FISH VACCINES – A SHORT, BUT REMARKABLE, JOURNEY

Professor Patrick Smith
Tethys Aquaculture Ltd

Veterinary Vaccinology Network Meeting
Birmingham ICC
February 16th-17th 2015
Aquatic Animal Health Research

An exciting new highway OR A ‘blind alley’

Patrick Smith
Tethys Aquaculture Ltd
Fish Vaccines and Fish Vaccine Research

“From Zero to Hero”
The Aquaculture Industry

- 65 million tonnes
- US$ 150 billion
- 40% of whole fish consumption
- Crossover (50/50) predicted between 2025 and 2030 already exceeded wild catch in Mediterranean
- Growth at 10-12% per annum (1% = capture fish; 2.3% = other animal production)
- Fin-fish (30+ species), shellfish, crustaceans, algae
- Becoming a key component of worldwide Food Security Programmes
Commercially-Available Fish Vaccines

1982
1 Enteric Redmouth (ERM) vaccine
2 Vibrio anguillarum vaccine
TOTAL = 2

2014
1 Enteric Redmouth (ERM) vaccine
2 Vibrio anguillarum vaccine
3 Furunculosis vaccine
4 Vibrio salmonicida vaccine
5 Combined Vibrio/Furunculosis vaccine
6 Combined Vibrio/Furunculosis/Coldwater Vibriosis/Moritella viscosa vaccine
7 Combined Vibrio/Furunculosis/Coldwater Vibriosis/Moritella viscosa/IPNV vaccine
8 IPN Virus vaccine
9 Pasteurella vaccine
10 Combined Pasteurella/Vibriosis vaccine
11 Vibriosis vaccine for cod
12 Shrimp Vibriosis vaccine
13 Warmwater Vibrio spp vaccine
14 SVC virus vaccine
15 Lactococcus garvieae/Streptococcus iniae vaccine
16 KHV vaccine
17 Aeromonas hydrophila vaccine
18 Carp Erythrodermatitis/Ulcer disease vaccine
19 Piscirickettsia salmonis vaccine
20 ISA virus vaccine
21 Gaffkaemia vaccine
22 Flavobacterium psychrophilum vaccine
23 Nodavirus vaccine
24 Pancreas disease virus vaccine
25 Edwardsiella ictaluri vaccine

TOTAL = 25 +
Benefits of vaccination

- Economic benefits
- Environmental benefits
- Animal welfare
- Reduction in the use of antibiotics
Reduction in levels of disease
Reduction in losses due to disease
# ERM Vaccine Field Trials

## Field Performance of ERM Vaccines

<table>
<thead>
<tr>
<th>Trial</th>
<th>Vaccinated Number</th>
<th>Mortality</th>
<th>%</th>
<th>RPS</th>
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<tr>
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<td>10,095,793</td>
<td>119,568</td>
<td>1.18</td>
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<tr>
<td></td>
<td>1,869,524</td>
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<td></td>
<td>1,521,516</td>
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<td>3</td>
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<td>86</td>
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<td>831,688</td>
<td>85,504</td>
<td>10.28</td>
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<td>4</td>
<td>82,500</td>
<td>3,878</td>
<td>4.7</td>
<td>78</td>
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<td>26,400</td>
<td>5,755</td>
<td>21.8</td>
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<tr>
<td>5</td>
<td>129,600</td>
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<td>100</td>
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<td>101,805</td>
<td>45,252</td>
<td>44.45</td>
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</tr>
</tbody>
</table>

*Weight at vaccination: 4.5g (100/lb)*

*Trials 1-3: weight vaccinated 103 tonnes*
External Symptoms
Mortalities due to Furunculosis as a % of total mortalities

% mortality

16
14
12
10
8
6
4
2
0

1992 1993 1994

Year
THESE TWO BOTTLES ARE WORTH £MILLIONS!

At least £15 million, for that's a conservative estimate of the financial burden imposed on the U.K. salmon industry by furunculosis.

Furogen immersion* and Furogen Injectable* – commercially proven in North America, Scandinavia and in Scottish trials – now offer solid, large-scale protection quickly, economically and cost-effectively.

*Manufactured by Aqua Health Ltd, Canada

Furogen Immersion
(Furunculosis Vaccine)

Furogen Injectable
(Furunculosis Vaccine)

1 Litre

Full information from sole UK distributors

Veterpharm Ltd
Dawton
Wilsford SP6 3QA
Telephone 07725 22330
Telefax 07725 20056

Tuesday 14
10.30
14.00

SALMON A
Chairman
14.30

15.05
16.15
16.45

17.15

TETTYS AQUACULTURE
Reduction in the use of antibiotics in the aquaculture industry following widespread adoption of vaccines

ESSENTIALLY A ‘GOOD NEWS’ STORY
**ERM VACCINE FIELD TRIALS**

**Medicated Feed Utilisation**

<table>
<thead>
<tr>
<th></th>
<th>Vaccinates</th>
<th>Controls</th>
<th>% Reduction</th>
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<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td>4.5</td>
<td>16.8</td>
<td>73</td>
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<td><strong>Trial 2</strong></td>
<td>2.27</td>
<td>15.9</td>
<td>86</td>
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<tr>
<td><strong>Trial 3</strong></td>
<td>6.3</td>
<td>22.0</td>
<td>72</td>
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</tbody>
</table>
ANTIBIOTIC USE IN EARLY DAYS OF FARMING ATLANTIC SALMON

• Introduction of a number of bacterial diseases - notably furunculosis.
• First response in absence of vaccine was to use a range of antibiotic treatments.
• “Dirty industry ?”
• Multiple resistance became commonplace.
• Effective vaccines developed and initial success led to widespread adoption of vaccination throughout industry.
Yearly Antibiotic Usage

- 1991: 1200 Kgs
- 1992: 400 Kgs
- 1993: 200 Kgs
SCOTLAND

AVL to show vaccines which saved Scottish farm salmon

ON ITS stand at the Scottish Fish Farming Exhibition in Avimore next month, Aquaculture Vaccines Ltd will show products which have resulted from an ambitious research program to develop vaccines against new diseases of farmed fish.

AVL supports its wide range of health care products with a comprehensive technical back-up service.

Many of its vaccine projects are carried out in collaboration with various universities, research institutes and government laboratories specialising in aquaculture.

Scottish Salmon Growers Association, the Institute of Aquaculture and University of Stirling, and the aquaculture team at the Marine Laboratory of the Scottish Office Agriculture and Fisheries Department.

“The vaccines employ new technology to produce novel and highly protective agents,” says Robin Wardle of AVL. “Field data collected over the past four years during the field testing and the widespread commercial use after licensing have shown that they produce high levels of protection of long duration.

AVL has developed a range of different formulations of the Furvac S range. The latest addition is an orally administered furunculosis vaccine, Aquavac Furvac S Oral, which has been granted an ATX Certificate by the UK licensing authorities.

Therefore combinations of immersion, applied, injection applied and oral vaccines can be used to protect against furunculosis during the life cycle of the salmon.

ATX Certificates have been granted for the orally administered forms of AVL’s furunculosis - (Aquavac Furvac S-Oral) and ERM (Aquavac ERM-Oral) vaccines. As reported earlier in FFI, AVL has also been able to test the oral version of its vibrio vaccine in sea bass and sea bream in Greece, France and the United Kingdom.

stomach of the fish, thus making them available to stimulate immunity in the lower gut.

The vaccine is in the form of a sterile emulsion which can be added to any feed pellets respective of size of manufacturer, either at the farm site or the feed mill.

Booster

Trials have shown that the oral vaccine can be used to regularly booster vaccinated fish which have been primarily vaccinated by immersion or injection. But experiments are in progress to evaluate vaccination and re-vaccination using only orally administered vaccine.

AVL’s product range includes the bacterial isolation and latex agglutination diagnostic kits, which it has sponsored package will be introduced at the Avimore show.

The product is in the form of a sterile solution which can be added to any feed pellets respective of size of manufacturer, either at the farm site or the feed mill.

Scottish salmon farm losses in 1989, most of these were due to furunculosis.

"The kit is robust and simple to use," Mr Wardle adds, "without the requirement for sophisticated laboratory equipment, thus making it ideal for the small diagnostic laboratory or for pond-side diagnosis. Results can be obtained within one hour of sampling the fish with short hands-on time of only ten minutes.”

Aquadip kits are available for the detection of the common bacterial diseases of farmed fish, including furunculosis, bacterial kidney disease, vibriosis, and enteric redmouth disease.

AVL says it has other research projects in an advanced stage for the development of pasteurised vaccines, streptococcal vaccines, testing of multivalent oral vaccines, and the evaluation of the pharmaceutical furamin in the prevention and treatment of myxosporean infections of salmon and trout.
There has been a recent focus on the use of antibiotics in animal husbandry throughout Europe

- “Over-use of antibiotics is an issue in animal husbandry, agriculture and fish farming”

- “The development of germs that are resistant to even the strongest of our current antibodies is one of the biggest health threats in the world.”

- “We continue to use them (antibiotics) in agriculture, fish farming and myriad other areas of life.”

- “......the typical farmed American salmon eats its own weight in antibiotics before it is sold.”

Dame Sally Davies, UK Chief Medical Officer

WHAT ARE THE FACTS?
Norwegian Salmon Production
Consumption of Pure Antibiotics and Effect of Vaccines

- Vibriosis Vaccine
- Furunculosis Vaccine
- Oil-based Fur Vaccine
- Combination Vaccine
Antibacterial agents

Antibacterial agents (metric tons)

Production (metric tons x 10^3)


Yellow: Production
Red: Antibacterial agents
Norway

- In 2013, the total sales of antimicrobial agents for therapeutic use in farmed fish were 972 kg of active substance of which quinolones accounted for 69%. The sales of antimicrobial VMPs in Norwegian aquaculture declined by approximately 99% from 1987 – 1996 and have thereafter remained relatively constant. This reduction is mainly attributed to the introduction of effective vaccines in salmonids.

NORM/NORMVET 2013

“Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway”
Bruk av antibiotika i Norge

1987
- Fisk: 58%
- Humant: 29%
- Andre dyr: 13%

2008
- Fisk: 14%
- Humant: 84%
Example: Use of Antibiotics on a French trout farm as vaccine use has increased over time.

### Quantity of medicinal feed distributed at Trout Farm in France

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<thead>
<tr>
<th>Year</th>
<th>Kg of antibiotics</th>
<th>% medicinal compared with feed</th>
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<td>2001</td>
<td>40</td>
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<td>5</td>
<td>0.5</td>
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<tr>
<td>2005</td>
<td>6</td>
<td>0.7</td>
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</table>

Kg of antibiotics

Medicinal quantity (tons) % medicinal compared with feed
Antibiotic Use in BC Salmon Aquaculture
1995 - 2009

Production is >90% Atlantic salmon (2006 - 2009)

Grams / MT salmon produced

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<thead>
<tr>
<th>Year</th>
<th>Grams / MT salmon produced</th>
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<tr>
<td>1995</td>
<td>456</td>
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<tr>
<td>96</td>
<td>336</td>
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<tr>
<td>97</td>
<td>516</td>
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<td>98</td>
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<td>07</td>
<td>109</td>
</tr>
<tr>
<td>08</td>
<td>68</td>
</tr>
<tr>
<td>2009*</td>
<td>57</td>
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</table>

* preliminary data
Total volume (kg) of active antimicrobial by year, 2003–2011.
Use of antibiotics against fish diseases in Iceland 1990 - 2014

- Introduction of vaccine against Atypical furunculosis
- Introduction of vaccine against Winter ulcers
- Introduction of vaccine against Cold water vibriosis

Graph showing the use of antibiotics against various fish diseases from 1990 to 2013.
Total use of Antibiotics per ton of slaughterfish in Icelandic aquaculture 1990 - 2013
Exceptions to the Rule?

- Ornamental fish industry
- SE Asia
- Latin America (mainly Chile)

Latter two must be regarded as ‘work in progress’- new industries, new emerging diseases and lateness in developing/adopting vaccination.
What about the use of other chemicals and anti-parasiticide?

- Possibly not such a ‘good news story’
- Use being reduced by integrated management systems.
- Much research into vaccine development
- Amoebic Gill Disease
Bruk av lusemiddel i oppdrett

Ectoparasitic agents and anthelmintics (metric tons)

Production of fish (thousand metric tons)

![Graph showing the relationship between ectoparasitic agents and anthelmintics and fish production over time.](image-url)
Bruk av lusemidlar

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<td>teflubenzuron</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2028</td>
<td>1080</td>
<td>26</td>
<td>751</td>
<td>1704</td>
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<td>emamektin</td>
<td>32</td>
<td>39</td>
<td>60</td>
<td>73</td>
<td>81</td>
<td>41</td>
<td>22</td>
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<td>36</td>
<td>51</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1413</td>
<td>1839</td>
<td>704</td>
<td>1611</td>
<td>3264</td>
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<td>23</td>
<td>29</td>
<td>39</td>
<td>62</td>
<td>61</td>
<td>54</td>
<td>121</td>
<td>136</td>
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<td>azametifos</td>
<td>55</td>
<td>45</td>
<td>49</td>
<td>30</td>
<td>32</td>
<td>88</td>
<td>107</td>
<td>48</td>
<td>232</td>
<td>211</td>
</tr>
</tbody>
</table>

Kjelde: www.FHI.no
Use of In-Feed Sea Lice Therapeutants in British Columbia (1996-2009)

Grains / MT of salmon produced

Emamectin Benzoate
Ivermectin

Year

* preliminary data Apr '10
The ‘blind-alley’

• Most ‘easy’ vaccines have been developed leaving vaccines against the more-difficult pathogens to be focused upon e.g. viruses, slow-growing bacteria, fungi, parasites.

• We need to focus on new technologies etc. to make these vaccines available and thus remove a considerable constraint on the continued development of the aquaculture industry.
Despite these new approaches to fish vaccine development, there are still a range of fish disease pathogens against which it is difficult/impossible to develop effective vaccines using conventional techniques:

- Viruses
- Slow-growing bacteria (BKD, SRS)
- Parasites
- Fungi
Development and application of new technologies
Manipulation of the growth medium
IROMP System

A-Layer

Cell Membrane

IROMP Antigens
Attenuated Vaccines
Attenuated Vaccines

- **Viral**
  - Channel Catfish Virus (CCV)
  - Infectious Haematopoietic Necrosis Virus
  - Infectious Pancreatic Necrosis (IPN) Virus
  - Spring Viraemia of Carp (SVC) Virus
  - Viral Haemorrhagic Septicaemia (VHS) Virus
  - Koi herpes virus (KHV)

- **Bacterial**
  - Flavobacterium psychrophilum
  - *Aeromonas salmonicida*
  - *Aeromonas hydrophila*
  - *Edwardsiella ictaluri*
  - *Flexibacter columnarae*
  - *Edwardsiella tarda*

- **Parasite**
  - *Crytobia salmositica*

However, it is the problems with licensing attenuated vaccines for use in the aquatic environment which has been a major constraint on their continued development.
Recombinant DNA Vaccines
Recombinant Fish Vaccines

• **Viral**
  Channel Catfish Virus (CCV)
  Infectious Haematopoietic Necrosis (IHN) Virus
  Infectious Pancreatic Necrosis (IPN) Virus
  Spring Viraemia of Carp (SVC) Virus
  Viral Haemorrhagic Septicaemia (VHS) Virus
  Infectious Salmon Anaemia (ISA) Virus
  Whitespot Virus of Shrimp (WSV)

• **Bacterial**
  Bacterial Kidney Disease (BKD)
  *Piscirickettsia salmonis* (SRS)

• **Parasite**
  *Ichthyophthirius multifiliis* (“Ich”)
  *Lepeophthirius salmonis* (Salmon louse)
DNA Vaccines
DNA-vaccination against VHS

Survival curves following challenge

Days after challenge

Survival (%)

50 ug G-plasmid
50 ug control-plasmid
Inactive virus
Unchallenged fish
Other ‘second generation’ vaccines/technologies which have shown promise under experimental conditions

- Virus-like Particle (VLP) Vaccines
- Chimera vaccines
- Molecular Decoys
- Molecular sponges
- Fish-derived anti-microbial peptides (broad-spectrum antibiotic properties. (No anti-microbial resistanc problems ?)
Virus-Like Particles (VLPs)
... The way it works – a “Ghost” virus

Yeast Cell (feed)

Yeast high copy plasmid

VP1

self assemble

VP2

Nuc

Empty VLP’s
(no nucleic acid)

“Ghost Virus”

Antigenic
... but not infective
... The way it works – a “Ghost” virus

“Effective, but not Infective”

Infective virus  Empty virus-like particle
Chimera Vaccines
... Making a bivalent vaccine using an IPNV VLP Platform
Gene Silencing
dsRNA

21-23 nt siRNAs
(small interfering RNAs)

RISC
(RNA-induced silencing complex)

mRNA target

perfect complementarity

Target cleavage and degradation

“miRNAs” with imperfect complementarity

Inhibition of translation
Molecular Decoys
Edible Shrimp “Vaccines”

... “Sponges” vs. “Decoys”

Diseased

Healthy

Virus binding to gut mucosa

Sponge binds up virus

Molecular Sponges

Healthy

Decoy blocks virus binding

Molecular Decoys

Both strategies presently in trials at SC-DNR
Oral Vaccines
<table>
<thead>
<tr>
<th>Pathogen and Reference</th>
<th>Fish Species</th>
<th>Challenge Route</th>
<th>% Mortality vaccinated (control)</th>
<th>Protection</th>
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<tbody>
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<td><em>Aeromonas hydrophila</em></td>
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<td>Post (1966)</td>
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<td>i.p.</td>
<td>60-90 (80-100)</td>
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<td><em>Aeromonas salmonicida</em></td>
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<td>Duff (1942)</td>
<td><em>S. clarkii</em></td>
<td>bath</td>
<td>25 (75)</td>
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<td>Sniezeko &amp; Fridle (1949)</td>
<td><em>S. fontinalis</em></td>
<td>i.p.</td>
<td>68 (90)</td>
<td>-</td>
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<td>Spence et al. (1965)</td>
<td><em>O. kisutch</em></td>
<td>scrape/bath</td>
<td>3 (22)</td>
<td>-</td>
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<tr>
<td>Overholser (1968)</td>
<td><em>O. kisutch</em></td>
<td>field</td>
<td>0 (58)</td>
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<td><em>S. fontinalis</em></td>
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<td>Udey &amp; Fryer (1978)</td>
<td><em>O. kisutch</em></td>
<td>field</td>
<td>35-62 (86)</td>
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<tr>
<td>Michel (1979)</td>
<td><em>S. gairdneri</em></td>
<td>i.m.</td>
<td></td>
<td></td>
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<tr>
<td>Smith et al. (1980)</td>
<td><em>S. arcuata</em></td>
<td>field</td>
<td></td>
<td></td>
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<tr>
<td><em>Flexibacter columnaris</em></td>
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<td>Fujiara &amp; Nakatani (1971)</td>
<td><em>S. gairdneri</em></td>
<td>field</td>
<td>19 (27)</td>
<td>+</td>
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<tr>
<td></td>
<td><em>O. kisutch</em></td>
<td>field</td>
<td>8 (48)</td>
<td>+</td>
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<tr>
<td><em>Vibrio anguillarum</em></td>
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<td>Hayashi et al. (1964)</td>
<td><em>S. gairdneri</em></td>
<td>field</td>
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<tr>
<td>Schreckenbach (1974)</td>
<td><em>Anguillaceae</em></td>
<td>salmonicida</td>
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<td><em>Salmonidae</em></td>
<td>field</td>
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<td>Bratton &amp; Hodgens (1976)</td>
<td><em>S. gairdneri</em></td>
<td>i.p.</td>
<td>7 (100)</td>
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<td>Hasted et al. (1977)</td>
<td><em>S. gairdneri</em></td>
<td>field</td>
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<tr>
<td>Prescott (1977)</td>
<td>Various marine tropical fish</td>
<td>sub cutaneous</td>
<td>9 (62)</td>
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<td>Kusalski et al. (1978)</td>
<td><em>P. alviss</em></td>
<td>cohabitation</td>
<td>10 (90)</td>
<td>+</td>
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<td>Gould et al. (1978)</td>
<td><em>O. nerka</em></td>
<td>field</td>
<td>23 (58)</td>
<td>+</td>
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<td>Nakajima &amp; Chikahara (1979)</td>
<td><em>P. alviss</em></td>
<td>cohabitation</td>
<td>22 (50)</td>
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<td>Bandin-LaRue &amp; Tangramipros (1980)</td>
<td><em>S. gairdneri</em></td>
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<td>32 (34)</td>
<td>+</td>
</tr>
<tr>
<td>Evelyn &amp; Ketcheson (1980)</td>
<td><em>O. nerka</em></td>
<td>field</td>
<td>18 (42)</td>
<td>+</td>
</tr>
<tr>
<td>Amend &amp; Johnson (1981)</td>
<td><em>O. kisutch</em></td>
<td>bath</td>
<td>27 (52)</td>
<td>+</td>
</tr>
<tr>
<td>Kawar et al. (1981)</td>
<td><em>P. alviss</em></td>
<td>cohabitation</td>
<td>27 (100)</td>
<td>+</td>
</tr>
<tr>
<td>Horne et al. (1982)</td>
<td><em>S. gairdneri</em></td>
<td>i.p.</td>
<td>94 (100)</td>
<td>+</td>
</tr>
<tr>
<td>Agius et al. (1983)</td>
<td><em>S. gairdneri</em></td>
<td>i.p.</td>
<td>40-100 (100)</td>
<td>+</td>
</tr>
<tr>
<td>Johnson &amp; Ahmed (1983b)</td>
<td><em>O. nerka</em></td>
<td>bath</td>
<td>15-45 (65)</td>
<td>+</td>
</tr>
<tr>
<td><em>Vibrio ruckeri</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ross &amp; Klontz (1965)</td>
<td><em>S. gairdneri</em></td>
<td>i.p.</td>
<td>10 (90)</td>
<td>+</td>
</tr>
<tr>
<td>Anderson &amp; Ross (1972)</td>
<td><em>S. gairdneri</em></td>
<td>sub cutaneous</td>
<td>10 (100)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>90 (100)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100 (100)</td>
<td>+</td>
</tr>
<tr>
<td>Johnson &amp; Amend (1983b)</td>
<td><em>S. gairdneri</em></td>
<td>bath</td>
<td>3 (65)</td>
<td>+</td>
</tr>
</tbody>
</table>

| a | oral intubation |
| b | anal intubation |
| c | fish were fed with chloroform killed cells |
| d | phenol killed cells |
| e | somiated formalin killed cells |
1. In the acid environment of the fish stomach, the feed pellets are digested. The *Antigens* themselves are protected by the APV and pass through intact.

2. *Antigens* are delivered to the area of the hind gut where they are absorbed and activate an effective immune response in the fish.
Microencapsulation

Problems associated with microencapsulation:

• Expensive
• Shelf-life/stability
• Difficulties with licensing
• Difficulty of incorporation into commercial feeds
• Palatability
Fig. 1. Scanning electron micrograph of PLGA (50:50) microparticles, incorporating HGG. 18 weeks post-incubation at 37°C (x 2700). Note that many of the microparticles appear pitted (P) and some have collapsed (C).
(c) Day 56, epithelial surface, supranuclear vacuoles (V) intensely stained (x400). DAB peroxidase development.

(d) Day 1, macrophages (M) showing immunoreactivity (x400). NiCl₂ enhanced DAB peroxidase development.
HOW TO SUCCESSFULLY MARKET THE BOOSTER

Part 1: SOWING THE SEEDS

DAVID HIGGINSON
of the Veterinary Health Centre, Blackburn gives a step-by-step guide to promoting vaccinations and regular pet health care check-ups

Stress 1. The need for annual boosters.
and 2. The benefits of an annual health check.

3. Explain that they will be reminded them by post.

4. Put the front of your vaccine certificate in bold (e.g. "SIX MONTHS PROTECTED UNTIL OCT 99").

5. We also inform clients that they will be re-called for a full health check at six months of age (more about this in the next issue).

6. Harvesting the crop - The recall.

1. Large attractive envelope with picture (preferably colour). Addressed to the PET owner.

2. Reminder of Annual Health Check and Booster (See Panel A). Also pictorial, and including a health check. This invites involvement; promises much more than just a 'gift', and brings the client in prime with problems to be solved.

3. Gift incentive (e.g. a paw-print teacup) if vaccinated within 14 days (See Panel B).

4. Information leaflet on a selected topic - e.g. 'Identifying'. Only one or two leaflets, don't overdo it. Overdue reminders are sent out to all boosters not done within three months of the date due.

Going through the mill (See Panel C) - Three grinding wheels to maximise your response.

1. What the client 'thinks' his pet needs.

2. Whatever else you do, you must deal with the problem appropriately in the client's mind - however trivial. Focus, observe, talk between the lines, and listen to your nurse. Nurses are often better placed to manage hidden tension while the client weights the pet, finds out what food is being fed, and when the animal was last wormed.

3. (All valuable ammunition to have before the consultation starts).

4. What you discover that the pet needs.

Discover is the important word here. The thrill and excitement of finding hidden treasure. Be enthusiastic about your thorough examination. Be seen to be examining the whole body, and talk your way through it. Say "I am looking in here because..." and checking this because..."

(Veterinary Life Assurance Services Limited)

Continued overleaf
Development of Improved Adjuvants
Speilberg Scale - Adhesion Scoring

GRADE 6

GRADE 6?
Adjuvants that specifically stimulate Th1 response in fish
Interleukins

Interleukins are produced by many cell types in response to damage and infection. So far in mammals 15 interleukins have been discovered. Of those Il-1 is the most important in the regulation and control of the immune response including:

• Killer-cell activity
• Polymorphonuclear leucocyte activity
• Activation of macrophages and macrophage killing
• B-cell proliferation
• Other functions
Recombinant Ovine Interleukin-1β as an Adjuvant for Multivalent Bacterial Vaccines

M. Elhay¹, G. Barcham², A. Cameron³, A. Andrews² and A. Nash²

¹TB Research Unit, Statens Seruminstitut, Artillerivej, 5, Copenhagen, 2300S, Denmark; ²Centre for Animal Biotechnology, The University of Melbourne, Parkville, Victoria 3052, Australia; ³CSL Ltd, 45 Poplar Road, Parkville, Victoria 3052, Australia

INTRODUCTION

The multifaceted nature of the immune response allows for development of protective immunity against a variety of pathogens, each of which may use a different strategy to infect and multiply within the host. To be effective, vaccination must induce an immune response of both sufficient magnitude and of an effector phenotype appropriate for rejection of the pathogen in question. With the exception of attenuated live vaccines, and some whole cell formulations, the magnitude and phenotype of the response to vaccination is determined primarily by the choice of adjuvant. Currently, aluminum based adjuvants such as aluminium hydroxide [Al(OH)₃] gel are the only adjuvants licensed for use in man and the most commonly used adjuvants for animal vaccines. While these compounds have been shown to be relatively safe, i.e. administration can be associated with nodule or granuloma formation (Gupta and Relyveld, 1991) and there may be other less well characterized systemic effects related to the use of aluminium-based compounds. In addition, induction of humoral and particularly cell-mediated immunity (CMI) by aluminium adjuvants is poor compared with induction by more reactive-oil-based adjuvants. The response induced by Al(OH)₃ is typical of the Th2 type (Grun and Maurer, 1989; Lise and Audibert, 1989) and while this may be appropriate for some pathogens this will not always be the case. Despite intense analysis of alternatives including mycobacterial fractions such as muramyl dipeptide, saponins and their derivatives, block-copolymer gels and hydrocarbon derivatives (reviewed in

Growth of Research
Increased interest in Finfish vaccination

Based on publications on vaccination efficacy trials: 1940’s to 1980’s
(Data from Newman [6])
TargetFish
TargetFish brings together a large number of leading European research groups that are experts on the fish immune system and enterprises from the Biotech and Veterinary sectors to advance the development of vaccines against important viral or bacterial pathogens in European aquaculture.

TargetFish is a large collaborative project funded by the European Commission under the 7th Framework Programme for Research and Technological Development.
The ‘blind-alley’

• Most ‘easy’ vaccines have been developed leaving vaccines against the more-difficult pathogens to be focused upon.
• We need to focus on new technologies etc. to make these vaccines available and thus remove a considerable constraint on the continued development of the aquaculture industry.
Registration of ‘second generation vaccines – a way forward or a ‘glass ceiling’ for aquatic animal health products?

• All veterinary vaccines for which the active ingredient has been derived by biotechnology must be registered through the Centralised Procedure

• Includes those containing genetically modified organisms (GMOs), recombinant but non-living organisms, virus-like particles, DNA vaccines, etc. (Definition in Directive 2001/18)
• Pros and cons:
  - Compared to National Procedures the Centralised route is the most predictable in terms of timelines, administration and interaction with the Rapporteur and Co-rapporteurs.
  - It can be expensive - > Euro 150,000 + Euro 32,000 maintenance fee. (but see later)
• The requirements for the registration dossier are fairly honerous
• An Environmental Risk Assessment (ERA) is required-expensive !!
• Potency test required
How can we utilize some of the incentives available for the registration of ‘second generation’ products?

• Not an easy answer and ‘level playing field’ arguments come into play.
• MUMS and SMEs can command a reduction in registration fees – Fees reduced by 90-100%!
• Rainbow trout = MUMS
  Sea Bass and Sea Bream = MUMS
• If we use SME status, how can large pharmaceutical companies work with them.
Disease/ Research Prioritisation
With the increase of disease pressures in aquaculture and funding constraints there needs to be a robust and regularly-applied \textbf{Prioritisation Exercise}
<table>
<thead>
<tr>
<th><strong>TROUT CONDITION</strong></th>
<th><strong>2013 v. 2012</strong></th>
<th><strong>5 year trend</strong></th>
<th><strong>Ranking/Cost</strong></th>
<th><strong>CONTROL</strong></th>
<th><strong>COMMENTS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative Kidney Disease (P.K.D.)</td>
<td></td>
<td></td>
<td>1</td>
<td>Exposure programmes</td>
<td>Failure of 2012 exposure programmes?</td>
</tr>
<tr>
<td></td>
<td>_arrow_up, arrow_down</td>
<td></td>
<td>£??</td>
<td>No treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Resistant strains?</td>
<td></td>
</tr>
<tr>
<td>Red mark Syndrome (R.M.S.)</td>
<td></td>
<td></td>
<td>2</td>
<td>Untreated recovery</td>
<td>Increase on restocking farms and sport fisheries</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>£??</td>
<td>Oxytetracycline</td>
<td>Now confirmed on brown trout</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Health diets</td>
<td>Rejections on table farms</td>
</tr>
<tr>
<td>Enteric Redmouth (E.R.M.)</td>
<td></td>
<td></td>
<td>3</td>
<td>Immersion/oral/injection vaccination</td>
<td>5 year trend down.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>£??</td>
<td>Oxolinic acid (Branzil)</td>
<td>Properly executed vaccination is controlling impact. Some farms injecting – 100% effective</td>
</tr>
<tr>
<td>‘Puffy Skin Condition’</td>
<td></td>
<td></td>
<td>4</td>
<td>None?</td>
<td>Mostly large triploid fish, increasing incidence and severity, CEFAS project</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>£??</td>
<td>Fish health diets?</td>
<td></td>
</tr>
<tr>
<td>Rainbow Trout Fry Syndrome (R.T.F.S.)</td>
<td></td>
<td></td>
<td>5</td>
<td>Florfenicol (Florocol), Amoxycillin (Vetremox)</td>
<td>Affecting larger fish.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>£??</td>
<td>Preventative health diets,</td>
<td>Resistance to florfenicol?</td>
</tr>
<tr>
<td>White Spot</td>
<td></td>
<td></td>
<td>6</td>
<td>Formalin, long term salt baths, increase water velocity</td>
<td>Increase v. 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>£??</td>
<td>Hot weather and low water levels</td>
<td>Future availability of formalin?</td>
</tr>
</tbody>
</table>
## DISEASE SCORECARD SEALICE

<table>
<thead>
<tr>
<th>Category</th>
<th>Weight</th>
<th>Score</th>
<th>Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. RISK &amp; IMPACTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1.1 Human health</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Is this a zoonotic disease? What is the risk?</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Risk of food poisoning?</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sum Human health</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1.2 Aquatic animal health</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk of territorial spread</td>
<td>2</td>
<td>4</td>
<td>12.0</td>
</tr>
<tr>
<td>Risk of spread across species</td>
<td>1</td>
<td>4</td>
<td>4.0</td>
</tr>
<tr>
<td>Sum health risk</td>
<td></td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td><strong>1.3 Environmental</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effect on wild populations</td>
<td>2</td>
<td>4</td>
<td>12.0</td>
</tr>
<tr>
<td>Ecological effects</td>
<td>3</td>
<td>6</td>
<td>6.0</td>
</tr>
<tr>
<td>Sum environmental score</td>
<td></td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td><strong>1.4 Financial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct loss from mortality</td>
<td>2</td>
<td>6</td>
<td>10.0</td>
</tr>
<tr>
<td>Direct loss due to high FCR/reduced growth/lower quality</td>
<td>2</td>
<td>6</td>
<td>10.0</td>
</tr>
<tr>
<td>Trade loss</td>
<td>2</td>
<td>3</td>
<td>3.0</td>
</tr>
<tr>
<td>Loss due to cost of official control measures</td>
<td>2</td>
<td>4</td>
<td>8.0</td>
</tr>
<tr>
<td>Sum Financial</td>
<td></td>
<td></td>
<td>7.8</td>
</tr>
<tr>
<td><strong>1.5 Customer view and societal impact</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Customer view</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Societal impact</td>
<td>2</td>
<td>4</td>
<td>4.0</td>
</tr>
<tr>
<td>Security of supply</td>
<td>1</td>
<td>4</td>
<td>4.0</td>
</tr>
<tr>
<td>Sum customer view and societal impact</td>
<td></td>
<td></td>
<td>1.7</td>
</tr>
<tr>
<td><strong>TOTAL RISK &amp; IMPACT SCORE</strong></td>
<td></td>
<td></td>
<td>26.4</td>
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<tr>
<td><strong>2. KNOWLEDGE GAP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Characterisation of agent / disease</td>
<td>0</td>
<td>4</td>
<td>4.0</td>
</tr>
<tr>
<td>Basic epidemiology</td>
<td>2</td>
<td>5</td>
<td>10.0</td>
</tr>
<tr>
<td>Immunology</td>
<td>2</td>
<td>5</td>
<td>10.0</td>
</tr>
<tr>
<td>Strain/species variation</td>
<td>2</td>
<td>5</td>
<td>10.0</td>
</tr>
<tr>
<td>Diagnostics</td>
<td>1</td>
<td>4</td>
<td>4.0</td>
</tr>
<tr>
<td><strong>SUM GAP SCORE</strong></td>
<td></td>
<td></td>
<td>7.8</td>
</tr>
<tr>
<td><strong>3. REQUIREMENT OF CONTROL MEASURES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Management/husbandry practices</td>
<td>1</td>
<td>6</td>
<td>5.0</td>
</tr>
<tr>
<td>Chemotherapeutants</td>
<td>2</td>
<td>3</td>
<td>9.0</td>
</tr>
<tr>
<td>Vaccines</td>
<td>3</td>
<td>5</td>
<td>15.0</td>
</tr>
<tr>
<td><strong>SUM CONTROL SCORE</strong></td>
<td></td>
<td></td>
<td>9.7</td>
</tr>
<tr>
<td><strong>OVERALL RATING</strong></td>
<td></td>
<td></td>
<td>75.3</td>
</tr>
</tbody>
</table>
Diagnostics

Reduction in the use of antibiotics, as well as relying on the use of vaccines, also depends on trained veterinary monitoring and the development and routine use of rapid diagnostic methods.
PRODUCTS

- AquaMab-P Detect pathogens IHC & IFAT
- AquaMab-F Detect fish species IgM ELISA
- Pre-coated ELISA plates
- HRP Conjugates
- Rapid Test Kits
ISAV RAPID KITS

- **Negative Result**

- **Positive Result**
There has been a recent focus on the use of antibiotics in animal husbandry throughout Europe

• “Over-use of antibiotics is an issue in animal husbandry, agriculture and fish farming”

• “The development of germs that are resistant to even the strongest of our current antibodies is one of the biggest health threats in the world.”

• “We continue to use them (antibiotics) in agriculture, fish farming and myriad other areas of life.”

• “......the typical farmed American salmon eats its own weight in antibiotics before it is sold.”

Dame Sally Davies, UK Chief Medical Officer

WHAT ARE THE FACTS ?
Chemical Use in Salmon Aquaculture: A Review of Current Practices and Possible Environmental Effects

Les Burridge, Judith Weis, Felipe Cabello and Jaime Pizarro


Salmon Production (Metric Ton) | Therapeutant Type | Kg (active ingredient) Used | Kg Therapeutant/Metric Ton produced
--- | --- | --- | ---
Norway | 509544 | Antibiotics | 805 | 0.0016
| | Anti-louse | 98 | 0.0002
| | Anaesthetics | 1201 | 0.0023
Chile | 280,481 | Antibiotics | 133800 | 0.477
| | Anti-louse | 136.25 | 0.0005
| | Anaesthetics | 3530 | 0.013
UK | 145609 | Antibiotics | 662 | 0.0045
| | Anti-louse | 110 | 0.0007
| | Anaesthetics | 191 | 0.0013
| | Disinfectants | 1848 | 0.013
Canada (includes data from Maine, USA) | 111,178 | Antibiotics | 30373 | 0.273
| | Anti-louse | 12.1 | 0.00011

# ERM VACCINE FIELD TRIALS

Medicated Feed Utilisation

<table>
<thead>
<tr>
<th>Medicated Feed (kg)/1000 Fish</th>
<th>Vaccinates</th>
<th>Controls</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>4.5</td>
<td>16.8</td>
<td>73</td>
</tr>
<tr>
<td>Trial 2</td>
<td>2.27</td>
<td>15.9</td>
<td>86</td>
</tr>
<tr>
<td>Trial 3</td>
<td>6.3</td>
<td>22.0</td>
<td>72</td>
</tr>
</tbody>
</table>
## Table 2: Distribution of antibiotic sales by species

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tonnes Active Ingredient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cattle Only Products</strong></td>
<td>9</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td><strong>Pig Only Products</strong></td>
<td>66</td>
<td>62</td>
<td>62</td>
<td>47</td>
<td>62</td>
</tr>
<tr>
<td><strong>Poultry Only Products</strong></td>
<td>18</td>
<td>31</td>
<td>37</td>
<td>50</td>
<td>23</td>
</tr>
<tr>
<td><strong>Sheep Only Products</strong></td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>Fish Only Products</strong></td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Pig and Poultry Combined Only</strong></td>
<td>216</td>
<td>195</td>
<td>205</td>
<td>252</td>
<td>162</td>
</tr>
<tr>
<td><strong>Multi Species Products In Food Animals Only</strong></td>
<td>22</td>
<td>28</td>
<td>31</td>
<td>29</td>
<td>29</td>
</tr>
</tbody>
</table>
Figure 2: Distribution of antibiotics sold by species
Figure 3: Sales of total antibiotics for fish only (tonnes active ingredient) by live weight (‘000 tonnes) of animals (fish) slaughtered for food use 1993–2011
Figure 4: Distribution of antibiotics sold (grams active ingredient) per tonne of live weight of animals slaughtered for food

- UK
Mortalities due to Furunculosis as a % of total mortalities

% mortality

Year

1992
1993
1994
Antibacterial agents

Production
(metric tons $\times 10^3$)

Antibacterial agents
(metric tons)


Production

Antibacterial agents

Yellow line: Production
Red line: Antibacterial agents
Antibiotic use
Danish Fish Farming

Mg active substances per kg produced meat

år

Fresh Water Farms
Marine Farms
Exceptions to the Rule?

- Ornamental fish industry
- SE Asia
- Latin America (mainly Chile)

Latter two must be regarded as ‘work in progress’- new industries, new emerging diseases and lateness in developing/adopting vaccination.