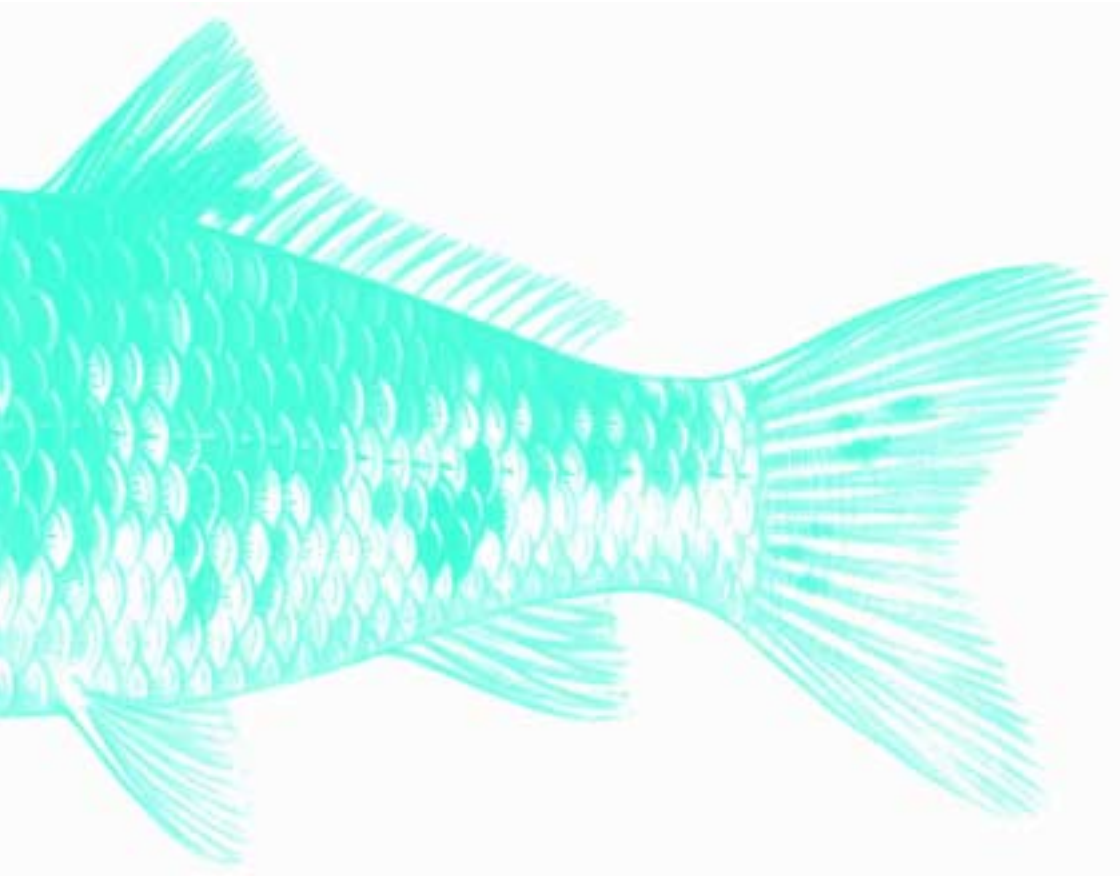
FISH VETERINARY JOURNAL

The Journal of the Fish Veterinary Society

Issue Number 4 • September 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.

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

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).

References:

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

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These are my final reflections before standing down as FVS President. Firstly, I would like to record my gratitude to my fellow committee members for their hard work on behalf of the Society. A great deal of effort goes into organisation of the scientific meetings, financial management and especially into production of the *Fish Veterinary Journal*, which is gaining recognition as a useful and worthwhile publication.

My thoughts in 1999, as in 1998, have been dominated by the continuing havoc being wrought on Scottish salmon farming by the government's pursuit of the policy of attempting to eradicate infectious salmon anaemia (ISA). I remarked in issue 3 of the Journal, that the objective is unlikely to be achieved, and certainly not without significantly damaging the industry. The policy must be reviewed.

The government has stated that a number of criteria will have to be met to trigger any change in policy; those criteria have surely been met several times over. It is quite possible that there will be an apparently 'successful' outcome of the present policy, namely that farms presently under suspicion are emptied and no others become suspicious; the government would thereby feel justified in maintaining the status quo. However, history suggests that recurrence of ISA is likely and without a policy change the same scenario would be repeated. The sword of Damocles would remain in place. We await with interest the results of the use of an experimental ISA vaccine in Canada which may offer an additional means of exercising control. It is clear that the management of ISA is far too complex to be addressed by the present draconian measures while maintaining a viable industry.

Since the issue of ISA is so pressing, I have formed a group within the Fish Veterinary Society (FVS) to meet on an *ad hoc* basis to discuss ISA and any other issues of particular relevance to salmon farming. The Salmon Health Group (SHG) membership is small and composed of FVS members with

significant interest in the Scottish industry but is open to any FVS member (*see page 68*). So far, we have directed our efforts towards those with the power to influence policy, and will continue to do so. It is my hope that FVS will make a constructive contribution to present and future fish diseases policy.

On a brighter note, cypermethrin (Excis®, Vericore Ltd) finally gained a marketing authorisation and, along with azamethiphos (Salmosan®, Novartis), will greatly improve sea lice management. It is to be hoped that medicines delivered in feed will soon join the armoury, finally opening the door to management of lice in a strategic manner. However, the difficulties of obtaining discharge consent for any medicine remain a formidable obstacle to effective control.

The trout industry's fortunes continue to track those of salmon, namely depressed! Rainbow trout fry syndrome (RTFS) is a major problem in small fish and resistance to the antibiotic of choice (amoxycillin) is present, while enteric redmouth (ERM) is a significant cause of loss in larger fish. The age-old problem of parasitic infestation remains and control will not be helped by the MRL deadline at the end of 1999.

Further afield, in Chile, the impact of disease is being felt by many producers as they experience problems similar to those of the Scottish industry in the late 1980s — hard lessons are being learned, as they were in Scotland.

A significant number of FVS members, some 60% of those who responded to a questionnaire, have an interest in ornamental fish and this is reflected in the content of this edition of the Journal. The ornamental fish industry is estimated to turn over about £300m annually and there is now some serious scientific research into health problems in pet fish. There are significant problems associated with the importation of fish into the UK with the accompanying potential for the introduction of novel pathogens.

Finally, I wish every success to the new committee which will be installed at the Annual General Meeting in December; I am confident that the Society will go from strength to strength in the years to come.

Editor's Comments

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

The last editorial was obviously written before the Journal was published. For someone who had produced nothing more elaborate than a practice newsletter, I have had to learn from experience, and by trial and error. The idea of introducing colour and photomicrographs, and using high quality materials (at three times the cost of issue 2) was a leap of faith for me. As I have discovered in the past, what looks fine on the computer screen at home often bears no resemblance to what comes off a commercial printing press. Therefore, I am greatly indebted to Mike Williams of Akalat Publishing for his assistance and help in producing a high quality, professional journal. As always, I am immensely grateful for the financial support of our advertisers and sponsors, without whom the Journal quite simply would not exist.

Despite the small size of the Fish Veterinary Society and its rather exclusive field of interest, many members are passionate about their work and its diverse nature. This issue contains some simple analyses of our members' interests and their distribution across the UK. Although the Society was established initially to bring together veterinarians working in the food-fish industry, it has also welcomed the growing interest in ornamental fish. As evidence of this, there are now regular presentations at the Society's biannual meetings, and it is reassuring to see that more scientific research is being carried out in this much-neglected field. Equally, it is good to see authors submitting articles describing their personal experiences in detail and it is hoped that this will continue to advance our knowledge of ornamental fish health.

The end of this year will see the election of a new committee and this will be the last issue under the guidance of our current president, Andrew Grant. Although he has given me a free rein with this publication, I wish to express my thanks to him for his encouragement and reassurance that my work is appreciated. His support and belief in the Journal has hopefully allowed me to develop new areas for the benefit of the Society's members and all those interested in veterinary aspects of fish health.

SCIENTIFIC PAPER

Fish Veterinary Journal (1999) (4) 1–16

Aerobic microflora of imported tropical ornamental fish from Singapore and South America. Part 1: Characterization

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Abstract

The primary aim of this study was to investigate the distribution patterns and the role of the aerobic bacterial flora associated with the importation of tropical ornamental fish. The samples examined included shipping water, fish tissue and bacteraemic fish from the two main supply regions of these fish on arrival into the UK and during the period of commercialization. Following determination of the bacterial patterns, the research focussed on the role of bacteria in fish mortality and the antibiogram of the most persistent microflora. The results enabled an assessment of the efficacy of the most effective antimicrobials during bath treatment trials, as well as an evaluation of the performance of antibiotics currently used by the industry. This is the first of three papers, and discusses the results of sampling the aerobic bacterial flora from imported tropical ornamental fish and their transportation water over a six-month period.

Introduction

The UK is the second largest importer of ornamental fish in the European Community. Despite this, little attention has been paid to the potential spread of harmful bacteria through the importation and sale of tropical ornamental fish. Approximately 35 to 40 million ornamental fish enter the UK every year: these arrive in 5,000 consignments consisting of over one thousand different species of tropical marine and freshwater fish. Tropical freshwater species constitute the bulk of this trade (Davenport 1996, Cheong 1996) with Singapore and South America being the two main exporting regions of these fish. It is known that ornamental fish are

produced massively in tank or pond systems in Singapore, but supplies from South America come mainly from the trapping of wild fish in the Amazon basin (Cheong 1996, Chao 1995).

In the early 70s, of the estimated 2 million cases of human salmonellosis in the USA, 300,000 were related to infection from pet turtles (*Chrysemys scripta*) (Wells and others 1973). This situation readily prompted the study of potentially harmful bacteria in other pet aquatic animals. Shotts and others (1975, 1976) initially reported one of the largest studies on bacteria associated with imported ornamental fish in USA. Gratzek and others (1978) concluded part of this study, but a more comprehensive paper including the antibiotic sensitivity patterns of the bacterial species was published six years later (Shotts and Gratzek 1984). Other large investigations on the microflora of ornamental fish have been undertaken in recent years. Dixon and Contreras (1992) found antibiotic multi-resistant motile aeromonads highly prevalent in tropical ornamental fish and other workers reported the prevalence of several bacterial species associated with ornamental diseased fish imported by wholesalers and retailers in Taiwan (Kuo and Chung 1994*a,b*).

Ornamental fish have also played a minor role in bacterial zoonoses. Isolated cases of septicaemias associated with strains of *Edwardsiella tarda*, cutaneous mycobacteriosis, liver abscesses and diarrhoea in infants have all been related to ornamental fish (Humphrey and others 1986, Vandepitte and others 1983, Vaishampayan and others 1996).

Government agencies and the ornamental fish industry are concerned about the possible introduction of pathogens through the importation of ornamental fish. The ornamental fish industry is also particularly concerned about the mortality rates of imported fish. Ferraz de Oliveira (1995) carried out a parasitology study of South American importations into the UK using a wide range of techniques. One of the results from the study suggested that mortalities are not significantly associated with parasites and may be due to bacterial disease. Recently, the investigation of bacterial diseases affecting coldwater ornamental fish has been undertaken by dedicated researchers in association with government officials. Bacteria would appear to play a very important role in disease problems in koi carp (*Cyprinus carpio*) causing significant economic losses to British wholesalers (Robertson and Austin 1994, Wildgoose 1998).

The current legislation which relates to the importation of ornamental fish varies from country to country. In the UK, the *Fish Health Regulations* and other related legislation control the importation of all live fish. However, warm-water ornamental species which are intended to be kept permanently in aquaria are not considered to represent a threat to food-fish or native fauna since they are unable to survive in the natural aquatic environment of the UK (MAFF 1995).

This paper discusses the findings of a six-month study intended to examine the patterns of the bacterial flora associated with the importation of tropical freshwater ornamental fish on arrival in the UK. This work was carried out in collaboration with the largest importer of tropical ornamental fish in Scotland and with the assistance of the Ornamental Aquatic Trade Association (OATA) Ltd., formerly known as the Ornamental Fish Industry (UK) Ltd.

Materials and methods

The sampling procedures used in this study were based on those of earlier workers (Gratzek and others 1978, Shotts and Gratzek 1984). The fish species chosen for this study were those for which there is the greatest demand in the UK trade and thus ensured a regular supply during the sampling period. Table 1 lists the species by source and methodology used in the sampling. Ten fish from each of the twelve species were selected at random, on a monthly basis for six months. The sample size was selected according to standard procedures for fish examination and sampling for prevalence and pathogen detection (des Clers 1994).

Media and identification kits

For the bacterial isolations, tryptone soy agar (TSA) and cytophaga agar (CA) were used and prepared according to the manufacturers' specifications. No attempt was made to culture fastidious organisms such as *Mycoplasma*, or *Mycobacterium* species, microaerophilic or anaerobic micro-organisms. Basic tests, that is Gram's stain, motility, morphology, oxidase test, catalase, glucose oxidation-fermentation (O-F) and susceptibility to vibriostat 0/129 (10 µg and 150 µg) were performed on all the isolates.

Further identification of Gram-negative micro-organisms was performed using API 20E (BioMerieux France, Enterobacteriaceae system). Isolates

TABLE 1: Fish species examined at the Institute of Aquaculture

	Origin	
Source	Scientific name	Common name
South America	*Paracheirodon axelrodi* [1]	cardinal tetra
	Canegiella strigata strigata [1]	marble hatchetfish
	Petitella georgiae [1]	rummy nose tetra
	Corydoras melanistius [2]	corydora catfish
	Corydoras punctatus [2]	corydora catfish
	Arius jordani [2]	Colombian shark
Singapore	*Paracheirodon innesi* [1]	neon tetra
	Brachydanio rerio [1]	zebra danio
	Capoeta tetrazona [1]	tiger barb
	Xiphophorus helleri [2]	sword tail
	Xiphophorus maculatus [2]	platy
	Colisa lalia [2]	dwarf gourami

[1] Fish slurry (small specimens) methodology
[2] Bacteraemic fish methodology

not readily identified by the API system were analysed using the Biolog™ system (Biolog Inc., Hayward, CA).

Water samples

At the wholesaler's premises, 100 ml of water from the transport bags of each of the species selected was placed in a sterile bottle; a total of twelve samples on each occasion. Upon arrival at the laboratory, each water sample was centrifuged (MSE Mistral 2000R®) at 5000 rpm (750 G) for twenty minutes. The sedimented material was resuspended in sterile saline solution (sodium chloride 0·85%) and 10-fold dilutions were made. Using a spread plate technique, 100 µl of these dilutions was inoculated onto TSA and CA. The plates were incubated at 25°C and colonies counted at 24, 48 and 72 hours after inoculation. Colonies were differentiated according to their basic characteristics (colour, form, shape, size and speed of growth), each type counted individually and sub-cultured for subsequent identification.

Fish slurry samples

Small fish species, measuring up to 3 cm in length, were selected for this process. After killing and individually weighing the fish (Mettler PC4400®)

the surface of the fish was sterilised by dipping them in 70% alcohol and flaming, followed by homogenisation in a blender with 50 ml of sterile saline solution (sodium chloride 0·85%). TSA and CA plates were inoculated with 200 µl of this suspension. Plating and characterization of the colonies was conducted as for water analyses. This protocol was intended to recover bacteria from inside the fish including the flora of the intestine and any other internal tissue.

Samples from internal organs

Bacteriological samples from the internal organs of the larger species were obtained by sterilising the external surface and dissecting the fish using an aseptic technique. The kidney was the main organ of preference, but for brackish water species such as swordtail (*Xiphophorus helleri*) and platy (*X maculatus*) liver and/or spleen samples were used since the kidney of these species is difficult to sample due to its small size compared with that of freshwater fish. TSA and CA plates inoculated with samples from internal organs were incubated at 25°C and checked daily for three days. Bacteraemic fish were defined as those individuals from which a medium to significant growth on the bacteriological media was obtained.

Results

Bacteria in the shipping water

In the shipping water from Singapore, 15 genera of the bacteria were recovered. These consisted of 19 different isolates, of which the most frequently encountered were *Alcaligenes faecalis*, *Pseudomonas* group 1 and *Pseudomonas* group 2 (homology groups) (Table 2). Eighty per cent of the isolates described here as *Pseudomonas* group 1, were identified as *Pseudomonas putida* and the rest as *Pseudomonas fluorescens*. *Pseudomonas* group 2 was a weak fermenter of sugars, which produced alkali in the open O-F tubes. The highest number of CFUs and most diverse in terms of number of species occurred in sample 2 (Table 3). Sample 3 contained the fewest number of species, three (data not included), and sample 5 the lowest number of CFUs. *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Shewanella putrefaciens, Acinetobacter*, *Flavobacterium*, *Bacillus* and *Klebsiella* species were recovered from water from Singapore, but not from the South American water. *Alteromonas putrefaciens*, a marine bacterium was only found in water containing *X helleri* and *X maculatus*, both brackish water species.

TABLE 2: Proportion of bacterial species (%) isolated from shipping water

	Origin	
	Singapore	South America
Bacterial species		
Alcaligenes faecalis	30·192 (5)#	8·302 (3)
Pseudomonas group 2	19·609 (3)	22·508 (5)
Pseudomonas group 1	12·160 (4)	0·979 (2)
Bacillus species	7·186 (3)	—
Chromobacterium violaceum	11·424 (2)	1·986 (2)
Acinetobacter species	10·799 (2)	—
Enterobacteriaceae	0·276 (2)	—
Aeromonas species	0·040 (2)	0·212 (2)
Sphingomonas paucimobilis	—	6·431 (2)
Cytophaga/Flexibacter species 2	—	55·796 (1)
Diplococcus species	—	0·149 (1)
Xhantomonas maltophilia	—	0·203 (1)
Proteus mirabilis	3·644 (1)	—
Alteromonas putrefaciens	1·822 (1)	—
Cytophaga/Flexibacter	1·193 (1)	3·323 (1)
Flavobacterium species	1·160 (1)	—
Pseudomonas aeruginosa	0·125 (1)	—
Enterobacter agglomerans/Erwinia herbicola	0·121 (1)	—
Klebsiella species	0·121 (1)	—
Escherichia coli	0·076 (1)	—
Pasteurella species	0·039 (1)	0·111 (1)
Enterobacter cloacae	0·003 (1)	—
Shewanella putrefaciens	0·008 (1)	—

NB. The sign minus (—) denotes no isolation during sampling by the method used.
Figures in parenthesis represent the number of times the organisms were found in pooled water samples (out of a total of 6 monthly samples).

TABLE 3: Average total bacterial count in the shipping water (CFU x 10^3/ml)

	Samples					
	1	2	3	4	5	6
Singapore	16,560	302,640	1,141	993	170	7,780
South America	669	65,040	400	500	3,460	3,950

In the shipping water from South America, bacteria from only 8 genera were recovered (11 different isolates). In this case, yellow-pigmented bacteria accounted for more than 50% of the presence (Table 2). However, this proportion is a function of the high number of these bacteria obtained from only one sample (data not included). In terms of frequency of isolation, *Pseudomonas* group 2 and *Alcaligenes faecalis* dominated the samples. Sample 2 produced the highest number of CFUs (Table 3). Sample 4 was the most diverse in terms of species present (data not included) but had one of the lowest total number of CFUs. *Sphingomonas paucimobilis*, *Diplococcus* species and an unidentified species of *Cytophaga/Flexibacter* were found in the South American shipping water but not in water from Singapore.

Shipping waters from Singapore and South America appeared to have similar bacterial loads on arrival at the wholesaler's premises, although there was a tendency for the Singaporean water to have a higher CFU count.

Bacteria from fish slurry

Eighteen different isolates were obtained from Singapore, from 16 genera. Four genera, *Pseudomonas* group 1, *Aeromonas* (motile aeromonads), *Bacillus* and *Acinetobacter* species were the most frequently isolated (Table 4). Samples 4 and 6 had the highest diversity of species (data not included).

From South America, 14 isolates were recovered from 11 genera (Table 4). The most common isolates were *Pseudomonas* group 2, *Alcaligenes faecalis* *Aeromonas* species and *Pseudomonas* group 1. However, the numbers of species in the last two samples were lower than in the previous samples, namely three in the sample 5 and two in sample 6 (data not included). As in the results from Singapore, more species were isolated from inside the fish than in the shipping water. The greatest diversity was observed in samples 2 and 4 (data not included). In terms of bacterial load, fish from Singapore tended to have one to two orders of magnitude more bacteria per gram than their counterparts from South America (Table 5).

Bacteria from internal organs

From both areas, more than one species of bacteria were found in the internal organs of the population sampled on every occasion. In a few cases (14 cases from Singapore and 20 cases from South America) monomicrobic

TABLE 4: Proportion of bacterial species (%) isolated from fish slurry

	Origin	
	Singapore	South America
Bacterial species		
Aeromonas species	26·549 (5)#	19·673 (3)
Pseudomonas species 1	20·299 (6)	12·020 (3)
Bacillus species	3·704 (4)	7·939 (2)
Acinetobacter species	10·426 (4)	—
Alcaligenes faecalis	4·932 (3)	6·083 (4)
Pseudomonas group 2	2·588 (3)	6·203 (5)
Diplococcus species	—	14·778 (2)
Micrococcus luteus	2·848 (2)	—
Gemella species	1·389 (2)	—
Pasteurella species	1·407 (2)	4·337 (1)
Micrococcus species	2·369 (1)	—
Vibrio species	7·204 (2)	—
Cytophaga/Flexibacter	7·923 (1)	8·471 (1)
Citrobacter freundii	0·599 (1)	—
Vibrio cholerae (non 01)	—	10·649 (1)
Xanthomonas maltophilia	0·584 (1)	4·994 (2)
Flavobacterium species	0·109 (1)	0·115 (1)
Chromobacterium violaceum	0·400 (1)	4·399 (1)
Plesiomonas shigelloides	3·097 (1)	—
Enterobacter sakasakii	0·065 (1)	—
Flavobacterium meningosepticum	—	0·281 (1)
Staphylococcus species	—	0·057 (1)

*The sign minus (—) denotes no isolation during sampling by the method used.
Figures in parenthesis represent the number of times the organisms were found in pooled tissue samples (out of a total of 6 monthly samples).

TABLE 5: Average total bacterial count in fish slurry (CFU x 10^3/gram)

	Samples					
	1	2	3	4	5	6
Singapore	1,859	2,141	1,085	1,723	2,738	2,434
South America	107	868	221	451	232	39

TABLE 6: Total prevalence of bacteraemic fish (%)

	Samples					
	1	2	3	4	5	6
Singapore	97	87	87	67	53	37
South America	67	60	85	53	95	25

infections were detected in individual fish. The prevalence of bacteraemic fish appeared higher from Singapore than from South America (Table 6).

For Singaporean fish, *Aeromonas* species were found in all the samples with a prevalence ranging from 3·3% to 43·3% (Table 7). The second most prevalent bacterium was *Pseudomonas* group 1 with a prevalence of 3·3% to 30%. Other species, such as *Citrobacter freundii*, were isolated on up to three occasions and with prevalence ranging from 3% up to 90%.

Similarly, *Aeromonas* species was found in all of the samples from South American fish, and on one occasion was present in all the individuals in the sample (Table 7, sample 5). *Flavobacterium* species, *Pseudomonas* group 2 and *Plesiomonas shigelloides* were isolated at low prevalence but with higher occurrences than other species of bacteria.

Discussion

Bacteria in the shipping water and fish slurry

The range of bacterial species recovered from the shipping water did not differ significantly from those present in waters of natural reservoirs containing fish, and from the pond water and fish reared for food (Olayemi and others 1991, Ogbondeminu 1993). Furthermore, this range of bacteria seems to be common in most types of aquaculture system for fish production, including intensive controlled systems such as recirculating systems (Nedoluha and Westhoff 1997*a,b*). In terms of bacterial patterns found in the fish slurry, this study corroborates the findings of previous authors on tropical ornamental fish and their shipping waters (Shotts and Gratzek 1984, Kuo and Chung 1994*a,b*). Pseudomonads and *Alcaligenes faecalis* dominated the water and were frequently present in the small specimens, but aeromonads (motile) were more prevalent in fish tissues.

TABLE 7: Individual prevalence (%) of bacterial isolates found in the internal organs

	Singapore						South America					
Sample	1	2	3	4	5	6	1	2	3	4	5	6
Bacterial species												
Aeromonas species	3·3	43·3	23·3	40	43·3	30	23	10	80	3	100	5
Pseudomonas group 1	–	–	3·3	10	26·6	30	–	–	–	–	20	–
Pseudomonas group 2	60	83·3	–	–	–	30	47	33	30	–	–	–
Citrobacter freundii	3	–	90	–	3·3	–	–	–	45	–	–	–
Flavobacterium meningosepticum	–	16·6	3·3	–	–	16·6	–	–	–	–	–	–
Bacillus species	7	10	–	–	13·3	–	2	3	–	7	–	–
Plesiomonas shigelloides	–	–	–	–	–	–	7	27	–	13	–	–
Enterobacter sakasakii	–	–	–	43·3	3·3	–	–	–	–	–	–	–
Shewanella putrefaciens	–	–	–	30	26·6	–	3	–	–	–	–	–
Pasteurella species	–	–	–	–	–	–	10	–	–	–	–	15
Alcaligenes faecalis	3·3	–	–	–	–	50	–	–	–	–	–	–
Micrococcus luteus	–	–	–	46·6	–	–	–	–	–	–	–	–
Enterobacter species	3·3	–	–	23·3	–	–	–	–	–	–	–	–
Enterobacter agglormer/Erwinia species	–	–	–	–	–	–	–	10	–	–	–	–
Flavobacterium species	–	–	–	–	–	16·6	13	–	–	7	50	10
Diplococcus species	–	–	–	–	–	–	7	–	–	–	–	–
Vibrio cholerae (non-01)	–	–	–	3·3	–	–	7	–	–	–	–	–
Micrococcus species	–	–	–	–	–	–	3	–	–	–	–	–
Chromobacterium violaceum	–	–	–	–	–	3·3	–	–	20	–	–	–
Sphingomonas paucimobilis	–	–	–	–	–	–	3	–	–	–	–	–
Acinetobacter species	–	–	–	–	13·3	–	–	3	–	–	–	–
Enterobacteriaceae	–	–	–	–	–	–	3	–	–	–	3	–

Similar patterns of bacterial microflora have also been reported in a diverse variety of fish including those for human consumption and wild populations (Tanasomwang and Muroga 1988, Ogbondeminu 1993, Olayemi and others 1991).

Bacteria in internal organs

The results of this study strongly suggest that bacteraemic fish are common in shipments of ornamental fish and that a range of bacteria are found in internal organs, often as a concomitant infection. Despite the high prevalence of aeromonads, important or obligate fish pathogens such as *Yersinia ruckeri*, *Edwardsiella tarda* or *Aeromonas salmonicida* were not found. There was no evidence of fish infected with these bacteria in any of the groups sampled here on arrival into the UK. However, the range of bacteria isolated in this study contained opportunist secondary invaders that cause disease in fish reared or maintained under stressful conditions. The sampling protocols used here were not designed to detect some aquatic pathogens such as *Mycobacteria* species. Similar results have been obtained when non-diseased and diseased tropical ornamental fish were sampled (Shotts and Gratzek 1984, Dixon and others 1990, Dixon and Issvoran 1993, Kuo and Chung 1994*a,b*) where aeromonads and pseudomonads prevailed in the internal organs.

Due to the size of the aquatic industry, recent reports have stressed the threat from bacterial pathogens and parasites introduced with imported ornamental fish (Robertson and Austin 1994, Ferraz de Oliveira 1995, Cheong 1996). Several species of *Aeromonas* have been associated with disease outbreaks causing economic losses in both aquaculture and the ornamental fish industries. In this study, the group with the highest prevalence, *Aeromonas* species, were left as an unidentified species-group since their controversial taxonomic position and species identification are under major revision (Carnahan and Altwegg 1996). The use of conventional identification kits, such as API 20E and Biolog™, on these isolates occasionally gave different results for the same isolate. Isolates identified by the API 20E as *A hydrophila* and ranked as 'excellent identification' were assigned as *A schubertii* by Biolog™. The complexity of the identification and considerable genetic diversity of this group can easily lead these kits to different outcomes.

Some researchers have suggested that the presence of aeromonads in the ornamental fish trade is of some concern and a review of the law relating to the importation of these animals is necessary (Ansary and others 1992, Hettiarachchi and Cheong 1994, Austin and Adams 1996, Wootten 1991). Gratzek and others (1978) suggested that the high incidences of bacteraemia in ornamental fish after shipment might be due to the severe stress during their transportation and handling. The authors suggest that the presence of bacteria in the internal organs of ornamental fish and other aquatic animals is normal; thus the definition of 'bacteraemic' used here may not necessarily be associated with disease or subclinical infection. Recent studies confirm that apparently clinically healthy aquatic animals may harbour bacteria within their internal organs. Crumlish and others (1997) observed bacteria in the spleen of farmed frogs (*Rana rugulosa*) kept in laboratory conditions. Attempts to recover these bacteria from suspensions of splenic tissue yielded a range of colonies whose identification has not yet been reported. Chowdhury and others (1997) isolated a range of bacterial species from the slime and kidney of farmed fish, *Puntius gonionotus*, but found *Aeromonas* species to be the dominant genus in the kidney.

It is possible that the presence of bacteria such as aeromonads is unavoidable due to their ubiquitous presence in the aquatic environment. Improving the survival of imported ornamental fish must involve optimising the environmental conditions during transportation and handling on arrival. However, the rational use of antimicrobials may also have a role to play. Antibacterial therapy is commonly used at present but there is insufficient experimental evidence to design rational strategies to control disease. According to the results obtained in this study, it would appear that control measures following importation should be designed to deal with opportunist infections.

The hazard of importing serious pathogens cannot be discounted on the basis of this or any other survey. However, surveys such as this may help to determine the risk associated with the importation of ornamental fish. A rational approach to reducing risk from imported fish through legislation or other controls must be balanced against the potential harm that could be caused to the ornamental fish industry by excessively strict controls.

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SCIENTIFIC PAPER

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Acid-fast bacteria found in granulomatous lesions in a koi carp (*Cyprinus carpio*)

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Abstract

This report describes the clinical investigation of a granulomatous lesion on the pectoral fin of a koi (Cyprinus carpio) and demonstrates the practical benefit of histological examination of a biopsy. In view of the chronic and potentially infectious nature of the disease, the fish was killed humanely and a detailed post mortem performed. The difficulties in isolating the acid-fast bacteria by culture, despite their presence in the granulomata, is highlighted. The epidemiology, control and zoonotic aspects are also discussed.

Introduction

Granulomatous diseases in fish are chronic progressive diseases which produce various clinical signs depending on the organs affected. The commonest organisms involved in these diseases of fish are acid-fast bacteria, *Mycobacterium* and *Nocardia* species. The infection may take several weeks or years to progress from an asymtomatic state to a clinical illness. Both bacteria produce similar clinical characteristics and identification of the pathogen by histological methods alone has resulted in confusion (Austin and Austin 1993). Attempts to isolate the aetiological agent by bacterial culture often fail, highlighting the fastidious nature of the organisms (Austin and Austin 1993).

Mycobacteria from fish are zoonotic and can cause granulomatous nodules in Man by contamination of wounds on the hands and fingers (Savin 1992). The first report of acid-fast bacteria in freshwater fish was published by Bataillon and others (1897) who described a tuberculous lesion in a common carp (*Cyprinus carpio*) from a lake polluted by a hospital for patients with tuberculosis. *Nocardia* species have occasionally been isolated

from lesions in man. Detailed reports of granulomatous diseases in ornamental fish are rare (Lansdell and others 1993) and only a few photographs of confirmed cases in koi (*Cyprinus carpio*) have been published (Butcher 1992).

This report describes the clinical and post mortem findings in a koi with systemic bacterial granulomata due to Gram-positive acid-fast bacteria and discusses the management of the case.

Case history

The fish, an orange ogon variety of koi, was 490 mm long and weighed 1,585 grams. It had been owned for 2½ years and was kept in a formal outdoor koi pond containing 13,500 litres with 19 other adult koi. It was bought from a local fish dealer in north west London and was thought to be about 5½ years old.

Clinical examination

The fish was presented on 14 July 1995 with small multiple swellings on the left pectoral muscle (Fig 1). The owner noticed that the koi had not been using the left pectoral fin for several weeks although the dark coloured swellings had only been present for 3 weeks and had increased in size during this time.

The koi was anaesthetised with tricaine methane sulphonate (MS-222, Thomson & Joseph) by immersion to allow closer inspection and for a biopsy to be taken for histological examination. On the dorsal aspect of the pectoral muscle, multiple dark cystic swellings measuring between 3 mm and 5 mm in diameter had developed beneath the skin. Some smaller cysts on the ventral aspect also appeared to originate from within the muscle mass. A biopsy containing a few cysts was taken from the dorsal surface and fixed in 10% formal saline. Many small light brown nodules were seen in the dermal tissues at the biopsy site, revealing the extent of the disease. It was considered to be impractical to remove the entire lesion without amputating the whole limb and so the wound was treated conservatively to restore integrity of the skin. Povidone-iodine (Pevidine® Surgical Scrub, Vericore) was applied to the surgical wound which was then packed with a waterproof protective paste (Orabase®, ConvaTec). The fish was given 62·5 mg sulfadoxine and 12·5 mg trimethoprim (Borgal® 7·5%, Hoechst)

by injection into the body muscle below the dorsal fin to prevent secondary bacterial infection.

Microscopic examination of a skin scraping of body mucus revealed a few non-motile trichodinids but these were not considered to be significant. No further treatment was considered necessary and the fish was returned to the pond.

Initial laboratory results

Histological examination revealed multifocal granulomatous lesions throughout the sampled tissue (Fig 2). The level of inflammatory cell infiltration and the classic appearance of epithelioid tissue surrounding the granulomata (Fig 3) suggested that the pathology was related to a chronic infectious agent. There was no neoplastic change. It was not possible to identify any causative agent in the lesions with the haematoxylin and eosin stain therefore differential staining with Ziehl-Neelsen (ZN) and Gram's stains were performed but still failed to reveal any bacterial organisms. A second opinion also suggested that the lesions were bacterial granulomas.

Although the biopsy site had healed completely after 10 days, the fish was lethargic and continued to swim with the left pectoral fin clamped against its body. In view of the chronic and potentially infectious nature of this disease the owner requested the fish to be killed.

Post mortem examination

The fish was killed with an overdose of anaesthetic on 30 July 1995. The koi's condition and body weight were good. The biopsy site had healed and locally there was some epidermal hyperplasia. The pectoral fin was resected at the 'shoulder' but the granulomata were only found at the distal end of the muscle mass (Fig 4).

Internally there was a good amount of body fat in the abdomen but no free ascitic fluid. This male fish had continued to eat well and the bowel was full of food. There were several light brown granulomata measuring between 2 mm and 4 mm in the stroma of the liver (Fig 5). These were firm, well circumscribed and could be easily expressed from the tissue (Fig 6). One granuloma was found on the posterior pole of the left kidney but no lesions were visible on the other organs. Routine samples were taken from the gill, heart, spleen, liver, skin and, anterior and posterior kidney for histological

FIG 1: Posterior aspect of the left pectoral fin at first presentation.

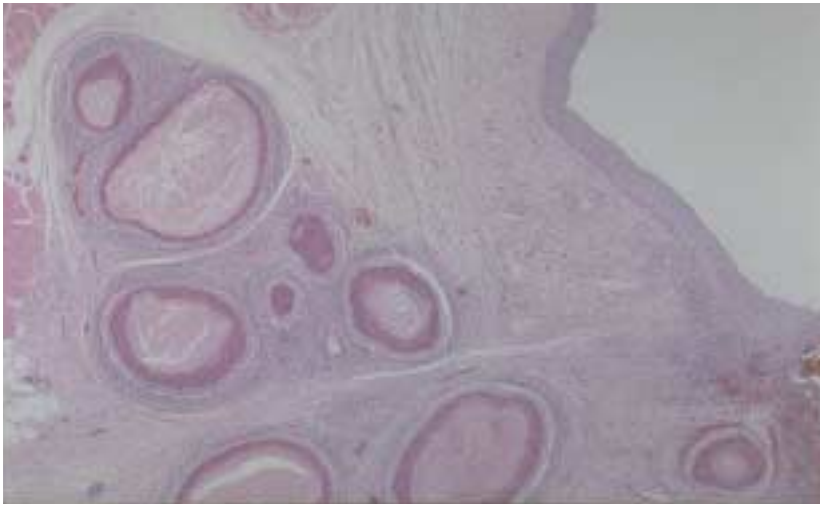

FIG 2: Histological section of the biopsy from site in Fig 1 revealing numerous granulomata with inflammatory cell infiltration in the dermis. (H&E) ×25

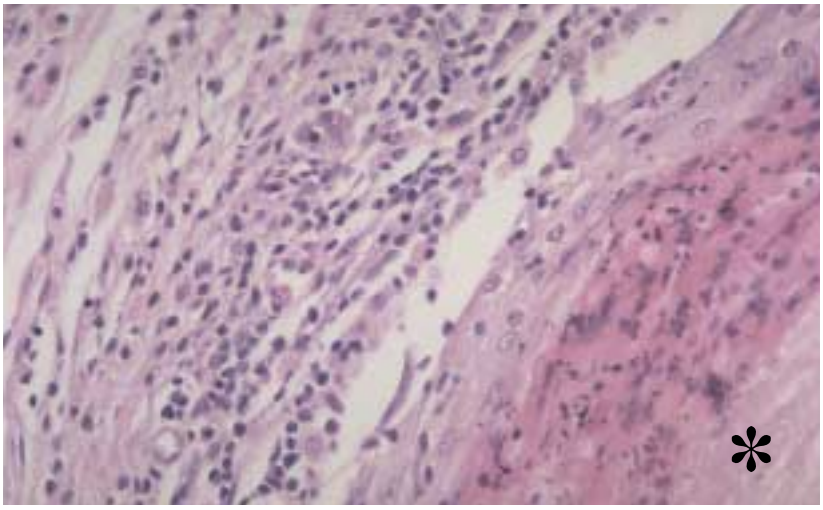

FIG 3: The periphery of a granuloma showing classical structure with central necrotic material (*) surrounded by fibroblasts and inflammatory cells. (H&E) ×400

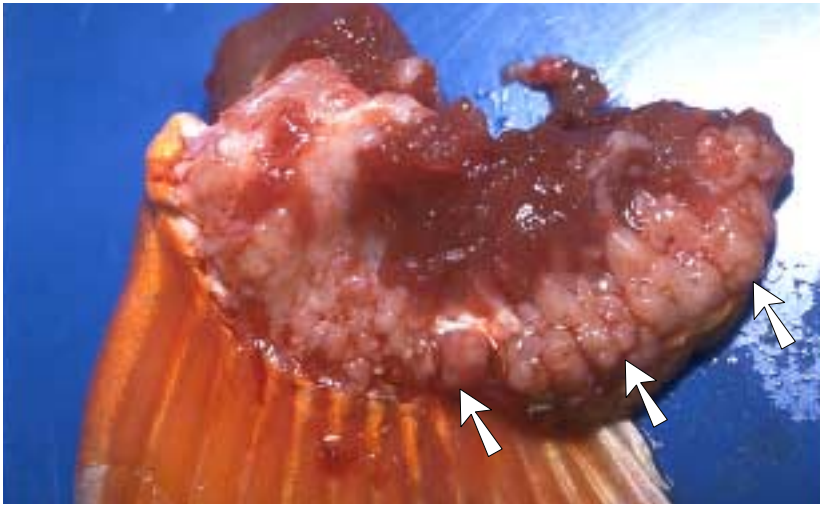

FIG 4: Extensive granulomatous formation (arrows) found at the biopsy site.

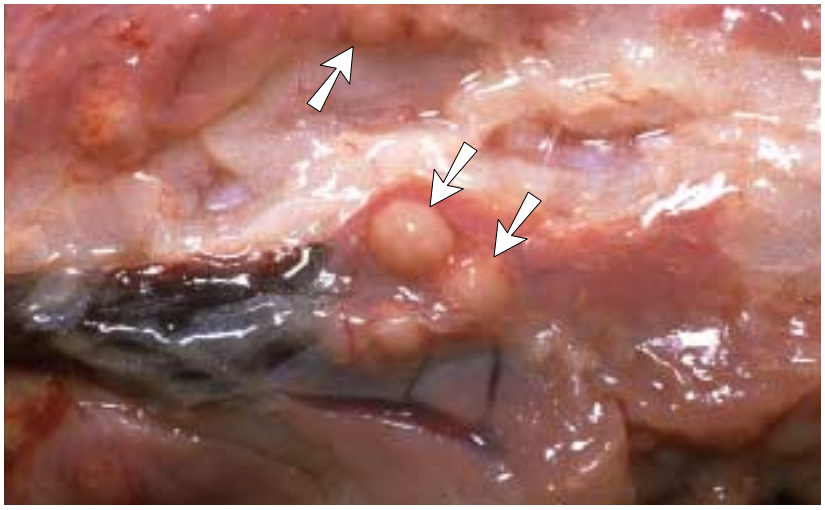

FIG 5: Granulomata (arrowed) in the liver and mesenteric tissues.

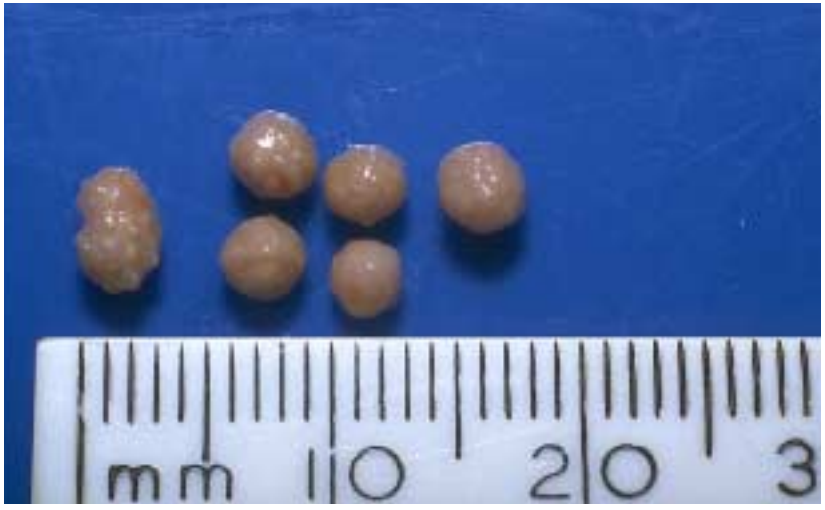

FIG 6: Granulomata removed from the liver (scale in millimetres).

examination. Further samples were taken from the left pectoral fin. Bacteriological samples were taken from a crushed granuloma with a standard swab and charcoal transport agar for culture on selective media and air-dried slides were prepared for differential staining. Entire granulomata were also sent for direct culture on to selective agar.

Final laboratory results

The results of differential staining of sections of the liver revealed masses of rod-shaped bacteria in a small number of granulomata. The ZN stain revealed weakly-stained acid-fast bacteria which were Gram-positive. Grocott silver and periodic acid-Schiff (PAS) staining techniques failed to demonstrate the presence of fungal elements in any of the sections.

Bacterial culture was unsuccessful despite the range of samples. Culture was attempted on Dorset egg medium and Löwenstein-Jensen slopes but these showed no growth after several weeks at 15° and 30°C. Differential staining of the granuloma smears confirmed the presence of acid-fast bacteria with ZN stain.

Recently, Gómez and others (1993) described the use of an immuno-cytochemical staining method using an avidin biotin complex (ABC) to reveal mycobacterial antigens in sections where bacilli were not seen when ZN stain was used. A sample of the granulomatous lesion on the fin was sent to Dr Gómez but no mycobacterial antigen was found.

Discussion

Granulomatous disease in fish can be due to infection with bacteria, fungi, protozoan or myxozoan parasites. Histological examination with differential staining methods revealed the presence of acid-fast bacteria in this case. Both mycobacteria and *Nocardia* species are acid-fast bacteria which produce granulomata and similar clinical signs. *Nocardia* species usually produce an abundance of bacterial filaments in the centre of lesions, irrespective of the stain used, whereas *Mycobacterium* species appear as rod-shaped bacteria as seen in this fish.

The most common species of mycobacteria pathogenic to fish are *M marinum, M fortuitum* and *M chelonae*. Of these, *M marinum* is the most commonly isolated species and has been found in tropical freshwater and

marine species (van Duijn 1981). *Mycobacterium fortuitum* is isolated less frequently and has been reported mainly from fish in freshwater tropical and temperate waters (Frerichs 1993). *Mycobacterium chelonae* has so far been identified only in coldwater salmonids (Frerichs 1993).

Epidemiology

Mycobacteria and *Nocardia* species are free-living and found in the soil and aquatic environment. In practice, mycobacteriosis is most frequently recognised in aquarium fish probably because they are maintained under a degree of captivity stress for long periods of time (Frerichs 1993). In the absence of natural predation, this allows the slow progressive infection to develop into a clinically diagnostic condition. Ambient temperatures may also play a role. The relatively low temperature of outdoor ponds in the United Kingdom by contrast to indoor room temperatures may account for a lower incidence of mycobacteriosis in pond fish. The degree of infection may therefore depend on the adaptability of the bacterial species to multiply inside coldwater fish where water temperatures are rarely above 20°C .

Mycobacterial infections of fish are probably transmitted naturally by ingestion of contaminated food or aquatic debris, although bacterial invasion through damaged skin or gill tissue may also be possible (Frerichs 1993). There had been no visible damage to the affected fin during the previous 2½ years of ownership but periodic infestation with ectoparasites had been suspected. Koi frequently scavenge the pond surfaces for algae and other food matter, and in this way may ingest various pathogenic organisms. However, the filter system appeared to be efficient at removing waste matter and debris from the bottom of this pond and should have reduced this risk.

A wide range of amphibians including European frogs (*Rana* species) are known to be susceptible to mycobacterial disease (Nigrelli and Vogel 1963). Frogs are often found in garden ponds particularly during their mating season in spring, and the owner reported that he had seen up to 30 frogs in the pond on some occasions. Here, the water is artificially heated in winter and this also attracts frogs into the pond. Occasionally, frogs would get caught in the pump and be killed, resulting in the disintegration of their body tissues and potential dissemination of infective organisms throughout the pond system. However, following investigation of unusual mortality in common frogs (*R temporaria*) in the UK, detailed post mortem and histo-

logical examinations were carried out on 59 frogs and no acid-fast organisms were found (Cunningham and others 1996).

The feeding of infected trash fish is known to spread disease to other fish (Ross and Johnson 1962, Chinabut and others 1990) but this practice is not relevant in koi ponds where the fish are usually fed on commercial pelleted foods. Although these foods usually contain 30% to 40% fish protein as herring meal, it is sterilised in the manufacturing process.

Ross and Johnson (1962) investigated trans-ovarian infection in chinook salmon (*Oncorhynchus tshawytscha*) and although their results did not demonstrate this under controlled conditions, it was not possible to exclude this route as a means of transmission. However, other researchers have confirmed that trans-ovarian infection occurs in viviparous platyfish *Xiphophorus maculatus* (Conroy 1966), guppies *Lebistes reticulatus* (Conroy and Conroy 1999) and in the eggs of Siamese fighting fish *Betta splendens* (Chinabut and others 1994). The origin and breeding history of this koi is unknown and the route of transmission cannot be confirmed here.

Clinical signs

The clinical signs of mycobacteriosis in fish depend on the species involved and if vital organs are affected. These signs may include some of the following: listlessness, anorexia, emaciation, dyspnoea, nervous disorders, unusual behaviour, exophthalmos, skeletal abnormalities, skin discoloration and external lesions such as ulceration and fin necrosis (van Duijn 1981, Frerichs 1993). Internally the lesions are similar in tropical and coldwater fish, and are seen as grey-white miliary granulomata which develop in the tissues of the spleen, liver and kidney in particular. The significance of the light brown colour of the granulomata found in this case is not known but may be related to the species of fish.

Laboratory methods

In the literature much diagnosis of mycobacterial infection is based on histopathology alone with little attempt to isolate and identify the organism. This results in confusion since both *Mycobacterium* and *Nocardia* species produce similar pathology.

Whereas the finding of acid-fast bacteria in granulomata on histological sections is strongly suggestive of mycobacterial infection, failure to find

them is not proof to the contrary (van Duijn 1981). In human lesions, when *M marinum* is present, examination of ten or more sections may be required before acid-fast bacteria are found (Chow and others 1983). Similar difficulties have been experienced in piscine samples (A. Holliman personal communication) and in older granulomata these bacteria are generally not visible (van Duijn 1981). This may account for the failure to find bacteria in most tissue sections in the present case.

Dorset egg and Löwenstein-Jensen agar slopes are now the standard recommended media for the isolation of *Mycobacterium* and *Nocardia* species (Austin and Austin 1993). All mycobacteria pathogenic to fish grow when incubated between 20° and 30°C although *M marinum* shows slow growth, often requiring up to 3 weeks. Cultures are not always obtained despite evidence of infection, and sometimes a large inoculum is required (Frerichs 1993). This may account for the failure to isolate any acid-fast bacteria in the present case.

Failure to find mycobacterial antigen using the immuno-cytochemical staining method may be due to the lack of organisms in the granulomatous sample from the fin since most were found in histological sections of the liver. It may also be due to the specificity of the antibodies since the antiserum was raised against *M paratuberculosis* and *M bovis*.

Recently, other researchers have developed monoclonal and polyclonal antibody probes to various strains of mycobacteria and these have been used in the development of enzyme-linked immunosorbent assay (ELISA), immunohistochemistry and immunofluorescence tests (Adams and others 1995, Adams and others 1996, Chen and others 1997). A polymerase chain reaction (PCR) test has also been developed (Knibb and others 1993).

Treatment

Due to the risk to human health, the treatment of fish with bacterial granulomata is questionable. Frerichs and Roberts (1989) unequivocally advise against treatment and advocate a slaughter and disinfection policy. However, the considerable value of the other koi precluded this approach. Various protocols and medications have been suggested but the prolonged use of antibiotic baths, up to three weeks, were impractical in this case (van Duijn 1981, Conroy and Conroy 1999). The bacteria are encapsulated within the granulomata and since the lesions have a poor blood supply, it is

difficult to achieve therapeutic drug levels at the site of infection.

Since the visible lesion on the pectoral fin appeared to be localised, the practicality of surgical amputation of the fin at the 'shoulder' joint was considered. However, this would have created a large cavity in the body wall making recovery and healing unpredictable.

Control

Prior to biopsy, the skin lesions on the affected koi had not ulcerated, theoretically limiting the spread of infection. The duration of the disease in this case and the extent of infection in the other koi is unknown. At present there are no suitable tests which may identify infected fish although the collection and concentration of faecal matter has been used to identify the presence of carrier fish (Post 1987).

The use of environmental disinfectants has been suggested by van Duijn (1981) and the owner has used chloramine-T on several occasions in the past to treat ectoparasites, the last treatment being given several months earlier. This chemical has some effect on external bacterial infections and may have some disinfectant activity at the dosages used here.

Vaccines against mycobacterial infections in fish are not available at present. However, experimental vaccines using *M tuberculosis* have been shown to produce a cell-mediated immune response to mycobacterial antigens in rainbow trout *Oncorhynchus mykiss* (Bartos and Sommer 1981). More recently, Chen and others (1996) have reported elevation of both the nonspecific and specific immune responses of rainbow trout to vaccination with extracellular products from *Mycobacterium* species. Although there are no reports of their use in practice, this suggests that Bacille Calmette-Guérin (BCG)-like vaccines for fish could be developed in the future.

The owner decided to wait for further cases to develop and have routine post mortem examinations performed on any fish dying in the pond in an attempt to assess the prevalence of the disease. At the time of writing there have been no further cases nor any deaths.

Zoonotic aspects

All species of mycobacteria from fish are capable of infecting man, of which *M marinum* is the most common. This produces cutaneous nodules,

commonly called 'swimming pool granuloma', usually on the hands, elbows or knees which are slow to heal, often taking many months (Black and Eykyn 1977). Although the owner himself has not had any such lesions, this aspect of the case has been fully discussed with him. The current medical approach to this disease in humans involves a six week course of therapy with co-trimoxazole, tetracycline (in particular minocycline) or rifampicin often in combination with ethambutol (Savin 1992).

Rare, and sometimes fatal, cases of disseminated systemic infections with *M marinum* have been reported in humans who are immunocompromised, such as those infected with human immunodeficiency virus (HIV) (Tchornobay and others 1992, Hanau and others 1994), receiving chemotherapy or following organ transplant (Gombert and others 1981).

Conclusion

This report has shown the practical benefit of successfully taking a biopsy of a lesion from a valuable fish for histological examination. In view of the potential of a systemic infection being present, euthanasia was considered to be more practical than local amputation of the limb. The difficulties in isolating acid-fast bacteria by culture, despite their presence in the granulomata have also been highlighted.

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SHORT COMMUNICATION *Fish Veterinary Journal (1999) (4) 33–37*

Use of Supaverm® for the treatment of monogenean infestation in koi carp (*Cyprinus carpio*).

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Infestation with monogenetic trematodes in koi carp (*Cyprinus carpio*) is a common problem. These are often referred to as flukes, and usually comprise of members from the genera *Gyrodactylus* (skin flukes) and *Dactylogyrus* (gill flukes). There are many proprietary medications available for the treatment of flukes, most of which contain formalin at varying dose rates and often in combination with other chemicals. Success with these treatments is variable although careful use of formalin at dose rates up to 25 mg/litre as a permanent bath, in combination with good aeration, has been found to give reliable results in the past. However, formalin used at these levels reduces dissolved oxygen, damages gill epithelial cells and can be lethal to fish with gill damage from whatever cause, including fluke infestation.

Supaverm® (Janssen) is an oral suspension containing closantel 50 mg/ml and mebendazole 75 mg/ml licensed for the control of fluke, gastro-intestinal nematodes and cestodes in sheep. It also controls the larval stages of the sheep nasal bot fly, *Oestrus ovis*. Both compounds have been used separately in the past to treat fluke infestations in fish, so it was thought that the combination could prove useful to control resistant flukes. This report details a trial where Supaverm® was used in an attempt to control a fluke infestation in koi.

Case history

In May 1996, a koi-keeper identified a heavy infestation of both skin and gill flukes in his fish. These parasites appeared to have a resistance to formalin since treatment with the chemical at dose rates up to 25 mg/litre failed to control the infestation, despite success in previous years. There was reluctance to use organophosphorus compounds therefore a trial using Supaverm®, a combination of closantel and mebendazole, was performed.

Approximately twenty koi, ranging in size from 15 to 60 cm, were kept in a pond containing 11,350 litres. The fish showed clinical signs of flicking, rubbing and had an excess of body mucus. Regular skin scrapings taken from behind the operculum revealed 5 to 6 flukes per microscope field (×100). Both skin and gill flukes were present. Trichodinids were found occasionally.

A single bath treatment with Supaverm® was used at a dose rate of 1 ml per 400 litres. This equates to 0·125 mg/litre of closantel and 0·1875 mg/litre of mebendazole. Supaverm® is an aqueous suspension which dilutes well in water and disperses rapidly. The measured dose was administered by dilution in a bucket of water prior to pouring into the pond.

An initial test was performed on a few koi, 10 to 15 cm size, in a separate small pond to assess any harmful reactions. Since no adverse effects were observed after two days, the main pond was dosed with Supaverm®. The fish were closely observed for signs of stress for several days and commercially available test kits (Tetra) were used to measure *p*H, ammonia and nitrite, daily for two weeks. Daily opercular scrapes were examined by light microscopy (×100 magnification) for two weeks and thereafter once weekly for 12 weeks. Particular attention was paid to providing good aeration during the test period. Routine pond management included performing 10% water changes twice per week. These were stopped for two weeks following treatment with Supaverm® then recommenced. Water temperatures during the twelve week trial period ranged from 20° to 23°C.

Results

The numbers of flukes found on microscopic examination remained unaltered until the fourth day following treatment and then dropped rapidly. None were seen after the sixth day. Subsequent scrapes revealed no flukes for 12 weeks when a few were occasionally found. Both *Dactylogyrus* and *Gyrodactylus* were present at this stage. The fish showed no obvious signs of stress, remaining active and continuing to eat throughout the trial period. Clinical signs of excess mucus and irritation resolved completely after four to five days. The water quality was unaffected. The *p*H remained between 7·2 and 7·8. The levels of ammonia and nitrite were both unaltered and remained at 0 mg/litre throughout the test period.

Three months after the first dose, in August 1996, when the first flukes began to reappear, a second dose of Supaverm® was used at the same rate with similar results. No further flukes were found until Spring 1997.

No objective attempt was made to monitor any adverse effects on other life in the pond during this trial but the snail population appeared to be unaltered throughout the rest of the year. No plants were kept in this pond.

Discussion

In this case, it was clear that an alternative remedy to formalin was required to treat the fluke infestation. Both the author and fish-keeper were reluctant to use organophosphorus compounds due to the potential hazard to both operator and the environment since the treated water would be discharged into the sewage system. In addition, the potential toxicity to the fish was considered. Although organophosphates are frequently used to treat ectoparasites in koi, some fish species such as orfe (*Leuciscus idus*) and rudd (*Scardinius erythrophthalmus*) are known to be sensitive to these compounds which can cause scoliosis and death. These species were not involved in this trial but it was felt that a safer treatment for the control of flukes should be investigated.

Both active ingredients of Supaverm® have been used separately as immersion treatments for flukes in fish. Closantel at 0·125 mg/litre as a 3 hour bath treatment has been used in trout against *Gyrodactylus* species (Stoskopf 1993). *Gyrodactylus elegans* infestation in goldfish (*Carassius auratus*) has been successfully treated with mebendazole at 0·1 mg/litre by immersion for 24 hours with no adverse effects, although higher doses of 0·4 mg/litre in combination with trichlorphon at 1·8 mg/litre was required to clear more resistant fluke species (Goven and Amend 1980).

The dilution rate of Supaverm® was chosen to produce a concentration of closantel of 0·125 mg/litre. The resulting concentration of mebendazole, 0·1875 mg/litre, was well within previously quoted dose rates. The drug was found to be safe and effective, and there was no observable adverse effect on the biological filtration system. Since this original trial, Supaverm® has been used on numerous occasions with similar results in various ponds containing mixed species of fish including koi, goldfish, orfe and sterlets (*Acipenser ruthenus*). However, some caution is necessary since the author

has received two anecdotal reports of deaths following the use of Supaverm® in discus (*Symphysodon discus*) and very small goldfish. These cases were not under veterinary supervision and no information is available concerning the accuracy of dosing or other relevant factors.

At the start of the trial, regular water changes were part of routine water maintenance. It was not known how quickly closantel and mebendazole would be effective, or how rapidly they would become inactivated in water. Therefore, water changes were withheld for an arbitrary period of two weeks. The results suggest that flukes were cleared from the fish between the fourth and sixth day following treatment, and as a result, it is recommended to withhold water changes for one week.

In this trial both egg-laying *Dactylogyrus* and larva-producing *Gyrodactylus* were successfully eliminated for 12 weeks, after which both species began to reappear. The author has found that, following the use of formalin, there is often a rapid reinfestation by *Dactylogyrus* flukes, suggesting that egg production makes this parasite more difficult to control. The manufacturer's data sheet for Supaverm® states that closantel has been shown to inhibit egg-laying by the mammalian liver fluke, *Fasciola hepatica,* for up to 13 weeks. This period appears similar to the suppression of both skin and gill flukes in this trial.

The suppression of egg-laying could be an important factor in controlling gill fluke for several months. Eggs released into the water by adult gill flukes take four days to hatch at 20°C (Andrews and others 1988). These eggs are a source for rapid reinfestation of fish, however this did not occur in this trial. The fact that both species gradually reappeared after 12 weeks suggests that most, but not all, adult flukes were killed by the treatment and that subsequent egg-laying by remaining gill flukes was suppressed for this period of time. It is suggested that Supaverm® has a direct inhibitory effect on eggs in the pond sediment or that the product remains active at these temperatures sufficiently long enough to kill the larvae when they hatch. Further work is required to investigate these aspects.

Flukes may enter the pond from outside sources such as newly introduced plants or fish but neither occurred during this trial. While treating other similar suburban ponds, the author has found that a single dose of Supaverm® in May or June suppresses fluke infestations for the rest of the

year. This suggests that reinfestation from external sources such as amphibians or birds is not a significant problem.

After dosing with Supaverm® it is frequently observed that one or two fish void tapeworms in their faeces. This interesting incidental finding suggests that infestation with tapeworm (species not identified) is not uncommon in koi although it may be of little clinical significance. This product appears to be an effective treatment against tapeworms in koi and could be useful for roundworm infestation although more research is necessary to evaluate its efficacy.

Another of the author's clients has claimed that Supaverm® treatment has also eliminated a mild infestation of fish lice, *Argulus* species, in his koi. Supaverm® has a wide range of antiparasitic activity including the larva of the sheep nasal bot fly. This raises the possibility that it could be used in the treatment of other metazoan parasites of koi although more investigation in this area is required.

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Availability of medicines for fish

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The problem

The market for fish medicines

Intensive fish culture, especially of salmonids, is a very recent development, much more recent than, for example, broiler chicken production. While fish farming of a sort dates back to mediaeval monasteries, this was at very low stocking rates. Furthermore, the fish were kept in ponds with low water flow rates; the rainbow trout, *Oncorhynchus mykiss*, (a member of the Pacific salmon genus) was unknown, and techniques for marine culture had not been developed. In consequence the main species farmed was the common carp, *Cyprinus carpio*.

Intensive production has inevitably created a requirement for medicines, but the legislative framework in the European Union (EU), for example Directives 81/851 and 81/852 and Regulation 2377/90, has made it uneconomic for pharmaceutical companies to develop medicines for a small market. The consequent lack of medicines has had a vicious circle effect and is one factor responsible for keeping the EU aquaculture industry small. Nevertheless, the industry has developed enormously in parts of the world where there are fewer constraints on medication, especially on the Pacific rim and, significantly, in Norway.

The range of fish species

The decline in stocks of wild fish is creating a need for alternative sources of fish. Husbandry techniques for rainbow trout and Atlantic salmon, *Salmo salar*, have been developed, and such market authorisations (MAs) as there are for fish medicines, are mainly for these species.

Among other species which are being farmed on a limited or experimental scale in the UK and for which there are no authorised medicines are:

Arctic char	– *Salvelinus alpinus*
brown trout	– *Salmo trutta*
Atlantic cod	– *Gadus morhua*
halibut	– *Hippoglossus hippoglossus*
Japanese flounder	– *Paralichthys olivaceus*
turbot	– *Scophthalmus maximus*

The European Medicines Evaluation Agency (EMEA), the EU body which co-ordinates the work of member state regulatory bodies such as the Veterinary Medicines Directorate (VMD), has published certain extrapolations it is prepared to make in assessing applications for medicinal products for fish, namely:

1. Salmonidae are regarded as a (single) major species
2. Other fin-fish species may be accorded the same maximum residue limits (MRLs) as salmonidae
3. If an MRL has been established for a substance in muscle in a major mammalian species it may be applied to salmonidae and other fin-fish as well.

However, these extrapolations apply to MRLs, not indications for use. The uses of drugs are subject to the 'cascade' laid out in Article 4 of Directive 90/676. This applies only to 'an animal or a small number of animals'; a literal interpretation of the phrase would exclude the several thousand fish normally kept in a single cage and so create a serious animal welfare problem. The wording of the directive has been incorporated into UK law but VMD has issued an interpretation of this law (AMELIA 8) which states:

> "Where a veterinarian is required to treat an infectious disease in, say, farmed fish, he may need to proceed on the assumption that all individuals in one cage in contact with one another are all equally and identically at risk, and the interpretation of 'a small number' may reflect this."

Where no specifically authorised drug exists the cascade preferences are:

- a medicine authorised for a different use in the same species;
- a medicine authorised for a different species;
- a medicine authorised for human use.

However, where fish farmed for food are concerned, a medicine authorised for a 'different species' must be for a different *food-producing* species, and the third option does not apply. So, while the VMD interpretation is helpful in relation to antibacterial and antiparasitic drugs, it does very little for the availability of anaesthetics or reproductive hormones for fish. In any case, the interpretation was produced in the interests of animal welfare, and it is questionable whether it could be extrapolated to reproductive manipulation.

There is a need to exploit additional aquatic ecological niches, and in some cases husbandry of the appropriate species requires medicines not currently authorised for any food-producing animal.

Discharge consents

In the UK, the economic problem for pharmaceutical companies is exacerbated by the requirements of quangos responsible for the purity of river water — the Environment Agency (formerly called the National Rivers Authority) in England and Wales and the Scottish Environment Protection Agency (formerly various river purification boards) in Scotland. Although companies applying for MAs are required to submit environmental safety data and assessments to the VMD, and these are taken into account by the Veterinary Products Committee (VPC), some of the environmental regulators refuse to acknowledge MAs for fish medicinal products. They demand separate submission of data, sometimes additional to the data submitted to the VMD; and some environmental regulators publish the data. Such publication is in effect theft of the intellectual property in which the applicant has invested; it would be an offence for VMD staff or VPC members (Medicines Act 1968, section 118). Both publication of data submitted to the VMD and the requirement for additional data are disincentives to companies investing in the development of fish medicines.

The right of environmental regulators to demand environmental safety data for a medicinal product which is the subject of a market authorisation should be statutorily defined. If environmental regulators are given such rights it should be a statutory offence for them to disclose the data to another person.

Awareness of the requirements

The curriculum at the veterinary schools in the EU concentrates on

domesticated mammals and poultry. Fish medicine is a post-graduate specialism studied by only a very few veterinarians. Fish are not included in the definition of animals in the Veterinary Surgeons Act 1966, and much of the diagnostic work in fish in the UK is currently undertaken by non-veterinarians.

In consequence, few veterinarians in the EU who are not involved in fish health are aware of the specific requirements for fish medicines. This was freely admitted by speakers, including representatives of the VMD and EMEA, at the session on 'Availability of Medicines' at the British Veterinary Association Congress, Nottingham in 1998.

Medicines currently authorised in the UK

Authorised active ingredients

The following are the active ingredients in medicinal products currently authorised for fish:

- antibacterial agents: amoxycillin
  oxolinic acid
  oxytetracycline
  sarafloxacin
  trimethoprim & sulfadiazine (co-trimazine)
- ectoparasiticides: azamethiphos
  cypermethrin
  hydrogen peroxide
- anaesthetic: MS-222 (tricaine mesilate)

In addition, MRLs specifically for fish have been determined for flumequine, (a fluoroquinolone antibacterial agent) and teflubenzuron (an ectoparasiticide) although there are no MAs for them in the UK at the time of writing (May 1999).

Oxolinic acid

This first generation quinolone antibacterial agent was originally developed for use in fish. It is presumed to cause arthropathy and a MA has never been sought for its use in mammals or birds in the past. However, oxolinic acid

has been granted a MRL for cattle, pigs, chickens and fin-fish which expires on 1 January 2001. It is listed under Annex 3 which includes substances for which a provisional MRL has been fixed.

Oxytetracycline

The other antibacterial agents listed above are likely to remain available for fish because the annex entries under Regulation 2377/90/EEC are for 'all food-producing species'. Nevertheless, oxytetracycline (OTC) is an unsatisfactory drug for use in farmed fish in respect of its environmental safety. In-feed medication is the only practical method of administering drugs to farmed fish, and the oral bioavailability of OTC in salmonids is of the order of 7% ; in carp it is an order of magnitude lower still. This means that it is necessary to administer in the feed, 14 times the dose which would be efficacious by injection in order to achieve systemic absorption of the minimum efficacious dose. As OTC is excreted unchanged in faeces or urine, the whole of these 14 doses eventually enters the environment. This may be compared to sarafloxacin and amoxycillin with bioavailabilities of the order of 67% and hence the need to use in feed only 1½ doses which would be efficacious by injection.

Sarafloxacin

The MA for sarafloxacin (SFX) is limited to one indication, furunculosis; one species, Atlantic salmon; and use only in sea water. However, the interpretation of the cascade given in AMELIA 8 means that the drug can be used more widely.

The problem is that very recently, questions have arisen over the veterinary use of fluoroquinolones. It has been suggested that they should be used only as a last resort and never prophylactically. In this context, it needs to be realized that virtually all antibacterial medication of farmed fish is prophylactic. As noted above, administration must of necessity be in-feed, but ill fish do not eat.

In the development of any code of practice for the use of fluoroquinolones in veterinary medicine, the use of SFX in farmed salmon should be specifically addressed.

Trimethoprim & sulfadiazine

The synergism between trimethoprim (TMP) and sulfonamides means that the efficacious dose of the combination contains less than an efficacious dose of either one component. Nevertheless, the synergism can only occur if the two components are at the site of pathogenesis at the same time: it is essential that the sulfonamide has the same pharmacokinetic profile as TMP. Sulfadiazine (SDZ) makes a satisfactory pharmacokinetic match with TMP in all mammal species studied. However, it makes a poor match in birds, which is why Tribrissen® Poultry Formula contained sulfaquinoxaline.

TMP-SDZ shows efficacy in salmonids at doses that clearly indicate that synergy is occurring, but the ideal sulfonamide to match TMP in fish is not known. Sulfamethylphenazole (SMP) has been shown to be the best of some seven sulfonamides tested in rainbow trout (McCarthy and others 1974), but this work was never followed up by the introduction of a commercial product. In any case, there is no reason to assume that either SMP or SDZ would be suitable in all genera of cultured fish.

There is an ever-present danger that one of the commercially available potentiated sulfonamides will one day be used in a species where the components have a significant mismatch. The pathogens will then be exposed, sequentially, to two different antibacterial agents each at less than an efficacious concentration.

There is a case for banning the use of potentiated sulfonamides in fish genera in which synergy has not been positively demonstrated.

Anaesthetics

There is an anaesthetic agent with an MA. So legally this drug, MS-222 (Thomson & Joseph), is the only one which may be used for anaesthesia of salmonids, for which it is authorised. Other fish species could be anaesthetised either with MS-222 or with an agent authorised for another food-producing species. Such other species are mammals or birds, and the formulations of anaesthetic agents authorised for them are quite unsuitable for fish. The only requirement for surgical anaesthesia in food-fish is for injection, although sedation is used in some fish species for husbandry procedures such as weighing, measuring and grading. Since injection takes

only a moment, rapid induction of, and recovery from, anaesthesia are desirable; and benzocaine is often used in preference to its authorised analogue, MS-222. For sedation, benzocaine or 2-phenoxyethanol (phenoxetol) are used. In practice therefore, the law is disregarded.

This situation applies as much to British research institutions as to fish farms. Even though the use of benzocaine may have been permitted under the Animals (Scientific Procedures) Act 1986, its use could be construed as an offence under the European Communities Act. This disregard for the law is a positive disincentive to pharmaceutical companies attempting to obtain MAs for fish medicines.

The British veterinary profession should exert some moral pressure to encourage the use of authorised medicinal products by research institutes. When offenders submit their research for publication, referees should draw their attention to the law; if the offenders are veterinary surgeons, then publishers should seek the advice of the Royal College of Veterinary Surgeons.

Unauthorised compounds

There are three compounds which are in routine use although they are not the active ingredients in any medicinal product which is the subject of an MA. They are formalin, malachite green and chloramine-T. All three compounds are used for the prevention and treatment of secondary infection of skin lesions. Chloramine-T is the standard treatment for bacterial gill disease, which may be a primary infection or secondary to monogenean fluke infestation.

Special mention needs to be made of the use of malachite green for the control of proliferative kidney disease (PKD) in freshwater salmonids. There is an extensive literature on the unique efficacy and method of use of this compound, and there is some indifferent literature on its toxicology. The legal position is comparable to that of oxolinic acid (see above), except that there never has been a product licence or MA (Alderman 1991). In Denmark and Germany it is specifically banned, although well known to be used. The British Trout Association has published advice strictly limiting its use. EU trout farmers have a choice between flouting the law and going out of business.

There is a serious need to make legal accommodation for the medicinal use of formalin, malachite green and chloramine-T in fish or licensed alternatives to be made available.

Summary

There are five antibacterial agents currently authorised for fish. Of these, the MA for one (oxolinic acid) may be withdrawn soon, and another (oxytetracycline) is undesirable for environmental safety reasons. Trimethoprim & sulfadiazine is suitable for salmonids but has not been adequately studied in other fish genera.

There are three authorised ectoparasiticides. The main use of these is for sea lice (Caligidae) on Atlantic salmon. Azamethiphos, an organophosphorus compound, is subject to some parasite resistance due to earlier widespread use of dichlorvos. Cypermethrin affects all stages of sea lice, currently has no known resistance and is safe to operators. Hydrogen peroxide is very expensive, but can have serious adverse effects on fish under treatment and is potentially hazardous to operators. The development of benzyl-urea compounds for in-feed administration deserves facilitation and encouragement.

One anaesthetic drug is authorised. Legal sanctions should be enforced against users of other drugs such as benzocaine, or they should be required to apply for an MA for this unlicensed drug.

There are no MAs in the UK for any anthelmintics or reproductive hormones for use in fish. While the interpretation of the cascade given in AMELIA 8 allows the use in fish of products authorised for mammals, the formulations of anthelmintics are usually unsuitable and mammalian reproductive hormones have low potency in fish.

Additional requirements

Antibacterial drugs

The range of antibacterial drugs authorised in the UK may be considered adequate, if minimal, for salmonids. Its suitability for other fish genera is questionable, especially in the case of trimethoprim & sulfadiazine.

Of the other agents which have been investigated for aquacultural use, florfenicol would appear to be the most suitable. It has a bioavailability in excess of 90%, thus minimizing the required oral dose, and it is depurated rapidly from the marine environment (Hektoen and others 1995).

In looking to the future, one particular disease which should be borne in mind is bacterial kidney disease (BKD), *Renibacterium salmoninarum* infection of salmonids. It is distinctive in that the causative organism is Gram-positive, whereas virtually all other bacterial pathogens of fish in the UK are Gram-negative, and it is an intra-cellular parasite. It is a notifiable disease in the UK. Were it ever to become widespread, necessitating antibacterial medication, the control method which has been found satisfactory is the use of erythromycin in broodfish to prevent vertical transmission (Brown and others 1990, Moffitt 1992).

In other parts of the world, erythromycin has proved useful for the control of streptococcosis and infection with *Chlamydia* and *Piscirickettsia* species. Any of these may eventually appear in the UK, especially in the warmer waters of the south coast, and a rickettsia-like organism has already been reported in farmed Atlantic salmon (Grant and others 1996).

Public funding should be made available for the preparation and submission of MA applications for additional antibacterial agents for use in fish. It is suggested that florfenicol premix and injectable erythromycin should be given priority.

Anthelmintics

The most important helminth parasites of fish in the UK are monogenean flukes — gill flukes (*Dactylogyrus* species) and skin flukes (*Gyrodactylus* species). Administration of anthelmintics is not easy because the helminths are actually ectoparasites and are best treated by immersion of the fish rather than oral or parenteral medication. Furthermore, the duration of immersion is as important for efficacy, if not more important, than concentration. Some, but not all, benzimidazole anthelmintics have shown activity against these parasites; fenbendazole, mebendazole and triclabendazole being the best. However, the best drug appears to be one used in mammals including Man, as a cesticide, praziquantel (Buchmann 1987). This drug is also efficacious against fish cestodes which could become important if intensive culture of carp were to be developed further.

Public funding should be provided for the development of a formulation of praziquantel for immersion administration to fish.

The need for reproductive hormones

The term reproductive hormones is used here to include not only gonadal steroids, for which there is limited use in fish, but also pituitary gonadotrophic hormones (GtH) and hypothalamic gonadotrophin-releasing hormones (GnRH). Many fish species also have endocrine production of a gonadotrophin-releasing inhibitory factor (GRIF) which has been identified with dopamine. Domperidone, which is a synthetic dopamine antagonist, is used in fish as a GnRH potentiator.

There are few, if any, fish species which will breed naturally under farmed conditions necessary for economic food production. Salmonids will ovulate and spermiate but not actually spawn (void their gametes); ripe broodfish have to be manually spawned and the gametes mixed in a bucket. Other species are inhibited at various earlier stages in gametogenesis and require hormone stimulation.

Pituitary gonadotrophic hormones

The traditional and still widely used drug is carp pituitary extract (CPE) or carp pituitary homogenate (CPH). The procedure is called hypophysation of broodfish. Acetone-dried, pulverized gland is available on the market in some countries but it may be contaminated with brain tissue so the potency is unreliable. The user can obtain a more reliable product by collecting glands himself from live fish in the spawning season. A method of obtaining pituitaries from carp and processing them, together with dosage and administration details were given in an official Food and Agriculture Organisation (FAO) publication twenty years ago (Woynarovich and Horvath 1980).

The author recently provided some evidence to the VMD that either CPE or, more probably, CPH was being imported into the UK, presumably mainly for use in carp. This importation is illegal; but without some hormonal assistance it will be impossible to breed carp in the UK.

Another widely used source of pituitary gonadotrophic activity for fish is human chorionic gonadotrophin (HCG) or Chorionic Gonadotrophin Ph Eur. This has been used for many years in both human and veterinary medicine as the routine source of luteinizing hormonal activity. In many freshwater species, HCG can be used to accelerate the pre-ovulatory phase of egg maturation, although actual ovulation cannot always be induced; in general, carnivorous species respond better than herbivorous ones. It is known that spawning can be induced with HCG in channel catfish, *Ictalurus punctatus*, striped mullet, *Mugil cephalus*, and the Chinese major carps, but these are sub-tropical species. There are no published reports of its use to induce breeding in the several marine species being cultured experimentally in the UK.

Although widely used in North America, HCG has no market authorisation there; and concerns have been expressed about the consumer safety of using a human hormone in food-fish. Apart from the fact that broodstock are rarely, if ever, food-fish, it has been shown that HCG disappears extremely rapidly from cooked fish flesh.

On the assumption that pituitary gonadotrophins may be species-specific in their chemical composition, some workers have experimented with the use of homologous pituitary extracts in breeding induction. It appears that gonadotrophins of fish origin are much more potent in fish than those of mammalian origin, such as HCG, but that fish gonadotrophins are not species-specific in potency. A partially purified salmon gonadotrophin has been produced commercially under the name SG-G100, and it is rather more potent than CPE.

Gonadotrophin-releasing hormones

There are three GnRH substances in routine use in the induced breeding of cultured fish, namely:

sGnRH – salmon gonadotrophin-releasing hormone

sGnRH-A – salmon gonadotrophin-releasing hormone analogue

LHRH-A – luteinizing hormone-releasing hormone analogue

The relative potencies of these vary with fish species. As might be expected, sGnRH-A is highly active in salmonids; it is also highly active in other cultured fish species and is about 10 times as potent as LHRH-A in the common carp and goldfish, *Carassius auratus*. LHRH-A is also used in mammals and this makes its synthesis and use in fish economic.

Domperidone has little activity by itself in fish but it potentiates GnRH and its analogues. Domperidone is available as a 0·1% injectable suspension for fish in some parts of the world, but of more importance is a combination of it with GnRH-A available commercially in Canada (Ovaprim®, Syndel Laboratories) which has been imported illegally into the UK for use in carp. Combined use of a GnRH analogue and a dopamine antagonist has been called the 'Linpe' method of breeding induction after its developers, Lin and Peter (Crim and others 1988). It is particularly useful in cyprinids where previously CPE or HCG were used. The advantages of the 'Linpe' drugs are known potency leading to accuracy in dosage, and greater stability on storage.

The regulatory position

The following substances have been assigned to Annex 2 of Regulation 2377/90/EEC, *ie* substances for which an MRL is not required:

- follicle stimulating hormone (natural FSH from all species and their synthetic analogues)
- human chorionic gonadotrophin (natural HCG and its synthetic analogues)
- luteinizing hormone (natural LH from all species and their synthetic analogues)
- gonadotrophin releasing hormone

The following points should be noted:

1. The two active ingredients of CPE and CPH are in Annex 2 but these glandular products are not.
2. While synthetic analogues of pituitary hormones are in the Annex, there is no mention of synthetic analogues of GnRH.
3. Domperidone is not in any Annex.

Thus, with the exception of HCG, there is no reproductive hormone likely to be of use in fish which is the subject of an MA, or even in an annex; and the efficacy of HCG in fish species cultured in the UK is unproven.

If the aquaculture of food-producing fish other than salmonids is to be developed there must be a legal means of making reproductive hormones available, and the first hurdle is Regulation 2377/90/EEC. The simplest approach would be to declare broodfish of all cultured species to be non-food producing animals — this does happen to be true! However, on the assumption that such thinking will be too radical and progressive for the EU Commission, the next approach would be to include fish pituitary glands and extracts of them, and possibly GnRH-A, in Annex 2.

Broodfish of all cultured species should be declared to be non-food producing animals for the purposes of Regulation 2377/90/EEC. Failing this, fish pituitary glands and extracts of them, and GnRH-A, should be assigned to Annex 2.

Investment is required for:

1. Research into the metabolism of domperidone and the toxicology of it or its major metabolites. This is with a view to obtaining an MRL for it on the assumption that broodfish cannot be declared non-food producing.
2. Research into methods of breeding induction in any species being experimentally cultured in the UK.
3. The preparation and submission of applications for MRLs and MAs as appropriate.

Very substantial sums of money are involved and they could not be afforded either by a fish farmer or a pharmaceutical company. They are, nevertheless, necessary if aquaculture is to develop in the UK (and EU).

Summary of recommendations

European Union Law

- broodfish of all cultured species should be declared to be non-food producing animals.

- failing this, fish pituitary glands and extracts of them, and GnRH-A, should be assigned to Annex 2 of Regulation 2377/90/EEC.
- legal accommodation must be made for the medicinal use of formalin, malachite green and chloramine-T in fish, or for licensed alternatives to be made available.
- consideration should be given to banning the use of potentiated sulfonamides in fish genera in which synergy has not been positively demonstrated.

Other recommendations

Research should be sponsored into:

- the metabolism of domperidone in fish, and the toxicology of it or its major metabolites. This is with a view to obtaining an MRL for it on the assumption that broodfish cannot be declared non-food producing.
- methods of breeding induction in any species being experimentally cultured in the UK.
- development of a formulation of praziquantel for administration to fish by immersion.

Public funding should be provided for the preparation and submission of applications for MRLs and MAs as appropriate for fish medicines. These might well include florfenicol premix and injectable erythromycin.

In the development of any code of practice for the use of fluoroquinolones in veterinary medicine, the use of sarafloxacin in farmed salmon should be specifically addressed.

The right of an environmental authority in the UK to demand environmental safety data for a medicinal product which is the subject of a market authorisation should be statutorily defined.

If environmental authorities are given such rights it should be a statutory offence for them to disclose the data to another person.

The British veterinary profession should exert some moral pressure to encourage the use by research institutes of authorised medicinal products.

Further reading

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Keith Treves-Brown graduated at Cambridge in 1961. After a brief spell in large animal practice he joined The Wellcome Foundation Ltd and worked in various marketing appointments for nearly 23 years. In 1987 he was appointed to the Medicines Unit of the Central Veterinary Laboratory, Addlestone which became the Veterinary Medicines Directorate. In 1991 he was given specific responsibility for fish pharmaceuticals. He retired at the end of 1994 but has continued his interests in fish medicines and has written a book on the subject.

This article is a modified version of one submitted to the Medicines Select Committee of the British Veterinary Association in February 1999. The original version was written in response to the invitation in the *Veterinary Record* (**144**, p130) of 30 January to submit written evidence to the Committee which had a remit 'to review medicines policy ... and to report to Council on any steps that could be taken to introduce greater flexibility into controls while accepting that protection of public health is paramount'.

Fish Veterinary Journal (1999) (4) 58–60

Infectious salmon anaemia in the United Kingdom: an update (June 1999)

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In early May 1998, infectious salmon anaemia (ISA) was confirmed in farmed Atlantic salmon (*Salmo salar*) on a marine site in Loch Nevis in the west of Scotland. During the summer and early autumn of 1998, further outbreaks of ISA were confirmed in Loch Nevis, Loch Greshornish on the Island of Skye, Loch Creran and Loch Linnhe in Argyll, and the Saint Magnus Bay area of the Shetland Islands. Additional marine farms were also placed under official suspicion of infection on the basis of results obtained during the ISA surveillance visits carried out by inspectors from the Fisheries Research Service (FRS), on behalf of the Scottish Office, Agriculture, Environment and Fisheries Department (SOAEFD).

During this period, strong circumstantial evidence suggested that the ISA virus had probably been spread from the original outbreak in Loch Nevis to all subsequently confirmed and suspected sites. Movement of live fish, boat movements and transfer of equipment or personnel appeared to be the most likely means of dissemination of infection. In December 1998, four new sites were placed under suspicion. Of these, a site in the Burra area of Shetland may have been exposed to untreated effluent discharged following processing of infected fish from Saint Magnus Bay. However, the route by which infection may have reached the remaining sites, two in Loch Linnhe and one in the north west of Skye, is less clear.

In April 1999, a further farm just north of Burra was declared suspect and ISA was confirmed there in late May. Although other farms in this area have not yet come under suspicion, it is possible that boat traffic and tidal flows may have allowed the ISA virus to spread from the originally suspect Burra site lying to the south. A more remote site in the Outer Skerries, Shetland was also declared suspect at this time and there is presently no information available to suggest how the ISA virus might have reached this area.

At the time of writing in June 1999, a total of eleven farms in Scotland have been declared infected with ISA and the stocks removed. A further eighteen

farms are suspected of being infected, of which ten are still in production. An epidemiological investigation into the outbreak of ISA in Scotland is still being carried out by the FRS, but the source of infection for the first confirmed case of ISA in Loch Nevis remains unknown.

ISA is a List 1 notifiable disease under European law and, with evidence of a point source of infection and links at least between the confirmed sites, SOAEFD have applied the legislation rigidly, in accordance with their obligation to eradicate the pathogen. In addition to the immediate, compulsory slaughter of confirmed stocks, for which no compensation is available, confirmed and suspected sites are also required to undergo a mandatory fallow period of six months, which has caused significant disruption to smolt placements and production plans. Investor confidence in the Scottish salmon farming industry appears to have been badly shaken.

SOAEFD has attracted considerable criticism from some sectors of the industry, who feel that the present policy cannot be sustained in the current economic climate. The approach taken by SOAEFD is seen to be at variance with Norway in particular, where ISA has been recognised since 1984 and where workers feel that the virus is endemic in the wild and cannot be eradicated. The control measures developed in Norway are intended to eliminate ISA as an economically important disease. Similarly in New Brunswick, Canada, where the effects of ISA left salmon farming in crisis, the direction taken was clearly aimed at controlling the clinical disease, while attempting to retain a viable salmon farming industry.

During the first half of 1999, it has become clear that SOAEFD have begun to consider contingency measures which may need to be implemented should the present ISA outbreak not be eradicated. It has been suggested that relatively rapid policy changes could be made in the areas of diagnosis and in fallowing, should certain criteria be fulfilled which indicate that the current outbreak has spread more widely, or that the virus has become endemic. Confirmation of the suspected infection in the Outer Skerries, with no demonstrable links to other affected areas would increase the pressure on the government to adopt a more flexible approach to the disease. More fundamental changes in the official approach to ISA, including the possibility of vaccination, would require a change in the notifiable status of the disease and would take longer to implement. However, it does seem that some of the groundwork for such a change could now be laid in advance.

Footnote:
Another marine salmon farm, situated near Ullapool on the Scottish mainland, was placed under official suspicion of ISA at the end of June 1999. The means by which the infection might have reached this area is unknown at present, but possible sources are being investigated by the FRS. The FRS have also indicated that shared net-washing facilities and shared use of divers could have introduced the ISA virus to the remote Outer Skerries site in Shetland, which was recently placed under suspicion. While more evidence would be needed to confirm ISA at these sites and to prove that cross-contamination with other infected areas had occurred, the clear implication is that disinfection procedures and measures to isolate salmon farms are still inadequate to control the spread of infectious agents. SOAEFD are still of the opinion that the present ISA outbreak has had a single source and that site-to-site contact has been responsible for the subsequent spread of disease. Under these circumstances, eradication of the ISA virus will remain the government's policy.

Tom Turnbull graduated from Glasgow Veterinary School in 1981 and obtained an M.Sc. 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.

This paper is based on a presentation given at the spring meeting of the Fish Veterinary Society in Edinburgh on 29 April 1999 and updates an earlier article published in issue Number 3 of the *Fish Veterinary Journal*. It was submitted for publication on 25 June 1999.

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The role of biosecurity in disease prevention: a poultry primary breeding company perspective

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Introduction

The major objective of any primary breeding company is to create nucleus groups of selected pedigree stock that may be multiplied and crossed over several generations to produce large numbers of commercial product. In the chicken industry, a five-year period is required to move genetically from pedigree to great grand parent, grand parent, parent and broiler stock. The power of the multiplication pyramid is such that one pedigree cockerel is responsible for the genetic input to approximately 20 million broilers.

The consequences of disease entering a primary breeding programme may be considered from two perspectives:

1. Infectious disease challenging the population horizontally leading to elevated mortality and/ or impaired biological performance. This influences the ability of the company to select the most desirable individuals.
2. Infectious disease passing vertically through the generations leading to vast multiplication of infection and the potential dissemination of that infection over large geographical areas dependant upon the breeding company's customer base.

Before any biosecurity strategy can be formulated, an exact specification of product is required. This usually encompasses freedom from specific diseases plus a defined level of maternally-derived antibodies, protecting against infection from a further set of pathogens.

A breeding company will begin by locating its farming base in an area of low poultry density to minimise the risks of disease spread by aerosols. A minimum distance of two kilometres should separate all farms in order to prevent cross infections with airborne pathogens.

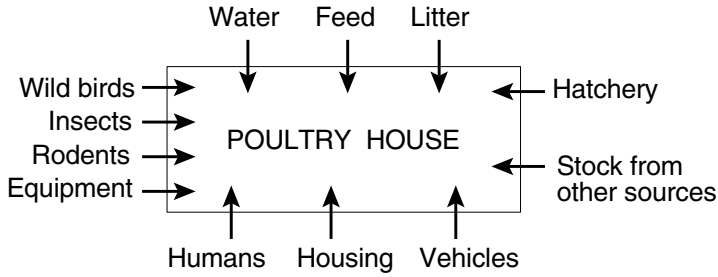

FIG 1: The potential sources of Salmonella in a poultry breeding unit

Although specific protocols may be adapted for certain diseases, a general approach may be designed by considering the infection model for *Salmonella* species. The policy of Ross Breeders Ltd states that no *Salmonella* serotype is tolerated. The possible sources of a *Salmonella* infection are illustrated in Fig 1.

Access to the poultry farm is restricted to those who have a genuine reason to be there. Examples of such personnel are farm staff, health monitoring teams, veterinarians, genetic selection team, company tradesmen and approved visitors. Movement books kept on the farm are required in order to trace personnel should any disease outbreak occur.

Whilst the visit of a potential customer is an important commercial consideration, it must not be allowed to compromise biosecurity. Visitors require advanced approval from management before entrance is granted. They must submit a stool (faecal) sample that tests negative for *Salmonella* isolation and not be in contact with livestock for the previous five days. Movements of the visitor are examined in a pre-visit questionnaire. Once on the farm, the visitor is required to shower and change into clothes, hat and shoes provided on the farm. There are two important points here:

1. Clothing should not leave the farm. If it is to be re-used, on-site washing facilities are required.
2. The use of the shower facility serves to ensure cleanliness and also delineate the start of the zone of elevated hygiene status. The showers must be in good order and comfortable to use. Staff and visitors alike will avoid the use of cold showers if given the chance. In some areas of

the world where education of farm workers in the principles of biosecurity is difficult, plunge pools are used. In this situation, a barrier is placed across a pool of water located after the changing facility. The only way to enter the farm is to completely submerge to pass under the barrier.

Since company farm staff will inevitably have access to the stock more often than external visitors, their on-going biosecurity is extremely important. Employment contracts place restrictions on the ownership of birds. Every member of staff who has regular contact with birds must submit a stool sample for *Salmonella* isolation once per week. They must report any gastro-intestinal sickness and access to farms is prohibited until a negative *Salmonella* test result is acquired. Foreign travel should be notified to the veterinary department and a further stool sample submitted on return before any farm or hatchery visit can be considered. These requirements do place restrictions and operational difficulties on staff movements but are essential if any trading guarantee on *Salmonella* freedom is to be honoured.

Animal feed is one of the major sources of *Salmonella* infection. The composition of feed and the testing of raw ingredients are an important part of any exclusion strategy. In this respect, Ross Breeders Ltd does not use poultry offal, feather meal or fats in their diets. This prevents the potential recycling of infected poultry products.

Sampling of raw feed materials is an integral part of the biosecurity protocol. Ross Breeders Ltd take 300 raw material samples per week and over the course of a year expect to see 4% positive *Salmonella* isolations. Hence all raw ingredients are assumed contaminated with *Salmonella* and extensive decontamination protocols have been developed for raw ingredient silos. Over the previous year, the five most common *Salmonella* isolations from UK raw feed ingredients have been, in descending order, *S mbandaka, S tennessee, S montevideo, S seftenberg* and *S binza*.

The use of a dedicated feed-mill with a heat-treatment facility is essential if *Salmonella*-free feed is to be produced. The system shown in Fig 2 follows the principles of a ‘contaminated-to-clean’ areas of the mill. There is a dedicated loading area used by dedicated vehicles that follow agreed route plans for deliveries. Feed-trucks should never drive past broiler farms on their way to pedigree facilities. Vehicle hygiene is monitored weekly using bacteriological techniques and staff knowledge of biosecurity must be

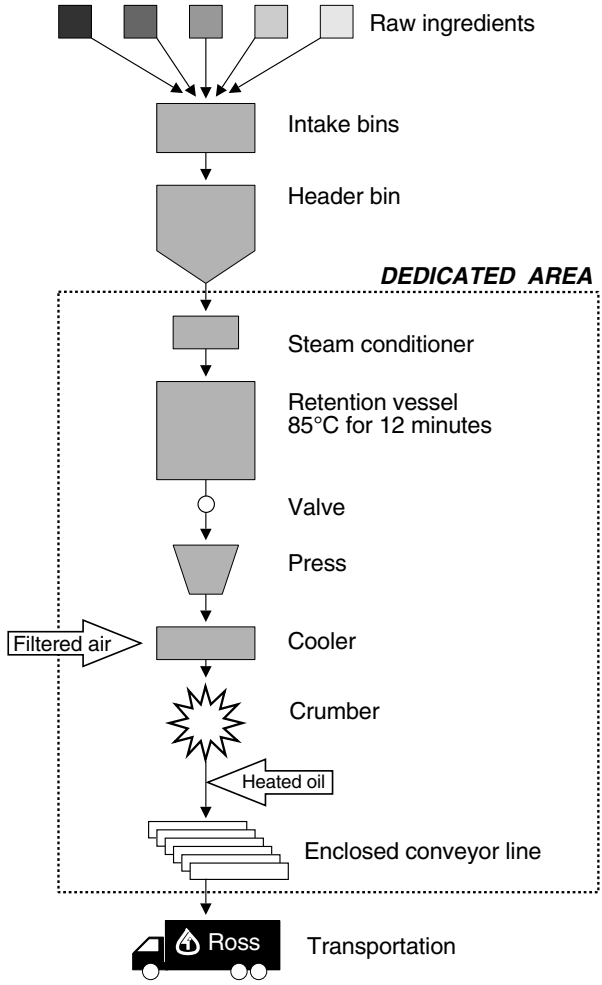

FIG 2: The diagrammatic layout of a dedicated poultry feed-mill

constantly reinforced. The most important part of the process revolves around the holding of all the feed at 85°C for 12 minutes. The exact temperature and time required are derived around the need to remove all traces of *S seftenberg* (the most heat-resistant *Salmonella*) with a high safety margin.

Short-chain organic acids (typically BP Bioadd® at 0·55% final inclusion) provide residual activity against *Salmonella* in the finished feed. The chicken is also able to metabolise these as an energy source and this is reflected in the feed formulation.

Farm staff must be aware of the constant risk posed by rodents, insects and wild birds. Pest control is an important part of any farming operation whilst the use of foot dips, clean boots and closing doors can prevent any wild birds or their droppings gaining access to the shed.

The movement of fomites between facilities should be discouraged and all such objects must be monitored bacteriologically to prevent transfer of any infection.

***Salmonella* Isolation**

The efficiency of detection of *Salmonella* in the laboratory is central to the running of the overall process. Ross Breeders Ltd use a dual isolation technique involving pre-enrichment in buffered peptone followed by the Rappaport and selenite techniques. Membership of the UK Poultry Health Scheme requires the use of the Rappaport technique. However, this is specifically targeted at *Salmonella pullorum* and *Salmonella gallinarum*. The reliability of the technique for detection of all *Salmonella* species is reinforced by the use of two methods to maximise detection of the more exotic serotypes commonly found in feed.

Testing takes place at both farm and hatchery levels. Following depopulation at the farm and thorough disinfection, swab samples are taken from feed bins, all areas inside the poultry house and from the water system. Specific bacteriological targets should be set for these samples and three examples are:

1. House floor — 5000 colony forming units/ 100cm^2 total viable count
2. House wall — 500 colony forming units/ 100cm^2 total viable count
3. No *Salmonella* isolation

If a farm fails to attain hygiene targets, it must be re-cleaned and re- tested.

At the hatchery, samples are taken from every order on all hatch-days from each source-flock. Bacteriological culture is attempted from plenum (fluff),

hatch-tray paper liners and cull chicks together with vaccine squirt and environmental samples taken throughout the hatchery.

At farm level, birds are constantly monitored to ensure that any breaches of biosecurity are detected as soon as possible. This involves taking swabs from the vent every six weeks, together with 'dust and drag' swabs every three weeks. All samples are cultured as described previously. This results in some 50,000 bacteriological samples per month.

Conclusion

Biosecurity provides the primary breeding company with a major barrier in the battle to prevent the dissemination of infectious disease both horizontally within a breeding operation and vertically down the breeding pyramid. The successful implementation of such a biosecurity programme requires commitment from senior company management together with a willingness to learn and implement new protocols at farm level. Adopting such an approach provides the customer with a product of superior health status and assists in providing the end consumer with a product compatible with the highest standards of food safety.

Richard Currie obtained a BSc in veterinary anatomy and physiology, and later, a PhD based on 'post translational control of luteinizing hormone secretion' which was intercalated between the third and fourth years of the veterinary curriculum. He finally obtained his veterinary degree from Edinburgh in 1996. He has worked with Ross Breeders for three years, initially as veterinarian responsible for the pedigree breeding programme and for the last year, as European veterinarian and regional technical manager responsible for customer health and technical services. His specialist research interests include avian cardiology and fertility.

This paper is based on a presentation given at the spring meeting of the Fish Veterinary Society in Edinburgh on 29 April 1999 and was submitted for publication on 23 June 1999.

The Fish Veterinary Society are grateful to the sponsors of the last scientific meeting held at Ross Breeders near Edinburgh on 29 April 1999.

Ross Breeders Ltd., Newbridge, Midlothian, Scotland EH28 8SZ
for providing the venue and lunch.

Intervet Norbio

Intervet Norbio AS, Thormøhlensgate 55, N-5008 Bergen, Norway
for sponsoring the evening meal.

The Society also wish to acknowledge the sponsors of previous meetings:

12 November 1998	at **Veterinary Laboratories Agency**, Penrith **Grampian Pharmaceuticals Ltd**, Leyland
23 April 1998	at the Queen's Moat House Hotel, Edinburgh **Alpharma**, Skøyen, Norway and **Vetrepharm Ltd**, Fordingbridge
28 November 1997	at Royal College of Veterinary Surgeons, London **Vetrepharm Ltd**, Fordingbridge
25 April 1997	at Ewos Technology Centre, Livingston **Ewos**, Livingston and **Novartis Animal Health UK Ltd**, Cambridge
27 September 1996	at the BVA Congress, Chester **British Veterinary Association**, London

Salmon Health Group

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The presence in Scotland of infectious salmon anaemia (ISA) has dominated the working lives of FVS members with significant involvement in Scottish salmon farming. Since May 1998 when the first case was diagnosed, the situation has evolved, often fairly rapidly, and frequent communication amongst us all has become necessary. A number of significant professional issues have been brought to the fore which are relevant beyond the confines of farmed salmon and indeed extend into many areas of veterinary medicine.

Since FVS meetings are only held twice yearly at present and most members will have just a passing interest in this area, I decided to form an informal group under FVS auspices to meet on an *ad hoc* basis in Scotland for those specifically with interests in salmon. The Salmon Health Group (SHG) first convened on 24 February 1999 and has ten members so far. The first meeting concentrated on ISA and produced a number of actions. A number of us subsequently met with Leslie Gardner, Assistant Chief Veterinary Officer in May to discuss the position of the State Veterinary Service in relation to notifiable diseases of fish, and this was followed by a meeting in June with Keith Baker, president of British Veterinary Association, on the issue of fish and the Veterinary Surgeons Act. An FVS policy position on this matter was produced in March 1992 and the BVA published its policy in the *Veterinary Record* on 10 August 1991. Our next action is to meet with with the Fisheries Research Service to discuss areas of mutual interest in relation to ISA and to farmed fish health management generally.

Membership of the SHG is open to any FVS member and anyone interested should contact the honorary secretary with their details and preferably an email address since this is the primary method of communication within the group. I hope that in the future, updates on SHG activities can be posted on the FVS web site, **http://www.greens.net/fishvet**. In any event, minutes of all meetings are copied to the FVS committee who will ensure that matters of general interest and importance are placed before the membership.

Fish Veterinary Journal (1999) (4) 69–70

Institute of Fisheries Management (IFM)

Fish Disease Discussion Group
Wednesday 10 March 1999

The inaugural meeting of this IFM Fish Disease Discussion Group was held at the Environment Agency (EA), National Fisheries Laboratory, Brampton in Cambridgeshire. It was attended by over thirty people and included representatives from various universities, the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), the EA, the IFM together with independent fish health consultants and veterinarians.

This group has been formed to bridge the gap between academia and business, using a discussion forum to facilitate the spread of ideas and information. The group will use this forum to identify problems and seek to resolve them by lobbying appropriate government and non-government organisations.

John Gregory, chairman of the IFM, initially chaired the meeting until the group committee was elected:

Chairman — David Bucke (consultant fish & shellfish pathologist)
Secretary — David Hawkins (fish health officer, EA, Brampton)
Press Officer — Sarah Chare (fisheries officer, EA, Brampton)
Committee Members — Steve Feist (pathologist, CEFAS, Weymouth)
David Hoole (parasitologist, Keele University)
Bernice Brewster (aquatic consultant)
Ian Wellby (fish health officer, EA, Brampton)
Peter Scott (Zoo & Aquatic Veterinary Group)

David Bucke chaired the meeting for the rest of the day. It was decided that the 'terms of reference' for the group would be:

1. To establish and maintain an appropriate discussion forum for the exchange of ideas and information. This will take the form of an e-mail bulletin board.
2. To identify problems affecting fisheries which relate to fish health and lobby appropriate organisations.
3. To provide comment and information to the IFM council in respect to its role as a consultee on fisheries issues.

4. To raise the profile of fish health issues and disseminate this information.
5. To promote the technical expertise and development of fisheries practitioners in relation to fish health issues and encourage continued professional development.

The group will aim to meet twice per year. The main meeting will be held at a central location in the spring while a second meeting will take place at the IFM study course in the autumn. The next IFM conference will be held at Sparsholt College near Winchester on 14–16th September 1999.

At this first meeting, a round-table discussion produced a list of fish health issues that might be discussed by the group which included:

- the IFM submissions to the Committee for the review of fisheries legislation and policy on 'introductions and transfers'
- the present legislation governing fish introductions into natural waters
- the fish health implications regarding the present trend for stocking large numbers of fish into inland stillwaters
- fish welfare concerns and fisheries management techniques
- the *Import of Live Fish (England and Wales) Act 1980* and the ecological issues related to this legislation
- *Gyrodactylus salaris*
- the potential threat to the UK fisheries from parasites and diseases currently in mainland Europe but not present in the UK
- nutritional problems affecting fish in stillwater fisheries.

Further details of the IFM Fish Disease Discussion Group can be obtained by writing to the secretary:

David Hawkins,
Environment Agency,
National Fisheries Laboratory,
Bromholme Lane,
Brampton,
Huntingdon,
Cambridgeshire PE18 8NE.

Editor's note: The meeting was attended by Fish Vet Society members, Edward Branson, Peter Scott, Chris Walster and William Wildgoose.

Fish Veterinary Journal (1999) (4) 71–74

Anaesthetic and Sedative Techniques for Aquatic Animals, 2nd edition

L.G. Ross and B. Ross (1999)
159 pages, hardback, £39.50
Blackwell Science Ltd, Oxford. ISBN 0 632 05252 X

If your approach to fish anaesthesia is like mine and you are a one-drug man (or woman), you may be wondering why you would want to buy this book. Indeed the authors wait until the concluding chapter before they discuss this very issue, although I had realised that my restrictive attitude had some limitations not long after I had opened this smart hardback.

The first edition was published by the Institute of Aquaculture, Stirling in 1984 following a presentation at the Veterinary Anaesthetists' Association meeting the previous year. It was originally based upon lecture notes and only had 35 pages with a few tables and rough line drawings. It sold out long ago and was in great demand — in fact, I was given a well-used copy by a koi-keepers' society. This new edition is a more polished product with professional graphics, clear illustrations and includes some black & white photographs. Much of the original text has been rewritten and expanded, with several new sections: an immense amount of research into fish anaesthesia has been published in the last decade. Despite the size of the subject, the authors have been selective and have successfully kept the contents practical, aiming their book at a world-wide readership in different areas of aquaculture and research.

There are several detailed contents pages and these are a good reference point, in many cases being of more use than the rather limited index. Most chapters have an introduction to the topic and conclude with a summary of important points and fully titled references. Following a brief introduction to related aspects of animal husbandry and pain in chapter 1, the second chapter discusses stress and the generalised stress response in detail, including physiological changes and effects of anaesthesia. Chapter 3 gives a general discussion on the nature of anaesthesia, sedation and analgesia, illustrating the wide scope of this book by including a brief mention of acupuncture and hypnosis. It is admitted that little is still known about the precise mode of action of anaesthetics in fish and invertebrates. The stages of anaesthesia are discussed and there is a brief comment on euthanasia.

The desirable features of anaesthetic agents and their legal use are described in chapter 4, with appropriate emphasis given to the legislative matters of food chain and environmental safety, animal welfare and experimentation. To illustrate the environmental issues, a recent survey is quoted, in which it was estimated that 2 tonnes of anaesthetic agents were used by Scottish aquaculture alone in 1992. The lack of approved fish anaesthetics in the UK and USA, and the commercial difficulties of licensing, is highlighted. Factors affecting the response of aquatic animals to anaesthesia is briefly discussed in the following chapter, explaining why some of my obese koi are slow to recover from fat-soluble agents, and why repeated anaesthesia is rarely a problem.

Chapter 6 is a new section and covers anaesthesia of aquatic invertebrates; mainly molluscs and crustaceans. Useful comments are given on a wide range of chemical agents that will give good results in a wide range of species. I now feel a little more comfortable about the not-inconceivable scenario where a spiny lobster that might appear in my consulting room requiring emergency surgical repair to its shell.

The next four chapters together form over one third of the book and discuss the procedures and drugs used in the anaesthesia of fish. Each chapter deals with different methods of anaesthesia namely, inhalation, inhalation using gases, parenteral and oral, and finally, non-chemical techniques. Of these, inhalation anaesthesia is the most detailed, with notes on all the drugs commonly used in practice, together with a method for artificially ventilated anaesthesia. The dose rates for a number of fish species are summarised in a table but it is emphasised that all drugs must be used carefully in unfamiliar situations. An extensive reference list is also given. A common problem in veterinary practice is that experienced by clients who wish to humanely euthanase their terminally ill pet fish. For various reasons they may not be able or willing to attend a veterinary surgery, and may not have access to fish anaesthetics. Clove oil, which may be purchased over the counter at most pharmacies, is a suitable agent and its anaesthetic use is discussed.

Inhalation anaesthesia using carbon dioxide is described, but also mentions the debate over the nature of the anaesthesia and analgesia produced during harvesting of salmon. Although the action of sodium bicarbonate is detailed, there is no mention of Alka Seltzer® which has been used by biologists working in the field and periodically appears in the fish keeping hobby

press. Similarly, fluorinated hydrocarbons such as halothane and methoxyflurane are included but isoflurane is omitted — it has been suggested that the latter may be of more use to veterinarians in general practice where the standard fish anaesthetics are not stocked.

There are limitations in the use of parenteral anaesthetics: fish must be sedated by inhalation to allow accurate weighing and calculation of the dose of the injectable drug. However, they avoid the need for a recirculating anaesthetic system and allow longer procedures to be performed. There is even mention of local anaesthetics given by intracranial injection and their use for spinal anaesthesia! Several commonly available veterinary products are described but the authors emphasise that the effects of some additives in fish is unknown.

Practical, non-chemical methods include hypothermia and electroanaesthesia. The former however, only immobilises the fish and does not produce true anaesthesia or analgesia and its use is therefore limited to calming fish for handling and transportation. Electroanaesthesia, on the other hand, has great potential in many circumstances and several pages are devoted to the theory, the equipment and the physiological effects of this topic. The authors feel that there is much scope for further work in this promising area and that this could be the method of choice for anaesthesia of air-breathing fish species.

Chapter 11 is another new chapter and covers anaesthesia of amphibians and reptiles. There are useful details about different agents and dose rates are summarised in two tables. However, the veterinary care of reptiles has advanced rapidly in recent years and the information presented here is rather dated, as shown by most of the references. Intravenous administration of propofol using the tail or jugular vein is considered the drug and routes of choice, followed by isoflurane inhalation where required. The use of hypothermia is also considered unethical.

Transportation and the role of anaesthetics is discussed in a further new chapter. It reviews the physical and biological problems associated with the transport of fish, and basic transport techniques are described. Various chemicals have been added to the water in order to maintain good water quality in containers and recently there has been interest in the protective effects of polyvinyl pyrolidone (PVP). The use of chemical sedation has

been shown to be of great benefit during transportation and the drugs used on invertebrates and fish are summarised.

The final chapter concludes briefly with remarks to persuade the reader to consider the various anaesthetic options available and invites comments about individual experiences. A final interesting tip is that, in emergencies, vodka or gin can be used with benzocaine to overcome solvent problems. There are two glossaries containing data on the common anaesthetic drugs and technical terminology used in the book.

As a veterinary surgeon in general practice, I am guilty of using only one fish anaesthetic. However, it is currently the only licensed product in the UK and professionally we should be aware of the implications of the *Medicines (Restrictions on the Administration of Veterinary Medicinal Products) Regulations 1994*. In addition, most of my patients are unhealthy and the anaesthetic risks are greater: there is a lot to be said about being totally familiar with the subtleties of your drug when dealing with a wide range of species under varying circumstances.

The authors state on more than one occasion that 'there are many disasters, unreported of course, which occur as workers rush headlong into untested methods'. In the veterinary profession, fish anaesthesia should not produce a mortality rate that is any worse than with anaesthesia of any other animal. It is hoped that the clarity and detail in this book avoids further unnecessary disasters since it is full of practical information, drawing together much of the data available on anaesthetic agents used on aquatic animals. It is a great improvement on the first edition and I would strongly recommend the book to everyone involved in this area of work.

William H. Wildgoose

Anaesthetic and Sedative Techniques for Aquatic Animals *is normally priced at £42.00 (£39.50 + postage), but readers can order it at the discounted price of £32.13, inclusive of postage and packing. Telephone Blackwell Science on 01865-206086 to place your order, quoting* Fish Veterinary Journal Offer *to claim your discount.*

Fish Veterinary Journal (1999) (4) 75–78

Colorguide of Tropical Fish Diseases: on freshwater fish
Gerald Bassleer (English, 1997)
272 pages, hardback, £20.96
Bassleer Biofish n.v., Stationsstraat 130, B-2235 Westmeerbeek, Belgium (tel: 00 32 16 69 6839 fax: 00 32 16 69 6831)

There are two major obstacles involved in the health care of tropical fish namely, size and value. Many of the common species are so small that in order for a lesion to become visible to the naked eye it is often enormous in proportion to overall size of the fish. Histological section of the whole body is sometimes the only practical method for post mortem examination, and methods of administering treatment are limited. The destruction and replacement of the individual is often cheaper than the cost of veterinary investigations, however, the financial and emotional value of the whole community tank is often significant.

This book aims to educate the hobbyist by providing a detailed description and clear summary of the important diseases in tropical fish, and offering advice on their treatment. Most of the book is suitable for the hobbyist but some areas of disease investigation require skill and experience: this includes identifying pathological lesions, performing routine post mortem examination and using the microscope to examine scrapes, tissue squashes and blood samples. It does not aim to cover all diseases but attempts to highlight the most common problems. The book is divided into distinct sections described as chapters in the text although not actually marked as such on the relevant pages.

The colorguide was originally published in German and Flemish in 1983, and has been translated and updated in 1997. This hardback with its attractive durable cover is packed with almost 400 colour photographs and line drawings to illustrate the subject.

The first chapter briefly discusses a few factors associated with diseases of fish and includes water quality, stocking density and nutrition. Although these factors and their role in disease is indicated, health problems directly due to these factors are not covered, presumably because cases of nutritional deficiency, gas bubble disease and congenital abnormality are not common. New fish should be quarantined and, according to the author, it is

recommended that a 'shotgun' treatment with a cocktail of nifurpirinol, formaldehyde, malachite green, methylene blue and metronidazole is used as a bath for two days.

The second section, Chapter B, briefly describes methods of examination and the associated terminology. The clinical signs and behavioural changes are explained and illustrated in photographs. Euthanasia of fish using an anaesthetic overdose and cervical dislocation is described. The microscopic investigations discussed include examination of skin and gill scrapes, and other samples taken during autopsy. The author states that it is essential for breeders, dealers and hobbyists to be able to use a microscope although instructions are not covered here. Unfortunately, during translation, the pancreas has been wrongly identified on the dissection diagram and should presumably be either the gall bladder or spleen.

Chapter C is a substantial discussion of the most common diseases of tropical fresh-water fish. Diseases are classified into viral, bacterial, fungal and parasitic infections. The latter form the bulk of this chapter, occupying 60 of the 82 pages of this section which deals entirely with infectious agents: there is no reference to neoplasia or other non-infectious diseases. The author gives us the benefit of his experience and comments on the frequency of some parasites whereas most hobbyist books are only too keen to list every known parasite and give no guide to their prevalence or difficulty in treatment. A few spelling errors (*Livoneca* for *Lironeca*) and the use of some uncommon names (*Ichthyosporidium* for *Ichthyophonus*) had me searching my other text books for information on these unfamiliar parasites. However, I failed to identify *Tetraonchidae* species, *Protopalina symphysodonis* and one of the 'best known nematodes', *Spirulina* species.

Chapter D fills almost half the book and gives an overview of common diseases in different species of tropical fish. The author has restricted himself to the 50 commonest fish species examined during a 20 year period up until 1997. Here he demonstrates the sheer wealth of his experience in this field by the vast numbers of fish involved. Interestingly, he starts with a comment on the differences between fish that have been bred in captivity and those caught in the wild. He finds that wild caught fish with bacterial disease respond more easily to antibiotic treatment than those that are reared in tanks and often have antibiotic resistant strains.

This unusual presentation of diseases may appear repetitive but there are subtle differences between the species which justifies this approach. There is an attempt to identify the most common and less frequent disease problems in each species and more usefully, give personal experience on species' sensitivities to medications. This is only a rough guide since the relative numbers of fish are not quantified and it may suggest incorrectly to the hobbyist that these are the only diseases which a particular fish may experience. The species of fish are listed by their Latin, scientific name but there is also a useful register of species to help find their common names. There are many interesting comments here: 'black stripe disease' was a term new to me and is used to describe a clinical sign associated with *Cryptobia* infection in Malawi cichlids.

Finally, chapter E, contains 18 pages on medicines used for the treatment of fish diseases. Various routes of administration are discussed but the author recommends using a permanent bath medication. Injections are considered a specialised procedure and are not discussed here. Although there is a passing reference to the zoonotic potential of *Mycobacterium* species, the accompanying photo is poor and unhelpful. There is further comment on the use of antibiotics and their indiscriminate use which has resulted in bacterial resistance to tetracycline, nitrofurazone and nifurpirinol.

There are some nuggets of information in the monographs on individual medicines. Under chloramphenicol it simply states in bold type that 'it is forbidden to use this antibiotic for treatments (*sic*) of animals'. Also, 'nifurpirinol is only available at your aquariumshop'. Itraconazole is suggested as a treatment for *Ichthyosporidium* (*Ichthyophonus*). FMC is a mixture of formaldehyde, malachite green and methylene blue developed by the author in 1978 and has an effect on a wide range of fungal, parasitic and bacterial diseases. Metronidazole, in the author's opinion, is more effective than dimetridazole for treating *Hexamita/Spironucleus*. Hobbyists excel at finding bizarre chemicals to add to fish tanks and diminazenacuterate *(sic)* to control external flagellates such as *Cryptobia* will certainly have you reaching for your medical formulary.

There is a bibliography containing 47 references and these have been divided into three categories according to level of expertise required and their degree of application to tropical fish. Most of these are from the 1970s or earlier, with only three published in the last decade.

The author concludes with some positive remarks about the future and suggests that the importation of tropical fish into every country should be supervised by specialists to help reduce the average mortality rate (up to 20% dead on arrival) to a more acceptable level of between 0·5% and 3%. He hopes that his book contributes to the welfare of tropical fish and urges co-operation at an international level.

It is unfortunate that the grammar and sentence structure were not improved during translation since this has resulted in text that has lost some of the subtleties of language and now appears rather stilted and distracting. Some expressions such as 'turbidity of the skin' do not immediately suggest 'excess mucus' or 'slime disease'. The spelling used in the book suggests that it is aimed at the American market. Some of the units of volume have been converted from litres into gallons, but it is only in a small note at the very end that it is made clear that these are US gallons (3·8 litres) and not imperial or UK gallons (4·5 litres)!

Although the photos are a good size (60mm × 100mm), it is difficult to visualise some lesions and the use of arrows would help enormously. Many of the fish are small but there is no guide to scale on the gross photos. The photomicrographs would have been improved by removal of the condenser to avoid the large black areas around the field of view. Although there are a vast number of photos, only a few are repeated in different sections of the book. However, it is sometimes difficult to see the benefit of seeing so many photos of, for example, fin-rot in different species.

Overall, this is a compact and useful book. It summarises experience gained over many years of working in this field and presents it in a simple manner. Although it is not intended for veterinarians and other professionals, there is much detail and information here for all those involved in the health care of tropical fish.

William H. Wildgoose

The names of local suppliers of this book can be obtained from the UK distributor, Tropical Marine Centre, Hertfordshire, tel: 01723-284151

Self-Assessment Colour Review of Ornamental Fish
G.A. Lewbart (1998)
192 pages, softback, £18.95
Manson Publishing, London. ISBN 1 874545 81 2

I came to this little paperback book having spent the greater part of my professional life immersed in farmed Atlantic salmon. It is refreshing and informative to be taken on a clinical tour amongst other species in a highly visual and accessible fashion.

The book opens with a comprehensive table of imperial/metric conversions and a list of common and Latin names for ornamentals; both very helpful to the reader new to the field. The author states in the preface his objectives, stressing the emphasis on clinical realism and the value of this approach becomes clear as you progress through the book. What is most immediately striking is the very high quality of the illustrations, mostly in colour and high definition; indeed the first patient presented is a most beautiful specimen with the clinical condition clearly visible. Visual information is often the the most valuable in solving problems in fish medicine since history is often poor or non-existent and the patient in an advanced state of decomposition!

The format of the book is question and answer paired on opposing sides of the same page to discourage cheating. The questions and illustrations give plenty of clues, and answers extend beyond the immediate solution to the problem, to include a wealth of additional information from basic husbandry and therapy to advanced imaging techniques. There is a comprehensive index which is arranged according to question and answer numbers rather than page number.

The author has managed to compress an enormous amount of knowledge and experience into a relatively small space in a way which should encourage the average practitioner to tackle unfamiliar species with confidence. I recommend this book to anyone with an enquiring mind and a willingness to learn about what is a fascinating area of veterinary medicine.

Andrew N. Grant

The publishers are pleased to offer this book to readers at the discounted price of £17.00 (inclusive of postage and packing) by phoning Manson Publishing on 0181-905-5150 to place your order, and quoting the Fish Veterinary Journal *to claim your discount.*

SPONSORS PAGE

The Fish Veterinary Society would like to thank the following companies who have generously contributed to the cost of publishing this Journal.

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Eastern Way, Bury St Edmunds, Suffolk IP32 7AH
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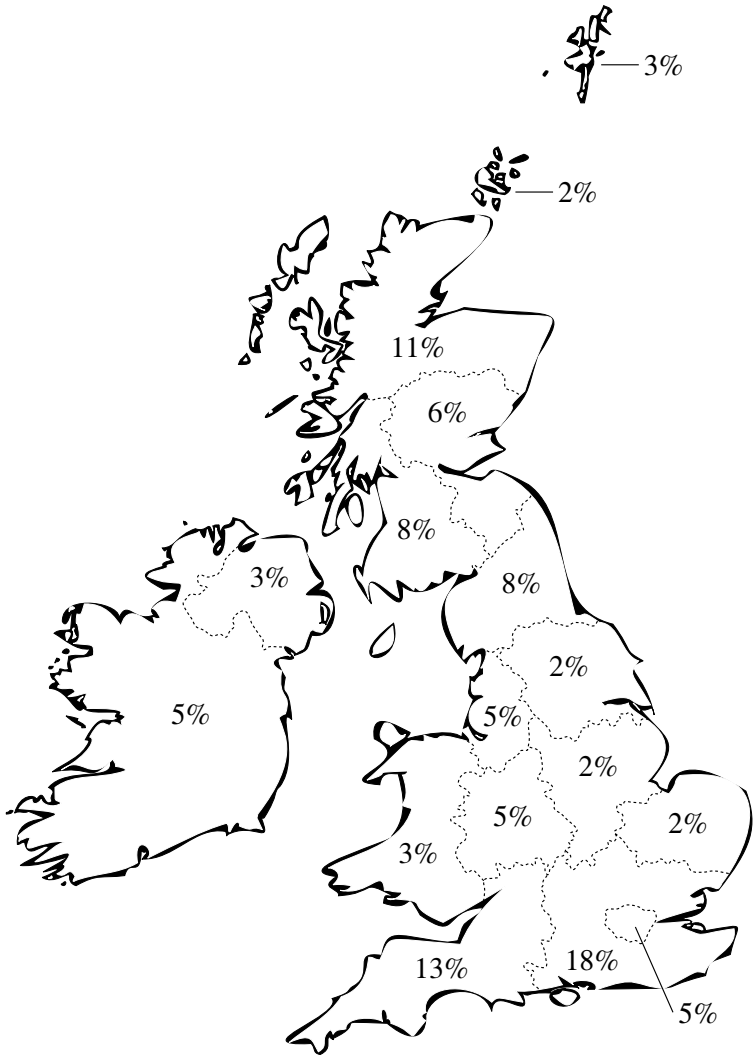
The Fish Veterinary Society would like to thank the following companies who have generously contributed to the cost of publishing this Journal.

Fish Veterinary Journal (1999) (4) 82–84

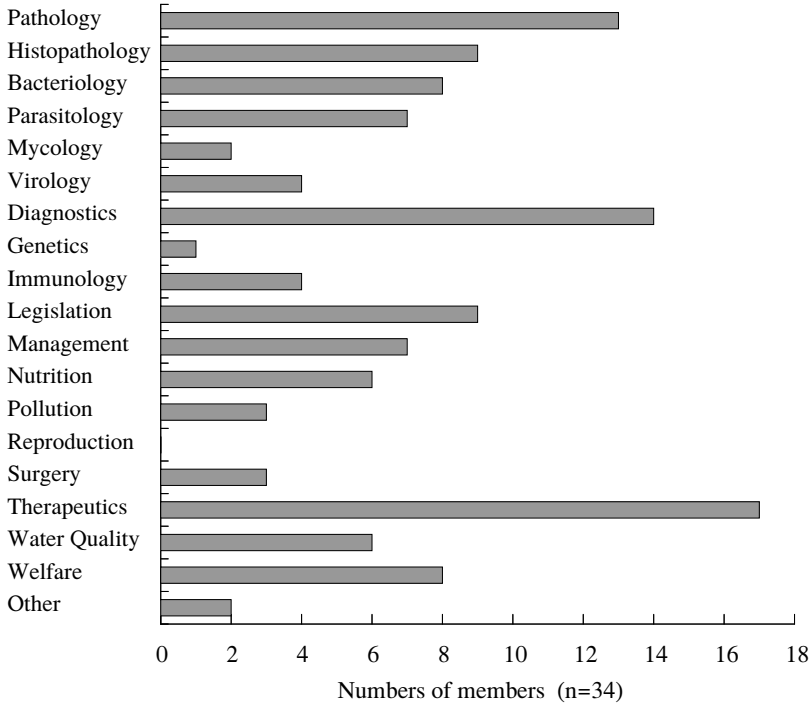
MEMBERSHIP ANALYSIS

This map shows the distribution of the current membership (69) in April '99 but does not include the five student members or the two individuals in the Channel Islands and the USA.



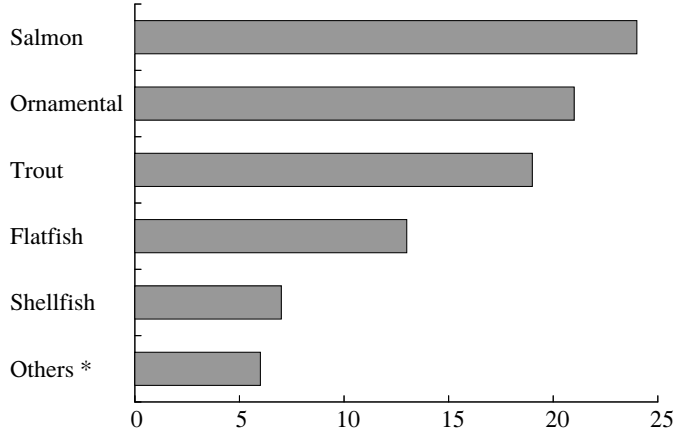
The following pages contain a simple analysis of the membership database held by the Fish Veterinary Society in April 1999. The information was submitted by 34 members, and represents 50% of the current membership of the Society. Some members have multiple interests in each category.

Areas of Interest



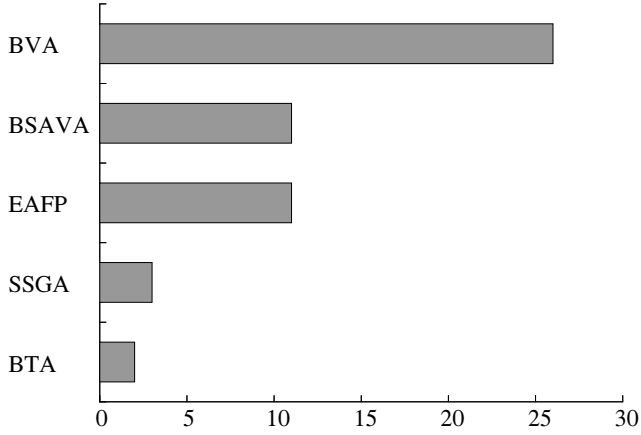
*Other specific areas of interest included ophthalmology and food hygiene

Species of Interest



*Other specific interests stated include all fin-fish, all farmed species, marine species, sea trout and sharks.

Membership of other organisations



BVA = British Veterinary Association
BSAVA = British Small Animal Veterinary Association
EAFP = European Association of Fish Pathologists
SSGA = Scottish Salmon Growers Association
BTA = British Trout Association

Fish Veterinary Journal (1999) (4) 85–86

Constitution of the Fish Veterinary Society

A. The Society should be called the Fish Veterinary Society

B. The purpose of the Society is to be a forum for professional and scientific discussion of fish health care and problems. An important function will be the exchange of information between Members

C. The Society shall consist of a Council and Elected Members

D. The Council shall consist of:

President – who shall preside at all meetings, or shall nominate an alternative if he is unable to attend

Honorary Secretary – who shall keep the minutes of, and be responsible for the safe custody of the Society's records. He will present a report at the Annual General Meeting (AGM) and be responsible for the organisation of meetings

Honorary Treasurer/Membership Secretary – who shall receive all monies on behalf of the Society, pay any expenses authorised by Council, and keep appropriate accounts. These will be audited and reported to the Annual General Meeting

Publications Officer – who shall be responsible for developing and arranging publication of all literature to be published by the Society

Elected Member

E. Council shall be elected for one year at the Annual General Meeting by all the Members present. The incumbent President shall be allowed to stand for re-election, but shall not be allowed to remain as President for a consecutive period of more than two years. All other members of council will be allowed to stand for re-election without time limit

F. All Council Members will be full members of the Society

G. Council will meet at least twice a year, at meetings held immediately prior to the scientific meeting

H. All Members of the Society will be fully qualified veterinary surgeons. Veterinary students will be elected as Associate Members

I. Members will pay an annual subscription set by council and ratified by the Membership at the AGM. Where more than one veterinary surgeon in a group is a member, then a group membership can be paid, which will be set by Council and ratified at the AGM. The definition of a group is determined by Council. There will be a joining fee which will include the first annual fee which will be set by Council

J. New Members of the Society may join by application to the Honorary Treasurer subject to the requirement that all members be qualified veterinary surgeons. Honorary membership shall only be conferred by a quorum of Full Members at an AGM

K. Honorary Members will not pay a subscription nor have any voting rights

L. There will be at least two scientific meetings annually. Other meetings can be arranged if the Council consider it to be necessary

M. The Annual General Meeting will be held during one of the day scientific meetings. Only Full Members of the Society will attend Annual General Meetings. The Annual General Meeting will be held at the second (Autumn) meeting of the Society

N. Any changes to the Constitution must be decided by ballot at an AGM following notification to all members at least three weeks prior to that meeting

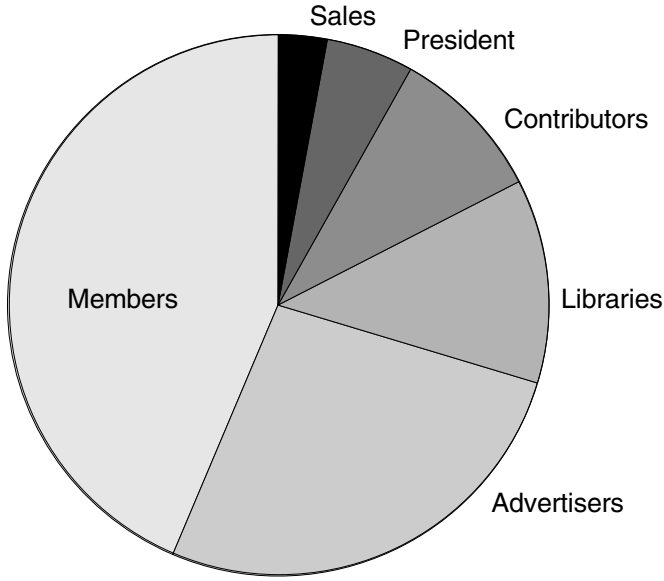
0. 33% of the Society or a minimum of 10 members shall be a quorum, whichever is smaller

This Constitution includes changes agreed at the Annual General Meetings of the Fish Veterinary Society on 14 October 1993, and on 4 October 1995.

A.N. Grant, President. April 1999

DISTRIBUTION ANALYSIS

This pie chart is a simplified account of the distribution of published copies of the Fish Veterinary Journal (Number 3).



The majority of copies (73) went to members of the Society who receive the Journal as part of their membership. Forty six copies went to advertisers, half of which were complimentary copies for companies that sponsored the issue, and the rest were sent some months later to others considered to be potential sponsors of this and future issues.

The Society are keen to circulate its scientific information and have ensured that complimentary copies were sent to 21 libraries at veterinary schools and other establishments involved in fish health. Other complimentary copies were sent to non-Society authors, reviewers and those associated with the publication, such as publishers of books reviewed in that issue. Several copies were given to the President for distribution to overseas fish veterinary societies and other related organisations. At present, only a small number of copies have gone to private sales.

BACK ISSUES

Copies of earlier issues, except number 1, are still available. These can be purchased using the order form overleaf, with preferential rates for packs of ten copies. The main contents of the last two issues are listed below.

Issue No. 2 (May 1998)

Management and control of proliferative kidney disease (PKD) in a freshwater Atlantic salmon (*Salmo salar* L.) farm in Ireland: a case history. *D.T.G. Quigley and J.F. McArdle*
Twelve month study of ulcer disease in a pond of koi carp (*Cyprinus carpio*). *W.H. Wildgoose*
Water quality and rainbow trout farming. *L.A. Kelly*
Meeting report
The Environment Agency and fish health: an overview. *A.G. Owen*
Zoonotic tuberculosis. *V. Blackwell*
Current overview of flatfish farming. *R.J. Slaski*
Rainbow trout fry syndrome: an update. *E.J. Branson*
RCVS certificate in fish health & production
Fish vets in cyberspace. *P.B. Green*
Book review:
Diseases in Marine Aquarium Fish (Bassleer 1996)

Issue No. 3 (February 1999)

Successful removal of a gastric foreign body from a red tailed catfish, *Phractocephalus hemioliopterus*. *W.H. Wildgoose*
Koi health care in the UK: a veterinary overview. *W.H. Wildgoose*
Preliminary investigations into the bacteriology of skin lesions of Atlantic salmon reared in seawater in Scotland. *L.A. Laidler, A.N. Grant & S. Wadsworth*
'Vibrio viscosus', the agent of winter sores. *E. Benediktsdóttir*
Parasites, resistance and control strategies. *M.A. Taylor*
Sea lice, medicines and a national strategy for control. *G.H. Rae*
Health care in a large public aquarium. *D.R. Gibson*
Sarafin®, a novel quinolone for bacterial disease. *P.J. Southgate*
Clinical observations of severe mortalities in koi, *Cyprinus carpio* with gill disease. *C.I.Walster*
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I wish to become a member of the Fish Veterinary Society, subject to the conditions governing the same as set out in the Constitution of the Society.

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