

Trends in therapy and prophylaxis 1991-2001

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Abstract

This overview attempts to chart the changes in approach to prevention and control of disease in aquaculture in Europe over the last ten years. In 1991 when the Office International des Epizooties organised a conference entitled "Chemotherapy in aquaculture: from theory to reality" concentration of interest was very much towards the problems of the use of chemotherapeutants, mainly antibiotics, to control aquatic disease. Major changes were underway in the way in which veterinary medicines were approved in Europe. Greater information on potential for drug resistance, both in the aquatic environment and in its possible importance for the consumer of aquacultural products was becoming essential. The "MRL Regulation" had just been published and work was going on towards the development of formal residue monitoring programmes for fish meat. As anticipated at that time, the availability of chemotherapeutants for use in aquaculture has become more restricted and very few new drugs have become available. Fortunately, also in the early 1990's another major change was taking place, in that effective fish vaccines began to become available, particularly for prevention of furunculosis in the rapidly expanding salmon industry. So rapidly did these vaccines become accepted that the market for antimicrobials in European aquaculture fell from many thousands to a few tens of kilograms per annum.

By the turn of the Century effective vaccines were available for control of most of the major bacterial pathogens and the first viral vaccines were reaching marketability. No vaccines are yet available for a few bacterial pathogens such as *Renibacterium* and *Flavobacterium psychrophilum*, both mainly problems with the lower value trout industry remain without effective vaccines. Outside of the bacterial pathogen field the principal advances have continued in chemotherapy, with oral drugs for the control of sea lice infestations becoming available to replace the use of organophosphates with their difficulties and hazards of use. Malachite green became recognised as a problem largely because of persistence of residues after exposure, which became evident as the residue monitoring pro-

grammes became established. In the last three or four years, new trends have become evident, with the publication of research into the use of probiotics to prevent or mitigate the effects of infectious disease. Interest in the use of immunostimulants has increased and these are now added to many commercial diets. Rigorous data substantiating the effects of these approaches is still not available.

Introduction

Ten years ago the Office International des Epizooties organised a major international conference entitled "Chemotherapy in aquaculture: from theory to reality" (Michel and Alderman, 1992). Although effective fish vaccines were then available, particularly for the control of furunculosis in salmon, concen-

tration of interest was very much towards the problems of the use of chemotherapeutants to control aquatic disease. In the ten years since that conference, the creation of the open market within the European Union has brought about major changes in legislation controlling fisheries medicines, particularly antibiotics. Control of sea lice in salmon culture, whilst still a major problem, has seen a change from organophosphates through hydrogen peroxide to oral agents. Use of malachite green which ten years or so ago was the universal remedy for fungal and topical ectoparasite problems in trout and salmon parr is now recognised as a source of residue problems and few if any farms use it even in the hatchery. Finally, the most significant change of all is that vaccines have now fully arrived, being universal in use in salmon culture and extending into trout and sea bass / bream culture.

During this period there have also been a number of significant changes in the pharmaceutical industry supplying drugs and vaccines to fish farmers. Small veterinary supply houses were the source of most drugs for aquaculture and fish vaccine manufacturers were generally small start up companies. Over the decade these small companies were replaced, usually by take-over by larger companies so that by 2001 most of the suppliers and manufacturers are now owned by the major pharmaceutical groups with major companies such as Akzo-Nobel (Intervet Norbio), Novartis (AquaHealth, Vericore), Alpharma (AL, Biomed) and Bayer (Microtek) involved.

Antimicrobials

The movement towards common harmonised legislation in the interests of freedom of trade has resulted in extensive and complex laws controlling the approvals of pharmaceutical and immunological products for use in veterinary medicine, most particularly for food species (Alderman, 1999) within the EU. Aquaculture is, except in Norway, a small market, relative to large animal land farming. It is thus unable to support the costs of gaining marketing approval for pharmaceuticals under the current legislative framework, except for well-established products. These are products for which in effect only the "additional" safety and efficacy data for use in the aquatic environment are required for approval. In many ways the availability of aquaculture pharmaceuticals in Europe has, despite these difficulties, remained much better than in N. America. This is partly because the changes in European legislation came later than in USA and Canada and because aquaculture was larger and longer established there. This meant that aquaculture pharmaceutical products had authorisation of right giving time to generate the data required and that the companies concerned were sure of an existing market.

The basic European legislation on harmonisation of laws on veterinary medicines dates back 20 years (CEC 1981a,b, 1987), but the change which has had most effect on the availability of aquaculture medicines is the combination of the MRL Regulation (2377/90/EEC) and the Residues Directive (96/23/EC). The effect of these has been to require that only veterinary medicines for which a Maximum Residue Level (MRL) has been set may be used in food animal species and that moni-

toring programmes to detect and prevent the presence of illegal residues and residues in excess of MRL must be introduced. Fortunately, few licensed products were lost to aquaculture use from the introduction and enforcement of these requirements although for some time it seemed likely that products such as oxolinic acid might be lost through lack of support. Indeed one new fluorinated quinolone, sarafloxacin did become available for aquaculture use during the period. The main effect of the changes in the legislation in the end was on the use of unauthorised products such as malachite green and ivermectin where the presence of any residue in fish at slaughter indicated either the use of an unauthorised product (malachite green) or failure to ensure sufficient withdrawal time (ivermectin). Changes in the way in which Marketing Authorisations (licenses) could be obtained, making applications in more than one member state easier, have been introduced too recently to have had any significant effect on the availability of pharmaceuticals in aquaculture.

The use of antibiotics in aquaculture has unquestionably led to selection for resistance in fish pathogens and an increasing number of studies have been published on different aspects of this (Alderman and Hastings, 1998; Barnes *et al.*, 1990; Hastings, 1997; Husevag, and Lunestad, 1995; Inglis *et al.*, 1991, 1993; Samuelson *et al.*, 1992b; Smith *et al.*, 1994; Tsoumas *et al.*, 1989). In some cases antibiotic resistance reached a point at which no authorised antibiotic was effective. This presented a very significant problem for the fish farmer that, for salmon at least, was overcome by wider availability of good vaccines. It also led to concern about risks that resistance

might be transferred to human pathogens and also in regard to the potential for adverse environmental impact. Many studies on environmental persistence and potential impact of antimicrobials perhaps unsurprisingly concentrated upon worst case use (Bjorklund *et al.*, 1991; Coyne *et al.*, 1994, Husevag and Lunestad 1995; Kerry *et al.*, 1994; Samuelson, 1992a, b; Smith, 1996; Smith *et al.*, 1994, 1995, 1996). The inevitable outcome of this was the development of a view that the use of antimicrobials in aquaculture was something to be avoided or prohibited. This was unsurprising since over the same time there had been an increasing anxiety over the use of antimicrobials in food animal species in general and evidence began to come forward which supported the view that such use could, in some circumstances, give rise to real risks for consumers.

The U.K.'s Advisory Committee for the Microbial Safety of Food's subcommittee on antibiotic resistance (1999) was one body to include aquaculture in its overview, as did the WHO (World Health organisation, 1999, Alderman and Hastings, 1998). Reports produced by both bodies largely exonerated the use of antimicrobials in aquaculture from creating consumer health problems. This view was perhaps potentiated by the changes, which had taken place in the salmon industry, which was the major user of antimicrobials. Although papers published on antimicrobials in salmon farming in peer review journals in first half of the 1990's indicated major use, selection for resistance and potential environmental impact, the dates of publication did not take account of the lead-time for taking research through to the publi-

cation stage. Problems there had been, particularly in worst case situations, but the introduction of effective vaccines against furunculosis and vibriosis had enabled nearly a 100 fold reduction in use of antimicrobials in less than 5 years whilst salmon production increased.

The introduction of effective vaccines had an inevitable effect on the aquaculture pharmaceuticals market where sales of antimicrobials fell rapidly in response. Although Europe had entered the last decade of the 20th Century with markedly more antimicrobials authorised for use in aquaculture than did N. America, towards the end of that period there was a risk that the situation might change drastically for the worse as drug suppliers considered ceasing to fund retention of Marketing Authorisations for some of their products. This cost pressure is exacerbated by the fact that the data generated for the original marketing authorisations no longer meets modern standards and regulatory authorities hence must request additional data generated to current standards from suppliers.

Products such as immunostimulants have entered use widely during the decade, normally as feed additives with the feed companies concerned not making claims that could be interpreted as medicinal claims and hence avoiding the costs putting such products through the strict regime of veterinary medicines licensing. This "escape" from regulatory control may now be coming to an end with the introduction of new legislation requiring strict safety and efficacy proof similar to that required for veterinary medicines (Commission Directive 2001/79/EC). This legislation comes into effect in early 2002 and

it will be of interest to see if such products will survive the potential costs involved.

Immunologicals

Although vaccines for prevention of enteric redmouth disease and against vibriosis were relatively easily developed and were established in the market in the 1980's, the critical disease for widespread acceptance of vaccination in the valuable salmon industry was furunculosis. By the beginning of the 1990s furunculosis vaccines were just becoming established, with effective products available for control of furunculosis, vibriosis and enteric redmouth. The major losses caused by furunculosis in the salmon industry accompanied by heavy use of antimicrobials and problems of drug resistance acted perhaps as the strongest driving force in the development of fish vaccines. The host species, salmon, was of sufficient individual value to make the investment in the extra cost and labour needed to vaccinate individual fish economic, provided the vaccine concerned offered a reasonable level of efficacy. Even the earliest vaccines with RPS values as low as 25% were worth using and the resulting sales may have given vaccine manufacturers the confidence to invest in development of more effective products. Also, the regulatory requirements for licensing of vaccines are less demanding than those for pharmaceuticals where consumer safety is paramount.

Although effective vaccines for most bacterial diseases of salmon have been introduced and indeed developed and improved, some bacterial diseases such as BKD still lack effective vaccines. The rapidly growing sea bass and bream industry of the Mediterranean is attracting vaccines for control of vibriosis and

pasteurellosis, viral vaccines are not yet widely available, although IPN is now a common antigen included in salmon vaccines. Where the disease concerned is notifiable to CEC there is currently a strong presumption against the use of vaccines. This is particularly the case with viral diseases where vaccinated fish might be expected to give false positive results with immunological or molecular test methods. Whether the convenience of diagnostic testing technology should over-ride the ability to protect fish against a disease is a moot point. In strict animal welfare terms, there is no case for restricting vaccination if the vaccine is effective and effective vaccines may require some aspects of the approved zone concept for fish health movement controls be reconsidered. Vaccination cannot however replace such biosecurity controls completely, since vaccines prevent or reduce the effect of disease and may well result in creation of levels of farmed fish carrier status sufficient to place wild fish stocks at risk. Such wild stocks cannot of course be vaccinated.

As yet, vaccines against systemic protozoans such as *Tetracapsula bryosalmonae* (PKX) and *Myxobolus* spp. or against external parasites such as *Ichthyophthirius* are little more than a "gleam" in the vaccine manufacturer's eye despite a significant amount of academic research.

Academic researchers also invested heavily in attempting to develop DNA vaccines in the last ten years with numbers of publications and conference contributions appearing. These although scientifically interesting and of high technical quality were in practical terms largely naive in approach. Academic

researchers seem to rarely be aware of the enormous hurdles that must be overcome in attempting to satisfy the requirements of the regulatory authorities of the safety of such biotechnology products in use. Any information generated which relates to vaccine safety must be generated in compliance with Good Laboratory Practice so that most of the academic research towards DNA vaccines which, although of high quality, has not been conducted to that formal quality standard and would have to be repeated. In Europe such biotechnology products can only be authorised for use via the centralised procedure by the European Medicines Evaluation Agency. Whether the aquaculture market can support the necessary costs to be first in the market with such a product remains to be seen.

Sea lice therapies

Although these are pharmaceuticals, the difference in the manner of use of the original sea lice control products justifies separate consideration. Sea lice infestations have been a major problem in salmon farming throughout its development. Although the original control agents, organophosphates such as dichlorvos were effective, they were difficult to use. The need to use canvas skirts to enclose treatment baths of toxic organophosphates, plus the subsequent discharge of considerable quantities after treatment presented user and environmental hazards, but the linkage of human health problems with use of OPs in sheep farming increased pressure for a need to find alternatives.

A more potent (but more toxic) OP, azimethiphos replaced dichlorvos. Hydrogen peroxide was found to be an effective alter-

native, but one that still required the use of canvas skirts on cages under treatment. Although environmentally friendly because it breaks down rapidly and leaves no residues, transport cost and transport hazards presented difficulties, (particularly for island based fish farms where hydrogen peroxide tanks could not be shipped on passenger ferries) and use at higher water temperatures revealed toxicity problems to the fish being treated. Cypermethrin a synthetic pyrethroid became available in the late 1990's, followed in Norway by Deltamethrin. These are much more environmentally persistent and still require the use of canvas skirts. Ivermectin a well-established insecticide also proved to be very effective, but the manufacturer has not been prepared to develop fish formulations and obtain a Marketing Authorisation. Oral formulations designed for use in swine can be used legally under the veterinary prescription cascade system, but not the cheaper cattle pour on formulations. Ivermectin proved to require a long withholding period, since even under the cascade system, no residues can be present in fish at slaughter because no in fish MRL exists. The residues monitoring programmes mentioned above continue to detect the presence of ivermectin from time to time in farmed salmon. Most recently oral anti sea lice agents such as teflubenzuron (Calicide, Nutreco) and emamectin benzoate (Slice, Schering Plough) have been developed that can be applied in feed and for which Marketing Authorisations have been obtained.

The use of quantities of insecticidal compounds in sea cages often in enclosed sea lochs and fjords present difficult environmental impact problems. Whilst assessment for

safety as a veterinary medicine requires a detailed consideration of aspects of environmental impact (Veterinary Medicines Directorate, 1996), this can only be generalised in nature. Impact assessment of individual fish farms and individual permits to discharge sea lice treatment chemicals (and most other aquaculture medicines) from the appropriate environment protection authority have become increasingly important and now play a considerable role in the availability of these products for use at each fish farm site.

With the development of improved sea lice treatments, and with the introduction of wide area cage farm management systems with programmed fallowing of sites, the sea lice problem is a more under control, but with still such a limited range of products, resistance remains a problem.

Malachite Green

Beyond the area of antimicrobials, in the 1980's the traditional fish fungicide and topical ectoparasiticide, malachite green, was recognised as being effective as a systemic therapeutant against the protozoan kidney parasite, PKX. This recognition led to the realisation that malachite green bioaccumulates in fish. With the introduction, as part of the harmonisation of European legislation on veterinary medicines, of a mandatory programme for monitoring veterinary residues in food fish species, it became very evident that, not only did malachite green bioaccumulate, but it persisted as a residue. With lack of modern toxicity data for an unauthorised veterinary medicine the widespread use of malachite green resulted in the detection of unauthorised residues in farmed trout in the new monitoring programmes (Veterinary Medicines Directorate, 2001). Its use

therefore rapidly became unacceptable and alternatives such as formalin were much less effective. Continued use in hatcheries on eggs could be tolerated, but with the risk of inadvertent contamination of fish from malachite green in the hatchery effluent. The trout farming industry made great efforts to limit its use, and rate and concentrations detected in fish tissues sampled in residues monitoring programmes fell to very low levels. However developments of analytical technology enable the detection of a metabolite, leucomalachite green that appears to persist even longer than the original substance and this has made malachite green's continuing use even in hatcheries problematical. Fortunately, at the time that these difficulties were continuing, bronopol (Pyceze, Novartis) was being developed as a replacement fisheries fungicide and this is now authorised for use on eggs and in final development for approval in fish. A major conceptual problem still remains that of cost. Malachite green was never a properly authorised veterinary medicine, it is a commercial dye, not a pharmaceutical product and its cost to farmers was therefore low. Any replacement product will be a veterinary medicine that has been subjected to an extensive safety and efficacy test programme to meet the requirements of European legislation and therefore will have a cost commensurate with such products. It will take some time for users to realise that had malachite green been approved as a veterinary medicine it too would have been a relatively expensive product.

Conclusions

The ten years to 2001 have been ones of major change for the aquaculture industry as re-

gards therapy and prophylaxis. On the pharmaceutical side, increases in regulatory requirements might well have proved disastrous with the loss of a number of critically important pharmaceuticals. This loss would partly have been due to increased regulatory costs and partly due to lack of sales sufficient to support those costs. This in turn would have resulted in most pharmaceutical use being by the cascade route so that heavy regulation in fact produced a reduction in regulatory control. The Regulatory authorities both in Europe and the USA have recognised that over heavy regulatory requirements may produce less effective regulation (Committee for Veterinary Medicinal Products, 1997, 1998; United States Food and Drug Administration, 1998). Designation of salmonids as major species and other fish as minor species and the recognition that no unexpected metabolites have come to light in fish so far have eased the need for costly safety testing. Worries over environmental impact and antibiotic resistance transfer appear to have waned. The range of authorised antimicrobials is still restricted, but at least no worse than ten years ago and major new products have become available to replace dichlorvos and malachite green.

The greatest success has been in the field of vaccines, with very effective multivalent vaccines now available for control of bacterial diseases in salmonids and sea bass. The economies of vaccination are now such that some parts of the trout industry are adopting vaccination even where the more labour expensive injection vaccines are needed. New vaccines and vaccine formulations continue to appear.

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