic loss and significant social impact. As at December 2003, estimated loss due to the disease was in the region of U$15 million. The disease outbreaks occurred in on-growing koi and common carp (*Cyprinus carpio*) of all ages and size, regardless stocking density and culture systems. The disease caused a total of mortality of up to 80-95%. The first outbreak occurred in koi carp that just been transported from Surabaya to Blitar, both in East Java. The fish was imported from Hong Kong through Surabaya Airport. Prior to the outbreak, there was a heavy rain. This phenomenon leads to the hypothesis that the causative agent was latent on the imported fish and became active under these particular circumstances, such as stress of transportation and handling and environmental changes, particularly water temperature fluctuations. Based on the clinical history, gross signs and histopathological changes, experimental infection and polymerase chain reaction (PCR) detection of naturally and experimentally diseased fish, it is strongly suspected that Koi Herpesvirus (KHV) is involved in the serious outbreaks among koi and common carp in Indonesia. As the outbreak continued, various clinical signs were observed or reported. These are lethargic, showed lost of balance, gasped for air, sloughing off the epithelium with loss of mucus, haemorrhages of operculum, fin, tail, and abdomen and blister-like lesion on the skin. However, the only consistent clinical sign of the outbreak was severe gill necrosis. Therefore, the diagnosis of the disease was based on clinical sign (diagnosis level 1) and PCR detection (diagnosis level 3). To prevent the spread of the outbreaks to other islands, the government of Indonesia has declared that Java and Bali Islands as isolated area of the disease and moving koi and carp from Java Island to other islands are strictly prohibited or should follow quarantine check for KHV. In addition, importing koi and common carp is permitted only from free KHV countries. The geographical distribution of the disease, the implementation of government regulations pertaining the

outbreak and efforts to control the disease in farm level are also discussed in this paper.

Tragedy claims life of Intervet specialist

Tribute has been paid to one of Intervet's aquatic animal health (AAH) specialists who died tragically along with his wife when an avalanche struck their mountain cabin retreat in the USA.

Robert (Bob) Busch (58), Chief Technical Officer of Intervet AAH who was based in Seattle, was vacationing with his wife at their much-loved cabin near the Soldier Mountain ski resort in Idaho when the avalanche hit during the early hours of January 2, 2004. He and his wife, Marsha Landolt - who was dean of the University of Washington's graduate school and also a renowned fish pathologist - were asleep at the time along with five other family members, who all survived the tragedy because they were sleeping in upstairs bedrooms.

The way we will remember him. Bob (right) and his friend and Intervet AAH colleague, Kurt Schuster, after a successful Chinook salmon fishing trip off the Western shore of Vancouver Island, BC, Canada in 2002. Kurt caught the biggest fish but Bob had him outnumbered!

"All those within Intervet who knew Bob were shocked and deeply saddened by the tragic news about him and his wife," said Alistair Brown, Director of Intervet's AAH Division. "He has been a valued colleague and friend to us in aquatic animal health over the last two-and-a-half years, and we will miss him dearly, both professionally and personally. He was a consummate professional. Once described as 'one of the grand old men of fish vaccines,' Bob leaves behind him a great legacy of significant contributions to aquaculture and aquatic animal health. These legacies, coupled with his enthusiastic and gregarious nature, mean that this larger-than-life character will live long and fondly in our memories. We wish all his family and friends our sincere condolences."

Intervet Aquatic Animal Health Newsletter

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