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Advances in disease diagnosis, vaccine development and other emerging methods to control pathogens in aquaculture

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Abstract: Disease is still regarded as a major constraint to aquaculture production globally. Rapid disease diagnosis and vaccination play a huge part in the control of bacterial diseases, and there has been significant progress in both of these areas. This chapter considers the limitations of existing methods and reviews recent advances made in pathogen detection technologies and vaccine development methodologies. Future directions are discussed, including nanotechnology and reversed vaccinology.

Key words: fish disease, fish health, disease diagnosis, vaccine development, pathogen detection technologies.

7.1 Introduction

Disease is still regarded as a major constraint to aquaculture production globally (Adams *et al*., 2005). As the industry continues to expand and diversify, the risk of new diseases emerging and old ones spreading to other geographical regions is a reality. The movement of eggs and fry between fish farms presents ideal circumstances for pathogens to adapt with their hosts and environment. Control of pathogens is complex and relies heavily on a combination of pathogen detection, disease diagnosis, treatment, prevention and general health management. Rapid disease diagnosis and vaccination play a huge part in this, and there has been significant progress in both of these areas since the 1980s. In the years since 2000 the pace has increased even more as methods developed for clinical and veterinary medicine are rapidly adapted and optimised for use in

aquaculture. Novel diagnostic methods are published frequently and the success of vaccination in reducing the use of antibiotics has been realised, at least in some countries. Innovative alternative methods for the control of fish diseases are also being researched and applied. This chapter aims to provide a review of the recent advances made in disease diagnosis, vaccine development and other emerging methods to control pathogens in aquaculture.

7.2 Key drivers to improve disease diagnosis and vaccine development

The main key driver to improve disease diagnosis and vaccine development is the continued significant losses to the industry caused by pathogens. Bacterial diseases cause a substantial economic burden to the aquaculture industry and, although antibiotics and chemotherapeutants are extensively used to control disease outbreaks, there is increasing concern about their use because of drug residues in food, the development of antimicrobial drug resistance and the detrimental effect on the aquatic microbial ecosystems and populations (Thompson and Adams, 2004). There is a concerted effort to move away from the use of antibiotics wherever possible. This was highlighted in September 2008 in Korea with the announcement that from next year the Korea Food and Drug Administration will ban the use of seven types of antibiotics in feed for livestock and fish raised in fish farms. In the UK, Norway and Japan there has already been a significant reduction in the use of antibiotics since the 1990s (Adams *et al*., 2005; Markestad and Grave, 1997).

There is a real need to increase production of fish globally through aquaculture and therefore health and welfare must be given a high priority if targets set are to be reached. Some vaccines, in particular multivalent vaccines, have led to welfare concerns in the past few years due to the presence of adhesions on internal organs, thought to result from oil-based adjuvants in the vaccines (Berg *et al*., 2007). Farming of new species will play a significant role in increasing production. Persistent disease problems in cod (Vibriosis in particular) have played a large part in this new industry stagnating in Scotland. As cobia farming increases globally, and with the first farms being licensed in Brazil (Eric Routledge, Special Secretariat for Aquaculture and Fisheries, SEAP, Brazil; pers comm), there will be a need for farmers to be one step ahead of potential disease threats.

7.3 Limitations of current diagnostic methods

Many of the current techniques for the detection of pathogens and diagnosis of diseases are actually very good. On the other hand, identification of

certain pathogens is difficult to achieve and some of the methods developed may be too complicated to apply or interpret. Conventional pathogen isolation and characterisation techniques, alongside pathology, still remain the methods of choice for the diagnosis of many diseases. However, these traditional methods tend to be costly, labour-intensive, and slow, and might not always lead to a definitive diagnosis being made. The rapid progress made in biotechnology since the 1990s has enabled the development and improvement of a wide range of immunodiagnostic and molecular techniques (Cunningham, 2004; Adams and Thompson, 2006, 2008), and reagents and commercial kits have become more widely available. These rapid methods both complement and enhance the traditional methods of disease diagnosis.

The *Manual of Diagnostic Tests for Aquatic Animals* (World Organisation for Animal Health, 2006) includes a variety of standardised methods (including traditional, immunological and molecular methods) for the identification of selected pathogens (causing notifiable diseases), and these will expand as new methods are developed and validated (Adams and Thompson, 2008). Most of these are, however, for viral diseases, and *Renibacteriun salmoninarum* and *Piscirickettsia salmonis*, the causative agents of bacterial kidney disease (BKD) and ricketsiosis, respectively, are the only bacterial pathogens included. The diseases caused by these pathogens do not meet the listing criteria, but they are included because reporting requirements for non-listed diseases apply in regard to significant epidemiological events. For those diseases not included in the Animal Health Code there are no set standards. It is important that reagents and methods used for detecting bacterial pathogens are standardised and rigorously tested for specificity and sensitivity. Commercial reagents and kit development (Adams and Thompson, 2008) have gone some of the way to achieving this, but there still is not a full range of reagents or kits available for use in aquaculture. The cost, speed, specificity and sensitivity of assays are all extremely important to end-users. The highest cost is often time, although labour costs do vary considerably between countries. Many of the new technologies require specialised equipment and highly skilled staff and few of the existing methodologies are suited to field testing, or use in rudimentary laboratories.

7.4 Advances in methods of disease diagnosis (mainly for bacterial diseases)

Disease diagnosis is currently made using a variety of methods, as reviewed by Adams and Thompson (2008). Traditional bacteriology, whereby the pathogen is isolated and identified biochemically (e.g. using API® strips), and observation of histological sections from diseased fish are widely used. Rapid methods that specifically identify the pathogen using antibodies

(immunodiagnostics) or by amplifying specific sequences of DNA or RNA using polymerase chain reaction (PCR) (i.e. molecular diagnostics) are also regularly used in many laboratories. In some instances molecular diagnostics has completely taken over from other methodologies. For many of the rapid methods live and dead pathogens cannot be distinguished; therefore, the inclusion of enrichment methods and the use of live/dead kits are useful supplementary methods (Vatsos *et al*., 2002). Interpretation of results using rapid methods of pathogen detection should be carefully considered with all the other clinical evidence, including histology and attempted culture of the pathogen.

Immunodiagnostic methods such as immunohistochemistry (IHC), the fluorescence antibody test (FAT) and indirect fluorescence antibody test (IFAT) enable rapid specific detection of pathogens in tissue samples without the need to first isolate the pathogen. The IHC method is a simple extension of histology allowing specific identification of pathogens in formalin tissue fixed sections (Adams and Marin de Mateo, 1994; Steiropoulos *et al*., 2002), while FAT/IFAT is extremely rapid and sensitive as well as being specific but requires a fluorescent microscope to read the results (Adams *et al*., 1995; Miles *et al*., 2003; Klesius *et al*., 2006). Both IHC and FAT/IFAT are technically easy to perform, and examples of the results obtained using these methods are shown in Fig. 7.1. (p. 201, see also colour section.) A variety of other antibody-based methods have also been developed for use in aquaculture. Some are very simple to use, but usually require pathogen isolation prior to use and lack in sensitivity (e.g. agglutination), while others are more complex, but with the added advantage of pathogen quantification (e.g. enzyme-linked immunosorbent assay, ELISA), or detection and characterisation of specific pathogen antigens to their molecular weight, e.g. Western blot (Rose *et al*., 1989; Adams and Thompson 1990; Adams, 1992, 2004). The ELISA also has the advantage of high throughput and automated equipment is available.

The ELISA can also be used for serology (detecting antibodies to specific pathogens). Although serology is an essential screening tool in clinical medicine and in most control programmes for the significant diseases of domestic animals (Palmer-Densmore *et al*., 1998; Yuce *et al*., 2001; Fournier and Raoult, 2003) it has not yet been validated for any bacterial diseases in fish. Serology is used effectively for detecting exposure to fish viruses, such as koi herpesvirus (KHV) (Adkinson *et al*., 2005; Adams and Thompson, 2008), but bacterial pathogens pose a much more complex picture with cross reactivities likely unless specific known proteins are used to coat the ELISA plates rather than whole pathogens.

The use of molecular technologies for the detection of fish bacterial pathogens is rapidly increasing and a vast array of methods has already been developed (Karunasagar *et al*., 1997; Cunningham, 2004; Adams and Thompson 2006, 2008; Wilson and Carson, 2003). Molecular methods

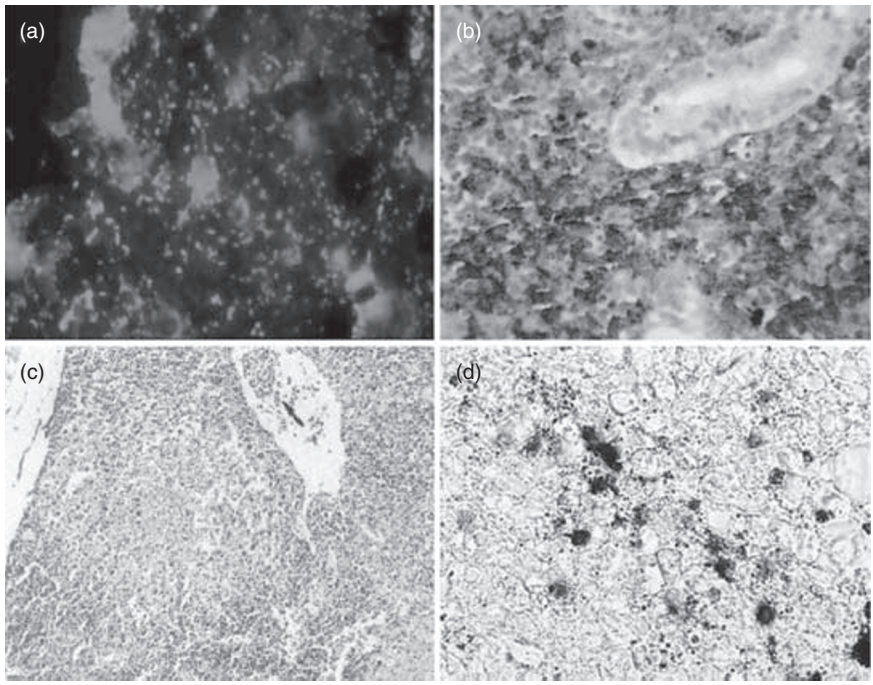


Fig. 7.1 (See also Plate I) Examples of immunodiagnostic methods to detect fish pathogens in fish tissue. Detection of *Renibacterium salmoninarum* using indirect fluorescent antibody test, IFAT (a). Detection of *Renibacterium salmoninarum* using True Blue as substrate (b), *Photobacterium damselae* subspecies *piscicida* using 3,3′-diaminobenzidine (DAB) as substrate (c) and *Streptococcus iniae* using Fast Red as substrate (d) by immunohistochemistry, IHC. (Photographs (a)–(c) and (d) courtesy of Dr K D Thompson and Dr P Klesius, respectively)

generally have the highest sensitivity and are therefore particularly useful for detecting microorganisms that are present in low numbers or for those that are difficult to culture. In addition, molecular methods can be used for the identification of pathogens to species level (Puttinaowarat *et al*., 2000; Pourahmed, 2008) and in epidemiology for the identification of individual strains and differentiating closely related strains (Cowley *et al*., 1999). The PCR is the best known method, although there are many useful variations, including nested PCR, random amplification of polymorphic DNA (RAPD), reverse transcriptase-PCR (RT-PCR), reverse cross blot PCR (rcb-PCR) and RT-PCR enzyme hybridisation assay (Puttinaowarat *et al*., 2000; Wilson and Carson, 2003; Cunningham, 2004). Colony hybridisation has also been used successfully for the rapid identification of *Vibrio anguillarum* in fish (Aoki *et al*., 1989) and has the advantage of detecting both pathogenic and environmental strains (Powell and Loutit, 2004). Real-time PCR (also known as qPCR) offers quantification and high sample throughput. Real-

time PCR methods have recently been developed for a variety of significant fish bacterial pathogens, such as *Aeromonas salmonicida* (Balcázar *et al*., 2007), *Piscirickettsia salmonis* (Karatas *et al*., 2008) *Renibacterium salmoninarum* (Jansson *et al*., 2008) and *Edwardsiella ictaluri* (US Patent 6951726), and it is likely that this range will expand rapidly. Polygenic sequencing following PCR of specific genes is being recommended for the identification of some pathogens where differentiation of closely related species is difficult. For example, Pourahmed (2008) found that sequencing of three different genes was necessary to classify certain mycobacteria species from fish.

A variety of novel rapid diagnostic methods are currently being developed that have potential for future application in the diagnosis of aquatic animal health. These were recently reviewed by Adams and Thompson (2008), and comparisons were made between these methods and existing technologies with regard to their advantages and disadvantages. Loop-mediated isothermal amplification (LAMP) is an emerging technology with potential for detection of fish and shellfish pathogens, and research tests to detect *Edwardsiella tarda*, *E. ictaluri*, *Nocardia seriolae*, and *Flavobacterium psychrophilum* (bacterial pathogens that cause edwardsiellois, enteric septicemia of catfish and nocardiosis, respectively), *Tetracapsuloides bryosalmonae* (the parasite that causes proliferative kidney disease, PKD) and infectious haematopoitic necrosis virus (IHNV) in fish and white spot syndrome virus (WWSV) in shrimp have already been developed (Savan *et al*., 2005; Manji, 2008). A commercial LAMP test kit is available for WSSV. The LAMP is a relatively new method for amplifying DNA which relies on autocycling strand displacement DNA synthesis and, since it is carried out under isothermal conditions, it can be performed without the use of a thermocycler. The method uses *Bst* DNA polymerase and a set of four specially designed primers (two inner and two outer primers) to recognise a total of six distinct sequences on the template DNA (Notomi *et al*., 2000). The main advantages of the method are the speed with which it can be performed, its sensitivity, and the fact that the results are read by eye, and, although the test requires the use of pipettes and an incubator, it does not require any other specialised equipment. Figure 7.2 (p. 203, see also colour section) shows the results of a LAMP test carried out to identify *Flavobacterium psychrophilum*, where a simple colour change in the tube from orange to green indicates a positive. Simple rapid field tests using lateral flow technology are also in development, and rapid kits are currently commercially available to detect infectious salmon anaemia virus (ISAV) and WSSV in shrimp. Although there are none yet for bacterial pathogens in fish, this type of technology has been successfully developed for clinical (Gatta *et al*., 2004) and veterinary use (Bautista *et al*., 2002) and offers simple field test capabilities.

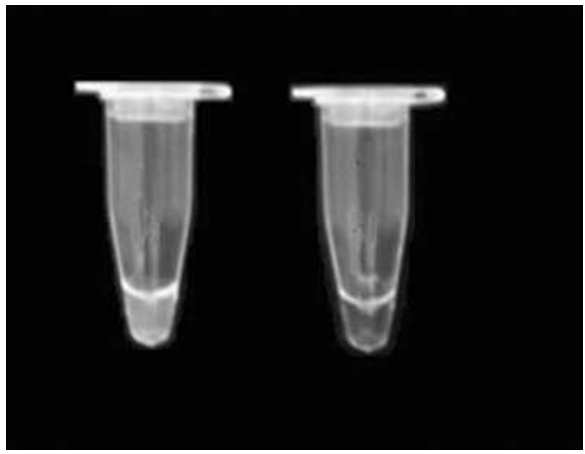


Fig. 7.2 (See also Plate II) Example of a loop-mediated isothermal amplification (LAMP) test carried out using purified *Flavobacterium psychrophilum* DNA. Positive samples turn green and negative ones remain orange. LAMP is highly sensitive and specific, and is performed under isothermal conditions, needing minimal instrumentation. (Photograph courtesy of F. Manji)

There is also interest in the development of multiplex tests to simultaneously detect different pathogens in a single sample. Multiplex technologies such as the Luminex xMAP™ (a bead array) and microarray both have huge potential in this area, but these are currently expensive and labour-intensive as assays are still being developed and optimised (Adams and Thompson, 2008). The xMap system theoretically offers simultaneous quantitative analysis of up to 100 different analytes from a single drop of sample in an integrated, 96-well formatted system (Dunbar, 2006). This is complex technology with huge potential as it can be used for vaccine development, through epitope mapping (Costa *et al*., 2007), as well as pathogen detection. This is flexible technology as antibodies, protein or DNA can be bound to the bead array. A number of research groups are also currently developing DNA and oligo microarray technology for diagnostics and these also will offer simultaneous detection of pathogens for the future (González *et al*., 2004; Matsuyama *et al*., 2006).

7.5 Advances in vaccine development

A wide range of commercial vaccines is available against bacterial pathogens, with most targeting salmon and trout, with additional vaccines available for channel catfish, European seabass and seabream, Japanese

amberjack and yellowtail, tilapia and Atlantic cod (Thompson and Adams, 2004; Adams *et al*., 2005; Sommerset *et al*., 2005). The salmonid market presently uses heptavalent vaccines containing *Listonella (Vibrio) anguillarum* serotypes O1 and O2, *V. salmonicida*, *Moritella viscosa*, *Aeromonas salmonicida*, the causative agents of vibriosis, Hitra disease, winter ulcer disease, furunculosis and infectious pancreatic necrosis, respectively, and infectious pancreatic necrosis virus (IPNV). Due to environmental and control concerns in most countries only two bacterial vaccines (*Edwardsiella ictaluri* and *Flavobacterium columnarae*, causing Columnarus, for Channel catfish in the USA) and one viral vaccine (KHV for Carp in Israel) are presently commercially available as 'live attenuated' vaccines (Adams *et al*., 2005). Most of the commercial vaccines are based on inactivated bacterial pathogens, with fewer available for viral vaccines and none against parasites yet. The major producers of fish vaccines are now Intervet-Schering Plough Animal Health (The Netherlands), Novartis Animal Health (Switzerland), Pharmaq (Norway) and Microtek International Inc. (Canada). There are a number of smaller companies producing vaccines locally (e.g. autogenous vaccines), in different countries. Vaccines used in Japan are mostly developed and distributed by Japanese companies.

Many new vaccines are in development (Thompson and Adams, 2004; Adams *et al*., 2005). The primary considerations for vaccines for aquaculture are cost-effectiveness and safety. Vaccines need to provide long-term protection against diseases on commercial fish farms. All the serotypic variants of the disease agent need to be considered, the time/age when the animal is most susceptible to disease, the route of administration and the method of vaccine preparation (i.e. inactivated whole cell, attenuated, subunit, recombinant). In order to develop an effective vaccine the protective antigens need to be identified and their protective response confirmed in the host species. The latter may be antibody mediated, cell mediated or both depending on the vaccine components. A practical method of administration and an inexpensive method of vaccine production also need to be established.

It is important in vaccine development to work with the antigens that are expressed during infection rather than antigens expressed in the laboratory. Many salmon vaccines from the past are based on inactivated (whole cell) cultures of the pathogenic organism (usually inactivated in formalin) grown *in vitro*. In these cases the vaccines gave good protection (e.g. *Vibrio* vaccine); however, many pathogens appear to switch off important protective antigens when cultured *in vitro*. In such cases alternative methods of culture (e.g. the inclusion of an iron-chelating agent) are required so that expression of the important 'protective' antigens is induced (Neelam *et al*., 1993). This can be achieved by modifying the culture medium of the pathogen *in vitro*, as shown by Bakopoulos *et al*., (2003) and Jung *et al*., (2007) for *Photobacterium damselae* subsp. *piscicida*,

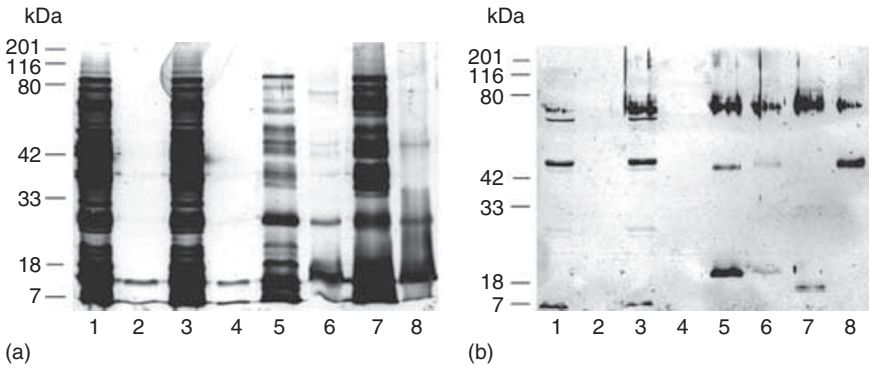
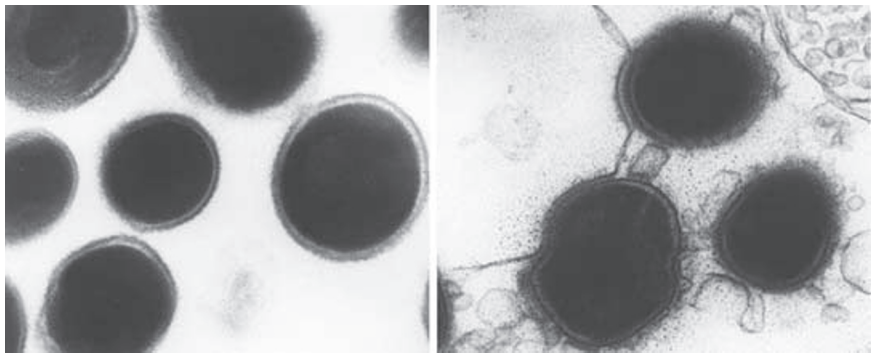


Fig. 7.3 Western blot analysis of *Photobacterium damselae* subspecies *piscicida* after culture of the bacterium in different media (1–8), showing that different proteins are expressed under different culture conditions. Blot (a) used rabbit serum while blot (b) was performed with fish serum where many fewer antigens were recognised. (Photographs courtesy of Dr V Bakopoulos)

the pathogen that causes pasteurellosis. The effects of altering the constituents of the media for culturing *P. damselae* subsp. *piscicida* in the laboratory on antigen expression are illustrated in Fig. 7.3, where differences are observed in the antigens expressed/recognised by fish sera between media 1, 7 and 8, for example (Fig. 7.3a, b). Some antigens appear to be up-regulated whilst others are down-regulated. This also serves to highlight the difference between mammalian and fish immune systems, as fish (Fig. 7.3b) recognise many fewer antigens than rabbits (Fig. 7.3a).

An alternative approach is to place the pathogen of interest inside the peritoneal cavity of fish, enclosed in sealed chambers (that permit the exchange of small molecules only), so that antigen expression *in vivo* can be determined (Bakopoulos *et al*., 2004; Poobalane, 2007; Jung *et al*., 2008). Figure 7.4 shows how *R. salmoninarum* alters the expression of surface molecules when the bacterium is cultured *in vivo* or *in vitro*. Bacterial cells appears smooth and rounded when cultured *in vitro* in contrast to those cultured inside the host fish. Application of sera from fish (that have been infected with the disease of interest and then recovered) in Western blot analysis on one- or two-dimensional gels (immunoprotomics) can then pinpoint potential vaccine candidates that can then be identified and vaccines produced (e.g. a recombinant vaccine against *Aeromonas hydrophila*, Poobalane, 2007). Of course these antigens may be expressed and the fish may respond by producing antibodies to them, but they may or may not be protective. Thus, challenge of vaccinated and non-vaccinated fish is then performed to establish if the vaccine is actually protective.



(a) (b)

Fig. 7.4 Electron micrographs (EM) of *Renibacterium salmoninarum* cultured *in vitro* and *in vivo*, illustrating the differential expression of bacterial surface molecules when bacteria are cultured in the laboratory or inside fish. (Photographs courtesy of Dr K D Thompson)

Fish vaccines have become much more sophisticated since the mid-1990s. Technologies such as recombinant and DNA vaccines are powerful tools for vaccine development (Leong *et al*., 1997; Smith, 2000) as these enable the isolation of potential protective antigens from suppressive ones. These are being developed because the simpler approach of using inactivated whole cell vaccines did not succeed for many of the important diseases, and attempts at attenuated vaccines in general have not been encouraged from a safety point of view (Benmansour and de Kinkelin, 1997). An IPNV vaccine based on a recombinant expressed viral protein has been developed (Frost and Ness, 1997) and has been on the market for several years for use in salmon in Norway, but the licensing of other recombinant vaccines has been slow. DNA vaccines for fish have been shown to be effective when based on DNA-sequences encoding for rhabdovirus glycoproteins (Lorenzen and La Patra, 2005) and the first DNA vaccine has been licensed in Canada against IHNV.

Vaccines for fish can be administered by a variety of different methods, i.e. injection (intramuscular or intra-peritoneally), immersion (bath or dip-vaccination) or orally. There is much interest in developing oral vaccines as this is the most practical method of administration. In the absence of natural exposure, booster vaccination is needed to maintain immunity. Oral vaccine boosters have been used successfully and are marketed commercially. For example, oral vaccines are available against enteric red mouth (ERM) and vibriosis in rainbow trout, and against furunculosis and IPNV in salmon (Meeusen *et al*., 2007). These vaccines employ an antigen protection vehicle to protect vaccines from the acid environment of the fish

stomach, and improved delivery systems are currently being researched for a variety of vaccines.

7.6 Other emerging methods to control pathogens

Close monitoring of stocks to ensure early detection of pathogens causing disease problems (and effective treatment) and vaccination to prevent infections are clearly not the only methods of disease control. Farmers can take a number of measures to manage the impact on healthy stocks, including improved nutrition/diet, and maintaining stocking densities at levels that optimise growth and avoid over-crowding that reduces the ability for the animal to resist infections. Immunostimmulants (e.g. glucans) added to diets have been reported to enhance the immune system of the fish in the short term, when applied either on their own or in vaccines as adjuvants, and are reported to be very affective at stimulating the non-specific defense mechanisms of the animal (Thompson and Adams, 2004; Peddie and Secombes, 2005). Other dietary additives are also being used, sometimes to target specific diseases, e.g. addition of vitamin E to target salmon pancreas disease (SPD). A wide range of modified diets and immunostimulants is available commercially and many new products are being researched (Bricknell and Dalmo, 2005; Peddie and Secombes, 2005). In addition, probiotics are widely used in some countries (Birkbeck, 2004).

Reducing the risk of exposure to, or impact on, healthy populations is also an effective alternative approach to pathogen control. Molecular methods (e.g. PCR) are ideal technologies to use for screening fish broodstock and eggs for the presence of pathogens, and as long as large enough sample sizes are tested (e.g. for eggs) this may prove to be an effective method of reducing the reservoir of specific pathogens on fish farms. Recently, Manji (2008) reported that, although the prevalence of *Flavobacterium psychrophilum* in rainbow trout eggs was very low (1–2.4 %) this still led to spread of disease (rainbow trout fry syndrome, RTFS) and mortalities when these eggs were grown on. In this study it was necessary to test at least 300 eggs per batch in the screening process.

The long-term approach to disease control is through the selection of disease-resistant strains or families of fish, and much research is currently focused on fish genotype and disease susceptibility (Biacchesi *et al*., 2007). Selective breeding programs have been shown to have large beneficial effects on production and quality traits in cold-water fish species, but only a small percentage of aquaculture production is based on genetically improved fish and shellfish (Gjedrem, 2000). Resistance is a complex quantitative trait that is likely to be affected by many genes (Gjedrem

et al., 1991; Grimholt *et al.*, 2003). To select for disease resistance it is necessary to challenge a large number of animals and measure each family's overall performance for the trait. Genes associated with disease and stress resistance are now being identified and their characteristics will be used as identifiers (markers) for selective breeding of disease- and/or stress-resistant individuals (Moen *et al.*, 2004). This approach should continue to be pursued, bearing in mind that increased resistance to one pathogen can result in decreased resistance to other pathogens.

As more nucleotide sequences become available for fish bacterial pathogens, this will provide opportunities to develop innovative alternative disease control methods. Recently, following the sequencing of *R. salmoninarum*, Sudheesh *et al.* (2007) described the identification of a new class of drug (phenyl vinyl sulfone) which inhibits the activity of a very important enzyme (sortase) in *R. salmoninarum*. Inhibition of this enzyme appears to dramatically reduce the virulence of the bacterium by interfering with the ability of the bacterium to adhere and colonise fish cells. This drug could offer a promising alternative to antibiotics to control bacterial kidney disease in fish, and this type of approach holds potential for future control of bacterial disease in general for fish.

7.7 Future trends

Technologies to assist with disease control are moving at a rapid pace. Careful consideration must be given to selecting which methods to take forward and apply in aquaculture. Vaccines need to be cost-effective and safe, and pathogen detection methods should be robust yet sensitive. There are many innovative technologies that may fulfil these criteria and provide new vaccines and useful diagnostics tools. It is important, however, that the diagnostics methods already developed are standardised and fully validated, if they are considered useful, and that new technologies do not supersede these just because they are novel methods. They need to have clear advantages over the existing methods for use in aquaculture. Nanotechnology is an area being explored for the detection of pathogens in food (Kim *et al.*, 2007) and in clinical and veterinary diagnostics, and this may prove extremely useful for application in diagnostics for aquatic animals. Nanotechnology is generally used when referring to materials of 0.1–100 nanometres; however, it is also inherent that these materials should display different properties from bulk (or micrometric and larger) materials as a result of their size. These differences include physical strength, chemical reactivity, electrical conductance, magnetism and optical effects. Such technology offers the ability to detect extremely low

levels of pathogens very quickly, and progress has already been made for the identification of foodborne pathogens (Joseph and Morrison, 2006). Recently, the focus is on different types of sensors to detect pathogens and immunomagnetic reduction (IMR) technology has been reported for the detection of low levels of the H5N1 virus that causes bird flu (Yang *et al.*, 2008). In this method magnetic nanoparticles were coated with antibody and a high-transition-temperature superconductive quantum interference device was used to sense the immunomagnetic reduction of the reagents.

Nanotechnology is also being used in conjunction with proteomics (Marko *et al.*, 2007) and may be a useful technology to identify markers of disease and vaccine antigens. Immunoprotomics, as discussed in Section 7.5, is also known as 'reversed vaccinology' and has recently been successfully used for the development of a recombinant vaccine against *A. hydrophila* for carp (Poobalane, 2007). Other technologies with the same approach are knockout technologies, which indicate whether specific antigens are essential or important for survival of the pathogen in the host. These methods have great potential for the future and include RNA interference where expression of certain genes is blocked by antisense RNA (Melamed *et al.*, 2002), *in vivo* expression technologies, IVET (Rainey and Preston, 2000), and signature tagged mutagenesis (Saenz and Dehio, 2005). The information obtained from a variety of these techniques is combined with data from existing literature to identify potential vaccine candidate antigens for cloning and for recombinant expression (Adams *et al.*, 2005).

Delivery of DNA vaccines using bacteriophages has been reported to be successful in a number of animals including fish (Skurnik and Strauch, 2006). The phage particles used are non-infectious and only grow on specialised laboratory strains of bacteria; the phage coat protects the vaccine from degradation and allows the host's immune system to process it more efficiently (March *et al.*, 2004; Clark and March, 2006). This technology may be extremely useful for oral delivery of DNA vaccines for fish.

Gene sequencing underpins many of the new technologies being developed both for diagnostics and vaccine development and, as gene sequences become available for both pathogens and the host fish species, microarrays can be developed. These will enable very rapid progress to be made in fish health control for the future.

7.8 Sources of further information and advice

In addition to the references cited there are numerous websites that provide useful information and advice. These are summarised in Table 7.1.

Table 7.1 Sources of further information and advice

Name of organisation or project	Website address
AquaFirst	http://aquafirst.vitamib.com
AquaNet	http://www.aquanet.ca
CEFAS (Centre for Environment. Fisheries and Aquaculture Science)	http://www.cefas.co.uk
DipNet (An EU funded project investigating disease interaction and pathogen exchange between farmed and wild aquatic animal populations)	http://www.dipnet.info
EAFP (European Association of Fish Pathologists)	http://www.eafp.org
EPIZONE – Network of Excellence for Epizootic Disease Diagnosis and Control	http://www.epizone-eu.net
EUROCARP	http://eurocarp.haki.hu
FAO (Food and Agriculture Organisation of the United Nations)	http://www.fao.org
FAO oneFish Community Directory Project (aquaculture/diseases)	http://www.onefish.org/id/10752
FEAP (Federation of European Aquaculture Producers)	http://www.feap.info/feap
FishEggTrade (An EU funded project appraising of the zoosanitary risks associated with trade and transfer of fish eggs and sperm)	http://cordis.europa.eu/life/src/control/qlk2-ct-2002-01546.htm
Fish Health Section of the American Fisheries Society	http://www.fisheries.org/units/fhs/
Fish Health Section of the Asian Fisheries Society	http://afs-fhs.seafdec.org.ph/
Fisheries Research Services	http://www.marlab.ac.uk/
IAAAM (International Association for Aquatic Animal Medicine)	http://www.iaaam.org
IMAQUANIM (EU funded project: Improved immunity of aquaculture animals)	http://imaquanim.dfvf.dk/info/
International Database for Aquatic Animal Diseases	http://www.collabcen.net
ISAAE (International Society of Aquatic Animal Epidemiology)	http://www.isaaepi.org/modules/news/
NACA (Network of Aquaculture Centres in Asia-Pacific)	http://www.enaca.org
OIE Aquatic Animal Health Standards Commission	http://www.oie.int/aac/eng/en_fdc.htm
OIE Designated Experts and Reference Laboratories for Aquatic Animal Diseases	http://www.oie.int/fdc/eng/Diseases/en_reflabslist.htm
PANDA (Permanent Advisory Network for Diseases in Aquaculture)	http://www.europanda.net
WAS (World Aquaculture Society)	http://www.was.org

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