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Intervet Aquatic Animal Health

Newsletter

Nov 2004

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INTERVET VIETNAM KEEPING PACE WITH VIETNAM'S AQUACULTURE BOOM

Intervet has been active in Vietnam since 1995, initially marketing poultry vaccines. In January 2004, a fully fledged local company was incorporated, Intervet Vietnam Ltd., which is headquartered in Ho Chi Minh City and managed locally by Tin NH. Intervet Vietnam is partnered by its primary distribution company, Van Quoc, whose Director is Nguyen VH.

The growth in Vietnam's aquaculture industry has been dramatic, averaging 10% per annum in recent years. This growth has not been accidental and is due to the far-sighted financial and legislative support provided by the Vietnamese government. In response to this, the export aquaculture industry, dominated by shrimp, tra catfish and basa catfish, has provided Vietnam with a huge source of foreign-earned revenue. A major knock-on effect of this surge in organized aquaculture is the growth in the professional aquafeed industry.



Tin NH (Manager, Intervet Vietnam on right) and Nguyen VH (Director, Van Quoc on left) at the office in Ho Chi Minh City.

During the last few years, Intervet AAH in Singapore and The Netherlands, and Intervet Vietnam have been working closely with research institutes and universities, farmers and feed companies to investigate disease pathogens and their epidemiology with a view to providing efficacious and quality products to help Vietnam's industry. The fact that Mr. Nguyen of Intervet Vietnam's sister company, Van Quoc, also acts as the technical support manager for Intervet's feed additives and aquaculture products, and that he has previously worked for 10 years in a public health laboratory as a bacteriologist, is a real advantage when it comes to bacteriological sampling, etc.

Intervet Vietnam's AAH activities include assistance in disease epidemiology studies and trial work related to product testing and registration. Intervet is working closely with the

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Right to left: Dr. Zilong Tan, Nguyen Vo Huang and Dr. Le Thanh Hung (UAF-HCMC)

Vietnamese authorities in carrying out the required field trial work in shrimp, tra catfish, etc. To date, Chlorasol, a disinfectant product, has been registered. With support from Intervet AAH colleagues in Singapore, namely Dr. Zilong Tan, Cedric Komar DVM and Dr. Luc Grisez, technical support and training on aquatic animal diseases and health management has been provided to the industry (farmers, university, feed mills, etc). In addition, Intervet has collaborated with various Vietnamese scientists to collect bacterial and viral strains, like for example, white spot syndrome virus (WSSV).



Mr. Nguyen (on left) recording body weight data in a tra catfish trial at Sadec in 2003

Recently, Mr. Nguyen has developed an electronic aquaculture-related newsletter (with news items of interest, primarily related to Vietnam) in English and Vietnamese (called "Ban Tin"), which is mailed on a frequent basis and is highly appreciated by customers, universities and institutes in Vietnam and overseas (**see Intervet AAH Newsletter no. 8**).

INTERVET STAFF ATTEND TILAPIA CONFERENCE IN THE PHILIPPINES

The 6th International Symposium on Tilapia Aquaculture took place this September in Manila and Dr. Zilong Tan and Cedric Komar DVM of Intervet Singapore attended. Delegates from over 30 different countries were at the conference. Tilapia is now the second most popular non-carp farmed fish family in the world after salmonids, with global production over 1.5 million metric tons in 2003. Today, the vast majority of world supply is still consumed in the producing countries of Asia and Latin America. However, exports to the USA and Europe have great potential and are growing fast. In this industry, where fingerling production is well understood and managed throughout the world, efforts are now focused on genetic improvement and nutrition in order to improve growth and quality of the fish. Health management and disease prevention are still new concepts in this species. But, with the intensification of the tilapia industry, they will soon become increasingly important as the means to improve survival and productivity.



Some attendees at the symposium. From right to left: Freek Huskens and Wayan Mudana (PT Aquafarm Nusantara, Indonesia); Martin Sukkel (Regal Springs Tilapia, Switzerland); Warren Turner (Nam Sai Farm, Thailand); Cedric Komar (Intervet Norbio Singapore); Neil Duncan (Institute of Agro-Food Research and Technology, Spain); Morris Villaroel (Universidad Politénica de Madrid, Spain).

INTERVET MARKET SURVEY IN INDIAN SUBCONTINENT

To investigate the fast-growing aquaculture industry in the Indian subcontinent, Dr. Zilong Tan and Dr. Sikka (see photo below) recently toured several areas in Sri Lanka, India and Bangladesh. The visits were informative and worthwhile. A number of technical seminars were given to local farmers and their support personnel. Valuable information was collected through interviews with farmers, feed manufacturers, research scientists, government officials, etc. The information will help Intervet local companies to formulate business strategy and provide better support to the industry.



Dr. Sikka (left; Chief Representative of Intervet International in Sri Lanka) and Dr. Zilong Tan (right; Intervet Norbio Singapore Regional Technical Manager) in front of the newly opened Intervet office in Colombo.



A black tiger shrimp farm in Nellore, India. From left to right: Mr. Patnaik (Intervet India AAH Executive), Dr. Zilong Tan, Mr. S. Reddy (farm owner) and Mr. Ashok Hegde (Intervet India Sales Manager).



Niribili Shrimp Hatchery at Cox's Bazar, Bangladesh. From left to right: Dr. Zilong Tan, Mr. Mohib Ullah (Hatchery Manager), Mr. A.K.M. Alamgir (Managing Director, Bengal Overseas Ltd., Intervet distributor), Dr. Sikka, Dr. S. Hossain (Senior Scientific Officer of Bangladesh Fisheries Research Institute) and Dr. Mostafa Alam (Marketing Executive, Bengal Overseas Ltd.).

NACA-SPONSORED DISEASE CARD ON INFECTION WITH KHV RELEASED

Further to our articles on KHV that appeared in Intervet AAH Newsletters no. 6, 7 and 8 (http://www.intervet.com/aah/newsletters.asp), a new publication "Infection with koi herpesvirus – disease card", written by M. Crane, M. Sano and C. Komar on behalf of NACA, was recently made available. It was developed to support the NACA/FAO/OIE regional quaterly aquatic animal disease (QAAD) reporting system in the Asis-Pacific region. It is a very informative aid for both scientists and producers and can be downloaded from the following link: http://www.enaca.org/modules/mydownloads/singlefile.php?cid=23&lid=557

INTERVET CHILE LAUNCH TWO NEW VACCINES

Intervet first entered the Chilean aquatic animal health market in 2000 with the monovalent vaccine against IPN, Compact™ IPN, protecting against infectious pancreatic necrosis virus. Due to the excellent results obtained in a large number of Chilean salmon farming companies, sales of this vaccine increased 10 fold in four years.

Today in Chile, almost all Atlantic salmon, along with a growing percentage of trout, are vaccinated against several pathogens of longstanding importance, like IPN. However, during the last few years, the Chilean aquaculture industry has experienced the appearance of new pathogens like atypical *Aeromonas salmonicida* (causing furunculosis) and *Vibrio ordalii*. This latter bacterial pathogen was first isolated and identified in 2003, by Intervet scientists in Norway and Chile, in active collaboration with ADL Diagnostic Chile Ltda. (one of the main aquatic animal health diagnostic laboratories). It is now clear that this



Professional injection vaccination team at work in Chile.

is the causative agent of vibriosis in Atlantic salmon in Chile (**see Intervet AAH Newsletter no. 8**). Both these new diseases are characterized by their aggressiveness and their ability to inflict serious economic losses for producers, which are manifested by high mortality levels coupled to a drop in quality of the final product.

In recent months, two new Intervet vaccines have been launched in the Chilean aquatic animal health market, namely, Compact™ FurIPN (a bivalent vaccine against furunculosis and IPN) and Compact™ VibIPN (a bivalent vaccine against vibriosis and IPN). These vaccines provide excellent protection against IPN and the two new pathogens. With the introduction of these new products, Intervet continues to be a major contributor to the increasing productive efficiency of the Chilean aquaculture industry.

SALMONID RICKETTSIAL SEPTICAEMIA (SRS)

SRS (a.k.a. Salmon Rickettsial Syndrome or Piscirickettsiosis or Coho salmon septicaemia or Huito disease) is considered to be the most important disease problem in the Chilean salmon farming industry, with economic losses of over US$100 million in some years. SRS was first reported, from Chile, in 1989, but (Pisci)rickettsia-like organisms (RLO) are now frequently associated with disease syndromes in both salmonid and non-salmonid fish from both fresh and saltwater worldwide. During 1989, this disease was considered to be the cause of death of an estimated 1.5 million Coho salmon, many near market-size. A year later, the disease was also found to occur in Atlantic salmon and up to 90% mortality was seen on some farms. Outbreaks of SRS in other countries have not reached the levels of the Chilean outbreaks. For example, variable and inconsistent mortality of 0.6 - 15% has been reported in Canada and Norway.

Causative agent

SRS is caused by the Gram-negative bacterium, *Piscirickettsia salmonis*. This was the first "rickettsia-like" bacterium to be recognized as a pathogen of fish. *P. salmonis* is a non-motile, obligate intracellular bacterium, pleomorphic but predominately coccoid, and 0.5-1.5 µm in diameter. It is currently placed in the class Gammaproteobacteria; order Thiotrichales; and family Piscirickettsiacaea, and has a closer relationship to, e.g., *Legionella* and *Coxiella*, than to members of the genera *Rickettsia*. *P. salmonis* replicates within membrane-bound cytoplasmic vacuoles in selected fish cell lines and in the cells of tissues throughout infected fish.

P. salmonis is the first of the RLO of fish to be fully characterized. Since it's recognition, the impact of RLO in fish has become increasingly

apparent. Growing awareness of the emergence of these intracellular organisms has led to the discovery of rickettsial diseases among diverse species of fish from different geographic locations and aquatic environments. The source, reservoir, and mode of transmission of many of these agents, as well as consistently effective methods of disease prevention and control, remain to be established.

Host range, geographic distribution
SRS disease in Chile typically occurs in marine waters during the on-growing process from smolt to harvest. It has also been isolated from freshwater cages of Coho salmon and trout. The disease was originally predominant in Coho salmon (*Oncorhynchus kisutch*) but is now recognized to cause serious losses in all farmed salmonid fish species including Atlantic salmon (*Salmo salar),* rainbow (steelhead) trout *(O. mykiss),* Chinook salmon *(O. tshawytscha),* pink salmon *(O. gorbuscha)* and masu salmon *(O. masou*). *Piscirickettsia sp*. are commonly found in fish worldwide (e.g., Chile, Canada, Ireland, Scotland and Norway) but are of major economic importance only in Chile to date. The distribution of *P. salmonis* and RLO is therefore wide spread. Several reports describing RLO infections in non-salmonid finfish exist. For example, about 10 years ago, a RLO was identified as the causative agent of an outbreak with mass mortality among pond-reared tilapia in Taiwan. Also, RLO-related mortalities in juvenile European sea bass at 12 – 15 °C in sea cages have been reported along the French Mediterranean coast.

Transmission and epidemiology
At present there are few reports of *P. salmonis* coming from wild salmonids, although it is likely that the bacterium is present in naturally occurring populations of marine fish. Horizontal transmission has been reported in marine-farmed salmon 2 weeks after the introduction of pathogen-free fish into infected sites. The extended extracellular survival time of this organism in salt water (several weeks at 5-20 °C) may be of sufficient duration to permit horizontal transmission without a vector. Experimentally it is documented that the bacterium can enter through the intact skin and gills although the mode of entry is still not clear.
The possibility of vertical transmission of *P. salmonis* now looks more and more likely due to recent research in Chile. Apparently there is an adhesion complex that allows the pathogen to enter the salmon egg. There is even a suggestion that this complex may be involved in fish to fish transmission.
Currently, no alternative host has been identified and the source, reservoir and means of transmission of *P. salmonis* remain important areas of research.



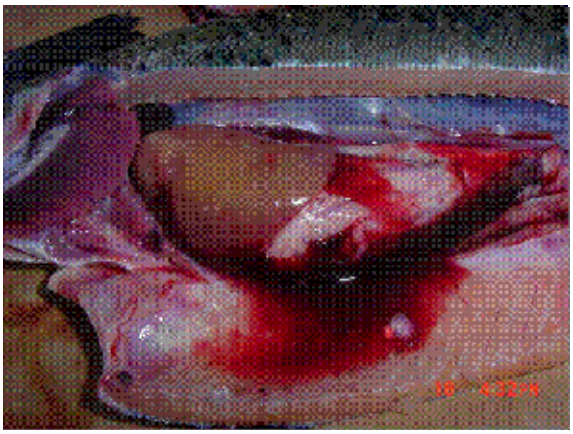
A beautiful and calm day at a fish farm on Tepuhueico Lake, Chile

The course of the clinical disease is typically chronic to subacute in nature with mortalities typically developing 10 - 12 weeks after the transfer of fish to seawater and lasting approximately 10 weeks before they diminish. Virtually all stocks become infected and usually experience more then one clinical episode, typically in the spring and autumn seasons.

Clinical signs and gross pathology
SRS can cause high economic losses to the farmers affected, particularly in Chile, as typical cumulative mortality averages 20% during the 18-month saltwater production time to harvest. Affected fish are lethargic, dark in colour, anorexic, anaemic with mottled focal lesions within the liver, show respiratory problems, and swim near the surface. The first signs observed are often haemorrhages and lesions of the skin. The lesions range from small areas to shallow ulcers up to 2 cm in diameter. Internally, the kidney is swollen and the spleen enlarged. Petechial haemorrhages are found on the swim bladder and viscera. Diagnostic ring-shaped, cream-coloured lesions are present on the livers of chronically infected fish. However, in acute cases, death may be the only gross sign of disease.

Histopathology
Histological changes have been classified into the broad category of necrosis and

inflammation. Inflammatory cells, fibrosis, a generalized coagulative necrosis, tubular degeneration and necrosis of the endothelium infiltrate the liver, spleen, intestine and haematopoietic cells of the kidney. Moribund fish are anaemic and haematocrit is often 20% to 50% of normal. The rickettsial organism infects a variety of cells, including circulating macrophages, in which they can replicate and cause cell lyses. It also enters brain tissue, thus affecting swimming ability. The mechanisms by which *P. salmonis* can enter target cells, avoid intracellular killing and survive inside the host are unclear.



SRS pathology in salmon. Note the severe inflammation and multifocal necrosis in liver and spleen. Haemorrhagic ascites are also observed.

Diagnosis

An initial diagnosis of piscirickettsiosis can be made from gross lesions and is supported by the examination of tissue sections. Confirmation of the diagnosis requires isolation and/or serological identification of the causative organism. Kidney tissue from affected fish is aseptically removed, homogenized and inoculated on a cell monolayer with an antibiotic-free growth media. *P. salmonis* has been cultured in many (mostly salmonid) fish cell lines maintained in buffered Eagle's minimum essential medium (MEM) supplemented with 10% foetal bovine serum. Optimal in vitro growth occurs at 15 – 18 °C but is retarded above 20 °C and below 10 °C. [Due in part to this thermal range, there is no indication that *P. salmonis* or other RLO of fish cause disease in humans or other mammals.] Typically, *P. salmonis* isolation and growth is determined by the gradual appearance of a typical cytopathic effect (CPE) in cell monolayers. The first signs of a CPE consist of the formation of cell clusters about 10 days post-inoculation. The infected cells in the clusters typically round up and develop one or more large vacuoles within the cytoplasm. Inoculated cell cultures should be observed for up to 28 days before they are considered negative.

An indirect fluorescent antibody technique (IFAT) and immunohistochemistry have been developed as alternative procedures to detect *P. salmonis*. These latter techniques are faster and more specific than histochemical staining. However, they require additional specialized equipment and are more expensive. The detection of *P salmonis* in cultivated salmonids via a nested PCR using universal primer is coming on stream and will be important for diagnosis of this disease.

Management and Prevention

Chemotherapy

In vitro, *P. salmonis* is sensitive to a variety of antibiotics including streptomycin, gentamicin, erythromycin, chloramphenicol and oxytetracycline, but shows resistance to penicillin, penicillin G and spectinomycin. However, the use of medicated feed to control intracellular pathogens, including *P. salmonis,* has been largely unsuccessful, possibly because antibiotic levels may not reach sufficient concentrations within the host cells *in vivo*. However, injection of broodstock with antibiotics before leaving seawater in order to control the typical "summer SRS outbreak" is common.

Vaccine development

Although commercial vaccines against *P. salmonis* are very recently available, there is little published information or field experience on their efficacy or economic value. However, several institutes and pharmaceutical companies, including Intervet, have active research programmes directed towards developing efficacious vaccines.

Management

Outbreaks frequently occur after smolt transfer to seawater, but good management practices do help. Such approaches include the early removal of mortalities and clinically diseased fish, with appropriate sanitary disposal of blood from harvested fish, reducing fish stocking density and providing periods of site fallowing. Other strategic measures include routine screening of broodstock, rejection of eggs from positive fish and individual incubation of egg

batches. Further information regarding horizontal and vertical transmission, pathogenesis, intracellular survival and immunogenesis is needed to support future control strategies. In addition, information on the geographic location and species distribution of *P. salmonis* among isolates and stocks of fish will be helpful in developing management and control strategies in the future.

Key References

Bruno DW, Woo PTK. Sporadic, emerging diseases and disorders. In: Diseases and disorders of finfish in cage culture. Woo PTK, Bruno DW, Lim LHS (eds.) CAB International. pp. 305-343, 2002.

Fryer JL, Mauel MJ. The Rickettsia: an emerging group of pathogens in fish. In: Emerging Infectious Diseases, Vol. 3, No. 2 April--June 1997 pp. 137-144.

Kent ML, Poppe TT. Infectious diseases of coldwater fish in marine and brackish water. In: Diseases and disorders of finfish in cage culture. Woo PTK, Bruno DW, Lim LHS (eds.) CAB International. pp. 61-105, 2002.

PROLIFERATIVE KIDNEY DISEASE

Charles McGurk, Institute of Aquaculture, University of Stirling, Scotland

Proliferative kidney disease (PKD) has been recognised as a parasitic disease of great economic significance to salmonid aquaculture. Although primarily regarded as a condition affecting first season rainbow trout (*Oncorhynchus mykiss*), all salmonids can become infected during freshwater stages with varying severity. Although the name PKD was first coined by Roberts & Shepherd in 1974, reports of a similar syndrome affecting trout date to at least 50 years previously. The disease is endemic in large areas of Western Europe and North America, but has not been recognised in the Southern hemisphere to date.

Aetiology

The identity of the enigmatic causative agent of PKD – originally denoted as PKX - remained elusive until the late 1990s when it was established to be a myxozoan whose spores possessed four distinctive polar capsules (Figure 1) and which also parasitized freshwater Bryozoa, invertebrates colloquially known as 'moss animals' (Figure 2). The parasite was consequently named *Tetracapsuloides bryosalmonae* in reference to its two known hosts. Various freshwater phylactolaemate bryozoans including *Fredericella sultana* have since been found to become infected with *T. bryosalmonae* resulting in the development of spherical sacs (Figure 3) which release spores. These are released into the surrounding water where they can infect salmonid fish. Massive numbers of spores can be produced from relatively small volumes of bryozoans, with recent research suggesting that very low numbers are capable of infecting fish to result in disease.



Figure 1: Spores of *T. bryosalmonae* dissected from *F. sultana*



Figure 2: Part of a bryozoan colony

Epidemiology

PKD has traditionally been perceived as a seasonal disease, outbreaks being largely limited to the warmer months of the year when the water temperature exceeds 12 ºC. As spores of *T. bryosalmonae* have been detected in infected waterways all year round, the temporal limits of disease manifestation have been attributed to temperature effects on parasite development in fish tissue. Although affected by water temperatures, the incubation

period in the fish is approximately 7 weeks, with a self-limiting clinical course of usually 2-3 months. On infected farms, morbidity levels can approach 100% while mortality levels varying widely depending on the impact of secondary factors such as concurrent diseases, environmental conditions and management practices. In addition to affecting first season rainbow trout, older year classes which are naïve to *T. bryosalmonae* exposure can also become infected. Those fish which survive clinical disease prove resistant to subsequent challenge, signifying the development of protective acquired immunity.

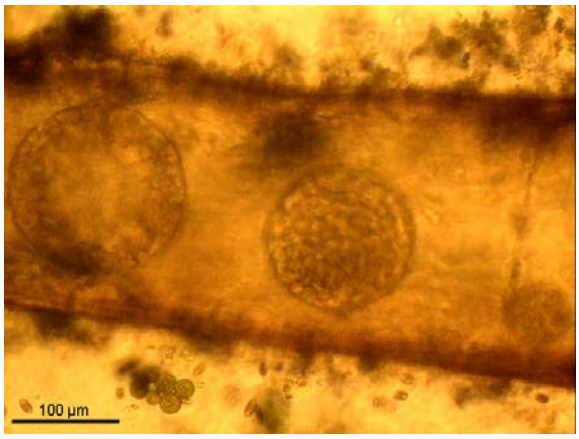


Figure 3: Spherical spore sacs of *T. bryosalmonae* within *F. sultana*

Clinical Signs of PKD

External signs are non-specific and may include:
Body darkening
Gross abdominal swelling
Exophthalmos
Gill pallor
Loss of equilibrium and respiratory distress

Internal signs:
Granulomatous renal swelling, particularly of the posterior (up to ×10 of the normal size)
Spenomegaly
Generalised pallor

Microscopic findings:
Renal haematopoietic hyperplasia followed by granulomatous interstitial nephritis
Presence in kidney of extrasporogonic and sporogonic parasite stages

Diagnosis

Although not pathognomonic, characteristic clinical and gross pathological signs could support a putative diagnosis of PKD in

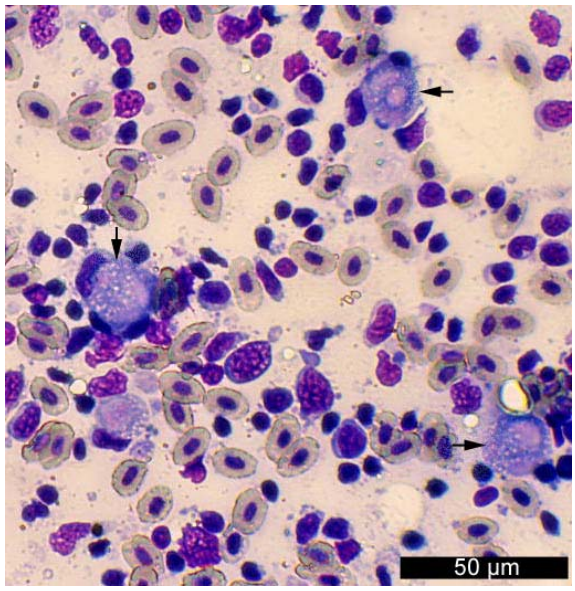


Figure 4: Kidney smear showing extrasporogonic stages of *T. bryosalmonae* (arrows) with adhered phagocytes

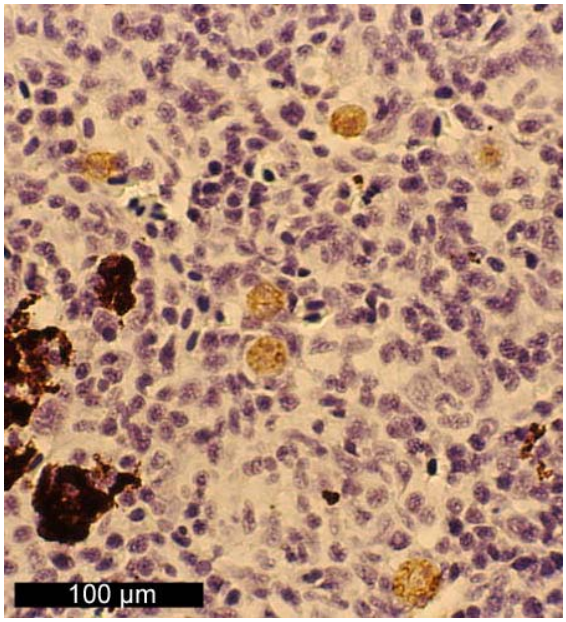


Figure 5: Immunohistochemistry of a kidney section showing extrasporogonic stages of *T. bryosalmonae* which are stained brown

conjunction with clinical history – especially the season of the year and records of previous farm outbreaks. However, definitive diagnosis relies upon the detection of *T. bryosalmonae* in samples of fish tissue. Extrasporogonic stages – often encircled by phagocytes - can be readily identified by methylene blue, May-Grünwald-Giemsa or Leishman-Giemsa staining of kidney smears (Figure 4), or in fixed paraffin-

embedded sections stained with haematoxylin and eosin or by immunohistochemistry (Figure 5) using *T. bryosalmonae* specific monoclonal antibodies such as P01 (Aquatic Diagnostics Ltd., Stirling, UK). Molecular techniques such as *in situ* hybridisation and polymerase chain reaction assays have also facilitated reliable diagnosis.

Prevention and Control

Currently, there are no licensed vaccines or treatments for PKD. In the past, malachite green, the antibiotic fumagillin DCH and its synthetic analogue TNP-470 have been used therapeutically with some efficacy, but concerns over toxicity to fish, residue levels and environmental issues have prevented wide adoption of these treatments. Husbandry measures including lowering summer water temperature (using bore-hole water), delaying transfer of naïve stocks to endemic waters until later in the year (to allow acquired immunity but not clinical disease to develop), eliminating secondary pathogens and reducing feeding rates have been implemented in attempting to limit economic losses. Much interest has focused on controlling bryozoan proliferation of inlet waters to farms, but the high level of infectivity of the innumerable spores released from relatively small colonies of bryozoans would mean that such control measures would have to be meticulously successful to confer protection to stock. Further research continues into vaccine and chemotherapeutant development with several possible candidates envisaged.

SUMMARIES OF SCIENTIFIC PUBLICATIONS

Relative virulence of three isolates of *Piscirickettsia salmonis* for coho salmon Oncorhynchus kisutch

Dis Aquat Organ. 35:107-113, 1999

House ML, Bartholomew JL, Winton JR, Fryer JL (USA)

Piscirickettsia salmonis was first recognized as the cause of mortality among pen-reared coho salmon Oncorhynchus kisutch in Chile. Since the initial isolation of this intracellular Gram-negative bacterium in 1989, similar organisms have been described from several areas of the world, but the associated outbreaks were not reported to be as serious as those that occurred in Chile. To determine if this was due to differences in virulence among isolates of P. salmonis, we conducted an experiment comparing isolates from Chile, British Columbia, Canada, and Norway (LF-89, ATL-4-91 and NOR-92, respectively). For each of the isolates, 3 replicates of 30 coho salmon were injected intraperitoneally with each of 3 concentrations of the bacterium. Negative control fish were injected with MEM-10. Mortalities were collected daily for 41 d post-injection. Piscirickettsiosis was observed in fish injected with each of the 3 isolates, and for each isolate, cumulative mortality was directly related to the concentration of bacterial cells administered. The LF-89 isolate was the most virulent, with losses reaching 97% in the 3 replicates injected with 10(5.0) TCID50, 91% in the replicates injected with 10(4.0) TCID50, and 57% in the fish injected with 10(3.0) TCID50. The ATL-4-91 isolate caused losses of 92% in the 3 replicates injected with 10(5.0) TCID50, 76% in the fish injected with 10(4.0) TCID50, and 32% in those injected with 10(3.0) TCID50. The NOR-92 isolate was the least virulent, causing 41% mortality in the replicates injected with 10(4.6) TCID50. At 41 d post-injection, 6% of the fish injected with 10(3.6) TCID50 NOR-92 had died. Mortality was only 2% in the fish injected with 10(2.6) TCID50 NOR-92, which was the same as the negative control group. Because the group injected with the highest concentration (10(4.6) TCID50) of NOR-92 was still experiencing mortality at 41 d, it was held for an additional 46 d. At 87 d post-injection, the cumulative mortality in this group had reached 70%. These differences in virulence among the isolates were statistically significant ($p < 0.0001$), and are important for the management of affected stocks of fish.

Rickettsial infection in farmed Atlantic salmon in Eastern Canada

Can Vet J. 43:435-440, 2002

Cusack RR, Groman DB, Jones SR (Canada)

The cause of death in a postsmolt, Atlantic salmon population with elevated levels of mortalities was investigated. Diagnosis of a rickettsia-like organism was based on gross pathology, histopathology, differential staining, electron microscopy and fluorescent antibody tests. The course of the infection and response to treatment are discussed. This is the first reported occurrence of salmon rickettsias in the Atlantic coast of North or South America.

Isolates of *Piscirickettsia salmonis* from Scotland and Ireland show evidence of clonal diversity
Appl Environ Microbiol. 70:4393-4397, 2004
Reid HI, Griffen AA, Birkbeck TH (UK)
Salmonid rickettsial septicemia, caused by *Piscirickettsia salmonis*, causes major mortalities in Chilean salmonid aquaculture and is an increasing problem in Atlantic salmon in Ireland and Scotland. Analysis of 16S-to-23S internal transcribed sequences and 16S ribosomal DNA (rDNA) shows that Irish isolates of P. salmonis form two new groups of the organism while Scottish isolates cluster together with Norwegian and Canadian isolates from Atlantic salmon.

Immunoresponse of Coho salmon immunized with a gene expression library from *Piscirickettsia salmonis*
Biol Res. 36:313-323, 2003
Miquel A, Muller I, Ferrer P, Valenzuela PD, Burzio LO (Chile)
We have used the expression library immunization technology to study the protection of Coho salmon *Oncorhynchus kisutch* to the infection with *Piscirickettsia salmonis*. Purified DNA from this bacterium was sonicated and the fragments were cloned in the expression vector pCMV-Bios. Two libraries were obtained containing 22,000 and 28,000 colonies and corresponding to approximately 8 and 10 times the genome of the pathogen, respectively. On average, the size of the inserts ranged between 300 and 1,000 bp. The plasmid DNA isolated from one of these libraries was purified and 20 micrograms were injected intramuscularly into 60 fish followed by a second dose of 10 micrograms applied 40 days later. As control, fish were injected with the same amount of DNA of the vector pCMV-Bios without insert. The titer of IgM anti-P. salmonis of vaccinated fish, evaluated 60 days post-injection, was significantly higher than that of the control group injected with the vector alone. Moreover, this response was specific against P. salmonis antigens, since no cross reaction was detected with *Renibacterium salmoninarum* and *Yersinia ruckeri*. The vaccinated and control fish were challenged 60 days after the second dose of DNA with 2.5 x 10(7) P. salmonis corresponding to 7.5 times the LD50. At 30 days post-challenge, 100% mortality was obtained with the control fish while 20% of the vaccinated animals survived. All surviving fish exhibited a lower bacterial load in the kidney than control fish. The expression library was also tested in Balb/c mice and it was found that the humoral immune response was specific to P. salmonis and it was dependent on the amount of DNA injected.

Experimental vertical transmission of *Piscirickettsia salmonis* and in vitro study of attachment and mode of entrance into the fish ovum
Dis Aquat Organ. 56:25-30, 2003
Larenas JJ, Bartholomew J, Troncoso O, Fernandez S, Ledezma H, Sandoval N, Vera P, Contreras J, Smith P (Chile)
Piscirickettsia salmonis is a pathogenic bacterial agent causing septicaemic disease in salmon. Since its isolation in Chile in 1989, P. salmonis has continually produced high mortality rates in salmon farms. Little information exists regarding the mechanisms of vertical transmission of this pathogen. Experimental vertical transmission was established in the present study by inoculation of male and female rainbow trout broodstock with P. salmonis. The bacterium was subsequently detected using indirect immunofluorescence in milt and coelomic fluid of the majority of inoculated broodstock (14/15). Bacteria were detected in the fry when 1 or both parents were inoculated, although none of the infected fry presented signs of the disease. P. salmonis was also detected in progeny obtained through fertilisation ova from non-inoculated females incubated in a medium containing a bacterial suspension, demonstrating transmission during the process of fertilisation. Ova infected in vitro were examined at sample periods from 30 s to 60 min using scanning electron microscopy. This demonstrated that the bacterium attaches to the ova by means of membrane extensions, structures which we have called 'piscirickettsial attachment complex' (PAC) and which would allow later penetration into the ovum.

***Piscirickettsia salmonis*: a Gram-negative intracellular bacterial pathogen of fish**
J Fish Dis. 26:251-262, 2003
Fryer JL, Hedrick RP (USA)
Piscirickettsia salmonis is the first Gram-negative, intracellular bacterial pathogen isolated from fish and is a significant cause of mortality in salmonid fish. Recent reports of P. salmonis or P. salmonis-like organisms from new fish hosts and geographic regions have increased the interest in the bacterium. In this review, the important characteristics of the bacterium including recent taxonomic changes,

features of the disease caused by the bacterium including transmission, hosts, reservoirs, diagnostic procedures, and current approaches for prevention and treatment have been discussed. The reader is also directed to other reviews concerning the bacterium and the disease it causes (Fryer & Lannan 1994, 1996; Almendras & Fuentealba 1997; Lannan, Bartholomew & Fryer 1999; House & Fryer 2002; Mauel & Miller 2002).

Piscirickettsiosis and piscirickettsiosis-like infections in fish: a review
Vet Microbiol. 87:279-289, 2002
Mauel MJ, Miller DL (USA)
Piscirickettsia salmonis was the first "rickettsia-like" bacteria to be recognized as a pathogenic agent of fish. Since the first reports of piscirickettsiosis emerged from Chile in the late 1980s, Piscirickettsia-like bacteria have been recognized with increasing frequency in a variety of fish species, from both fresh and saltwaters around the world. Although the first reported incidents of Piscirickettsia were in salmonids, Piscirickettsia-like bacteria are now being frequently associated with disease syndromes in non-salmonid fish. Mortalities have occurred in white seabass (Atactoscion noblis), black seabass (Dicentrarchus sp.), tilapia (Oreochromis, Tilapia and Sarotherodon spp.) and blue-eyed plecostomus (Panaque suttoni). Piscirickettsiosis and piscirickettsiosis-like diseases have affected aquaculture productivity, profitability, the species of fish compatible with commercial rearing, and transportation of fish from site to site. Piscirickettsiosis and syndromes caused by similar bacteria are an emerging disease complex that will increasingly inhibit fish production.

The expression of immune-regulatory genes in rainbow trout, *Oncorhynchus mykiss*, during a natural outbreak of proliferative kidney disease (PKD)
Parasitology Suppl. S95-102, 2003
Holland JW, Gould CR, Jones CS, Noble LR, Secombes CJ (UK)
Proliferative kidney disease (PKD) is a parasitic infection of salmonid fish characterized by an apparently abnormal immune response to the presence of the myxozoan parasite, *Tetracapsuloides bryosalmonae*. In order to examine the nature of the immune response at the molecular level, the expression of a range of immune regulatory genes, including cytokines and cyclooxygenase (COX)-2 was examined in naive unexposed fish and in naive fish exposed to parasite-infected water at three points during the course of a natural outbreak of PKD. Since fish with advanced PKD pathology generally exhibit increased susceptibility to secondary infections which is typical of stress/cortisol-mediated immune suppression, a further aim of this work was to examine in vitro the influence of the glucocorticoid cortisol on the bacterial lipopolysaccharide (LPS)-induced expression of the trout cytokine genes studied. Two weeks after the initial sampling, naive exposed fish showed a specific profile of up-regulated tumor necrosis factor (TNF)-alpha2, COX-2 and, to a lesser extent, transforming growth factor (TGF)-beta1 expression. As the disease pathology increased, TNF-alpha2 and COX-2 expression returned to normal levels. Stress levels of cortisol suppressed the LPS inducibility of pro-inflammatory cytokine genes, although TGF-beta1 and TNF-alpha2 appeared to be refractory. These data demonstrate that specific immune responses at the molecular level are affected during PKD infection, with the cortisol suppression of cytokine expression in vitro providing a possible link to PKD-mediated cytokine down-regulation and immune suppression.

Infection of bryozoans by *Tetracapsuloides bryosalmonae* at sites endemic for salmonid proliferative kidney disease
Dis Aquat Organ. 57:221-226, 2003
Tops S, Okamura B (UK)
Laboratory-reared colonies of the bryozoans *Fredericella sultana* and *Plumatella fungosa* were placed upstream of 2 fish farms endemic for salmonid proliferative kidney disease (PKD) to assess rates of infection of bryozoans by *Tetracapsuloides bryosalmonae*, the causative agent of PKD. Colonies were deployed in the field for 8 trial periods of 2 wk each throughout the summer of 2001. Following each trial, bryozoan colonies were maintained in laboratory culture for 28 d and were regularly monitored for infection by searching for sac stages of T. bryosalmonae. Infections were never identified by observations of sac stages, however positive PCR results and sequencing of cultured material confirmed that cryptic infections were present in colonies of both species deployed at one site. The possibility that PCR results reflected contamination of surfaces of bryozoans can be excluded, given the short period of spore viability of T. bryosalmonae. Highest rates of infection occurred when 4 of 23 colonies of F. sultana

and 1 of 12 colonies of P. fungosa were infected during the period 10 to 24 July. No infections were detected from mid-August to late October at this site. None of the colonies at the other site became infected throughout the period of study. Our data provide the first estimates of infection rates of bryozoans by T. bryosalmonae. Additionally, they provide evidence that a cryptic stage can be maintained within bryozoan hosts for a period of 4 to 6 wk.

NEW RESPONSIBILITIES AT INTERVET NORBIO

Earlier this year, Dag H. Knappskog was appointed Director of R&D for Intervet Norbio a.s. (Bergen, Norway) which left his old position as Director of the Commercial Operations Department (better known as COD) vacant. This position has now been filled by Roy Olav Hovlid who was promoted from his previous role as Sales and Marketing Manager. During the year the structure of COD and some responsibilties have been changed to portray the importance of the Intervet Norbio remit covering all salmonid markets. Eivind Isdal is now responsible for global customer service in addition to his position as Assistant Director of COD and Niels Petter Maaseide has taken over all veterinary support and customer service functions for the Nordic countries. Last, but by no means least, we are very happy to welcome Roar J. Tomassen as our new Sales and Marketing Manager. Roar joined the team on 1st October from his previous position as Marketing Director for Intrafish Media a.s. /Fiskaren a.s.

Intervet Aquatic Animal Health Newsletter

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We wish them all plenty of fun and a lot of success in their new postions!



And here is Roy Olav (right of his new buddy, the Portuguese football team mascot) having fun at Euro 2004 in Portugal. Well, if Norway wasn't there, I suppose you had to find some other team to support!

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It is our hope and intention that all the information contained in this Newsletter is accurate; however, the Newsletter is intended solely to supply useful information to the aquaculture industry. Thus, Intervet is not liable for any inaccuracies in this Newsletter.

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