



REVIEW

Viral and bacterial diseases of Atlantic cod *Gadus morhua*, their prophylaxis and treatment: a review

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ABSTRACT: 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.

KEY WORDS: Atlantic cod · Diseases · Prophylaxis · Treatment

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INTRODUCTION

The increased focus on aquaculture of Atlantic cod *Gadus morhua* (Svåsand et al. 2004) has brought renewed attention to the diseases of this species, and to the treatment and prophylaxis of these diseases. There are many reports on cod diseases, mainly on wild stocks (see for instance the review on vibriosis by Egidius 1987), but a side-effect of domestication of a 'new' species is the unavoidable increased focus on its diseases, their prophylaxis and treatment (Bergh et al. 2001).

The parasites of Atlantic cod were reviewed by Hemmingsen & MacKenzie (2001). This subject is therefore not included in this review. The purpose of the present paper is to review the viral and bacterial

diseases of Atlantic cod, as well as the present knowledge on the pharmacokinetics of antibacterial agents, treatment and prophylaxis.

OCCURRENCE AND COMMERCIAL PRODUCTION

Atlantic cod *Gadus morhua* is one of the best-known cold-water species in the world, has been traded for a millennium on 4 continents (Kurlansky 1999), and has now also become a candidate for aquaculture (Svåsand et al. 2004).

Atlantic cod are distributed on both sides of the Atlantic. In the west Atlantic, cod are found from Cape Hatteras north to the ice edge and, in the east Atlantic, from the Bay of Biscay in the south to the northern part

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of the Barents Sea (Bergstad et al. 1987). Atlantic cod occur as several stocks, each of which has its own distinct life-history characteristics and migration patterns (Jakobsson et al. 1994).

In European waters, as in the rest of the world, wild stocks of Atlantic cod have declined steadily throughout the past few decades (Fig. 1). In parallel with the declining wild stocks, considerable efforts have been put into developing Atlantic cod farming (Svåsand et al. 2004). This research has its roots as far back as the 1880s, when attempts were initiated in Norway, the USA and Canada to stock the sea with millions of newly hatched yolk sac larvae. This activity was continued for nearly 90 yr, although no benefits of the releases were ever determined (Shelbourne 1964, Solemdal et al. 1984). However, this laid the foundations for later successes in the farming of marine species.

From the 1970s onwards, there was renewed interest in the cultivation of Atlantic cod, both as a species for farming and for stock enhancement (Dahl et al. 1984). Finally, in 1983, researchers at the Institute of Marine Research in Norway managed to produce 70 000 juvenile cod in a seawater enclosure (Øiestad et al. 1985). In the following years, juvenile Atlantic cod were produced both for cod farming and for stock enhancement, using seawater enclosures or semi-natural rearing systems. Interest in cage rearing was, however, less in the late 1980s, due to low profitability, mainly caused by early maturation and low prices for cod (Svåsand et al. 2004). From 1990 to 1999, the main focus shifted to sea ranching. However, sea ranching of Atlantic cod also turned out to be unprofitable (Svåsand et al. 2000), and further activities were stopped.

Under culture conditions Atlantic cod mature 2 yr after hatching. In the late 1990s, researchers at the Institute of Marine Research in Norway succeeded in postponing the maturation of cod by means of artificial

light (Hansen et al. 2001), thereby removing an important bottleneck to viable cod farming. After several years, during which time interest in cod aquaculture remained low, a series of new research projects was launched, and private companies built large-scale hatcheries for the rearing of Atlantic cod (Svåsand et al. 2004). While the previous juvenile production used semi-natural rearing systems, production was now shifted to intensive rearing, which provided more control over the rearing environment (Fig. 2).

VIRAL DISEASES AFFECTING ATLANTIC COD

Nodaviruses

Nodaviruses are small viruses (25 to 30 nm) with a very simple architecture. The genome consists of 2 positive-sense, single-stranded RNA segments: RNA1 (3100 nt), which encodes the *RNA-dependent RNA polymerase* responsible for the replication of the genome, and RNA2 (1400 nt), which encodes the capsid protein that builds up the icosahedral capsid into which the genome is packaged. In addition, there is a third RNA segment, RNA3, generated sub-genomically from RNA1 and encoding a polypeptide of unknown function. RNA3 is present only in the infected cells and is not packaged into the viral particles (Mori et al. 1992, Nishizawa et al. 1995, Grotmol et al. 2000, Sommerset & Nerland 2004).

Viruses with this architecture were first isolated from insects (denoted alpha-nodavirus) (Scherer & Hurlbut 1967), later from fish (denoted beta-nodavirus) (Mori et al. 1992) and more recently from Crustacea (Bonami et al. 1997). However, the homologies of the genomes at the nucleotide or amino acid level between the alpha-, beta-nodaviruses and those isolated from Crustacea are remarkably low. It may therefore be that these viruses do not have a common origin, but just happen to have a similar organisation.

The main target organ for nodavirus infection of fish is the central nervous system (CNS), including the brain, spinal cord and retina, where it causes extensive cellular vacuolation and neuronal degeneration (Mori et al. 1992). The disease is therefore referred to as either viral encephalopathy and retinopathy (VER) or viral nervous necrosis (VNN). In general, the clinical signs relate to neurological distortion: abnormalities of movement such as uncoordinated swimming, tonic spasms of myotomal musculature, lethargy, change of pigmentation and loss of appetite.

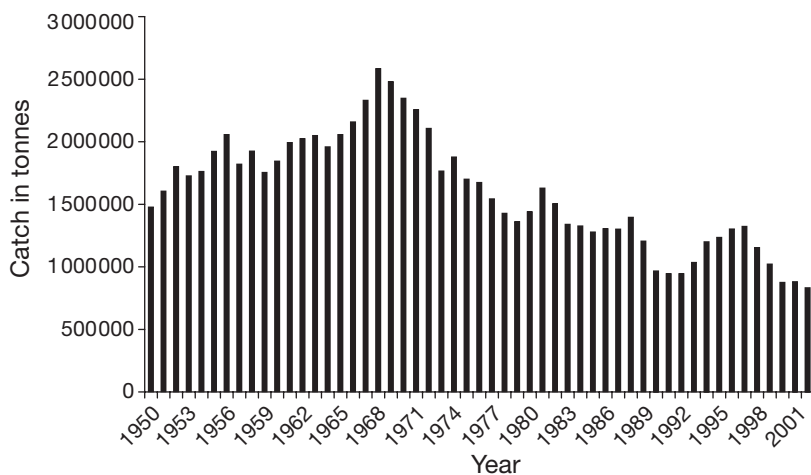


Fig. 1. *Gadus morhua*. Wild catch of Atlantic cod in Europe from 1950 to 2005 (www.fao.org)

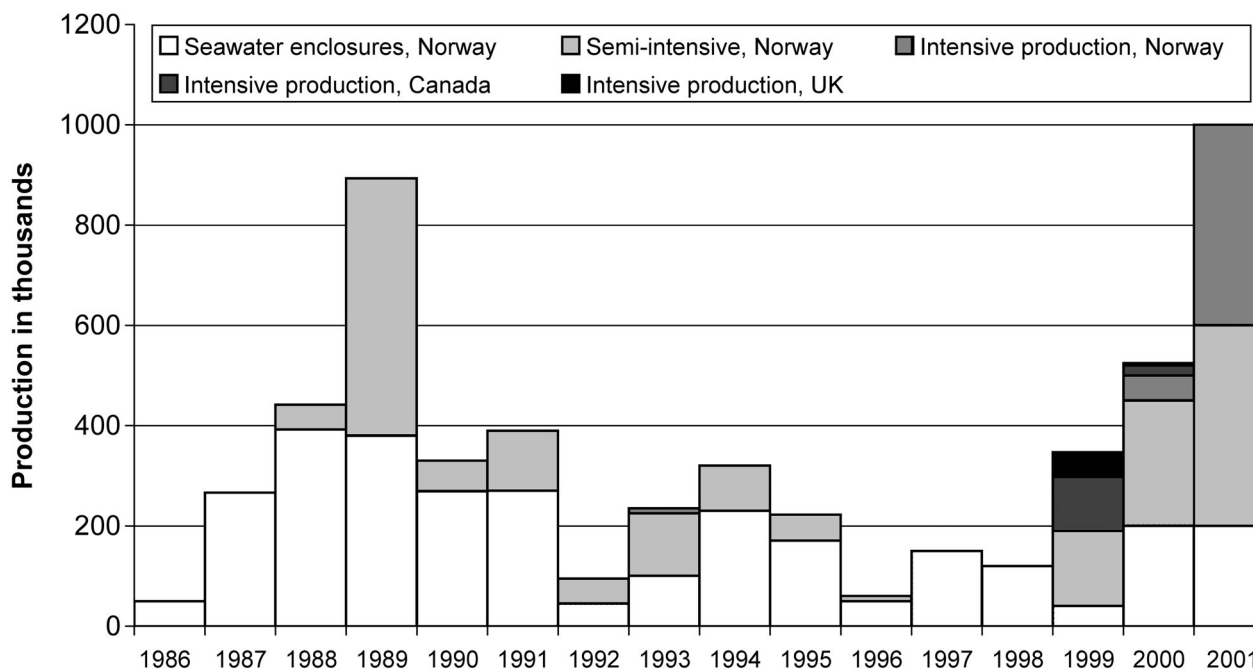


Fig. 2. *Gadus morhus*. Production of juvenile cod (>10 g) in Norway, UK and Canada. Redrawn from data by Svåsand et al. (2004)

Nodavirus infection particularly affects the larval or juvenile stages of fish, in which mortality may be very high. In the Atlantic halibut *Hippoglossus hippoglossus* outbreaks of this disease are most often seen during first feeding and weaning (40 to 100 d post-hatching), although yolk-sac stages may also be affected (Grotmol et al. 1997, 1999). However, in recent years, significant mortalities have occurred in older fish up to harvesting size (Fukuda et al. 1996, LeBreton et al. 1997, Aspehaug et al. 1999).

The simple organisation of the nodavirus particle may explain why viruses from this family are able to replicate in cells from a variety of species. Aside from insect cells, alpha-nodavirus has been shown to be able to replicate in mammalian (Ball 1992), plant (Selling et al. 1990) and yeast (Price et al. 1996) cells as soon as the virus particles have entered the cells. It is the only virus originating from higher eukaryotic organisms that has been found able to replicate in yeast cells. This is in agreement with the broad host range of beta-nodavirus, infections of which have been described in around 40 different fish species from all over the world (Munday et al. 2002). Although strains of nodavirus have been referred to according to the species from which they were first isolated (like Atlantic halibut nodavirus, striped jack nodavirus), recent observations indicate that the strains tend to be geographically rather than species related (Gagne et al. 2004).

Beta-nodaviruses have been categorised into genotypes based on the formation of clusters in phyloge-

netic analyses of the variable region of the viral capsid gene (Nishizawa et al. 1995, 1997, Dalla Valle et al. 2001).

How nodaviruses are transmitted is not fully understood. In theory, the virus can be transmitted vertically from the broodfish via the eggs or sperm, or may enter the rearing unit through the inlet water or from the feed and then spread horizontally. Recent reports have shown that the virus can persist for a long time in subclinically infected fish and still be infectious (Johansen et al. 2004). The virus has been detected in gonads and in association with eggs, and experimental vertical transmission has been demonstrated in sea bass (Breuil et al. 2002). We have detected very high concentrations of nodavirus in rearing units in which infected halibut larvae were held, indicating that horizontal transmission may take place. It is therefore tempting to speculate that normal transmission is vertical, from subclinically infected parents to the larvae, and as a few of these acquire the disease the virus is shed into the water and then spread horizontally. Nodavirus has been shown to be very stable under extreme environmental conditions (Frerichs et al. 2000). Once the virus has entered a farm or rearing unit it may be very difficult to exterminate.

The main diagnostic methods for nodavirus infections are immunohistochemistry (IHC) and reverse transcriptase–polymerase chain reaction (RT-PCR). Although nodaviruses can be cultivated in cell culture, several passages may be necessary before the virus replicates properly, and it is therefore difficult to use

cultivation for diagnostic or screening purposes. IHC is very time-consuming and therefore not very suitable for screening. The sensitivity of the method depends on the available antibodies. RT-PCR is less laborious and is most sensitive if suitable primers are used. However, if the primers do not match the virus strain, the sensitivity drops dramatically. The high sensitivity of RT-PCR methods may cause false positives. One possibility would be to use RT-PCR as the primary screening method and IHC to confirm positive findings. Samples for both of these methods are normally taken from brain or eye tissue. Neither of these methods is therefore suitable for testing broodfish for latent infections. Such screenings have been performed by testing sera from the fish for antibodies against nodavirus by means of the ELISA technique. However, the results from ELISA may be difficult to interpret, as there is not always a clear-cut difference in titre between infected and non-infected individuals (Mushiake et al. 1994, Breuil & Romestand 1999).

No commercial vaccines against nodavirus are currently available, although vaccination with recombinant vaccines have shown high protection of turbot vaccinated at a weight of about 5 g and challenged 7 wk later (Húsgard et al. 2001, Sommerset et al. 2003, 2005). Induction of innate immunity by means of a DNA vaccine based on the gene encoding the G-protein from VHSV has been shown to achieve high, but short-term, protection against nodavirus infection (Sommerset et al. 2003). The vaccination of larvae or juvenile fish is difficult for 2 reasons: the virus affects the larvae/juveniles at a stage in which the immune system is not well developed, and the small size of the fish makes injections impractical. However, one possibility could be to immunise the broodfish before spawning, in order to prevent vertical transmission.

Nodavirus has been one of the major limiting factors in the culture of marine fish species all over the world during the past decade. This may also be the case for Atlantic cod. In North America, outbreaks of disease caused by nodavirus have been reported in hatchery-reared Atlantic cod and/or haddock *Melanogrammus aeglefinus* in Newfoundland, Nova Scotia, New Brunswick and along the east coast of the USA (Johnson et al. 2002). These disease outbreaks have resulted in high levels of morbidity and mortality. Nodavirus infection has also been reported in cultured juveniles of Atlantic cod from the UK, with low mortality rates (approximately 2%) observed over a 3 mo period (Starkey et al. 2001). Signs of disease in affected cod included loss of equilibrium, as the fish are seen swimming on their sides; lethargy, when the fish lie on the bottom of the tanks; and abnormal swimming behaviour, such as looping and spiralling. Sequencing has shown that nodavirus isolated from cod shows high

similarity to that isolated from Atlantic halibut (Johnson et al. 2002).

The first step towards control of nodavirus infection is to select and screen the broodfish to avoid allowing the virus into the facility. The presence of nodavirus in several wild marine fish species, including Atlantic cod, has been reported (Barke et al. 2002, Gagne et al. 2004), and the ability of the virus to persist subclinically suggests that wild-caught broodfish may be a source of the virus. Keeping broodfish in separate, smaller units will prevent transmission of the virus between individual fish. Stressing the fish should be kept to a minimum. The fish should not at any life stage be fed by fresh or frozen feed of marine origin that has not been properly checked. In order to minimise vertical transmission to the offspring, the eggs can be disinfected by ozonation, while to reduce horizontal transmission extensive mixing of batches of larvae/juveniles should be avoided. The water should not be recirculated, and inlet water should be disinfected by UV-treatment or ozonation. In the future, vaccination of broodfish prior to spawning might be an alternative.

Infectious pancreatic necrosis virus

IPNV is a member of the family Birnaviridae, in which the virions are non-enveloped icosahedrons, about 60 nm in diameter, containing a genome consisting of 2 segments of RNase-resistant, double-stranded RNA. The shorter segment (~2800 nt) encodes the RNA-dependent RNA polymerase. The larger segment (~3100 nt) encodes a protein that is cleaved cotranslationally into 3 components, 2 of which will form part of the virion (Duncan & Dobos 1986, Duncan et al. 1987). In addition, a fourth short polypeptide inhibiting apoptosis of the infected cell is transcribed from a different reading frame (Essbauer & Ahne 2001).

The disease caused by IPNV is principally associated with salmonids, in which acute infections occur in 1- to 4-mo-old fish and may cause mortalities up to 90%. Susceptibility decreases with increasing age. Factors like stress appear to increase susceptibility, as Atlantic salmon smolts often develop the disease shortly after transfer to seawater.

IPNV seems to be widespread in the marine environment, having been isolated from many species of freshwater and marine fish, crustaceans and molluscs. The geographical distribution is worldwide; it has been reported from Europe, North and South America, Asia, South Africa and New Zealand (Wolf 1988).

The external clinical signs of IPN are abdominal distension, uncoordinated swimming and trailing, dark pigmentation, pale gills and white faecal casts. Further examination of the fish reveals that the spleen, kid-

neys, liver and heart are abnormally pale and the body cavity may contain ascitic fluid (Biering et al. 1994).

The intestine may be the primary organ for virus entry and replication (Biering & Bergh 1996). The virus is shed via faeces, sexual fluids and probably urine and can therefore be transmitted horizontally through the water route by ingestion of infected material or by direct contact with infected fish. Likewise, infected transport water, contaminated nets, containers and other equipment are potential transmission channels. Infectious virus may also be transported and excreted by fish-eating birds and mammals (Wolf 1988).

IPNV has been shown to persist subclinically and later be transmitted vertically (via fertilised eggs) from carrier broodfish. Survivors of an IPNV outbreak become IPNV carriers and can shed the virus for the rest of their lives (Wolf 1988).

Several methods have been developed for diagnosis of IPNV infection. The presence of viral RNA can be detected by RT-PCR. The presence of viral particles in fish tissues can be detected by immunological techniques such as IHC or IFAT (indirect-fluorescent antibody test). The presence of infectious viral particles can be detected by using permissive cell lines like BF-2, CHSE-214, or RTG-2, followed by inspection for pathological changes or examination for the presence of virus by one of the methods mentioned above. The presence of antibodies directed against the virus in the fish serum can be detected by ELISA and indicate that the fish are or have previously been infected by the virus.

Vaccines against IPNV infections based upon inactivated virus as well as recombinant antigens are available, the latest being the first recombinant vaccine commercially available in aquaculture. However, the degree of protection offered by the vaccines has been difficult to evaluate, as reproducible challenge tests have not been available until quite recently.

Screening of wild marine fish from the North Sea detected IPNV in several species, including Atlantic cod (Skall et al. 2000). An outbreak of disease caused by IPN in reared Atlantic cod fry has been reported from Denmark and the Faroe Islands, indicating that this may be a problem for intensive farming of Atlantic cod.

Viral haemorrhagic septicaemia virus

Although VHSV has been mostly regarded as a threat to freshwater species, particularly rainbow trout *Onchorhynchus mykiss*, reports exist of the isolation of VHSV from cod. In fish disease surveys VHSV has been isolated from a small proportion of the cod displaying lesions, and the isolates were identified as

VHSV by immunofluorescence or ELISA (Mortensen et al. 1999, Smail 2000, King et al. 2001). VHSV has also been found in wild-caught Pacific cod *Gadus macrocephalus* (Meyers et al. 1992).

Nucleotide sequencing of the glycoprotein gene of VHSV from different geographical areas has confirmed a link between VHS in farmed salmonids and viruses isolated from cod (Stone et al. 1997). Two virus isolates recovered from wild-caught cod off Shetland and farmed turbot in Scotland showed 99.4% nucleotide sequence similarity with a virus associated with VHS in rainbow trout. The susceptibility of juvenile cod to VHSV isolated from wild-caught cod was tested in challenge experiments by Snow et al. (2000), who found that intraperitoneal administration of the virus caused clinical disease and mortality in excess of 80%. However, no virus was recovered from cod cohabiting with intraperitoneally challenged fish at a ratio of 1:1, and, likewise, no VHSV-associated mortality was demonstrated following bath challenge. External signs of the disease were the presence of exophthalmia and ascites. VHSV was recovered from brain, kidney, liver and spleen and identified by ELISA. The fact that cod were not susceptible to VHSV following waterborne exposure raises questions surrounding the propagation, maintenance and impact of a naturally occurring reservoir of virus in the marine environment. Snow et al. (2004) investigated the genetic population structure of marine VHSV isolates, concluding that the rainbow trout isolates constitute a separate group, Baltic isolates a second, whereas marine isolates from around the British Isles and from North America constituted the third and fourth group, respectively. These authors stated that their findings suggested a marine origin of VHSV in aquaculture.

Challenge experiments with turbot have also confirmed the aetiology of the disease, with typical signs of the disease in challenged turbot including darkening of the skin and the presence of haemorrhaging around the head and fin bases (Snow & Smail 1999).

BACTERIAL DISEASES AFFECTING ATLANTIC COD

Bacteria associated with early life stages

Bacteria may be present in large numbers on the surface of fish eggs. This epiflora seems to be dominated by members of the *Cytophaga/Flavobacterium/Flexibacter* group, while *Vibrio* spp. are not frequent (Hansen & Olafsen 1989). In the early days of cod aquaculture, the filamentous *Leucothrix mucor* was regarded as a problem, since the overgrowth of eggs, with consequent problems such as immobilisation or

possible oxygen deficiency, occurred (Hansen & Olafsen 1988). The psychrotrophic *Tenacibaculum ovolyticum* (formerly named *Flexibacter ovolyticus*) was originally isolated from halibut eggs with high mortality (Hansen et al. 1992), and is able to penetrate the egg shell (Bergh et al. 1992). It resembles the fish pathogen *T. maritimum*, but differs from the latter in several biochemical and physiological characteristics. This bacterium has not been found on hosts other than halibut, in spite of the fact that it may cause mortality in cod eggs and larvae in challenge experiments (Bergh 2000).

While the composition of the intestinal bacterial flora associated with the yolk-sac larvae of fish generally resembles the egg epibiota, a shift in the intestinal microbiota from a generally nonfermentative to a fermentative flora dominated by the *Vibrio/Aeromonas* group coincides with the onset of exogenous feeding (Vadstein et al. 2004). During first feeding, yolk-sac larvae in general are subject to a massive inflow of bacteria from the live feed organisms *Brachionus plicatilis* and *Artemia* spp., and suspected pathogens, like *Listonella anguillarum*, may be accumulated in the live feed (Korsnes et al. 2006). The mucosal surfaces of fish serve as a substrate for bacterial adhesion (Hansen & Olafsen 1989). Uptake of intact antigen from bacteria in the intestine of 4- to 6-d-old cod larvae has been demonstrated, and it has been suggested that this uptake may play a role in the immune development, or in nutrition (Olafsen & Hansen 1992).

Vibrio sp.

Egidius (1987) cites the earliest report of a disease caused by a *Vibrio* sp. in cod, where Bergman (1912) described an infectious eye disease primarily affecting the cornea in cod along the south coast of Sweden during the autumn of 1910. The prevalence of the disease in trawl-caught fish was about 16%, while in traps it was around 10%, and all sizes of cod seemed to be affected. In the early stages of the disease, the cornea became greyish and opaque; in later stages, the cornea disintegrated and the entire eye was damaged, and in some cases both eyes were affected. From the diseased eyes, Bergman (1912) isolated a *Vibrio*, but he was unable to isolate bacteria from the blood. Bergman (1912) was able to reproduce the disease by injecting cod with a culture of the bacterium in the corpus vitreum or the conjunctiva.

In cod, vibriosis has long been the most important disease (Egidius & Andersen 1984), and *Listonella anguillarum* is dominant among pathogenic isolates (Knappskog et al. 1993, Wiik et al. 1995). In traditional semi-extensive systems ('polls') outbreaks of vibriosis often occurred when fish were moved from the enclo-

tures in July to September at 10 to 20 cm length. The main clinical signs are fin erosion and haemorrhages in the head region, especially in and around the eye. The infection is general, and bacteria can readily be isolated from the blood and kidney (Egidius & Andersen 1984). In modern intensive aquaculture systems, vibriosis with the same clinical signs frequently occurs during or after weaning of the fish onto artificial diets, or at various stages during ongrowth.

Isolates of *Listonella anguillarum* from Norwegian marine fish with vibriosis were found to be free of plasmids, strongly indicating that their virulent properties were chromosome mediated (Wiik et al. 1989).

Ben-Haim et al. (2003) have advanced the hypothesis that aquaculture sites serve as foci or reservoirs of pathogenic *Vibrio* strains: during certain times of the year, pathogenic vibrios could endure adverse environmental conditions within farms, and when favourable environmental conditions are re-established, they would be able to cause disease in wild animals. On the other hand, there is no doubt that vibrios are ubiquitous on/in marine organisms, and that diseases caused by vibrios commonly occur in wild marine animals, including cod and other gadoids, and did so well before the development of modern aquaculture (Egidius et al. 1983, Colwell & Grimes 1984, Egidius 1987). We would therefore argue that it is more likely that wild populations constitute reservoirs for pathogens. However, favourable conditions for proliferation of pathogens in aquaculture may be caused by high host density and/or suboptimal rearing conditions (Vadstein et al. 2004).

Aeromonas spp.

Both typical and atypical *Aeromonas salmonicida* have been found in wild and cultured Atlantic cod (Wiklund & Dalsgaard 1998, Magnadóttir et al. 2002b). The causative agent of furunculosis in Atlantic salmon *A. salmonicida* ssp. *salmonicida* can, for practical purposes, be regarded as apathogenic to halibut. In a field survey during a major outbreak of furunculosis in an Atlantic salmon stock, all dead halibut at the station were studied (Hjeltnes et al. 1995), but no indications of a transfer were found. An experimental bath challenge with typical *A. salmonicida* ssp. *salmonicida* to yolk-sac larvae gave a more complex result (Bergh et al. 1997). Significant mortality did take place. However, this was probably a result of production of toxic exudates by the bacterium, as histological and immunohistochemical examinations of the larvae revealed no evidence of bacteria in affected tissues.

In contrast to *Aeromonas salmonicida* ssp. *salmonicida*, which comprises a homogenous group of strains, atypical *A. salmonicida* strains are heterogeneous with

respect to serological and biochemical characteristics (Austin et al. 1998, Dalsgaard et al. 1998, Wiklund & Dalsgaard 1998). Atypical strains of *A. salmonicida* are occasionally isolated from cod, although at present we cannot rule out the possibility that some of these infections may be secondary. Magnadóttir et al. (2002b) reported isolating atypical *A. salmonicida* from naturally infected reared cod. The strains were homogeneous with respect to biochemical characteristics, and similar to the *achromogenes* sp. type strain (NCIMB 1110, National Collection of Industrial and Marine Bacteria, UK) and to reference strains isolated from salmonids and halibut in Iceland. The histopathology consisted mainly of widespread granuloma formation, with and without internal bacteria. In challenge experiments with 1-yr-old farmed cod kept at 9°C, mortalities were induced by the isolates mentioned above (Magnadóttir et al. 2002b). Pathological changes in the experimentally challenged cod differed from those in naturally infected individuals, and haemorrhage, early stages of granuloma formation and necrotic changes were found in several organs. Gills, skin and the serosa of the alimentary canal, especially of the lower intestine, were all affected. Fish that received intramuscular injection of the bacteria tended to display a more intense response than intraperitoneally infected fish, and external signs of infection, for example lesions at the site of injection, were more intensive.

COD ULCER SYNDROME

In this syndrome, reviewed by Jensen (1983) and occasionally affecting wild cod, various skin lesions are observed all over the fish. The lesions start as papules, and their development into ulcers takes some time. In later ulcerative stages, the stratum compactum is always discontinuous. The primary cause of the ulcer syndrome is probably of rhabdoviral nature; however, large amounts of secondary *Listonella anguillarum* can be isolated from the ulcers. In fish disease surveys in the North Sea for marine rhabdoviruses in 1993 and 1995, the cod ulcer syndrome was widely seen in all ages of cod, especially in the 2 to 5+ yr classes (Smail 2000).

OTHER BACTERIAL DISEASES

Pathogenic organisms within the *Tenacibaculum/Flexibacter/Cytophaga* group have been isolated from wild-caught cod in the German Wadden Sea (Hilger et al. 1991). The so-called 'yellow pest' affected juvenile cod only. Long slender bacterial rods were demonstrated frequently in histological preparations of the yellow nodules typically associated with the ulcerative

lesions. In most cases there was severe ulcerative destruction of the integument and of cartilage and bones, especially of the jaws. The disease occurred in the early winter and spring, soon after the cod had entered the estuaries of the main river tributary to the North Sea. Other bacterial pathogens described in cod include *Streptococcus parauberis* (Romalde 1999) and a *Mycobacterium* sp. (B. Hjeltnes, National Veterinary Institute, Norway, pers. comm.). Furthermore, *Vibrio salmonicida*, a well-known pathogen in salmonids, has also been shown to cause mortality in net-pen captured cod (Jørgensen et al. 1989).

In 2005, a novel systematic granulomatous inflammatory disease was discovered in several farms and associated with a facultatively intercellular bacterium belonging to the genus *Francisella* (Nylund et al. 2006, Olsen et al. 2006). A large fraction of the fish in these farms seemed to be affected. Moribund fish caught for examination showed extensive internal gross lesions, with moderate to massive occurrence of white, partly protruding nodules of various size in the spleen, heart, kidney and liver. Histological examinations revealed numerous granulomas in these organs. An isolated bacterium showed high 16S rDNA similarity to *Francisella philomagia* (Olsen et al. 2006).

PROBIOTICS

Probiotics, which may be defined as 'live microorganisms which when administered in adequate amounts, confer a health benefit on the host' (Anonymous 2001), have attracted significant interest in aquaculture (Gatesoupe 1999). Modern development of probiotics for fish has proved that protection against disease may be achieved (Gram et al. 1999), but probiotics must be investigated for different probiotic-host-pathogen combinations (Gram et al. 2001). Recent data suggest that marine larvae may be protected against vibriosis by addition of probiotics to rearing water or via live feed (Hjelm et al. 2004, Planas et al. 2006). Reports with probiotics in cod rearing are scarce. Strøm & Ringø (1993) were able to demonstrate a significant improvement in the survival of cod larvae following addition of *Lactobacillus plantarum* to rearing water. Gilberg et al. (1997) and Gilberg & Mikkelsen (1997) showed a limited improvement of resistance in challenge experiments following the supplement of lactic acid bacteria to the feed of cod fry.

VACCINATION

Unlike in other teleosts, immunisation of Atlantic cod with *Vibrio* bacteria, or hapten-carrier antigens (NIP-

LPH, LPH and TNP-SRBC) has been reported by several authors to produce limited or no increases in the level of specific antibodies (Espelid et al. 1991, Israelsson et al. 1991, Schröder et al. 1992, Magnadóttir et al. 2001). Extensive studies of the immune system of cod have failed to reveal any simple explanation for this (see below).

Although specific antibody responses are weak or absent, adult Atlantic cod respond well to vaccination, and memory is retained against the bacterial diseases classical vibriosis and cold-water vibriosis after either intraperitoneal (i.p.) or immersion vaccination (Espelid et al. 1991, Schröder et al. 1992, Mikkelsen et al. 2004). Classical vibriosis, caused by *Listonella anguillarum*, is the major bacterial disease in cod farming. More than 23 serotypes of *L. anguillarum* have been described, but, unlike in salmonids, which mainly are affected by Serotype O1 and O2 α , Serotypes O2 α and O2 β are most commonly associated with cod (Larsen et al. 1994, Pedersen et al. 1999). As culture of cod has increased, Serotype O2 β has become more dominant (Santos et al. 1995). For unknown reasons, outbreaks of classical vibriosis still cause mortality in farmed cod in Norway, even when the fish have been vaccinated with bacterins of *L. anguillarum* Serotypes O1, O2 α and O2 β (K. Gravningen pers. comm.).

Intraperitoneal vaccination with atypical *Aeromonas salmonicida* bacterin in mineral oil or in aqueous suspension has recently been shown to elicit good protection against homologous bacteria (administered i.p.), and low, but significant, protection against *Listonella anguillarum* was obtained following bath administration (Mikkelsen et al. 2004). The challenges were performed 11 to 12 wk after vaccination, and no reduction in weight gain was observed during this period. However, side effects such as intra-abdominal adhesions were observed in fish vaccinated with vaccines containing oil. A commercial polyvalent vaccine developed for salmon (Alphaject 5200) has also been tested in Atlantic cod, and this provided good protection in fish experimentally infected with classical vibriosis (*L. anguillarum* O2 α) but not against atypical furunculosis (*A. salmonicida* ssp. *achromogenes*) and winter ulcer disease (*Moritella viscosa*) (Gudmundsdóttir et al. 2004a).

Since marine larvae and juveniles share their environment with the pathogens, early prophylactic treatment is required. Atlantic cod are poorly developed at the time of hatching and undergo a long larval period before metamorphosis (Kjørsvik et al. 1991, Pedersen & Falk-Petersen 1992). During this period, the immunological organs are immature and no immunoglobulin producing cells are detected. The first plasma cells were observed in cod juveniles at 33 mm body length, 58 d post-hatching (Schröder et al. 1998). Recently,

vaccination experiments have revealed that cod juveniles vaccinated at weights of 1 and 1.7 g are poorly protected after bath as well as dip vaccination against classical vibriosis (relative percent survival: RPS = 32 to 47 at 6 wk post-vaccination and RPS = 16 to 36 at 14 wk post-vaccination). On the other hand, juveniles vaccinated at a weight of 2.2 g obtained good protection when challenged by bath 7 wk after vaccination (RPS = 77). The long-term protection after vaccination was not evaluated at this size, while cod vaccinated at 5 g were highly protected (RPS = 83) for as long as 28 wk after dip vaccination (Schröder et al. 2006). Vaccines administered by dip (immersion in a 1:10 diluted vaccine for 30 s) to 10 g cod also resulted in significantly better protection against classical vibriosis than bath vaccination (vaccine diluted 1:200 and the fish immersed for 30 min) (Schröder et al. 2006).

Stimulation of the innate immune system might be an alternative method to vaccination (before the adapted immune system has matured), and studies of halibut larvae have shown that administration of immunomodulators via water or live food can improve larval survival (Dalmo et al. 1998, Skjermo & Bergh 2004). In Atlantic cod fry, oral administration of immunostimulants (fish protein hydrolysate or cationic proteins from cod milt) or probiotics (*Carnobacterium divergens* isolated from the intestines of Atlantic salmon or Atlantic cod) has resulted in some reduction of mortality after bath challenge with *Listonella anguillarum* (Gildberg & Mikkelsen 1997, Pedersen et al. 2004). Furthermore, cod larvae bath treated with bacterial lipopolysaccharide (LPS) or a potentially probiotic bacterium (*Vibrio* sp., isolated from cod) displayed significantly improved survival, but no protection was observed after experimental infection with *Aeromonas salmonicida* ssp. *achromogenes* (Gudmundsdóttir et al. 2004b).

ANTIBODY RESPONSES AND GENES

Although Atlantic cod produces immunoglobulin class IgM antibodies against pathogens such as *Listonella anguillarum* (LPS and surface proteins, see above), the level of the responses, as measured in the ELISA assay, was much lower than that in Atlantic salmon (Espelid et al. 1991, Israelsson et al. 1991, Schröder et al. 1992, Magnadóttir et al. 2001). These findings raised several basic questions about the immune system in cod and, along with other results, prompted studies of the genes encoding the antibody repertoire and the organisation of these genes, both in salmon and cod (Solem & Stenvik 2006).

The antibody repertoire or the number of antibody specificities that an individual can make is determined

by the number of V-genes encoding the variable part of the antibody heavy (H) and light (L) chains. In addition, mutations made during reorganisation of multiple V-gene segments (V, D and J loci) and the active site structures resulting from recombination of the H and L chains are important as well.

With respect to the V-gene repertoire, the number of V-genes was shown to be similar in cod and salmon, but the variety is somewhat more limited in cod. This appeared when the V-genes in cod and salmon were sequenced and sorted into V-gene families. A V-gene family is defined as genes having >80% internal sequence similarity, and salmon had at least 9 V-H families, whereas the cod V-H sequences were sorted into 4 families (Solem et al. 2001, Stenvik et al. 2000). Furthermore, cod mainly expressed 1 (covering 80%) of the 4 families as judged by cDNA sequencing and gene expression studies (Stenvik et al. 2001). This implies that the functional heavy-chain V-gene repertoire in cod is somewhat restricted in comparison with that in salmon. A similar restriction was, however, not seen in the light-chain genes (Daggfeldt et al. 1993). Moreover, additional characteristics, such as the variability in the hyper-variable regions (the Complementarity Determining Regions, CDRs) and the number and composition of the amino acid residues in the third CDR, suggest that cod and salmon possess a similar ability to create antibody-active sites (Stenvik et al. 2000).

Although the total variety of antibody V-genes expressed in cod is smaller than that in salmon, this hardly explains the lack or weak humoral responses in the cod species. A similar reduction in or lack of antibody responses was also seen in other cod species, such as the saithe (*Pollachius virens*, Solem & Stenvik unpubl. data) and haddock (Dacanay et al. 2004), immunised with model antigens (hapten-carrier) or bacteria. Thus, the poor antibody responses seen in cod, haddock and saithe to some antigens are probably a trait inherited by the whole gadoid family.

A second peculiar property of the cod antibody genes is the organisation of the 'second' immunoglobulin class, IgD. This class was first discovered in the American channel catfish (Wilson et al. 1997) and later also in Atlantic salmon (Hordvik et al. 1999). This new class has similarities with IgD in mammals, except that the number of structural domains varies. While the channel catfish and salmon IgD heavy chains (δ) possess 10 domains, the mouse and human δ contain 2 and 3 domains, respectively (Putnam et al. 1982).

This also implies that the phylogeny and gene organisation of IgD (δ) in cod is different from that in catfish and salmon, as the δ chain is smaller, is duplicated differently and contains only 5 δ domains (Stenvik & Jørgensen 2000). This additional 'discrepancy' between salmon and cod immunoglobulin genes does

not explain the limited production of antibodies in cod, as the IgM and IgD use the same V-genes to express their antibody repertoire. Furthermore, the IgD class in cod is not produced in the form of serum antibodies, but is only expressed as a membrane-bound Ig receptor on B-cells.

Also, the difficulties finding MHC Class II genes in cod has been connected with a lack of Class II molecules, an inadequate antigen presentation and stimulation of T helper (TH2) cells and a corresponding absence of T-cell-dependent antibody responses (Pilstrom et al. 2005). In this context, it is probably relevant that the Atlantic cod can mount a significant antibody response towards the LPS moiety of the *Aeromonas salmonicida* pathogen (supposed to be T cell independent), although immunisation with the LPS-containing *Listonella anguillarum* did not elicit similar antibody production (Lund et al. 2006).

Another trait of the 3 cod species mentioned is that they express relatively high natural immunoglobulin levels in the blood (Israelson et al. 1991, Magnadóttir et al. 2002a, Dacanay et al. 2004, Solem & Stenvik unpubl. data). Although individual variation exists, serum concentrations in cod are about 10 times as high as in salmon. What actually stimulates such high production of natural immunoglobulin is not known, although bacterial antigens taken up from the intestinal flora are one possibility. Alternatively, the immunoglobulin-producing B-cells in cod might develop autonomously into plasma cells without the involvement of particular antigens. The latter hypothesis is quite unorthodox, as there is no experimental evidence for such a mechanism, either in other fish species or mammals. At any rate, potent activation of B-cells and the high concentration of natural antibodies may activate strong negative feedback mechanisms and subsequently suppress the production of 'new' (and specific) antibodies and thereby inhibit antibody production after immunisation.

Thus, all the genetic data considered, the immunological differences between cod and salmon are still poorly understood. The high immunoglobulin level in cod probably indicates that cod use a different strategy against pathogens than salmon. Cod may rely more on 'first-line' mechanisms and molecules, and 'up-front' production of natural antibodies may facilitate un-specific or multi-specific binding to pathogens and thereby elicit a general protection against disease.

The fact that vaccinating cod against pathogens such as *Listonella anguillarum* and *Aeromonas salmonicida* elicits specific protection against the corresponding diseases (see above) clearly demonstrates that the cod immune system possesses an effective memory and appropriate mechanisms sufficient for protection, at least against some diseases. What new diseases the

cod will face as a forthcoming aquaculture species is, of course, unknown, but it is to be hoped that optimisation of the currently available vaccines and valid information about the cod immune system will save the cod farming industry from future epizootics and disasters.

ANTIBACTERIAL AGENTS

Administration

Several antimicrobial agents have found application in the treatment of bacterial diseases of fish (Treves-Brown 2000). The most important groups in this respect are the tetracyclines, quinolones, sulphonamides, aminopyrimidines and amphenicols.

The quinolones flumequine and oxolinic acid act by interfering with the enzyme DNA-gyrase. This enzyme is essential to the negative supercoiling of bacterial DNA and to the process of packing the DNA molecule within the cell. Oxytetracycline and florfenicol, an amphenicol, both affect bacterial growth by inhibiting bacterial protein synthesis. Sulphadimethoxine competitively inhibits the incorporation of p-aminobenzoic acid into folic acid, thereby preventing folic acid synthesis and bacterial growth. Ormethoprim, an aminopyrimidine, potentiates the anti-folate effect of sulphadiazine by competitively inhibiting the enzyme dihydrofolate reductase (Treves-Brown 2000).

The use of antibacterial agents in aquaculture is limited by several practical constraints. In theory almost all treatments can be administered by injection, as is otherwise usual in veterinary practice. However, individual injection of fish is costly in time and labour, requiring fish to be caught and anaesthetised, with the inevitable associated stress. Injection is therefore only used in the treatment of fish with high individual value such as brood-stock, or ornamental fish. Bath treatment, although easy to perform with agents of high water solubility, is restricted to recirculating systems or tanks of limited size and is therefore used mainly in the treatment of small fish. Bath treatment has demonstrated high efficacy in treating fish suffering from systemic infections and skin or gill infections. An important limitation, however, is the ability of cations in the seawater to complex with certain antibacterial agents, such as oxytetracycline, oxolinic acid and flumequine, and thereby inactivate and reduce the ability of the fish to absorb the antibacterial agent. The dose and treatment regimens are therefore very different in freshwater and seawater. A higher dose and longer period of treatment is required in seawater.

Oral administration makes the treatment of large quantities of adult fish relatively easy at a low cost in labour and has become the prime route of medication

for fish. Nonetheless, there are also limitations to oral administration. Oral treatment is intended to control the level of infection in the group rather than infections in individual fish. Since loss of appetite is frequently observed in those fish that are suffering from a bacterial infection, oral treatment of a population will mainly deliver the drug to those individuals that are still uninfected. The treatment may therefore be considered prophylactic rather than therapeutic in that it aims to prevent further spreading of an existing infection.

Furthermore, the absorption of the drug from the gut may be inadequate, the drug may be unpalatable, and palatability may differ from species to species. Therefore, it is important that therapeutic regimes be designed to maximise the efficacy, thereby minimising the risk of the development of resistant pathogens. Ideally, therapy should only be carried out when the target pathogen and its sensitivities to specific drugs have been identified. The antibacterial agent is administered in the form of medicated food pellets, whereby the drug is either coated on the outside of the pellets or incorporated into the pellet by mixing it with the food ingredients prior to the pelleting process.

Pharmacokinetics

The study of the pharmacokinetic properties of drugs is, in combination with minimum inhibitory concentration (MIC) determinations, an important tool for the establishment of optimal dosage regimes and thus the promotion of their correct use. However, as the pharmacokinetic properties of antibacterial agents may vary significantly between species, the use of a drug should be investigated in the particular species in which it is intended to be used (Kleinow et al. 1994, Martinsen et al. 1994, van der Heiden et al. 1994).

Pharmacokinetic information on antibacterials in cod is limited, since only a few studies have addressed this subject.

Using bath treatment for 2 h, the absorption and excretion of flumequine were studied in cod with an average weight of 171 g (Hansen & Horsberg 2000). The temperature in the bath was 8°C. At the end of treatment, the average plasma concentration was 0.13 µg ml⁻¹, showing that flumequine is poorly absorbed from seawater; therefore, to obtain adequate concentrations, a higher dose needs to be administered over a longer period of time. The need for a higher concentration in the bath and an extended treatment period were shown in small turbot by Samuelsen (2003). Using a bath concentration of 150 mg l⁻¹ for 72 h, muscle concentrations of 7 µg g⁻¹ after 12 h, increasing to 10.2 µg g⁻¹ after 72 h, were obtained in turbot of 1 to 2 g.

The pharmacokinetic properties of flumequine were also studied in cod following oral (per 0.5, p.o.) administration using medicated feed (10 mg kg^{-1}) and intravenous (i.v.) injection (5 mg kg^{-1}) (Hansen & Horsberg 2000). The fish were 1+ generation, weighed $171 \pm 72 \text{ g}$ and were held in seawater with a temperature of 8°C and salinity of 32‰. The distribution volume, V_{dss} , of 2.4 l kg^{-1} and the total body clearance, Cl_T , of $0.024 \text{ l kg}^{-1} \text{ h}^{-1}$ were all much lower than the values for oxolinic acid found by Samuelsen et al. (2003a), while the plasma elimination half-lives ($t_{1/2\beta}$) values were similar to those of oxolinic acid, with 75 h for the i.v.-injected group and 74 h for the p.o.-administered group. Flumequine obtained an oral bioavailability of 65% in cod. The group of orally treated fish had a C_{max} of $3.5 \mu\text{g ml}^{-1}$ at T_{max} 24 h. In humans, 7-hydroxy-flumequine is a common urinary metabolite of flumequine and is also found in plasma (Harrison et al. 1984, Decolin & Nicolas 1987). This metabolite was not searched for in cod by Hansen & Horsberg (2000), but, in previous studies, it was found only in minor amounts in fish (van der Heiden et al. 1994, Samuelsen & Ervik 1997).

Pharmacokinetic studies in cod include single-dose studies of p.o. (25 mg kg^{-1}) and i.v. (12.5 mg kg^{-1}) administered oxolinic acid and p.o. administration (25 mg kg^{-1}) of the carbitol ester of oxolinic acid (vetoquinol) (Samuelsen et al. 2003a). The fish ranged in weight from 100 to 150 g, and the studies were performed in seawater at 8°C . Bioavailability was calculated as 55% after administration of oxolinic acid as medicated feed (p.o.), while oral administration of oxolinic acid in the form of the carbitol ester raised the bioavailability of oxolinic acid to 72%. Furthermore, the peak plasma concentration (C_{max}) of oxolinic acid increased from 1.2 to $2.5 \mu\text{g ml}^{-1}$, while the time to peak plasma concentration (T_{max}) declined from 24 to 12 h following administration of vetoquinol compared to oxolinic acid. A V_{dss} of 5.5 l kg^{-1} suggests wide distribution of oxolinic acid from plasma into the tissues. Cl_T was measured at $0.047 \text{ l kg}^{-1} \text{ h}^{-1}$, whereas the $t_{1/2\beta}$ values of oxolinic acid in plasma were not influenced by the administration route and were calculated to be 84, 82 and 79 h, respectively, following i.v., p.o. (given as oxolinic acid), or p.o. (given as vetoquinol) administration. If these results are compared with pharmacokinetic data for oxolinic acid and vetoquinol in Atlantic salmon and Atlantic halibut, it is shown that oral administration of oxolinic acid produced higher bioavailability in cod than in salmon and halibut and that vetoquinol enhanced the pharmacokinetic properties such as C_{max} , T_{max} and bioavailability compared to oxolinic acid in all 3 species (Samuelsen & Ervik 1999, Samuelsen et al. 2000, 2003a). Improved clinical efficacy following the administration of vetoquinol should therefore be expected. The slow plasma elimination of

oxolinic acid, especially when compared with Atlantic salmon, suggests a dosage regime with medication every second day and an extended withholding period following medication.

In summary, when comparing the pharmacokinetic properties of oxolinic acid and flumequine in cod with those of other fish species, the most profound differences are the higher bioavailability of the quinolones and the extended elimination.

In a study by Samuelsen et al. (2003b) cod were given florfenicol by intravenous injection (10 mg kg^{-1}) and via oral administration at a dose of 10 mg kg^{-1} in feed. The fish, which ranged in weight from 100 to 200 g, were held in seawater at $8 \pm 0.5^\circ\text{C}$, with a salinity of 33‰. Bioavailability following oral administration was 91%, and the plasma concentration reached a C_{max} of $1.4 \mu\text{g ml}^{-1}$ at a T_{max} of 7 h. A distribution volume of 1.1 l kg^{-1} is similar to those reported for Atlantic salmon and indicates concentrations in tissue equal to those obtained for plasma. When compared with elimination in Atlantic salmon ($\text{Cl}_T = 0.086 \text{ l kg}^{-1} \text{ h}^{-1}$ and $t_{1/2\beta} = 12.2 \text{ h}$) (Martinsen et al. 1993), the elimination of florfenicol was slow in cod, with a Cl_T value of $0.015 \text{ l kg}^{-1} \text{ h}^{-1}$ and $t_{1/2\beta}$ values of 43 and 39 h, respectively, following i.v. and p.o. administration. Florfenicol amine, the deacetylated derivative of florfenicol, is described as a main metabolite of florfenicol in Atlantic salmon and was found in a higher concentration in plasma than florfenicol itself from 48 h after administration and throughout the study (Horsberg et al. 1996). In cod, however, florfenicol amine was not detected in measurable amounts in either plasma or tissues (Samuelsen et al. 2003b). This may indicate that deacetylation is a less important metabolic pathway in cod than in Atlantic salmon and may contribute to the slower elimination of florfenicol in cod.

Oral administration of a combination of ormethoprim and sulphadimethoxine, Romet³⁰, produced rapid absorption and elimination of ormethoprim ($C_{\text{max}} = 1.44 \mu\text{g ml}^{-1}$, $T_{\text{max}} = 7 \text{ h}$, $t_{1/2\beta} = 7 \text{ h}$) and slow absorption and elimination of sulphadimethoxine ($C_{\text{max}} = 20.93 \mu\text{g ml}^{-1}$, $T_{\text{max}} = 59 \text{ h}$, $t_{1/2\beta} = 53 \text{ h}$) (Samuelsen 2006). For a synergistic antibacterial action to occur it is essential that both drugs be available at the site of infection in sufficient amounts at the same time. Therefore, in order to have a synergetic effect the sulphonamide and the enhancer should ideally possess similar pharmacokinetic profiles. Samuelsen's study revealed that a combination of ormethoprim and sulphadimethoxine is not optimal in cod, and he suggested that the pharmacokinetics of alternative sulphonamides should be investigated. It should also be noted that the pharmacokinetics of ormethoprim and sulphadimethoxine in cod differ significantly from the pharmacokinetics in Atlantic salmon described by Samuelsen et al. (1995).

EFFICACY

Only a few papers have been published on the efficacy of antibacterials in treating bacterial infections in cod. The efficacy of oxolinic acid was demonstrated against experimentally induced vibriosis (*Listonella anguillarum*) in cod maintained at 12°C. The fish were challenged by bath using Strain HI-610 and treated orally with a daily dose of either 10 or 20 mg kg⁻¹ on Days 1, 2, 4, 6, 8 and 10 following initiation of treatment (Samuelsen & Bergh 2004). The treatment was initiated 3 d post-challenge. In challenged, unmedicated fish, mortality began on Day 3 post-challenge, reaching a final cumulative mortality of 87.5% at Day 22, when the experiment was terminated. In the medicated groups, the majority of deaths occurred from Day 3 to 5 post-challenge, reaching final cumulative mortalities of 34 and 28%, respectively, for the fish treated with 10 and 20 mg kg⁻¹. Applying the procedure in Inglis et al. (1991), corresponding RPS values were calculated at 61 and 68%, respectively. Using an identical experimental set-up, the efficacy of 10 d of treatment with 10 or 20 mg kg⁻¹ d⁻¹ of florfenicol were studied (Samuelsen & Bergh 2004). Final cumulative mortalities of 31 and 20%, with corresponding RPS values of 64 and 77%, were obtained for the 10 and 20 mg kg⁻¹ dosages, respectively. However, observations made at farms using florfenicol (10 mg kg⁻¹ d⁻¹) showed low efficacy in spite of the isolation of florfenicol-sensitive pathogens. Therefore, due to the discrepancy between the field observations and the laboratory studies, the authors suggest that additional field studies should be performed before a final recommendation is made. The mechanism underlying this problem is unknown.

Flumequine was delivered at 2.5, 5, 10, 15 and 25 mg kg⁻¹ d⁻¹ for the treatment of experimentally induced vibriosis (*Listonella anguillarum*) in cod in a laboratory efficacy study (Vik-Mo et al. 2005). The fish were held in seawater at a temperature of 12°C and challenged by bath using Strain HI-610. The administration started on Day 3 following challenge, and the doses were administered on Days 1, 2, 4, 6, 8 and 10 following initiation of treatment. Mortality was significantly lower in all treatment groups compared to in controls, with a cumulative mortality of 81.7%. The 25 mg kg⁻¹ dose produced a cumulative mortality of 22.8%, which was significantly more efficacious than the dosage of 5 mg kg⁻¹ with a mortality of 48.7%. However, with cumulative mortalities of 42% (2.5 mg kg⁻¹ d⁻¹) and 37% (10 and 15 mg kg⁻¹ d⁻¹), no significant differences in efficacy were observed between the other treatment regimes. Calculated RPS values were 48.8, 40.3, 54.4, 55.1 and 72.1%, respectively, for the 2.5, 5, 10, 15 and

25 mg kg⁻¹ dosages. The authors concluded that a dosage of 25 mg kg⁻¹ d⁻¹ was to be recommended in order to obtain proper treatment.

LITERATURE CITED

- Anonymous (2001) FAO/WHO: evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Food and Agriculture Organization of the United Nations and World Health Organization expert consultation report, FAO, Rome
- Aspehaug D, Devold M, Nylund A (1999) The phylogenetic relationship of nervous necrosis virus from halibut (*Hippoglossus hippoglossus*). Bull Eur Assoc Fish Pathol 19: 196–202
- Austin B, Austin DA, Dalsgaard I, Thornton JM, Larsen JL, O'Hici B, Powell R (1998) Characterization of atypical *Aeromonas salmonicida* by different methods. Syst Appl Microbiol 21(1):50–64
- Ball L (1992) Cellular expression of a functional nodavirus RNA replicon from vaccinia virus vectors. J Virol 66: 2335–2345
- Barke DE, MacKinnon AM, Boston L, Burt MD and 6 others (2002) First report of piscine nodavirus infecting wild winter flounder *Pleuronectes americanus* in Passamaquoddy Bay, New Brunswick, Canada. Dis Aquat Org 49:99–105
- Ben-Haim Y, Thompson FL, Thompson CC, Cnockaert MC, Hoste B, Swings J, Rosenberg E (2003) *Vibrio coralliilyticus* sp. nov. a temperature-dependent pathogen of the coral *Pocillopora damicornis*. Int J Syst Evol Microbiol 53: 309–315
- Bergh Ø (2000) Bacterial pathogens associated with early life stages of marine fish. In: Bell CR, Brylinski M, Johnson-Green P (eds) Microbial biosystems: new frontiers. Proceedings of the 8th international symposium on microbial ecology. Atlantic Canada Society for Microbial Ecology, Halifax, p 221–228
- Bergh Ø, Hansen GH, Taxt RE (1992) Experimental infection of eggs and yolk sac larvae of halibut, *Hippoglossus hippoglossus* L. J Fish Dis 15:379–391
- Bergh Ø, Hjeltnes B, Skiftesvik AB (1997) Experimental infection of turbot, *Scophthalmus maximus* and halibut *Hippoglossus hippoglossus* yolk sac larvae with *Aeromonas salmonicida* subsp. *salmonicida*. Dis Aquat Org 29:13–20
- Bergh Ø, Nilsen F, Samuelsen OB (2001) Diseases, prophylaxis and treatment of the Atlantic halibut, *Hippoglossus hippoglossus*: a review. Dis Aquat Org 48:57–74
- Bergman AM (1912) Eine ansteckende Augenkrankheit, Keratomalacie, bei Dorsch an der Südküste Schwedens. Zentbl Bakteriell Parasitenkd Infektionskr Hyg Abt 1 Orgin 62:200–212
- Bergstad OA, Jørgensen T, Dragesund O (1987) Life history and ecology of the gadoid resources of the Barents Sea. Fish Res 5:119–161
- Biering E, Bergh O (1996) Experimental infection of Atlantic halibut, *Hippoglossus hippoglossus* L, yolk-sac larvae with infectious pancreatic necrosis virus: detection of virus by immunohistochemistry and *in situ* hybridization. J Fish Dis 19:405–413
- Biering E, Nilsen F, Rødseth O, Glette J (1994) Susceptibility of Atlantic halibut *Hippoglossus hippoglossus* to infectious pancreatic necrosis virus. Dis Aquat Org 20:183–190
- Bonami JR, Hasson KW, Mari J, Poulos BT, Lightner DV (1997) Taura syndrome of marine penaeid shrimp: characterization of the viral agent. J Gen Virol 78 (2):313–319

- Breuil G, Romestand B (1999) A rapid ELISA method for detecting specific antibody level against nodavirus in the serum of the sea bass, *Dicentrarchus labrax* (L.): application to the screening of spawners in a sea bass hatchery. *J Fish Dis* 22:45–52
- Breuil G, Pepin JPF, Boscher S, Thiery R (2002) Experimental vertical transmission of nodavirus from broodfish to eggs and larvae of the sea bass, *Dicentrarchus labrax* (L.). *J Fish Dis* 25(129):697–702
- Colwell RR, Grimes DJ (1984) *Vibrio* diseases of marine fish populations. *Helgol Meeresunters* 27:265–287
- Dacanay A, Bentley EB, Brown LL, Johnson SC (2004) Studies of immunoglobulin structure and diversity in non-salmonid marine fish. In: Proceedings of the 6th international symposium on fish immunology. Turku, p 30
- Daggfeldt A, Bengtén E, Pilström L (1993) A cluster type organization of the loci of the immunoglobulin light chain in Atlantic cod (*Gadus morhua* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum) indicated by nucleotide sequences of cDNAs and hybridization analysis. *Immunogenetics* 38:199–209
- Dahl E, Danielssen E, Moksness E, Solemdal P (eds) (1984) The propagation of cod *Gadus morhua* L. Flødevigen Rapp 1(2):1–895
- Dalla Valle L, Negrisolo E, Patarnello P, Zanella L, Maltese C, Bovo G, Colombo L (2001) Sequence comparison and phylogenetic analysis of fish nodaviruses based on the coat protein gene. *Arch Virol* 146:1125–1137
- Dalmo RA, Seternes T, Arnesen SM, Jørgensen TØ, Bøgvold J (1998) Tissue distribution and cellular uptake of *Aeromonas salmonicida* lipopolysaccharide (LPS) in some marine fish species. *J Fish Dis* 21:321–334
- Dalsgaard I, Gudmundsdóttir BK, Helgason S, Hoie S, Thoresen OF, Wichardt UP, Wiklund T (1998) Identification of atypical *Aeromonas salmonicida*: inter-laboratory evaluation and harmonization of methods. *J Appl Microbiol* 84(6):999–1006
- Decolin D, Nicolas A (1987) Determination of flumequine and its 7-hydroxy metabolite by reversed-phase high-performance liquid chromatography. *J Chromatogr* 414: 499–503
- Duncan R, Dobos P (1986) The nucleotide sequence of infectious pancreatic necrosis virus (IPNV) dsRNA segment A reveals one large ORF encoding a precursor polyprotein. *Nucleic Acids Res* 14:5934
- Duncan R, Nagy E, Krell PJ, Dobos P (1987) Synthesis of the infectious pancreatic necrosis virus polyprotein, detection of a virus-encoded protease, and fine structure mapping of genome segment A coding regions. *J Virol* 61:3655–3664
- Egidius E (1987) Vibriosis: pathogenicity and pathology. a review. *Aquaculture* 67:15–28
- Egidius E, Andersen K (1984) Disease problems in cod rearing. In: Dahl E, Danielsen D, Moksness E, Solemdal P (eds) The propagation of cod *Gadus morhua* L. Flødevigen Rapp 1(2):103–105
- Egidius E, Braaten B, Andersen K, Lohne Gokstad S (1983) Vibriosis in saithe (*Pollachius virens*) populations off the Norwegian coast. *Rapp P-V Reun Cons Int Explor Mer* 182:103–105
- Espelid S, Rødseth OM, Jørgensen TØ (1991) Vaccination experiments and studies of the humoral immune responses in cod, *Gadus morhua* L., to four strains of monoclonal-defined *Vibrio anguillarum*. *J Fish Dis* 14:185–197
- Essbauer S, Ahne W (2001) Viruses of lower vertebrates. *J Vet Med Ser B* 48:403–475
- Frerichs GN, Tweedie A, Starkey WG, Richards RH (2000) Temperature, pH and electrolyte sensitivity, and heat, UV and disinfectant inactivation of sea bass (*Dicentrarchus labrax*) neuropathy nodavirus. *Aquaculture* 185:13–24
- Fukuda Y, Nguyen HD, Furuhashi M, Nakai T (1996) Mass mortality of cultured sevenband grouper, *Epinephelus septemfasciatus*, associated with viral nervous necrosis. *Fish Pathol* 31:165–170
- Gagne N, Johnson SC, Cook-Versloot M, MacKinnon AM, Olivier G (2004) Molecular detection and characterization of nodavirus in several marine fish species from the north-eastern Atlantic. *Dis Aquat Org* 62:181–189
- Gatesoupe FJ (1999) The use of probiotics in aquaculture. *Aquaculture* 180:147–165
- Gildberg A, Mikkelsen H (1997) Effects of supplementing the feed of Atlantic cod (*Gadus morhua*) fry with lactic acid bacteria and immuno-stimulating peptides during challenge trial with *Vibrio anguillarum*. *Aquaculture* 167:103–113
- Gildberg A, Mikkelsen H, Sandaker E, Ringo E (1997) Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (*Gadus morhua*). *Hydrobiologia* 352:279–285
- Gram L, Melchiorsen J, Spanggaard B, Huber I, Nielsen TF (1999) Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* strain AH2—a possible probiotic treatment of fish. *Appl Environ Microbiol* 65:969–973
- Gram L, Løvold T, Nielsen J, Melchiorsen J, Spanggaard B (2001) *In vitro* antagonism of the probiont *Pseudomonas fluorescens* strain AH2 against *Aeromonas salmonicida* does not confer protection of salmon against furunculosis. *Aquaculture* 199:1–11
- Grotmol S, Totland GK, Thorud K, Hjeltnes BK (1997) Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: a probable cause of mass mortality of cultured larval and juvenile Atlantic halibut *Hippoglossus hippoglossus*. *Dis Aquat Org* 29:85–97
- Grotmol S, Bergh Ø, Totland GK (1999) Transmission of viral encephalopathy and retinopathy (VER) to yolk-sac larvae of the Atlantic halibut *Hippoglossus hippoglossus*: occurrence of nodavirus in various organs and a possible route of infection. *Dis Aquat Org* 36:95–106
- Grotmol S, Nerland A, Biering E, Totland G, Nishizawa T (2000) Characterisation of the capsid protein gene from a nodavirus strain affecting the Atlantic halibut *Hippoglossus hippoglossus* and design of an optimal reverse-transcriptase polymerase chain reaction (RT-PCR) detection assay. *Dis Aquat Org* 39:79–88
- Gudmundsdóttir BK, Björnsdóttir B, Magnadóttir B, Gudmundsdóttir S (2004a) Experiments to vaccinate Atlantic cod (*Gadus morhua*) by *Aeromonas salmonicida*, *Listonella anguillarum* and *Moritella viscosa*. In: Proceedings of the 6th international symposium on fish immunology. ?, Turku, p 49
- Gudmundsdóttir S, Gudmundsdóttir BK, Björnsdóttir B, Magnadóttir B (2004b) Increased survival of cod larvae bathed with LPS or a bacterium of a *Vibrio* sp. In: Proceedings of the 6th international symposium on fish immunology. Turku, p 34
- Hansen GH, Olafsen JA (1988) *Leucothrix mucor*—en skadelig påvekstorganisme i intensivt marint oppdrett. *Norsk Fiskeoppdrett* 7:62–63
- Hansen GH, Olafsen JA (1989) Bacterial colonisation of cod (*Gadus morhua* L.) and halibut (*Hippoglossus hippoglossus*) eggs in marine aquaculture. *Appl Environ Microbiol* 55:1435–1446
- Hansen GH, Bergh Ø, Michaelsen J, Knappskog D (1992) *Flexibacter ovolyticus* sp. nov., a pathogen of eggs and larvae of Atlantic halibut, *Hippoglossus hippoglossus* L. *Int J Syst Bacteriol* 42(3):451–458

- Hansen MK, Horsberg TE (2000) Single-dose pharmacokinetics of flumequine in cod (*Gadus morhua*) and goldsinny wrasse (*Ctenolabrus rupestris*). *J Vet Pharmacol Ther* 23: 163–168
- Hansen T, Karlsen Ø, Taranger GL, Hemre GI, Holm JC, Kjesbu OS (2001) Growth and sexual maturation of Atlantic cod (*Gadus morhua*) reared under different photoperiods. *Aquaculture* 203:51–67
- Harrison LI, Schuppan D, Rohlfing SR, Hansen AR, Hansen CS, Funk ML, Collins MS, Ober RE (1984) Determination of flumequine and a hydroxy metabolite in biological fluids by high-pressure liquid chromatographic, fluorometric and microbiological methods. *Antimicrob Agents Chemother* 25:301–305
- Hemmingsen W, MacKenzie K (2001) The parasite fauna of the Atlantic cod, *Gadus morhua* L. *Adv Mar Biol* 40:2–60
- Hilger I, Ullrich S, Anders K (1991) A new ulcerative flexibacteriosis-like disease ('yellow pest') affecting young Atlantic cod *Gadus morhua* from the German Wadden Sea. *Dis Aquat Org* 11:19–29
- Hjelm M, Bergh Ø, Nielsen J, Melchiorson J and 6 others (2004) Selection and identification of autochthonous potential probiotic bacteria from turbot larvae (*Scophthalmus maximus*) rearing units. *Syst Appl Microbiol* 27: 360–371
- Hjeltnes B, Bergh Ø, Wergeland H, Holm JC (1995) Susceptibility of Atlantic cod *Gadus morhua*, halibut *Hippoglossus hippoglossus* and wrasse (Labridae) to *Aeromonas salmonicida* subsp. *salmonicida* and the possibility of transmission of furunculosis from farmed salmon *Salmo salar* to marine fish. *Dis Aquat Org* 23:25–31
- Hordvik I, Thevarajan J, Samdal I, Bastani N, Krossøy B (1999) Molecular cloning and phylogenetic analysis of the Atlantic salmon immunoglobulin D gene. *Scand J Immunol* 50:202–210
- Horsberg TE, Hoff KA, Nordmo R (1996) Pharmacokinetics of florfenicol and its metabolite florfenicol amine in Atlantic salmon. *J Aquat Anim Health* 8:292–301
- Hús-gard S, Grotmol S, Hjeltnes BK, Rødseth OM, Biering E (2001) Immune response to a recombinant capsid protein of striped jack nervous necrosis virus (SJNNV) in turbot *Scophthalmus maximus* and Atlantic halibut *Hippoglossus hippoglossus*, and evaluation of a vaccine against SJNNV. *Dis Aquat Org* 45:33–44
- Inglis V, Richards RH, Varma KJ, Sutherland IH, Brokken ES (1991) Florfenicol in Atlantic salmon parr: tolerance and assessment of efficacy against furunculosis. *J Fish Dis* 14: 343–351
- Israelsson O, Peterson A, Bengtén E, Wiersma EJ, Andersson J, Gezelius G, Pilström L (1991) Immunoglobulin concentration in Atlantic cod, *Gadus morhua* L.; serum and cross-reactivity between anti-cod-antibodies and immunoglobulins from other species. *J Fish Biol* 39:265–278
- Jakobsson J, Astthorsson OS, Beverton RJH, Björnsson B and 6 others (eds) (1994) Cod and climate change. Proceedings of a symposium held in Reykjavik. *ICES Mar Sci Symp* 198:1–699
- Jensen NJ (1983) The ulcer syndrome in cod (*Gadus morhua*): a review. *Rapp P-V Reun Cons Int Explor Mer* 182:58–64
- Johansen R, Grove S, Svendsen A, Modahl I, Dannevig B (2004) A sequential study of pathological findings in Atlantic halibut, *Hippoglossus hippoglossus* (L.), throughout one year after an acute outbreak of viral encephalopathy and retinopathy. *J Fish Dis* 27:327–341
- Johnson SC, Sperker SA, Leggiadro CT, Groman DB, Griffiths SG, Ritchie RJ, Cook MD, Cusack RR (2002) Identification and characterization of a piscine neuropathy and nodavirus from juvenile Atlantic cod from the Atlantic coast of North America. *J Aquat Anim Health* 14:124–133
- Jørgensen T, Midling K, Espelid S, Nilsen R, Stensvåg K (1989) *Vibrio salmonicida*, a pathogen in salmonids, also causes mortality in net-pen captured cod (*Gadus morhua*). *Eur Assoc Fish Pathol* 9:42–43
- King JA, Snow M, Smail DA, Raynard RS (2001) Distribution of viral haemorrhagic septicaemia virus in wild fish species of the North Sea, north east Atlantic Ocean and Irish Sea. *Dis Aquat Org* 47:81–86
- Kjørsvik E, van der Meeren T, Kryvi H, Arnfinnson J, Kvenseth PG (1991) Early development of the digestive tract of cod larvae, *Gadus morhua* L., during start-feeding and starvation. *J Fish Biol* 38:1–15
- Kleinow KM, Jarboe HH, Shoemaker KE (1994) Comparative pharmacokinetics and bioavailability of oxolinic acid in channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*). *Can J Fish Aquat Sci* 51: 1205–1211
- Knappskog DH, Rødseth OM, Slinde E, Endresen C (1993) Immunochemical analyses of *Vibrio anguillarum* strains isolated from cod, *Gadus morhua* L., suffering from vibriosis. *J Fish Dis* 16:327–338
- Korsnes K, Nicolaisen O, Skår CK, Nerland AH, Bergh Ø (2006) Bacteria in the gut of juvenile cod *Gadus morhua* fed live feed enriched with four different commercial diets. *ICES J Mar Sci* 63:296–301
- Kurlansky M (1999) Cod: a biography of the fish that changed the world. Vintage, London
- Larsen JL, Pedersen K, Dalsgaard I (1994) *Vibrio anguillarum* serovars associated with vibriosis in fish. *J Fish Dis* 17: 259–267
- Le Breton A, Grisez L, Sweetman J, Ollevier F (1997) Viral nervous necrosis (VNN) associated with mass mortalities in cage-reared sea bass, *Dicentrarchus labrax* (L.). *J Fish Dis* 20:145–151
- Lund V, Børdal S, Kjellsen O, Mikkelsen H, Schrøder MB (2006) Comparison of antibody responses in Atlantic salmon (*Gadus morhua* L.) to *Aeromonas salmonicida* and *Vibrio anguillarum*. *Dev Comp Immunol* 30:1145–1155
- Magnadóttir B, Jonsdóttir H, Helgason S, Björnsson B, Solem ST, Pilström L (2001) Immune parameters of immunised cod (*Gadus morhua* L.). *Fish Shellfish Immunol* 11:75–89
- Magnadóttir B, Crispin M, Royle L, Colominas C, Harvey DJ, Dwek RA, Rudd PM (2002a) The carbohydrate moiety of serum IgM from Atlantic cod (*Gadus morhua* L.). *Fish Shellfish Immunol* 12(3):209–227
- Magnadóttir B, Bambir SH, Gudmundsdóttir BK, Pilström L, Helgason S (2002b) Atypical *Aeromonas salmonicida* infection in naturally and experimentally infected cod, *Gadus morhua* L. *J Fish Dis* 25:583–597
- Martinsen B, Horsberg TE, Varma KJ, Sams R (1993) Single dose pharmacokinetic study of florfenicol in Atlantic salmon (*Salmo salar*) in seawater at 11°C. *Aquaculture* 112:1–11
- Martinsen B, Horsberg TE, Ingebrigtsen K, Gross IL (1994) Disposition of ¹⁴C-sarafloxacin in Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) as demonstrated by means of whole-body autoradiography and liquid scintillation counting. *Dis Aquat Org* 18:37–44
- Meyers TR, Sullivan J, Emmenegger E, Follett J, Shorts S, Batts WN, Winton JR (1992) Identification of viral hemorrhagic septicemia virus isolated from Pacific cod *Gadus macrocephalus* in Prince William sound, Alaska, USA. *Dis Aquat Org* 12:167–175
- Mikkelsen H, Schrøder MB, Lund V (2004) Vibriosis and atypi-

- cal furunculosis vaccines; efficacy, specificity and side effects in Atlantic cod, *Gadus morhua* L. *Aquaculture* 242:81–91
- Mori KI, Nakai T, Muroga K, Arimot M, Mushiaki K, Furusawa I (1992) Properties of a new virus belonging to nodaviridae found in larval striped jack (*Pseudocaranx dentex*) with nervous necrosis. *Virology* 187:368–371
- Mortensen HF, Heuer OE, Lorenzen N, Otte L, Olesen NJ (1999) Isolation of viral haemorrhagic septicaemia virus from wild marine fish species in the Baltic Sea, Kattegat, Skagerrak and the North Sea. *Virus Res* 63:95–106
- Munday B, Kwang J, Moody N (2002) Betanodavirus infections of teleost fish: a review. *J Fish Dis* 25:127–142
- Mushiaki K, Nishizawa T, Nakai T, Furusawa I, Muroga K (1994) Control of VNN in striped jack: selection of spawners based on the detection of SJNNV gene by polymerase chain reaction (PCR). *Fish Pathol* 29:177–182
- Nishisawa T, Mori K, Furuhashi M, Nakai T, Furusawa I, Muroga K (1995) Comparison of the coat protein genes of five fish nodaviruses, the causative agents of viral nervous necrosis in marine fish. *J Gen Virol* 76:1563–1569
- Nishizawa T, Furuhashi M, Nagai T, Nakai T, Muroga K (1997) Genomic classification of fish nodaviruses by phylogenetic analysis of the coat protein gene. *Appl Environ Microbiol* 63:1633–1636
- Nylund A, Ottem KF, Watanabe K, Karlsbakk E, Krossøy B (2006) *Francisella* sp. (family Francisellaceae) causing mortality in Norwegian cod (*Gadus morhua*) farming. *Arch Microbiol* 185:383–392
- Øiestad V, Kvenseth PG, Folkvord A (1985) Mass production of Atlantic cod juveniles (*Gadus morhua*) in a Norwegian saltwater pond. *Trans Am Fish Soc* 114:590–595
- Olafsen JA, Hansen GH (1992) Intact antigen uptake in intestinal epithelial cells of marine fish larvae. *J Fish Biol* 40:141–156
- Olsen AB, Mikalsen J, Rode M, Alfjorden A, Hoel E, Straum-Lie K, Haldorsen R, Colquhoun DJ (2006) A novel systemic granulomatous inflammatory disease in farmed Atlantic cod, *Gadus morhua* L, associated with a bacterium belonging to the genus *Francisella*. *J Fish Dis* 29:307–311
- Pedersen GM, Gildberg A, Olsen RL (2004) Effects of including cationic proteins from cod milt in the feed of Atlantic cod (*Gadus morhua*) fry during a challenge trial with *Vibrio anguillarum*. *Aquaculture* 233:31–43
- Pedersen K, Grisez L, van Houdt R, Tiainen T, Ollevier F, Larsen JL (1999) Extended serotyping scheme for *Vibrio anguillarum* with the definition and characterization of seven provisional O-serogroups. *Curr Microbiol* 38:183–189
- Pedersen T, Falk-Petersen IB (1992) Morphological changes during metamorphosis in cod (*Gadus morhua* L.), with particular reference to the development of the stomach and pyloric caeca. *J Fish Biol* 41:449–461
- Pilström L, Warr, GW, Strömberg S (2005) Why is the antibody response of Atlantic cod so poor? The search for a genetic explanation. *Fish Sci* 71:961–971
- Planas M, Perez-Lorenzo M, Hjelm M, Gram L, Uglenes Fiksdal I, Bergh Ø, Pintado J (2006) Probiotic effect in vivo of *Roseobacter* strain 27-4 against *Vibrio* (*Listonella*) *anguillarum* infection in turbot (*Scophthalmus maximus* L.) larvae. *Aquaculture* 255:323–333
- Price B, Rueckert R, Ahlquist P (1996) Complete replication of an animal virus and maintenance of expression vectors derived from it in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 93:9465–9470
- Putnam FW, Takahashi N, Tetaert D, Lin LC, Debuire B (1982) The last of the immunoglobulins. Complete amino acid sequence of human IgD. *Ann NY Acad Sci* 399:41–68
- Romalde JL (1999) Genetic analysis of turbot pathogenic *Streptococcus paruberis* strains by ribotyping and random amplified polymorphic DNA. *FEMS Microb Lett* 179:297–304
- Samuelsen OB (2003) Administration of the antibacterial agents flumequine and oxolinic acid to small turbot (*Scophthalmus maximus* L.) by bath. *J Appl Ichthyol* 19:55–58
- Samuelsen OB (2006) Absorption, tissue distribution, metabolism and excretion of ormetoprim and sulphadimethoxine in cod (*Gadus morhua*) after oral administration of Romet. *J Appl Ichthyol* 22:68–71
- Samuelsen OB, Bergh Ø (2004) Efficacy of orally administered florfenicol and oxolinic acid in the treatment of vibriosis in cod (*Gadus morhua* L.). *Aquaculture* 235:27–35
- Samuelsen OB, Ervik A (1997) Single dose pharmacokinetic study of flumequine after intravenous, intraperitoneal and oral administration to Atlantic halibut (*Hippoglossus hippoglossus*) held in seawater at 9°C. *Aquaculture* 158:215–227
- Samuelsen OB, Ervik A (1999) A single-dose pharmacokinetic study of oxolinic acid and vetoquinol, an oxolinic acid ester, in Atlantic halibut (*Hippoglossus hippoglossus*) held in seawater at 9°C. *J Fish Dis* 22:13–23
- Samuelsen OB, Ervik A, Wennevik V (1995) Absorption, tissue distribution, metabolism and excretion of ormetoprim and sulphadimethoxine in Atlantic salmon (*Salmo salar*) after intravenous and oral administration of Romet 30. *Xenobiotica* 25(11):1169–1180
- Samuelsen OB, Ervik A, Pursell L, Smith P (2000) Single-dose pharmacokinetic study of oxolinic acid and vetoquinol, an oxolinic acid ester, in Atlantic salmon (*Salmo salar*) held in seawater and *in vitro* antibacterial activity against *Aeromonas salmonicida*. *Aquaculture* 187:213–224
- Samuelsen OB, Bergh Ø, Ervik A (2003a) A single-dose pharmacokinetic study of oxolinic acid and vetoquinol, an oxolinic acid ester, in cod (*Gadus morhua* L.) held in seawater at 8°C and *in vitro* antibacterial activity of oxolinic acid against some *Vibrio* sp. isolated from diseased cod. *J Fish Dis* 26:339–347
- Samuelsen OB, Bergh Ø, Ervik A (2003b) A single-dose pharmacokinetic study of florfenicol in cod (*Gadus morhua* L.) held in seawater at 8°C and *in vitro* antibacterial activity against some *Vibrio anguillarum* strains isolated from diseased cod. *Dis Aquat Org* 56:127–133
- Santos Y, Pazos F, Bandin I, Toranzo AE (1995) Analysis of antigens present in the extracellular products and cell-surface of *Vibrio anguillarum* Serotypes O1, O2 and O3. *Appl Environ Microbiol* 61:2493–2498
- Scherer WF, Hurlbut HS (1967) Nodamura virus from Japan: a new and unusual arbovirus resistant to diethyl ether and chloroform. *Am J Epidemiol* 86:271–285
- Schröder MB, Espelid S, Jørgensen TØ (1992) Two serotypes of *Vibrio salmonicida* isolated from diseased cod (*Gadus morhua* L.); virulence, immunological studies and vaccination experiments. *Fish Shellfish Immunol* 2:211–221
- Schröder MB, Villena AJ, Jørgensen TØ (1998) Ontogeny of lymphoid organs and immunoglobulin producing cells in Atlantic cod (*Gadus morhua* L.). *Dev Comp Immunol* 22:507–517
- Schröder MB, Børdal S, Lund V, Gravningen K, Mikkelsen H (2006) Early vaccination and protection of Atlantic cod (*Gadus morhua* L.) juveniles against classical vibriosis. *Aquaculture* 254:46–53
- Selling BH, Allison RF, Kaesberg P (1990) Genomic RNA of an insect virus directs synthesis of infectious virions in plants. *Proc Natl Acad Sci USA* 87:434–438
- Shelbourne JE (1964) The artificial propagation of marine fish. *Adv Mar Biol* 2:1–83

- Skall HF, Møllergaard S, Olesen NJ (2000) Isolation of Birnavirus serogroup B in wild and aquacultured fish species. *Bull Eur Assoc Fish Pathol* 20:229–236
- Skjermo J, Bergh O (2004) High-M alginate immunostimulation of Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae using *Artemia* for delivery, increases resistance against vibriosis. *Aquaculture* 238:1–4
- Smail DA (2000) Isolation and characterisation of viral haemorrhagic septicaemia (VHS) viruses from cod *Gadus morhua* with the ulcer syndrome and from haddock *Melanogrammus aeglefinus* having skin haemorrhages in the North Sea. *Dis Aquat Org* 41:231–235
- Snow M, Smail DA (1999) Experimental susceptibility of turbot *Scophthalmus maximus* to viral haemorrhagic septicaemia virus isolated from cultivated turbot. *Dis Aquat Org* 38:163–168
- Snow M, Cunningham CO, Bricknell IR (2000) Susceptibility of juvenile Atlantic cod *Gadus morhua* to viral haemorrhagic septicaemia virus isolated from wild-caught Atlantic cod. *Dis Aquat Org* 41:225–229
- Snow M, Bain N, Black J, Taupin V, Cunningham CO, King JA, Skall HF, Raynard RS (2004) Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). *Dis Aquat Org* 61:11–21
- Solem ST, Stenvik J (2006) Antibody repertoire development in teleosts—a review, with emphasis on salmonids and *Gadus morhua* L. *Dev Comp Immunol* 30:57–76
- Solem ST, Hordvik I, Killie JEA, Warr GW, Jørgensen TØ (2001) Diversity of the immunoglobulin heavy chain in the Atlantic salmon (*Salmo salar* L.) is contributed by genes from two parallel IgH isoloci. *Dev Comp Immunol* 25:403–417
- Solemdal P, Dahl E, Danielssen DS, Moksness E (1984) The cod hatchery in Flødevigen—background and realities. In: Dahl E, Danielssen ES, Moksness E, Solemdal P (eds) The propagation of cod (*Gadus morhua* L.). Flødevigen Rapp 1:17–45
- Sommerset I, Nerland A (2004) Complete sequence of RNA1 and subgenomic RNA3 of Atlantic halibut nodavirus (AHNV). *Dis Aquat Org* 58:117–125
- Sommerset I, Lorenzen E, Lorenzen N, Bleie H, Nerland A (2003) A DNA vaccine directed against a rainbow trout rhabdovirus induces early protection against a nodavirus challenge in turbot. *Vaccine* 21:4661–4667
- Sommerset I, Skern R, Biering E, Bleie H, Fiksdal I, Grove S, Nerland A (2005) Protection against Atlantic halibut nodavirus in turbot is induced by recombinant capsid protein vaccination but not following DNA vaccination. *Fish Shellfish Immunol* 18:13–29
- Starkey WG, Ireland JH, Muir KF, Jenkins ME, Roy WJ, Richards RH, Ferguson HW (2001) Nodavirus infection in Atlantic cod and Dover sole in the UK. *Vet Rec* 149:179–181
- Stenvik J, Jørgensen TØ (2000) Immunoglobulin D (IgD) of Atlantic cod has a unique structure. *Immunogenetics* 51:452–461
- Stenvik J, Lundbäck A, Jørgensen TØ, Pilström L (2000) Variable region diversity of the Atlantic cod (*Gadus morhua* L.) immunoglobulin heavy chain. *Immunogenetics* 51:670–680
- Stenvik J, Schrøder MB, Olsen K, Zapata A, Jørgensen TØ (2001) Expression of immunoglobulin heavy chain transcripts (VH-families, IgM, and IgD) in head kidney and spleen of the Atlantic cod (*Gadus morhua* L.). *Dev Comp Immunol* 25:291–302
- Stone DM, Way K, Dixon PF (1997) Nucleotide sequence of the glycoprotein gene of viral haemorrhagic septicaemia (VHS) viruses from different geographical areas. A link between VHS in farmed fish species and viruses isolated from North Sea cod (*Gadus morhua* L.). *J Gen Virol* 78:1319–1326
- Strøm E, Ringø E (1993) Changes in the bacterial composition of early developing cod, *Gadus morhua* (L.) larvae following inoculation of *Lactobacillus plantarum* into the water. In: Walther BT, Fyhn HJ (eds) Physiological and biochemical aspects of fish development. Zoological Institute, University of Bergen, p 226–228
- Svåsand T, Kristiansen TS, Pedersen T, Salvanes AGV, Engelsen R, Nævdal G, Nødtvedt M (2000) The enhancement of cod stocks. *Fish Fish* 1:173–205
- Svåsand T, Otterå H, Taranger GL (2004) Atlantic cod (*Gadus morhua*). In: Moksness E, Kjørsvik E, Olsen Y (eds) Culture of cold-water marine fish. Fishing News Books, Blackwell Scientific, London, p 433–444
- Treves-Brown KM (2000) Applied fish pharmacology. Kluwer Academic Publishers, Dordrecht
- Vadstein O, Mo TA, Bergh Ø (2004) Microbial interactions, prophylaxis and diseases. In: Moksness E, Kjørsvik E, Olsen Y (eds) Culture of cold-water marine fish. Fishing News Books, Blackwell Scientific, London, p 28–72
- van der Heiden MHT, Keukens HJ, van den Nieuwboer WHFX, Mengelers MJB, Boon JH (1994) Plasma disposition of flumequine in common carp (*Cyprinus carpio* L., 1758), African catfish (*Clarias gariepinus* Burchell, 1822) and European eel (*Anguilla anguilla* L., 1758) after a single per oral administration. *Aquaculture* 123:21–30
- Vik-Mo F, Bergh Ø, Samuelsen OB (2005) Efficacy of orally administered flumequine in the treatment of vibriosis caused by *Listonella anguillarum* in cod *Gadus morhua*. *Dis Aquat Org* 67:87–92
- Wiik R, Hoff KA, Andersen K, Daae FL (1989) Relationships between plasmids and phenotypes of presumptive strains of *Vibrio anguillarum* isolated from different fish species. *Appl Environ Microbiol* 55:826–831
- Wiik R, Stackebrandt E, Valle O, Daae FL, Rødseth OM, Andersen K (1995) Classification of fish pathogenic vibrios based on comparative 16S rRNA analysis. *Int J Syst Bacteriol* 45:421–428
- Wiklund T, Dalsgaard I (1998) Occurrence and significance of atypical *Aeromonas salmonicida* in non-salmonid and salmonid fish species: a review. *Dis Aquat Org* 32:49–69
- Wilson M, Bengtén E, Miller NW, Clem LW, Du Pasquier L, Warr GW (1997) A novel chimeric Ig heavy chain from a teleost fish shares similarities to IgD. *Proc Natl Acad Sci USA* 94:4593–4597
- Wolf K (1988) Fish viruses and fish viral diseases. Cornell University Press, Ithaca