Institution: University of Stirling

Unit of Assessment: A6 Agriculture, Veterinary and Food Science

Title of case study: Improving fish health and the aquatic environment through vaccination.

1. Summary of the impact

Research by the Institute of Aquaculture has made a significant contribution to the development of effective fish vaccines, some of which have been commercialised and are used widely within the aquaculture industry. The majority of farmed fish in the UK are vaccinated (44 million salmon and 7.5 million rainbow trout in 2012 alone) with vaccines developed at Stirling, resulting in vast improvements in survival and fish health, and a *sustained minimal use in antibiotics* through mass vaccination. Vaccines have been developed for all the major farmed species in Europe, and recently the first vaccine for *Pangasius* catfish in Vietnam (>2 million tonne).

2. Underpinning research

While aquaculture is rapidly expanding worldwide the single most important limiting factor on continued growth is loss due to disease. In some sectors of the aquaculture industry disease losses can reach up to 40% of a fish population, threatening the very existence of certain sectors. Prior to the introduction of vaccines, antibiotics were the only method of controlling bacterial diseases.

Fish immunology research at the Institute of Aquaculture has contributed essential tools and methods to assist in the development of fish vaccines since 1993. The group has close links with industry and much of the research has been undertaken with funding from major pharmaceutical companies, leading to commercialisation of the vaccines developed (Furunculosis, Pasteurellosis, Bacillary Necrosis).

In order to develop an effective vaccine the protective antigens need to be identified and their protective response confirmed in the host species. As very few reagents were available to monitor the immune response or to identify pathogens in fish, this became one of the main tasks for the group at Stirling (1993-date) and a wide range of monoclonal antibodies (mAbs) and standard operating procedures were developed for this purpose[1].The fish health group has also made significant contributions to the elucidation of host-pathogen interactions and characterisation of pathogens for a number of fish species and diseases leading to vaccine development *e.g.* Furunculosis[2] in salmonids, Pasteurellosis[3,4] in sea bass and sea bream, Aeromonad septicaemia in carp and Tilapia[5], and Bacillary Necrosis in *Pangasius* catfish[6].

Development of methods to identify the antigens expressed by pathogens during infection was also important so that potential vaccine candidates could be isolated and tested. The team focused on differential expression of pathogen antigens under different culture conditions leading to the successful development of vaccines. This was first approached in 1995 using iron restriction conditions to culture *Aeromonas salmonicida in vitro*[2] and a vaccine to protect against Furunculosis was subsequently developed. A similar approach was used to develop a Pasteurellosis vaccine for use in Mediterranean fish species (1997-2003)[3,4]. *Aeromonas hydrophila* is very heterogeneous and therefore vaccine development was particularly difficult, so more recently the team took a different approach in that the bacteria were actually cultured *in vivo* in culture chambers rather than manipulating the *in vitro* culture media. In addition, immunoproteomics was used to identify common vaccine antigens between the field isolates. This time a recombinant vaccine was successfully developed (2005-2010)[5]. Finally, the first commercial fish vaccine for use in *Pangasius* catfish was recently developed. This vaccine protects against bacterial necrosis from *Edwardsiella ictaluri* which is the most significant disease affecting the catfish sector in Asia. The research performed at Stirling (2006-2011) provided the original disease diagnosis, pathogen identification and screening of vaccine candidates[6]. This is an extremely important development as the use of antibiotics in Vietnamese aquaculture has been a cause for concern.

Key staff working on vaccine development include Adams (1995-present), Thompson (1993-present), Richards (1979-present) and Crumlish (1999-present).

3. References to the research

The results of the research have been published widely in scientific papers and disseminated in numerous trade magazines and through many oral and poster presentations at scientific conferences.

1. Adams A, Thompson K D (2006) Biotechnology offers revolution to fish health management. *Trends in Biotechnology* 24, 201-205.
2. Neelam, B., Thompson, K.D., Price, N.C., Tatner, M.F., Adams, A., Ellis, A.E. and Stevens, L. (1995) Development of monoclonal antibodies to iron-regulated outer membrane proteins and lipopolysaccharide of *Aeromonas salmonicida*. *Diseases of Aquatic Organisms*, 21, 201-208.
3. Bakopoulos, V., Adams, A. and Richards, R.H. (1997) The effect of iron limitation growth conditions on the cell and extracellular components of the fish pathogen *Pasteurella piscicida*. *Journal of Fish Diseases*, 20, 297-305.
4. Bakopoulos V., Pearson M., Volpatti D., Gousmani L., Adams A., Galeotti M, Richards R.H. and Dimitriadis G.J. (2002) Investigation of media formulations promoting differential antigen expression by *Photobacterium damsela* ssp. *piscicida* and recognition by sea bass, *Dicentrarchus labrax* (L.), immune sera. *Journal of Fish Diseases* 26, 1-13.
5. Poobalane S, Kim D. Thompson, László Ardó, Noel Verjan, Hyun-Ja Han, Galina Jeney, Ikuo Hirono, Takashi Aoki, Alexandra Adams (2010) Production and efficacy of an *Aeromonas hydrophila* recombinant S-layer protein vaccine for fish. *Vaccine* 28, 3540-3547.
6. Crumlish, M., Thanh P.C., Koesling J., Tung, V.T and Gravningen K. (2010) Experimental challenge studies in Vietnamese catfish *Pangasianodon hypophthalmus* (Sauvage), exposed to *Edwardsiella ictaluri* and *Aeromonas hydrophila*. *Journal of Fish Diseases* 33, 717–722.

Grants supporting the underpinning research include:

1. 1998-2002**:** FAIR/EU - Fish Pasteurellosis vaccine development. £424k, (PI Adams)
2. 1998**:** Aquaculture Vaccines Ltd - *Aeromonas hydrophila* vaccine development. £21k, (PI Adams)
3. 2006: Schering Plough Aquaculture, *Aeromonas hydrophila* vaccine development. £35k, (PI Adams/Thompson)
4. 2006-2011 Pharmaq Norway: Vaccine development against *E. ictaluri* in farmed Pangasius species. £280k (PI Crumlish).
5. 2009-2010 Intervet Schering Plough Developing control strategies for *Aeromonas hydrophila.* £40k (PI Adams/Thompson)

4. Details of the impact

Fish health and welfare

The vaccines developed and commercialised were to protect salmonids against Furunculosis, sea bass/bream against Pasteurellosis, catfish against *Edwardsiella ictaluri* and carp against *Aeromonas hydrophila.* Field trials have been performed for the latter and patents filed in the US (12/678,078) and Europe (008806225.2). Impact is coroborated by Professor Patrick Smith, founder of the first fish vaccine company (Aquaculture Vaccine Limited) 'University of Stirling has a pivotal role in the area of aquatic animal health research and its work over the years has had a major impact on the worldwide aquaculture industry which is so crucial for future world Food Security programmes*'.*

Aquatic environment

Use of vaccines led to a dramatic reduction in use of antibiotics in the late 80s and this has been sustained to the present day with continued wide-spread use of vaccines in UK and Norway. Thus, the use of antibiotics in the salmonid aquaculture industry is very low in comparison to use in other farmed animals. Impact on the aquatic environment is again corroborated by Professor Patrick Smith *'….*antibiotic usage in fish farms declined rapidly to the position we are at today where Norwegian farmed salmon production has reached approximately 800,000 tonnes (a near 7-fold increase) while the usage of antibiotic has declined to less than 100kg (a near 50-fold reduction)!'*.*

Commerce

New fish vaccines have been developed and commercialised – this includes vaccines to prevent Furunculosis, Pasteurellosis with 51.5 million doses administered in 2012 (>£5m). The Strategic Marketing Director of MSD Animal Health confirms *'the key role which research and testing undertaken by the Institute of Aquaculture at University of Stirling has played in the development of vaccines…….I and my team are directly involved in commissioning this work for the company'*.

The first commercial fish vaccine against Bacillary Necrosis for use in *Panagasius* catfish was launched in October 2011 by Pharmaq. ALPHA JECT Panga 1 was granted a license in Vietnam in April 2013. Vaccination and training in good practice in the vaccination process has been initiated.

Antibodies to fish immunoglobulins have been marketed through Aquatic Diagnostics Ltd. Impact is confirmed by Professor Brian Dixon, University of Waterloo, Canada *'Indeed, antibodies to fish immunoglobulins for various species are one commercial product that has emerged from this line of research, with companies such as Aquatic Diagnostics in Stirling, Scotland, marketing these.'*

Industry has invested in research and development. Schering Plough Aquaculture, now MSD Animal Health, have provided >£500,000 in funding on fish vaccines (1998-2009), while Pharmaq has invested £280,000 (2006-11). Recently we were awarded £1,390,000 by Zoetis (Pfizer)/Technology Strategy Board to develop a sea louse vaccine.

Public policy

The position of contributing scientists on key committees and working groups has ensured transfer of the knowledge generated to industry and government and has influenced public policy. For example, Richards is the veterinary advisor to the Scottish Salmon Producers Organisation and chairs the Scottish Government Ministerial Group on Sustainable Aquaculture Working Group on Fish Health. Adams is a member of the Asia-Europe Meeting (ASEM) Aquaculture Health Steering Group, a multi-stakeholder platform for networking and coordination concerning sustainable aquaculture between EU and Asia. She has also recently joined the Veterinary Medicines Directorate (VMD) Aquaculture Working Group on control of antibiotic use. Richards and Adams are also facilitator and immunology group leader of the European Aquaculture Technology and Innovation Platform (EATIP) for Fish Health, which sets priorities for commercially-focused research in aquaculture.

International development

Introduction of the first fish vaccine in Vietnam has benefited fish health, changed methods of fish husbandry in Vietnam, reduced the use of antibiotics, and has benefited local people though creation of jobs and wealth through increased productivity. Numerous workshops have been conducted in disease diagnosis, microbiology, immunology and vaccine development for a wide range of end-users including the scientific community and farmers in a variety of countries including Chile, China, Korea, Thailand and Vietnam. For example, in Vietnam workshops (translated into Vietnamese and other languages) were given to 1,400 fish farmers and a diagnostic facility and wet lab were established.

5. Sources to corroborate the impact

1. Patents lodged in Europe (008806225.2) and USA (12/678,078) for *A. hydrophila* vaccine.
2. First fish vaccine in Vietnam; *www.pharmaq.no/updates/the-first-/*
3. Evidence that new antibodies to fish immunoglobulins for various species have been developed:
   http://www.scielo.cl/scielo.php?pid=S0717-34582012000500014&script=sci_arttext
4. www.aquaticdiagnostics.com. University of Stirling spin-out company marketing antibodies developed within the University.

In addition, letters corroborating the impact are available from MSD Animal Health, Aquatic Vaccines Ltd, General Director of Pharmaq Vietnam, the Food and Agriculture Organisation and Aquaculture Underwriting and Insurance Management Services Ltd