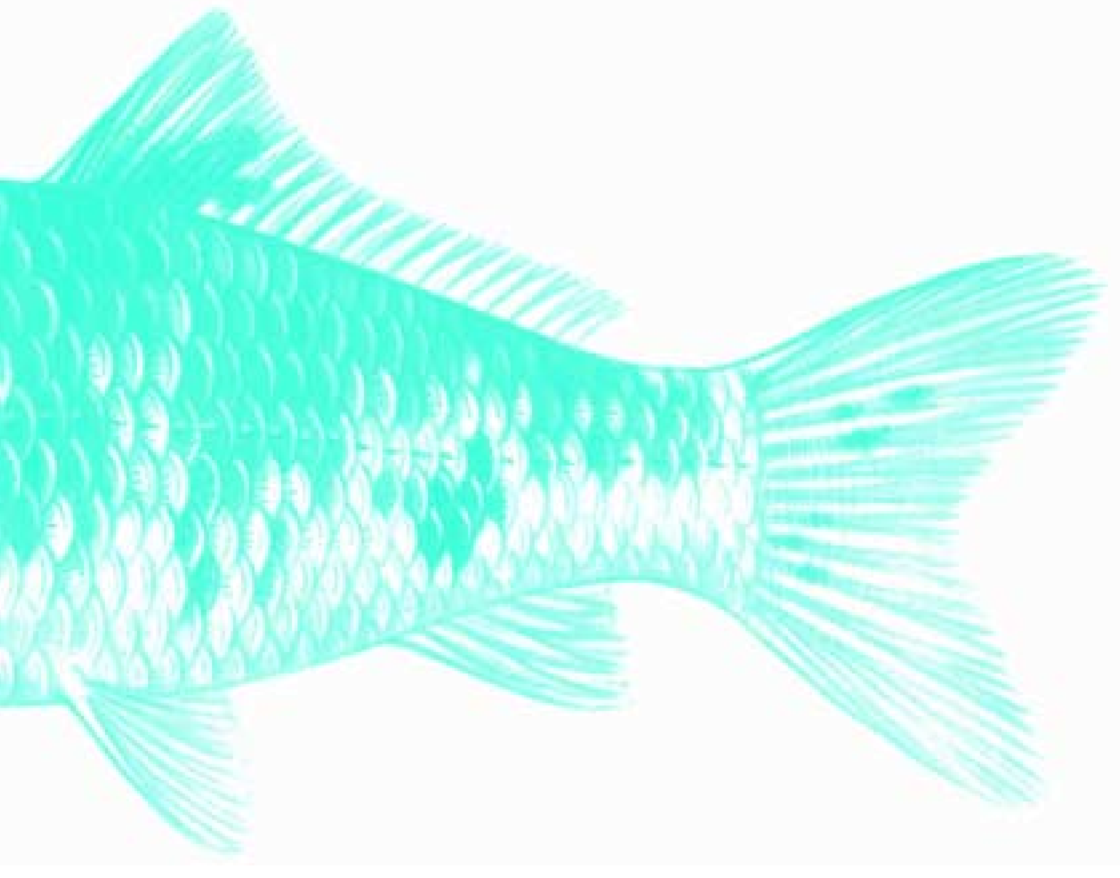
FISH VETERINARY JOURNAL

The Journal of the Fish Veterinary Society

Issue Number 3 • February 1999



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The *Fish Veterinary Journal* is published by the Fish Veterinary Society and provided free to the society's members. Additional copies can be obtained from the editorial department for £15 (UK) and £25 (overseas).

The FISH VETERINARY SOCIETY was formed in July 1990, with the intention of bringing together veterinarians with an interest in fish, so that they may benefit from mutual experiences and discussions, and help to advance the veterinary care and welfare of fish.

FISH
veterinary
SOCIETY

The society provides:

- two scientific meetings, held annually
- publication of the *Fish Veterinary Journal*
- publication of policy documents on fish health and welfare
- political lobbying and representation on behalf of the members' interests

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MEMBERSHIP of the Fish Veterinary Society is open to members of the Royal College of Veterinary Surgeons, although the Society will consider applications from overseas veterinarians. Currently membership costs £20 per annum and there is an additional £30 joining fee for new members. Veterinary students may become associate (non-voting) members of the Society; they pay no fee until they graduate, at which point they will become full members if they so wish. Enquiries regarding membership of the Fish Veterinary Society should be addressed to the treasurer — see the membership form at the back of this Journal.

Notes for contributors

The *Fish Veterinary Journal* invites contributions from members and other professional colleagues and is keen to publish original research, review articles and clinical case histories on all aspects of fish health. Letters, book reviews and other comment on relevant topics are also welcomed.

Scientific articles submitted to, or published in, other refereed journals will not be considered for publication. Papers and short communications submitted for publication are subject to peer review. The editor has the final decision on publication and if accepted, the copyright becomes the property of the Fish Veterinary Society.

Manuscripts and all communications should be sent to W.H. Wildgoose, 655 High Road Leyton, London E10 6RA. Manuscripts should be submitted in duplicate, typewritten using a Times or Roman font (double line spaced) on one side of A4 paper with wide margins. Scientific articles may also be submitted as an ASCII file on a 3½" diskette (MS-DOS format). The Journal cannot accept responsibility for loss or damage of manuscripts.

Format:

Papers should be headed with the full title, which should describe accurately the subject matter. The initials and surnames of the authors, with full postal addresses should follow. Each paper should have a self-contained summary (maximum of 150 words) which embodies the main conclusions.

Abbreviations should be avoided. Where they must be used, the word or phrase must be given on the first occasion, *eg* infectious pancreatic necrosis (IPN). All units of measurement should be given in the metric system and temperatures in °C. Blood biochemistry values should be expressed in standard SI units. Medicinal products should be referred to by their generic name followed by proprietary name and manufacturer in brackets when first mentioned, *eg* amoxycillin (Vetremox®; Vetrepharm). The full Latin name for each species should appear at least once when mentioned in the text.

Length of papers:

Papers should be concise. As a guide, the maximum length for scientific articles is 3,000 words; for review articles up to 4,000 words; for short communications and clinical case reports up to 1,500 words.

Tables and illustrations:

The minimum number of figures necessary to clarify the text should be included and should contain only essential data. Tables must be typewritten on separate sheets and numbered. Illustrations should be drawn in black ink on white paper and should be suitable for direct photographic reproduction.

Legends should be typed on a separate sheet. Photographs should be clear and sharp, and in colour where possible (transparencies should be accompanied by one set of prints). Photomicrographs must state magnification and stain technique. Each illustration or photograph should bear the author's name and figure number in pencil (or on a label) on the back and an arrow used to identify the top edge. All photographs will be printed in black & white but may be reproduced in colour at the expense of the author(s).

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Only papers closely related to the author's work should be mentioned. These should be stated in chronological order in the body of the text and should be listed in alphabetical order and include the full title thus:

Hanson, L.A. & Grizzle, J.M. (1985) Nitrite-induced predisposition of channel catfish to bacterial diseases. *Progressive Fish-Culturist* **47**, 98–101

Morrison, C.M., Cornick, J.W., Shum, G. & Zwicker, B. (1984) Histopathology of atypical *Aeromonas salmonicida* infection in Atlantic cod, *Gadus morhua* L. *Journal of Fish Diseases* **7**, 477–494

Roberts, R.J. (1993) Motile aeromonad septicaemia. In: *Bacterial Diseases of Fish.* (eds V. Inglis, R.J. Roberts & N.R. Bromage). Blackwell Scientific Publications, Oxford. pp143–155

If three or more authors are quoted, then all must be listed in the references and should be written as 'Morrison and others 1984' in the body of the text.

Personal communications should be cited as such.

Miscellaneous:

A brief personal profile of academic achievements and the current position of the author(s) is also required as a foot-note (maximum of 100 words) for scientific articles.

The *Fish Veterinary Journal* is covered by the CAB abstracts database.

President's Reflections

Andrew N. Grant
Marine Harvest McConnell, Farms Office, Blar Mhor Industrial Estate
Fort William, Highland, Scotland PH33 7PT

1998 has been the *annus horribilis* for Scottish salmon farming with the first appearance in an EU member state of infectious salmon anaemia (ISA). An account of the early stages of the outbreak appears on page 64.

The disease has been officially recognised in Norway since 1984 and appeared subsequently in Canada in 1997. The impact in all three countries has been devastating and the full effects in Scotland have yet to be seen. At present ISA is a List 1 disease under EU law and the policy for such a disease is eradication with no options for alternative action at the discretion of the member state concerned, and no compensation for loss suffered by farmers. Who could argue that eradication of any disease is desirable but is it realistic in the context of extensive aquaculture? The unfolding story in Scotland has demonstrated how problematic this approach can be and there is no certainty that the stated objective can be achieved while retaining a viable industry in the present economic climate. The Norwegian industry has lived with ISA for 15 years but has failed to eliminate it completely.

The ramifications of the present outbreak extend well beyond the strict confines of science and it seems that any issue affecting salmon farming in Scotland is destined to become a political football attracting any pressure group with an axe to grind; the facts soon become lost in rhetoric and misinformation.

The only ultimately satisfactory and practical way of managing infectious disease in aquaculture is by effective vaccination combined with best practice in husbandry. However, the present policy excludes consideration of vaccination as an alternative or supplement to the actions stipulated by law. This places both farmer and regulator in an invidious position from which there seems at present, little prospect of relief. The Canadians have taken the decision, after a brief but disastrous acquaintance with ISA, to embark on a large scale trial with a vaccine. There is no certainty that this strategy will offer complete protection for the next input of smolts but a start has been made and available technology can further refine the vaccine. It does seem that there is a tacit acceptance in Canada that eradication is not possible.

Beyond ISA, fish medicine in general faces the usual issue of medicines availability and the continuing pressure, in the case of food fish, from customers and consumers for reduced use of medicines combined with greater concern for welfare and environmental impact. The Fish Veterinary Society (FVS) can, by demonstrating concern and competence, offer assurance in both areas and we will continue to press the case for easing the licensing process.

Revision of the Veterinary Surgeons Act 1966 continues to feature in the correspondence columns of the veterinary press. FVS is committed to making the case for inclusion of farmed fish in any revision of the Act.

Finally, I urge members to take advantage of the FVS web site (**http://www.greens.net/fishvet**) to communicate widely with others who have similar interests and to attend our meetings; the next will be in April 1999.

Happy 1999

Editor's Comments

William H. Wildgoose, 655 High Road, Leyton, London E10 6RA

The constraints of space and time have limited me to giving my thanks to the impressive contributions from all authors and contributors. The bulk of this issue is a collection of proceedings from both the Society's spring and autumn scientific meetings. Both were well attended and attracted speakers involved in a wide range of subjects, reflecting the diversity of the field of fish health care. I am indebted to the prompt efforts from the anonymous reviewers and also to Keith Treves Brown for ensuring that the respected standard of this Journal is maintained. With greater financial support from our advertisers and the help from Mike Williams of Akalat Publishing, we hope that the introduction of colour is also appreciated by our readers.

Fish Veterinary Journal (1999) (3) 1–18

Koi health care in the UK: a veterinary overview

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655 High Road, Leyton, London E10 6RA

The veterinary investigation of ornamental fish diseases is fraught with problems. It is time-consuming and often requires a lengthy visit to the site with detailed examination of the facility, fish and laboratory samples. The latter may involve bacteriology and histopathology, taking several days for a reasonably accurate diagnosis to be made. Sick fish are usually seriously ill before veterinary attention is sought and the sacrifice of affected cases to obtain pathological samples is rarely possible due to their emotional and financial value. Successful treatment involves not only curing the patient and eradicating the pathogen but correcting the under-lying environmental factors to avoid the problem recurring. Most koi are kept in outdoor ponds where seasonal temperatures influence the rate of recovery from disease, which may take several weeks in cold weather. Despite the high cost of a well-stocked pond and filter system, koi-keepers are frequently unwilling to pay appropriate fees for a comprehensive investigation and treatment. Consequently, treating these fish is often a considerable challenge but, despite this, their health problems are often fascinating and curing them can be immensely rewarding.

Historical background

Fish are not included in the Veterinary Surgeons Act (1966) and therefore non-veterinarians can investigate, diagnose, treat, and perform surgery on fish. As a result, there has been a significant involvement by dealers or retailers, biologists and hobbyists in the field of ornamental fish health. However, few dealers, if any, offer more than basic water quality tests and examination of skin and gill scrapings under the microscope. Despite this, hobbyists are often willing to entrust dealers to inject their fish and prescribe treatments.

One national group of hobbyists, the British Koi Keepers Society (BKKS), have formed a sub-committee, the Koi Health Forum (KHF), and a network of hobbyists, Health Liaison Officers, to assist other members on koi health matters. These volunteers are examined at the start and end of a casual two-year training program organised by a ‘fish health consultant’, a biologist who has been actively involved in the trade for several years. In addition to routine water quality testing, these officers ‘will assist with the identification of parasites and advise on early treatments with the appropriate medications and dosages advised by the KHF. They will advise on the use of anaesthetic (MS-222) and how to topically treat a wound or injury: also on the correct use of antibiotics once the appropriate medication has been identified by a professional source’ (BKKS web-site: www.bkks.co.uk).

In theory, these activities will be limited by the experience of the individual concerned and in some instances by legislation (Protection of Animals Act 1911, Medicines Act 1968). However, there are occasionally illegal sales of antibiotics and other prescription-only medicines at koi shows and retail outlets. In most cases the drugs are of an unknown age, unknown purity and the packs rarely contain any details of how to use the product: some drugs are imported and only have instructions in Japanese! A failure to understand the principles of therapeutics and pharmacokinetics has resulted in widespread misuse of drugs and may be partly responsible for the increasing problem of bacterial resistance to some antibiotics. Inaccurate information, such as the following examples, appear regularly in the hobbyist press:

> "Aeromonas Infections... these should respond to antibiotics, but you may need to try several before you hit the right one" *Editorial, BKKS magazine (Oct 91) p5*

> Regular feeding of antibiotic as a preventative measure....(from a BKKS member) "of 14 days oxolinic acid, 14 days oxytetracycline and 14 days penicillin in that order" *Hotline, BKKS magazine (Mar 93) p23*

In the past, hobbyists had a poor attitude towards the veterinary profession. Vets were often seen to be lacking in knowledge about fish, perceived as expensive (despite the value of fish) and restrictive in the supply of some medicines such as antibiotics. Although there were often unjustified attacks on the lack of veterinary interest, I personally feel that this was no worse in fish-keeping than it is with many other minority pets such as birds and reptiles. Articles written by veterinarians and other fish health professionals appear in the hobbyist press and one publication, the *Koi Health Quarterly*, had two veterinary advisers who contributed regularly and attempted to bridge the divide between the hobby and the health professions.

As with most other aspects of veterinary business, it is often a matter of economics. The cost of time and effort to maintain a working knowledge of ornamental fish must be balanced against the financial return. All vets genuinely want to help clients and their animals, regardless of species, but individual vets can no longer be expected to satisfy all clients' demands. We now live in an age of specialisation within the veterinary profession and while we can, and are professionally obliged to, offer first aid to all species, many areas are now very specialised.

Legislation

There are several laws in current UK and European Union (EU) legislation which affect veterinary surgeons involved in koi health.

The Veterinary Surgeons Act 1966. This restricts the practice of veterinary surgery to members of the Royal College of Veterinary Surgeons. It includes the diagnosis of diseases in animals, giving advice based on that diagnosis, providing medical treatment and performing surgical procedures on animals. However, fish are not included in the definition of 'animals' in this Act and, as discussed earlier, this has resulted in the involvement of non-veterinarians in fish health matters.

Medicines Act 1968. Veterinary drugs are classified under this Act into various categories including prescription-only medicines (POM) and pharmacy and merchants list medicines (PML). Dispensing these medicines to fish clients requires satisfying the requirement that the animals are under the care of the veterinarian. This is generally defined by the statement that 'the veterinary surgeon should have been given responsibility for the health of the animal or herd in question by the owner or his agent' and that this care 'should be real and not merely nominal'. This restricts the prescribing of POM (*eg* antibiotics, Aquagard®, Ivomec®) and PML (*eg* Supaverm®) products and requires direct veterinary involvement. Consequently, it is not uncommon for some antibiotics (*eg* Terramycin® Fish Formula, Pfizer Japan) and organophosphates (*eg* Masoten®; Bayer Japan) to be imported with consignments of fish and sold illegally by dealers. Veterinary surgeons are occasionally approached for POM and PML drugs and are seen solely as suppliers of medicines rather than for their diagnostic skills and services. In some cases, accidental over-prescribing has resulted in the excess or unused medicines being illegally sold-on by hobbyists.

Currently, there is only one anaesthetic licensed for use in fish in the UK, namely MS-222 (Thomson & Joseph, Norwich). This contains tricaine methane sulphonate and is categorised as a PML drug. Because of its restricted availability and expense, hobbyists have used other anaesthetic agents such as benzocaine, 2-phenoxyethanol and eugenol (oil of cloves).

The Medicines (Restrictions on the Administration of Veterinary Medicinal Products) Regulations 1994 requires that only veterinary medicines which have been granted a marketing authorisation (product licence) may be used to treat particular conditions in the species for which it is licensed. The Regulations apply to both food-producing and non-food producing animals. Amoxycillin, co-trimazine, oxolinic acid, oxytetracycline and sarafloxacin are the only antibiotics currently licensed in the UK for the treatment of bacterial infections in fish. Only two of these are licensed for use in ornamental fish, namely oxolinic acid 100% (Aquinox®; C-Vet Livestock Products) and oxytetracycline 50% (Tetraplex®; C-Vet Livestock Products). However, the small number of animals limitation, and the requirement to follow the three stages of the 'cascade' do not apply to non food-producing animals of minor or exotic species. Ornamental fish are interpreted by the Veterinary Medicines Directorate (VMD) as an exotic species and permitted a greater degree of freedom with the choice of drugs. However, great care should always be taken when prescribing an unfamiliar drug to any species for which a product is not licensed.

The Medicated Feedingstuffs Regulations 1998 controls the manufacture of medicated fish foods by registered feed compounders although this does not apply to fishkeepers who 'surface coat' their own fish food with antibiotics. In the UK, only one company (King British, Bradford) manufactures medicated food for ornamental fish, both as a pellet and flaked food. This is only available on receipt of a Medicated Feeding Stuffs prescription (MFS) (formerly called a Veterinary Written Direction [VWD]) from a veterinary surgeon and at present, only oxolinic acid is included in this medicated food. EC Regulation 2377/90 establishes Maximum Residue Limits (MRL) for medicines used in food-producing animals. However, an MRL for oxolinic acid has not yet been established due to suspicion of toxicological problems and the cost is likely to be prohibitive for any pharmaceutical company. As a result, this antibiotic may be withdrawn from the market in the future and an alternative may need to be found for inclusion into medicated food for ornamental fish.

The Diseases of Fish Acts (1937 and 1983) are designed to control the entry and spread of fish diseases through the UK by import controls and enforced movement restrictions. This is administered by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth and the Fisheries Research Services (formerly known as the Scottish Office Agriculture, Environment and Fisheries Department [SOAEFD]), Aberdeen. Spring viraemia of carp (SVC) is the only notifiable disease of koi in the UK and is on List III of EU legislation 91/67/EEC giving Member States the option to establish their own national control programs. Recent outbreaks of this disease in the UK in 1994 and 1995 were thought to be due to an increase in illegal shipments of carp following a reduction of EU border controls. Statutory investigation and monitoring programs are now used to control and eradicate this viral disease.

The Zoo Licensing Act 1981 applies to all places where fish are displayed to the public and is primarily concerned with public safety. However, animal welfare is also covered in this legislation and facilities are required to be inspected by a suitably qualified or experienced veterinarian on a regular basis.

The Veterinary Checks Directive 91/496/EEC requires that consignments of fish which are imported into the UK are subjected to a health inspection and physical identification. This is carried out by officers of the State Veterinary Service and on the basis of reducing stress to the fish, an EU derogation permits this to be performed on a random basis at the importers' premises.

Training and further qualifications

Currently, veterinary undergraduate training in fish health is limited to only a few hours tuition at most of the seven veterinary colleges in the UK and Ireland. Formal lectures on basic biology, husbandry, disease and treatment are usually given by experienced practitioners but only limited practical tuition is available at some colleges.

The Institute of Aquaculture at Stirling offers a Master of Science (M.Sc.) degree in aquatic veterinary studies by attending a comprehensive full-time one-year course or by pursuing a suitable part-time research project. Further degrees and a Ph.D. can be taken by arrangement with the Institute.

On the other hand, specific training in ornamental fish health has been very limited in the UK. A two-day course has been organised by a trade organisation and the Fish Veterinary Society (FVS) in 1992 and another by the Institute of Aquaculture, Stirling in 1993. A one-day course was held by the British Small Animal Veterinary Association (BSAVA) in 1994 and a further four hours of presentations were given at the BSAVA Congress in 1998. Lectures in fish health also form part of various MSc courses, such as that in wild animal health held at London Zoo. Special interest lectures are occasionally presented at other meetings and conferences such as those held by the FVS and the European Association of Fish Pathologists (EAFP). However, most veterinarians are self-taught through personal experience and by adapting knowledge and techniques used in food-fish farming.

As a result of an increase in the level of specialisation in the profession, the Royal College of Veterinary Surgeons (RCVS) now offers both a certificate and diploma in fish health and production. These are self-study programmes open to members of the RCVS, requiring the submission of a case-book or dissertation and formal written, practical and oral examinations. Certificate level indicates a degree of competency that would be expected from a new graduate who has worked for two years in that field, whereas the diploma indicates a high standard of academic and professional expertise. To date, only two candidates have passed the certificate examinations but none has registered for the diploma.

The RCVS also maintains a list of recognised specialists who have a post-graduate qualification such as a diploma and can meet certain requirements. These include demonstrating an expertise and being recognised in the subject area for five years, during which 50% or more of their full-time veterinary work involves the speciality. Other criteria include having advanced the subject by publishing refereed papers and giving presentations at international meetings. Retention on the specialist list requires payment of an annual administration fee (£60 in 1998) and reapplication (£120) is necessary every five years. At present there are only three veterinarians on the specialist list for fish health among a total of 170 for other disciplines.

Veterinarians in practice

At present there are approximately 14,000 veterinary surgeons registered in the UK, of which 6,000 are in general practice and about 70 are members of the Fish Veterinary Society. The majority of vets working full-time in fish health are involved with food-fish production: some are employed by the large salmon-producers, fish food manufacturers and pharmaceutical companies. One veterinary practice in Scotland, dedicated to fish health, employs three full-time vets and services various fish producers.

The British Veterinary Association (BVA) maintains a list of about 90 veterinary surgeons willing to treat ornamental fish. Application to this list does not imply any specialist knowledge or experience but is used to assist members of the public and other organisations to find a local veterinarian interested in ornamental fish health. However, few vets in general practice see more than the occasional pet fish, and most fish-keepers usually approach their local fish retailer for advice and treatment before contacting a veterinary practice.

Importation and trade

The main countries of origin for koi imported into the UK are Israel and Japan. These account for sales of £2 million and £1·7 million respectively, although Japanese koi are individually more expensive. Other countries, such as USA, south-east Asia and South Africa, form a small but important source of this trade.

Mag Noy Ltd, the consortium of Israeli fish farms, annually exports approximately 14 million fish to the UK, worth in excess of £2·5 million. They now employ a full-time veterinary surgeon in Israel and have established a comprehensive fish health monitoring programme. In addition, they retain the part-time services of a veterinary surgeon in UK who investigates problems associated with the importation of Israeli koi.

A few large importers and retailers in the UK employ experienced vets on an occasional basis, but this is usually for the investigation and treatment of urgent cases rather than in an advisory capacity.

The two large trade organisations, the Ornamental Fish International (OFI) and Ornamental Aquatic Trade Association (OATA) Ltd., formerly known as the Ornamental Fish Industry (UK) Ltd., employ veterinary surgeons on an occasional basis. Their role is to comment on veterinary matters and give advice on fish health issues that affect the industry.

The construction of several new public aquaria in recent years has seen an increase in veterinary involvement in this sector. Until recently, one organisation employed a full-time veterinary surgeon to supervise their 16 national sites. Other independent aquaria engage vets on a part-time basis.

Professional involvement

The investigation of diseases in koi by vets in practice is similar to that performed in food-fish and follows the same principles. Most koi in the UK are kept in enclosed ponds or tanks which use a re-cycling system with mechanical and biological filtration. Poor water quality in these facilities is probably responsible for up to 90% of koi health problems. It is therefore essential that water tests for pH, ammonia, nitrite and nitrate are performed at the start of an investigation and that the filtration system is thoroughly inspected. *Eliminating the underlying environmental problems is crucial to the success of treating most koi health problems.*

The microscopic examination of mucus smears taken from the skin and gills is used to assess ectoparasite levels. Postmortem examinations will be carried out if suitable cases are available and biopsies taken for further tests. Samples from most organs (gill, heart, anterior kidney, posterior kidney, liver, spleen, skin and muscle) and any specific lesions will be sent in neutral buffered formal saline for routine histological examination by experienced pathologists. The normal histology of fish has occasionally been misinterpreted by laboratories more familiar with mammalian samples. In a few cases, the normal tissue of the posterior kidney has been mistaken as lymphoma and subsequent discussion has raised unnecessary alarm about possible retrovirus involvement.

Swabs of external ulcers, and from the kidney at autopsy, are often taken for bacterial culture and sensitivity tests. If the practice is equipped with incubators for the culture of fish bacteria, then the samples are plated out on to tryptone soya agar (TSA) or similar media suitable for fish pathogens. In many cases these samples are sent in transport agar to a fish disease laboratory by first class post, aiming to arrive the following day. However, this delay and transport by post can affect mixed populations of bacteria, resulting in the over-growth of some bacteria such as *Aeromonas hydrophila* and suppression of more sensitive species. It is not yet known how significant this is in terms of the final culture and sensitivity tests.

Serological studies and virus culture are rarely performed other than for the investigation of SVC and in research programmes. Electron microscopy of koi samples is only carried out in exceptional cases that are of specific interest such as suspected viral disease and detailed tissue examination.

There are 24 laboratories in the UK which form the Veterinary Laboratories Agency, an executive agency of the Ministry of Agriculture Fisheries and Food (MAFF) and investigate disease problems in all species. Among the 60 veterinary staff at these centres only two have a post-graduate qualification in fish health. Due to changes in funding arrangements, fish health investigations are unlikely to be encouraged and only a limited service will be available for ornamental fish in future.

The primary role of the CEFAS laboratory in Weymouth and the Fisheries Research Services in Aberdeen is in the surveillance and monitoring of notifiable diseases in all species of fish. Although the staff include several highly respected fish pathologists there is little veterinary involvement at either laboratory. Both centres are fully equipped for disease investigation but there are no diagnostic departments that provide a commercial service for small-scale ornamental fish work.

At present, the Institute of Aquaculture at Stirling is one of the few laboratories offering specialised fish disease investigation on a commercial basis. There are several veterinary surgeons on the staff and two are engaged full-time in the Veterinary Investigation Service. Most of their work is related to servicing the salmon farming industry in Scotland but there is a small and increasing volume of work involving ornamental fish from private veterinary practices and some public aquaria.

There are three experienced fish pathologists in the UK who provide a diagnostic service, including histopathological interpretations, on a private basis. Two of these are veterinarians and, although most of their work is related to salmonids, are willing to take on ornamental fish cases.

Current Research

Recently, a one-year project carried out by CEFAS, Weymouth investigated bacteria associated with ulcer disease in koi. This studied various aspects of the bacterial flora and antibiotic sensitivity with a view to identifying the species involved in this serious disease of ornamental carp (Barker and others 1997).

At present, there are very few formal projects researching disease problems in ornamental fish. Some small-scale projects are occasionally carried out by students at the Institute of Aquaculture at Stirling and other colleges as part of a degree course. One project involves the study of *Mycobacterium* species of acid-fast bacteria which cause tuberculosis in fish and the development of various diagnostic tools including polymerase chain reaction (PCR) methods (Adams and others 1997).

Common Diseases

Diseases commonly seen by veterinarians in practice are probably different to those commonly encountered by hobbyists. For example, I rarely see cases of white spot because it is easily identified by the owner and treated effectively with many proprietary products. Regardless of the cause, sick fish are often treated with a variety of these products by owners prior to contact with a veterinary surgeon and usually without any specific diagnosis being made. Therefore conditions which fail to respond to these products are sometimes presented for further investigation and treatment. This often reveals misdiagnosis of the pathogen or a failure to treat the underlying environmental problems. Many ornamental fish are elderly and as a result neoplasia and other age-related problems are common in geriatric fish. In some cases, surgical removal of external tumours can be performed successfully and to the benefit of the patient.

Environmental factors contribute to the majority of health problems in koi. It is therefore essential to have a good knowledge of recycling filtration systems and the principles of water quality in order to avoid recurrence of these problems. A breakdown of the nitrogen cycle with subsequent build-up of ammonia and nitrites are common in newly established ponds and where there are poor or inadequate filter systems. Similar problems can occur following change or damage to a biological filter by antibacterial medications. In addition to physically blocking filter units, algal blooms in summer can produce dramatic fluctuations in pH, with high levels at midday, and give rise to low dissolved oxygen levels at night. Poor maintenance of filter systems and a failure to measure water quality with simple water test kits are common shortcomings of hobbyists. Rather than test water quality regularly themselves, hobbyists will occasionally take water samples to dealers once or twice per year.

Bacterial disease which produces septicaemia with or without body ulcerations is one of the most common disease problems affecting koi in the UK. It is not yet certain if this is a true primary disease or the result of several other factors. However, ulcers often require vigorous debridement and wound cleaning under general anaesthesia, followed with antibiotic treatment by injection and in the diet. Other disease problems are usually due to secondary parasitic or fungal infection and in many cases associated with the introduction of new fish or changes to the pond and filter system.

In many cases, histological investigations reveal significant damage to major organs such as the gills and kidneys. Due to the expense of this procedure and the lack of visible evidence during postmortem examination, there may be internal pathology at a cellular level which goes undetected. It is often difficult to identify the exact cause of these lesions and assess their significance but it is always surprising to see the extent of serious tissue damage in some fish. This may account for the lack of visible lesions found at autopsy following unexplained mortalities in koi. *It is hoped that greater use of this important investigative tool will yield a better understanding of some disease processes in koi*.

Common treatments

Proprietary products which are available 'over the counter' and without a prescription are commonly used by owners as a first line of approach to a fish health problem. At present there are almost 100 such products available in the UK, and these are manufactured or distributed by 13 companies. A few products are imported from Germany and the USA.

Unfortunately, there has been considerable commercial secrecy surrounding the ingredients of the products. However, the latest edition of the *Veterinary Formulary* (1998) contains information on 80 of these proprietary medicines from 11 companies. Only a limited range of chemicals are used in the products and many contain formaldehyde and various biological dyes. Information about most of these chemicals can be found in the literature. Although the easy-dose containers reduce the risk of accidental over-dosing, few owners know the exact volume of water in their pond system.

As discussed earlier, all veterinary medicines must be granted a Marketing Authorisation by the Veterinary Medicines Directorate before they can be marketed in the UK. However, manufacturers of these fish medicines did not apply for a Marketing Authorisation since they required expensive trials for safety, quality and efficacy. It is anticipated that in the near future, EU legislation will introduce a scheme which will exempt these products from fulfilling the Marketing Authorisation requirements.

The lack of any regulation in this area of the market has resulted in various anomalies. While many of the products may have been tried and tested over years of use in the hobby, manufacturers have used their own claims for efficacy. The majority of known ingredients, except malachite green, are only likely to act as external medications yet some claim to treat internal bacterial infections, viraemia and 'dropsy'. Equally, the efficacy of some products against fish lice (*Argulus* sp.), anchorworm (*Lernaea* sp.) and leeches is questionable since none of them contains organophosphate or similar insecticide. While many of the claims have improved recently and are more realistic, some are extracted from the literature. One product containing methylene blue quotes in error from Herwig (1976) that it can be 'used as a substitute oxygen source in cases of respiratory distress, allowing fish to absorb oxygen through the skin independently of gill condition'. In this case, an oversight of a semi-colon after 'respiratory distress' and misinterpretation that the methylene blue is absorbed through the skin, not oxygen, has distorted the information.

My own approach to treating koi is to offer a diagnostic service, and in cases of ectoparasite infestation advise owners on using a proprietary product containing a mixture of formalin and malachite green. However, the larger metazoan parasites such as fish lice, leeches and anchorworm are often problematic and most proprietary medicines are ineffective. There has been a report of using ivermectin by injection in goldfish with anchor-worm (Hyland and Adams 1987), but this is time-consuming and stressful to the fish: its use as an in-feed treatment in ornamental fish has not been reported. Due to the life-cycle of these parasites, an environmental approach is required rather than chemical dips or treatment of the individual. The use of an organophosphate, trichlorphon, is the treatment of choice commonly stated in the literature. However, there are no products licensed in the UK for fish treatment which contain this chemical, although it is found in various insecticides used on crops (*eg* Dipterex®; Bayer). Trichlorphon is licensed for use in fish in Japan as Masoten® (Bayer) and this is occasionally imported into the UK as a powder or liquid formulation. In water, trichlorphon breaks down into the active ingredient, dichlorvos. Although Aquagard® (Novartis) contains dichlorvos and is licensed for the treatment of sea-lice in salmonids, there is limited information about its use in koi in enclosed ponds. As a result of this and the hazards of using organophosphates, I have used cypermethrin (Excis®; Grampian) on a trial basis in a few cases and found it to be effective against *Argulus* and *Lernaea* sp. However, it may be necessary to repeat the treatment periodically since egg development of *Argulus* may take up to 6 months at 15°C and cypermethrin may not kill all stages in the life cycle. (W.H. Wildgoose, unpublished data).

Successful treatment of ulcer disease in koi requires a vigorous approach. Severely affected cases which are unlikely to recover should be euthanased to reduce the spread of the disease and for the welfare of the fish. Experience of failure is required to assess which fish should be destroyed at the outset but I feel that if the ribs or bones of the spine are exposed then treatment is unlikely to succeed. Some cases treated by hobbyists have recovered without the use of antibiotics but from a veterinary point of view I feel that they should always be given to ulcerated koi. In my experience, oxytetracycline has a limited effect and long-acting injectable formulations of this drug can produce adverse reactions with the formation of fluid-filled swellings at the injection site. My own preference is to use injections of enrofloxacin (Baytril®; Bayer) and sulphadoxine and trimethoprim (Borgal®; Hoechst) followed by in-feed medication for 10–14 days. Due to the normal adhesions between the abdominal organs and the body wall, I never use the intraperitoneal route and prefer to use deep injections into the dorsal musculature using long (0·7 mm × 40 mm) needles.

I have found that the use of antibiotics alone has a limited effect and debridement with topical dressing of the ulcers with povidone-iodine (Tamodine®; Vetark) and waterproof paste (Orabase®; ConvaTec) to be an important step to success. In addition, using salt in the water at a rate of ¼ ounce per gallon (1·5 gram/litre) reduces the osmotic stress on the exposed tissues and has a mild antiseptic effect which is an important aid to recovery. Where possible, keeping the affected fish in a suitable large indoor isolation facility where the water temperature can be raised to 25–28°C has also proved valuable. Constant monitoring of water quality in these facilities is essential.

Practical problems

Some of the difficulties in treating koi are due to practical limitations and the unrealistic expectations of saving all the clients' fish. In some cases, the health problems can be severe at the outset and advice from a veterinarian is often requested after several fish have died. Inevitably the disease process is slow to arrest and further mortalities are not uncommon. Apart from visible skin diseases, sick koi can show few other obvious clinical signs of disease and a degree of educated guesswork is often required. The high emotional and financial value of these fish requires an approach where sacrifice of fish for investigation is rarely possible and treatment of individual fish is often requested.

When ponds are being setup and stocked, there is a constant acquisition of fish from different sources which may result in the introduction of various infectious diseases. It is rare for hobbyists to quarantine new stock and most fish are put straight into the main pond. Although uncommon, in some instances a total loss of stock has followed the introduction of new fish.

The value of culture and antibiotic sensitivity of samples from ulcers is questionable due to the unavoidable environmental contamination. Ulcer disease is primarily an external disease and systemic infection may not always be present. Therefore samples taken from the kidney using an aseptic technique are often sterile. Isolation of the suspected causal organism, *Aeromonas salmonicida*, is often difficult and its sensitivity pattern may be of limited value if the predominant organism is *A hydrophila*. Bacterial resistance, particularly to oxolinic acid and oxytetracycline, is now an increasing problem in the treatment of ulcer disease in koi.

Postmortem investigations must be performed immediately following, or within one hour of death so that tissue autolysis is minimal. This is not always convenient in a busy, general practice routine. In addition, some lesions, such as renal disease and gill hyperplasia, may not always be visible to the naked eye, particularly in small specimens. Fish which die suddenly and show no external signs of disease are occasionally presented by owners on suspicion of poisoning. The carcases are often frozen and therefore of very limited value. The cost and technical difficulties of identifying an unknown poison usually make these investigations unrealistic from the outset. Toxic products from the anaerobic rotting of barley straw used to control algae, and styrene from improperly cured glass reinforced plastic (fibreglass) used to line ponds, are two common examples of chemicals that adversely affect ornamental fish.

There is often a mix of species, size and ages in a fish pond which makes accurate treatment a challenge. The uptake of oral medication may be variable and occasionally there are species differences in the effects of some medications (*eg* organophosphates). Many cases will have been treated previously by the owner with proprietary medications, often complicating the clinical picture, and the residual amounts of some chemicals may limit further treatment. Anorexic fish will probably require injection with antibiotics and careful handling of all patients is essential, particularly with larger koi which may weigh over 5 kg.

Future developments

Surgical procedures commonly performed on ornamental fish, including koi, are limited to external body areas. These include debridement and topical treatment of skin ulcerations, and manual removal of anchorworm parasites. There are anecdotal reports in the hobby press of repairs to damaged fins. The biopsy and excision of tumours and other localised masses, followed by topical treatment of the site, has proven to be an effective approach to these lesions. Enucleation of badly damaged eyes in koi is relatively easy to perform but often carries a poor prognosis which, in my own experience, may be due to other underlying problems. However, enucleation and experimental implantation of a prosthesis in cultured hybrid striped bass, *Morone* sp., has been successfully carried out and reported in the USA (Nadelstein and others 1997). There are also reports on the use of cryosurgery for the treatment of body ulcers and tumours in koi (Reynolds 1993).

The practical use of advanced diagnostic procedures such as radiography, ultrasonography, nuclear scintigraphy and computed tomography (CT scan) in koi have been investigated (Love and Lewbart 1997, Bakal and others 1998). While radiography is useful for identifying skeletal deformity and abnormalities of the swim bladder, it can also be used to demonstrate localised intra-abdominal tumours. The contrast between various soft tissues is limited, and this may be more usefully investigated with ultrasound. There is very little data in the literature about normal and abnormal biochemical values in koi. Despite the growing use of dry-chemistry blood analysers in veterinary practice, this area requires a significant amount of investigation and validation before they may be of practical benefit in koi health. There appears to be few specific heart diseases in koi and therefore there may be a limited use for electrocardiography.

The organised theft of valuable koi from retailers' and hobbyists' premises does occur in the UK. It is theoretically possible to implant a microchip into the musculature of these fish but few owners have this done. Although the use of microchips is is common in dogs and cats, a central register of microchip numbers and the owner's details, together with the wide distribution of suitable scanning equipment has been essential in the marketing of this service. The lack of any external sign that an animal has a microchip requires the routine scanning of all stray and lost pets for this to be of any benefit. It is unlikely that this will extend to koi in the near future and owners will probably prefer to rely on photographic evidence of colour patterns as currently used at koi shows.

There is a small amount of commercial breeding of koi in the UK although details are rarely published. This tends to follow carp farming methods, adapting natural spawning techniques, and with some use of specific carp pituitary extract. At present, dedicated koi-keepers continue to favour high quality, Japanese-bred koi rather than the hardier (climate adjusted) British-bred fish.

Ulcer disease in koi is the most common and serious infectious problem in koi. Although further research is required, it has been shown that atypical *Aeromonas salmonicida* can initiate this disease and produce ulcers which rapidly become infected with *Aeromonas hydrophila* (G. Barker, personal communication). Until recently, a commercial dip vaccine, Cyprivac® CE (Aquaculture Vaccines Ltd), was licensed for use in temperate species of cyprinid fish, including koi. My experience of its use in a few cases suggested that it had a limited effect on the subsequent outbreak of ulcers. This vaccine has been withdrawn (due to cattle brain extract used in the growth medium) although another vaccine is currently being developed.

Various immunostimulants are being investigated for use in aquaculture and two manufacturers currently include these in some of their koi foods. It is difficult to assess their full benefit at present, but hopefully they will not be regarded as a substitute for good husbandry by koi-keepers.

Conclusions

The veterinary profession has a lot to offer koi-keepers. Our knowledge of epidemiology and disease processes combined with an understanding of medicine and surgery puts vets in a unique position to provide a comprehensive diagnostic and therapeutic service. However, it is advisable to gain experience and an insight into the hobby by joining a local koi club and offering to perform some work free of charge, such as gross postmortem examinations, or give a talk to club members. It is essential to follow up all cases, regardless of the difficulty of facing up to failures. This will quickly help establish the limits of what can be achieved and often reward you with the surprise of success. Establishing a relationship with a well-run local koi retailer and referring clients to them for the correct proprietary medicines is usually of mutual benefit. Due to the rarity of veterinarians willing to treat ornamental fish, it helps to announce your interest in fish to local practices, particularly the receptionists, since they are the ones who answer the phones. Ensuring that your name is on the BVA ornamental fish vet list is equally helpful.

For koi health to progress further, a greater level of scientific investigation and wider publication of case studies is required. This effort needs to be carried out by all professionals involved in fish health in order to raise the standard of care to the same level that can be expected in other species. Veterinarians cannot claim to be instantly familiar with all aspects of a subject that they have neglected for so long but can now use their unique skills in a positive direction for the benefit of the client and their koi.

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Further reading

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This article is based on a presentation given at a German veterinary group seminar on koi health in Munich on 25 June 1998 and also at the autumn meeting of the Fish Veterinary Society in Penrith on 12 November 1998. It was submitted for publication on 6 November 1998.

SHORT COMMUNICATION

Fish Veterinary Journal (1999) (3) 20–26

Successful removal of a gastric foreign body from a red tailed catfish, *Phractocephalus hemioliopterus*

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Many species of fish ingest foreign matter to identify its edibility or graze on small food particles then spit it out. As a result some foreign bodies may become trapped in the digestive system and require removal. In general veterinary practice these unusual accidents are rarely presented for treatment.

Case history

A juvenile red tailed catfish, *Phractocephalus hemioliopterus*, called 'Red' was referred for a second opinion and treatment on 8 July 1992, having swallowed a fragment of decorative slate 2 days earlier. The fish was 12 cm long, weighed 20 grams and had been kept in an indoor aquarium for three months by a young schoolboy. The slate had lodged in the stomach with a sharp cranial point protruding through the ventral body wall (Fig 3). The abdomen was swollen and the bulk of the slate was easily palpated. The weight of the slate made it difficult for the fish to swim and achieve neutral buoyancy.

The catfish was carefully anaesthetised with tricaine methane sulphonate (MS-222®; Thomson & Joseph) and removed from the water. Radiographs were taken to establish the size and location of the foreign body. Lateral and dorso-ventral views (Figs 1 and 2) revealed a large radio-opaque mass in the ventral abdomen measuring 35 mm by 15 mm. A large pocket of gas and some radio-dense intestinal contents were also visible.

The sharp pointed cranial end of the slate had penetrated the body wall in such a way as to make it impossible for the fish to regurgitate the mass. An auroscope was used to explore the buccal cavity and oesophagus to assess the feasibility of *per oral* removal of the foreign body (Fig 4). On opening the mouth, the large size of the oral cavity made it easy to inspect the internal bulk of the slate fragment. Using forceps, the internal piece of slate

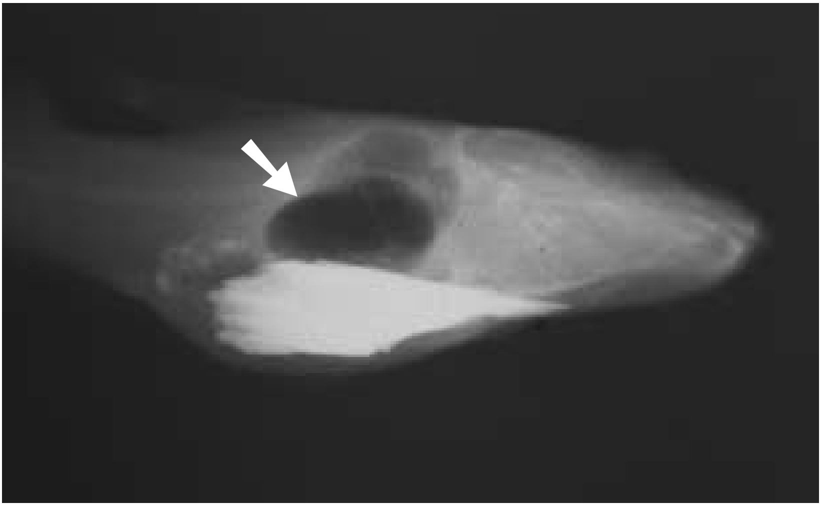

Fig 1 Lateral view radiograph showing the large slate fragment, over-inflated swim bladder (arrow) and radio-dense material in the bowel.

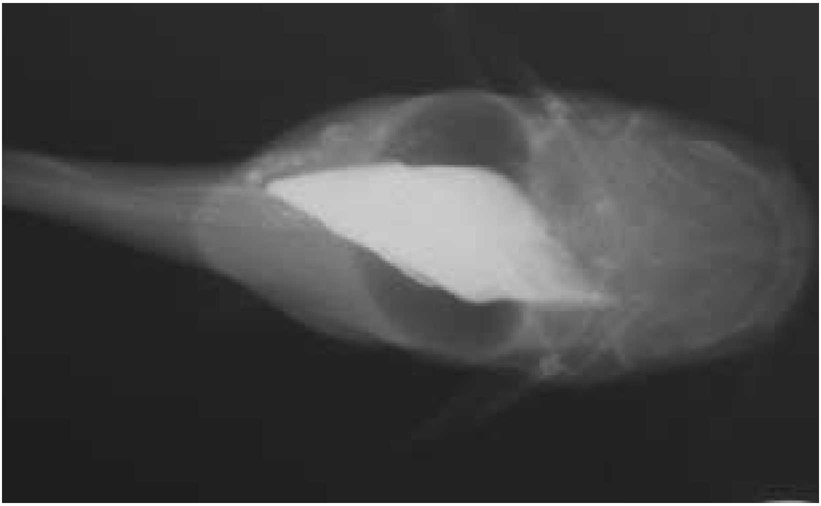

Fig 2 Dorso-ventral view radiograph of the catfish.

Fig 3 Lateral view of catfish with protruding piece of slate arrowed.

Fig 4 Using an auroscope to inspect the oral cavity and oesophagus.

Fig 5 Use of forceps to grasp the slate fragment internally.

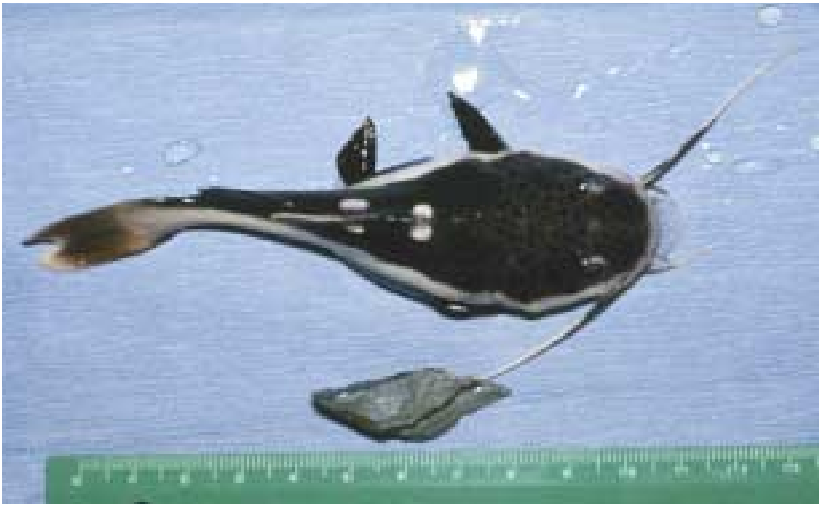

Fig 6 The juvenile red tailed catfish following successful removal of the foreign body (scale in centimetres).

was grasped firmly and pushed caudally to disengage the point from the body wall and release the mass (Fig 5). The whole fragment was then easily extracted through the mouth. There was no visible bleeding and the blunt caudal end of the slate did not appear to cause any further damage. The fish was given a single intramuscular injection of 3 mg sulfadoxine and 0·6 mg trimethoprim (0·05 ml Borgal® 7·5%, Hoechst).

On returning the fish to the water, it was seen to be excessively buoyant. From the radiographs, the large pocket of gas appeared to be due to over-inflation of the swim bladder (gas bladder). While still semi-conscious, 1 ml of gas was removed by careful paracentesis through the body wall using a needle and syringe. This procedure reduced the buoyancy but there was still a tendency for the fish to float on the water surface despite its efforts to swim to the bottom. In order to keep the fish submerged and thus avoid the skin drying out from exposure, a clear polythene bag was submerged in the tank. This acted as a smooth barrier forcing the fish underwater and avoided causing further trauma to the dorsal skin surface.

Commercially prepared medicated food pellets (King British) containing 0·1% oxolinic acid (Aquinox® 50%, C-Vet LP) were dispensed for feeding for the next 10 days.

Three days later, the fish was active and had recovered well. It had regained buoyancy control and no longer required support from the polythene bag. However, the fish refused to eat the medicated pellets but it would eat earthworms. Although the 5 mm extruded pellets were small enough to swallow, this species is usually carnivorous and not accustomed to pelleted foods. As a result a further injection of sulfadoxine and trimethoprim was given and the pellets discontinued.

Four years later the fish had grown to 90 cm and had shown no longterm problems as a result of its unusual experience.

Discussion

Brief reports of foreign bodies, mainly pieces of gravel becoming lodged in the mouth of goldfish, *Carassius auratus*, have been published (Clark 1988, Macholc 1988, Earl 1988). In these cases the fish had difficulty swimming and required manual removal of the gravel with forceps. The author has seen one such case previously (unpublished report) but the gravel was dislodged during transportation to the practice and the goldfish showed no physical damage following the event.

It is recognised that gravel should be avoided in aquaria since most red tailed catfish will swallow substantial quantities (Sands 1995). This species will also move objects around the tank and this is considered to be due to boredom or territorial display. A survey by the Red Tailed Catfish Club records a variety of non-food items swallowed including filter pipes, rubber suckers and even a pair of sunglasses (Scott 1992). Most items were regurgitated within a week although the sunglasses were removed manually under anaesthesia.

Catfish are physostomous and control the amount of gas in the swimbladder by the pneumatic duct which lies on the right side of the oesophagus near the stomach. Any interference or obstruction of this duct may result in over- or under-inflation of the swim bladder and interfere with buoyancy control. It is also possible that the fish attempted to improve its buoyancy by actively over-inflating the swim bladder to compensate for the additional weight of the slate (5 grams). However, the release of some gas by paracentesis in this case is thought to have assisted the fish's recovery.

In September 1996, a 45 cm red tailed catfish which swallowed a smooth pebble measuring 105 mm by 50 mm and weighing 287 grams was reported to the author (J.F. Moloney, personal communication). Following radiography, a similar technique was used to massage the foreign body forward into the mouth to allow manual removal. The fish also experienced excessive buoyancy post-operatively but this resolved over five days without the need for paracentesis.

This unusual case demonstrates the usefulness of radiography in fish medicine and shows how a simple technique with post-operative care can achieve a successful outcome.

Acknowledgements: I am grateful to Phillip Goddard MRCVS for referring this unusual and interesting case and to the Grover family for their patience with the treatment of their pet fish. Jerry Moloney MRCVS provided details of his case and Dr David Sands of the Aquarian Advisory Service added useful remarks.

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Author's note :
No references were found on the subject of foreign bodies in fish by searching the CD-ROM databases (Medline Express 1983 to present, Zoological Record 1993 to present, CAB Abstracts 1987 to present) at the Science Reference Section of the British Library, London on 8th May 1996.

William Wildgoose graduated from Glasgow Veterinary School in 1977 and has worked in small-animal practice in London since then. He has a special interest in exotic pets and ornamental fish in particular, and obtained his RCVS Certificate in Fish Health and Production in 1997.

This paper was presented as one of the six case studies required for the casebook which formed part of the author's examination for the RCVS Certificate in Fish Health & Production. It was submitted for publication in August 1998.

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Preliminary investigations into the bacteriology of skin lesions of Atlantic salmon reared in seawater in Scotland

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Introduction

In Atlantic salmon, *Salmo salar*, L., focal lesions caused by ulceration of the skin and underlying muscle are a feature of a number of bacterial diseases, especially those caused by members of the Vibrionaceae (Inglis and others 1993). Ulceration associated with the colder water temperatures encountered in winter has been described in Norway (Lunder and others 1995) and Iceland (Benediktsdóttir and others 1998). These studies showed that the isolated bacteria largely fell into two groups, termed species 1 and 2 (Lunder and others 1995) and phena 1 and 2 (Benediktsdóttir and others 1998). Although not as yet completely described, these isolates have been referred to as 'Vibrio viscosus' (species 1; phenon 1) and 'Vibrio wodanis' (species 2; phenon 2).

Skin lesions are quite commonly found on salmon in Scotland in the winter months and can cause significant morbidity and mortality. Photoperiod-manipulated smolts which are put to sea as the water temperature is declining often appear to be particularly susceptible and the healing response can be overwhelmed. The aim of the present study was to isolate and identify organisms from lesions on salmon in Scotland and to determine their pathogenicity.

Materials and Methods

Salmon were sampled on eight marine farms on the Scottish mainland and the Inner and Outer Hebrides between December 1997 and March 1998. The populations had been affected typically for 3 to 4 months but exceptionally up to 9 months. The morbidity rate was up to 15% and up to 30% of total mortalities were attributed to lesions. Photoperiod-manipulated smolts were present on six out of the eight farms.

Bacterial cultures were inoculated onto tryptone soya agar supplemented with 1·5% sea salts and 5% horse blood. Kidney and lesion transport swabs were taken into Amies charcoal medium containing 2% sea salts. Fragments of tissue from lesions were homogenised in an equal volume of 1·5% saline, diluted in ten-fold steps to 10^{-8} and 0·02 ml drops inoculated onto the surface of dried agar plates. Isolated bacteria that were Gram-negative, oxidase-positive and sensitive to either 10 µg or 150 µg of vibriostat 0/129 were presumptively classified as *Vibrio* spp. Further identification was carried out by agglutination test with a $^{1}/_{32}$ dilution of antiserum to *Vibrio* phenon 1 (Benediktsdóttir and others 1998) and presence or absence of colonial viscosity and haemolysis.

Two isolates, one forming viscous colonies and the other non-viscous colonies (isolates 3905 and 3895 respectively) were used to infect healthy 60 g salmon held in disinfected seawater at 7·5°C. Two groups of 20 fish received 2×10^5 colony forming units (CFU) of isolate 3905 either by subcutaneous injection or by dropping onto a 2 cm^2 area of skin scarified by removal of scales with a sterile hypodermic needle. Twenty fish were injected subcutaneously with 8×10^4 CFU of isolate 3895. Groups were split into duplicates of 10 fish and maintained in separate tanks with equal numbers of untreated cohabitant salmon.

Results

Table 1 shows the results of sampling lesioned fish from the eight marine

Table 1: Bacteria isolated from skin lesions or kidneys of Atlantic salmon from eight farms in Scotland.

Bacteria	**Fish with lesions (n=51)**			
	Skin lesions (n=51)		Kidneys (n=34)	
	No.	(%)	No.	(%)
Vibrio spp.[a] — viscous colonies	1	(2%)	5	(15%)
Vibrio spp.[b] — non-viscous colonies	41	(80%)	7	(21%)
Vibrio spp. — both viscous and non-viscous	6	(12%)	0	
Other bacteria	3	(6%)	11	(32%)
No bacteria	0		11	(32%)

[a] Isolated in pure culture

[b] Isolated in pure culture or mixed with other taxa

farms. Presumptive *Vibrio* isolates with colonies which were viscous (as evidenced by a sticky thread forming when attempting to remove them from agar with a bacteriological loop), haemolytic and positive to phenon 1 antiserum were isolated from lesions in 7 out of 51 (14%) of fish and 5 out of 34 (15%) of kidneys. Presumptive *Vibrio* isolates with colonies which were non-viscous, haemolytic and failed to agglutinate with phenon 1 antiserum were isolated from lesions in 47 out of 51 (92%) fish and 7 out of 34 (21%) kidneys.

Infection challenge trials resulted in 100% mortality of fish receiving isolate 3905 by scarification or injection; mortalities beginning six and eight days post-inoculation respectively and affected fish all exhibited lesions at the site of inoculation (Fig 1). There was only one mortality amongst the cohabitant salmon; survivors were free from lesions and when culled 19 days post-inoculation were negative for isolate 3905 by kidney culture. In contrast, all fish cohabiting with the scarified trial group survived but kidney culture of the culled fish showed that all were infected with isolate 3905. Fish injected with isolate 3895 exhibited no lesions or mortalities for the duration of the trial and no bacteria were cultured from the kidneys when culled 19 days post-inoculation.

Fig 1 Experimental lesion produced four days after injection with a suspension of viscous-colony *Vibrio* sp.

Discussion

The results of the present study show that from a wide geographical area, skin lesions in Scottish salmon are associated with the isolation of *Vibrio* species.

Detailed characterization of isolates was outside the scope of the present study. However, the physiological and antigenic properties of the viscous-colony vibrios and the pathogenicity of a representative of the group suggest that they may be similar to the ‘species 1; phenon 1’ isolates from cold water skin lesions in Norway (Lunder and others 1995) and Iceland (Benediktsdóttir and others 1998). From the standpoint of salmon farming operations, it may be significant that the present study has shown that a representative of the viscous-colony vibrios caused mortalities when infection occurred via a small area of skin from which the scales had been removed. Transfer of salmon smolts to sea during the winter months, when mechanical abrasion of skin may occur, thus provides a possible route of infection. Salmon injected with a representative of the non-viscous vibrios (isolate 3895) showed this organism to be non-pathogenic. It may therefore be analogous to the non-pathogenic ‘species 2; phenon 2’ isolates from Norway (Lunder and others 1995) and Iceland (Benediktsdóttir and others 1998).

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Tony Laidler's interest in fish diseases began while working at the MAFF Veterinary Investigation Centre near Penrith, Cumbria. While there, he had the opportunity to gain membership of the Institute of Biology and obtain an MSc by research into the isolation and detection of Renibacterium salmoninarum. Moving to Marine Harvest McConnell in 1989, he is now Laboratory Services Manager in charge of laboratories at Lochailort and Stornoway. Apart from fish diseases his professional interests encompass quality assurance microbiology and salmon flesh quality analysis.

Andrew Grant obtained a first degree in Developmental Biology at Aberdeen and an MSc at Bangor, and graduated in Veterinary Medicine from Cambridge University in 1980. He worked in a number of practices before joining Marine Harvest McConnell in 1988 as Health Services Manager. He has overall responsibility for the company's diagnostic and advisory services with the objective of maximising fish health, welfare and survival. He manages a multi-disciplinary team of 15 and his diverse remit includes representing the company to regulatory authorities and on industry working groups, and liaising with pharmaceutical companies in the planning and execution of field trials of novel medicines.

Simon Wadsworth holds a BSc in environmental science and an MSc in applied fish biology. He has 10 years experience working in the aquaculture industry and is currently employed as Development Manager responsible for research and development at Marine Harvest McConnell. Trials include all aspects of salmonid health as well as nutritional work and developing the farming of new species. He is in the process of submitting a part-time PhD to Aberdeen University on sea lice control.

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'Vibrio viscosus', the agent of winter ulcers in salmonids

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'Winter ulcer' disease is characterized by small and large, shallow skin lesions. Diffuse or petechial haemorrhages are often observed on internal organs. The disease may produce considerable mortality, between 10–30%, and has been recognised in Iceland and Norway for more than 10 years. In two studies performed in these countries, totally independent of each other, it was found that two species of vibrio bacteria are most often isolated from diseased fish (Benediktsdóttir and others 1991,Lunder 1992). A biochemical study of Icelandic strains, that also included reference strains isolated in Norway, revealed that the strains isolated in the two countries fall into the same phena. The two groups of bacteria were initially given the names 'Vibrio viscosus' and 'Vibrio wodanis' by Tor Lunder in 1992. Both species have also been isolated from winter ulcers in Scotland. Challenge tests have indicated that 'V. viscosus' is virulent to Atlantic salmon, *Salmo salar,* but 'V. wodanis' is not. (Lunder and others 1995, Benediktsdóttir and others 1998).

Blood agar supplemented with a final concentration of 2% sodium chloride is the medium of choice for initial isolation of 'V. viscosus'. Samples should be incubated at 15°C for up to one week. In most cases, it takes two or three days for the colonies to grow to a visible size, and these are recognized by haemolysis and by their viscosity: a thread is formed when they are picked up from the agar. Strains that fail to grow on conventional test media supplemented with sodium chloride may give better results when marine broth (Difco) is used as a base medium in biochemical tests. This is presumably because of its content of marine salts and peptone. 'V. viscosus' strains are sensitive to 150 µg of the vibriostat 0/129, but resistant to 10 µg. They grow at 4°C, 15°C and 21°C, but not at 26°C, and are dependent on 2% sodium chloride for growth. They are indole-negative and nitrate-positive. The strains are rather inactive in sugar tests, but active in the production of degradative enzymes. They produce amylase, gelatinase, deoxyribonuclease (DNase) and lipase. Differences between subgroups of

'V. viscosus' are noted in the production of acid from maltose and mannose, and in the production of lysine decarboxylase.

A comparison between the strains of 'Vibrio viscosus' and 'Vibrio wodanis' isolated at different geographical locations was made using genotypical and phenotypical methods. A novel DNA-fingerprinting method, AFLP, is able to differentiate between groups within species. Using this method, the results show that the Icelandic and Norwegian strains of 'V. viscosus' cluster into homogeneous groups according to the geographical origin of the strains. SDS-PAGE discriminates between strains of the same genus and groups them according to species and sometimes subspecies level. When the protein profiles of 'V. viscosus' are studied, Norwegian, Scottish and south-west Icelandic strains have the same uniform profile, and confirm that they all belong to the same homogenous species.

When strains of 'V. wodanis' are subjected to genotypical and phenotypical tests, they indicate that this group is a defined species. An overall heterogeneity is observed within this species which is not dependent on the place of isolation. This heterogeneity indicates that the 'V. wodanis' strains consist of many different clones, an observation more common in strains isolated from the environment rather than among pathogens. However, separate findings from Norway and Iceland show that bacteria belonging to this group are often isolated from outbreaks of winter ulcer where no other suspect or known pathogenic or opportunistic microbe is found, suggesting that 'V. wodanis' might be an opportunistic pathogen.

The homogeneity found within subgroups of 'V. viscosus' strains indicates that a few clones of 'V. viscosus', which share a common origin within each geographical location, are isolated from salmonids suffering from 'winter ulcers'. This is a pattern often seen among pathogenic bacteria, and supports the results from challenge tests that 'V. viscosus' is a primary pathogen.

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Sarafin®, a novel quinolone for bacterial disease in salmon

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Sarafloxacin hydrochloride (Sarafin®; Vetrepharm) is a member of a new generation of aryl fluoroquinolones, a group of extremely potent oral antimicrobial compounds. Older members of the group include the non-fluorinated oxolinic acid which is still widely used as an antibiotic in aquaculture. The newer fluoroquinolones include ciprofloxacin, difloxacin, enrofloxacin and sarafloxacin. In general, these have been shown to have improved efficacy at lower doses, a broader spectrum of activity and a lower potential for bacterial resistance than the older quinolones.

The principal mechanism of action of the quinolones is to inhibit DNA synthesis by blocking the activity of an essential bacterial enzyme, DNA gyrase. The presence of fluorine on the molecule greatly enhances this activity and produce a rapid bactericidal effect. The ability of bacteria to mutate into a form which resists this mode of action appears to be very low and suggests a low risk of bacterial resistance. No plasmid resistance has ever been demonstrated.

Sarafin® has been under development for use in aquaculture for several years and was granted a full marketing authorisation early in 1998. The indicated use is for infection with *Aeromonas salmonicida* in Atlantic salmon, *Salmo salar*, held in seawater. If it is used for any other indication it becomes necessary to fulfil the requirements of the prescribing 'cascade'.

An advantage of the fluoroquinolones is that they demonstrate a high bioavailability and a low binding to plasma proteins. This results in rapid distribution from the plasma into tissues to attain higher levels than the minimum inhibitory concentration (MIC) for a large number of fish pathogens. All isolates with an MIC of 2 µg/ml or lower, are sensitive to Sarafin®:

Bacterial species	Mean MIC (µg/ml)	Range	(n)
Aeromonas salmonicida	0·48	0·0156–2·0000	45
Yersinia ruckeri	0·023	0·0156–0·0312	2
Flavobacterium psychrophilum	0·00195	0·000195–8·000	47
Vibrio sp.	0·36	0·0078–0·500	3
Aeromonas hydrophila	0·0625		1

The very rapid tissue distribution and relatively rapid clearance (half life of Sarafin® is 12–24 hours) gives a quick clinical response, a short treatment period and a short withdrawal period. Residue and metabolism studies have established a withdrawal period of 150 degree days, the compound being excreted as the parent molecule with no quantifiable metabolites detected.

A wide range of efficacy studies both in laboratory challenge trials using immersion and cohabitant models and in-field outbreaks of furunculosis were carried out. These established and confirmed the optimum dose rate of 10 mg/kg bodyweight of fish per day and a treatment period of five days.

In antibiotic sensitivity tests, the *Aeromonas salmonicida* isolated from the field outbreaks was found to be resistant to oxolinic acid and demonstrated that sarafloxacin is effective against oxolinic acid-resistant strains of *A salmonicida* (if the MIC of sarafloxacin is below 2µg/ml).

Immersion challenge study

Sarafin® dose	% Mortality	
	5-day treatment	10-day treatment
0 mg/kg	53	53
2 mg/kg	59	49
6 mg/kg	37	19
10 mg/kg	0	0
14 mg/kg	0	0

Sarafin® is pure (100%) sarafloxacin hydrochloride

Injection challenge models confirmed that 10 mg/kg Sarafin® for 5 days was the optimum dose and that no benefit was seen when the period of medication was increased to 10 days in these studies.

Three field trials carried out on commercial salmon farms in Scotland compared a five-day treatment with a ten-day treatment against a positive control antibiotic. These trials confirmed that Sarafin® was effective in controlling clinical furunculosis and that a five-day treatment period was as effective as a ten-day treatment in reducing furunculosis mortalities. For a five-day treatment course, the reduction in mortalities following treatment ranged from 56·2% to 100% (mean 81·1%). For a ten-day treatment course, the reduction in mortalities following treatment ranged from 76·9% to 100% (mean 89·4%) and for the positive controls the reduction in mortalities following treatment ranged from 56·1% to 94% (mean 77·8%).

In each of these trials the Sarafin® was surface coated on to feed pellets on the farm using either vegetable or fish oil to aid adherence. No problems with activity or homogeneity have been experienced with either on-site surface coating or mill mixing when the data sheet recommendations are followed.

Target animal safety tolerance studies produced no abnormal behaviour, mortality or pathology in Atlantic salmon when fed with two and four times the recommended dose. No palatability problems were experienced, with the fish showing no reduction in feeding response when the medicated diet was administered. This observation was supported in the efficacy studies.

An acute 96-hour toxicity study to Atlantic salmon fry using the highest concentration of sarafloxacin hydrochloride which can be obtained in fresh water resulted in no observable toxic effects.

Sarafin® is a useful addition to the limited therapeutic armoury available to the fish clinician. It is active against a range of fish bacteria using a low dose over a short treatment period. It is tolerated well by fish, commands a short withdrawal time and the potential for generating bacterial resistance appears to be low.

Peter Southgate graduated from the Royal Veterinary College, London in 1979 and obtained his MSc in Aquatic Veterinary Studies at the Institute of Aquaculture, Stirling in 1983. He is a partner in the Fish Vet Group and recognised by the RCVS as a specialist in fish health and production.

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Health care in a large public aquarium: some case studies

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Deep Sea World is a major public aquarium located in an old quarry site beneath the Forth Rail Bridge north of Edinburgh. It houses around 3,500 fish of approximately 160 species in 23 displays ranging from tropical freshwater fish of the Amazon region through native marine fish and tropical marine fish. It's main attraction is the longest acrylic tunnel in the world at 112 metres long. This runs through the largest marine system outside of North America holding around 5·5 million litres of seawater which is pumped in directly from the Firth of Forth before being circulated through gravity sand filters. We currently receive around 400,000 visitors per year.

Sea lice

In October 1994, the 400+ ballan wrasse, *Labrus bergylta*, in our main display became infected with the sea lice, *Caligus centrodonti,* which were accidentally introduced with new fish. The infection quickly reached 100% prevalence in the ballan wrasse. No mortalities were experienced during the outbreak but many of the fish exhibited severe epidermal erosion, especially around the base of the fins and the head. Observations by Potts (1973) and others showed that in the wild goldsinny wrasse, *Ctenolabrus rupestris*, and rockcook wrasse, *Centrolabrus exoletus*, display symbiotic cleaning behaviour with ballan wrasse.

It was decided to stock the tank with both of these cleaning species in an attempt to reduce the incidence of infection with the sea lice in the ballan wrasse. Around 250 goldsinny and 150 rockcook wrasse were placed in the tank. Within two days the new wrasse had established cleaning stations and the ballan wrasse were observed to visit these stations and display the typical cleaning posture of head down with fins and operculum flared. Within a further few days the level of infection was seen to reduce and two weeks later no lice could be seen on the ballan wrasse. The lesions caused by the lice soon healed. Four years later there are still some of the original goldsinny and rockcook wrasse in the tank and we have had no recurrence of infection.

Aeromonas salmonicida

One of the most popular displays at Deep Sea World are the wolf-fish, *Anarhichas lupus*. This a very large member of the blenny family which is native to the east Scottish coast. They can reach over 1 metre in length and have a very dramatic appearance due to their impressive dentition which they use to feed on crustaceans and echinoderms. We receive our wolf-fish from local fishermen who catch them in lobster creels. After starting to lose up to one third of the fish which we had brought into the aquarium we contracted our veterinarians at the Institute of Aquaculture in Stirling to do some bacteriological work on the fish. It soon became apparent that almost all the wild caught fish were carrying an atypical strain of *Aeromonas salmonicida*. We contacted Aquatic Vaccines Limited in Saffron Walden and they supplied, free of charge, some of their furunculosis vaccine (Aquavac® Furovac 5, injection) for trial with the wolf-fish. The fish were inoculated with 0·2 ml of the vaccine as soon as they arrived at our aquarium. Since we began using the vaccine two and a half years ago, we have not had any wolf-fish die due to furunculosis.

Vibrio sp.

One of the problems encountered in the first few years of operation was uncontrollable temperature rises in main display during the summer-time. In the first year the temperature exceeded 20°C for a short period. This caused severe stress to native marine fish especially the local gadoids. During times of temperature stress we regularly observed outbreaks of unspecified *Vibrio* sp. affecting mainly the native gadoids. Only once in five years have we isolated Vibrio anguillarum. These outbreaks were treated using antibiotics after the bacterial sensitivities had been confirmed. Typically, oxytetracycline (Terramycin®; Pfizer) would be fed daily at the rate of 150 mg/kg body-weight for ten days but other antibiotics have been used according to the sensitivities reported. These were administered by surface-coating pellets using gelatine. Fortunately, due to improved temperature control, we have now reduced the annual number of vibrio-related deaths to a few per summer. We have also been considering using one of the newly developed oral vibrio vaccines to combat the disease.

Marine Oodinium (*Amyloodinium*)

During my talk I also made reference to an outbreak of *Oodinium* in our tropical marine system which we successfully treated using a commercially available aquarium treatment Octozin® (active ingredient is undisclosed) from Waterlife Research Ltd., West Drayton.

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David Gibson graduated with a BSc in Zoology from Glasgow University in 1991. Following his interest in fish he obtained a PhD from the Institute of Aquaculture at Stirling University. His degree studied the diseases of native wrasse species in relation to the salmon farming industry. After completing his PhD he started work with Deep Sea World, a public aquarium in North Queensferry, Fife where he is currently employed as the Curator.

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PROCEEDINGS

Fish Veterinary Journal (1999) (3) 43–45

Parasites, resistance and control strategies

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Fish can be infected with a diverse range of parasitic infections that include helminths, crustaceans and protozoa for which many different chemical treatments are used. A range of in-water proprietary medications containing one, or several, ingredients are available for the treatment of external parasites. Endoparasites, and some ectoparasites of fish, can be treated with a number of antiparasitics which are routinely used in higher vertebrates, in particular, domestic farmed livestock such as grazing ruminants. In this latter group, livestock intensification has lead to an increased reliance on the use of antiparasitics to control parasite burdens. One of the inevitable consequences of increased drug usage is the development of drug resistance. This has been seen with many parasitic infections of which anti-malarial resistance in *Plasmodium* infections of man, and anthelmintic resistance in sheep nematodes are prime examples. The emergence of resistant parasites limits the choice of effective compounds with a resultant adverse effect on animal welfare and loss in animal productivity. Lessons learned from the use of antiparasitics in birds and mammals should apply equally in fish medicine.

Antiparasitic resistance can be described as the heritable ability of a parasite to tolerate the therapeutic dose of a chemical compound. This assumes that the drug is fully effective in the first place. Some parasites may be unaffected by certain antiparasitic compounds and can be considered to have a natural or innate resistance which must be distinguished from genetically acquired resistance through repeated drug exposure. Antiparasitic *resistance* should not be confused with antiparasitic *failure* — the decreased efficacy of anthelmintic treatment which can occur under certain situations such as:

- under-dosing
- failure to follow the manufacturer's instructions
- return of animals to a heavily contaminated environment or
- use of the incorrect drug for the target species.

Taking anthelmintics for use in cattle and sheep as an example, three main (broad spectrum) anthelmintic groups exist which differ in their modes of action. The three main anthelmintic groups are:

1. **benzimidazoles** and **probenzimidazoles** (thiabendazole, oxibendazole, fenbendazole, mebendazole, oxfendazole and albendazole; febantel, thiophanate and netobimin)
2. **imidazothiazoles** and **tetrahydropyrimidines** (levamisole and tetramisole; morantel and pyrantel)
3. **avermectins** and **milbemycins** (ivermectin, doramectin, eprinomectin and abamectin; moxidectin).

When resistance develops to a single compound it is often associated with side resistance to related compounds in the same class rendering the whole group ineffective. Cross resistance between compounds of differing classes has occasionally been reported, but of greatest concern is multiple resistance to all groups which has been reported in some nematode species from sheep and goats in southern hemisphere countries.

Parasite resistance can only be detected using suitable diagnostic aids. For this purpose a number of in-vivo and in-vitro assays have been devised. In-vitro assays for detecting anthelmintic resistance in nematodes of ruminants for example, involve the use of the free-living stages (eggs and larvae) and have the potential to be adapted for use on fish parasites.

The rate of development of resistance is influenced by management practices (stocking rates, husbandry practices, and drug usage patterns). One of the most important factors is, perhaps, climate which influences parasite fecundity, seasonality and incidence. Selection pressure may be increased if parasites are exposed to sub-lethal doses of drug, as in accidental under-dosing, or through repeated exposure to a single antiparasitic type.

Again taking anthelmintic resistance in sheep nematodes as an example, a number of measures have been recommended to delay the development of resistance and include; ensuring full and effective dosing; introducing regular screening checks to ensure anthelmintic efficacy is being maintained; alternation of anthelmintic class on a yearly basis; reduced frequency of anthelmintic usage by strategic planning; dosing all new animals and quarantine for an appropriate period of time; and if possible use of narrow spectrum drugs for specific situations. Such control measures could be equally applied to treatments used for fish parasites.

In conclusion, livestock intensification can lead to a heavy reliance on chemicals to control parasite burdens. Over reliance or inappropriate use of these drugs can lead to the development of parasite resistance limiting the choice of effective treatments. Veterinarians involved in the control of fish parasites should be aware of the consequences of resistance to available medications developing and advise accordingly.

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Mike Taylor qualified from Glasgow University Vet School in 1976 with distinction in parasitology. He joined the Central Veterinary Laboratory (CVL) in 1984 after spending several years in general practice and the State Veterinary Service. At the CVL he has specialised in parasitology and in 1989 was awarded a PhD from London University for work on anthelmintic resistance. He is now Head of Parasitology and Ecotoxicology at the CVL where his main interests are in parasite chemotherapy and resistance, and the environmental toxicity of antiparasitic compounds.

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Sea lice, medicines and a national strategy for control

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Sea lice are well known parasites of wild and farmed salmon. Numbers can build up to epizootic proportions and the damage caused can seriously affect fish welfare and the profitability of salmon farms. Treatment is required but the choice of authorised medicines is very limited and those in use are only partially efficacious, while often causing their own problems by killing fish.

Sea lice are therefore, the Scottish Salmon Growers Association's (SSGA) number one research priority and to date, farmers have spent £1·7 million to fund a wide-ranging programme of investigation into all aspects of parasite control. This money has attracted funds from elsewhere and the total spent on researching the problem in Scotland is nearer £5 million.

While a vaccine is the ultimate goal, it is unrealistic to expect trial products within the next five to ten years and therefore a range of medicines is required in the interim. The SSGA continues to support a screening programme for new active ingredients at the Institute of Aquaculture at the University of Stirling. This project has been running since 1989 and has worked on azamethiphos (Salmosan®; Novartis) and hydrogen peroxide (Salartect®; Solvay Interox) and was the first to identify the properties of cypermethrin, soon to be licensed as Excis® by Grampian Pharmaceuticals.

Currently, there are only three active ingredients which can be prescribed for salmon and treatment is administered by bath or in-feed. These are dichlorvos, an organophosphate to which some lice have now developed resistance and is restricted by the Scottish Environment Protection Agency (SEPA); hydrogen peroxide, to which SEPA allows unrestricted access; and ivermectin, which is licensed (on a temporary basis) on a few farms only.

Bath treatments have been the mainstay of control over the last twenty years but they are weather-dependent, difficult to carry out and have a tendency to kill fish. They are only partly efficacious resulting in a need to repeat treatments. Hydrogen peroxide-based medicines are unsuitable at high water temperatures in summer.

In-feed treatments are now preferred but there are none presently licensed for use in salmon. A few farms have SEPA consents for ivermectin but most applications have been referred to the Scottish Office, Agriculture, Environment, Fisheries Department (SOAEFD) for a decision and this has effectively stopped its use in Scotland. However, in-feed treatments can be effective in controlling lice and two insect growth-regulators and a novel avermectin are currently in the process of gaining approval for use in fish.

In order to avoid the development of resistance, salmon farmers would like access to different molecules for administration by bath and in-feed and look forward to the time when the product range includes Salmosan®, Excis®, Salartect®, Paramove®, Calicide®, Lepsidon® and the novel avermectin for use in an integrated pest management strategy.

The licensing procedure in the UK is complex and expensive, and the fact that three authorities have to be approached, contributes to a slow and frustrating process. The Veterinary Medicines Directorate (VMD) looks at data on safety, quality and efficacy before granting a Marketing Authorisation (MA). The European Medicines Evaluation Agency will look at similar data but is particularly interested in consumer safety and will establish a Maximum Residue Limit (MRL). An MRL must be established before an MA can be granted. The next step is for the salmon farmer to apply to the SEPA for a consent to discharge the medicine. This is a public procedure and can result in long delays. Meanwhile, our Norwegian competitors have access to all the modern sea louse medicines and the Irish have a pragmatic approach to the use of ivermectin and cypermethrin.

While the Scottish farmers wait for the regulators to make up their minds about the new medicines, the SSGA has developed a strategy for dealing with lice which will get the best from the few that are available. This has become the ‘National Treatment Strategy for the Control of Sea Lice’ and it has been adopted by the industry as a Code of Practice. Its evolution is described below.

At the SSGA Technical Seminar in November 1997, industry scientists reported on significant work regarding a strategic approach to control of sea lice. They have demonstrated that the reproductive capacity of female lice is compromised in the spring, resulting in reduced survival in their offspring. A late winter treatment offensive aimed at lowering the number of adult females to the lowest possible levels can have significant benefits. When data from the 1993 year class were compared with that of the strategically treated 1995 year class, the results showed that they could reduce fish mortalities by up to 83%, reduce fish being downgraded at harvest by 80%, have a 62% reduced mobile louse infestation level and reduce treatments by as much as 46% (Wadsworth and others 1998).

It was suggested that control of sea lice at a national or regional level will be improved if salmon farmers adopt a collaborative policy of coordinated treatments much like those that were recently proposed for the eradication of sheep scab by the crofters of Skye (West Highland Free Press 1997).

Progress with this initiative depended on the maximum cooperation from all Scottish salmon farmers. In order to initiate improved control of lice, farmers were invited to try out the strategy on a regional basis in the first instance. This involved working with neighbouring salmon farmers to form co-operative groups and all treating simultaneously. The way that this was achieved is outlined below.

Code of Practice for Salmon Farmers

1. **Define the area to be managed.** Farmers first identified the area to be managed and to help in this, an attempt was made to divide Scotland into manageable hydrographic areas. Twenty two areas were defined.

2. **Identify all the salmon farming operatives in the area and elicit a commitment from them towards the National Strategy.** Farmers know who their neighbours are. In the case of non-SSGA members, it fell upon the chairman of the Management Group to identify them and approach them to find out what their views were after seeing the evidence presented.

3. **Form a local area Management Group and include veterinarians.** A positive response meant that local area Management Groups were formed which included the veterinarians who have responsibility for the fish. Seventeen groups were formed early in 1998.

Each Management Group had two main tasks to agree and oversee. All lice populations required monitoring at agreed intervals to a standard protocol and regional treatments had to be carried out according to agreements drawn up between neighbouring farms.

4. Agree the monitoring protocol and frequency. Lice numbers on all the farms in one area required regular monitoring. A protocol was suggested and is as follows:

- starting in February, two pens on each site will be monitored weekly.
- ten fish should be sampled from each pen and the number of chalimus, 'mobiles' (pre-adult and adult stages) and 'females with eggs' (gravid females) recorded. A useful article on this subject, titled 'Optimal timing for lice treatments' was recently published in *Fish Farmer* (Treasurer and Grant 1997).
- results should be audited by neighbouring farmers or whoever the Management Group decides to nominate.

5. Agree timing and threshold of strategic treatments. It is expected that monitoring will show that the population of lice is low in March and the Management Group will then decide when to treat. The main aim of the treatments is to prevent the large settlement of chalimus stage (which has been shown to occur between weeks 20–25) and the subsequent build up in mobile lice. The idea is therefore, to treat all the lice in the management area at the agreed time (probably in March) in an attempt to remove all the gravid females. A follow-up treatment should then be given as required, to kill any lice which had grown to the pre-adult stage. Thereafter, lice will be treated in order to keep 'females with eggs' at the lowest possible level to stop recruitment, the re-infestation of fish by the infective copepodid stage. It is important to synchronise the early treatments.

Some farmers and veterinarians may feel that the level of lice is too low to warrant treatment, however, in order for the strategy to be successful, lice must be treated at the crucial time before numbers build up. Suggested thresholds for mandatory treatment during the crucial period are as follows:

(a) for any age of fish, one adult female louse with eggs in the ten fish sampled

(b) for fish in the sea for less than six months, as in (a), or with an average of one 'mobile' louse per fish

(c) for growers, as in (a), or with an average of two mobile lice per fish.

The Management Group may wish to coordinate treatments throughout the year but, to be successful, must revert to this code before the numbers of lice increase in the spring.

6. **Carry out the strategic treatments.** For those Management Groups organised in time to initiate the strategy in 1998, the only available medicines are Aquagard® and hydrogen peroxide. Though less than perfect, these medicines were effective on the trial sites and farmers were encouraged to repeat the work using them (provided the farmer is confident of achieving a treatment that is safe to the fish).

   The success of the strategy will be improved when new, fully authorised medicines become available. These new bath and in-feed treatments should be used wherever possible. In-feed treatments will be particularly useful for small fish but are unlikely to become available for strategic use until 1999.

   It is envisaged that, by the winter of 1999/2000, the strategy will be well established and it will become increasingly effective as these new, safe and effective medicines become available. The result will be an overall reduction in the numbers of lice around the Scottish coast and farm louse numbers will be at their lowest when wild salmon and sea trout smolts are migrating.

7. **Continue Monitoring.** It is important to continue monitoring in order to keep control of the lice but also to report to the Management Group so that the success of the strategy can be recorded and used to make plans for the following year.

8. **Continue consultation with stakeholders.** In preparing this Code of Practice, the SSGA has taken account of constructive comments from salmon farmers, shellfish farmers, regulatory authorities, fisheries trusts,

anglers and fishermen. Management Groups should continue this process in order to take advantage of their collective experience and to take account of their concerns. For example, farmers should be mindful that, due to the presence of juvenile stages of commercial species in the plankton, fishermen would like strategic treatments to be complete before week 13 each year.

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Footnote:
Since writing this article, Salmosan® (Novartis) and Excis® (Grampian Pharmaceuticals Ltd.) have been consented for use by SEPA on some farms. It is expected that their use will increase as more Discharge Consents are granted.

Gordon Rae graduated in 1970 with a BSc (Hons) in zoology from Aberdeen University and joined Unilever Research at Lochailort in 1971 to work on the developmental aspects of salmon farming. He joined Unilever's salmon farming company, Marine Harvest in 1978 as fish health manager and held this post until 1990 when he was seconded to the Scottish Salmon Growers Association to work on an industry response to the bacterial disease, furunculosis. As research and development manager, he has been a permanent member of the SSGA staff since 1993 and his priority is now control of sea lice.

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Clinical observations of severe mortalities in koi carp, *Cyprinus carpio*, with gill disease

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Introduction

In 1998 there were many incidents of a high mortality in koi carp, *Cyprinus carpio*, with severe gill disease in the United Kingdom. At the time of writing, November 1998, no specific pathogen has yet been identified. Most cases occurred following the introduction of new fish although in several instances, this took place either months or years prior to the outbreak. Similar patterns of mortality have occurred in Holland, Belgium, Germany, Israel and the USA that have shown a similar clinical picture.

Clinical history

The disease frequently occurs following the recent introduction of new fish and may result from as few as one fish, and after a period of quarantine. In many cases, water quality and stocking density are at acceptable levels although higher stocking densities increases the severity of the disease. A typical outbreak will show high morbidity (80–100%) and high mortality (greater than 50% but typically 70–80%). In ponds with mixed populations of fish, only *Cyprinus carpio* show clinical signs while other species remain unaffected. The disease appears more prevalent in hard water conditions.

The incubation period is typically between two and three weeks. However, if unaffected fish are placed in a system with an established infection then clinical signs can be seen within three days. The worst affected fish are often between 25–30 centimetres in size.

Most outbreaks occur when water temperatures are between 20°C and 23°C but there are reports of some occurring over a wider range from 15°C to 28°C. Mortalities will occur within 48 hours of gill lesions being noted at these temperatures. At lower temperatures the disease process is slower and appears latent at exceptionally low or high temperatures.

Clinical signs

Initially, fish are lethargic, anorexic and have increased respiratory movements. Anorexia may be noticed for up to 10 days before other clinical signs develop. Lethargy is particularly obvious and characteristically profound. Affected fish will either congregate on the bottom of the pond or in the middle of the water column.

Extensive gill necrosis is always present although some fish may only show small, localised lesions. Increased gill and body mucus production is often evident. The gill mucus can be very thick and brown in colour, often seen trailing from the gills. Excess body mucus may be one of the first clinical signs, typically producing an additional sheen on the surface of the skin. If the fish survive long enough, then mucus production ceases and the skin develops a rough texture, like sandpaper.

Other external signs include ulceration, skin haemorrhages, petechiation, ecchymosis and fin rot. A white lesion may be seen on the skin of light-coloured fish while swimming in the water and resembles a mild fungal infection with *Saprolegnia* sp. However, no fungal elements or tissue debris is found on microscopic examination. In darker varieties of koi, such as chagoi, these lesions may appear black.

In addition to lethargy and anorexia, other abnormal behaviour may include sporadic hyperactivity, loss of co-ordination and aimless swimming. However, this may only be observed in a small number of affected fish.

Prior to the outbreak, there may also have been evidence of ectoparasite infestation that failed to respond to treatment with routine medications. Microscopic examination of skin mucus may reveal numerous ectoparasites or nothing at all. Fresh gill squash preparations will often show necrosis and hyperplasia.

Internally, affected fish have clinical lesions similar to septicaemia but often there are no obvious gross lesions. The internal organs have been described both as more pale or darker than normal. Occasionally petechial haemorrhages are found on the liver and kidney, and sometimes there is an accumulation of abdominal fluid.

Histopathology

Several batches of fish have been sent to the CEFAS laboratory, Weymouth. All have shown inflammatory changes in most tissues. The gills often have extensive pathological changes and the kidney has shown moderate damage. Minimal changes have been found in the liver and spleen. Overall the tissue changes are that of a non-specific inflammation.

In addition, inflammatory changes have been found in the brain and in the ganglion cells in particular. This could explain the lack of muscular coordination seen in some fish and may be a diagnostic marker. Further work on brain tissue has been carried out but the results have not proved useful.

Causative agent

At the time of writing, no causative agent has been isolated from affected fish in the UK. Various bacteria have been cultured from cases, including *Aeromonas hydrophila*, *A sobria*, *A caviae*, atypical *A salmonicida* and *Flavobacterium psychrophila*. None of these have been recovered in sufficient quantity to suggest that they are the cause of this disease. No virus has been found by culture or examination by electron microscopy (EM).

Reports from Israel and Germany indicate that unidentified viral particles have been seen using electron microscopy. In Israel, these particles were seen in brain and gonadal tissue but, as yet, these findings have not been reproduced by other workers. Transmission studies have been carried out in both countries and suggest that an infectious agent is involved. The early indications are that a viral infection may be responsible for this disease.

In the USA, a Herpesvirus has been implicated in an outbreak in New York. This was found in EM sections of gill tissue. Transmission studies are now being carried out at the University of California, Davis.

Diagnosis

The clinical and pathological signs are non-specific and variable, therefore diagnosis is by exclusion of all other causes. As yet there is no diagnostic test available.

Control

There is no specific treatment available. Altering the water temperature can stop the disease but there is evidence that the disease will recur when water returns to the original temperature.

It is helpful to perform continuous water changes and remove debris from the pond on a daily basis during an outbreak since this reduces the number of infectious organisms present in the environment. Strict hygiene measures should be taken on infected sites to limit the spread of infection.

From studies done in Israel, it would appear that fish surviving an outbreak cannot transmit the disease to other susceptible fish.

Discussion

The disease has been identified in Europe and in the USA this year, but this link is only based on clinical findings. With hindsight, and from my own clinical records, I feel that this disease has been present in the UK since 1996. Other veterinary surgeons and producers of koi have suggested that this disease may have been present for at least four to six years.

From cases seen in 1998, the disease would appear to be highly infectious. It has been passed between systems on infected sites by contamination of nets, on hands and by aerosol. Fish lice, *Argulus* sp., were present in seventeen cases recorded in hobbyists' ponds just prior to outbreaks of this disease. *Argulus* has been implicated in the transmission of viral infections in the past (Ahne 1985).

In the USA a Herpesvirus has been implicated in a similar outbreak and in Japan, a Coronavirus causing 'non-bacterial ulcer disease' has been isolated (Ra'anan Ariav, personal communication). Although a virus could be involved here, as yet, neither of these have been shown to produce the severe gill disease seen in outbreaks in the UK.

'Spring mortality of carp syndrome' describes a similar high mortality in angling carp. This is believed to have been seen in coarse fisheries in the UK for almost twenty years. It is often related to rising water temperatures in spring and early summer. Again, there is no clear pattern of pathology and no specific pathogen has been identified. Research into this syndrome is currently being carried out in association with CEFAS, Weymouth. The syndrome appears equally mysterious and although there are interesting similarities to the high mortalities in koi, they are dissimilar in pathological appearance and water temperatures.

It is hoped that continued research and investigation will reveal the agent responsible for these severe mortalities in koi.

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Chris Walster qualified from Glasgow Veterinary School in 1983. He is the senior partner of a 12-vet practice based in the Midlands. He has been a fish health consultant for Mag Noy, Israel Ornamental Fish in the UK since 1996.

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PROCEEDINGS

Fish Veterinary Journal (1999) (3) 59–63

Description of a myxosporean disease in cultured turbot (*Scophthalmus maximus*)

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Abstract

In the summer of 1996, and again in 1997, losses occurred in a Spanish on-growing turbot farm. Fish of all sizes were affected. Tanks were randomly affected, but in affected tanks mortality was almost 100%. Clinically affected fish were dark and anorexic. Typically, eyes were sunken and a prominent bony ridge was present on the head. Internally there was ascites and pallor of the internal organs, and the gut was fluid-filled. The most obvious post mortem finding was haemorrhage of the terminal part of the gut, although the entire gut could be involved. These symptoms have become almost pathognomonic of this disease. Histological examination of H&E stained tissue sections revealed a severe enteritis in the haemorrhagic areas of gut. Foreign cells, typical of myxosporean trophozoites, were present in the epithelium of these areas, and free within the gut lumen. Although initial infestation of the fish was probably due to water-borne actinospores, development of the disease suggests the possibility of direct transmission from fish to fish.

Introduction

In the summer of 1996, and again in 1997, losses occurred in a Spanish on-growing turbot farm. The farm is a pump-ashore site on the Atlantic coast of Galicia. Fish are put into the farm as juveniles all year round, and harvested after approximately 1½ years. In 1997 affected tanks were randomly distributed around the farm but, within affected tanks, mortality was almost always 100%. Fish of all sizes were affected.

Investigations indicated that the cause of the losses was an infestation with a myxosporean parasite. These investigations are detailed here.

Materials and methods

Many clinically affected fish were examined. All fish were subjected to a full postmortem examination. Sampling for bacteria and viruses was carried out, with a range of different media and cell lines being used. Samples taken for histological examination were preserved in 10% neutral buffered formalin and prepared in the usual way, sections being stained with haematoxylin and eosin (H&E).

Results

Clinically affected fish were dark in colour and anorexic. Typically, the eyes were sunken and the bony ridge on the head was very pronounced.

On postmortem examination there was ascites and pallor of the internal organs, and the gut was fluid-filled. The most obvious finding was haemorrhage of the terminal part of the intestine, although the entire gut could be involved.

On histological examination of the H&E stained tissue sections, the most significant finding was severe enteritis in the haemorrhagic areas of the gut. In the worst affected areas there was complete loss of gut epithelium. In less affected areas inflammatory cells were present in the sub-epithelial tissues. These changes were accompanied by the presence of foreign cells in the epithelium, and free within the gut lumen associated with sloughed epithelium (Figs 1 & 2). The foreign cells were typical of myxosporean trophozoites. Although occasional spores were seen in later cases, these were not a common feature of the condition (Alvarez-Pellitero, personal communication). There were no other consistent changes seen in other tissues from affected fish.

No bacteria, viruses or other parasites were consistently found associated with the condition. Nutritional factors were ruled out as the fish were fed commercial diets which were not associated with problems on other sites.

Spontaneous recovery has apparently occurred in some fish taken from production tanks for further study. In these cases, oedema of the gut was seen on histological examination, but no other changes were present. No myxosporean parasites were seen in these cases.

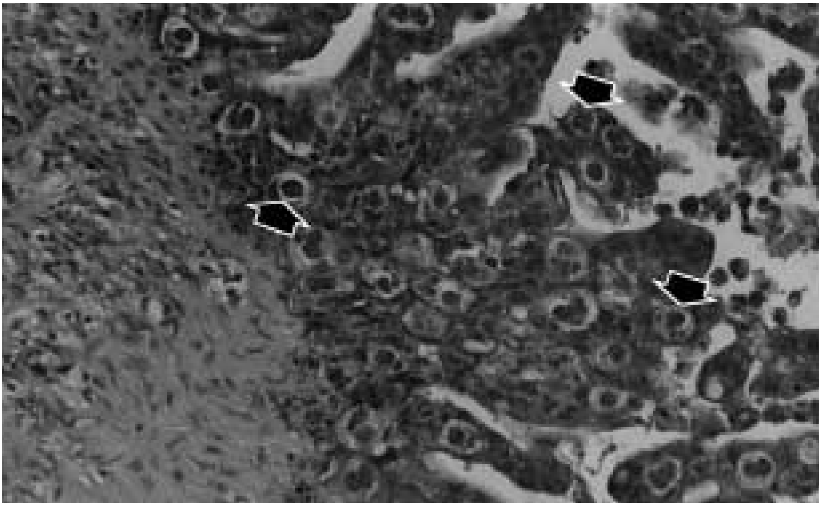

Fig 1 Myxosporean trophozoites (arrowed) within the gut epithelium (H&E) ×400

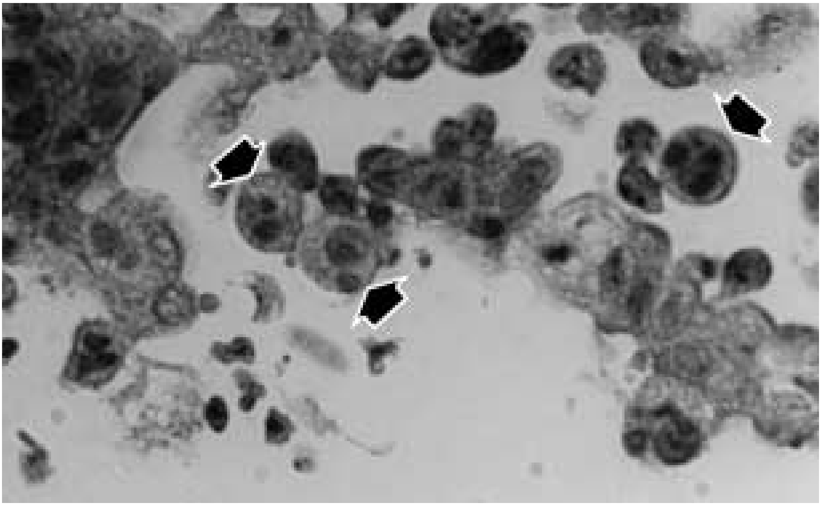

Fig 2 Trophozoites (arrowed) in the gut lumen with sloughed epithelial tissue (H&E) ×1000

Discussion

The presence of myxosporean trophozoites in clinically affected fish, and the lack of other significant pathological findings, indicate that the losses in these fish were related to a myxosporean infestation. The external appearance of clinically affected fish might be found in chronically sick fish with other diseases but, with the gut changes described above, it has become almost pathognomonic of this disease.

Myxosporean infestations are not uncommon in fish, both in the fresh water and marine environment (Lom 1987, Lom 1990). They are thought to have an indirect life-cycle with the final host, the fish, infected by an actinospore arising from an intermediate host (Kent and others 1994). The random distribution of affected tanks on the site suggests that initial infestation of the fish in this case probably arose from water-borne actinospores. However, the subsequent high mortality (almost 100%) in affected tanks suggests a direct fish to fish transfer.

Kent and others (1994) state that almost all attempts at direct transmission of myxosporeans with spores between fish have failed. This fact and the failure to find spores in all cases of this disease suggests that transfer has occurred by some means other than via spores.

Losses with similar pathology to that reported here, have been recorded in the gilt head sea bream, *Sparus aurata*, (Diamant 1992, Diamant and others 1994, Diamant and Wajsbrot 1997) due to infestation with *Myxidium leei*. Transmission between fish was shown to occur due to gut fragments infected with *M leei*, presumably containing pansporoblasts. It seems likely that this same process may be occurring here. Work continues to identify the parasite and its life cycle.

Conclusions

Myxosporean infestation can be a cause of significant loss in cultured turbot. Although initial infestation is probably by actinospore, fish to fish transmission also seems to occur. The similarities of the pathology described here to that seen in other farmed species infested with *Myxidium leei*, suggest that the organism reported here may be the same or a closely related species, but only further work will elucidate this.

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Ana Riaza obtained a degree in biology at the Universidad Complutense de Madrid in 1982, a national diploma in aquaculture at the Ministry of Education, Madrid in 1984 and an MSc in fish nutrition in 1986. After working at the Oceanographic Institute of Murcia (1985) and IFREMER, Brest from 1986–7, she joined Prodemar in 1988. Since 1998 she has been research and development manager, with responsibility for R&D projects, fish health and workers' education.

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Infectious salmon anaemia in the United Kingdom

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In early May 1998 an outbreak of infectious salmon anaemia (ISA) was confirmed in farmed Atlantic salmon, *Salmo salar,* held at a marine site in Loch Nevis on the west coast of Scotland; the first record of this infection within the European Union (EU). ISA is a List 1 notifiable disease under current Diseases of Fish (Control) Regulations and measures for its control are defined in Council Directive 93/53/EEC. Following confirmation, action was taken by the Scottish Office Agriculture Environment and Fisheries Department (SOAEFD) in accordance with the Directive, to contain the outbreak and eradicate sources of infection. Subsequent surveillance of Scottish sites by SOAEFD scientists has led to additional sites being confirmed as infected with ISA, or placed under suspicion of infection.

The 'Nevis B' sea site was stocked with approximately 1·8 million photoperiod manipulated S½ smolts during October and November 1997. Non-smolted fish and poor handling resulted in higher than anticipated post-transfer losses, but mortalities dropped to 0·14% per week during December. An ulcerative syndrome associated with 'Vibrio viscosus' infection persisted from input until April 1998, accounting for a loss of approximately 3% of the stock during this period. During April and early May, mortalities attributed to a new systemic infection increased very rapidly, with a peak of nearly 25% of the stock lost during one week in early May. Notification of the suspicion of ISA was made to SOAEFD on 5 May 1998 and confirmation of infection was given by them on 15 May. Compulsory slaughter of the remaining stock began an 21 May.

Ulcerated, moribund fish suffering from 'V. viscosus' infection, could be seen in variable numbers in most of the cages during the winter and spring period, but occasional non-ulcerated individuals were also noticed from the beginning of March. The non-ulcerated fish were either undamaged, or showed some skin congestion and raised scales on ventral surfaces and most of these exhibited hyphaema. Internally, affected fish typically had petechial haemorrhages over the pyloric caecae, and some had petechiation or patchy congestion of the liver. An increase in abdominal and pericardial fluid was also noted but gross pathology was not considered diagnostic. Initial histological findings were variable, with a low level of cardiac myopathy, variable focal hepatic necrosis and congestion of the spleen and kidney. Phagocytic cells were consistently seen adhering to endothelial linings of vessels in the internal organs and erythrophagocytosis was noted, particularly in the spleen.

During the period when mortalities were increasing rapidly at the end of April, some of the moribund fish exhibited profound anaemia, blood-stained ascites and had swollen congested livers which were almost black in colour. Haematocrits at this time averaged less than 15%, with a range of 2% to 25%. Histologically, necrosis and haemorrhage of the liver, spleen and kidney became more obvious as the disease progressed. Focal haemorrhages in the lamina propria of the caecae and intestine were also common. The liver in some cases had extensive focal and confluent haemorrhagic necrosis of the parenchyma, sometimes with a zonal appearance, surrounding areas of normal hepatic tissue around central vessels. By mid-May, most moribund fish were in poor condition and showed scale loss and exophthalmos. At this stage, enlarged dark livers, ascites and internal haemorrhages were less frequently seen and anaemia was generally less severe.

The results of additional investigations carried out earlier in the course of the disease, tended to be inconsistent and were not diagnostic. Significant anaemia was not found until the end of April. Blood biochemistry was performed by a commercial veterinary laboratory but the results were inconclusive. Aspartate transaminase (AST) was often raised, as was creatine phosphokinase (CPK) in some fish. Plasma sodium was often increased and potassium levels were decreased. One, from a sample of ten, apparently normal fish from this stock had antibodies to salmon pancreas disease virus (SPDV) at levels greater than 1/20. Routine bacteriology failed to reveal any significant organisms other than occasional isolates of untyped vibrios.

Virology samples were processed at the Fish Disease Laboratory, Department of Agriculture for Northern Ireland (DANI) and at the Institute of Aquaculture, University of Stirling. Several cell lines were used and a cytopathic effect (CPE) was eventually observed after blind passages on a salmon head kidney cell line (SHK-1) at both laboratories. A transmission study performed at Stirling, using a crude tissue extract inoculated by intraperitoneal injection into salmon parr, produced 100% mortality. Pathology identified in these fish included anaemia, caecal haemorrhages, swollen spleens and cardiac congestion. A virus was visualised by transmission electron microscopy (TEM) of infected SHK-1 cells at DANI and this appeared morphologically similar to the orthomyxo-like virus responsible for ISA.

Kidney impression smears made on poly-L-lysine coated slides were screened by a direct immunofluorescent antibody test (IFAT) using an anti-ISA monoclonal antibody by Dr Knut Falk at the National Veterinary Institute, Oslo. Additionally, kidney samples in 70% alcohol were tested by reverse transcriptase polymerase chain reaction (RT-PCR) by Dr Are Nylund at the Department of Fisheries and Marine Biology, University of Bergen. Positive reactions were obtained from both laboratories.

There is circumstantial evidence to suggest that ISA has been spread from Loch Nevis to other farm sites in Scotland. During February 1998, before any signs of the new infection had been seen, post-smolts from 'Nevis B' were transferred by well-boat to a site in the north of the Island of Skye. This site was subsequently diagnosed as infected with ISA. Later in the spring and before disease was suspected, a well-boat delivered additional unrelated smolts to this site on Skye and was tied up adjacent to the cages holding the 'Nevis' stock for several hours while it discharged part of its cargo. The remaining smolts in the boat's wells were then taken directly to a site in the Shetland Islands, which was later diagnosed as ISA-positive. It seems reasonable to assume that these fish became infected through water exchange while the boat was unloading on Skye.

The 'Nevis B' smolt input also overlapped production of large grower salmon at the nearby 'Nevis A' site. Throughout the autumn and winter of 1997, fish from 'Nevis A' were transported by well-boat for slaughter at the harvest station on Loch Creran, near Oban. Two production sites on Loch Creran were also confirmed as ISA-positive shortly after the confirmation of ISA in Loch Nevis. This suggests that ISA may have become established in Loch Creran following transfer of sub-clinically infected grower fish from Loch Nevis. At the time of writing (December 98), all remaining sites which have subsequently been confirmed as ISA-positive, or those which are officially under suspicion of infection, can all be shown to have a link with either Loch Nevis or Loch Creran, through boat movements or by transfer of equipment or personnel. Contact of this nature between sites is likely to increase the risk of spreading ISA from an infected population.

At the beginning of December 1998, a total of ten marine sites had been confirmed as infected with ISA by the Scottish Office and the stocks removed as required by Directive 93/53/EEC. Eleven other sites have been placed under official suspicion of infection on the basis of laboratory results obtained during surveillance by Scottish Office scientists. Seven of these sites are still in production and no clinical signs of ISA have been detected at the time of writing. Loch Nevis is presumed to have been first infected between April 1997 and February 1998 but the original source remains unknown. An epidemiological survey into the outbreak is being conducted by the Scottish Office, but no results have been made available at this time.

'Fallowing zones', based on local tidal flows, have now been placed around all confirmed and suspected sites. Mandatory fallow periods have been set for these zones and re-stocking will not be permitted until all farms in the zone have completed the appropriate fallow period. Cage fittings and equipment must be disinfected to SOAEFD's requirements before fallowing can begin. The original high-risk zones, determined on the tidal overlap between sites, remain in place and farms within these zones are also subject to fallowing and controls on the movement of fish and equipment. The effectiveness of these control measures may not be fully apparent until the summer of 1999 at the earliest, given the recorded autumn and spring seasonality of ISA outbreaks in Norway and Canada. While the original source remains unclear and given the experimental evidence that herring *Clupea* sp., sea trout, *Salmo trutta,* and rainbow trout, *Oncorhynchus mykiss,* can maintain the ISA virus for variable periods, opinion remains divided as to the likelihood that ISA can now be eradicated from Scottish waters.

Footnote:
At the end of December 1998, SOAEFD placed a further four marine farms under suspicion of infection with ISA virus. Three of these sites appear to have no obvious connection with the confirmation of ISA initially made in Loch Nevis. While all discussions on the source and transmission of the ISA virus within Scotland remain speculative, the early assumption that infection was transferred from Loch Nevis to Skye is now less certain.

Tom Turnbull graduated from Glasgow Veterinary School in 1981 and obtained an MSc in aquatic veterinary studies at the Institute of Aquaculture in Stirling in 1991. Following four years in the Veterinary Diagnostic Service at the Institute of Aquaculture he joined Hydro Seafood GSP as a veterinary adviser in 1995.

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Halamid® = Biosecurity

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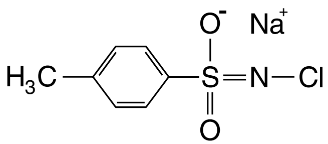
Halamid® and Biosecurity

Biosecurity may be defined as all measures managemental, nutritional, immunological, therapeutic, genetic and environmental that protects fish from disease. Buzzwords are popular especially if they are politically correct and suggest that they are environmentally friendly. Anything beginning with 'bio-' is good (*eg* biocide, biosecurity). Anything beginning with 'dis-' is bad (*eg* disease).

Halamid® (Vetrepharm Ltd.) is an aid to good biosecurity and depending on the concentrations used, it is a selective biocide. Biosecurity is the holistic approach to disease control. It encompasses all those measures that you need to undertake to ensure the health and welfare of the fish under your care. The level of security will vary with the specific operation being considered. Sometimes the environment can be closely controlled whilst on other occasions you ensure the best level of immunity in the stock to enable them to thrive in near natural conditions.

What is Halamid® ?

Chemical name : Sodium-N-chloro-para-toluenesulfonamide
Molecular formula : $C_7H_7ClNNaO_2S.\ 3H_2O$
Chemical formula :



Better known as chloramine-T, it is 100% pure and has many more uses than as simple treatment for bacterial gill disease.

Indications for use

Fish health	Water sanitation
Water disinfection	Equipment disinfection

Effectively, increasing the working concentration of the product changes the use for which it is applicable. When used as a fish medication, it is used at low doses (in milligrams per litre) whereas much higher concentrations are required for disinfection (in the order of kilograms per 100 litres water). Over the years, more information has emerged about the diverse uses for Halamid®, its safety and its lack of damage to the environment.

Mode of action

In solution, the chloramine-T ion is formed. This is the active ingredient, which reacts with cellular material. It is an irreversible oxidative reaction and consequently, it is not possible for microbes to adapt or develop resistance. It does not form hypochlorous acids as stated in some literature, nor does it rely on the formation of free, reactive oxygen or chlorine radicals. It acts through the formation of the chloramine-T ion in solution which then reacts with the organic material with which it comes into contact. The compound is a relatively poor 'chlorine donor' and does not form organochlorines in the environment.

Factors affecting biocidal activity

- Concentration
- Contact-time
- Temperature
- *p*H
- Water hardness
- Organic load
- Additives (*ie* foaming agents)

Effect of *p*H

Fig 1 demonstrates the stability of the product over a range of different *p*H conditions. Halamid® reacts faster in acidic environments and this may be beneficial in some cases, an attribute that can be adjusted to suit individual circumstances. However, this may be more difficult to achieve in aquaculture than in land-based operations because of the additional constraints of the environment.

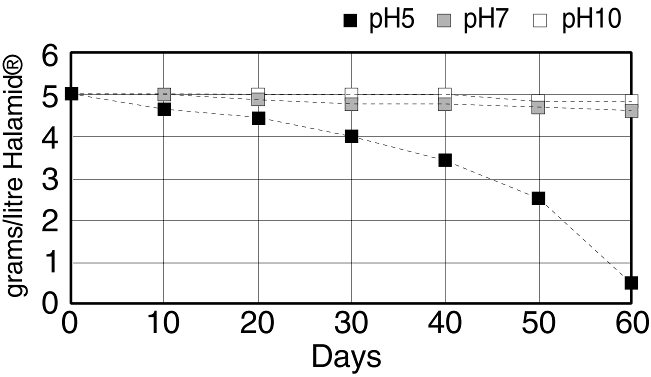


Fig 1 The effect of *p*H on the stability of Halamid® solutions.

Environmental aspects

Halamid® is more environmentally friendly when compared to many similar products because of it's following characteristics:

- Readily bio-degradable
- Low soil adsorption
- No accumulation in the environment
- Low toxicity to aquatic species
- Very low ability to form chlorinated hydrocarbons.

Range of efficacy

Halamid® has shown a wide range of efficacy against many different groups of organisms (data on file):

	Number of species tested
Viruses	49
Bacteria	94
Fungi	22
Algae	6
Yeasts	4
Parasites	4

Halamid® has been shown to kill the following aquatic pathogens:

Aeromonas salmonicida	*Vibrio anguillarum*
Vibrio salmonicida	*Yersinia ruckerii*
Saprolegnia parasitica	*Chlorella vulgaris*
Gyrodactylus salaris	Infectious pancreatic necrosis (IPN) virus

Toxicity to aquatic species

In laboratory studies, the following species have responded to the effects of Halamid® over various time intervals at the concentrations listed below:

Daphnia magna	48 hour NOEC[1]	1·8 mg/litre
	21 day NOEC	1·1 mg/litre
Pimephales promelas	96 hour LC_{50}[2]	7·3 mg/litre
	35 day NOEC	1·5 mg/litre
Rainbow trout[3]	96 hour LC_{50}	2·8 mg/litre
	1 hour NOEC	100 mg/litre
	8 hour NOEC	50 mg/litre
	24 hour NOEC	10 mg/litre

[1] NOEC = No observable effect concentration.

[2] LC_{50} = Lethal concentration that kills 50% of the organisms in the test per unit time.

[3] Rainbow trout, *Oncorhynchus mykiss,* can survive concentrations from 10 to 100 mg/litre but this is likely to be harmful to bio-filters.

Halamid® — additional uses

Fig 2 illustrates the range of activities involved in salmon production and highlights the areas where transfer of disease may occur. Halamid® is useful as a disinfectant in the following areas of aquaculture:

- Hatchery disinfection, foot-baths, tanks, floors and working utensils
- Feed dust build-up on walls and ceilings, an ideal bacterial environment
- Disinfection of service vehicles
- Cleaning down and disinfection of graders and vaccination tables
- Sea sites: cages and nets, foot-baths, diving suits, protective clothing.

Halamid® is an important partner in biosecurity

It is active against bacteria, viruses, fungi, algae, fungi and parasites.
It is stable in solution, has a prolonged action and has good storage stability.
It is safe to handle, has a low toxicity, is bio-degradable and non-corrosive.

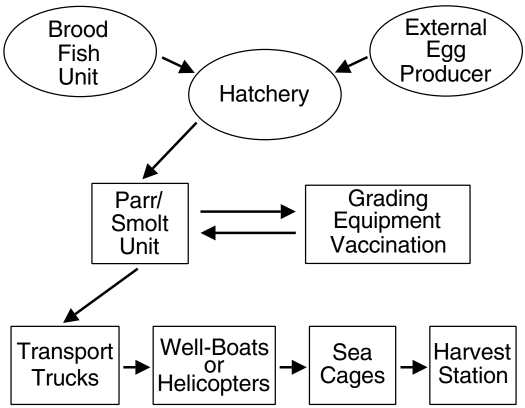


Fig 2 Areas of salmon production where disease transfer can occur.

A practical programme for disinfecting well-boats

In view of the recent concerns that well-boats may have been a factor in the transmission of infectious salmon anaemia (ISA), a practical and effective method of disinfecting well-boats was investigated. The following is a summary of the important aspects of such a disinfection program.

1. Identify the areas in special need of attention and those parts which come into contact with fish. These will include contaminated nets, inlet valves, working areas, ropes and reels. Other areas of high-risk include rough surfaces, protective clothing and the oxygenation pipes on the floor of the well. The pumps will also need to be cleaned and disinfected if they have been hired.

2. Cleaning prior to the use of any biocide is vitally important. Remove all organic material using a foam to allow the biocide to work effectively. The foam acts as a wetting agent and increases the contact-time with the surface to which it has been applied. This enables the operator to see which areas have been covered or missed since many different objects are easy to miss when working to time limits. Cover everything with foam and leave for half an hour before washing off.

3. Disinfect using 2 kg of Halamid® with 25 ml of a foaming agent in 200 litres of sea or fresh-water. Use a calibrated container to ensure accurate measurement of the products and that they are mixed correctly. The mixed solution should then be applied to surfaces using a pressure washer. Afterwards, rinse off or leave to dry on to the treated surfaces.

4. Prevent recontamination by placing a foot-bath at every entry and exit to the boat.

Notes for cleaning well-boats

In cold conditions, it is helpful to premix Halamid® in a little tepid water to help it dissolve. Start on the top decks and ropes, working down to the working decks. Ensure that all the cranes, lifting gear and equipment is covered with the product. Working down to the well, starting with the roof, walls and down to the floor. Make sure that the inlet valves and pumps are covered in Halamid®. Even the most inaccessible spots must be done. This product is no more corrosive than seawater.

David Parsons graduated from the Royal Veterinary College, London in 1975 and obtained the RCVS Certificate in Poultry Medicine & Production in 1990. He has worked in general practice, as a research officer at the Central Veterinary Laboratory, Weybridge and as company veterinarian to one of the UK's leading poultry integrations. David joined Vetrepharm Ltd in 1996 as veterinary advisor, having been consultant to the company for several years, and has responsibilities for their full range of products and regulatory affairs.

Donald Campbell comes from an agricultural background involved in arable sheep and beef production in Central Scotland. He joined Vetrepharm Ltd in 1996, having previously worked in the wholesaling business. He is the divisional manager responsible for the supply of products to Scotland and Ireland.

This article is based on a presentation given at the autumn meeting of the Fish Veterinary Society in Penrith on 12 November 1998 and submitted for publication on 2 December 1998.

Fish Veterinary Journal (1999) (3) 74–78

Avian influenza ecology: a brief review

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Recent work has suggested that the virus responsible for infectious salmon anaemia (ISA) should be placed in the Family Orthomyxoviridae with the influenza viruses and Thogoto-like viruses. This has raised interest among those involved in the veterinary aspects of fish disease and in the epidemiology of other orthomyxoviruses in other hosts. The objective of this article is to briefly review the ecology of the enormous pools of antigenically diverse influenza A viruses found in birds.

Aetiology

Influenza viruses are segmented, negative strand RNA viruses that are placed in the family Orthomyxoviridae. They are divided into three types A, B and C. Only influenza A viruses have been reported to cause natural infections of birds. Type A influenza viruses are further divided into subtypes based on the antigenic relationships in the surface glycoproteins haemagglutinin (HA) and neuraminidase (NA). At present 15 HA subtypes have been recognised (H1–H15) and nine NA subtypes (N1–N9). Each virus has one HA and one NA antigen, apparently in any combination.

Although the range of influenza A subtypes and combinations occurring naturally in mammals appears to be restricted, H1, H2 and H3 in humans, H1 and H3 in pigs, H3 and H7 in horses, and occasional isolated infections of other mammals such as seals, whales and mink, all HA subtypes and the majority of possible combinations with NA subtypes have been isolated from avian species.

Influenza A viruses infecting poultry can be divided into two distinct groups on the basis of their ability to cause disease. The very virulent viruses cause a disease formerly known as fowl plague and now termed highly pathogenic avian influenza (HPAI) in which mortality may be as high as 100%. These viruses have been restricted to subtypes H5 and H7, although not all viruses of these subtypes cause HPAI. There have been 17 reported primary isolates of such viruses from domestic poultry since 1959. All other viruses cause a much milder disease consisting primarily of respiratory disease, depression and egg production problems in laying birds. Sometimes other infections or environmental conditions may cause exacerbation of influenza infections leading to much more serious disease.

Influenza in birds

In 1955 it was demonstrated that the viruses responsible for 'fowl plague' and other, milder, diseases of domestic poultry were influenza A viruses. The first isolation of influenza virus from feral birds was in 1961 from common terns, *Sterna hirundo*, in South Africa. However, it was not until the mid-1970s that any systematic investigations of influenza in feral birds were undertaken. These investigations revealed the enormous pools of influenza viruses that are now known to be present in the wild bird population.

Influenza viruses have been isolated from avian species representing most of the major Families of wild birds throughout the world. Reviews of these surveillance studies list 90 avian species, covering 22 different Families and 12 Orders, from which virus has been isolated. The actual number of fully susceptible species is likely to be much greater, and to some extent this is demonstrated by the recorded susceptibility of a wide variety of birds in laboratory experiments or investigations of captive birds in quarantine or in ornamental collections.

Virus isolations from other wild birds have been completely overshadowed by the number, variety and widespread distribution of influenza viruses in waterfowl, Family Anatidae, Order Anseriformes. In the surveys undertaken in the USA during the 1970s and 1980s a total of 21,318 samples from all species resulted in the isolation of 2,317 (10·9%) viruses. However, 14,303 of these samples were from birds of the Order Anseriformes which yielded 2,173 (15·2%) isolates. The next highest isolation rates were 2·9% and 2·2% from the Passeriformes and Charadriiformes, respectively; but these compare with an overall isolation rate of 2·1% from all birds other than ducks and geese. In fact the overall isolation rate falls to 2·2% if birds of the genus *Anas* are excluded and 6·2% if those from *Anas platyrhyncos* (mallard) are ignored.

Each year waterfowl congregate in huge flocks, usually on lakes, before migratory flights are undertaken and in studies of ducks at this time isolation rates from juvenile ducks have exceeded 60%.

Although occasionally viruses of low and high pathogenicity for poultry have spread among populations of domestic poultry, usually by the agency of man, the majority of reports of influenza infections have been of viruses of low virulence and the occurrence of such infections has been directly related to their contact with wild birds, especially migratory waterfowl. Usually outbreaks are sporadic and infrequent, but in parts of the USA, such as in Minnesota where turkey farms are heavily concentrated, situated on migratory waterfowl flyways and birds are reared mainly on open range influenza virus infections have been seen more consistently. Of the 22 separate outbreaks recorded in turkeys in Great Britain between 1963 and 1993, 17 were on farms in Norfolk, a county that includes important ‘stop-over’ areas for migratory waterfowl. Similarly domestic duck flocks, particularly fattening flocks, in most parts of the world, are kept on range or on ponds, often in areas on migratory routes and influenza viruses are frequently present and probably enzootic.

It seems highly likely that influenza viruses are perpetuated in feral waterfowl by the passage of virus from adult to juvenile birds on lakes where the birds congregated before migration. Considerable quantities of the virus are excreted with the faeces into the lake or pond water, to the extent that virus may be isolated from untreated lake water where large numbers of waterfowl are found. In one study it was shown that influenza virus may remain infective in lake water for up to 4 days at 22°C and over 30 days at 0°C. While in another infectivity was retained for up to 207 days at 17°C and 102 days at 28°C from an initial concentration of 10^6 $TCID_{50}$ per millilitre (median tissue culture infective dose). Contaminated lake or drinking water may therefore result in infection by the faecal/oral route, or possibly by the faecal/cloacal route as a result of ‘cloacal drinking’. For all birds the ingestion of infective faeces appears to be the most important mode of transmission.

Avian influenza viruses in other animals

In general terms the host range of influenza viruses appears to be restricted by a number of factors and genetic studies of viruses from different species have revealed distinct lineages of viruses associated with the species from which they are normally isolated. The exception appears to be pigs and in experimental infections these have been shown to be readily infected with viruses of avian and human origin as well as those from the 'pig' genetic lineage. Interestingly although viruses clearly of avian origin can be isolated from pigs as a result of natural infection and appear to be established in pig populations these are still restricted to H1 and H3 subtypes. Similarly in the 1989 outbreak in horses in China the influenza virus responsible was genetically most closely related to avian viruses rather than those normally isolated from equine species, but was of H3N8 subtype, a subtype combination that has circulated in horses since 1963.

In 1979–1980 respiratory disease resulted in the death of about 20% of the harbour seals on the north east coast of the USA. An influenza virus of H7N7 subtype was implicated in the disease and genetic analysis showed this virus to have close identity with avian influenza viruses. Avian influenza viruses of H4N5 subtype in 1983, H4N6 in 1991 and H3N2 in 1992 were later isolated from dead seals found on the NE coast of the USA. Avian influenza viruses of H13N2 and H13N9 subtype combinations were isolated from a stranded whale. Similarly disease on a coastal mink farm in Sweden was associated with an H10N4 influenza virus showing close identity with viruses of similar subtype isolated contemporaneously from waterfowl. In each of these instances genetic analyses led to the conclusion that the infections of these mammals was a result of direct spread of the virus from avian species.

Early volunteer experiments suggested that avian influenza viruses were unlikely to cause more than transitory infections in humans. In two reports of infections of laboratory workers in Australia in 1977 (with a virulent chicken isolate) and USA in 1981 (with the seal virus) where virus was introduced directly in to the eye, clinical signs were restricted to conjunctivitis. An avian influenza virus was also isolated in England in 1996 from the eye of a woman with conjunctivitis who kept ducks and this was thought to be the first substantiated natural infection of humans with an influenza virus in which all the genes were most closely related to avian influenza viruses. In Hong Kong in 1997 virus of H5N1 subtype showing close identity in all genes to virus known to be present in local chickens was isolated from 18 people (6 of whom died) mainly showing severe respiratory illness. Unlike the disease in chickens there was little evidence of secondary spread in humans.

Conclusion

There are huge pools of influenza A viruses showing marked antigenic diversity in many avian species, especially waterfowl in which influenza viruses appear to be perpetuated. Recent evidence suggests that occasionally avian influenza viruses may be able to infect other species without undergoing dramatic genetic modification.

Further reading

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Webster, R.G. (1998) Influenza: An emerging microbial pathogen. In: *Emerging Infections* (ed R.M. Krause). Academic Press, London pp275–300

Dennis Alexander graduated from Brunel University in 1968 and then worked as a postgraduate student at the Royal Postgraduate Medical School, London University obtaining a PhD in 1971. Apart for a very brief interlude as an Associate Professor at the University of Wisconsin, USA from 1986–1987, he has worked, primarily on viruses of birds, at the Central Veterinary Laboratory (CVL), Weybridge since 1972. He obtained a DSc from London University in 1986, was elected Fellow of the Institute of Biology in 1988 and elected Fellow of the Royal College of Pathologists in 1997. He is currently Head of the Avian Virology and Mammalian Influenza Section in the Virology Department at CVL and Director of the European Union International Reference Laboratories for Newcastle Disease and Avian Influenza. He is also a member of the EU Scientific Committee on Animal Health and Animal Welfare.

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Fish Veterinary Journal (1999) (3) 79–81

Bronopol: an alternative fungicide to malachite green?

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Malachite green has been used for many years as an antifungal treatment on fish farms. However, it is now considered to be a carcinogen and teratogen. There are additional concerns about the suitability of malachite green on residue and environmental grounds. Also under new regulations (Product type 3 in Annexe 5 to EC98/8) it will be illegal to use unregistered biocides after 31 December 1999 for veterinary health purposes.

Grampian Pharmaceuticals, with the co-operation of various research institutes has developed bronopol (Pyceze®) as a potential replacement for malachite green. Following the necessary series of trials a product licence application has been submitted to the Veterinary Medicines Division (VMD), initially for use in the treatment of fungal infections on salmonid eggs. Further experiments have demonstrated that it may also be suitable as a fish treatment, so an application for an animal test certificate (ATC) has been submitted for treatment trials on commercial fish farms.

Materials

The formulation contains bronopol, a biocide which has a broad spectrum of efficacy, including antibacterial and antifungal activity. Bronopol has been used for at least 25 years in many human products (*eg* toothpaste, babycare products, wet wipes, cosmetics, ophthalmic preparations) at much higher concentrat-ions than it will be used in aquaculture. During this use it has had a very good human safety profile. The environmental safety package has been accepted by various groups, including the Environment Protection Agency (EPA), the Food and Drug Administration (FDA) and the Canadian authorities.

Trials and results

As a first step in the development, efficacy against *Saprolegnia parasitica* was confirmed by *in vitro* trials using experimental models. Two protocols were used. One involved the culture of Saprolegnia parasitica on agar gels and looked at both sporulation and vegetative growth. The other used a hemp seed in water culture technique. The results from both showed that bronopol was worth further investigation as a potential topical treatment for fungal infections on fish eggs; so *in vivo* trials were undertaken. The safety for, and treatment of eggs was investigated initially as this is a less difficult experimental model than *Saprolegnia parasitica* infection on fish.

The safety for salmonid eggs was shown to be satisfactory even when the formulation was used at higher final concentrations (2–4 times) and for longer periods (3 times) than the anticipated therapeutic regime. The efficacy for control of fungal growth on salmonid eggs was then investigated. Initially this involved trials in a research unit and showed efficacy at an appropriate concentration which was at least as good as that achieved with malachite green. These results were then confirmed by 5 trials at commercial farms after an ATC had been obtained. In these trials large numbers of eggs were treated in various types of incubators and at various temperatures. This series of trials showed that using the anticipated treatment regime, bronopol had the same efficacy as a typical malachite green treatment.

Following those trials, the next development was trials to investigate and assess the use on salmonid fish. So far, these trials have all been in research facilities, to allow us to generate sufficient data for an application for an ATC. If this is granted it will permit treatment trials on commercial farms. The laboratory trials have shown that this formulation of bronopol is well tolerated by both Atlantic salmon, *Salmo salar*, and rainbow trout, *Oncorhynchus mykiss*, at final concentrations greater than the anticipated use rate.

To reliably assess efficacy, reproducible disease models had to be developed for rainbow trout and salmon. When the models, which involved applying a controlled stress to the fish, and a known challenge with *Saprolegnia parasitica*, had been developed, dose-response trials in both species were started. These showed that bronopol treatment is at least as effective as treatment with malachite green. An application for an ATC has been submitted for treatment trials involving fish on commercial farms.

Conclusion

The information which is already available about the biocide, and results of the trials mentioned here together with other experimental results suggest that Pyceze® will be an environmentally sound, therapeutically suitable formulation. It has several potential uses on fish farms, without the hazards of malachite green.

Acknowledgements: I would like to thank my colleagues, and scientists at CEFAS, IFE and at Stirling University who have been involved with the development studies for this project.

Graham Cawley graduated from Glasgow University Veterinary School and gained an MSc in Aquatic Pathobiology at the Institute of Aquaculture, Stirling. His career has included experience in general practice, the Veterinary Investigation Service and the pharmaceutical industry. He is currently the manager of veterinary services at Grampian Pharmaceuticals Limited.

This article is based on a presentation given at the autumn meeting of the Fish Veterinary Society in Penrith on 12 November 1998 and submitted for publication on 30 November 1998.

Fish Veterinary Journal (1999) (3) 83–86

RCVS Diploma in fish health and production

There are currently 23 speciality subjects in which graduates may obtain further veterinary qualifications and fish health and production is one of the latest. The purpose of these qualifications is to develop a process of self-advancement in the profession and in a formal manner so that achievement can be properly acknowledged. A standing committee of the Royal College of Veterinary Surgeons, the Speciality and Further Education Committee (SFEC), has developed a number of speciality Subject Boards which are responsible for the development and administration of their subject.

Two levels of qualification are recognised, the Certificate (which was described in the last issue of the *Fish Veterinary Journal*) and the Diploma.

- A Certificate holder is a competent clinician who has proved their experience and expertise in the subject.
- A Diploma indicates that the holder is someone who has achieved a high standard of academic and professional expertise, and who would be eligible, with the necessary experience and contributions to the subject as defined in the specialist criteria, for inclusion on the list of specialists recognised by the RCVS.

It is believed that these further qualifications will result in the improvement of clinical service to the public and to the profession.

There are specific requirements for veterinary surgeons who wish to apply for enrolment for the Diploma and initial approval of experience. These are as follows:

① Candidates must be members of the Royal College of Veterinary Surgeons or hold an approved veterinary qualification for a minimum of five years.

② In most cases, applicants should have obtained the Certificate in fish health and production before being eligible to enrol for the Diploma. Exemption from the Certificate examination, with a view to proceeding directly to the Diploma examination in the same subject is possible but rarely granted. This option is only available in the early stages while subject examinations are being established and is offered to Candidates who have obtained qualifications and experience above the standard required for passing the Certificate examinations and wish to proceed directly to the Diploma exams. Now that fish health and production examinations have been available for several years, exemption will only be granted in exceptional cases.

③ Practical experience in the subject can be obtained through work at an ‘approved practice’ and an ‘approved centre’. These are defined by the following:

- An approved practice usually means a general veterinary practice or similar facility approved by the Board.
- An approved centre is a centre where the candidate works and is primarily concerned with fish health. At these centres there will be one or more appropriately qualified colleagues (such as RCVS Specialists, Diplomates or others considered by the Board to have equivalent experience or qualifications) with a substantial workload and variety of relevant cases where advanced veterinary work in fish health is practised. Appropriate support facilities and access to adequate library material will also be available.

Prior to entry to the examination, candidates must provide evidence that they have spent the appropriate minimum period of time gaining experience with a substantial involvement in fish health for:

- at least four years (or modules equivalent to four years taken over a maximum period of eight years) at an approved centre; OR
- at least five years at an approved practice, which includes 200 days at an approved centre; OR
- at least six years if gaining experience solely at an approved practice.

‘Substantial involvement’ is defined as a minimum of 30 hours per week dealing with and advising on health, production, diagnostic work and epidemiological investigations. Some of this time should also involve research and writing reports, literature reviews, scientific papers, and other articles in the subject.

④ Attendance at suitable short courses and conferences will be expected. Candidates are required to submit a copy of their CPD record card with their application for final approval of experience. In addition, it is recommended that candidates should become members of relevant societies and specialist associations.

No specific training is organised by the College but the detailed syllabus and reading list which are provided will serve as a guide for self-study towards the examinations. The standard of the Diploma examination will be high and consequently candidates will need to engage in extensive private studies. An adviser or supervisor, usually a senior colleague, should be approached so that they are able to advise on the candidate's studies and preparation for the examination.

The examination for the Diploma includes:

- a dissertation of 5,000 to 10,000 words based on original material and data, and including personal observations on a subject approved by the Board. This may be a research project in any aspect of fish disease and management, or the evaluation of preventative medicine or disease control schemes in which the candidate is personally involved.
- two 3-hour written papers. One of these papers will give candidates the choice whether to be examined in ornamental or farmed fish.
- an oral examination which may also include practical or clinical tests and questions asked in relation to the dissertation, and on any part of the syllabus. This exam may last up to three hours for each candidate.

Successful candidates will be invited to receive their Diploma parchment at a presentation during the Annual General Meeting. They are then permitted to use the abbreviation DFHP as appropriate, after their names in the RCVS Register and on practice stationary.

At present there no applicants for the Diploma in fish health and production. A significant amount of work and effort has gone in to establishing both the Certificate and Diploma by the four veterinarians on the Board and they are now keen to encourage colleagues to enrol for these qualifications. For application forms and further information, candidates should write to:

The Royal College of Veterinary Surgeons
62–64 Horseferry Road,
London SW1P 2AF

Please note that from time to time, and following Board meetings, these requirements and application dates will be revised, therefore readers should refer to the latest information pack from the RCVS.

Diploma in Fish Health & Production

REQUIREMENTS

be member of RCVS for 5 years
4–6 years experience at an approved centre or practice
minimum of 30 hours per week involvement in fish work
attend regular meetings & short courses, etc.

APPLY for enrolment and initial approval of experience before 1st. May

↓

APPLY for final approval of experience with final choice of dissertation subject before 1st. May in year prior to examination

↓

both above stages may be combined

APPLY for entry to examination before 1st. October

Dissertation to be submitted before 1st. November

↓

EXAMINATIONS held annually in April

two written papers
clinical, oral and practical exam

Fish Veterinary Journal (1999) (3) 88–90

Fish Stress and Health in Aquaculture
eds G K Iwama, A D Pickering, J P Sumpter and C B Schreck (1997)
Society for Experimental Biology Seminar Series 62.
280 pages, hardback, £60.
Cambridge University Press, Cambridge. ISBN 0521555183

This is a welcome addition to the literature on stress in fish, particularly since it focuses on fish as food animals. In the developed world, the consumer is becoming ever more aware and concerned about the conditions in which food animals are reared, and the Farm Animal Welfare Council (FAWC) has recently (September 1996) submitted its report on the welfare of farmed fish to the Government. The report contained 131 recommendations, some of which will lead to legislation. What will be crucial to the debate is the availability of objective methodology for assessing the impact of stressors in the farm environment and their significance for the animals themselves. This will help dispel some of the more extreme and uninformed comment on the subject which finds its way into the public domain.

Fish Stress and Health in Aquaculture opens with an historical perspective on stress in fin-fish, identifying at the outset the difficulties inherent in defining what stress is. Research activity up to the present day is reviewed, describing how ideas about stress have evolved and shaped approaches to the subject. The major focus has been on biochemical and physiological responses to stressors. However, there is a move to find indices which are more relevant to aquaculture *ie* bioenergetics, immunity and reproduction, all three of which are of great importance to the farmer.

This theme is taken up in the succeeding chapters which provide, in every case, an exhaustive and authoritative review of their subject matter. As well as being welfare issues, disease and mortality are of great economic importance to the fish farmer. The significance of stress, whether environmental or man-made, to the pathological process is now better understood and most progressive farmers account for this fact in production processes. Chapter 2 contains a wealth of data, but also provides much practical advice on alleviating stress in the real world of aquaculture. Particularly useful, are the recommended limits for water chemistry parameters, summarized in one table for easy reference. These are of great importance in modern recirculation production units which depend for their success on accurate control of water quality. Behavioural interactions between fish are also covered, as well as the role of stress in infectious disease which is a key performance indicator in production.

Chapter 3 addresses the role of stress in the reproduction and growth of fish. These topics have, perhaps, been somewhat neglected in the field of stress research but are now receiving more attention. They are obviously of central importance to the aquaculturist.

The following two chapters give a detailed and comprehensive description of the biochemistry and physiology of the stress response, again with reference to real situations in aquaculture. The data presented point to the complexity of the stress response in a number of species, as mediated through the hypothalamo-pituitary-interrenal axis. The details of this cascade are clearly presented in a logical fashion which guides the reader through what can be unfamiliar territory to the non-specialist.

Chapter 6 deals with behavioural responses to stress which may be highly significant to the farmer and to the fish. Dominance, aggression and feeding behaviour deserve careful scrutiny as feed prices rise and profit margins fall, demanding close attention to efficient feeding. Such social interactions also have implications for fish welfare in that stocking density is known to have effects on fish behaviour.

The possibility of selection for stress-tolerance in fin-fish is considered in Chapter 7. Aquaculturists with experience in breeding for performance have long recognized that some fish strains adapt better than others to domestication (with its unavoidable stress). In addition, the impact of stress on flesh quality is beginning to receive more attention. The authors draw interesting parallels between attempts to manipulate the stress-adaptive response in poultry and the limited work which has been undertaken in fish. The importance of stress in relation to disease is also touched on here and enlarged upon in the Chapter 8 which examines, in detail, immune-endocrine interactions. Its author provides a comprehensive summary of the current state of knowledge in this emerging field. This work is likely to assume greater importance in the future, since the range of therapeutics available for fish is small and unlikely ever to be significantly expanded for what is a relatively small market in animal health. There will, therefore, be pressure to develop novel ways of managing health in fish populations which do not rely on medicines.

There is a growing interest in enhancing the ability of farmed fish to withstand disease challenge indirectly, with feed additives and oral delivery of immunostimulants and vaccines. Chapter 9 explores this theme along with discussing ‘production’ diseases (*ie* those which may have a basis in dietary imbalance). These are very significant in other livestock and likely to become increasingly important in aquaculture as fish are pushed to perform. The author takes us through the macro- and micro-nutrients, the minerals and vitamins before concluding that much remains to be done to elucidate whether dietary manipulation can indeed afford significant protection against disease.

The final chapter is a very useful and practically directed résumé of current experimental techniques which are readily applicable in the field. The authors offer sound advice on technique and interpretation of results, often difficult in ectotherms in which normal ranges are not easy to determine.

In summary, this is a most useful text for anyone interested in the health and welfare of fin-fish. The chapters flow in a logical fashion and integrate the themes of the book well. Illustrations are relevant to the text and generally easily understood. The index and reference list are comprehensive, though I looked, but was unable to find any sections on stress at slaughter. I would recommend the book as a source text for readers seeking a thorough overview of the subject in a very readable format.

Andrew N. Grant

This review was originally published in *Animal Welfare* (1998) volume 7, number 3, pages 327–328. The *Fish Veterinary Journal* is grateful to the Universities Federation for Animal Welfare (UFAW) for permission to reproduce this article.

Fish Veterinary Journal (1999) (3) 91–92

Handbook of Trout and Salmon Diseases, 3rd edition

R.J. Roberts & C.J. Shepherd (1997)
256 pages, hardback, £39.50
Fishing News Books, Oxford. ISBN 0 85238 244 8

The publication of the new edition of the *Handbook of Trout and Salmon Diseases* is a welcome event. Earlier editions of this book have been an important aid to all people wishing to learn about the basics of fish disease, and can be found on many bookshelves world-wide. Nevertheless there were shortcomings.

The third edition follows in the footsteps of its predecessors in giving a good solid grounding in the essentials of what normal fish are and the basics of disease diagnosis, and has addressed many of the shortcomings of the previous editions.

A comprehensive rundown of salmonid genera gives an interesting insight into the diversity of this group of fish. The review of the normal anatomy of the salmonid is, of necessity, superficial, but the information included is sufficient to explain the impact caused by the diseases dealt with later.

Basic husbandry and an outline of the main types of fresh water and marine farming systems are discussed. The essentials of water quality and elementary nutrition are addressed. It is good to see that the welfare of the farmed fish is also given due regard. Because of the nature of the book, these areas are dealt with very superficially, but they are extremely important as they put into context a lot of the information on diseases which comes later.

The main body of the book is devoted to disease, infectious and otherwise. The main infectious diseases encountered in salmonids are reviewed well in a systematic way, although the organisation of the protozoan diseases was rather puzzling, being arranged neither phylogenetically nor in order of importance. Many new diseases which have appeared since the previous editions are included, but the failure to deal more fully with rainbow trout fry syndrome (RTFS), an extremely important disease in the European trout industry, is unfortunate.

Separate sections on nutritional diseases, fish kills and diseases of wild fish and broodstock complete the diseases section.

A particular strength of this book is the way in which diseases are not just listed as is the way with many standard texts, but are also grouped according to where they might be found within the farming environment. This makes life much easier for the seeker with a particular area of interest, and enables production-related diseases to be dealt with in context.

The book concludes by addressing the basics of disease prevention and treatment. Examples of calculations of treatments are particularly helpful, and an appendix containing conversion factors is always a godsend.

This book is well laid out, and is a valuable and accessible source of information about normal salmonids, methods of husbandry in the different farming systems and common diseases which afflict fish kept in these systems. Use of excellent colour photographs greatly enhances its value for people seeking help with identification of diseases, and corrects one of the main weaknesses of its predecessors, the reliance on black and white photographs. This is an excellent book for the veterinary surgeon with little knowledge of the farming of salmonids, but who would like to know more.

Edward Branson

This review is based on one originally published in *Journal of Fish Biology* (1998) volume 53, number 6, pages 1373–1374. Further information about this journal can be found on their website at http://www.academicpress.com

Fish Veterinary Journal (1999) (3) 93–96

Koi Health and Disease
Erik L. Johnson D.V.M. (1998)
Video cassette, $38US plus post & packing.
Johnson Veterinary Services, 3125 Rosewell Road, Suite H, Marietta, Georgia 30062, USA (tel: 010 770 977 5377 fax: 010 770 973 0301 website: www.koivet.com)

'This video may contain autopsy material which is unsuitable for children or for folks who may have just had lunch'. This warning introduces the 90-minute video aimed at the hobbyist and is designed to augment the information in his book of the same name. However, the content is also a good basic introduction for veterinary surgeons embarking on the treatment of fish since a certain amount of basic medical knowledge is required to fully appreciate the video.

The first 15 minutes gives a brief overview of fish health, using some tables from the author's book and the familiar Venn diagram to illustrate the relationship between host, pathogen and environment in relation to disease. He maintains that the average lifespan of koi in the US is about two weeks, taking into account the many small fish that die within days of ownership and the few that live to a ripe old age. This is followed by demonstrations of skin and gill scrapes (or biopsies). There are useful tips on sampling, but I'm not sure how many hobbyists would recognise iris tenotomy scissors for taking gill snips. To obtain a meaningful interpretation of parasite levels, it is recommended that at least three samples should be taken from three fish and that samples should always be taken from the gills. A few minutes are spent describing the features of a microscope and how it should be set up correctly, and it is emphasised that regular practice is the key to success.

There is 30 minutes discussion of the various groups of pathogens in koi, starting with parasites. Examples of most common ectoparasites are shown with comments on various treatments used in each case. However, there is no indication of the magnification used and the field of view is limited by the condenser. Nevertheless, showing images of motile parasites to hobbyists who may be unfamiliar with microscopy is infinitely more useful than still photographs. The use of potassium permanganate to treat parasitic, fungal and bacterial disease is favoured by the author and a clear, simple protocol for using this potentially hazardous chemical is described.

Pertinent comments are made about checking parasite levels after treatment to assess the efficacy of the medication.

Bacterial diseases and various antibiotics are discussed at length. It is the author's opinion that Gram-negative bacteria are relatively resistant to penicillin, tetracycline, amoxycillin and first generation cephalosporins. More success has been obtained from using Amiglyde-V® (amikacin), Azactam® (aztreonam), Baytril® (enrofloxacin), Naxcel® (ceftiofur) and Nuflor® (florfenicol). Various injection techniques are demonstrated although I am apprehensive about owners injecting their own fish, particularly after experiencing a few mortalities following intramuscular injection by owners. The author favours the intraperitoneal route and gives instructions on how this should be done.

Various anaesthetics used in koi are discussed including isoflurane, eugenol, 'oil of cloves', and the American brand of MS-222, Finquel®. Anaesthesia using isoflurane is demonstrated and it would appear to be a useful drug to use by veterinary practices in an emergency and do not stock MS-222. Although isoflurane is a prescription only medicine in the UK, I would be rather apprehensive about dispensing this to any client because of the potential for human abuse.

There is a short section on the topical treatment of wounds on koi with the use of tincture of iodine, mercurichrome, Panolog® and hydrogen peroxide being mentioned. The author prefers using a paste made from potassium permanganate crystals but warns that eye protection should be worn.

The final 15 minutes is spent on practical demonstrations of an autopsy. Removal of the operculum and opening the body cavity is shown, and the various organs are pointed out. Some experience and familiarity of normal tissue appearance is required by the viewer to appreciate this section: a 'running commentary' of the actual procedure would have been useful. I found surgical removal of the posterior kidney in cases of hydronephrosis an interesting suggestion and wondered how I would ever make the diagnosis in a live fish.

The video concludes with an acknowledgement to the author's clients and staff. There are a few additional minutes of photomicrography intended as a parasite identification challenge for viewers although no answers are given.

Dr Johnson is to be congratulated for putting together an informative video that should appeal to a wide audience including koi-keepers, dealers, fish biologists and veterinarians. Hobbyists in particular may benefit from viewing the video a few times, and even (as suggested by the author) listen to the sound-track on its own without the distraction of the picture.

Understandably, this video is made for the American market and viewers should be aware of the difference between British and US fluid measures (1 US gallon = 0·833 UK gallon) since several chemical dose rates for immersion are quoted.

Several proprietary medications manufactured in the US are mentioned and discussed at various points in the presentation. Some of these include interesting chemical combinations including Fluke Tabs® (carbamate and organophosphate), Furazone Green® (three different nitrofurans and methylene blue) and Heath Guard® (di-glutaric aldehyde). It is even possible for hobbyists to obtain antibiotics from various suppliers and have these compounded into a medicated feed. Although it is interesting to see relaxed American controls on drug availability it inevitably suggests to hobbyists that the same products are also on sale in the UK and that antibiotics are easily obtained on this side of the Atlantic.

Video presentation of technical information is very different to written text in more than just its medium. There are many benefits, particularly in demonstrating the movement of live parasites and manual techniques, but it is too easy to compare Dr Johnson's first video endeavour to the polished commercial productions that we see on television. Colour is important to convey the subtle changes that take place on skin lesions and internal organs at autopsy. Unfortunately my video picture was rather dull and this limited the detail that could be seen. Despite this, the sound is clear and the script is well presented, with Erik's American accent adding a touch of Hollywood. Inevitably there is some unfamiliar American phraseology which challenges the European listener such as 'power outage' and 'junker fish'. There are a few moments of humour with, among others, the over-dubbed sound of sawing during the autopsy which have been criticised for compromising the professional image but are injected to maintain the viewers' interest. The electronic background music is too *allegro* at times and rather distracting but is preferable to total silence.

It is easy to criticise minor issues such as spelling mistakes in the tabulated text and some of the personal mannerisms. However, current competition in this field of video presentation pales in comparison. Some of these have made me cringe in embarrassment at the misunderstanding of basic medical information, particularly since they are intended to educate viewers. It is a relief to see a video that has few inaccuracies, emphasises basic principles of correct husbandry and demonstrates a professional approach to koi care.

William H. Wildgoose

Although both the video and book complement one another, the content of the video is complete enough to stand alone. It can be ordered direct from the secure web server but make sure that you request the correct video format for the UK, namely PAL. The 166-page book, *Koi Health and Disease* (1997), is available separately for $37US plus post & packing.

The William Hunting award is presented annually in recognition of the best paper by a practitioner in *The Veterinary Record*. The winner for 1998 was **Tony Wall** from the Fish Vet Group in Inverness, for his paper 'Cataracts in farmed Atlantic salmon (*Salmo salar*) in Ireland, Norway and Scotland from 1995 to 1997' (*Veterinary Record* **142**, 626–631).

Abstract:

> From 1995 to 1997 cataracts were observed in Atlantic salmon (*Salmo salar*) in Ireland, Norway and Scotland at around the time of smoltification (when freshwater fish become adapted to the hypertonic seawater) in both fresh- and seawater fish. Over 38,000 fish were screened for the presence of cataracts. Posterior cortical cataracts were the earliest and most consistent change, followed by perinuclear, equatorial and anterior cortical cataracts. On histological examination vacuolation of the lens fibres was consistently present in the posterior cortex. The pattern of the outbreak suggested that a nutritional factor was involved although the variable incidence and severity of the condition indicated that a number of modifying factors may have been involved in the expression of the condition.

The prize of an engraved medal and a substantial cheque was awarded at the British Veterinary Association Congress in Nottingham. Tony was president of the Fish Veterinary Society from 1991 to 1993 and has held an interest in ophthalmology for many years and was one of the first vets to study for the RCVS Certificate in Veterinary Ophthalmology in 1983. Later, he wrote his thesis, 'An investigation into some aspects of cataracts in fish' and obtained an MSc by research at the Institute of Aquaculture at Stirling in 1996.

An article entitled 'Skin diseases in ornamental fish: identifying common problems' by **William Wildgoose** was published in May 1998 in the journal of veterinary postgraduate clinical study, *In Practice* **20**, (5), 226–243. This was written at the suggestion of the Fish Veterinary Society and was partly funded by the Society following a presentation given at the autumn meeting in Cambridge, November 1994.

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