FAO
FISHERIES AND
AQUACULTURE
TECHNICAL
PAPER

547

ISSN 2070-7010

Improving biosecurity through prudent and responsible use of veterinary medicines in aquatic food production

Cover photograph:
Art work depicting fish vaccination by Ms Manuela D'Antoni, Marine and Inland Fisheries Service (FIRF), Department of Fisheries and Aquaculture, Rome, Italy

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2012

ISBN 978-92-5-106975-2

Preparation of this document

Under the Aquatic Animal Health and Aquatic Biosecurity Project, and building on a number of consultations that dealt with veterinary medicines,[1] the FAO/AAHRI Expert Workshop on Improving Biosecurity through Prudent and Responsible Use of Veterinary Medicines in Aquatic Food Production was convened in Bangkok, Thailand, from 15 to 18 December 2009, in order to understand the current status of the use of antimicrobials in aquaculture as a basis for improving biosecurity through responsible use of veterinary medicines in aquaculture production.

The project culminated in the publication of this document, which is presented in two parts. Part 1 contains 15 technical papers presented during the expert workshop and contributed by 29 specialists. Part 2 of this document contains the highlights of the expert workshop, which was participated by a total of 39 experts from some of the major aquaculture-producing countries, including experts from the Association of Southeast Asian Nations, the European Commission, the World Organisation for Animal Health and the World Health Organization, as well as experts from the private sector (producers, producer organization, and pharmaceutical and feed companies).

The expert workshop and publication, technically supervised by Dr Melba B. Reantaso, Aquaculture Officer, and Dr Rohana P. Subasinghe, Senior Aquaculture Officer, both from the Aquaculture Service, Fisheries and Aquaculture Resources Use and Conservation Division of the Food and Agriculture Organization of the United Nations (FAO) Fisheries and Aquaculture Department (FI), were made possible with financial assistance through the Programme Cooperation Agreement of Norway under B.1 and D.1 objectives administered through the FishCode Programme of FI and the Nutrition and Consumer Protection Division of the FAO Agriculture and Consumer Protection Department, respectively.

[1] Expert Meeting on the Use of Chemicals in Aquaculture in Asia (May 1996); GESAMP Ad-Hoc Meeting of the Joint Group of Experts on the Scientific Aspects of the Marine Environmental Protection Working Group on Environmental Impacts of Coastal Aquaculture (May 1996); Workshop on International Harmonization for Aquaculture Drugs and Biologics (February 1997); Workshop and Round Table of the European Association of Fish Pathologists (EAFP) (September 1997); World Health Organization (WHO) Consultation (with FAO and the World Organisation for Animal Health, OIE) on Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food (June 2000); First Joint FAO/OIE/WHO Expert Workshop on Nonhuman Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment (December 2003); Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL (August 2004); and Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance (June 2006).

Abstract

The current trend towards increasing intensification and diversification of global aquaculture has led to its dramatic growth, thus making aquaculture an important food-producing sector that provides an essential source of aquatic protein for a growing human population. For both developed and developing countries, the sector is recognized as creator of jobs and an important source of foreign export earnings. The expansion of commercial aquaculture, as is the case in commercial livestock and poultry production, has necessitated the routine use of veterinary medicines to prevent and treat disease outbreaks owing to pathogens, assure healthy stocks and maximize production. The expanded and occasionally irresponsible global movements of live aquatic animals have been accompanied by the transboundary spread of a wide variety of pathogens that have sometimes caused serious damage to aquatic food productivity and resulted in serious pathogens becoming endemic in culture systems and the natural aquatic environment. The use of appropriate antimicrobial treatments is one of the most effective management responses to emergencies associated with infectious disease epizootics. However, their inappropriate use can lead to problems related to increased frequency of bacterial resistance and the potential transfer of resistance genes in bacteria from the aquatic environment to other bacteria. Injudicious use of antimicrobials has also resulted in the occurrence of their residues in aquaculture products and, as a consequence, bans by importing countries and associated economic impacts, including market loss, have occurred. As disease emergencies can happen even in well-managed aquaculture operations, careful planning on the use of antimicrobials is essential in order to maximize their efficacy and minimize the selection pressure for increased frequencies of resistant variants. The prudent and responsible use of veterinary medicines is an essential component of successful commercial aquaculture production systems.

The FAO/AAHRI Expert Workshop on Improving Biosecurity through Prudent and Responsible Use of Veterinary Medicines in Aquatic Food Production was convened in Bangkok, Thailand, from 15 to 18 December 2009, in order to understand the current status of the use of antimicrobials in aquaculture and to discuss the concerns and impacts of their irresponsible use on human health, the aquatic environment and trade. Such discussions became the basis for drafting recommendations targeted for both government and private sectors and for developing guiding principles on the responsible use of antimicrobials in aquaculture to be considered as part of future FAO Code of Conduct for Responsible Fisheries (CCRF) Technical Guidelines on Prudent and Responsible Use of Veterinary Medicines in Aquaculture.

Because aquaculture is expected to continue to increase its contribution to the world's production of aquatic food, offer opportunities to alleviate poverty, increase employment and community development and reduce overexploitation of natural aquatic resources, appropriate guidance to aquaculture stakeholders on the responsible use of veterinary medicines has become essential. Safe and effective veterinary medicines need to be available for efficient aquaculture production, and their use should be in line with established principles on prudent use to safeguard public and animal health. The use of such medicines should be part of national and on-farm biosecurity plans and in accordance with an overall national policy for sustainable aquaculture.

This publication is presented in two parts: Part 1 contains 15 technical background papers presented during the expert workshop, contributed by 29 specialists, and which served as a basis for the expert workshop deliberations; Part 2 contains the highlights of the expert workshop.

Bondad-Reantaso, M.G., Arthur, J.R. & Subasinghe, R.P., eds. 2012.
Improving biosecurity through prudent and responsible use of veterinary medicines in aquatic food production.
FAO Fisheries and Aquaculture Technical Paper. No. 547. Rome, FAO. 207 pp.

Contents

Preface

Modern aquaculture, through the intensification of culture systems and the diversification of both the species cultured and the culture methods employed, often creates an ideal environment for pathogens to flourish. The expanded and occasionally irresponsible global movements of live aquatic animals have been accompanied by the transboundary spread of a wide variety of disease agents that have sometimes caused serious damage to aquatic food productivity and resulted in serious pathogens becoming endemic in culture systems and the natural aquatic environment. Traditionally, the threats to aquaculture posed by aquatic pathogens have been addressed through the use of antimicrobials, including chemotherapeutants, disinfectants, antibiotics and vaccines. However, the inappropriate use of antimicrobials can lead to problems related to increased frequency of bacterial resistance, with negative impacts on the efficacy of these agents to control infectious diseases in aquaculture and the potential transfer of resistance genes in bacteria from the aquatic environment to other bacteria and the possibility of resistance extending to human pathogens. Injudicious use of antimicrobials has also resulted in the occurrence of their residues in aquaculture products, resulting in commodity bans by importing countries and associated economic impacts.

By themselves, antimicrobials cannot fully prevent losses due to disease. A holistic approach is required by modern aquaculture, and this can be achieved only through effective biosecurity programmes whereby pathogens are excluded from the culture environment. The Food and Agriculture Organization of the United Nations (FAO) is promoting a holistic approach to modern aquaculture through effective biosecurity actions taken at different levels ranging from more responsible international trade in aquatic organisms to better on-farm practices. The responsible use of antimicrobials is an important part of farm biosecurity, as this helps ensure that pathogen challenges are minimized, that the natural defence mechanisms of the cultured stocks are maximized, and that disease and mortality, including costs of containing, treating and/or eradicating diseases, are reduced. The injudicious and/or incorrect use of antimicrobials poses a great concern to successful and sustainable aquaculture. In order to develop appropriate strategies or guidelines that will enable the rational and prudent use of antimicrobials, particularly by small-scale aquaculturists, we need to assess the current situation with regard to the extent of their use and misuse, and to have a good general understanding of how these substances are being applied in aquaculture.

The FAO/AAHRI Expert Workshop on Improving Biosecurity through Prudent and Responsible Use of Veterinary Medicines in Aquatic Food Production was convened in Bangkok, Thailand, from 15 to 18 December 2009, in order to understand the current status of the use of antimicrobials in aquaculture and to discuss the concerns and impacts of their irresponsible use on human health, the aquatic environment and trade. Such discussions became the basis for drafting recommendations targeted for both government and private sectors and for developing guiding principles on the responsible use of antimicrobials in aquaculture to be considered as part of future FAO Technical Guidelines for Responsible Fisheries on Prudent and Responsible Use of Veterinary Medicines in Aquaculture.

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Acknowledgements

This publication was an outcome of the contributions of the many individuals who participated in this project, beginning with the desk study, through to the expert workshop, and then to the final publication of this document. They are all gratefully acknowledged.

Many kind thanks are due to the officials of the Inland Aquatic Animal Health Research Institute (AAHRI), Department of Fisheries of Thailand, and the FAO Regional Office for Asia and the Pacific for gracing the opening and closing sessions, and to AAHRI staff for logistic arrangements. Special thanks also go to the various companies, institutions and organizations (Bayer-Thailand, the Association of Southeast Asian Nations, the European Commission, Intervet/Schering-Plough Animal Health, Skretting-Spain, Surerath Farms-Thailand, Thai Aquaculture Business Association, the World Health Organization, the World Organisation for Animal Health, United States Food and Drug Administration) that provided support for the participation of experts. The authors of contributed papers and all workshop participants are sincerely acknowledged for making this publication possible.

The editors would like to thank officials of the Fisheries and Aquaculture Department (FI) – J. Jia, I. Karunasagar and W. Miao for support, guidance and encouragement and E. Irde for the organization and conduct of the expert workshop and contribution to this publication. The kind assistance of T. Farmer, M. Guyonnet and M. Panzironi, also of FI, and S. Arthur (desktop publisher) for various types of assistance during the final production of this document is much appreciated.

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Abbreviations and acronyms

AAHP	aquatic animal health practitioner
AAHRI	Aquatic Animal Health Research Institute
ADI	acceptable daily intake
AHD	1-aminohydantoin
AMDUCA	Animal Medicinal Drug Use Clarification Act (United States of America)
AMOZ	3-amino-5-morpholinomethyl-1,3-oxazolidin
AMR	antimicrobial resistance
AMU	antimicrobial use
AO	Administrative Order (Philippines)
AOZ	3-amino-oxazolidinone
BAI	Bureau of Animal Industry (Philippines
BFAD	Bureau of Food and Drugs (Philippines)
BFAR	Bureau of Fisheries and Aquatic Resources (Philippines)
BKD	bacterial kidney disease
BMP	best management practice; better management practice
BW	body weight
CAC	Codex Alimentarius Commission
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods
CEDMA	Centre for Environment and Disease Monitoring in Aquaculture (Viet Nam)
CLSI	Clinical and Laboratory Standards Institute
CNY	Chinese yuan
CoC	Conduct of Conduct for Responsible Aquaculture Farming
CO_{WT}	wild type cut-off value
DA	Department of Agriculture (the Philippines)
DAH	Department of Animal Health (Viet Nam)
DG SANCO	Directorate General for Health and Consumer Affairs
DOA	Department of Aquaculture (Viet Nam)
DOF	Department of Fisheries (Thailand)
DOH	Department of Health (the Philippines)
DOSTE	Department of Science, Technology and Environment (Viet Nam)
EMB	Emamectin benzoate
ELISA	enzyme-linked immunosorbent assay
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration (Thailand)
FDA-CVM	Food and Drug Administration's Center for Veterinary Medicine (United States of America)
FDA-DOH	Food and Drug Administration-Department of Health (Philippines)
FFDCA	Federal Food, Drug, and Cosmetic Act (United States of America)
FIQAS	Fish Inspection and Quality Assurance Service (Philippines)
FOO	Fisheries Office Order (the Philippines)
FSANZ	Food Standards Australia New Zealand
FVO	Food and Veterinary Office
GAqPs	good aquaculture practices
GFI	Guidance for Industry
GMO	General Memorandum Order (Philippines)
GMO	genetically modified organism

GMP	good management practice; good manufacture practice
HMP	health management programme
HACCP	Hazard Analysis and Critical Control Point
H_2O_2	hydrogen peroxide
IGO	intergovernmental organization
IPN	infectious pancreatic necrosis
ISA	infectious salmon anaemia
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LCMSMS	liquid chromatography tandem mass spectrometry
LMG	luecomalachite green
MARD	Ministry of Agriculture and Rural Development (Viet Nam)
MIC	minimum inhibitory concentration
MOFI	Ministry of Fisheries (Viet Nam)
MRL	maximum residue level
MRL	maximum residue limit
MRPL	minimum required performance limit
NACA	Network of Aquaculture Centres in Asia and the Pacific
NARMS	National Antimicrobial Resistance Monitoring System (United States of America)
NGO	non-governmental organization
NOAEL	no-observed adverse effect level
NRI	normalized resistance interpretation
NWT	non-wild type
OIE	World Organisation for Animal Health
PD	pharmacodynamics
PHP	Philippine peso
PK	pharmacokinetics
ppb	parts per billion
ppm	parts per million
QC	quality control
RAHO	Regional Animal Health Office (Viet Nam)
RA	Republic Act (Philippines)
RIA 1	Research Institute for Aquaculture No. I (Viet Nam)
SAG	Agriculture and Livestock Service (Chile)
SEM	semicarbazide
SERNAPESCA	National Fisheries Service (Chile)
SFR	specific feeding ratio
SPF	specific pathogen free
SPIC	Single Plate Internal Control
SPS Agreement	Sanitary and Phytosanitary Agreement of the World Trade Organisation
SRS	salmon rickettsial syndrome
SUBPESCA	Undersecretariat of Fisheries (Chile)
USDA	United States Department of Agriculture
VHML	*V. harveyi* myovirus-like
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
VNN	viral nervous necrosis
WHO	World Health Organization
WSSV	whitespot syndrome virus
WT	wild type
WTO	World Trade Organization

PART 1

CONTRIBUTED PAPERS ON UNDERSTANDING THE USE OF VETERINARY MEDICINES IN AQUACULTURE

Public health and trade impact of antimicrobial use in aquaculture

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ABSTRACT

Detection of residues of certain banned antibiotics in fish and crustaceans in international trade during 2001–2002 led to greater attention on the public health risks owing to the use of antimicrobial agents in aquaculture. The risk of residues with respect to antimicrobials that are permitted for use in aquaculture is managed by enforcing a maximum residue limit (MRL), but there are very few antimicrobials for which MRLs have been established by international agencies. Most fish importing countries adopt a zero tolerance approach regarding residues of antimicrobials that are banned for use in food animals. In such cases, residue levels that attract regulatory action are based on analytical capability rather than toxicology of the residues. Development and spread of antibiotic resistance has been a cause of concern, although this issue is complicated by possible multiple origins of resistance traits found in aquatic bacteria. Work done in this area by international agencies such as the Food and Agriculture Organization of the United Nations, the World Organisation for Animal Health, the World Health Organization and the Codex Alimentarius Commission is reviewed in this paper.

INTRODUCTION

The importance of antimicrobial agents in protection of animal health has been widely acknowledged, but the negative impacts of the use of these agents in animals raised for food have been a cause of concern. The Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) and the World Organisation for Animal Health (OIE) have organized several expert consultations and technical meetings to review the global situation and develop recommendations. While the issue of selection and spread of antibiotic-resistant bacteria in aquaculture has been deliberated upon for quite some time, the issue of antimicrobial residues in aquaculture products came to the fore in 2001 following marked improvements in laboratory methods to detect residues. This was followed by disruptions of trade in aquaculture products. According to the World Trade Organisation's Sanitary and Phytosanitary Agreement (SPS Agreement), countries have the right to establish measures to protect the life and health of their population and also to determine the level of protection that is appropriate for the country; however, available scientific evidence should be used when

establishing control measures, and these measures should not be taken only to favour the domestic industry. Measures adopted by countries with respect to antibiotic residues and antibiotic-resistant bacteria would be within the framework of the SPS Agreement.

At the international level, the responsibility of providing advice on risk management concerning veterinary drug residues lies with the Codex Alimentarius Commission (CAC) and its subsidiary body, the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). The primary responsibility for risk assessment is with the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The CCRVDF determines the priorities for consideration of residues of veterinary drugs, and JECFA provides independent scientific advice by evaluating the available data on veterinary drugs prioritized by CCRVDF. The Risk Assessment Policy for the the Setting of MRLs in Food established by the CAC defines the responsibilities of CCRVDF and JECFA and their interactions. For the establishment of the priority list, CCRVDF identifies, with the assistance of Members, the veterinary drugs that may pose a consumer safety problem and/or that may have potential adverse impacts on international trade. Veterinary drugs meeting some or all of the following criteria could appear on the priority list:

- a Member has proposed the compound for evaluation;
- a Member has established good veterinary practices with regard to the compound;
- the compound has the potential to cause public health and/or trade problems;
- it is available as a commercial product; and
- there is commitment that a dossier will be made available (CAC, 2010).

JECFA uses a risk assessment process to establish acceptable daily intake (ADI) and maximum residue limits (MRLs). Veterinary drugs that are toxic or have carcinogenic potential are not evaluated by JECFA and, therefore, no ADI or MRL are established. Chloramphenicol and nitrofurans, compounds that caused disruptions in trade in aquaculture products, belong to this category and are banned for use in food-producing animals in most countries. Currently, there is a Codex MRL only for chlortetracycline/oxytetracycline/tetracycline in fish and shrimp (CAC, 2009). However, there are national/regional MRLs for several other antimicrobial agents. In the European Union (EU), the Commission Regulation (EC) No. 1181/2002 establishes MRLs for veterinary drugs in foods of animal origin, including aquaculture products (EC, 2002). Lack of Codex MRLs for veterinary drugs could be a problem for many developing countries that adopt Codex MRLs as national MRLs. This situation has led FAO (2004) to recommend that for veterinary drugs that have been evaluated by national governments and are legally used in many countries, a comprehensive approach needs to be adopted to expedite harmonization. JECFA evaluation of substances may be constrained by lack of sponsors. FAO (2004) recommended that with the assistance of JECFA and based on national/regional MRLs, an initial list of temporary/operative MRLs could be adopted by CCRVDF. This list could be made permanent by CAC, if the national/regional risk assessments are not questioned, or if JECFA could establish ADI using the data used by the country/region to propose MRL. Substances that do not fulfil these requirements could then be moved to the list of compounds not to be used in food animals.

For veterinary drugs without ADI/MRL, regulatory authorities generally adopt a zero tolerance approach. In this situation, as the analytical capability improves, levels that were not detectable by earlier technology become detectable and hence reportable. Therefore, independent of any toxicological risk posed by the food product, the residues would attract regulatory action. The countries taking a zero tolerance approach argue that the products are not acceptable because they have evidence of use of a banned drug in animal production and, therefore, represent violation of regulations. However, when the levels that can be detected by extremely sensitive analytical techniques reach concentrations approaching environmental concentrations, there could be problems for the aquaculture operators. As discussed in later in this paper, there is increasing evidence that this point is being reached for at least for a couple of the residues.

Table 1 shows the Rapid Alerts that appeared in the European Union market owing

to residues of antibiotics in fish and fishery products. The major veterinary drugs involved are chloramphenicol, nitrofuran metabolites and malachite green. Following the trade disruptions caused by detection of residues, a Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs Without ADI/MRL was held in 2002. This technical meeting recommended that for residues of drugs without ADI/MRL, CCRVDF should request JECFA to perform and report, if possible, an estimate of the risks associated with the exposure to residues, as such risk estimates would be useful in risk management, and that CAC should include consideration of cost-benefit and risk comparisons in their risk analysis process (FAO, 2004). Use of alternate risk management approaches that reflect the toxicological risk of the residue for regulatory analytical methods, such as recommended performance level or a control strategy chosen by the competent authority, were also recommended (FAO, 2004). They further emphasized that the illegal use of veterinary drugs cannot be condoned. A Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance was held in Seoul during 2006 (WHO, 2006). This Expert Consultation used a risk assessment approach to address the public health impacts of antimicrobial use in aquaculture. The hazards recognized were (a) development and spread of antimicrobial resistance; and (b) antimicrobial residues in fish.

TABLE 1
Rapid Alerts owing to detection of residues of veterinary drugs in the European Union

Veterinary drug	2001	2002	2003	2004	2005	2006	2007	2008	2009	Total
Chloramphenicol	44	102	9	8	1	1	4	2	3	174
Nitrofuran (including all metabolites)	0	89	51	27	30	41	31	48	89	406
Malachite green	0	2	11	18	50	17	9	2	5	114

ANTIMICROBIALS OF CONCERN IN AQUACULTURE PRODUCTS – EVALUATION BY JECFA AND NATIONAL RISK ASSESSMENTS

Chloramphenicol

Chloramphenicol was evaluated by JECFA at its twelfth, thirty-second and forty-second meetings and further commented upon in its sixty-second meeting. Bone marrow depression is the major manifestation of chloramphenicol toxicity in humans. Dose-related bone marrow depression is the most common form in humans, when the daily dose of chloramphenicol is >4 g (WHO, 2004). A more serious and unpredictable reaction is aplastic anaemia (with >50 percent mortality) that can occur at a frequency of 1 in 24 000 to 40 000 courses of treatment with chloramphenicol, but the incidence has been reported to be associated with certain risk factors (WHO, 2004). Chloramphenicol has ophthalmic use in human medicine, and JECFA evaluation concluded that such use is unlikely to be associated with aplastic anaemia (WHO, 2004). JECFA also considered the human health risk associated with low levels of chloramphenicol detected in chicken and aquaculture products during 2001–2003. Based on levels reported by the Food Standards Agency of Ireland, the median concentration in aquaculture products was estimated to be 0.5 ppb. The committee noted that for preferential eaters of fish and shellfish containing a median of 0.5 ppb chloramphenicol, the exposure would be one order of magnitude lower than exposure from a daily ophthalmic formulation used in human medicine (WHO, 2004). There are no reported cases of aplastic anaemia associated with ophthalmic use of chloramphenicol. Eckert (2006) carried out a survey of chloramphenicol residues in imported crab meat in South Australia during 2006. Six of 17 samples tested had residues at levels ranging from 0.1 to 0.3 ppb. After reviewing chloramphenicol toxicity data and JECFA review data, the report concluded that the levels found in crab meat were unlikely to cause human health problems. There

are no epidemiological records of aplastic anaemia in any country attributable to the residues of chloramphenicol in foods. The levels of chloramphenicol residues found in fish and crustaceans in international trade are generally low (Table 2). The highest number of Rapid Alerts in the EU for chloramphenicol residues was in 2002 (Table 1). During early periods of residue testing, a positive reaction triggered Rapid Alerts irrespective of the levels detected. To harmonize the reporting by member countries, the European Commission established minimum required performance limits (MRPLs), the analytical methods used for detection of residues of banned antimicrobials (EC, 2003). As seen from Table 2, during 2002, about one-third of the alerts were for levels <0.3 ppb, which was adopted by the EU as the MRPL for the assay used for detection of chloramphenicol residues. Rapid Alerts during recent years have been triggered by levels exceeding MRPL.

TABLE 2
Levels of chloramphenicol reported in European Union Rapid Alert System for Food and Feed alerts for crustaceans in the EU during 2002 (n = 92)

Range (ppb)	Number of cases	Comments
<0.3	32	Lowest level for which alert was issued was 0.07 ppb. In 18 cases, levels were not indicated, but reported as "positive"
0.3 to 1.0	39	
>1.0 to 5.0	13	
>5.0	8	Highest level detected was 297 ppb

Nitrofurans

Nitrofurans are synthetic antimicrobials that are rapidly metabolized in animals. The four nitrofuran groups of antimicrobials and their metabolites are shown in Table 3. Furazolidone and nitrofurazone were evaluated by JECFA in 1993 (WHO, 1993). Based on the positive effects of furazolidone in genotoxicity tests *in vitro* and the increased incidence of malignant tumours in rats and mice, JECFA concluded that furazolidone is a genotoxic carcinogen and did not establish an ADI. Nitrofurazone was also evaluated by JECFA in the same meeting, which noted that although this compound is tumourogenic in rats and mice, the tumours produced were benign and restricted to endocrine organs and the mammary gland (WHO, 1993). Mutagenicity studies suggest that nitrofurazone is mutagenic *in vitro* but not *in vivo*. However, JECFA did not establish ADI, as no-effect levels have not been established for tumourogenic effects. Consequent to JECFA evaluation, use of nitrofurans in animals raised for food was banned in many countries.

TABLE 3
Nitrofurans and their metabolites

Nitrofuran antimicrobials	Metabolites
Furazolidone	3-amino-2-oxazolidinone (AOZ)
Furaltadone	3-amino-5-morpholinomethyl-1,3-oxazolidin (AMOZ)
Nitrofurantoin	1-aminohydantoin (AHD)
Nitrofurazone	Semicarbazide (SEM)

Following detection of residues of nitrofurans in prawns, Food Standards Australia New Zealand (FSANZ) performed a toxicological review and risk assessment (FSANZ, 2005). Data from the Australian Quarantine and Inspection Service and the Queensland Health Department showed levels of 3-amino-2-oxazolidinone (AOZ) in the range of 1.1 to 40 ppb, one sample with 2.2 ppb 3-amino-5-morpholinomethyl-1,3-oxazolidin (AMOZ), and one sample with 8.9 ppb semicarbazide (SEM). FSANZ noted that there are no long-term dietary studies on AOZ that would enable comparison between levels at

which AOZ would produce tumours in animals and the level of human dietary exposure to AOZ. Nevertheless, the risk associated with exposure to AOZ was characterized by determining the margin of exposure between the known levels of AOZ residues in prawns for mean and high consumers of prawns and the level of the parent compound furazolidone shown to cause tumours in animal studies. They noted that there was an approximate 4 million-fold difference between the dietary exposure for high consumers of prawns as compared with the dose shown to cause tumours in animal studies. At mean exposure level, the margin between the dietary exposure and the dose causing tumours in animals was 12 million. FSANZ concluded that even with a worst-case scenario, the public health and safety risk from nitrofuran residues in prawns was very low.

Data in Table 1 show that the EU Rapid Alerts for chloramphenicol dropped sharply after 2002. This could be because many fish-exporting countries took measures to control use of banned antimicrobials in aquaculture and instituted residue control programmes and monitoring of residues in aquaculture products as required by EU regulation. However, the problem with nitrofurans seems to have continued or even to have increased (Table 1). Examination of the data presented in Table 4 suggests that alerts owing to the metabolite AOZ, which were highest in 2002, have been declining, while alerts owing to SEM have been increasing. Therefore, there is a need to examine the issue with SEM. The increased number of cases could also be the result of increased frequency of testing of products from some countries that have yielded positive results.

TABLE 4
Trends in the detection of nitrofuran metabolites in fish and fishery products in the European Union during the last three years as compared with 2002

Nitrofuran metabolites	Number of cases			
	2002	*2007*	*2008*	*2009*
AOZ	50	21	18	11
AMOZ	0	1	0	0
AHD	0	0	0	0
SEM	0	12	32	76
Unspecified	13	0	2	1

Note: AOZ = 3-amino-2-oxazolidinone; AMOZ = 3-amino-5-morpholinomethyl-1,3-oxazolidin; AHD = 1-aminohydantoin; SEM = semicarbazide.

Malachite green

Malachite green was evaluated by the 17th Report of JECFA (WHO, 2009). The Committee noted that, although the available short- and long-term studies point to a no-observed adverse effect level (NOAEL) on the order of 10 mg/kg body weight (bw) per day, the study on teratogenicity in rabbits, albeit of low quality, raises concern regarding the potential developmental toxicity of malachite green. It further noted that since a NOAEL could not be identified, additional studies would be needed to properly address the potential reproductive and developmental hazards of malachite green. Scientific studies indicate that following ingestion, malachite green is expected to be extensively reduced to luecomalachite green (LMG), primarily by the gastrointestinal microflora, before absorption, and it cannot be ruled out that LMG, the major metabolite of malachite green, induces hepatocellular adenomas and carcinomas in female mice via a mutagenic mode of action. Based on these considerations, the committee considered it inappropriate to establish an ADI for malachite green and did not support the use of malachite green in food-producing animals.

CAN LOW LEVELS OF SEMICARBAZIDES AND MALACHITE GREEN BE ENVIRONMENTAL CONTAMINANTS?

Recent scientific evidence shows that SEM may be naturally found in several aquatic organisms. Saari and Peltonin (2004) detected SEM in fresh and cooked crayfish meat in Finland, where crayfish are not medicated with nitrofurazones. Levels of up to 12 ng/g were detected, and the source was not known. Studies by Hoenicke *et al*. (2004) have shown that SEM are naturally present in seaweeds (1–3 ng/g) and shrimp (0.9 ng/g) from the North Sea. Following increased detection of SEM in crustaceans imported into Belgium, the Seafood Importers and Processors Alliance of Belgium sponsored a study by the University of Ghent. Giant freshwater prawn, *Macrobrachium rosenbergii*, grown under controlled conditions at the university had SEM in the shell (Moser, 2009), showing that this compound may be naturally present in crustaceans.

Schuetze, Heberer and Juergensen (2008) noted that traces of malachite green and LMG could be detected in wild eels caught from waters downstream from municipal sewage treatment plants. Levels of 0.765 ppb were found in tissues of 25 of 45 eels caught from different lakes, a river and a canal in Germany. Since malachite green has multiple uses, they suggested that residue may originate from uses such as wash off from clothes or paper towels coloured with malachite green or even from private aquaria, as malachite green is legally used for treatment of ornamental fish. However, the levels detected by Schuetze, Heberer and Juergensen (2008) were within the MRPL value of 2 ppb (for sum of malachite green and LMG).

HEALTH HAZARD OWING TO ANTIBIOTIC RESISTANCE

Bacterial resistance to antimicrobial agents is a great public health concern. The widespread use of antibiotics in different sectors such as animal husbandry, agriculture and human medicine has contributed to selection and spread of antibiotic-resistant bacteria in the environment. Antibiotic resistance genes can spread among unrelated bacteria without any phylogenetic, ecological or geographical barriers. The 1996 Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance identified two types of hazard with respect of antimicrobial resistance:

- Development of acquired resistance in bacteria in aquatic environments that can infect humans. This can be regarded as a direct spread of resistance from aquatic environments to humans.
- Development of acquired resistance in bacteria in aquatic environments whereby such resistant bacteria can act as a reservoir of resistance genes from which the genes can be further disseminated and ultimately end up in human pathogens. This can be viewed as an indirect spread of resistance from aquatic environments to humans caused by horizontal gene transfer.

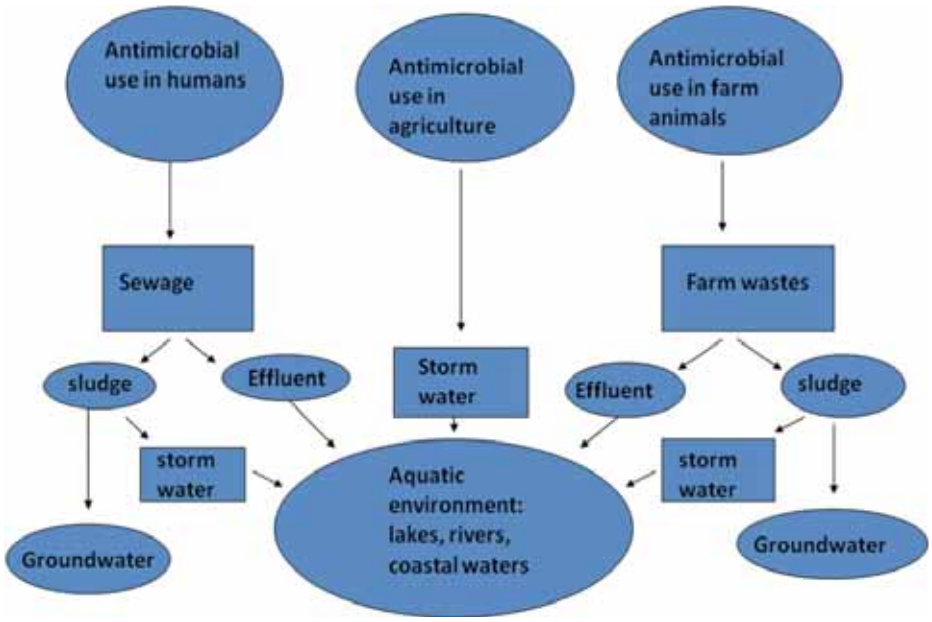
The consequences of antimicrobial resistance in bacteria causing human infections could include increased severity of infection and increased frequency of treatment failures (WHO, 2006). However, there are no recorded cases of human infections caused by antibiotic-resistant bacteria from aquaculture products.

There are few human pathogenic bacteria that are commonly found in the aquatic environment (e.g. *Vibrio parahaemolyticus*, *V. vulnificus*, *V. cholerae*, motile *Aeromonas* spp., *Edwardsiella tarda*). Antibiotic resistance that cannot be linked to the use of antimicrobials in aquaculture may be found in these aquatic bacteria. Baker-Austin *et al*. (2008) found antibiotic resistance in *V. parahaemolyticus* isolated from water and sediment along the coast of Georgia and South Carolina (United States of America), and resistance frequency was slightly reduced among virulent strains compared with non-virulent strains. Baker-Austin *et al*. (2009) examined antibiotic resistance in *V. vulnifucus* from different sites and found no difference in antibiotic resistance frequency in isolates from pristine and anthropologically impacted areas and suggested that the resistance traits are naturally derived rather than from human-derived sources. A recent FAO/WHO

risk assessment has shown that the risk of transmission of cholera through warmwater shrimp in international trade is very low (FAO/WHO, 2005). Motile *Aeromonas* spp. and non-O1 *V. cholerae* are rarely involved in gastrointestinal infections that are mostly self-limiting, and such infections do not require antibiotic therapy.

Indirect spread of antibiotic resistance from aquatic bacteria and human pathogens has been considered a possible hazard. Although some authors (e.g. Cabello, 2006) have tried to link the antibiotic resistance seen in V. cholerae involved in the cholera outbreak in Latin America in 1991 with bacteria present in shrimp farms in Ecuador, Smith (2007) presented evidence that resistance plamids found in these bacteria were earlier reported from pandemic V. cholerae strains in other countries and concluded that no link to the pool of resistance genes in the aquaculture environment could be established. Conclusions based on similarity of genetic determinants found in aquatic bacteria and human pathogens need to be evaluated carefully because of the fact that the aquatic environment receives effluents from various sectors of antimicrobial use, e.g. human medicine (hospital effluents), agricultural use, animal husbandry and aquaculture (fish-farm effluents). Thus, the water source used in aquaculture may be contaminated with antibiotic residues or antibiotic-resistant bacteria derived from different sectors (Figure 1). FAO (2008) noted that a risk analysis of the release of human and animal effluents into aquatic environments serving as water sources for aquaculture needs to be performed, particularly with respect to the antimicrobials identified as critically important by WHO and OIE. Such a risk analysis would determine the appropriate management options through which improved effluent management measures should be implemented (e.g. measures dealing with hospital effluents). Thus, the issue of antimicrobial resistance cannot be addressed for one sector (e.g. aquaculture) alone, but requires a comprehensive approach involving all sectors of antimicrobial usage.

FIGURE 1
Pathways for spread of antimicrobial residues and resistant bacteria in the aquatic environment



CONCLUSIONS

Detection of low levels of residues of certain banned antibiotics in aquaculture products in international trade has focused attention on the public health and trade impacts of the use of antimicrobials in aquaculture. All measures need to be taken to ensure that the

illegal use of antimicrobials does not take place in aquaculture. At the same time, the institution of regulatory action based on analytical capability rather than toxicological review of the residues, the lack of Codex MRLs for antimicrobials that have been evaluated at national/regional level, and the need to adopt harmonized risk management measures with respect to residues of veterinary drugs without a Codex ADI/MRL are some of the issues that need to be resolved at the international level. The current lack of epidemiological data on the perceived public health risks and the cost of implementing regulatory measures based on analytical capability emphasize the need for more innovative approaches to manage this problem.

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Environmental impacts and management of veterinary medicines in aquaculture: the case of salmon aquaculture in Chile

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ABSTRACT

Salmon are the most important species for aquaculture in Chile, and diseases are the main threat to this economic activity. The most important pathogens in freshwater are *Saprolegnia* sp., *Flavobacter* spp. and infectious pancreatic necrosis virus, while in seawater *Piscirickettsia salmonis*, *Caligus rogercresseyi* and infectious salmon anaemia (ISA) virus cause the most serious economic losses. Veterinary medicines have been used since the beginning of the salmon industry to control *Piscirickettsia salmonis* and *Caligus rogercresseyi*. However, the development of resistance has been recorded in the last few years, with a negative effect on the industry. After the ISA virus outbreaks reported in 2007, the Chilean Government implemented a contingency plan for emerging diseases and a surveillance programme for ISA and sea lice. Biosecurity and management plans emphasizing good aquaculture practices have been also adopted by the Chilean salmon industry to minimize the outbreaks of infectious disease.

INTRODUCTION

Aquaculture is an important economic activity for Chile, with 18 species under commercial production. In 2008, the revenue generated by exportation was around US$2.693 million, 93 percent of which was generated by salmon production, 3 percent by mussel production and 2 percent by seaweed culture. The other 2 percent was generated by the culture of other species, including scallops and oysters (www.subpesca.cl).

Salmon are not native to the southern hemisphere; the first stocks were introduced in Chile in 1875 to develop recreational fisheries. Salmon farming in Chile started at the end of the 1970s, and by 1992 Chile had become the second-largest salmon producer after Norway, with a growth rate of about 20 percent per year.

In comparison with the 500 tonnes produced in 1984 in Region X, Chilean salmon

production reached 630 653 tonnes in 2008. Three salmon species contributed to this total: Atlantic salmon (*Salmo salar*) – 60 percent; coho salmon (*Oncorhynchus kisutch*) – 20 percent; and rainbow trout (*O. mykiss*) – 20 percent (Figure 1). In 2007, 71 percent of the salmon was produced in Region X, 28 percent in Region XI, and 1 percent in Region XII (Figure 2).

FIGURE 1
Annual salmon production in Chile by salmon species, 1985–2008 (thousand tonnes)

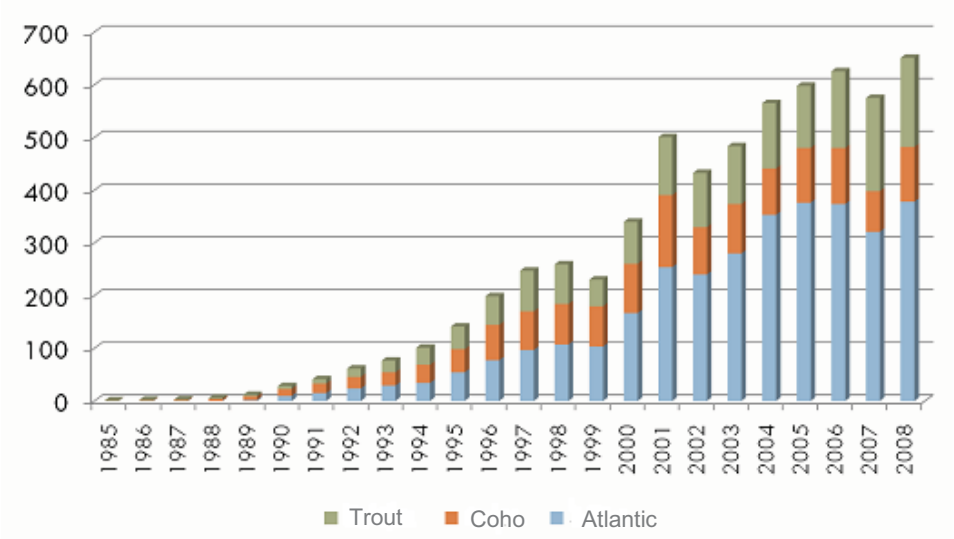
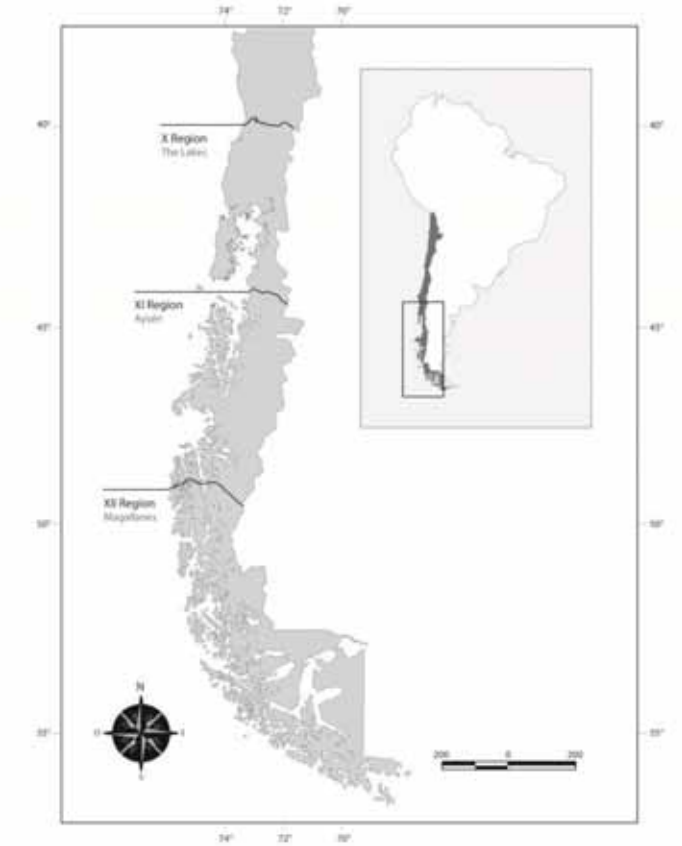


FIGURE 2
The three regions where salmonids are farmed in Chile (Region X; XI and XII)



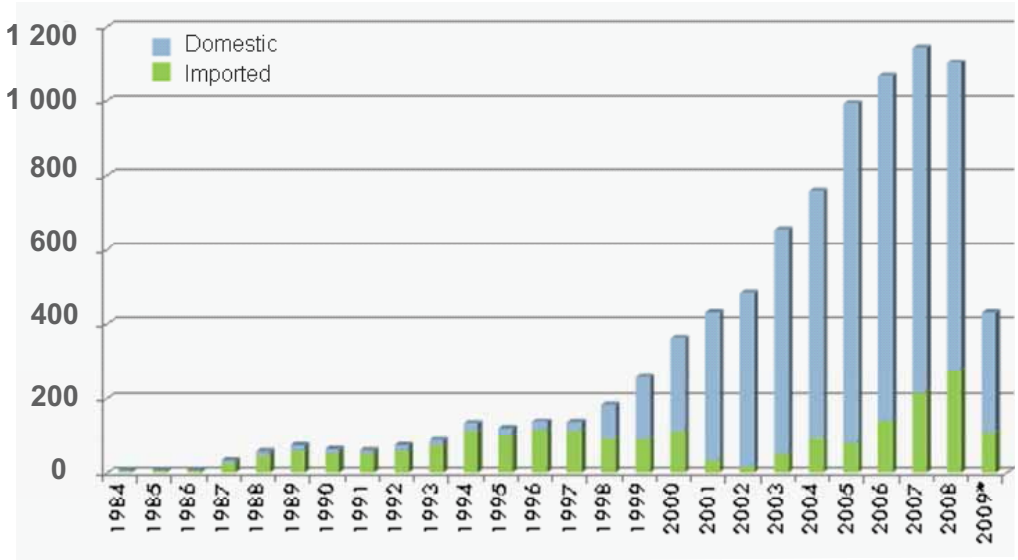
Source: SERNAPESCA.

SANITARY SITUATION OF THE SALMON INDUSTRY IN CHILE

Since the beginning of salmon farming activity, diseases have always presented a major threat. In the beginning, the important pathogens affecting farmed salmon were *Renibacterium salmoninarum* causing bacterial kidney disease, which was introduced from the United States of America to Chile through the movement of infected eggs of coho salmon (Wood, 1970); *Piscirickettsia salmonis* causing salmon rickettsial syndrome (SRS), reported in 1989 (Bravo and Campos, 1989); and *Saprolegnia* sp. and the copepod parasite *Caligus* (Reyes and Bravo, 1983). The two last pathogens are present in wild fish and transmitted to farmed salmon.

This fledgling industry was mainly supported by salmon eggs imported from different countries of the northern hemisphere and from New Zealand (Table 1). However, because of the increase in the number of pathogens introduced since 1992 (Table 2) and the evidence that the movement of eggs is an important risk factor for the introduction of pathogens transmitted vertically from infected parents, since 1998 the salmon industry has been mainly supplied by domestically produced salmon eggs. This is despite the importation of over 298 million salmon eggs in 2007 and 2008, which corresponds to the highest importation of eggs during the entire period (Figure 3). In 2008 about 85 percent of the imported eggs were those of Atlantic salmon, with the remaining 15 percent being those of rainbow trout; a difference from the previous years when 60 percent of the imported eggs came from Atlantic salmon.

FIGURE 3
Salmon eggs supplied to Chile by year and source (thousand tonnes)



Note: * indicates only partial information available for 2009.
Source: SERNAPESCA.

The salmon industry in Chile was severely affected when the virus causing infectious salmon anaemia (ISA) was reported in July 2007. The main factors that contributed to the sanitary crisis were:

- the salmon industry was partially supplied by eggs imported from countries of the northern hemisphere where ISA is present;
- weak sanitary regulations without clear penalties;
- lack of a contingency plan for emerging diseases;
- high concentration of farms in limited areas and high stocking of salmon per farm (>3 000 tonnes/farm);
- many farms of different owners sharing the same area, close to each other, and without agreements of "all in-all out" policy, and a lack of coordination in stocking of smolts;

- sites operating for more than 20 years without fallowing;
- high number of transportation routes for well boats;
- inefficient biosecurity practices without focus on fish farming activities;
- lack of treatment of effluents and wastes from the processing plants and slaughterhouses; and
- poor management of mortalities in farms and hatcheries.

TABLE 1
Countries of origin from which salmon eggs have been imported to Chile

Country	Atlantic salmon	Coho salmon	Rainbow trout	Chinook salmon
Denmark	x		x	
Scotland	x			
Ireland	x		x	
Norway	x		x	
United Kingdom	x		x	
Sweden	x		x	
Faroe Islands	x			
Finland			x	
Iceland	x		x	
United States	x	x	x	x
Canada	x	x		x
New Zealand				x

Source: SERNAPESCA.

TABLE 2
Exotic pathogens/diseases reported from farmed salmon in Chile, 1983–2007

Year	Pathogen/disease	Reference
1970	Renibacterium salmoninarum (bacterial kidney disease)	Wood, 1970
1983	Infectious pancreatic necrosis virus (IPN VR-299)	McAllister & Reyes, 1984
1984	Piscirickettsia salmonis (salmon rickettsial syndrome)	Bravo & Campos, 1989
1992	Yersinia ruckeri (enteric redmouth disease)	Bravo, 1993
1993	Flavobacter psychrophilus (rainbow trout fry syndrome)	Bustos et al., 1995
1995	Aeromonas salmonicida atypica	Bravo, 1999
1995	Nucleospora salmonis	Bravo, 1996
1998	Infectious pancreatic necrosis virus (IPN sp.)	Unpublished
2000	Streptococcus phocae	Unpublished
2003	Vibrio ordalii	Colquhoun et al., 2004
2004	Listonella (Vibrio) anguillarum	Unpublished
2006	Francisella sp.	Birkbeck et al., 2007
2007	Infectious salmon anaemia (ISA)	Godoy et al., 2008

REGULATIONS APPLIED TO AQUACULTURE IN CHILE

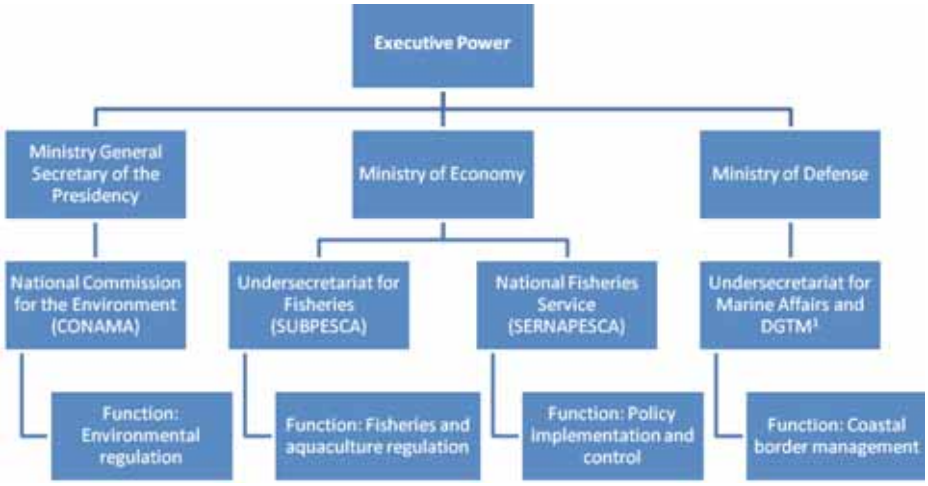
The first regulations on fish health were implemented in 1984 by the Undersecretariat of Fisheries (SUBPESCA). In 1995, the National Fisheries Service (SERNAPESCA) implemented regulations governing pharmaceutical products for veterinary use. All pharmaceutical products, as well as generic products allowed to be marketed in Chile, have to be approved by the Agriculture and Livestock Service (SAG) (Table 3). SERNAPESCA belongs to the Ministry of Economy, SAG to the Ministry of Agriculture, and the Institute of Public Health of Chile to the Ministry of Health. The organizational structure and interrelationships of the bureau units involved in the aquaculture regulations are shown in Figure 4.

TABLE 3
Antimicrobial agents permitted by the official authority for application in aquaculture in Chile

Antimicrobial agent	Withdrawal period	Antiparasitic agent	Withdrawal period
Oxolinic acid	450 °d	Emamectin benzoate	1 500 °d
Flumequine	300 °d	Hydrogen peroxide	0 °d
Oxytetracycline	600 °d	Deltamethrin	130 °d
Erythromycin	500 °d	Diflubenzuron	350 °d
Florfenicol	300 °d	Bronopol	70 °d
Amoxycillin	70 °d	Saprofin	0 °d
Sulfatrimethoprim	300 °d	Benzalkonium chloride	0 °d
		Cloramine–T	50 °d

°d: degree-days
Source: www.sag.ch

FIGURE 4
Organizational structure of the governmental institutions involved in aquaculture activity



[1]DGTM = General Direction of Marine Territory.
Source: SUBPESCA.

Through the years, SUBPESCA has implemented the following regulations in order to maintain a sustainable aquaculture sector:

- 1984: First Fish Health Regulation;
- 1991: General Act for Fisheries and Aquaculture;
- 1994: General Act for Environment;
- 2001: Sanitary Regulations for Aquaculture; and
- 2001: Environmental Regulations for Aquaculture.

In addition, SERNAPESCA has established several norms in the framework of the Sanitary Regulations for Aquaculture for farmed salmon (Table 4) and farmed shellfish (Table 5). SERNAPESCA also has implemented the following programmes in order to minimize outbreaks of disease:

- 2007: surveillance programme for sea lice;
- 2007: surveillance programme for ISA; and
- 2007: biosecurity measures.

ANTIMICROBIAL AGENTS USED BY THE AQUACULTURE SECTOR IN CHILE

Since 2007, farmers must declare to SERNAPESCA (National Fisheries Service) the types and amounts of medicine used to control diseases in aquatic organisms. However, SAG is the institution that gives the authorization for the marketing of veterinary medicinal products intended for aquatic species (Table 3). The withdrawal period values correspond to the accumulated degree days established by the respective pharmaceutical

companies to meet the maximum residual limits (MRLs) imposed by the international markets (Table 6) and authorized by the Institute of Public Health of Chile.

Salmon are the main aquaculture species reared in Chile, and the three major pathogens for which medicines are applied are *Piscirickettsia salmonis*, *Caligus rogercresseyi* and *Saprolegnia* sp. Antibacterial medicines are mainly administered in the feed, while a variety of antiparasitics and pesticides may be used either by bath or by addition to the feed to control parasitic infestations.

Since 1995, veterinarians are the only professionals allowed to prescribe medicines for aquatic organisms in Chile, and the diagnosis of fish health has been mainly conducted by private laboratories, with the authorization of the official authority. To date, Chile has not established a National Reference Laboratory.

TABLE 4
Sanitary regulations for farmed salmon

Programme	Resolution	Code
Programa Sanitario General de Vacunaciones	Nº 60	PSGV-60
Programa Sanitario Específico de Vigilancia Activa de Enfermedades de Alto Riesgo en Peces (EAR)	Nº 61	PSEVA-61
Programa Sanitario General de Investigación Oficial de Enfermedades	Nº 62	PSGI-62
Programa Sanitario General de Registro de Datos y Entrega de Información de Laboratorio	Nº 63	PSGL-63
Programa Sanitario General de Procedimiento de Transporte	Nº 64	PSGT-64
Programa Sanitario General de Desinfección de Ovas de Salmonídeos	Nº 65	PSGO-65
Programa Sanitario General de Manejo de Mortalidades	Nº 66	PSGM-66
Programa Sanitario General de Manejo de Enfermedades	Nº 67	PSOE-67
Programa Sanitario General de Manejo de Desechos	Nº 68	PSGD-68
Programa Sanitario General de Procedimientos de Cosecha	Nº 69	PSGR-69
Programa Sanitario General de Manejo Sanitario de la Reproducción de Peces	Nº 70	PSGR-70
Programa Sanitario General de Manejo de Alimentos	Nº 71	PSGA-71
Programa Sanitario General de Limpieza y Desinfección aplicable a la producción de peces	Nº 72	PSGL-72
Programa Sanitario General de Control de Residuos	Nº 1925	PSGRES-1925
Programa Sanitario Específico de Vigilancia y Control de la Anemia Infecciosa del Salmón	Nº 2638	PSEC-ISA
Programa Sanitario Específico de Vigilancia y Control de la Caligidosis	Nº 2117	PSECV-Caligidosis

Source: www.sernapesca.cl.

TABLE 5
Sanitary regulations for farmed shellfish

Programme	Resolution	Code
Programa Sanitario General de Limpieza y Desinfección aplicable a la producción de Moluscos	Nº 1803	PLDM-1803
Programa Sanitario General de Procedimientos de Cosecha para Moluscos	Nº 1804	PMC-1804
Programa Sanitario General de Manejo de Desechos de Moluscos	Nº 1805	PDM-1805
Programa Sanitario General de Enfermedades de Moluscos	Nº 1806	PEM-1806
Programa Sanitario General de Procedimiento para Transporte de Moluscos	Nº 1807	PSGTM-1807
Programa Sanitario General de Investigación Oficial de Enfermedades de Moluscos	Nº 1808	PIOM-1808
Programa Sanitario Específico de Vigilancia Activa para Enfermedades de Alto Riesgo (EAR) en Moluscos	Nº 1809	PVM-1809

Source: www.sernapesca.cl.

Maximum residue limits (MRLs)

The main market for Atlantic salmon is the United States of America, whereas the market for coho salmon and rainbow trout is Japan. Chilean products have to fulfil the requirements established by the international markets (Table 6).

TABLE 6
MRLs in flesh and skin of fish allowed for the different markets

Antimicrobial agent	United States	European Union	Japan	Chile
Oxytetracycline	2 000 µg/kg	100 µg/kg	200 µg/kg	100 µg/kg
Oxolinic acid	Absence	100 µg/kg	Absence	100 µg/kg
Flumequine	Absence	600 µg/kg	Absence	600 µg/kg
Sulfadiazine	Absence	100 µg/kg	Absence	Absence
Trimetropim	Absence	50 µg/kg	Absence	Absence
Florfenicol	Absence	1 000 µg/kg	Absence	1 000 µg/kg
Erythromycin	Absence	200 µg/kg	Absence	200 µg/kg
Enrofloxacin	Absence	100 µg/kg	Absence	Absence
Amoxycillin	Absence	50 µg/kg	Absence	Absence
Spiramycin	Absence	Absence	200 µg/kg	Absence
Ivermectin	Absence	Absence	Absence	Absence
Emamectin benzoate	Absence	100 µg/kg	Absence	100 µg/kg
Diflubenzuron	Absence	100 µg/kg	100 µg/kg	–
Deltamethrin	Absence	10 µg/kg	30 µg/kg	–

Source: SERNAPESCA.

Antibacterials

Although a wide range of bacterial pathogens have been reported in Chile, Piscirickettsia salmonis is the main pathogen for which a broad range of antibacterial drugs available in Chile are being used (Table 7).

TABLE 7
Antibacterial products used to control bacterial diseases in Chile

Disease	Antibacterial agent	Administration	Dose	Administration period
		Seawater		
Salmon rickettsial syndrome	Flumequine	In feed	20–30 mg/kg fish/day	14–21 days
	Oxolinic acid	In feed	20–30 mg/kg fish/day	14–21 days
	Oxytetracycline	In feed	100–120 mg/kg fish/day	14–21 days
	Oxytetracycline	Injection	30–35 mg/kg fishay	
	Florfenicol	In feed	20 mg/kg fish/day	10–14 days
Bacterial kidney disease	Erythromycin	In feed	100 mg/kg fish/day	21 days
	Erythromycin	Injection	20 mg/kg fish	
	Oxytetracycline	In feed	100–120 mg/kg fish/day	21 days
Atypical furunculosis	Flumequine	In feed	20–30 mg/kg fish/day	14–21 days
	Oxolinic acid	In feed	20–30 mg/kg fish/day	14–21 days
Vibriosis	Flumequine	In feed	20–30 mg/kg fish/day	14–21 days
	Oxolinic acid	In feed	20–30 mg/kg fish/day	14–21 days
	Oxytetracycline	In feed	100–120 mg/kg fish/day	14–21 days
Streptococcosis	Oxytetracycline	In feed	100–120 mg/kg fish/day	21 days
	Erythromycin	In feed	50–100 mg/kg fish/day	12–21 days
	Florfenicol	In feed	20 mg/kg fish/day	10–14 days
		Freshwater		
Flavobacteriosis	Florfenicol	In feed	20–25 mg/kg fish/day	10–14 days
	Florfenicol	Bath	20 ppm	1 hour
	Oxytetracycline	In feed	100–120 mg/kg fish/day	14–21 days
	Oxytetracycline	Bath	40–100 ppm	1 hour
	Amoxycillin	In feed	80–100 mg/kg fish/day	7–10 days
	Amoxycillin	Bath	80 ppm	1 hour
Yersiniosis	Sulfatrimethoprin	In feed	33 mg/kg fish/day	7–10 days

Source: Aquatic Health Laboratory (2004).

To determine the amount of antibacterial products that has been used in aquaculture, information was collected from the results obtained through the project FIP 2003-28, where a survey of the chemicals used by Chilean aquaculture was carried out for the period 1999–2003 (Bravo *et al.*, 2005). The information for the years 2007–2008 was

supplied by the Ministry of Economy. No official information was available for the period 2004–2006 (Table 8). The amount of antibacterial products increased from 0.276 kg of active ingredient/tonne of salmon produced in 2003 to 0.648 kg of active ingredient/tonne of salmon produced in 2007.

TABLE 8
Amount of antibacterial products (active ingredient) used to control bacterial diseases in Chile during the period 1999–2008

Active ingredient (kg)	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Oxolinic acid	19 222	19 180	25 168	39 829	37 940	–	–	–	78 582	25 325
Amoxycillin	0	0	0	0	0	–	–	–	1 732	349
Enrofloxacin	502	228	76	114	51	–	–	–	0	0
Erythromycin	1 810	1 950	1 464	1 720	937	–	–	–	2 139	7 981
Florfenicol	211	396	597	299	5 484	–	–	–	143 009	184 715
Flumequine	19 779	30 046	61 364	51 738	70 005	–	–	–	74 773	32 293
Oxytetracycline	16 738	18 251	44 962	26 100	19 644	–	–	–	89 309	74 931
Sulfatrimethoprin	93	198	185	118	103	–	–	–	91	22
Total	58 354	70 249	133 815	119 917	134 163	–	–	–	385 635	325 617
Salmon production	230 189	342 407	504 422	482 392	488 256	569 146	614 139	647 263	600 835	630 647
Kg active ingredient/tonne	0.25	0.21	0.27	0.25	0.28	–	–	–	0.64	0.52

Source: Bravo *et al.*, 2005

Antiparasitic agents

In freshwater, several protozoan parasites transmitted from wild fish have been reported in salmon reared in lakes. Most of them are controlled with formalin applied by bath. However, the most important pathogen is the aquatic fungus *Saprolegnia* sp., for which several antifungal products have been tested after the use of malachite green was banned. Among these, salt (NaCl) is the most popular product used in hatcheries, followed by bronopol.

Since the first confirmed report of *Caligus* affecting coho salmon reared in seawater (Reyes and Bravo, 1983), several medicinal products have been used in an attempt to keep the parasite under control. As in the northern hemisphere (Costello, 1993; Roth, Richards and Sommerville, 1993), bath treatments followed by oral treatments were initially used. Metriphonate (Neguvon™) was the first product used to control sea lice between the years 1981 and 1985. This was replaced by dichlorvos (Nuvan™) from 1985 to 2000. Ivermectin administered in feed was introduced in Chile at the end of the 1980s.

Emamectin benzoate (EMB) was exclusively used during the period from 2000 to 2007 until evidence of resistance was reported in *Caligus rogercresseyi* (Bravo, Sevatdal and Horsberg, 2008). The product Slice™, developed by Schering-Plough Animal Health, was initially the only emamectin product available to control this parasite. However, three standard products of emamectin supplied by other pharmaceutical laboratories became rapidly available at a lower cost.

Table 9 contains information about the amount of chemicals used to control sea lice in Chile during the period 1999–2008. The information for the period 1999–2003 was generated by the project FIP 2003-28 (Bravo *et al.*, 2005), while the information for the years 2007–2008 was supplied by SERNAPESCA. The information for the period 2004–2006 was supplied by a pharmaceutical company in Chile. The amount of EMB utilized increased significantly in 2007, reaching 1 503g/tonne of salmon produced.

Because of the inefficiency of EMB in the treatment of *C. rogercresseyi*, hydrogen peroxide (H_2O_2) was the only alternative sea lice treatment in the period from February to September 2007. Pyrethroid deltamethrin was introduced in September 2007. Two years later (April 2009), the chitin synthesis inhibitor diflubenzuron was introduced. This was done in order to facilitate the rotation of antiparasitic agents with a view to reducing the development of resistance.

TABLE 9
Amount of antiparasitic products (active ingredient) used to control sea lice in Chile during the period 1999–2008

Active ingredient (kg)	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Emamectin benzoate (EMB)	25	52	77	121	127	149*	212*	326*	906*	285.0
Salmon production (tonnes)	230 159	342 406	504 422	482 392	486 837	569 146	614 139	647 263	600 835	630 647
g EMB/tonne (salmon)	0.109	0.152	0.153	0.251	0.260	0.262	0.345	0.504	1.503	0.452
Ivermectin	7	20	10	3	3	–	–	–	0	0
Diflubenzuron	0	0	0	0	0	–	–	–	.99	162.0
Cypermethrin	0	0	0	0	6	–	–	–	0	0
Deltametrin	0	0	0	0	0	–	–	–	5.2	105.2
Dichlorvos	0	1.6	3.4	0	0	–	–	–	0	0

Note: * data obtained from a pharmaceutical company
Source: Bravo et al., 2005

In contrast to Norway and Scotland where several wrasse species (family Labridae) are used as a successful biological control (Kvenseth and Andreassen, 2003; Treasurer, 2005), no wild marine fish that could act as cleaner fish have been identified in Chilean waters. Furthermore, a number of alternative control measures such as the use of onions in pens and garlic in feed have been tested in the past without evidence of any effect.

Sensitivity of *Piscirickettsia salmonis* to control treatments

Although *P. salmonis* is the most important pathogen affecting the salmon industry in Chile and for which a broad range of antibacterial drugs are available, there is no official information about the sensitivity of this intracellular pathogen to the antibacterial products used for its control.

At the beginning of salmon production in Chile, just two outbreaks of SRS were recorded during the whole production cycle of the affected salmon species, one in autumn and the other in spring. Antibacterial agents used to control the disease were relatively effective, their effectiveness depending on the level of infection. However, since 2005 six outbreaks of SRS per production cycle have been reported in several sites, and the concentrations and period of application of the antibacterial products have increased through the years. That increase is in some way an indicator of the loss of sensitivity developed by *P. salmonis* to the antibacterial agents used in its control. In fact, Table 8 shows that the total amount of antibacterials used in salmonid production increased from 0.25 kg active ingredient/tonne in 2002 to 0.65 kg active ingredient/tonne in 2007.

To date, the only information available about the sensitivity shown by *P. salmonis* to antibacterial agents is presented in a thesis on veterinary medicine (Barria, 2008). The results presented in this thesis showed that there is no standardization of the methodology and procedures used to evaluate sensitivity among the different fish health diagnostics laboratories in Chile. Besides, the criteria used to define sensitivity/resistance through the disc diffusion size or minimum inhibitory concentration corresponds to the standards developed by the National Committee for Clinical Laboratory Standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals and were not developed for aquatic animals.

Sensitivity of *Caligus rogercresseyi* to control treatments

Efficacy of oral treatments

Ivermectin was used in Chile from the end of the 1980s until the end of the 1990s to control *Caligus rogercresseyi.* At the end of the 1990s, EMB was introduced onto the Chilean market. Both products showed high efficacy against all developmental stages of *C. rogercresseyi.* In 2000, EMB was approved by SAG as the only chemotherapeutant permitted for use in the control of sea lice. This compound was exclusively used up until

2006, when resistance of *C. rogercresseyi* to EMB began to become apparent. Bioassay methodology was implemented to assess the sensitivity of *C. rogercresseyi* to EMB, as developed for the pyrethroid deltamethrin by Sevatdal and Horsberg (2003) and adapted for EMB in *C. rogercresseyi* (Bravo, Sevatdal and Horsberg, 2008) in the framework of the project FONDEF- D04I1255 (Bravo *et al.,* 2008).

The loss of sensitivity in both Atlantic salmon and rainbow trout was recorded in farms distributed throughout Region X in 2006. The recommended dosage of 50 µg active ingredient/kg fish/day for a period of 7 days did not give the same effect as that recorded when the product was initially introduced. As a result, some farmers decided to increase the dosage up to 100 µg of active ingredient/kg fish/day and also to increase the treatment period up to 10 days. The number of treatments per production cycle was also increased (Bravo *et al*., 2008). This facilitated the development of resistance by allowing selection of resistant strains. The loss of effect was attributed to the use of similar products for at least 16 years, 10 years with the avermectin ivermectin followed by 6 years with another avermectin, EMB (Bravo, Sevatdal and Horsberg, 2008). Results of the bioassay applied to seven successive generations from a presumably resistant population of *C. rogercresseyi* that was cultivated in the laboratory without the selection pressure of the antiparasitic drug showed that there was no recovery of sensitivity over the seven inbred generations studied. This may point to a long-standing resistance problem against EMB (Bravo, Sevatdal and Horsberg, 2010).

Efficacy of bath treatments

Bath treatments were initially used in Chile to control sea lice in the salmon industry. The organophosphate metriphonate (Neguvon™) was first used and later replaced by dichlorvos (Nuvan™). Both products were only effective against the adult stages, having no effect on the chalimus stages. However, in contrast to Norway, Scotland and Ireland, where resistance problems were reported towards organophosphates (Jones, Sommerville and Wooten, 1992), the parasites were kept under control in Chile without evidence of resistance problems, perhaps because salmon production was relatively low during the first years.

Because of the development of resistance of *C. rogercresseyi* to EMB in 2007, alternative treatments for controlling sea lice in the Chilean salmon industry were explored. H_2O_2 applied as a 20-minute bath treatment at a concentration of 1 500 ppm showed a significant reduction in the number of adult parasites 24 hours after treatment. However, results obtained from the field and from laboratory studies indicated that H_2O_2 did not give adequate results (Bravo *et al*., 2010). Most of the parasites detached from the fish were able to recover completely after 10 minutes and were capable of reinfesting the fish. Similar observations were reported by Johnson, Constible and Richard (1993) under laboratory conditions.

The pyrethroid deltamethrin (AlphaMax™) was authorized to control sea lice in Chile in September 2007. As treatment failures against *Lepeophtheirus salmonis* with this compound had been reported in Norway in 1998 (Denholm *et al*., 2002; Sevatdal and Horsberg, 2003), the sensitivity of this compound was tested on *C. rogercresseyi* before introducing the deltamethrin treatment to Chile. The tests revealed values of sensitivity (EC_{50}) of 0.36 ppb, similar to sensitivities seen in pyrethroid-sensitive *L. salmonis*. After 14 months of use, a decrease of sensitivity of *C. rogercresseyi* to deltamethrin was recorded, similar to the findings of Sevatdal (2005) for *L. salmonis* in Norway. In Norway, Sevatdal *et al.* (2005) reported reduced sensitivity to pyrethroids in geographically isolated areas, but this disappeared after fallowing of the sites. A similar situation should be recorded in Chile, although the distances between fish farms are shorter than in Norway.

VACCINES

Vaccines have proven effective tools to prevent outbreaks of bacterial diseases in fish (Midtlyng, 1997, 2005), and are seen as the main reason for the dramatic reduction of

antimicrobial drug use in salmon culture in Norway (Grave *et al.*, 1990, 1999). The use of fish vaccines in Chile began in the early 1980s when the first stocks of coho salmon were vaccinated against vibriosis before transfer to seawater (Bravo and Midtlyng, 2007). However, after some years, this practice was discontinued because there was no evidence of the presence of this disease in Chile. Yersiniosis vaccines came into use again in 1995 following the first occurrence of enteric redmouth disease in Atlantic salmon in 1992 (Bravo, 1993). In Chile, animal vaccine products (including fish vaccines) require a marketing authorization issued by SAG.

Altogether, 41 vaccine product licences were obtained by eight pharmaceutical companies between 1997 and 2009 (Annex I). Among these were five vaccines against SRS, one of them recombinant and one administered in feed; four vaccines against ISA, one of them recombinant and one administered in feed; and eight vaccines against infectious pancreatic necrosis (IPN), one of them administered by immersion and one administered in feed. Twenty-two were combination vaccines containing two or more antigens in the same formulation; all of them included the IPN antigen and 11 included the SRS antigen.

The first bivalent fish vaccine for immersion, Fryvac 2 (against *Flexibacter columnaris* infection and yersiniosis), was launched in 1999. The first trivalent injectable vaccine, Alpha Ject 3-2 (against IPN, furunculosis and vibriosis), was introduced in 2004. In addition, authorization to produce and sell autogenous vaccines against infections with *Flavobacter psychrophilum, Streptococcus* spp. and *Vibrio* spp. was issued, and this is likely to reduce the amount of antimicrobial drugs used for control of these diseases. SAG issued the first authorization to market a recombinant vaccine against SRS in 2004, and vaccines against ISA were introduced in 2008.

BIOSECURITY MEASURES

SERNAPESCA has implemented biosecurity measures since 2007, when outbreaks of the ISA virus were declared for the first time, to keep this disease under control. The measures include:

- coordinated stocking and fallowing of sites;
- only one generation in each site: all in-all out;
- no movement of fish between sites;
- no contact between sites – equipment used in one site at a time;
- disinfection of well boats;
- cleaning and disinfection of equipment;
- regulations on disinfection of wastewater from slaughterhouses and processing plants;
- regulations on mortality disinfection and disposal;
- zonation of areas for sanitary management; and
- vaccination of stocks before transfer to seawater.

CONCLUSIONS

In Chile, antimicrobial treatments have been mainly used in an attempt to control *Piscirickettia salmonis* and *Caligus rogercresseyi*. Evidence of the development of resistance in the field has been recorded for both pathogens. However, until now only *C. rogercresseyi* has been the object of studies to assess the sensitivity of the pesticides used for its control. In fact, a surveillance programme has been implemented since 2007, which focuses on an Integrated Pest Management (IPM) approach with rotation of the available treatments through an annual plan and plans for area fallowing and sanitary actions to reduce the dispersal of sea lice.

Considering the intracellular nature of the infection caused by *P. salmonis*, the effectiveness of the antimicrobial agents used for its control is relative and some of the agents may never have been efficacious. It is suggested for this reason that *P. salmonis* would have to be treated as clinically resistant (P. Smith, personal communication). Inactivated

vaccines against SRS have been available in Chile since September 1998. However, their efficacy has not been completely successful, which has forced the continued use of antibacterial drugs to control the disease despite vaccination. It is believed that a rapid progress in vaccination against SRS is the single strongest factor that may contribute to a rapid reduction of antibiotic use and resultant residue emissions in the coastal areas of southern Chile.

Since the first ISA outbreaks were reported in 2007, the Chilean salmon farmers have understood that prevention and good management practices based on biosecurity measures are the best tools to minimize outbreaks of disease, and that the use of veterinary medicines is not a solution to management problems.

ACKNOWLEDGEMENTS

I would like to thank Catherine Hall and Jim Treasurer for their review of the manuscript, and the organizers for their invitation to participate in the Aquachem workshop as an FAO expert.

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ANNEX I
Fish vaccine products supplied to the Chilean market as of December 2009[1, 2]

Product Name	Formulation	Disease	Manufacturer
Alpha Ject 3-3	Inactivated/Injection	Atypical furunculosis + Vibriosis + IPN	Pharmaq AS Chile Ltda.
Compact VIAS	Inactivated/Injection	Vibriosis + Furunculosis + IPN	Intervet Chile Ltda
Alpha Ject 1000	Inactivated/Injection	IPN	Pharmaq AS Chile Ltda.
Agrovac SRS	Inactivated/Injection	SRS	Agrovet Ltda.
Alpha Ject 2-3	Inactivated/Injection	Vibriosis + IPN	Pharmaq AS Chile Ltda.
Compact IPN	Inactivated/Injection	IPN	Intervet Chile Ltda
Rickettvac Oleo	Inactivated/Injection	SRS	Recalcine S.A.
Bayovac SRS	Recombinant protein/ Injection	SRS	Bayer S.A.
Aquavac IPN	Inactivated/Injection	IPN	Schering Plough Cia. Ltda.
Aquavac IPN Oral	Inactivated/Oral	IPN	Schering Plough Cia. Ltda.
Birnagen Forte	Inactivated/Injection	IPN	Novartis Chile S.A.
Birnagen Forte V	Inactivated/Injection	IPN + Vibriosis,	Novartis Chile S.A.
Birnagen Forte AV	Inactivated/Injection	IPN + Vibriosis +Furunculosis	Novartis Chile S.A.
Agrovac IPN	Inactivated/Injection	IPN	Agrovet Ltda.
Vibriosis	Inactivated/Injection	Vibrio ordalii	Centrovet Ltda.
Ipe-Vac	Inactivated/Immersion	IPN	Veterquímica Ltda.
	Inactivated/Injection	IPN	Centrovet Ltda.
Birnagen Forte 3	Inactivated/Injection	IPN + Vibrio ordalii + SRS	Novartis Chile S.A.
	Inactivated/Injection	SRS	Centrovet Ltda.
Alpha Ject 4-1	Inactivated/Injection	Atypical Furunculosis + Vibriosis + SRS + IPN	Pharmaq AS Chile Ltda.
Agrovac 4	Inactivated/Injection	SRS + IPN+ Vibriosis + Furunculosis	Agrovet Ltda.
Birnagen Forte 4	Inactivated/Injection	IPN + Vibrio ordalii + Atypical Furunculosis + SRS	Novartis Chile S.A.
Birnagen Forte 2	Inactivated/Injection	IPN + SRS	Novartis Chile S.A.
Agrovac 3	Inactivated/Injection	SRS + IPN + Vibriosis	Agrovet Ltda.
Alpha Ject Micro 3	Inactivated/Injection	Vibriosis + SRS + IPN	Pharmaq AS Chile Ltda.
	Inactivated/Injection	IPN + Vibrio ordalii + SRS	Centrovet Ltda.
Aquavac Vibrio Oral	Inactivated/Oral	Vibrio ordalii,	Schering Plough Cia. Ltda.
	Inactivated/Injection	IPN + SRS	Centrovet Ltda.
Agrovac 2	Inactivated/Injection	IPN + Vibriosis	Agrovet Ltda.
	Inactivate /Injection	Vibrio ordalii + IPN	Centrovet Ltda.
Alpha Ject Micro 2	Inactivated/Injection	SRS + IPN	Pharmaq AS Chile Ltda.
Bayovac 3,1	Recombinant protein/ Injection	IPN+ SRS	Bayer S.A.
	Inactivated/Oral	SRS	Centrovet Ltda.
	Recombinant protein/ Injection	ISA	Centrovet Ltda.
	Inactivated/Injection	SRS + IPN + Vibrio ordalii + Atypical furunculosis	Centrovet Ltda.
	Inactivated/Injection	SRS + IPN + Vibriosis	Recalcine S.A.
	Inactivated/Injection	ISA + IPN + Vibriosis + SRS	Recalcine S.A.
	Inactivated/Injection	ISA	Novartis Chile S.A.
Agrovac-ISA	Inactivated/Injection	ISA	Agrovet Ltda.
Agrovac 3 + ISA	Inactivated/Injection	SRS + IPN + ISA	Agrovet Ltda.
	Inactivated/Oral	ISA	Centrovet Ltda.

[1]*Source*: www.sag.cl, December 2009.
[2]IPN = infectious pancreatic necrosis; ISA = infectious salmon anaemia; SRS = salmon rickettsial syndrome.

Good aquaculture practices to minimize bacterial resistance

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Koonse, B. 2012. Good aquaculture practices to minimize bacterial resistance. *In* M.G. Bondad-Reantaso, J.R. Arthur & R.P. Subasinghe, eds. *Improving biosecurity through prudent and responsible use of veterinary medicines in aquatic food production*, pp. 25—28. FAO Fisheries and Aquaculture Technical Paper No. 547. Rome, FAO. 207 pp.

ABSTRACT

In all aspects of health care, the first step toward minimizing the risk of any disease or malady is prevention. In aquaculture, the preventive measures intended to reduce the risk of disease occurring in the produced animals can be called good aquaculture practices, best management practices, biosecurity measures, etc., but they all have the intended purpose of preventing diseases from occurring and having to use a chemotherapeutic agent to treat the animals. Therefore, developing and implementing a preventive measures programme is the first step in the prudent and responsible use of veterinary medicines (antimicrobials) in aquatic food production. Overall basic GAqPs include good hatchery management practices, good grow-out conditions, good environmental controls and good primary processing. For this paper, those GAqPs that can also help minimize bacterial resistance will be discussed. They are generally those that will reduce stress and promote animal health and quality, thus reducing the need for chemotherapeutic intervention. In this case, they can be put in three basic categories: (i) GAqPs for hatcheries and farms; (ii) GAqPs for regulators; and (iii) GAqPs for academia, health providers and biologists.

INTRODUCTION

Good aquaculture practices (GAqPs) can generally be defined as those preventive measures that once developed and properly implemented – according to the species cultured, the local environmental conditions and the aquaculture system being used – can help assure that aquacultured animals are maintained healthy, are safe to consume and do not impede trade, are produced in a sustainable manner, and are a high-quality product.

GAQPS FOR HATCHERIES AND FARMS

Specific GAqPs for hatcheries and farms are a combination of preventive measures that are intended to reduce stress and maintain a healthy animal.

It is normally understood that the occurrence of disease is a combination of the health of the animal, the condition of the environment and the presence of a pathogen. Klesius, Shoemaker and Evans (2003) described how disease was the result of a weakened immune system of the culture animal that causes neuroimmune changes resulting in stress and infection. Therefore, if infectious agents are excluded and stress is reduced, disease outbreaks are much less likely to occur.

Lightner (2003) identified ways of excluding pathogens from stock (i.e. postlarvae and broodstock), especially through the use of quarantine and specific pathogen-free certified stocks, excluding vectors and external sources of contamination, and preventing internal cross-contamination.

Horowitz and Horowitz (2003) described the physical, chemical and biological precautionary measures to be taken, as well as a second line of defense against potential disease outbreaks. From this concept, as well as others, some preventive GAqPs for hatcheries and farms can be summarized as follows:

- *Physical*. These are, generally, measures that are intended to prevent disease-carrying vectors from entering a hatchery or farm site, and include, among other things, physical barriers, water treatment and quarantine. *Physical* can also be those measures that reduce stress, such as not overcrowding animals in pens or ponds; proper locating of farming operations; and avoiding inconsistent or improper temperatures, consistent dissolved oxygen problems, excessive handling, physical abuse and inadequate diets.
- *Chemical.* These include those measures that are used to prevent the introduction of pathogens or vectors by treating materials before they enter a facility. For example, chlorination or ozonization can be used to treat incoming water, and iodine and chlorine can be used to treat other potential vectors such as tools, footwear and clothing.
- *Biological.* These include those measures that prevent or treat infections, such as the prompt and effective use of proper chemotherapeutics or the use of vaccines. They may also include the use of specific pathogen-free shrimp. Biological GAqPs for hatcheries and farms also include management practices to prevent pathogen or bacterial contamination of aquaculture products, grow-out ponds or cages, and the environment. This includes having proper toilet facilities, sanitary waste removal, healthy workers, effluent treatment and site security.

Other GAqPs for hatcheries and farms include practices that prevent the spread of pathogens, as well as the agents used for prevention (e.g. sanitizers, vaccines) or treatment of animals (e.g. antibiotics, antifungal medications), from contaminating the environment. This also includes keeping accurate and complete records of grow-out parameters, the start and end dates of chemotherapeutic use, withdrawal times, and bought and sold information.

GAQPS FOR REGULATORS

GAqPs for regulators are generally those meant to ensure that only approved chemotherapeutic agents are available and used, that they are used properly (i.e. particularly that withdrawal times are properly calculated and followed), and that records are kept. This includes: conducting farm, hatchery, inspecting feed mill and veterinary medical product locations; restricting the entry of chemotherapeutic agents and their components at the border; ensuring that only those properly trained in chemotherapeutic diagnosis and application have access to drugs; collecting and analyzing verification samples for residues; checking records; and training and education.

GAQPS FOR ACADEMIA, HEALTH PROVIDERS AND BIOLOGISTS

The proper use of chemotherapeutants begins with an accurate diagnosis of the disease and the causative agent. This information should be used with a thorough understanding of the animal (and the species) being reared, the biological and production system the animal is being reared in, and the intended purpose of the aquaculture product (e.g. shrimp for human consumption for the United States of America market).

These are broad GAqPs that are intended to assure chemotherapeutics are used wisely. This generally includes proper training/certification/licensing of aquaculture professionals, having and using adequate detection and diagnostic tools/programmes/methods, using only the correct drugs and that they are used properly for effectiveness

and that the drugs do not leave an undesirable residue, and that health providers or biologists are participating in disinfection and pathogen eradication programmes.

Some common questions health-care providers should ask are:

- Is the drug registered for use in aquaculture against the etiological agent?
- What is the toxicity of the drug to the host animal?
- Will the available methods of treatment deliver effective levels of the drug to the site of infection?
- What hazards does the drug pose to the user?
- How will the drug affect desirable biota or biological filter systems?
- Will the drug leave harmful or undesirable residues in the flesh of treated animals?

RECOMMENDATIONS

To the Food and Agriculture Organization of the United Nations (FAO):

- FAO should pull together the appropriate stakeholders to discuss a strategy to develop specific GAqPs and biosecurity guidelines that the aquaculture sector (particularly farmers) can use to both minimize aquatic animal susceptibility to disease and to help prevent the spread and proliferation of diseases.
- The stakeholders should consider the feasibility of developing an international chemotherapeutic use network. The goal of this network or programme would be to harmonize drug use standards; develop uniform training, certification and licensing requirements; establish mechanisms for data sharing on what drugs are being used, approval data, drug application data, etc.; and establish protocols for responding rapidly and effectively when a disease problem occurs. In addition, some type of oversight and accountability system should be considered for this network and include funds to sustain the programme.

To national governments:

- Each country should develop and implement its own GAqP programme appropriate for its country, including the legal regulatory structure, the aquaculture industry and the environmental conditions. The programme should include those measures that will reduce the need for chemotherapeutic interventions, measure the use of chemotherapeutic agents (e.g. maintain proper records), and assure there are no unacceptable levels of unapproved chemotherapeutic agents.
- National competent authorities should have and implement the national GAqP programme. Their agents should be trained in GAqPs and should conduct inspections of farms to assure GAqPs are being properly implemented (e.g. proper densities are being maintained, wastewater is not impacting source water, farms and hatcheries are keeping drug use records, and samples are being collected and tested).

To the aquaculture industry:

- The aquaculture industry should assume responsibility for minimizing the need for and use of chemotherapeutic agents and should properly implement the national GAqP programmes.

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Survey on the use of veterinary medicines in aquaculture

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ABSTRACT

The survey aimed at understanding the current status of the use of veterinary medicines (i.e. antimicrobials and other chemotherapeutants was conducted through e-mail distribution and face-to-face consultation with a wide range of stakeholders (e.g. fish farmers, government staff, feed manufacturers, feed and drug sellers and extension officers) in selected countries. A total of 197 survey returns were collected from 21 countries (in addition to three unknown country sources), and a survey database was created. The methods of analysis used were: (i) percentages and medians to describe the distribution of categorical (e.g. geographic location of respondents) and continuous variables (e.g. number of substances reported); (ii) species-wide comparisons using only data from e-mail respondents and for both species being considered in the analysis; (iii) Bartlett's Test for Inequality of Population Variances to establish whether an ANOVA (parametric) or Kruskal-Wallis (non-parametric) test was most appropriate to compare continuous variables (e.g. the number of substances reported for different species). All tests were conducted using Epi Info 3.5 and the use of a p-value of 0.05 to identify significant associations. Survey results provided an insight on the use of treatments and prophylactic measures used in aquaculture. Despite the small number of respondents, some valuable associations were identified and some recommendations were put forward.

INTRODUCTION

As in any other animal production sector, veterinary drugs are used in aquaculture during production mainly to prevent and treat bacterial, fungal and parasitic diseases. The use of these products has been taken up progressively by the industry with the learning and understanding of health management and the application of biosecurity to aquaculture. The gains that have been made in aquaculture production capacity would not have been possible without the use of these products.

More than ten years ago, the Food and Agriculture Organization of the United Nations (FAO), in cooperation with the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC-AQD) and the Canadian International Development Agency (CIDA), organized the Expert Meeting on the Use of Chemicals in Aquaculture in Asia or "Aquachem" from 20–22 May 1996 at the SEAFDEC-AQD headquarters in Tigbauan, Iloilo, the Philippines. That meeting generated information on the pattern of use of veterinary drugs at that time through national reports. In 2009, it was again considered timely to assess the current usage of these products. For this purpose, a survey was prepared in an attempt to assess the current situation.

OBJECTIVES OF THE STUDY

The objective of the survey was to understand the current status of the use of veterinary medicines, i.e. antimicrobials and other chemotherapeutants in aquaculture conducted through an internet-based survey and interview-based survey in selected countries.

METHODOLOGY

Survey structure and process

A survey questionnaire was developed with seven sections, briefly described below

- Section 1: Respondent profile (academic background and professional activity of the respondent)
- Section 2: Types of antimicrobials used for therapeutic purposes (antimicrobials used for treating disease (therapeutic application) in different host species groups)
- Section 3: Types of antimicrobials used for prophylactic purposes (antimicrobials used for prevention of diseases (prophylactic application) and the stages when they are applied (broodstock, hatchery and grow out).
- Section 4: Application (percentage at the different stages of culture (broodstock, hatchery and grow out) and dosage and duration of antimicrobial treatments for prophylactic and therapeutic use)
- Section 5: Use of chemotherapeutants (type, mode of application and for which diseases, source and availability), as well as other veterinary products (i.e. anesthetics, sex control aids, spawning aids, etc.) used in aquaculture
- Section 6: Impact (perceived positive and negative impacts) and efficacy (possible reasons for failure)
- Section 7: Recommendations for actions to improve effectiveness and responsible use in aquaculture

For each of the questions, a list of aquaculture species was given. These include the main cultured species groups such as shrimp, salmon, trout, tilapia, pangasius, carp and marine fish. Additional space was provided for other species. A list of veterinary drugs was also provided, as can be seen in Table 1.

Considering that the use of veterinary products and antimicrobials in particular is a sensitive matter because of its implications to the product and national image or product market access, responses were kept anonymous and analyzed regardless of the geographic origin. There was no intention to quantify the use of these products, as information on quantities is only reliable in highly regulated countries.

TABLE 1
List of active ingredients of veterinary medicines listed in the survey.

Antibiotics	External treatments (bacteria, parasites and fungi)	Sea lice control	Antihelmintics
Flumequine	Benzalkonium chloride	Emamectin benzoate	Ivermectin
Oxolinic acid	Chloramine-T	Teflubenzuron	Diflubenzuron
Trimethoprim-sulfadiazine	Copper sulphate	Azamethiphos	Praziquantel
Florfenicol	Diquat bromide	Cypermethrin	
Amoxycillin	Formalin	Dichlorvos	
Erytromycin	Hydrogen peroxide	Acyl urea	
Oxytetracycline	Potassium permanganate	Hydrogen peroxide	
Enrofloxacin	Dichlorvos		
Fosfomicine	Trichlorfon		
	Malachite green		
	Methylene blue		
	Trifularin		

Distribution of the questionnaire

The survey targeted interested stakeholders involved in the production, sales, use and application of veterinary products such as fish farmers, government staff, academicians, feed manufacturers, feed and drug sellers, extension officers, aquatic animal health and aquatic veterinary professionals.

Two approaches were used to distribute the questionnaire. One approach used was through Internet correspondence to participants of major conferences (e.g. Larvi 2009, World Aquaculture Society (WAS) Aquaculture America – Seattle 2009, Asia-Pacific Conference – Australia 2008, and European Aquaculture Society – Poland 2008), databases of Latin American and North American aquaculture pathologists and veterinarians and country stakeholders (Chile), web-based discussion groups (Sarnissa, Sociedad Latino Americana de Aquacultura, Asian Fisheries Society), professional associations (Fish Veterinary Society, World Aquatic Veterinary Medical Association, Fish Health Section of the Asian Fisheries Society, Mexican Aquaculture Sanitary Committee), international organizations (Network of Aquaculture Centres in Asia and the Pacific (NACA) and Centre international de hautes études agronomiques méditerranéennes (CIHEAM)) and newsletters (American Veterinary Medical Association (AVMA), AQUATIC-L listserv).

The second approach was through direct interviews. Face-to-face individual and group interviews were done in selected major aquaculture-producing countries (i.e. China, the Philippines, Thailand and Viet Nam). Every effort was made to include all the stakeholder groups involved in the production and use of veterinary drugs (e.g. seed producers, fish farmers, extension workers, drug manufacturers, aquatic animal health and aquatic veterinary professionals). The distribution of the questionnaire was done between July and October 2009.

STATISTICAL METHODS

All the statistical analyses were conducted using Epi Info 3.5.1.

Descriptive analysis was conducted using frequency tables for both continuous and categorical variables. Continuous variables were described also using medians and ranges.

Pair-wise comparisons on the number of substances reported for different species groups were conducted using only information from e-mail respondents and from respondents answering questions for both the species groups being considered in the comparison. This approach was adopted for all comparisons with the exception of the pangasius-catfish comparison, which used all the e-mail submissions for the two species groups.

ANOVA and the Kruskal-Wallis Test were used to assess the significance of univariate associations when the population variances were tested to be equal or unequal, respectively. The inequality of population variances was tested using the Bartlett's Test. Multivariable analysis was conducted by developing linear regression models.

A p-value of 0.05 was used to identify significant associations. When non-statistical associations were identified, p-values were reported to allow a deeper understanding of the association

RESULTS

Section 1. Respondent profile

In total 197 responses were gathered. These included 54 (27.4%) email and 143 (72.6%) interview-based submissions.

E-mail responses were submitted by people located in all the continents (Table 2) and in 24 countries (Table 3). Particularly represented were Mexico (13 respondents) and Colombia and Spain (both with 5 respondents). Twenty-eight (51.9%) submissions were in Spanish, while 26 (48.1%) were in English. The country-wise distribution of the interviews showed that most respondents were located in China and Viet Nam (Table 4). The academic level of respondents was very diverse, with the majority of them having a Bachelor of Science degree or higher degree (Table 5).

The majority of respondents (i.e. 33.9%) were primarily involved with farming or worked for the government (20.9%), although data were also gathered from considerable numbers of respondents involved primarily in other activities (Table 6). Respondents were often involved with more than one activity, with a relatively higher proportion of respondents being involved with farming (Table 7).

There were considerable differences between the e-mail submissions and the interview results. In fact, people submitting information by e-mail covered a wider range of species groups when compared with people interviewed, who in 59.4% of cases submitted information only about one species (Table 8)

The species-wise distribution also differed considerably between the two groups of respondents, being wider in e-mail submission (Table 9) when compared with interview results (Table 10), which were largely focused on five species groups, namely shrimp, tilapia, pangasius, carps and marine fish.

TABLE 2
Region-wise distribution of the email respondents

Region	Frequency	Percent
Asia	11	20.4%
Europe	12	22.2%
North America	17	31.5%
Oceania	2	3.7%
South America	9	16.7%
Unknown	3	5.6%
Total	54	100.0%

TABLE 3
Country-wise distribution of the e-mail respondents

Country	Frequency	Percentage	Country	Frequency	Percentage
Australia	2	3.7%	Norway	1	1.9%
Bangladesh	1	1.9%	Peru	1	1.9%
Bosnia &Herzegovina	1	1.9%	Philippines	3	5.6%
Brazil	1	1.9%	Saudi Arabia	1	1.9%
Canada	1	1.9%	Spain	5	9.3%
Chile	1	1.9%	Switzerland	1	1.9%
Hong Kong SAR	1	1.9%	Thailand	2	3.7%
Colombia	5	9.3%	United Kingdom	3	5.6%
Croatia	1	1.9%	Unknown	3	5.6%
India	1	1.9%	United States of America	3	5.6%
Bahrain	1	1.9%	Venezuela	1	1.9%
Mexico	13	24.1%	Viet Nam	1	1.9%

TABLE 4
Country-wise distribution of the interviewees

Country	Frequency	Percent
China	50	35.0%
Philippines	35	24.5%
Thailand	9	6.3%
Viet Nam	49	34.3%
Total	143	100.0%

TABLE 5
Distribution of academic level among respondents

Academic level	Frequency	Percent
Primary (Elementary)	18	9.8%
Secondary (High School)	13	7.1%
Professional education	35	19.0%
Bachelor of Science	69	37.5%
Post-graduate	47	25.5%
Other	2	1.1%
Total	184	100.0%

TABLE 6
Distribution of the main activities among respondents

Main Activity	Frequency	Percent
Farming	60	33.9%
Clinical	13	7.3%
Administration	19	10.7%
Government	37	20.9%
Private sector	9	5.1%
Research	16	9.0%
Suppliers	22	12.4%
Other	1	0.6%
Total	177	100.0%

TABLE 7
Distribution of involvement of the respondents in different activities

Activity	Total respondents	No. people involved in activity	Percentage
Farming	190	85	44.7%
Clinical	190	55	28.9%
Administration	190	42	22.1%
Government	190	55	28.9%
Private	190	52	27.4%
Research	190	43	22.6%
Suppliers	190	43	22.6%

TABLE 8
Frequency distribution of the number of species for which respondents submitted information on treatment or preventive measures

No. of Species groups	Treatment		Preventive measures	
	Frequency	Percentage	Frequency	Percentage
0	3	5.6%	4	2.8%
1	11	20.4%	85	59.4%
2	10	18.5%	15	10.5%
3	11	20.4%	11	7.7%
4	6	11.1%	24	16.8%
5	7	13.0%	4	2.8%
6	6	11.1%	0[1]	0.0%
Total	54	100.0%	143	100.0%

[1] "0" indicates that no information on products was submitted, although answers to other questions were provided.

TABLE 9
Species-wise frequency distribution of responses in the e-mail submission group and by region

	Shrimp	Salmon	Trout	Tilapia	Panga	Carp	Marine	Catfish	Ornamentals	Seabass	Frog	Crab
Asia	7	3	0	5	2	5	9	2	2	1	1	1
Europe	2	3	7	1	1	2	9	1	1	1	0	1
North America	4	3	11	11	3	9	7	4	1	0	0	0
South America	5	1	5	7	0	2	3	1	0	0	0	1
Oceania	1	1	2	1	0	1	1	1	1	0	0	0
Unknown	1	0	0	0	0	0	1	1	1	0	0	1
TOTAL	20	11	25	25	6	19	30	10	6	2	1	4

TABLE 10
Species-wise frequency distribution of responses in the interviewed group and by country

	Shrimp	Salmon	Trout	Tilapia	Panga	Carp	Marine	Catfish	Ornamentals	Seabass	Frog	Crab
China	19	1	0	16	0	41	12	0	0	0	0	0
Philippines	19	1	0	19	4	1	15	0	0	0	0	0
Thailand	7	0	0	7	2	5	4	0	1	0	1	0
Viet Nam	21	1	0	13	40	8	6	0	0	0	0	0
TOTAL	66	3	0	55	46	55	37	0	1	0	1	0

Section 2. Types of antimicrobials used for therapeutic purposes

For most species, external treatments and antibiotics prevailed as methods for disease treatment (Table 11). Oxytetracycline was the product most reported for treatment of diseases in all major species, e.g. shrimp, tilapia, pangasius, marine fish, trout and salmon (Table 12). Oxytetracycline and trimethoprim-sulfadiazine were the antibiotics with the wider species coverage. Concerning external treatment, formalin was used for more species.

Praziquantel was the antihelmintic with the widest species coverage, while for sealice control, emamectin benzoate and hydrogen peroxide were widely used (Table 13).

There appeared to be significant differences in the number of agents used as a whole or as treatment for different species. A significantly higher number of agents were used for shrimp when compared with carp. The association between the number of treatments in trout versus tilapia and the one between catfish and pangasius were also significant (Table 14).

TABLE 11
Number of substances used for treatment for each species or species group and by type of use

Species	Antibiotics	Antihelmintic	External treatment	Sea lice control	Other
Carp	7	4	14	4	1
Catfish	5	4	10	1	
Crab		1	3		
Frog	3	2	3		
Marine fish	9	4	14	5	1
Ornamentals	5	2	6		
Pangasius	10	4	13	2	
Salmon	8	1	7	5	
Seabass	2		4	1	
Shrimp	9	3	14	4	1
Tilapia	9	4	14	4	1
Trout	9	1	12	5	

TABLE 12
Number of times an antibiotic was reported as treatment by species

Substance	Carp	Catfish	Crab	Frog	Marine fish	Ornamentals	Pangasius	Salmon	Seabass	Shrimp	Tilapia	Trout
Amoxycillin	2	2	0	0	5	1	15	2	0	13	12	2
Enrofloxacin	9	3	0	0	6	2	13	0	0	9	9	3
Erytromycin	0	0	0	0	5	0	4	1	0	14	4	6
Florfenicol	15	5	0	0	18	0	23	4	0	23	21	16
Flumequine	0	0	0	0	6	0	4	3	0	3	1	6
Fosfomicine	0	0	0	1	0	1	1	0	0	0	0	0
Others	10	0	0	0	9	0	16	3	0	17	11	3
Oxolinic acid	2	0	0	0	7	0	2	4	0	6	3	5
Oxytetracycline	38	9	0	1	38	1	34	7	1	49	44	21
Trimethoprim-sulfadiazine	13	6	0	2	9	1	17	1	1	9	16	8

TABLE 13
Number of species for which a substance was used for treatment by type of use

Substance	Antibiotics	Antihelmintics	External treatments	Sea lice control	Other
Acyl urea				1	
Amoxycillin	9				
Azamethiphos				2	
Benzalkonium chloride			7		
Chinese Herb medicine					4
Chloramine-T			8		
Copper sulphate			7		
Cypermethrin				6	
Dichlorvos			8		
Dichlorvos				2	
Diflubenzuron		5			
Diquat bromide			7		
Emamectin benzoate				7	
Enrofloxacin	8				
Erytromycin	6				
Florfenicol	8				
Flumequine	6				
Formalin			12		
Fosfomicine	3				
Freshwater bath			1		
Hydrogen peroxide			9	7	
Ivermectin		6			
Malachite green			6		
Methylene blue			9		
Others	7	9	8	6	
Oxolinic acid	7				
Oxytetracycline	11				
Potassium permanganate			10		
Praziquantel		10			
Salt			6		
Trichlorfon			8		
Trifularin			8		
Trimethoprim-sulfadiazine	11				

TABLE 14
Pair-wise comparison of the median number of substances used as treatment for different species

Species	No. agents	p-value	No. agents (treatment)	p-value
Tilapia vs Trout	5 vs 7	0.4445	3.5 vs 6	0.1016
Carp vs Tilapia	7 vs 6	0.6134	5 vs 5	0.5056
Carp vs Shrimp	0 vs 5	0.0381	0 vs 3	0.0152
Catfish vs Tilapia	0 vs 1	0.6455	0 vs 1	0.6259
Marine vs Tilapia	4 vs 6	0.1692	3 vs 5	0.2829
Carp vs Trout	7 vs 8	0.3758	5 vs 6	0.1448
Carp vs Marine fish	6.5 vs 4	0.3680	4.5 vs 3.5	0.5849
Shrimp vs Trout	4.5 vs 0	0.1986	3 vs 0	0.3605
Catfish vs Pangasius	9 vs 4.5	0.2442	6 vs 5	0.0706

Section 3. Types Of Antimicrobials Used For Prophylactic Purposes

External treatments and treatment with antibiotics were the most commonly reported methods for disease prevention for most species (Table 15).

Oxytetracycline was the most-reported antibiotic used for prophylactic treatment for most species (Table 16). Oxytetracycline was also the most-reported antibiotic used for prevention for the largest number of species (i.e. 10), while formalin was the external treatment with the highest species coverage, and also the treatment reportedly used in the prevention of diseases for ten species (Table 17). Ivermectin was the antihelmintic with the widest species coverage, while hydrogen peroxide was the treatment mostly used on many species for sea lice infection (Table 17).

Although there appeared to be differences in the number of agents used as prophylactic measures for different species, none of the associations were significant (Table 18).

TABLE 15
Number of substances used for prevention for each species and by type of use

Species	Antibiotics	Antihelmintic	External treatment	Sea lice control	Other
Carp	9	3	12	1	1
Catfish	4	2	7		
Crab	2		2		
Frog	3	1	2		
Marine fish	8	3	12	3	1
Ornamentals	3	2	6		
Pangasius	8	4	13	2	
Salmon			5	1	
Shrimp	8	3	12	2	1
Tilapia	8	3	13	2	1
Trout	6	2	12		

TABLE 16
Number of times an antibiotic was reported as prophylactic method by species

Substance	Carp	Catfish	Crab	Frog	Marine fish	Ornamentals	Pangasius	Salmon	Sea-bass	Shrimp	Tilapia	Trout
Amoxycillin	2	0	0	0	1	0	7	0	0	7	4	0
Enrofloxacin	5	2	0	1	2	2	3	0	0	7	8	2
Erytromicyn	1	0	0	0	1	0	3	0	0	6	2	1
Florfenicol	6	2	0	0	7	0	6	0	0	14	10	2
Flumequine	0	0	0	0	0	0	0	0	0	0	0	0
Fosfomicine	1	0	0	0	0	0	0	0	0	0	0	0
Others	5	1	1	0	7	0	1	0	0	11	9	1
Oxolinic acid	1	0	0	0	1	0	1	0	0	1	1	0
Oxytetracycline	21	5	1	1	19	2	18	0	0	36	17	2
Trimethoprim-sulfadiazine	4	0	0	1	3	2	8	0	0	2	5	2

TABLE 17
Number of species for which a substance was used for prevention by kind of use

Substance	Antibiotics	Antihelmintic	External treatment	Sea lice control	Other
Amoxycillin	5	-	-	-	-
Benzalkonium chloride	-	-	7	-	-
Chinese herb medicine	-	-	-	-	4
Chloramine-T	-	-	7	-	-
Copper sulphate	-	-	8	-	-
Dichlorvos	-	-	6	-	-
Diflubenzuron	-	2	-	-	-
Diquat bromide	-	-	1	-	-
Emamectin benzoate	-	-	-	4	-
Enrofloxacin	9	-	-	-	-
Erytromicyn	6	-	-	-	-
Florfenicol	7	-	-	-	-
Formalin	-	-	10	-	-
Fosfomicine	1	-	-	-	-
Freshwater bath	-	-	2	-	-
Hydrogen peroxide	-	-	6	5	-
Ivermectin	-	7	-	-	-
Malachite green	-	-	6	-	-
Methylene blue	-	-	7	-	-
Others	8	8	7	2	-
Oxolinic acid	5	-	-	-	-
Oxytetracycline	10	-	-	-	-
Potassium permanganate	-	-	9	-	-
Praziquantel	-	6	-	-	-
Salt	-	-	8	-	-
Trichlorfon	-	-	8	-	-
Trifularin	-	-	4	-	-
Trimethoprim-sulfadiazine	8	-	-	-	-

TABLE 18
Pair-wise comparison of the median number of substances used for prophylaxis for different species

Species	N agents (total)	p-value	N agents (prophylaxis)	p-value
Tilapia vs Trout	5 vs 7	0.4445	1 vs 2	0.6341
Carp vs Tilapia	7 vs 6	0.6134	2 vs 2	0.7659
Carp vs Shrimp	0 vs 5	0.0381	0 vs 2	0.1308
Catfish vs Tilapia	0 vs 1	0.6455	0 vs 0	0.6892
Marine vs Tilapia	4 vs 6	0.1692	1 vs 2	0.1415
Carp vs Trout	7 vs 8	0.3758	1 vs 2.5	0.7177
Carp vs Marine	6.5 vs 4	0.3680	1 vs 1	0.6951
Shrimp vs Trout	4.5 vs 0	0.1986	1.5 vs 0	0.1231
Catfish vs Pangasius	9 vs 4.5	0.2442	2 vs 2	0.7127

Section 4. Application

Treatments were done throughout all stages of production for most species (Table 19). No significant trend could be detected in the number of stocks subjected to treatment with considerable variability in responses.

TABLE 19
Percentage of stocks treated at different stages of culture

Species	Stage	N. of respondents	Average %
Shrimp	Broodstock	15	53.1%
Shrimp	Hatchery	30	67.0%
Shrimp	Grow-out	24	48.3%
Salmon	Broodstock	2	11.0%
Salmon	Hatchery	3	43.3%
Salmon	Grow-out	3	46.3%
Trout	Broodstock	4	8.4%
Trout	Hatchery	13	54.4%
Trout	Grow-out	10	42.9%
Tilapia	Broodstock	12	53.3%
Tilapia	Hatchery	23	63.7%
Tilapia	Grow-out	30	56.9%
Pangasius	Broodstock	3	41.7%
Pangasius	Hatchery	7	29.9%
Pangasius	Grow-out	11	39.9%
Carp	Broodstock	15	29.1%
Carp	Hatchery	20	27.4%
Carp	Grow-out	28	43.6%
Marine fish	Broodstock	8	62.6%
Marine fish	Hatchery	13	45.8%
Marine fish	Grow-out	23	50.8%
Catfish	Broodstock	4	55.3%
Catfish	Hatchery	6	29.3%
Catfish	Grow-out	7	34.3%
Ornamentals	Broodstock	0	
Ornamentals	Hatchery	0	
Ornamentals	Grow-out	2	25.0%
Seabass	Broodstock	1	1.0%
Seabass	Hatchery	1	2.0%
Seabass	Grow-out	1	2.0%

Section 5. Use of chemotherapeutants in aquaculture

Use of chemotherapeutants in water was the preferred method of application for most species, although reporting of substance application directly on to the animal or eggs or on to inert materials was also common (Table 20).

Most products were considered freely available, including products that are banned for use in aquaculture in most countries, e.g. malachite green (Table 21).

There was a strongly significant difference between regions with respect to the percentage of products reported to be freely available (Table 22); Asia and South America reported to have more products freely available in the market than other regions (98.5% and 89.2%, respectively).

Similarly, when analyzing only the interview-based submissions, there was a significant difference between countries reporting availability of the agents. For example, Thailand reported a lower percentage compared with other countries, where the percentage of products freely available in the market was almost 100% (Table 23). There was a significant difference in the percentage of products reported to be freely available with respect to whether the respondent was involved in farming or not, with fish-farmer respondents reporting a significantly higher percentage (Table 24).

When a multivariable linear regression model was developed to take into account the potential confounding effect of the involvement in farming or region versus the percentage of products freely available, the associations with region and farming involvement remained significant, with a correlation coefficient of 0.41 (Table 25).

Vaccine use was reported primarily for tilapia, marine fish, pangasius, trout and salmon. Vaccines were most commonly addressed for the prevention of aeromonids and vibrios, although vaccines for *Streptococcus*, *Yersinia*, *Edwardsiella* and *Pasteurella* were also common (Table 26).

Two respondents reported the use of bacterins (*Aeromonas* sp. bacterin; Autogenous bacterins; *Edwardsiella ictaluri* bacterin; *Streptococcus* sp. bacterin; *Vibrio* sp. bacterin; *Vibrio anguillarum* bacterin; *Vibrio parahaemolyticus* bacterin) for carp.

Fifteen respondents also mentioned that vaccines were being used for shrimp diseases, with four respondents specifying that these "vaccines" were in reality probiotics.

Among other products used, anesthetics were most commonly reported for most species. Spawning aids were most common for pangasius, carp and catfish, while sex control aids were the most frequently reported type of product used in tilapia farming (Table 27).

TABLE 20
Frequency distribution of the methods of application of chemotherapeutants

Species	Method of application	Total N. respondents	N. positive respondents	Average %
Shrimp	Animals/eggs	57	20	35.1%
Shrimp	Water	57	54	94.7%
Shrimp	Inert Materials	57	17	29.8%
Salmon	Animals/eggs	7	5	71.4%
Salmon	Water	7	6	85.7%
Salmon	Inert Materials	7	4	57.1%
Trout	Animals/eggs	21	16	76.2%
Trout	Water	21	15	71.4%
Trout	Inert Materials	21	10	47.6%
Tilapia	Animals/eggs	40	14	35.0%
Tilapia	Water	40	36	90.0%
Tilapia	Inert Materials	40	14	35.0%
Pangasius	Animals/eggs	14	3	21.4%
Pangasius	Water	14	13	92.9%
Pangasius	Inert Materials	14	1	7.1%
Carp	Animals/eggs	34	7	20.6%
Carp	Water	34	31	91.2%
Carp	Inert Materials	34	9	26.5%
Marine fish	Animals/eggs	33	14	42.4%
Marine fish	Water	33	29	87.9%
Marine fish	Inert Materials	33	11	33.3%
Catfish	Animals/eggs	5	1	20.0%
Catfish	Water	5	5	100.0%
Catfish	Inert Materials	5	2	40.0%

TABLE 21
Reported availability of different products

Product	No. respondents	No. freely available	% freely available	No. only on prescription	% only on prescription
Antibiotics					
Oxytetracycline	128	117	91.4%	11	8.6%
Trimethoprime-sulfadiazine	46	37	80.4%	9	19.6%
Amoxiciline	56	45	80.4%	11	19.6%
Florfenicol	64	50	78.1%	14	21.9%
Enrofloxacina	54	42	77.8%	12	22.2%
Erythromycin	48	37	77.1%	11	22.9%
Oxolinic acid	32	24	75.0%	8	25.0%
Fosfomicina	15	10	66.7%	5	33.3%
Flumequine	22	14	63.6%	8	36.4%
External treatments					
Dichlorvos	20	20	100.0%	0	0.0%
Benzalkonium chloride	54	53	98.1%	1	1.9%
Copper sulphate	80	78	97.5%	2	2.5%
Hydrogen peroxide	64	62	96.9%	2	3.1%
Trichlorfon	60	58	96.7%	2	3.3%

TABLE 21 (CONTINUED)
Reported availability of different products

Product	No. respondents	No. freely available	% freely available	No. only on prescription	% only on prescription
Potassium permanganate	82	79	96.3%	3	3.7%
Trifularin	25	24	96.0%	1	4.0%
Formalin	99	95	96.0%	4	4.0%
Methylene blue	43	41	95.3%	2	4.7%
Malachite green	34	32	94.1%	2	5.9%
Chloramine-T	36	32	88.9%	4	11.1%
Diquat bromide	16	14	87.5%	2	12.5%
Sealice control					
Hydrogen peroxide	32	30	93.8%	2	6.3%
Dichlorvos	14	13	92.9%	1	7.1%
Acyl urea	7	6	85.7%	1	14.3%
Teflubenzuron	8	6	75.0%	2	25.0%
Cypermethrin	15	11	73.3%	4	26.7%
Azamethiphos	11	7	63.6%	4	36.4%
Emamectin benzoate	13	7	53.8%	6	46.2%
Antihelmintics					
Ivermectin	29	28	96.6%	1	3.4%
Diflubenzuron	9	8	88.9%	1	11.1%
Praziquantel	17	13	76.5%	4	23.5%
Other					
Hydrogen peroxide	39	39	100.0%	0	0.0%
Chlorine	52	52	100.0%	0	0.0%
Phenolic compounds	14	14	100.0%	0	0.0%
Iodophors	35	35	100.0%	0	0.0%
Formaldehyde	53	52	98.1%	1	1.9%
Isopropyl alcohol	30	29	96.7%	1	3.3%
Quaternary ammonia	30	27	90.0%	3	10.0%
Vaccines	18	11	61.1%	7	38.9%

TABLE 22
Comparison of the reported percentage of products freely available in the market in different regions (p-value <0.0001)

Region	Observations	Total	Mean	Variance	Std Dev
Asia	130	128.0948	0.9853	.0053	.0729
Europe	10	7.1843	0.7184	.0952	.3085
North America	15	12.6810	0.8454	.0898	.2997
Oceania	2	.7027	0.3514	.2469	.4969
South America	9	8.0286	0.8921	.0388	.1969
Unknown	2	1.7391	0.8696	.0340	.1845

TABLE 23
Comparison of the reported percentage of products freely available in the market in different countries (p-value <0.0001)

Region	Observations	Total	Mean	Variance	Std Dev
China	48	47.8108	0.9961	.0007	.0273
Philippines	33	32.9375	0.9981	.0001	.0109
Thailandia	7	6.2846	0.8978	.0289	.1701
Viet Nam	32	31.9000	0.9969	.0003	.0177

TABLE 24
Comparison of the reported percentage of products freely available in the market in respect to the involvement of the responder in farming (p-value = 0.0151)

	Observations	Total	Mean	Variance	Std Dev
Not involved in farming	93	85.7108	0.9216	.0388	.1969
Involved in farming	71	69.4785	0.9786	.0154	.1240

TABLE 25
Results of multivariable analysis to assess the association between the reported percentage of products freely available in the market in respect to both the involvement of the responder in farming and the region where the responder was located

Variable	Coefficient	Std Error	F-test	p-value
Involved in farming	0.052	0.022	5.6596	0.018570
Region (Europe/Asia)	-0.257	0.045	33.2707	0.000000
Region (North America/Asia)	-0.130	0.037	12.2237	0.000615
Region (Oceania/Asia)	-1.017	0.136	56.2957	0.000000
Region (South America/Asia)	-0.102	0.046	4.7976	0.029985
Region (Unknown/Asia)	-0.147	0.097	2.3284	0.129060
CONSTANT	0.965	0.016	3840.8372	0.000000

TABLE 26
Species-wise distribution of pathogens against which vaccines were used

Species	Pathogen	Total No. of respondents	No. of positive respondents	Average %
Salmon	*Aeromonas*	9	8	88.9%
Salmon	Vibrios	9	8	88.9%
Salmon	*Yersinia*	9	5	55.6%
Salmon	IPN	9	3	33.3%
Salmon	*Streptococcus*	9	2	22.2%
Salmon	VHS	9	1	11.1%
Salmon	*Piscicricketsia*	9	1	11.1%
Salmon	*Renibacterium*	9	1	11.1%
Trout	*Aeromonas*	12	9	75.0%
Trout	Vibrios	12	7	58.3%
Trout	*Yersinia*	12	7	58.3%
Trout	*Streptococcus*	12	2	16.7%
Trout	IPN	12	2	16.7%
Trout	*Piscicricketsia*	12	1	8.3%
Tilapia	*Streptococcus*	18	15	83.3%
Tilapia	*Aeromonas*	18	4	22.2%
Tilapia	Vibrios	18	2	11.1%
Tilapia	IPN	18	1	5.6%
Tilapia	*Edwardsiella*	18	1	5.6%
Tilapia	*Pasteurella*	18	1	5.6%
Pangasius	*Edwardsiella*	12	10	83.3%
Pangasius	*Aeromonas*	12	8	66.7%
Pangasius	Vibrios	12	3	25.0%
Pangasius	IPN	12	1	8.3%
Pangasius	*Streptococcus*	12	1	8.3%
Marine fish	Vibrios	18	13	72.2%
Marine fish	*Streptococcus*	18	5	27.8%
Marine fish	*Pasteurella*	18	4	22.2%
Marine fish	*Edwardsiella*	18	2	11.1%
Marine fish	*Aeromonas*	18	1	5.6%
Marine fish	*Tenacibaculum*	18	1	5.6%
Marine fish	*Flexibacterium*	18	1	5.6%
Marine fish	*Photobacterium*	18	1	5.6%

TABLE 27
Percentage of respondents reporting the use of anesthetics, sex control aids and spawning aids by species

Species	No. of respondents	Percentage of respondents		
		Anesthetics	Sex control aids	Spawning aids
Carp	42	31.0%	7.1%	83.3%
Catfish	6	66.7%	16.7%	66.7%
Marine fish	27	66.7%	14.8%	63.0%
Ornamentals	5	80.0%	0.0%	20.0%
Pangasius	22	18.2%	9.1%	95.5%
Salmon	8	87.5%	25.0%	12.5%
Tilapia	52	19.2%	92.3%	15.4%
Trout	18	94.4%	11.1%	11.1%

Section 6. Impact and efficacy

Among the perceived negative impacts of antibiotics, (i) building up of resistance and (ii) the presence of residues of food safety concern were the most frequently reported. While (i) toxicity to the environment, (ii) residues of food safety concern and (iii) toxicity to farmers were reported to be the main negative impacts of the use of chemotherapeutants. On the use of both antibiotics and chemotherapeutants, (i) reduction in mortality during disease events and (ii) an overall better survival were the most frequently reported positive impacts (Table 28). Fish/shrimp welfare, development of new technology, reduction in the use of other treatments and better quality products were also reported equally as some of the positive impacts more or less for both use of antibiotics and chemotherapeutants. The most common reasons for treatment failure were poor diagnosis and the pathogen not being the primary cause of the disease, although other reasons were also given at a considerable frequency (Table 29).

TABLE 28
Perceived impacts of the use of antibiotics and chemotherapeutants

Impact	Total No. respondents	No of respondents (antibiotics)	N. respondents (chemotherapeutants)
Negative impacts			
Build up of clinical resistance in fish/ shrimp	177	110	60
Residues of food safety concern	177	107	83
Toxicity to the environment	177	63	92
Build up laboratory bacterial isolates resistance	177	54	17
Toxicity to fish/shrimp	177	47	76
Toxicity to farmers	177	42	81
Total		423	409
Positive impacts			
Reduction in mortality in disease events	177	143	118
General increase of survival	177	128	113
Fish/shrimp welfare	177	51	50
Allow the development of new farming technology	177	49	51
Reduction on the use of other treatments	177	47	58
Better quality product	177	42	42
Mask knowledge gap in husbandry	177	20	20
Others:	177	2	1
Total		482	453

TABLE 29
Reported reasons for failure of treatments

Reason	1 least	2	3	4	5 most
Poor diagnosis	34	10	16	19	93
Pathogen not primary cause	23	27	23	32	56
Unsure/unproven quality	36	36	33	21	23
Poor info on fish stock	58	24	26	18	21
Subtherapeutic	26	34	24	35	36
Inappropriate duration	29	33	26	40	31
Few products available	45	31	23	15	29
Inadequate storage	51	28	26	19	10
Used isolated	34	27	15	25	32

Section 7. Recommendations for actions

Among the recommendations, training of farmers, practitioners and laboratory personnel, primarily on accurate diagnosis, but also on proper and prudent use, and awareness on the negative impact and health management also appeared to be training priorities (Table 30).

TABLE 30
Recommendations for action

Training for farmers	**1 most**	**2**	**3**	**4**	**5 least**
Accurate diagnosis	143	10	6	3	8
Proper and prudent use	108	42	8	2	9
Negative impact of their use	70	31	32	7	6
Training for practitioners	**1 most**	**2**	**3**	**4**	**5 least**
Accurate diagnosis	133	12	2	0	13
Proper and prudent use	94	46	8	1	12
Negative impact of their use	61	26	35	8	11
Training for laboratory personnel	**1 most**	**2**	**3**	**4**	**5 least**
Accurate diagnosis	139	8	3	4	11
Determination of resistance	82	46	16	2	12
Determination of proper dosage	79	36	26	7	8
Training on health management practices	**95**	**22**	**9**	**8**	**6**
Training on alternative treatments	**71**	**44**	**21**	**5**	**15**
Consultation to set priorities for research	**49**	**36**	**36**	**11**	**8**
Provide information on residue testing	**55**	**30**	**38**	**12**	**5**
More information on pharmacokinetics	**51**	**32**	**28**	**27**	**12**

DISCUSSION

The survey provided an insight on the use of treatments and prophylactic measures used in aquaculture. The limited number of submissions showed that Internet-based surveys may not be the most suitable way to conduct this type of survey. While the survey reached more than 2 000 professionals, there was very low response. It is also possible that the low response can be attributed to the fact that the use of veterinary drugs is a sensitive issue that might reflect negatively of the country, the product image and the respondents. While responses were received from all regions, because of the small response rate, care should be taken when interpreting the results. Nonetheless, despite the small numbers, some valuable associations were identified. These include:

1. Active participation of stakeholders is evident when a country has a well-organized industry. This is evident in Mexico, where there was a stronger response rate to the survey owing to the well-organized shrimp industry sector with a committee that regulates the industry from a sanitary standpoint.
2. More emphasis was given on treatment rather than on prevention. This was revealed by pair-wise comparison and the question on training needs, where a large percentage of respondents felt that diagnosis vs health management being the priority need.
3. Oxytetracycline was the most reported product widely used for both treatment and prevention for most species or species froups. Such wide use of this product may mean that it is effective and, considering the regular use of this drug for many years now, may also mean that widespread resistance against this drug has not been developed.
4. Most species or species groups had similar range of products, with some differences in the number of substances used. Comparison made only among respondents with similar responses showed more products reportedly used for some species (e.g. shrimp vs carps) but it was not possible to assess the appropriateness of the use of such substances.
5. Sanitary bottlenecks of the aquaculture sector are prevalent in both grow out and hatchery operations, but differ among species or species groups. More treatments were used during grow-out operations for marine fish, carp, pangasius and tilapia; more treatments were applied during hatchery operations in shrimp and trout; and in both grow-out and hatchery phases of salmon. These observations can guide in drawing health management strategies aimed at the proper use of veterinary medicines during the different aquaculture production phases.

6. The most commonly perceived reasons for treatment failures were wrong diagnosis and the pathogen not being the primary cause of the disease.
7. In terms of availability of veterinary medicines, differences exist between regions and between countries.

One of the issues that have been raised during this study was the few number of veterinary drugs approved for aquaculture use, except for specific countries such as Japan. This limitation that the industry faces will lead to a decrease in the efficiency of the current products. The main reason for such a deficiency is the high cost involved in obtaining the approval of a veterinary product for food-producing animals. Considering the small size of the aquaculture market, very few companies can afford to go through this procedure for new or existing products. The availability of veterinary products for a relatively new and developing industry should not be at the left to the economic interest of manufacturing companies.

CONCLUSIONS AND RECOMMENDATIONS

Internet-based survey can provide useful insight on the use of veterinary drugs used in aquaculture, although care should be taken when interpreting and generalizing results. Interview-based surveys appear to improve considerably the response rate, although they require more resources.

It is suggested that, if resources allow, future efforts to gather this information be done through direct contact with stakeholders. This would also allow overcoming language barriers. Internet-based surveys should also be used, although this should be done as an ongoing data collection method (i.e. surveys kept open for long periods of time) to improve the response rate and allow the identification of trends.

National plans for better enforcement of veterinary drugs regulations are necessary in certain countries. Such plans should target the different stakeholders involved, e.g. farmers, fish health advisors, veterinary drug shops, veterinary drug producers. Emphasis should be placed on both the farmers and on the fish health advisors.

The most effective way to reduce the use of veterinary drugs is to minimize their need. This can be achieved through training of both farmers and fish health advisors:

- on health management and biosecurity,
- on diagnostics, and
- on the proper use of veterinary drugs.

As diagnostic support is crucial to proper fish health management and as different countries and different industries have uneven access to such services, national Competent Authorities of aquaculture nations should have diagnostic support services as a priority.

Not enough veterinary products are available for health management in aquaculture. Government agencies should consider approving certain products based on the needs of the industry and efficacy of the products, instead of relying on the economic interests of the veterinary product manufacturers.

Antimicrobial use and resistance in selected zoonotic bacteria in aquaculture: preliminary findings of a survey of aquaculture-allied professionals

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Tuševljak, N., Rajić, A., Dutil, L., Uhland, F.C., Reid-Smith, R, and McEwen, S. 2012. Antimicrobial use and resistance in selected zoonotic bacteria in aquaculture: preliminary findings of a survey of aquaculture-allied professionals. *In* M.G. Bondad-Reantaso, J.R. Arthur & R.P. Subasinghe, eds. *Improving biosecurity through prudent and responsible use of veterinary medicines in aquatic food production*, pp. 45—50. FAO Fisheries and Aquaculture Technical Paper No. 547. Rome, FAO. 207 pp.

ABSTRACT

Antimicrobial resistance (AMR) is an important public and animal health issue and is globally recognized as a priority for the aquaculture sector. However, existing knowledge regarding the extent of AMR and its association with antimicrobial use (AMU) in aquaculture remains to be assessed. The main study objective was to develop and administer an online questionnaire to aquaculture-allied professionals and solicit their views on this issue. The questionnaire included five sections: expert demographics, importance of AMR in selected bacteria/aquatic species, the extent of AMU in various aquatic species, monitoring or surveillance programmes, and AMR laboratory testing in the respondents' jurisdictions. The questionnaire was administered in English and Spanish to 586 professionals distributed around the world and with various expertise in aquaculture. The response proportion was 32.9 percent. Over half of the participants had more than ten years of experience in aquaculture; 70.4 percent (140/199) were involved in fish health/clinical management and their experience was primarily associated with salmon, tilapia, trout and/or ornamental fish. Tetracyclines were reported as "frequently" or "almost always" used in salmon (22/43 responses), followed by "frequently" used for potentiated sulfonamides (11/41). With respect to shrimp/prawn, all antibiotics except tetracyclines were more often reported as "never used". In ornamental fish, catfish, shrimp/prawn, trout, salmon and tilapia, quinolones were reported to be used "frequently" or "almost always" by 51, 24, 21, 16, 15 and 13 percent of respondents, respectively; and quinolone resistance was "frequently" or "almost always" observed by 16, 7, 30, 7, 9 and 10 percent of respondents, respectively. Furthermore, inappropriate duration of treatment, utilization

of subtherapeutic dosages and absence of accurate diagnosis (73.9, 71.4 and 71.2 percent, respectively) were reported as the most important contributors to the development of AMR. Around 20 percent of respondents identified knowledge gaps as the effect of the runoff of antimicrobial agents from farms into aquaculture and the risk posed by aquatic AMR to human health.

INTRODUCTION

Over 50 percent of human pathogens are zoonotic in nature and 31 percent are zoonotic bacteria that pose a threat to human health (Taylor, Lathan and Woolhouse, 2001). Over the past decade, substantial international initiatives have been implemented in surveillance of zoonotic pathogens (WHO, 2006).

Antimicrobial agents are widely used to treat and prevent infections in humans and animals, but the extent to which antimicrobial use (AMU) in the agri-food sector contributes to the overall problem of antimicrobial resistance (AMR) is not well understood. AMR has been explored and well documented in some food-animal commodities (McEwen *et al*., 2008) and some bacterial isolates from humans. Desirable attributes of an AMR surveillance programme include, among others, the ability to monitor trends in patterns of resistance in pathogens and commensal bacteria, as well as detection of emerging AMR problems within targeted populations (McEwen, Aarestrup and Jordan, 2006). In Canada, the Canadian Integrated Program for Antimicrobial Resistance Surveillance has focused on the main food-animal commodities such as pork, beef and chicken at the on-farm, abattoir and retail levels, as well as in humans (CIPARS, 2005). To date, however, Canadian aquaculture or imported seafood is not incorporated within routine surveillance. Yet, Canada is actively involved in aquaculture and is globally competitive in terms of production and import/export, and there is a desire to better understand monitoring needs.

The objective of this study was to elicit the opinions of aquaculture-allied professionals regarding AMU in fish and AMR in aquatic zoonotic bacteria, including seafood and ornamental fish, through the use of an online questionnaire. The results of the questionnaire will be used in tandem with a comprehensive, transparent and replicable systematic review of the existing literature to prioritize potential research and monitoring activities in these areas within the Canadian context. In this paper, only very preliminary results are presented, as data analysis and interpretation are in progress.

MATERIALS AND METHODS

Questionnaire description

A core research team (Drs Andrijana Rajić, Lucie Dutil and F. Carl Uhland) developed a draft questionnaire that was further refined by a research team consisting of members with various types of expertise. The final questionnaire included five sections: respondent demographics, extent of AMU in aquaculture, frequency of AMR observations in aquaculture, AMR monitoring and surveillance, and antimicrobial susceptibility testing in various jurisdictions. The questionnaire was pretested by five professionals with a broad range of expertise and modified based on pretester comments to improve clarity and consistency. The final version of the questionnaire included 26 questions: 25 closed and 1 open. Closed question formats included multiple choice and yes/no and rating using a five-point ordinal scale (e.g. “not at all important” to “very important”). Four of the yes/no questions were designed to navigate the skip logic of the questionnaire so that respondents would answer only questions/sections pertaining to their experience. Additional space was provided in open format at the end of the questionnaire for respondent comments.

The questionnaire was translated into Spanish for administration in Spanish-speaking regions (South and Central America and the Caribbean). In addition, a brief questionnaire was designed for non-responders to the main questionnaire to aid in the assessment of non-response bias. It consisted of five questions: four were demographic

in nature and the fifth elicited reasons for non-participation. Ethical approval for the surveys was received by the University of Guelph Ethics Review Board.

Database of aquaculture-allied professionals

An initial list of aquaculture-allied professionals was developed using various aquaculture-related mailing address lists or publications. These individuals were contacted by e-mail and asked to provide names and/or contacts of other colleagues and professionals with expertise in aquaculture, selected zoonotic bacteria, AMU and AMR. In addition, a blog on AquaVetMed e-news was used to advertise the project and solicit additional participants. All received contact information was entered into our "target respondents" database (Microsoft Excel, Microsoft Corporation, Redmond, Washington, United States of America), specifying the name, field, country, organization, e-mail and telephone when available.

The final "potential respondent" database included individuals with a wide range of professional experience from North America (n = 551), Latin America (n = 41), Europe (n = 51), Asia (n = 48), Africa (n = 1) and Australia (n = 23).

Survey administration

The questionnaire and reminders were administered using Survey Monkey, a web-based application (Survey Monkey, Portland, Oregon, United States of America). Two weeks prior to initial administration, a letter was sent by e-mail to all individuals from the above-mentioned database inviting them to consider participation in the questionnaire. The initial questionnaire was administered on 12 June 2009 with four reminders sent at two-week intervals.

Participants were provided with a unique link that allowed them to access the questionnaire on more than one occasion, if desired, and enabled them to save responses. To respect the Survey Monkey "terms of use", each participant had a choice to refuse and opt-out of further communication by being removed from the mailing list.

Data analysis

The completed online questionnaire and non-response data were exported separately to spreadsheets (Microsoft Excel, Microsoft Corporation, Redmond, Washington, United States of America), cleaned and imported into Stata 10 for statistical analysis (Stata Corporation, College Station, Texas, United States of America). Frequency tabulations were performed and basic descriptive statistics were generated; further statistical analyses are in progress.

RESULTS

From the initial questionnaires sent to 715 individuals (including 41 in Spanish), 78 and 21 invitations bounced back for the English and Spanish versions, respectively. Twelve individuals who received the English questionnaire chose to opt-out of receiving further communication. Consequently, the potential pool of the questionnaire respondents was 584 for the English version and 20 for the Spanish version. The overall response proportion was 32.9 percent. Most participants (62.5 percent) (110/199) had more than ten years of experience in aquaculture, and 70.4 percent (140/199) were involved in fish health/clinical medicine. The majority of respondents had the highest level of experience with salmon, tilapia, trout, ornamental fish and wild fish populations and the lowest experience corresponded to crawfish, clams and lobster (Figure 1).

With respect to the AMU and AMR sections, most responses were provided for salmon, tilapia, shrimp/prawn and ornamental fish. For salmon, tetracyclines were the antimicrobials reported most often as "frequently" and "almost always" used (22/43), followed by potentiated sulfonamides (11/41 "frequently" category) (Figure 2). Resistance to tetracyclines was also reported the highest with "occasionally", "frequently" or "almost always" categories (14/18), followed by potentiated sulfonamides (9/14) and

then penicillins (4/11). Quinolones were reported being used by 25/43 respondents, including "frequently" or "almost always" (6/43), while resistance was reported by 7/11 respondents.

FIGURE 1
Respondents rating of their experience per fish/shellfish species

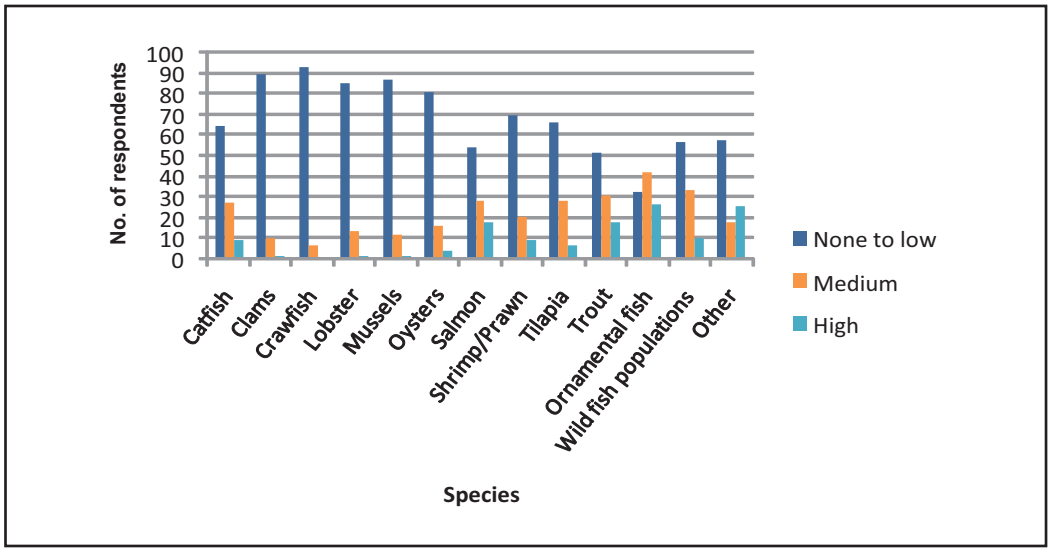
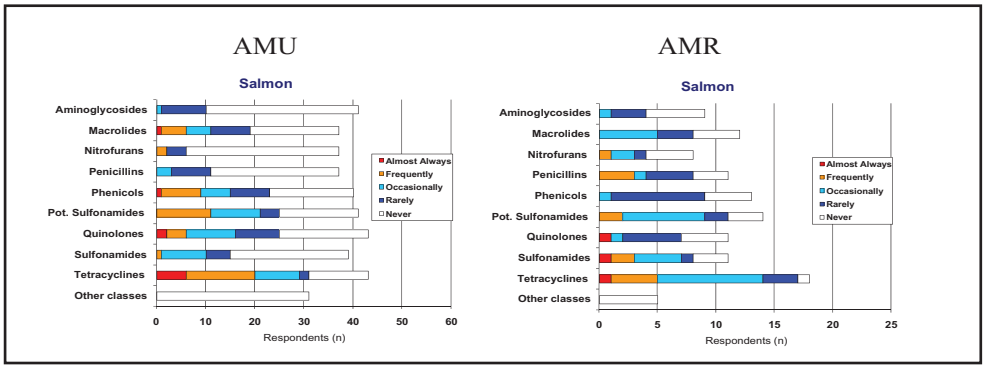


FIGURE 2
Survey respondent opinion on the frequency of antimicrobial use (AMU) and antimicrobial resistance (AMR) in salmon

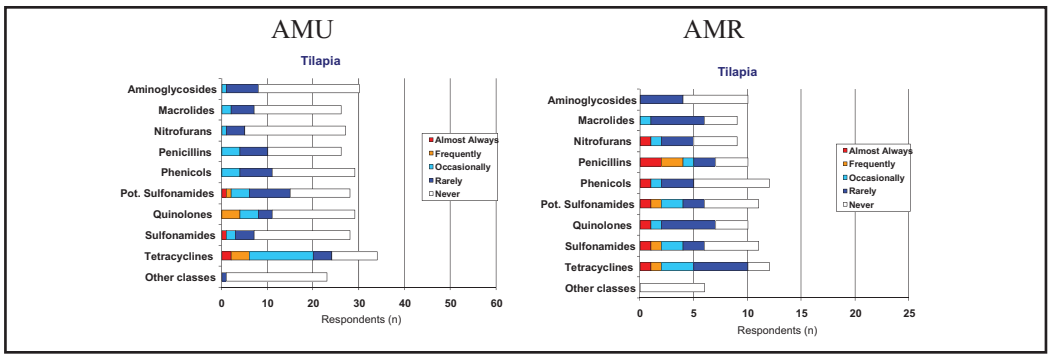


For tilapia, aside from tetracyclines, most antibiotics were reported as "never" or "occasionally" used, and so were the observations of antimicrobial resistance. (Figure 3). Quinolone use and quinolone resistance were reported "occasionally" or more frequently by 8/29 and 2/10 respondents, respectively.

With respect to shrimp/prawn, tetracycline use was most frequently reported, while quinolone use ranked second. Fewer respondents provided answers related to AMR (9 to 11), but the majority of respondents reported observing AMR "occasionally" to "almost always", except for the animoglycosides category.

Lastly, for ornamental fish, antibiotics reported as being used "occasionally" or more frequently were quinolones (56/66), tetracyclines (43/59), aminoglycosides (23/58) and nitrofurans (33/58). Similarly, AMR was "frequently" and "almost always" observed across all antibiotic classes, with the highest frequency of resistance observed for tetracyclines (20/35). Quinolone use and resistance was "occasionally" or more frequently observed by 46/66 and 16/36 respondents, respectively. Ornamental fish had the highest number of responses for the AMU and AMR sections of all species.

FIGURE 3
Survey respondent opinion on the frequency of antimicrobial use (AMU) and antimicrobial resistance (AMR) in tilapia



When asked which practice is contributing the most to the development of AMR, participants responded by selecting inappropriate duration of treatment, utilization of subtherapeutic dosages, and absence of accurate diagnosis (73.9 percent, 71.4 percent and 71.2 percent, respectively). In addition, when asked what they feel are the most important knowledge gaps, only 66 respondents identified those gaps, with 18.2 percent listing the potential runoff of antimicrobials into aquaculture settings from other facilities (e.g. hospitals, large animal production), as well as the risk of AMR from aquaculture to human health.

DISCUSSION

The data presented here are only preliminary findings and are subject to further analysis and interpretation. Moreover, the study and its results have yet to be peer reviewed. Therefore, considerable caution is urged in the use of these findings. In light of this, we elected, for the purposes of this meeting, to simply present the summary data as they are available to date, and do not discuss further their meaning or importance to the industry or the public.

Overall, the questionnaire achieved a response rate of 32.9 percent, which is fairly good for this type of survey, but there is still potential for non-response bias, which we are in the process of evaluating. Because of the nature of the questions asked in the survey, it is important to remember that in most cases the responses represent opinion and belief rather than fact. Moreover, since participants could choose not to answer questions or entire sections, depending on their expertise, the response rate for some questions is much lower than the 32.9 percent overall. Evaluation of the non-response bias may provide more insight into this aspect.

The highest number of respondents answered the AMU and AMR sections for salmon, tilapia, shrimp/prawn and ornamental fish; hence, only the results for these species were presented in this paper. Not surprisingly, respondents also indicated the highest experience with salmon, tilapia, shrimp/prawn and ornamental fish. Generally, the number of respondents for the AMR section was lower than for the AMU section. For example, when looking at the AMU and AMR data for ornamental fish, the largest number of people that responded for AMU was 65, whereas 36 responded to the AMR section. Further analysis will reveal whether individuals responding to the AMU section were the same as those responding to the AMR section.

Future analysis of the questionnaire results will focus on interpreting the antimicrobial drug usage and AMR information in light of the responses from the demographic section. In addition, non-response evaluation may provide a view into increasing the response in under-represented regions. The systematic review will provide further information to complement that of the questionnaire with the goal of using both to identify components (i.e. fish/shellfish species, antimicrobial drug, bacteria) that would be of interest for surveillance system design and implementation in Canada.

ACKNOWLEDGEMENTS

The authors would like to thank pretesters, Drs Victoria Alday and Roy Yanong, and collaborators, Drs Carol McClure and Sophie St. Hilaire, who have generously dedicated their time in development of the project, as well as the Public Health Agency of Canada for funding.

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Use of veterinary medicines in Chinese aquaculture: current status

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ABSTRACT

Aquaculture is a rapidly developing industry in China since the 1980s and currently accounts for about 70 percent of total national fishery production. Polyculture and intensive management are the two main characteristics that describe the sector. There are more than 60 aquatic species cultured in China, with carp comprising about 70 percent of the total aquaculture production and shrimp and tilapia ranked as the most important species for exportation. For this survey on the use of veterinary medicines in Chinese aquaculture, Jiangsu and Guangdong provinces were selected as survey sites because of their importance to Chinese aquaculture production. The survey was conducted in Taixing City, Jiangsu Province, for carp farming and in Zhanjiang City, Guangdong Province, for shrimp and tilapia farming. During the survey, extension officials, fish farmers, drug sellers and feed company technicians were interviewed, with 50 questionnaires from different respondents being collected. The survey results are analyzed to provide an overview of the use of veterinary medicines in Chinese aquaculture and some recommendations for improving fish health management are given.

INTRODUCTION

Aquaculture is the fastest developing industry in China. Commercial fish farms were developed through the intensification and diversification of modern fish farming. The resulting aquaculture systems have created a good environment for disease-causing organisms to flourish. The growing international trade in aquatic animals paved the way for the transboundary spread of many pathogens together with the movement of their hosts, and thus many have caused serious damage to aquatic food productivity.

Among the effective management tools, use of veterinary medicines was widely promoted in the Chinese aquaculture practices since 1980's. There were many commercial factories producing veterinary medicines for aquaculture and using commercial veterinary medicines became a convenient way oftreating fish diseases. In recent years, there were

frequent occurrence of serious fish diseases with high mortality, and there were reports of increasing bacteria resistance. With extensive application of antibiotics, bacterial residues were reported from the final products before the marketing, these leading to a further impact on the human consumption and health.

In order to achieve the practice of responsible use of veterinary medicines and pursuing sustainable development of aquaculture careful planning and prudent use of veterinary medicines became essential to industry development. In China, guidance on limitation of the use of veterinary medicines was published and research on alternatives to antibiotics was encouraged. After years of studies and other related investigations, improvement in fish health management protocols and effective approaches fpr maintaining good culture environment for aquatic animals were promoted to Chinese fish farmers.

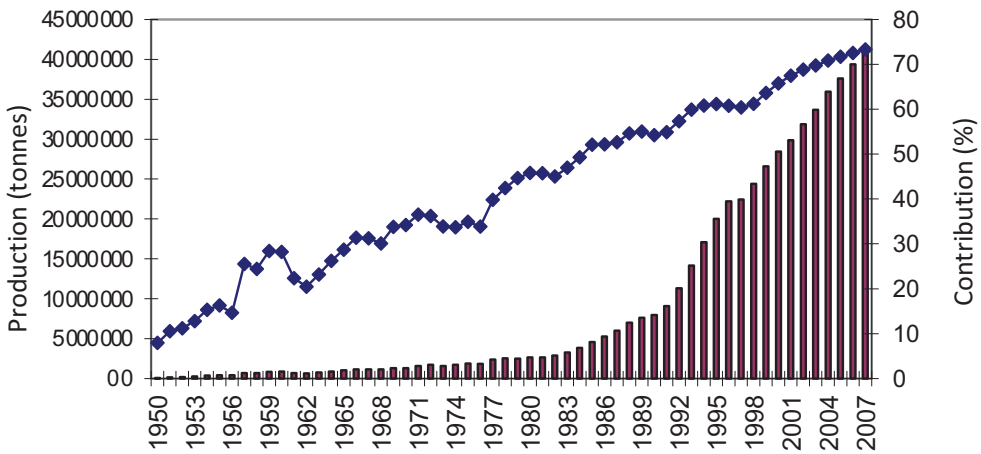
This study will help to understand the current status of the use of veterinary medicines in Chinese aquaculture, which had rapidly expanded along with the development of aquaculture, and to identify the problems and effective and meaningful alternatives to chemical treatments for aquatic animal diseases, as a means of enhancing the sustainability of the sector, conserving aquatic biodiversity and maintaining the safety of aquaculture products.

OVERVIEW

Aquaculture development in China

China has a long history in aquaculture that dates back some 2500 years. Since the 1970s, under the reform policies of the government and driven by the potential economic benefits, the rapid development of Chinese aquaculture in both freshwater and marine systems has greatly contributed to world aquaculture production. Globally, China is currently ranked as the top aquaculture producer, contributing more than 70 percent of total fishery production (Figure 1). In 2008, the production of aquatic products by volume was 48.96 million tonnes, which is 108 times that of production in 1949. The contribution of fisheries production (from both aquaculture and capture fisheries) to the whole agricultural sector has increased from 0.2 percent by value in 1949 to 10 percent in 2008. The export value of Chinese aquatic products was US$10.6 billion, accounting for 26 percent of the total exportation of agricultural products (Bureau of Fisheries, 2009).

FIGURE 1
Aquaculture production and its contribution to the total national fishery production



The top-ten species harvested in inland aquaculture were grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio carpio*), bighead carp (*H. nobilis*), Crucian carp (*Carassius carassius*), Nile tilapia (*Oreochromis niloticus*), Wuchang bream (*Megalobrama amblycephala*), Chinese mitten crab (*Eriocheir sinensis*), whiteleg shrimp *(Litopenaeus vannamei*) and black carp (*Mylopharyngodon*

piceus). Over 100 000 tonnes of other species were also recorded in 2008, including yellow catfish (*Tachysurus fulvidraco*), snakehead (*Channa argus argus*), Japanese eel (*Anguilla japonica*), Asian swamp eel (*Monopterus albus*), mandarin fish (*Siniperca chuatsi*), largemouth black bass (*Micropterus salmoides*), oriental river prawn (*Macrobrachium nipponense*) and softshell turtle (*Trionyx sinensis*). The top-ten species harvested in marine culture were giant cupped oyster (*Crassostrea gigas*), Asiatic hard clam (*Meretrix meretrix*), scallop (*Chlamys nobilis*), Japanese kelp (*Laminaria japonica*), blue mussel (*Mytilus edulis*), Chinese razor clam (*Sinonovacula constricta*), whiteleg shrimp (*L. vannamei*), granular ark (*Tegillarca granosa*), wakame (*Undaria pinnatifida*) and giant mud crab (*Scylla serrata*). For marine-cultured fish, Japanese seabass (*Lateolabrax japonicus*) has the highest production, followed by large yellow croaker (*Larimichthys crocea*), turbot (*Scophthalmus maximus*), blackhead seabream (*Acanthopagrus schlegelii schlegelii*) and red drum (*Sciaenops ocellatus*).

Aquatic animals produced through aquaculture not only supply animal protein to the domestic market, but are also exported to the European Union, the United States of America, Japan and other international markets. In 2008, the total volume of China's international trade was 6.848 million tonnes, with a value of US$16.02 billion. The export amount was 2.965 million tonnes with a value of US$10.61 billion, while imports amounted to 3.884 million tonnes with a value of US$5.4 billion.

MANAGEMENT OF VETERINARY MEDICINES FOR AQUACULTURE

Development history

After the founding of the People's Republic of China, the development of veterinary medicines for use in aquaculture can be divided into three phases:

- The first phase (1950–1970) was characterized by the use of single products from agriculture for the prevention and treatment of fish diseases following the guidance provided by traditional medicine, such as the application of teaseed cake or *Croton tiglium* for pond cleaning, the use of quicklime or bleaching powder for sterilization and removal of bacterial pathogens, and the use of copper sulfate and ferrous sulfate to eliminate parasites.
- The second phase (1970–1990) included the use of human and veterinary medicines, agriculture chemicals and water-treatment agents for the prevention and treatment of aquatic animal diseases, with some drugs that showed good results and low toxicity proving effective for use in aquaculture.
- The third phase (after 1990) has seen the development of drugs specifically for use in fish and shellfish. With unique therapeutic effects and special guidance to aquaculture, they have been used to treat serious diseases or to reduce the pathogen biomass in the aquaculture environment.

The present research on veterinary medicines for use in aquaculture began with the investigation of fish diseases and the identification of pathogens affecting the major cultivated fish species in China. Pharmaceutical studies focused mainly on the actual effect of drug application in the early stage. Many studies have been conducted on drug selection, efficacy testing, etc. In order to improve the efficiency of drugs application, increased attention was paid to pharmacokinetics, pharmacodynamics, drug residues, toxicity, impacts on the environment, among others (Cui *et al.*, 2005).

In China, the main research organizations for the study and development of veterinary medicines for use in aquaculture are the universities, the Chinese academies of fishery sciences, the national institutes, and the research units of some enterprises producing veterinary medicines. The enterprises have played an important role in developing new medicines, while the national academies, institutes and universities have focused mainly on the dynamics and scientific analysis on the results of application of veterinary medicines in aquaculture (Bao and Chen, 2005; Wang Q.X., 2005; Wang T.J., 2005; Wang and Guo, 2005; Ma, 2006).

Administrative organization

The Bureau of Animal Husbandry and Veterinary Medicines and the Bureau of Fisheries of the Ministry of Agriculture are responsible for the management of veterinary medicines used in aquaculture. Their tasks and responsibilities are divided according to the "Bulletin of Fish Drugs Management in China".[1]

- The Bureau of Animal Husbandry and Veterinary Medicines is responsible for centralizing management of veterinary medicines (including those used in aquaculture), while the Bureau of Fisheries is responsible for the management of veterinary medicines used in aquaculture.
- The Appraisal Group of Fish Drugs, established under the Appraisal Committee of Veterinary Medicines of the Ministry of Agriculture, participates in the appraisal of standards for veterinary medicines used in aquaculture, examines and comments on them (including new and imported drugs), certifies academies and institutes to undertake safety tests for veterinary medicines used in aquaculture, and is a member of the Commission of Chinese Veterinary Pharmacopoeia, the Committee of Bio-Products for Veterinary of the Ministry of Agriculture and the Experts Committee of Veterinary Drug Residues.
- For the task of managing veterinary medicines, the Bureau of Animal Husbandry and Veterinary Medicines takes charge of drafting the laws and regulations dealing with the management of veterinary medicines (with the endorsement of the Bureau of Fisheries), and the Bureau of Fisheries takes charge of specified regulations and technical guidance on veterinary medicines used in aquaculture, prepares lists of permitted and forbidden drugs, and submits appraisal proposals to the Committee of Veterinary Medicines (with the endorsement of Bureau of Animal Husbandry and Veterinary Medicines).
- The approval of veterinary medicines for use in aquaculture (including new and imported drugs) is administered by the Bureau of Fisheries, with requests for permission being submitted by the Appraisal Committee of Veterinary Medicines, with the endorsement of the Bureau of Animal Husbandry and Veterinary Medicines. The approval of veterinary medicines for use in aquaculture is administered by the Bureau of Animal Husbandry and Veterinary Medicines, with the endorsement of the Bureau of Fisheries.
- Licences for enterprises for fisheries drug production are issued by the Bureau of Fisheries, with endorsement by the Bureau of Animal Husbandry and Veterinary Medicines. The licensing of veterinary medicines manufacturers engaged in the production of drugs for use in aquaculture is supervised by the Bureau of Animal Husbandry and Veterinary Medicines and endorsed by the Bureau of Fisheries.
- The Bureau of Fisheries proposes the annual work scheme for the surveillance and the sampling for drug residues in aquatic products under the national veterinary drug residue sampling scheme, confirms the main species and areas to be sampled, and implements the annual work plan and enforcement.

REGULATIONS AND STANDARDS

In China, the drugs and chemicals used for fish disease control fall under the management of veterinary medicines (see Table 1 for the list of veterinary medicines that are banned for use in Chinese aquaculture). There are two main national regulations, i.e. the Regulation on Feed and Feed Additives (revised in 2001) and the Regulation on Veterinary Medicines (revised in 2004). There are also several regulations published by the Ministry of Agriculture to ensure the prudent use of veterinary medicines in aquaculture (Table 2) (Ministry of Agriculture, 2005).

1 In keeping with the terminology used in China, the original draft provided by the authors made frequent use of the term "fish drugs". During editing and in keeping with the agreed upon definitions used in this paper, where possible, these instances were replaced with "veterinary medicines for use in aquaculture".

TABLE 1
List of drugs banned for use in aquaculture

Sn.	Drugs	Sn.	Drugs
1	Fonofos	17	Sulfathiazolum ST, norsultazo
2	Benzine hexachloride (BHC), benzem, bexachloridge, (HCH)	18	Sulfaguanidine
3	Lindane, agammaxare, gamma-BHC, gamma-HCH	19	Furacillinum (nitrofurazone)
4	Camphechlor	20	Furacillinum (nifulidone)
5	Dichloro-diphenyl-trichloroethane (DDT)	21	Furanace, nitrofurazone
6	Calomel	22	Chloramphenicol
7	Mercurous nitrate	23	Erythromycin
8	Mercuric acetate	24	Zinc bacitracin premin
9	Carbofuran	25	Tylosin
10	Chlordimeform	26	Ciprofloxacin, (Cipro)
11	Amitraz	27	Avoparcin
12	Flucythrinate	28	Olaquindox
13	Sodium pentachlorophenoxide (PCP-Na)	29	Fenbendazole
14	Malachite green	30	Diethylstilbestrol, stilbestrol
15	Tryparsamide	31	Methyltestosterone, metandren
16	Antimony potassium tartrate		

TABLE 2
List of regulations and standards published in China

Year	Regulation/standard	Contents
1992	"Deactivated vaccine against grass carp haemorrhagic disease" (SC1001-1992); "Testing method of inactivated vaccine against grass carp haemorrhagic disease" (SC1002-1992); "Injection manual of inactivated vaccine against grass carp haemorrhagic disease" (SC1003-1992)	The first fish vaccine used in China and its application manual
1999	"Regulation on fish drugs used in eel culture for exportation"; "List of forbidden veterinary medicines in eel culture for exportation"	7 drugs in eel culture and 21 forbidden drugs were listed
2002	Revised "Fish Drugs Guideline for Environment-friendly Food Production"	The usage, dosage and precautions for 26 permitted fish drugs are listed; the withdrawal periods for 7 drugs and 32 forbidden drugs are also listed. This is the first published guidelines for the application of fish drugs in China
2002	"List of banned veterinary medicines and their compound of food animal" in Bulletin No. 193	Drugs for aquatic animals are included
2003	The withdrawal period of veterinary drug in Bulletin No. 278	The withdrawal periods for 202 drugs are listed in national standards and specialized standards for veterinary drugs, including 7 fish drugs
2004	Revised "Veterinary medicines management regulation"	Defines the administration and management scheme for veterinary medicines in China

SURVEY ON THE USE OF VETERINARY MEDICINES IN CHINESE AQUACULTURE

In order to understand the present status of the use of veterinary medicines in Chinese aquaculture, a country-level survey on the prudent and responsible use of veterinary medicines in aquaculture was conducted and effective alternative therapeutic treatments for aquatic animal diseases were identified.

Survey sites

Jiangsu and Guangdong provinces were chosen as the survey sites because of their importance in Chinese aquaculture production (Figure 2). The survey on the use of veterinary medicines was conducted in Taixing City, Jiangsu Province, for carp farming, and in Zhanjiang City, Guangdong Province, for shrimp farming. Extension

officials, fish farmers, drug sellers and feed company technicians were interviewed during the survey.

Guangdong is the leading fishery province in China, having a total aquaculture area of about 540 000 hectares in 2008 and a total production of 5.14 million tonnes valued at Chinese yuan (CNY) 59.1 billion. The production value for shrimp and tilapia was about CNY 15.0 billion. Zhanjiang City has about one million labourers involved in shrimp culture, and the sector is the city's most important industry.

Jiangsu is a traditional fishery and aquaculture province; the total production in 2008 was about 4.25 million tonnes with a total value of CNY 66.5 billion. With an average net income of about CNY 8 960 per fisher in 2008, aquaculture became the most important industry in Jiangsu. Taixing City was ranked as the top city in Jiangsu for freshwater aquaculture development.

FIGURE 2
Location of the survey sites

SURVEY METHODOLOGY

Survey form

A draft survey questionnaire provided by the Food and Agriculture Organization of the United Nations (FAO) project team was modified after the preliminary survey in China. The list of veterinary medicines and other chemicals used in Chinese aquaculture was added (including treatments such as quicklime and Chinese herbs) and the questionnaire translated into Chinese for the convenience of conducting interviews with Chinese farmers and stakeholders. The content of the questionnaire form included: a description of the respondents; types of antimicrobials, chemotherapeutants, vaccines, and other products used; their application, dosage, availability, and perceived impacts (positive and negative); and treatment failures and recommendations.

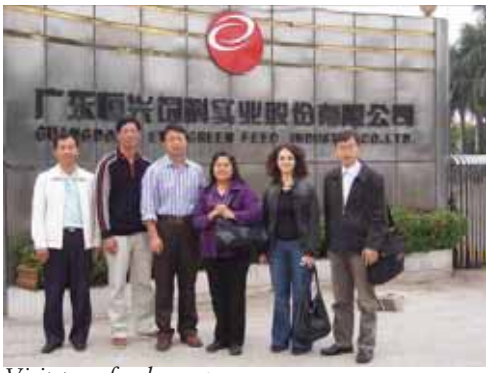
Conducting the survey

The survey on the use of veterinary medicines in Chinese aquaculture had two components: (i) a survey carried out in Taixing City, Jiangsu Province, on freshwater carp farming systems; and (ii) a survey conducted in Zhanjiang City, Guangdong Province, on shrimp aquaculture. During the survey, stakeholder meetings (including participation by fish farmers, drug manufacturers and drug retailers/sellers) were organized. During each stakeholder meeting, a brief description of the survey and its purpose was presented,

followed by free discussion, group presentation, and the filling out of the questionnaire form (Figure 3). Fifty completed questionnaire forms were collected during the survey.

Visits to stores selling drugs used in aquaculture, to fish farms, a feed company, a local fishery technical extension station and with authorities were well organized for the collection and confirmation of survey information. The FAO project team consisting of Dr Melba B. Reantaso and Ms Elena Irde participated in the stakeholder meetings and field visits.

FIGURE 3
Photographs taken during the survey on the use of veterinary medicines in Chinese aquaculture



Visit to a feed company

Interviews with stakeholders

Group discussion

Meeting with local officers

SURVEY RESULTS

Use of veterinary medicines in Chinese aquaculture

In China, the veterinary medicines were classified by their functions and ingredients. Five different kinds of veterinary medicine were reported to be used in aquaculture, i.e. disinfectants, antiparasitics, water-quality treatments, antimicrobial agents and herbal treatments (Yang and Zheng, 2007).

- Quicklime is a traditional disinfectant that is most widely used in Chinese aquaculture. Some new chemical disinfectants include chloride and bromide.
- Antiparasitics generally have a wide spectrum action in killing parasites. They can effectively kill anchor worms, fish lice, *Trichodina*, *Gyrodactylus*, *Dactylogyrus*, tapeworms, etc. The most commonly used antiparasitics include Dipterex and copper sulfate.
- With improved understanding of the relationship between the environment and fish disease outbreaks, water-quality treatments are more frequently used in aquaculture. Some chemicals such as zeolite and calcium peroxide help to improve water quality and pond silt condition, and some effective micro-organisms such as photosynthetic bacteria and *Paenibacillus* sp. help balance the bacterial population.
- Antimicrobial agents are used to treat infectious diseases caused by bacteria by inhibiting their growth or reducing the pathogen biomass. They can be

classified as natural antibiotics, semisynthetic antibiotics and synthetic antibiotics. Negative impacts of antibiotic use may include drug residues and resistance.

- As Chinese herbs have few side effects and do not cause drug resistance, they are now widely used as ingredients in veterinary medicines. Many reports have been made indicating the successful use of herbal extracts to treat and prevent fish diseases.

Production and distribution of veterinary medicines

Toward the end of the 1980s, the first commercial fish drug – "Yu Fu Kang A and B" – appeared on the Chinese market and was sold to fish farmers. Some 50 manufacturers of veterinary medicines for use in aquaculture were reported to exist in China in 1992, and more than 150 drugs have been approved by the Bureau of Veterinary Medicine Authority. In 2008, there were more than 450 manufacturers of aquaculture drugs in China. Among these were 150 producers specializing in the manufacture of veterinary medicines for use in aquaculture and 300 who were producers of veterinary medicines that included production of medicines for use in aquaculture (Bureau of Fisheries, 2009).

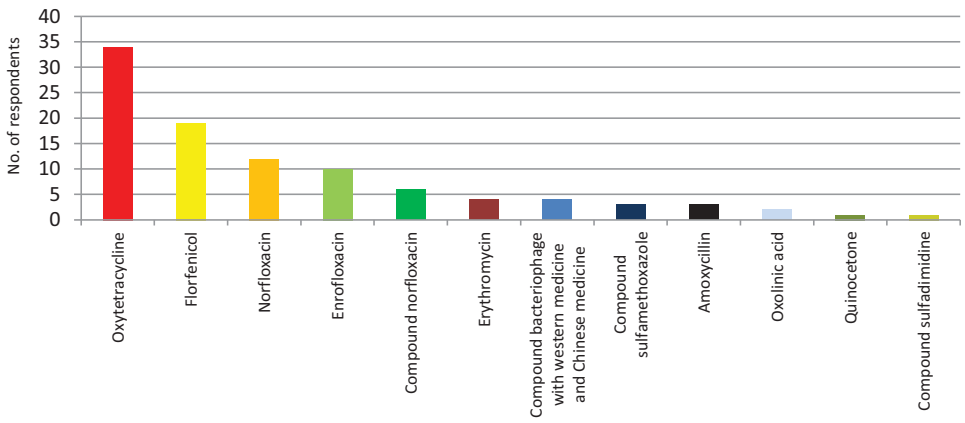
The manufacturers of veterinary medicines for use in aquaculture are mainly located in Shanxi, Jiangsu, Hubei, Hunan, Guangdong, Zhejiang and Beijing. It was reported that there were about 70 kinds of veterinary medicines for use in aquaculture produced in Jiangsu Province.

With the increasing public concerns for the quality and safety of aquatic products, since 2005 all manufacturers of veterinary medicines used in aquaculture should be qualified with the good manufacturing practices standard. The Chinese government has given high attention to food quality and safety management and strict regulation of drug residues in aquatic products. A list of drugs banned for use in aquaculture was published to ensure the quality and safety of aquatic products (Table 1).

Types of veterinary medicines used in aquaculture

In the survey, some antimicrobial agents were reported as being used to inhibit pathogen growth or to eliminate the pathogen biomass in aquaculture systems. By analyzing the survey results (Figure 4), oxytetracycline was found to be the antibiotic having the highest frequency of application in aquaculture, while the second most widely used antibiotic was florfenicol, which is used as an alternative to chlorophenicol.

FIGURE 4
Frequency of antibiotic use in aquaculture in China



The most commonly used chemotherapeutants in aquaculture were methyl bromide and povidone-iodine, which are used as disinfectants to eliminate the pathogen biomass in aquaculture ponds (Figure 5).

FIGURE 5
Frequency of chemotherapeutant use in aquaculture in China

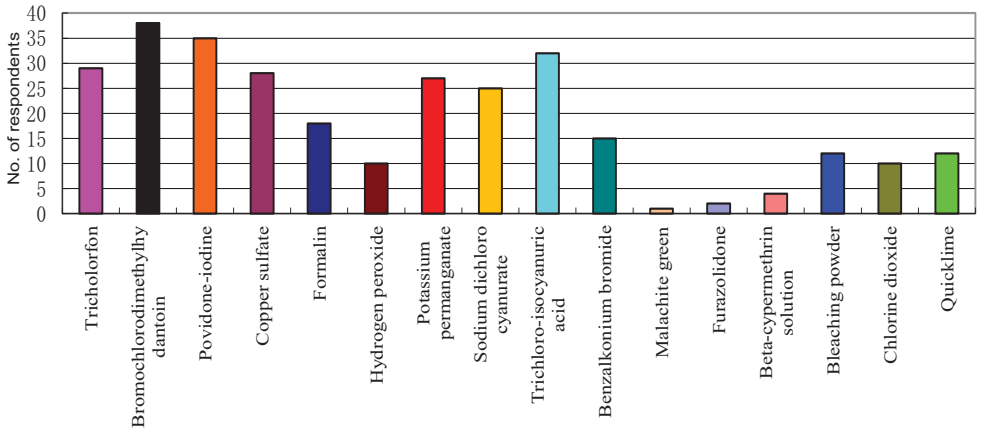


Table 3 presents a list of the antimicrobial agents used in Chinese aquaculture along with their dosages and applications.

TABLE 3
Antimicrobial agents used in Chinese aquaculture

Antibiotic	Target pathogen or disease	Dosage and application
Oxytetracycline	Enteritis, bacterial disease	4 ‰ in fish feed for 3–5 days or 2–10 mg/kg fish weight for 3–7 days
Norfloxacin	Bacterial disease	Prevention: 100 g/80–100 kg feed, 1 time per day for 3–5 days; Treatment: 100 g/50–60 kg feed, 2 times per day for 5–7 days
Enrofloxacin	Bacterial disease	Treatment: 200 g/80 kg feed
Florfenicol	Broad spectrum antibiotic, bacterial infection	10–15 mg/kg fish weight for 3–5 days, once per day; 0.5 ppm for 3 days
Compound bacteriophage with western medicine and Chinese medicine	Bacterial, fungal and viral infections	1–2.5 g/kg feed for 3–5 days
Compound Norfloxaci	Bacterial infection, Mycoplasma infection	20 g/kg feed for 3 days, once per day
Quinocetone	Gastrointestinal diseases	40–50 ppm
Compound sulfamethoxazole	Bacterial infection	2–3 mg/kg fish weight for 3–5 days
Compound sulfadimidine	Redfin disease, red skin disease, lepidorthosis, enteritis, etc.	1.5 g/kg fish weight for 6 days, twice per day
Erythromycin	White head-mouth disease, gill rot disease, etc.	0.5 g/100 kg fish weight for 6 days; 1 ppm for 5 days
Amoxycillin	Infectious diseases of fish	0.2 ppm for 5 days
Oxolinic acid	Redfin, red skin disease, etc.	10–20 mg/kg fish weight for 4–7 days
Ivermectin	Parasites	Treatment: 20–30 ml/mu fish pond
Abamectin	Parasites	Treatment: 20–30 ml/mu fish pond

A list of veterinary medicines and disinfectants, including traditional herbal treatments that are commonly used in preventing and treating diseases in Chinese aquaculture, is given in Table 4 along with their target pathogens/use.

In China, Chinese herbs are often used for the prevention and treatment of aquatic animal diseases. Researchers have found some traditionally used herbs are effective in controlling disease. Table 5 lists some of the common herbs used in Chinese aquaculture.

Three vaccines are currently registered and licensed by the State, i.e. deactivated vaccine for grass carp haemorrhagic virus, deactivated vaccine for *Aeromonas hydrophila*, and marine fish antibodies vaccine for Japanese flounder.

TABLE 4
List of veterinary medicines and disinfectants and their target pathogens

Veterinary medicine/disinfectant	Target pathogen/use
Quicklime (calcium oxide)	Pond cleaning, elimination of predators and bacteria
Bleaching powder	Pond cleaning, improving water quality, bacteria causing red skin, gill rot, septicaemia
Sodium dichloroisocyanurate	Pond cleaning, bacteria causing skin ulcer, gill rot, septicaemia
Trichlorosisocyanuric acid	Pond cleaning, bacteria causing skin ulcer, gill rot, septicaemia
Chlorine dioxide	Bacteria causing red skin, gill rot, septicaemia
1,3-Dibromo-5,5-dimethylhydantoin	Bacteria causing red skin, virus disease prevention
Sodium chloride	Bacteria, fungi, parasitic diseases
Copper sulfate	Ciliates giardiasis
Ferrous sulfate	Ciliates giardiasis
Potassium permanganate	Anchor worms
4 alkyl quaternary ammonium salts	Viruses, bacteria, ciliates, algal-caused diseases
Crow's treacle (garlic)	Enteritis
Garlic powder (10%)	Enteritis
Medicinal rhubarb	Enteritis, gill rot
Raikai skullcap	Enteritis, gill rot, red skin, septicaemia
Amur corktree	Enteritis, septicaemia
Chinese sumac	Bacteria causing gill rot, red skin, white skin, furuncle
Common andrographis	Bacteria causing enteritis, gill rot, red skin
Lightyellow sophora	Bacteria causing enteritis, vertical scale disease
Oxytetracycline	Enteritis, vibriosis
Oxolinic acid	Enteritis, red fin, vibriosis of ayu and shrimp, sarcoidosis of perch, furuncle of herring
Sulfadiazine	Red skin, enteritis of carps, streptococcosis of marine species
Sulfamethoxazole	Enteritis of carps
Sulfamonomethoxine	Vertical scale disease, red skin, vibriosis of carps
Florfenicol	*Edwardsiella*, red skin of eel
Povidone-iodine	Bacterial gill rot, vibriosis, red head of eel, prevention of septicaemia of grass carp, infectious pancreatic necrosis virus, infectious hypodermal and haematopoietic necrosis virus, septicaemia

TABLE 5
Chinese herbal medicines used in aquaculture

Chinese herbal medicine	Application
Mix of rhubarb, *Scutellaria baicalensis* and *Phellodendron*	Used against viral and bacterial diseases of cultured aquatic animals
Rhubarb	
Scutellaria baicalensis	
Coptis chinensis	
Phellodendron	
Allantolin Allicin	
Astragalus membranaceus	Used to improve the immunological function of aquatic animals
Pine needle (*Pinus* sp.)	Used against parasitic diseases of aquatic animals
Calamus	

Marketing of veterinary medicines

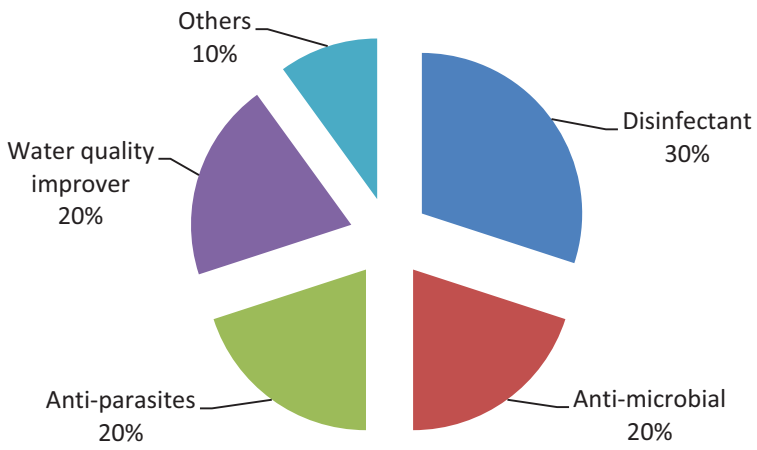
An analysis of the relative market composition for veterinary medicines used in Chinese aquaculture shows that disinfectants comprise 30 percent, followed by water-quality improvers, antimicrobial agents and antiparasitics, all comprising about 20 percent of the market (Figure 6). Projections for the next ten years show that the demand for veterinary medicines will be about 350 000 tonnes, and that the annual sales value will be on the order of CNY 6–8 billion (Yang and Wang, 2008).

From a market-chain perspective, the distribution channel includes direct selling by drug manufacturers, sales by drug stores and feed stores, and sales by registered dealers via contracts. According to a report issued by the Bureau of Fisheries (2009), there are 1 460 drug stores in Fujian Province for veterinary medicines used in aquaculture.

Surveillance of veterinary medicines used in aquaculture

In 2004, the new Regulations on Administration of Veterinary Medicines was published by the State Council, becoming the fundamental law for the regulation of veterinary medicines in China. The regulations outline the administrative organization for the development, production, marketing, import and export, application in farms, surveillance, legal responsibilities, etc., for veterinary medicines. The county and higher-level governments are responsible for implementing the law for surveillance.

FIGURE 6
Relative composition of veterinary medicines for aquaculture on the Chinese market



In early 2005, the Fish Drug Surveillance Department was established under the National Fishery Technology Extension Centre with the approval of the Ministry of Agriculture. This has greatly enhanced the surveillance of veterinary medicines use in Chinese aquaculture (Wang, 2005).

In China, the surveillance of veterinary medicines is very strict, with high attention paid to food safety concerns. The Bureau of Veterinary Medicines, Bureau of Quality, Inspection and Surveillance and the Fishery Technical Extension Station are the organizations assigned responsibility for surveillance of veterinary medicines. Regular inspections are made to examine the drug stores and the availability of drugs in the fish farms. To ensure the implementation of the regulation on quality and safety of veterinary medicines used in aquaculture, the Ministry of Agriculture has organized a national inspection team to conduct inspections in major aquaculture-producing regions of the country (Xiao, 2005).

Impacts of the application of veterinary medicines in aquaculture

Positive impacts

Respondents to the survey reported several positive impacts of the application of veterinary medicines in fish and shrimp farms (Table 6). Antimicrobial agents and chemotherapeutants were reported to be useful in reducing mortalities during disease events and generally increased the survival of the cultured stocks. Veterinary medicines were considered important in allowing the development of new farming technology.

TABLE 6
Positive impacts of the application of veterinary medicines in fish and shrimp farms

Positive impact	Number of respondents	
	Antimicrobials	Chemotherapeutants
Reduction in mortality during disease events	42	38
General increase of survival	40	37
Reduction of the use of other treatments	12	19
Better quality product	11	8
Fish/shrimp welfare	7	5
Mask knowledge gap in husbandry	5	6
Allow the development of new farming technology	21	19

Negative impacts

Some negative impacts resulting from the use of veterinary medicines in fish and shrimp farms were also reported (Table 7). The most important of these are the build up of clinical resistance in fish and shrimp, the presence of residues of food safety concern, and the fact that some veterinary medicines are toxic to cultured animals.

TABLE 7
Negative impacts of the application of veterinary medicines in fish and shrimp farms

Negative impact	Number of respondents	
	Antimicrobials	Chemotherapeutants
Toxicity to farmers	15	23
Toxicity to fish/shrimp	22	21
Toxicity to the environment	15	21
Residues of food safety concern	28	29
Build up of clinical resistance in fish/shrimp	35	29
Build up of resistance in laboratory bacterial isolates	0	0

Reasons for failure of disease control

An analysis of the survey results showed that the reasons for failure of disease control included the absence of an accurate diagnosis and the fact that the pathogen was often not the primary cause of the disease process (Table 8). The use of antimicrobial agents in isolation and the use of subtherapeutic dosages were ranked as the other important reasons for the failure of disease control.

TABLE 8
Reasons for failure of disease control

Reason for failure	Score
Pathogen was not the primary cause of disease	41.8
Absence of accurate diagnosis	41.6
Use of antimicrobials in isolation (i.e. without improvement in farming practices, environment, etc.)	30.0
Utilization of subtherapeutic dosages	29.6
Inappropriate duration of treatment	27.4
Use of antimicrobials of unsure/unproven quality	22.2
Lack of approved medications (few products available)	17.8
Lack of information concerning the fish stock (e.g. biomass)	16.4
Inadequate storage of chemicals	11.2

Needs of the industry

The recommendations made by the farmers and extension officers indicate that training on accurate disease diagnosis for farmers, veterinary practitioners and laboratory technicians is urgently needed (Table 9). There is also a need to establish more demonstration projects to help the aquaculturists in areas such as improving the eco-aquaculture model, developing better fishery community facilities and exchanging information on rural aquaculture development. There is high demand to establish diagnostic, examination and quality testing centres in communities where aquaculture is an important industry to help farmers

have rapid and accurate disease diagnoses and examinations. A local network for epidemic forecasting and providing information services to aquaculturists is also needed.

The respondents reported a high demand for research on new veterinary medicines and alternative approaches. The new approaches included environment improvement for disease prevention, better fish strains having disease resistance, water purification, environmentally friendly farm reformation, and the use of wetland integrated pond systems.

New trends

During the survey, some new trends in the prevention and control of aquatic animal diseases in China were reported. Some of these trends have already shown promising results.

TABLE 9
Recommendations for actions on disease control Needs of the industry

Recommendations for actions	Score
Training for farmers	
– Accurate diagnosis	47.6
– Proper and prudent use	44.6
– Negative impacts of use	33.4
Training for veterinary and paraveterinary practitioners	
– Accurate diagnosis	48.2
– Proper and prudent use	44.2
– Negative impacts of use	32.6
Training for laboratory technicians	
– Accurate diagnosis	46.0
– Alternatives to antimicrobial agents and chemotherapeutants	37.2
– Determination of resistance	33.4
– Determination of proper dosage	38.2
Training in health management practices	29.6
– Consultation to set priorities for research	31.2
– Provide information on laboratory testing for residues in feeds and tissues	32.8
– Increase production of information on pharmacokinetics	31.8

Licensed fishery clinic pilot programme

In order to implement the "Animal Epidemic Prevention Law" and the "Regulations on the Management of Veterinary Medicines" to ensure the safety of aquatic products, a pilot programme for an aquatic animal licensed fishery clinic was conducted in Guangdong, Fujian, Jiangxi and Jiangsu provinces.

Based on the "Action Plan to Promote Healthy Aquaculture" ([2007] No. 30) of the Ministry of Agriculture, the Center for Aquatic Animal Epidemic Disease Prevention and Control of Guangdong Province has established the Fish Vet Doctor Training Programme, including the compilation of a "Fish Veterinary Doctor's Manual" for training purposes. The first batch of fish veterinary doctors was comprised of technicians from fishery technical extension stations, aquatic animal epidemic prevention and quarantine stations, leading aquaculture farms, fisheries drug manufacturers and fisheries drug stores. Trainees who passed the examination were certified as licensed fish vet doctors. In August 2007, 149 fish vet doctors and assistants were licensed.

The training programme for fish vet doctors was also successfully conducted in Jiangsu Province, where 304 trainees were licensed as fish vet doctors. In Fujian Province, more than 100 fish vet doctors were approved, while 154 fish vet doctors were issued licenses in Jiangxi Province. The fish vet doctors play an important role in helping aquaculturists control disease outbreaks.

Fish disease clinics in fishery communities

Fish disease clinics offer services to farmers, including disease diagnosis, prescription of

veterinary medicines and guidance for drug application. The clinics are equipped with excellent facilities for diagnosis and a computer-based distant diagnosis system, and are staffed by licensed fish vet doctors. The clinics can issue prescriptions for aquatic animal disease treatments, with difficult cases being quickly referred to the distant diagnosis system for support. In emergency cases, fish vet doctors will go to the farms for inspection and to provide on-site technical guidance.

Fishery technical extension plays an important role in fish disease prevention and the improvement of aquaculture models. Through technical training, television and radio programmes, newspapers, the publications of fishery associations, and mobile phone message service networks, information on disease prevention is effectively disseminated to aquaculturists. Through demonstration farms, eco-friendly aquaculture models are transferred to the local fishery communities.

Veterinary medicines for aquaculture are available in drug stores, which are licensed by the Bureau of Veterinary Medicines of the local government. The sellers should have a college education with a background in aquaculture or a certificate on fish disease diagnostics issued by the Bureau of Veterinary Medicines or a Fishery Technical Extension Station.

Drugs sold in the stores are registered by the Administration Bureau of Veterinary Medicine of the Ministry of Agriculture and carry labels indicating the ingredients, dose, usage and precautions. Drugs produced by different factories may have different contents of ingredients and combination, and thus also differ in their usage.

Criteria for development of prescribed veterinary medicines for use in aquaculture

Because of the large number of aquaculture farms and the frequent occurrence of disease outbreaks, there is a high demand for veterinary medicines. The criteria proposed below for the approval of prescribed and non-prescribed veterinary medicines for use in aquaculture will help farmers to treat easy cases of disease, avoiding the high demand for prescriptions from fish vet doctors.

The proposed criteria for classification as a prescribed drug for use in aquaculture are:

- approved new drugs within the observation period;
- low safety for aquacultured animals;
- high risk to food safety and the environment;
- likely to cause drug resistance;
- specified usage and the need to have specific guidance; and
- not included among the non-prescribed drugs.

The proposed criteria for classification as a non-prescribed drug for use in aquaculture are:

- low toxicity;
- no drug resistance caused;
- has a good record of safe usage;
- no high risk to food safety or the environment;
- no specific usage required; and
- no professional guidance needed.

Ecological aquaculture model

Ecological aquaculture is a practice in which the by-products (wastes) from one species are recycled to become inputs (e.g. fertilizers, food) for another species. Fed aquaculture (e.g. fish, shrimp) is combined with inorganic extractive (e.g. seaweed) and organic extractive (e.g. shellfish) aquaculture to create balanced systems for environmental sustainability (biomitigation), economic stability (product diversification and risk reduction) and social acceptability (better management practices).

Farmers reported that they used to pay high attention to disease prevention rather than treatment. Disinfectants such as quicklime and chloride were used to reduce the pathogen

biomass in the water and on the fish. In epidemic situations, drugs recommended by the extension officials and technicians would be applied to fish ponds.

Polyculture and the use of the environmentally friendly model has contributed greatly to aquatic disease prevention. The polyculture stock model is a successful approach to ecological aquaculture in China. The polyculture of Chinese carps, tilapia cum shrimp, etc., are common practices in China.

In the ecological aquaculture model, integrated water purification units were constructed within the fish farm, a constructed wetland was established to remove nutrients in the effluent using a biological approach, and biofilters were used to purify the water discharged from aquaculture ponds, which was then pumped back to the ponds. The results showed that organic matter can be effectively removed by this process.

Improved information network service to fish farmers

A new information distribution network "Nong Xin Tong" has been established to help fish farmers. The Technical Extension Station distributes information on fish disease prevention and treatment through the use of mobile text messaging so that aquaculturists can easily obtain help by sending messages and receiving answers through the network. The technical information is organized by the extension experts, and a database on disease prevention and veterinary medicines has been constructed at the service centre. With strong support from the local government, fish farmers pay only about CYN 5 per month in service charges, showing this method to be an inexpensive but effective way to establish an information network.

There is also demand for training on awareness of biosecurity and the use of organic products in aquaculture. Through more effective technical training and extension programmes, the aim is to improve sustainable and responsible aquaculture development.

RECOMMENDATIONS

The following recommendations are made based on the survey findings

- *Training in fish disease diagnosis and health management.* There is a high demand for training in disease diagnosis and on emerging epidemic diseases of aquatic animals. With the development of new aquaculture species and changes to the culture environment, the knowledge gap between farmers and extension officers has increased. Training for both farmers and extension officers is thus recommended; the training methodology can use different approaches, such as newspapers, radio broadcasts and formal training courses. It is urgent that scientific guidance on the use of veterinary medicines is given based on the results of monitoring pathogen tolerance, and the use of scientific and effective methods to select and use medicines correctly, ensuring efficacy of treatment, keeping cultured animals healthy, reducing residues and improving the quality and safety of aquatic products.
- *Research on alternative approaches to disease control.* Changes in cultured species and the aquaculture environment have led to the appearance of more complex diseases. More effective quarantine is needed for broodstock and seed, and rapid and accurate methods for disease detection need to be developed. To satisfy the high demand of aquaculture operations for healthy seed, well-equipped and effective quarantine systems and laboratories are required. Specific pathogen-free seed and specific pathogen-resistant broodstocks need to be developed. Research is needed to develop new veterinary medicines with low toxicity and high efficacy for treating diseases in aquaculture. Food quality and safety should be maintained through the development of new drugs to treat aquatic animal diseases. The development of effective alternatives for disease control will help to avoid technical barriers to trade based on the presence of residues.
- *Quality and safety concerns.* In the marketing and sale of veterinary medicines,

aquaculturists are often misled by commercial names and advertising. A uniform indication of drug ingredients and contents is needed. The drugs manufacturers need practical guidance on the correct application of veterinary medicines under different water quality conditions and for different culture species. To meet the requirements related to maximum residue levels and quality control, more intensive surveillance on the use of veterinary medicines in aquaculture systems is needed. Facilities for quality control and testing are needed at the level of the fishery community or the leading fishery village. Although a traceability system has been installed to monitor export-oriented aquaculture facilities, more support is needed for establishing a traceability system to ensure aquatic food quality and safety.

- *Improve the infrastructure of fish culture*. In China, fish pond management is mainly done by farmers through contracts. As most contracts pay more attention to production without addressing the need to improve infrastructure, pond conditions have deteriorated after years of aquaculture practice. This has resulted in problems related to heavy pond siltation, poor water source and inadequate flood disaster prevention. There is an urgent need to improve the condition of ponds, including the pond dykes, water source canals, roads and effluent treatment. More support from the government is needed to improve the infrastructure via guidance or programmes on ecological aquaculture, constructed wetlands, deepening of ponds, removal of pond silt, etc.
- *Apply good aquaculture practices (GAqPs) to improve aquaculture management*. GAqPs are commonly recognized protocols for improved management of aquaculture. GAqPs are fundamental guidelines with practical outlines for developing standards in conformance with codes of conduct. To produce good quality and safe aquatic products for consumers, farms must be standardized, sanitary and generate no environmental impacts. Furthermore, aquatic animal health management must avoid the use of antimicrobial agents and other substances that lead to residues in aquatic animal products. The use of veterinary medicines must be recorded and minimum withdrawal periods before harvesting respected. There is a need to promote GAqPs to aquaculturists, and extend these guidelines to include feeds, veterinary medicines and the management process for the entire production chain.

Many factors cause diseases in aquaculture production facilities, including the aquatic animal, the pathogen, and the environment and their inter-relationships. As any single technical solution cannot solve all these problems, they need to be addressed through a combination of actions, including improvement of the culture environment, disease surveillance and monitoring, the use of immunostimulants and new breeding technologies.

ACKNOWLEDGEMENTS

We would like to express our gratitude to all those who provided help essential to accomplishing the survey in November 2009, especially Mr Huang Aiping, Director of the Fishery Technical Extension Station of Taixing City, Jiangsu Province, and Mr Huang Tianwen, Director of the Fishery Technical Extension Station of Zhanjiang City, Guangdong Province, and their staff, who provided us with the opportunity to complete this survey. We also thank the Aquaculture Management and Conservation Service of the Fisheries and Aquaculture Department of FAO for giving us the chance to undertake the survey, with special thanks to Dr Melba Reantaso and Ms Elena Irde for their participation and guidance during the survey and report preparation.

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Use of veterinary medicines in Philippine aquaculture: current status

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Somga, S.S., Somga, J.R. and Regidor, S.E. 2012. Use of veterinary medicines in Phillippine aquaculture: current status. *In* M.G. Bondad-Reantaso, J.R. Arthur & R.P. Subasinghe, eds. *Improving biosecurity through prudent and responsible use of veterinary medicines in aquatic food production*, pp. 69—82. FAO Fisheries and Aquaculture Technical Paper No. 547. Rome, FAO. 207 pp.

ABSTRACT

A survey on veterinary medicines currently used in Philippine aquaculture was conducted covering different key players in aquaculture production. Veterinary medicines, particularly antimicrobial, antiparasitic and antifungal agents and disinfectants, are applied in aquaculture and more commonly used in hatcheries rearing shrimp, tilapia and milkfish/marine fish to prevent and treat health problems associated with stress and diseases owing to bacteria, external parasites and fungi. Their use increases survival rates and improves resistance to health problems in fry during transport and stocking. In grow-out, there is minimal application of antimicrobial agents; instead probiotics are being used. Other environmentally friendly approaches such as greenwater technology, crop rotation and the application of biosecurity measures are employed for disease prevention. However, there are cases where antibiotics are incorporated into the feeds as a disease preventive approach. The presence of residues in aquaculture products brought about by the irresponsible use of veterinary medicines is now an international food safety issue affecting trade. Products with residues beyond the maximum residue limit can be rejected for export. Consequently, farm registration may be suspended, resulting in the affected farms no longer being permitted to supply their products to accredited processing plants. Although the industry is now becoming aware of this issue, there is still a need for continued efforts by the government to strengthen programmes promoting the responsible use of veterinary medicines in aquaculture.

INTRODUCTION

The Philippine aquaculture sector provides the highest contribution to the country's total fisheries production. In 2008, this contribution was about 48 percent of total production, amounting to 2 407 697.9 tonnes valued at PHP 81.67 billion (BFAR, 2008). Among the most commonly cultured species are shrimp, milkfish and tilapia. These are being cultured either in ponds or cages using extensive, semi-intensive or intensive production systems.

With the rapid growth of the aquaculture industry, intensification of the culture systems has required a more advanced farming technology and management. Diversification

through the introduction of new farmed species and the potential offered by the highly competitive global seafood market have also attracted the interest of farmers seeking more profitable markets for their harvest. Maintaining the optimum condition of the aquatic environment for productivity and sustainability has become a big challenge for the industry. More often than not, health problems occur because of the poor condition of the culture environment, with diseases being one of the major constraints to successful aquaculture production.

The use of veterinary medicines is an option to maintain animal health. Antimicrobial agents and other substances are being used to treat and prevent diseases, and at low dosages may serve as growth promoters. However, they only provide a short-term benefit, as their continuous use may lead to the development of resistance in pathogens.

The presence of residues of antimicrobial agents in aquaculture products beyond the maximum residue level (MRL) can be grounds for rejection of products by importing countries. Thus, producers must ensure that their products are both safe for human consumption and of high quality. This is verified by the competent authority through inspection and sampling along the production chain for compliance with the set standards.

This paper presents the results of a survey on the current application of veterinary medicines in Philippine aquaculture. An overview of the regulation of veterinary medicines in the Philippines and other control programmes being implemented to promote the prudent use of veterinary medicines in aquaculture production is also presented.

OVERVIEW OF THE CONTROL OF VETERINARY MEDICINES

The authorization of veterinary medicinal products in the Philippines is the mandate of the Food and Drug Administration-Department of Health (FDA-DOH), formerly the Bureau of Food and Drugs. Through enactment of the Republic Act No. 9711 in 2009, the FDA-DOH has strengthened regulatory powers, resources and capabilities to perform its mandate. The new law provides for the ordering of the ban, recall and withdrawal of health products that cause or have the potential to cause death, serious illness or injury to consumers. It also authorizes the FDA-DOH to retain all income in addition to its annual budget. This is expected to provide sufficient resources for its operation, including the establishment of testing laboratory facilities in Luzon, Visayas and Mindanao, as well as the operating expenses of the existing central office laboratory in Metro Manila, which will be provided with the necessary laboratory equipment. At present, the agency is developing the law's implementing rules and regulations.

To effectively implement Republic Act No. 9711, the FDA-DOH, through an Order, has deputized the Department of Agriculture through the Bureau of Animal Industry (BAI) and the Bureau of Fisheries and Aquatic Resources (BFAR) to assist in the registration and monitoring of veterinary drugs and other products used in the rearing of terrestrial and aquatic animals, respectively. The Order is now being finalized after a series of consultations with the concerned agencies for clear delineation of responsibilities.

Veterinary drugs and products must be registered before they can be placed on the market. They are evaluated and registered based on the specific requirements and standards. Their distribution and sale are also monitored through inspection of outlets, aquaculture farms and feedmills. These activities are controlled by several Republic Acts (RAs) and their corresponding Administrative Orders (AOs) and Memoranda. The RAs include:

- RA No. 9711 – the Food and Drug Administration Act;
- RA No. 1556 – the Livestock and Poultry Feeds Act;
- RA No. 3720 – the Foods, Drugs and Devices and Cosmetics Act;
- RA No. 6675 – the Generics Act;
- RA No. 1071 – an Act to Regulate the Sale of Veterinary Biologics and Medicinal Preparations;
- RA No. 8550 – the Philippine Fisheries Code; and
- RA No. 7394 – Consumers Act of the Philippines.

The AOs and Memoranda include:

- Special Order No. 167, Series 2004 – Creation of Aquatic Feeds Monitoring Task Force;
- Department of Agriculture (DA) Special Order No. 69, Series of 2004 – Deputation of BFAR Fish Health Officers and DA Regional Veterinary Personnel as Aquatic Animal Feed and Veterinary Drug and Product Control Officers Following the Terms of Agreement in the Memorandum of Agreement Between BAI and BFAR;
- BAI-Memorandum Circular No. 6, Series of 2003 – Guidelines Governing the Disposal and Destruction of Banned Veterinary Drugs and Products Used in All Food-producing Animals;
- DA Special Order No. 23, Series of 2002 – Deputation of BFAR Fish Health Officers as Aquatic Animal Feed and Veterinary Drug and Product Control Officers;
- DA-BFAR and BAI Memorandum of Agreement (2001) – Regulation on Animal Feed, Veterinary Drugs and Products in Aquaculture;
- Animal Industry AO No. 9, Series of 1994 – Guidelines Governing the Conduct of Clinical Trials of Veterinary Drugs and Products;
- Animal Industry AO No. 27, Series of 1993 – Minimum Requirements for Determining/Evaluating the Efficacy and Safety of Veterinary Drugs to Target Animals;
- Animal Industry AO No. 35, Series 1975 – Rules and Regulations Governing the Manufacture, Importation, Labelling, Advertising, Distribution and Sale of Livestock and Poultry Feeds and Feeding Stuffs;
- DA AO No. 3 and Department of Health (DOH) AO No. 118, Series of 1992 – Rules and Regulations on the Process of Review and Evaluation of Questioned Veterinary Drugs or Veterinary Drugs Combinations; and
- DOH-AO No. 111-A and DA-AO No. 33, Series of 1991 – Rules and Regulations on Registration of Veterinary Drugs and Products.

The fish health officers of BFAR are deputized as Aquatic Animal Feed and Veterinary Drug and Product Control Officers through DA Special Order No. 23, Series of 2002 and Special Order No. 69, Series of 2004, to conduct inspection and sampling at aquaculture facilities, fish ports, fish processing plants and markets to monitor the use of veterinary drugs and products in aquaculture. They are also authorized to conduct disease diagnosis and recommend medications for use in aquatic animals. However, the application of restricted veterinary drugs requires a prescription by a duly licensed veterinarian, and their use must comply with the applicable regulations, particularly for drugs requiring a minimum withdrawal period.

The following products have been banned through joint DOH and DA Administrative Orders (AOs):

- *Beta-agonist.* DA AO No. 14, Series of 2003 – Ban on the Use in Food Animals of Beta-agonist Drugs Used in Humans as Bronchodilators and Tocolytic Agents.
- *Nitrofurans.* DOH and DA Joint AO No. 2, Series of 2000 – Declaring a Ban/Phase Out of the Use of Nitrofurans in Food-producing Animals.
- *Olaquindox and carbadox*. DOH AO No. 4-A and DA AO No. 1, Series 2000 – The Banning and Withdrawal of Olaquindox and Carbadox from the Market.
- *Chloramphenicol.* DOH AO No. 91 and DA AO No. 60, Series of 1990 – Declaring a Ban on the Use of Chloramphenicol in Food-producing Animals.

CONTROL OF RESIDUES IN AQUACULTURE

BFAR is the responsible agency and takes the lead in implementing the Republic Act No. 8550, otherwise known as the Philippine Fisheries Code of 1998, and its subsequent implementing Orders, which include the regulation of the use of veterinary medicines in aquaculture, health conditions for production and food safety control of fishery and

aquaculture products.

Pursuant to Sections 62, 65, 67 and 85 of Republic Act No. 8550, concerning the country's commitment to ensure that the safety of aquaculture products for human consumption is at par with the international standard, several regulations were enacted specifying the powers and functions of regulatory officers for safety and quality assurance of aquaculture products, including the right to enter farms and take corrective action in the event of non-compliance.

Legislation applicable for the residue control programme includes:

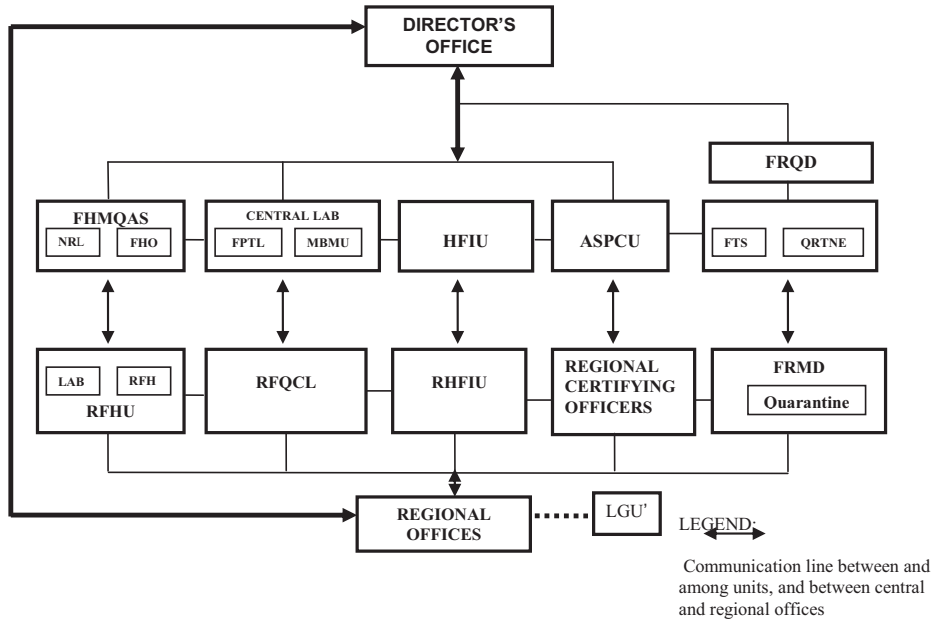
- FAO No. 210, Series of 2001 – Regulations for the Exportation of Fresh/Chilled and Frozen Fish and Fishery Aquatic Products;
- FAO No. 212, Series of 2001 – Guidelines on the Implementation of HACCP System;
- FAO No. 211, Series of 2003 – Amendment to Fisheries Office Order 147-01, Series of 2001: Designation of Regional Fish Health Officers of BFAR;
- Fisheries Office Order (FOO) No. 210, Series of 2003 – On-farm Residue Monitoring;
- General Memorandum Order (GMO) No. 225, Series of 2004 – Continued Implementation of the Commission Decision 2003/858/EC by the Fish Health Officers;
- Memorandum Circular Order No. 01, Series of 2005 – Sanitary and Phyto-sanitary Requirements for Exportation of Aquaculture Products for Food Safety and Quality Assurance;
- GMO No. 1, Series of 2005 – Implementation of Memorandum Circular on Sanitary and Phyto-sanitary Requirements for Exportation of Aquaculture Products for Safety and Quality Assurance;
- Memorandum Circular No. 01, Series of 2005 – Sanitary and Phyto-sanitary Requirements for Exportation of Aquaculture Products for Food Safety and Quality Assurance;
- Special Order 310, Series of 2005 – Designation of Fish Health Section as the National Reference Laboratory for Veterinary Residues for Aquaculture Products;
- FOO No. 155, Series of 2005 – Creation of the Fish Inspection and Quality Assurance Service (FIQAS);
- FOO No. 152, Series of 2005 – Creation of Fishery Inspection and Quality Assurance Service: Residue Monitoring and Disease Surveillance;
- FOO No. 247, Series of 2006 – Powers and Functions of Regulatory Officers (Fish Inspectors, Fish Health Officers, Fisheries Quarantine Officers and Certifying Officers) for Safety and Quality Assurance of Fishery and Aquaculture Products Intended for Human Consumption;
- DA-AO No. 24, Series of 2009 – Implementing Guidelines on the National Veterinary Drug Residues Control Program in Food Pursuant to Administrative Order No. 14, Series of 2006; and
- DA-AO No. 14, Series of 2006 – Implementation of the National Veterinary Drug Residues Control Program and Creation of the Inter-agency Committee.

Its implementation was also strengthened by virtue of DA-AO No. 14, Series of 2006, on the implementation of the national veterinary drug residues control programme and the creation of an inter-agency committee, and DA-AO No. 24, Series of 2009, as its implementing rules and regulations. This defines the roles of the competent authority, farmers and suppliers.

To strengthen and facilitate the implementation of food safety control programmes, the Fish Inspection and Quality Assurance Service (FIQAS) was created. It consists of several units in BFAR involved in food safety control and operates under the direct supervision of the BFAR's Director. The FIQAS implements a Hazard Analysis and Critical Control Point (HACCP)-based inspection programme, a residue monitoring

programme and a coordinated certification programme. The structure of FIQAS is presented in Figure 1.

FIGURE 1
Organizational structure and interrelationship of BFAR units involved in the National Fish Inspection and Quality Assurance Service (FIQAS)[1]



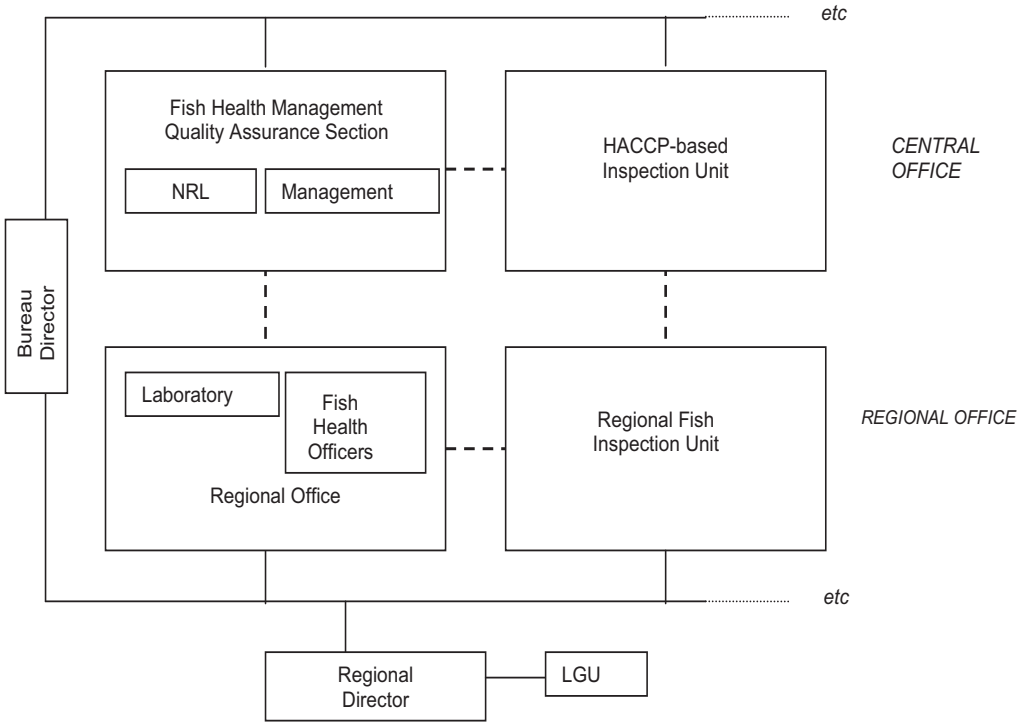
[1]Legend:
ASPCU – Administrative Support and Product Certification Unit
FIQAS – Fish Inspection and Quality Assurance Service
FRQD – Fisheries Regulatory and Quarantine Division
FPTL – Fishery Product Testing Laboratory
FHMQAS– Fish Health Management and Quality Assurance Section
FTS – Foreign Trade Section
FRMD – Fisheries Resource Management Division
HFIU – HACCP-based Fish Inspection Unit
LGU – Local Government Unit
MBMU – Marine Biotoxin Monitoring Unit
NRL – National Reference Laboratory
QRTNE – Quarantine Section
RFQCL – Regional Fish Quality Control Laboratory
RFHU – Regional Fish Health Unit
RHFIU – Regional HACCP-based Fish Inspection Unit

The National Residue Control Program is implemented by the Fish Health Management and Quality Assurance Section (FHMQAS) in coordination with the 15 BFAR regional offices. The organizational structure and coordination between regional and central offices is presented in Figure 2.

The National Residue Control Program includes:

- aquaculture farm registration scheme;
- monitoring of hygiene of production;
- disease surveillance and reporting;
- information dissemination and education of the aquaculture food chain operators on the need for aquatic animal feeds and veterinary drugs and products registration prior to their marketing and usage;
- surveillance and monitoring of aquatic animal feeds, veterinary drugs and products by the Aquatic Animal Feed and Veterinary Drug and Product Control Officers;
- regulatory actions on any violations of policies and guidelines on the registration, manufacture, distribution and use of veterinary drugs and aquatic animal feeds; and
- assistance in planning, directing and supervising national programmes on aquatic feeds, veterinary drugs and products control.

FIGURE 2
Organizational structure for residue monitoring and coordination between central and regional offices[1]



[1]Legend:
LGU – Local Government Unit
NRL – National Reference Laboratory

USE OF VETERINARY MEDICINES IN AQUACULTURE

The survey on the use of veterinary medicines and other related products in aquaculture in the Philippines was conducted in November 2009 to determine the current range of veterinary medicines applied in aquaculture and to assess the perception of the respondents on their impacts. The survey form used was developed by the Food and Agriculture Organization of the United Nations (FAO). The survey consists of information about the respondents, types of veterinary medicines and other products used, species and culture system, dosages, and availability, efficacy and impact, reasons for treatment failure and recommendations.

Respondents

A total of 69 respondents were interviewed, either in groups or individually, or provided with the survey form through e-mail. The respondents were from different key subsectors of aquaculture production as follows: operators of shrimp hatcheries and grow out (18), milkfish/marine fish grow out (5) tilapia hatcheries and grow out (24); government field workers (9); and feed and aquatic products suppliers (13). Respondents came from Regions III, IV-A, NCR, VI and VII, which are the major aquaculture-producing areas in the country.

Veterinary Medicines and Other Products

The veterinary medicines and other products used in Philippine aquaculture are presented below in Tables 1–8, based on their classification as antibiotics, antiparasitic agents, antifungal agents, disinfectants, vaccines, sex control aids, probiotics and immune enhancers. These were available in the market and were recommended by sales agents and government field workers who provide technical assistance to farmers or were obtained directly by the farmers themselves, who used knowledge gained through years of farming experience to resolve health-related problems in their farms.

The results of the survey showed that among the antibiotics listed in Table 1, oxytetracycline is the most popular and commonly used in all species (Figure 3). Thirty respondents mentioned that they use oxytetracycline. It is usually a practice in hatchery operation to apply antibiotic as preventive measure against bacterial infection. For them, oxytetracycline is effective, affordable and readily available. Erythromycin, amoxicillin, florfenicol and trimetoprim-sulfadiazine are also applied in all species, but with lower frequency. Oxolinic acid, rifampicin and sulfamonomethoxine are only applied in shrimp hatcheries. Other antibiotics mentioned by respondents were applied less frequently.

The antiparasitic agents used are presented in Table 2. According to the results of the survey, formalin is the most frequently used because it not only treats external parasites but is also an excellent remedy for external fungal and bacterial infections. For freshwater fish, sodium chloride for external treatment is preferred, as it is accessible, practical and safe.

Antifungal agents such as formalin, methylene blue and trifuralin are consistently used in hatchery as routine practice to prevent fungal infection in eggs and fry (Table 3). It is noted that malachite green is still being applied, however, it is no longer recommended for food fish.

A range of disinfectants are being used and are available in the market (Table 4). Chlorine, formaldehyde and iodophores are commonly used. Chemicals listed in Table 4 provide and maintain good sanitary and hygienic conditions of facilities, particularly the hatcheries. The disinfectants listed are also used for water conditioning; cleaning of tanks and farm implements; routine disinfection; and disinfection of broodstock, eggs and larvae.

TABLE 1
Antibiotics used in Philippine aquaculture

Antibiotic	Species and culture system	Dosage
Amoxicillin	Shrimp hatchery Tilapia hatchery, marine fish grow-out	Not indicated 80 mg/kg fish for 7 days
Doxycycline	Tilapia hatchery	10 mg/kg fish for 3–5 days
Erythromycin	Shrimp hatchery, tilapia hatchery Marine fish grow out	2–3 ppm for 3 days Not indicated
Enrofloxacin	Tilapia hatchery, marine fish grow-out	Not indicated
Florfenicol	Shrimp hatchery, milkfish hatchery Tilapia grow-out, marine fish grow-out	2 ppm 10 mg/kg fish for 10 days
Neomycin sulphate	Marine fish grow-out	Not indicated
Norfloxacin	Tilapia hatchery Tilapia grow-out, marine fish grow-out	50 ml/100 litre of water for 10 days 2.5–5 mg/kg fish for 5 days
Oxytetracycline	Shrimp hatchery,tilapia hatchery marine fish grow-out Tilapia grow-out	1–2 ppm for 7 days 2–5 g/kg feed for 10 days 7-27 g/kg feed/day
Oxolinic acid	Shrimp hatchery	20 mg/kg for 7 days
Rifampicin	Shrimp hatchery	1–2 ppm for 7 days
Sulfamonomethoxine	Shrimp hatchery	2–4 ppm daily
Sulfaquinoxaline	Tilapia hatchery Marine fish grow-out	3 g/kg feed 4-14 g/kg feed/day
Trimethoprim-sulfadiazine	Shrimp hatchery,tilapia hatchery Marine fish grow-out	Not indicated 15–20 g/kg feed for 7 days

FIGURE 3
Antibiotics used in different aquaculture species and their culture system and the frequency of use according to respondents.

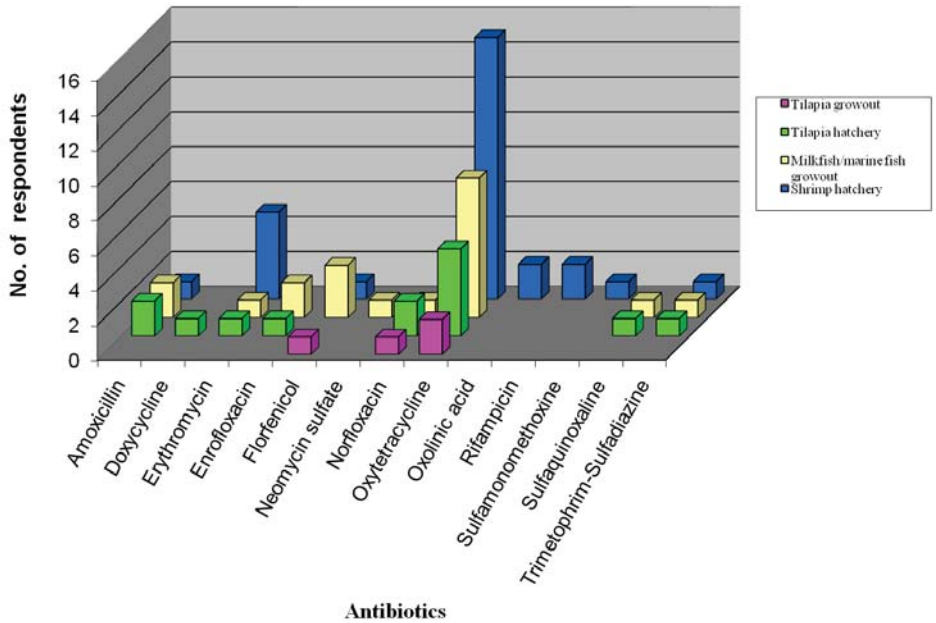


TABLE 2
Antiparasitic agents used in Philippine aquaculture

Antiparasitic agent	Species and culture system	Dosage
Belzalkonium chloride	Shrimp hatchery Tilapia hatchery	0.5 –1 ppm 1 ppm
Copper sulphate	Tilapia and shrimp hatchery, marine fish grow-out	2–5 ppm as 30 min bath
Formalin	Shrimp hatchery Tilapia hatchery Marine fish grow-out	1–2 ppm 200 ppm as 10–30 min bath (also for tail rot, fin rot) 20 ppm as 30-minute bath (also for tail rot, fin rot)
Hydrogen peroxide	Marine fish grow-out	2–5 ppm as 30-minute bath
Omnicide	Shrimp hatchery	1 –1.25 ppm for *Zoothamnium*
Praziquantel	Tilapia and marine fish grow-out	Not indicated
Potassium permanganate	Shrimp hatchery and grow-out,tilapia hatchery and grow-out,marine fish grow out	1 ppm
Quinacrine hydrochloride	Shrimp hatchery	2–3 ppm (mysis) 3–5 ppm (PL)
Sodium chloride	Tilapia hatchery and grow-out	0.25–1 ppt as indefinite bath
Trichlorfon	Marine fish grow-out	0.5–1 ppm indefinite bath 30 ppt as short bath

TABLE 3
Antifungal agents used in Philippine aquaculture

Antifungal agent	Species and culture system	Dosage
Formalin	Shrimp hatchery,tilapia hatchery, marine fish grow-out	40–60 ppm as indefinite bath
Malachite green	Shrimp hatchery, tilapia hatchery, marine fish grow-out	20 ppm as 20 min bath (not advisable to apply)
Methylene blue	Tilapia hatchery, shrimp hatchery	3–5 ppm as indefinite bath
Trifuralin	Shrimp hatchery Tilapia hatchery and grow-out marine fish grow-out	0.05–0.1 ppm for 24 h over 2–3 days 0.5 ppm for 14 days

TABLE 4
Disinfectants used in Philippine aquaculture

Disinfectant	Species and culture system	Dosage
Chloramine-T	Shrimp hatchery	Not indicated; for shrimp egg disinfectant
Chlorine	Shrimp hatchery and grow-out, tilapia hatchery and grow-out, milkfish/marine fish grow-out	20–100 ppm for disinfection of water, tanks, pipes and equipment
Cypermethrin	Shrimp hatchery	125–200 ml/1 000 m^3 water
Dichlorvos	Shrimp grow-out	1.5–2 ppm for pond preparation
Formaldehyde	Shrimp hatchery, tilapia hatchery, marine fish grow-out Shrimp hatchery	50 ml/liter for disinfection of tanks and equipment 8 ppm for water disinfection before stocking, then stock shrimp nauplii after 3 days
Hydrogen peroxide	Tilapia hatchery Shrimp hatchery	70 ppm as 2-hour flush for disinfection of tanks, pipes and equipment Not indicated
Iodophores	Shrimp hatchery and grow-out, marine fish grow-out	1–2 ppm for water conditioning
Omnicide	Shrimp hatchery and grow-out	For routine disinfection and aerial fogging: 1:400 For wheel/foot bath: 1:100
Potassium monopersulphate	Shrimp hatchery Shrimp grow-out Tilapia hatchery and grow-out, marine fish grow-out	50 ppm as 1-minute dip 3–6 kg/ha at 1 m water depth 0.3 ppm as 24-hour bath
Potassium permanganate	Shrimp hatchery and grow-out	10 ppm, for disinfection of surface, spray; use in foot/vehicle tire bath in shrimp grow-out pond
Povidone-iodine	Shrimp hatchery	200 ppm for 30 seconds (egg washing) 20 ppm (broodstock disinfection upon arrival)
Trichlorfon	Shrimp grow-out	0.5–1 ppm for preparation prior to stocking

TABLE 5
Vaccines used in Philippine aquaculture

Vaccine	Species/system	Dosage
Streptococcus sp. Bacterin	Tilapia hatchery	1 000 ml:100 kg fingerlings by immersion one time

TABLE 6
Sex control aids used in Philippine aquaculture

Hormone	Species	Dosage
17 Alpha methyltestosterone	Tilapia fry	60 mg/kg feed until 21 days

TABLE 7
Probiotics used in Philippine aquaculture

Probiotics	Species/system	Dosage
About 20+ products available on the market	Shrimp hatchery and grow-out, tilapia hatchery and grow-out, milkfish/marine fish hatchery and grow-out	Depends on product; applied either in pond or via feeds

TABLE 8
Immune enhancers used in Philippine aquaculture

Immune enhancer	Species/system	Dosage
Ergosan (Extract of *Laminaria digitata*, 99% and *Ascophylum nodosum*, 1%)	Shrimp hatchery	0.1–0.7 g per tonne of larval rearing tank daily
	Tilapia hatchery and grow-out,milkfish hatchery and grow-out, shrimp grow-out	2—5 g/kg feed daily
Shrimp Activa (Glucan and mannan polysacharides)	Shrimp hatchery	
	Zoea	12.5 g/100 000 fry
	Mysis	19 g/100 000 fry
	Early PL1–7	25 g/100 000 fry
	PL 8–15	60 g/100 000 fry
	Shrimp grow-out	2 g/kg of feed

There is only one vaccine registered for aquaculture use in the Philippines. This vaccine is used against *Streptococcus* spp. in tilapia (Table 5). Fingerlings are vaccinated prior to stocking in ponds. However, very few hatcheries are using it, as for them it is just an additional cost. They have not yet recognized the need for vaccination and do not fully appreciate its benefits.

The use of the sex control aid 17 alpha methyltestosterone for sex reversal in tilapia fry is common. Sex-reversed tilapia are preferred for their faster growth and shorter culture period. Some farmers also stock genetically improved farmed tilapia (GIFT) fingerlings (Table 6).

Aquaculturists are now inclined towards the application of probiotics, both in hatcheries and in grow-out facilities (Table 7). There are many probiotics available on the market, and through their use farmers benefit by maintaining good environmental culture conditions, animal health and productivity.

Immune enhancers are available in the market and are currently more popular in shrimp culture (Table 8).

The survey noted some differences in the kinds of antibiotic used in aquaculture as compared with the results of the earlier survey conducted by Cruz-Lacierda, de la Peña and Lumanlan-Mayo (1996). Some previously used antibiotics such as nitrofurans and chloramphenicol are now banned for use in food-producing animals, including aquatic animals as per DOH and DA Joint AO No. 2, Series of 2000, and DOH-AO No. 91 and DA-AO No. 60, Series of 1990, respectively. Most of the antibiotics currently being used are regulated so that producers must follow the minimum withdrawal period and the standards for MRLs in products. The farmers are now more aware of consumer food safety concerns, particularly on possible residues in meat if veterinary medicines are not properly used.

Compliance with food safety requirements started in 2004 when BFAR implemented the residue control programme. The initial years of implementation have traced non-compliance in feeds where antibiotics were incorporated to prevent disease and help promote growth. Since then, control over the aquatic feeds production has improved such that this is no longer a practice.

The residue control programme has been continuously disseminated to the stakeholders. It includes a farm registration scheme through which farms undergo inspection of their production hygiene, monitoring of their use of antimicrobial agents and sampling for laboratory analysis. However, laboratory capability to support the programme is a challenge, since additional substances need to be monitored.

Efficacy and Impacts

Antimicrobial agents are more commonly used in hatchery operations for shrimp, tilapia and milkfish/marine fish, where they are applied to prevent common problems such as stress-related bacterial and fungal infections as well as parasitic infestations. The

use of antimicrobial agents results in higher survival rates due to increased resistance against diseases brought about by the stressful conditions that occur during transport and stocking. The respondents have identified some positive impacts resulting from their application of antimicrobials, including antibiotics, antiparasitics, antifungals and disinfectants (Table 9).

TABLE 9
Positive impacts of antimicrobial usage

Positive impact	No. of Respondents
General increase survival	60
Reduction in mortality in disease events	59
Better quality products	34
Reduction on the use of other treatments	32
Fish/shrimp welfare	32

Treatments do not always provide good results. Some of the farmers observed that antimicrobial agents, particularly antibiotics, are no longer effective when continuously used. In grow-out operation, application of antimicrobial agents is more expensive and choosing the route of administration is difficult. Medication through feed is no longer applicable in diseased animals, whereas bath treatment at this stage is not practical.

The respondents have recognized some negative impacts of using antimicrobial agents (antibiotics, antiparasitics and antifungals) (Table 10) and disinfectants (Table 11).

TABLE 10
Negative impacts of antimicrobial usage

Negative impact	No. of respondents
Residues of food safety concern	28
Build up of clinical resistance in fish/shrimp	24
Toxicity to environment	23
Toxicity to farmers	18
Build up of laboratory bacterial isolates	15

TABLE 11
Negative impacts of disinfectants

Negative impacts	No. of respondents
Toxicity to environment	21
Toxicity to farmers	16
Toxicity to shrimp	15
Residues in food concern	12
Build up of clinical resistance to shrimp	5

These negative effects are known but in many instances ignored by farmers and caretakers. Big farms usually employ safety measures in using antimicrobials such as using protective gear during handling and application, storage and disposal. This is not given much attention by the small farmers who comprise the majority of the aquaculture sector.

In shrimp grow-out, the use of antibiotics is minimal. Farmers have experienced the short-term benefits of using antibiotics and the development of bacterial resistance. Intensive shrimp farming involves the use of closed culture systems and the application of biosecurity measures. Other farmers have adopted the integration of finfish culture within the shrimp culture system or the use of greenwater technology for maintaining optimal water quality and controlling the occurrence of diseases such as vibriosis (Paclibare *et al.*, 2001). Crop rotation improves the pond sediment's bacterial load and reduces the incidence of disease. The adoption of these approaches by some farmers has most likely lessened the need for antimicrobial agents.

The Philippine Government has been promoting good management practices such as the *Code of Practice for Sustainable Shrimp Farming* (DA-BFAR, 2008), which was adapted from a document developed by the Global Aquaculture Alliance based on the FAO's *Code of Conduct for Responsible Fisheries* (FAO, 1995). All sectors of the industry are committed to support its implementation. In shrimp culture, white spot syndrome (WSS) is still the most devastating disease. The manual *Best Farm Practices to Reduce Risk of White Spot Syndrome Virus (WSSV) Infection in Shrimp Culture* was written by Usero, Albaladejo and Albaladejo (2009) based on the experiences of the shrimp farming industry in Negros. This manual provides management strategies and precautionary measures to minimize the occurrence of the disease. Recently, *HACCP in aquafarms. A Practical Handbook* was developed by Regidor and Dabbadie (2010) as been published as the result of a study conducted in different types of aquaculture systems such as tilapia cage culture, semi-intensive shrimp monoculture and extensive shrimp polyculture. This handbook will assist farmers in understanding and implementing a risk-based management approach, identifying hazards, determining the level of risk and managing the risk. The development and improvement of farming technologies has paved the way for reduction of antimicrobial usage.

Treatment Failure

The perceptions of respondents as to the reasons for unsuccessful treatment were also studied. For example, there are cases where treatment initially improved the condition of the stocks but the stocks subsequently experienced a relapse. In some instances, treatment was not effective such that the condition of the sick animals was not improved. According to the farmers surveyed, failure of treatments can be attributed to: (a) absence of accurate diagnosis, (b) pathogen not being the primary cause of disease, (c) lack of information concerning stocks, (d) use of antimicrobial in isolation, (e) inappropriate duration of treatment and (f) lack of approved medication.

Successful treatment depends on the accuracy of diagnosis. There are many factors to consider to be able to come up with a correct diagnosis. The information provided by the farmer is important to determine the root cause of the problem. Information on factors such as water quality, stocking density, feeding, size/age, clinical signs, mortality and other observations are necessary. Farmers have been encouraged to keep records of their monitoring activities so that events can be retraced. This is useful in identifying the causal factors associated with a health problem. The results of laboratory analyses confirm the pathogen involved in the problem.

The regional fish health laboratories, which have different levels of capability based on the needs of the industry in their areas of jurisdiction, can provide the services that the industry needs (Regidor, Albaladejo and Somga, 2004). Fish health officers continuously receive training to improve their expertise and capabilities and have already developed a network.

Very few veterinary medicines are registered for use in aquaculture; thus, farmers have opted to use veterinary drugs registered for livestock and poultry. Although available in the market, they are sometimes difficult to purchase because some veterinary medicines require a prescription or veterinary drug order issued by a veterinarian. However, very few veterinarians are working in aquatic animal health. In most cases, treatments are done by fish farmers based on their practical experiences and on the recommendations made by sales agents. Government field workers usually recommend good aquaculture practices and responsible use of veterinary drugs.

CONCLUSIONS AND RECOMMENDATIONS

The results of the survey led to the following conclusions and recommendations:

- The respondents provided recommendations to address their issues and concerns. There is a need to improve the effectiveness and responsible use of veterinary medicines and other products. Training is needed for farmers, veterinary and paraveterinary practitioners in aquaculture, and fish health officers on accurate diagnosis, the prudent use of antimicrobials and the negative impacts of the use of veterinary medicines and other products. The farmers need to have a support laboratory with diagnostic capability and competent laboratory technicians that can provide the services they need.
- National regulations on the use of veterinary medicines are already established and have long been implemented for terrestrial animals, unlike in aquaculture, where their application is relatively new. In previous years, the BAI has included registration of veterinary medicines for aquaculture in their system. However, with the recent developments, the FDA will delegate this task to BFAR. Therefore, there is a need for capacity building so that BFAR will be able to efficiently assist in the registering and monitoring of these veterinary drugs.
- There is a need for fish health management programmes to be continuously strengthened, particularly with regard to the capability to diagnose important and emerging diseases.
- The existing information and education campaign should be continued through seminars and fora on the responsible use of veterinary medicines. Suppliers also have a responsibility to educate farmers on the prudent use of veterinary drugs.
- The Philippine Government should continue to promote good aquaculture practices, the Code of Practice for Sustainable Shrimp Farming, and other sound management techniques to promote the prudent and responsible use of veterinary medicines.
- Given the limited information that is available on the use veterinary medicines in aquaculture, research should be done on their safety, efficacy, withdrawal periods, MRLs, fate and effects on non-target species and the build-up of resistance.

ACKNOWLEDGEMENTS

We thank Ms Carol Lopez, Ms Carmencita Agustin and Mr Gonzalo Coloma, Regional Fish Health Officers; Mr Chris Mitchum Ganacial and Mr Neil Raphael Jamon for their help during the conduct of the survey; and the respondents who provided the information. Our gratitude goes to Dr Melba B. Reantaso for the opportunity to participate in this project. Also, the support of the BFAR management is highly appreciated.

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Use of veterinary medicines in Thai aquaculture: current status

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ABSTRACT

The shift in Thai aquaculture towards the use of super-intensive culture systems during the last decade has led to several serious problems, particularly the misuse of veterinary medicines and chemicals used for the treatment of disease outbreaks, consequently raising concern with regard to the food safety of aquaculture products. As one of the world's top aquaculture producers, Thailand recognizes this global concern and makes efforts to ensure the quality and safety of Thai aquaculture products. This paper reviews the current status of the use of veterinary medicines, antimicrobial agents, chemicals and aquatic animal feeds in Thai aquaculture, including the regulatory framework and legislation that the Department of Fisheries employs to control the quality of our aquaculture products.

INTRODUCTION

Thailand is one of the world's major aquaculture producers and exporters, ranking fourth globally in 2006 in terms of production, with a volume of 1.3 million tonnes (FAO, 2009). The rapid growth of the aquaculture sector and the production of fish and shellfish during the last decade is due to a shift from semi-intensive to intensive and super-intensive culture systems. This change in culture systems has occasionally led to improper farm management, especially in terms of the misuse of veterinary medicines and chemicals used for the treatment of disease, raising serious concerns with regard to the food safety of aquaculture products. In order to ensure the quality and safety of Thai aquaculture products, the Department of Fisheries (DOF) continuously controls the use of veterinary medicines and chemicals throughout the production chain. Basic legislation, such as the Fisheries Act B.E. 2490 (1947) and other related laws and

regulations, are employed by the DOF as a regulatory framework to control aquaculture production. DOF also has policies and programmes for aquaculture farm management to reduce or avoid the use of drugs and chemicals in the production of aquatic food. This paper reviews the current status of the use of veterinary medicines in Thai aquaculture and the framework for their management, including legislation dealing with the use of antimicrobial agents and other chemicals used in the rearing of cultured aquatic animals and the manufacture of aquatic animal feeds.

USE OF ANTIMICROBIAL AGENTS IN THAI AQUACULTURE

Regulations and the role of the Department of Fisheries

The Food and Drug Administration (FDA), Ministry of Public Health, is assigned the responsibility to regulate the use of antimicrobial drugs in Thailand under the Drug Act, B.E. 2510 (1967) and amendment B.E. 2518 (1978). This act lays down the requirements for the manufacturing, importing and marketing of veterinary medical products used in food-producing animals and requires that any person who wishes to commence such activities shall be authorized by the authorities (Section 12). All veterinary medicines are required to be registered and authorized before manufacturing, importation or placing on the market, including the use of proper labelling and storage, as specified in Sections 25 and 26. All drugs must be registered through the FDA, Thailand. Wholesalers/distributors and pharmacies must obtain a licence from the FDA for the sale of veterinary medicines. However, the Ministerial Notification of the Ministry of Public Health No. 33 B.E. 2545 (2002) has appointed DOF officers to execute Section 91 of the Drug Act B.E. 2518 (1978) and empowers officers to enter places of manufacturing, marketing and storing of veterinary medicines for inspection with regard to compliance.

In summary, DOF's role in regulating the use of veterinary medicines in aquaculture is as follows:

- Experts from the DOF, along with others from the FDA, the Department of Livestock, the universities and the Veterinary Council of Thailand, are appointed as members of the committee that oversees approval of aquatic animal drugs for registration.
- DOF staff are authorized to control the distribution of veterinary medicines and are able to inspect drugs available in the market.
- Although veterinary medicines can only be prescribed by a licensed veterinarian, either veterinarians or DOF officers can provide assistance to aquaculturists during disease outbreaks and on the appropriate use of drugs.
- Drug residues in live aquatic animals and aquaculture products must be regularly examined under surveillance and monitoring programmes that are operated by (i) the Inland Fishery Research and Development Bureau; (ii) the Coastal Fishery Research and Development Bureau; and (iii) the Fishery Inspection and Quality Control Division.

Approved antimicrobials and treatments

Currently, few antimicrobial agents are approved for use in aquaculture. Registered drugs such as oxytetracycline, tetracycline, sulfadimethoxine, trimethoprim, sulfadimethoxine and ormetoprim are mainly used in Thai aquaculture, particularly for bacterial treatments (Table 1). For viral outbreaks, which occur mainly in shrimp culture, prevention and management are the preferred methods for disease control in this country. Only approved antimicrobial agents are legally used for therapeutic treatment in aquaculture. The only extra-label drug approved by the FDA, Thailand, for use in aquaculture is amoxycillin.

TABLE 1
Antimicrobial agents approved for treatment of bacterial diseases

Diseases	Pathogens	Antimicrobials
Columnaris	Flavobacterium columarae Flavobacterium spp.	Enrofloxacin, oxytetracycline
Edwardsiellosis	Edwardsiella tarda, E. ictaluri	Oxytetracycline, sulfadimethoxine/ormetoprim
Motile Aeromonas septicaemia	Aeromonas hydrophila, A. sorbia, A. caviae	Enrofloxacin, oxytetracycline, sulfadimethoxine/ ormetoprim
Streptococcosis	Streptococcus agalactiae, S. iniae	Amoxycillin, oxytetracycline
Vibriosis	Vibrio spp.	Enrofloxacin, oxytetracycline, Sulfadimethoxine/ ormetoprim

Prohibited antimicrobial agents

Several antimicrobial drugs had been widely used because of their excellent antibacterial properties; however, long-term studies with experimental animals have shown detrimental characteristics such as carcinogenic and mutagenic properties. Therefore, many drugs have been banned from use in food animal production. Since 2002, the Ministry of Agriculture and Cooperatives and the DOF have decreed that the use of the following drugs in aquaculture is prohibited:

- Nitrofurans:
  - nitrofurazone
  - furazolidone
  - furatadone
  - nitrofuratoin
  - nitrovin
  - nifurpirinol
  - nifuraldezone
  - nifurparzine
- Nitroimidazoles:
  - ronidazole
  - dimetridazole
  - ipronidazole
  - metronidazole
- Glycopeptides:
  - vancomycin
  - avoparcin
- Beta-agonists:
  - salbutamol
  - clenbuterol
  - cimaterol
  - mabuterol
- Carbadox
- Olaquindox
- Dapsone
- Chlorpromazine
- Chloroform
- Colchicine
- Diethylstilbestrol
- Aristolochia spp.
- Chloramphenicol and derivatives

Therapeutic failure of antimicrobial agents in aquaculture

Although antimicrobial agents used in aquatic animal farms must be prescribed by a veterinarian, therapeutic failure sometimes occurs owing to the limited number of antimicrobials available, incorrect diagnosis, antimicrobial resistance (or reduced

susceptibility) and incorrect drug administration. This circumstance can lead to the serious problem of antimicrobial resistance; thus, encouraging the appropriate use of antimicrobial agents is essential.

Surveillance and monitoring programmes

It is the DOF policy that aquatic animal products from Thailand must be examined for drug residues under a monitoring programme. A number of drugs and chemicals are investigated using enzyme-linked immunosorbent assay (ELISA)/high-performance liquid chromatography/ liquid chromatography tandem mass spectrometry (LCMSMS), as shown below:

- Oxytetracycline
- Oxolinic acid
- Nitrofurans and metabolites:
  - furazolidone and 3-amino-2-oxazolidinone
  - furatadone and 3-amino-5-morpholinomethyl-2-oxazolidinone
  - nitrofurazone and semicarbazide
  - nitrofuratoin and 1-aminohydantoin
- Chloramphenicol
- Malachite green, leucomalachite green

USE OF CHEMICALS IN THAI AQUACULTURE

Regulations and the role of the Department of Fisheries

The Ministry of Industry empowers the Ministry of Public Health and the Ministry of Agriculture and Cooperatives to enforce the Hazardous Substance Act, B.E. 2535 (1992). This act provides for the control of chemical substances, including detergents and sanitizers, for use in food production. Requirements set out by the act include those related to the importation, manufacturing, marketing, use and discard of substances. Instruction on usage is required to appear on the label. The DOF through the Fishery Environmental Group is appointed to control the use of chemicals in aquaculture in accordance with this act. The chemicals used in aquaculture are classified according to the need for control into four types – I, II, III and IV –(Uttarapong, 2007) that are under the jurisdiction of DOF (Table 2). Importers, exporters, producers and possessors of the four types of chemicals must follow the requirements of the act.

To deal with Type II and III chemicals, registration must be approved by the members of the DOF Hazardous Substance Committee. Anyone dealing with Type II and III chemicals is also required to inform and notify the DOF. A licence for Type III only chemicals must be obtained from the DOF. Type IV chemicals for any purpose are prohibited.

USE OF AQUATIC ANIMAL FEEDS IN THAI AQUACULTURE

Regulations and role of the Department of Fisheries

Aquatic animal feeds are controlled by the DOF's Feed Research and Development Institute following the Animal Feed Quality Control Act B.E. 2525 (1982) and amendment B.E. 2542 (1999). The most important aspect of feed regulation is to ensure the safety of feed used for food-producing animal culture. Aquatic animal feeds, whether produced domestically or imported, must be registered and approved by the members of the DOF's Aquatic Animal Feed Committee. According to Drug Act B.E. 2510 (1967), Section 27 bis, non-registered drugs or medicinal products shall not be allowed for import into Thailand. Illegal importation or the distribution and sale of non-registered drugs will result in the persons involved being subjected to a fine and/or imprisonment. This includes the manufacturing of medicated aquafeed containing any feed additives with antibiotic and coccidiostatic action. Currently, medicated feed is not allowed to be

used in Thai aquaculture. In addition, the use of chemicals not yet proven safe for human health is not permitted.

TABLE 2
Classification of chemicals used in Thai aquaculture

Type I	Type II	Type III	Type IV
• Calcium hypochlorite • Sodium hydroxide ≤ 20% W/W[1] • Sodium hypochlorite	• Formaldehyde • Micro-organisms[2] • Products containing active ingredients intended for use in preventing, destroying or controlling micro-organisms, parasites or unwanted plants and animals	• Acetic acid • Benzalkonium chloride • Fentin acetate • Hydrochloric acid ≤ 15% W/W • Rotenone • Trichlorfon • Chlorine • Trifluralin • Chlorine and chlorine-releasing substances • Glutaraldehyde • Peracetic acid • Malachite green hydrochloride[3] • Malachite green oxalate[3]	• Trichloroisocyanuric acid and its salts • Malachite green hydrochloride • Malachite green oxalate

[1]Weight per weight
[2]Regulation of use of micro-organisms in Thai aquaculture is included under the Hazardous Substance Act.
[3]These two chemicals are authorized only for use in ornamental fish culture. Regulations and the role of the Department of Fisheries.

Apart from registration, DOF also conducts quality control for aquatic animal feeds via feed mill inspection. DOF certifies the voluntary sanitary programmes used by aquatic feed producers, such as good management practices or the Hazard Analysis Critical Control Point (HACCP) system. The DOF also undertakes routine inspection of aquafeeds by sampling of feed from feed mills, exporters, importers and farms to analyze for biochemical composition (protein, lipid, fiber and moisture), pathogens (Salmonella sp.), alpha toxin, melamine and drug contamination.

Drug residue monitoring programme

Aquafeeds collected from several sources as mentioned above are examined for contamination for the following drugs:

- Tetracycline:
  - oxytetracycline
  - chlortetracycline
- Nitrofurans and metabolites:
  - furazolidone
  - furatadone
  - nitrofurazone
  - nitrofuratoin
- Chloramphenicol

Two types of tests are used for drug examination: screening and confirmation test. The screening test used for tetracycline and nitrofurans is a colour test, while chloramphenicol is investigated through the use of ELISA. LCMSMS is used as a confirmation test for all listed drugs.

CONTROL OF THE USE OF VETERINARY MEDICINES AND CHEMICALS IN AQUACULTURE FARMS BY THE DOF

Registration, farm inspection and training

Following Fisheries Act B.E. 2490 (1947), all aquaculture farms are required to be registered with DOF. Upon approval, DOF staff have to inspect the farms using a checklist that was developed from the Codex Alimentarius Commission's guidelines. Inspectors from DOF must ensure that guidelines for site selection, proper farm design, operational standards, disease control, records of drugs/chemicals/feeds used in farm, water quality and water treatment are followed. We encourage farmers to carry out good farm management actions to avoid the use of drugs and chemicals, such as the use of good aquaculture practices (GAqPs), the Code of Conduct for Responsible Aquaculture Farming (CoC), and standards for organic farms and biosecurity systems.[1] GAqPs are currently applied for the farming of several fish and shrimp species, while the CoC has been initially implemented in marine shrimp culture. The systems for organic farming and biosecurity have already been introduced to several marine shrimp farms in Thailand. In addition, DOF constantly offers training programmes on the appropriate use of drugs and chemicals in aquaculture.

Environmental monitoring

DOF carries out the environmental monitoring programme through water quality examination. The water from natural resources close to aquaculture farms is routinely collected and analyzed for heavy metals, pesticides and other basic parameters.

Antimicrobial agents, chemicals and aquafeed control

At the farm level, we also inspect for drug and chemical contamination in aquatic foods and aquafeeds used in registered farms. Samples of fish and shrimp from registered farms are collected at random for the drug residue monitoring programme as described in DOF (2006). Such samples must be collected by authorized and well-trained persons. At least 10 percent of the registered farms in each area must be sampled. At each sampling, samples are taken from four different locations in the same pond, with up to 500 g/sample collected for grow-out shrimp/fish and 3–10 g/sample for fry. Shrimp are prioritized for sampling in the following manner:

1. shrimp at least 90 days old from grow-out farms;
2. fry at PL (Post-larvae) 12 or older from hatcheries; and
3. weak shrimp or shrimp showing signs of disease.

CONCLUSIONS

Concern about the use of drugs and chemicals in the rearing of aquatic animals destined for human consumption has been raised worldwide. As the government agency responsible for regulating aquaculture production, DOF has made an effort to control the drugs, chemicals and feeds used in aquaculture. DOF also has several policy plans to improve the prudent use of veterinary medicines and chemicals in Thai aquaculture. We intend to improve the knowledge of extension officers and aquaculturists on the appropriate use of antimicrobial agents. In addition, we are trying to increase the number of diagnostic service units. The development of accurate and simple diagnostics tests must be continued. The use of alternative approaches to avoid the use of drugs and chemicals, such as GAqPs, organic farm standards, improved biosecurity measures, the development of disease resistant strains of aquatic animals and the use of vaccines, should be given priority in long-term strategies for aquaculture development. Finally, international standards on the application of antimicrobial agents in aquaculture production should be harmonized to

1 These include the Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003) and the Code of Practice on Good Animal Feeding (CAC/RCP 54-2004).

facilitate trade in aquaculture products.

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Use of veterinary medicines in Vietnamese aquaculture: current status

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ABSTRACT

Aquaculture production and its management by government have changed greatly and so has its use of drugs and chemicals. This study presents a summary of a survey of chemical use conducted in 2003–2004; the results of recent consultative workshops with stakeholders and a desktop review are also presented in this report assessing the development and current status of veterinary medicines usage in aquaculture in Viet Nam. By 2004, at least 1 893 commercial products were available, with 1 262 registered products, including 223 antibiotics. There were 220 domestic companies involved in production and trade, and nine foreign countries had 238 products imported into Viet Nam, with 61.7 percent of these originating from Thailand. Between 2007 and the end of 2009, there were 4 100 products registered, including 2 945 domestic products and 1 155 imported ones. Because of the withdrawal of circulation permission for a number of products, the total number of registered products available in the market is now 2 193, with 813 being veterinary drugs and 2 100 being chemicals for environment improvement. There are 230 domestic companies, 136 of which produce drugs and/or chemicals, and more than 15 importing countries. The study also revealed that there is a need for measures to reduce both the number of companies and the number of products available in the market. The research, monitoring and extension systems and local partnerships among stakeholders, particularly between farmers and retailers, play important roles, and there should be capacity building and incentives for them to cooperate in practicing more prudent use of veterinary medicines.

INTRODUCTION

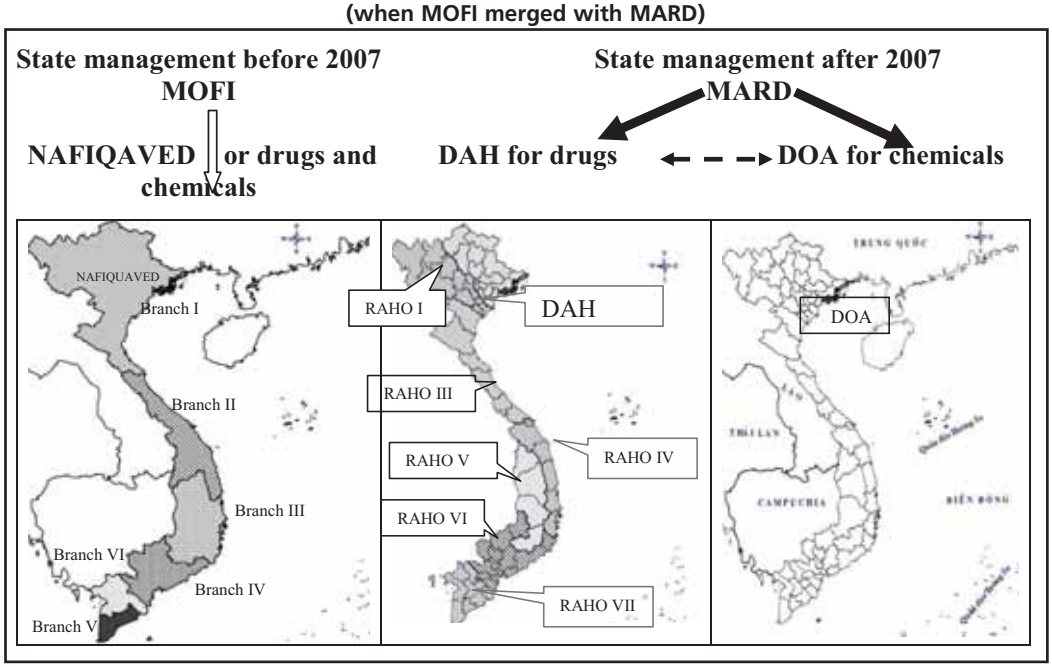
Aquaculture and its use of veterinary medicines and other drugs and chemicals has developed rapidly over the last ten years in Viet Nam. This report presents the status of chemical use and associated management perspectives based on a survey conducted in 2003–2004 and the findings of recent consultative workshops and a desktop review. The survey was a government project implemented with the purpose of enumerating the drugs and chemicals used in aquaculture to improve their practical use by aquaculturists and their management by state agencies. Six recent consultative workshops were recently

held with the participation of aquaculturists, supply companies, agents and retailers, and government officers in two representative areas in the north and south. The workshops presented the current applications, delivery systems and state management at the local levels for veterinary medicines and other drugs and chemicals used in aquaculture. Desktop reviews examined the government management systems, mainly within the Ministry of Fisheries (MOFI) before 2007 and within the Ministry of Agriculture and Rural Development (MARD) after 2007, in terms of their mandates and those of the various subordinate regulatory bodies within the ministries and at the provincial levels.

STATE MANAGEMENT OF DRUG AND CHEMICAL USE IN AQUACULTURE

Figure 1 shows the critical institutional change in the management of drugs and chemicals used for aquaculture that occurred when MOFI merged with MARD in 2007. The management mandates for both drugs and chemicals were vested in the National Fisheries Quality Assurance and Veterinary Directorate (NAFIQAVED), with six regional branches, and the provincial departments before 2007. NAFIQAVED is now responsible only for assuring the quality of post-harvest products. The mandate for drug management was then taken up by the Department of Animal Health (DAH), with seven Regional Animal Health Offices (RAHOs) and provincial DAHs in 63 provinces. Similarly, the mandate for chemical management was shifted to the Department of Aquaculture (DOA) and its provincial subordinates.

FIGURE 1
State management for drug and chemical use in aquaculture in Viet Nam before and after 2007 (when MOFI merged with MARD)



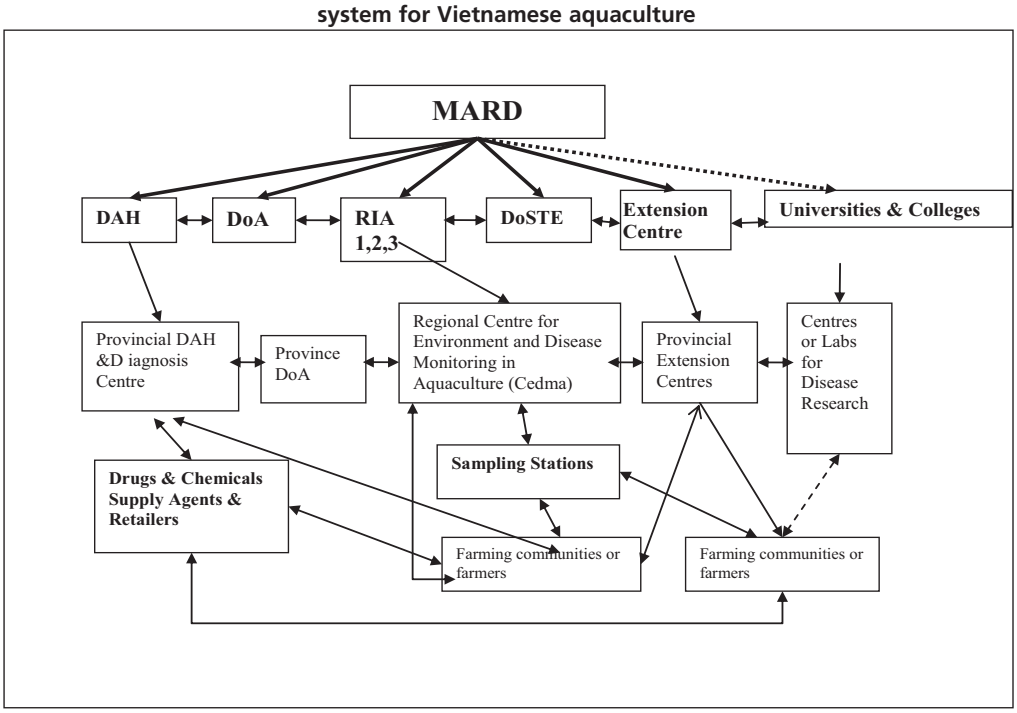
Note: DAH = Department of Animal Health; DOA = Department of Aquaculture; MOFI = Ministry of Fisheries; MARD = Ministry of Agriculture and Rural Development; NAFIQAVED = National Fisheries Quality Assurance and Veterinary Directorate; RAHO = Regional Animal Health Office.

There is no clear delineation of mandates between the DAH and the DOA with regard to the management of drug and chemical production, their distribution, and the testing of new products and their practical use in aquaculture at both the ministerial (MARD) and provincial (Department of Agriculture and Rural Development) levels. In general, DAH is responsible for drugs used for disease treatments, while DOA is responsible for chemicals used in environmental treatments. DoH and DOA under MARD are responsible for management of drug and chemical producers, while provincial DAHs and DOAs are responsible for regularly or randomly investigating supplying companies and agents in their province. However, small village or district drug retailers are not compulsorily

checked. DAH can also authorize their regional offices to implement their investigations.

Figure 2 presents the monitoring, research and extension system with the main stakeholders associated with the aquatic environment and disease, as well as the drugs and chemicals used in aquaculture.

FIGURE 2
Schematic representation of the environment and disease research, monitoring and extension system for Vietnamese aquaculture



Note: DAH = Department of Animal Health; DOA = Department of Aquaculture; DoSTE = Department of Science, Technology and Environment; MARD = Ministry of Agriculture and Rural Development; NAFIQAVED = National Fisheries Quality Assurance and Veterinary Directorate; RIA = Research Institute for Aquaculture.

The first level of the system includes five institutions under MARD, namely the DAH; the DOA; the Research Institutes for Aquaculture (RIAs) No. 1, 2 and 3; the Department of Science, Technology and Environment (DoSTE) and the Extension Centres; and some universities and colleges that provide aquaculture training involving the aquatic environment and disease monitoring and prevention. There is no clear cut separation of functions and practices among the above institutions. However, the DAH has more responsibility for disease control; the DOA has more responsibility for aquaculture management in general; RIA1, 2 and 3 and the regional Centres for Environment and Disease Monitoring in Aquaculture (CEDMAs) are mainly oriented towards research and monitoring on the environment and disease; and the Extension Centre is mainly responsible for training and technology transfer.

The second level consists of provincial-level agencies that are subordinates of the above-mentioned institutions and whose main responsibilities are the practical state management and technical operation of the system. The monitoring system is vested in DoSTE, but it is operated by three regional CEDMAs under the three RIAs. The centres have sampling stations in the main farming areas, where they work in partnership with the farming communities and farmers. The provincial DAHs, the Diagnosis Centres and the DOA are involved in disease control, the practical use of drugs and chemicals by farmers and in carrying out investigations on supplying agents. The provincial DOAs and DAHs are responsible for harvest product quality before processing occurs. Farm records and aquatic product samples in concentrated farming areas are checked for residues and traceability in determined farms or by random investigations.

The third level, which is also the basis of the system, includes the suppliers and

retailers of drugs and chemicals, the farming communities and the farmers. Farmers often buy drugs and chemicals from retailing village or district agents and shops. Retailers often also sell other kinds of products for aquaculture use, such as feeds. In many cases, agents and retail shops also sell drugs for use in the farming of terrestrial animals that, because of poor understanding, are sometimes purchased by aquafarmers for use on their fish. Besides providing products, retailers often supply fish farmers with information on government regulations, technical guidance and advice on treatment. Small retailers and farmers note that many products lack Vietnamese language labels, and that the price of products is increasing, preventing them from proper use in terms of selecting the right product, treatment time and dose.

Depending on the available institutions and the local relationship between the farmers and these institutions, farmers may consult the local DOA, DAH, Agriculture Extension Centres or sampling stations, as well as the drug-supplying companies or agents for solutions to the disease outbreaks and any environmental problems occurring in their ponds. These institutions, companies and agents often provide training courses for famers and small retailers in aquaculture management, including drug and chemical use.

To date, there have been few studies on the environmental impacts of the drugs and chemicals used in aquaculture, particularly on the occurrence of antimicrobial resistance in pathogens and the presence of residues in the environment and wild animals. Therefore, research and the monitoring and prevention of disease related to antimicrobial use should be given a high priority in building capacity for aquatic biosecurity in the key institutions.

Small-scale or household aquaculture accounts for approximately 70 percent of aquatic production in Viet Nam. Both large-scale and small-scale farmers have become more prudent in using drugs and chemicals. They mainly use registered products, and use less antibiotics and more probiotics, premix, vitamins, minerals and locally mixed herbs for health improvement, and environmentally friendly chemicals for enhancing water-quality management. However, the availability of too many products can confuse farmers. There is a trend towards both formal and informal cooperation among farmers, drug and chemical production and supply companies, retailers and processing plants in certain areas for the purpose of traceability and applying standards based on good aquaculture practices, better management practices, etc. (Mai, 2009).

DRUG AND CHEMICAL USE IN AQUACULTURE

Before 2004

According to Mai (2004), by 2004 there were 14 chemicals for water and bottom treatment, 6 fertilizers, 86 disinfectants, 138 antibiotics, 47 probiotics, 13 vitamins, 57 additives, 10 hormones and 5 unidentified substances used in Vietnamese aquaculture. Shrimp farming used 186 different products, including 32 antibiotics; production of shrimp larvae used 98 products, including 39 antibiotics; marine finfish culture used 29 (14 antibiotics); freshwater cage culture used 74 (41 antibiotics); pond culture used 67 (31 antibiotics); and freshwater seed production recorded 85 (37 antibiotics). There were at least 1 893 commercial products used in Vietnamese aquaculture, of which only 1 262 were registered. There were 223 antibiotics (Table 1) and 99 drugs containing a combination of more than three antibiotics, with 70 percent of them containing fluoroquinolone. In 2002, a number of chemicals were banned for use in aquaculture by MOFI, including chloramphenicol, chloroform, nitrofuran, furazonidone and metronidazone, which were known to be used prior to 2004.

TABLE 1
Number of registered veterinary products (drugs and chemicals) used in aquaculture before 2002 and in 2003

	Before 2002	2003	Total
Antibiotics	223	83	306
Probiotics	82	37	119
Chemicals	105	55	160
Mix of vitamins and minerals	206	166	372
Minerals	140	146	286
Fertilizers	1	18	19
Total	757	505	1 262

Source: NAFIQAVED, 2004 (internally circulated document).

During the period from 2007 to 2009

The current trend in the use of veterinary medicines, including drugs, chemicals and probiotics, is shown in Table 2. In the government documents providing the data summarized in this table, registered products used in aquaculture are grouped into two general categories: drugs and environment treatment chemicals. Drugs are used for the purpose of disease prevention, treatment and health improvement, while chemicals for environmental treatment are used for improving water and bottom quality. In the period between 2007 and 2009, 4 100 products were registered, with 2 945 products originating from domestic producers and 1 155 products being imported. Veterinary drugs comprised a total of 1 091 products, while there were 3 009 products sold for environment treatment. However, in 2007, there were 278 drugs, and 909 chemicals had only been available for the previous six months. As a result, the total number of registered products available in the market is now 2 193, with 813 veterinary drugs and 2 100 chemicals for environment improvement.

TABLE 2
Registered of drug and chemical products used in aquaculture from 2007–2009

	2007[1]				2008[2]		2009[3]	
	Drugs		Environment treatment chemicals		Drugs	Environment treatment chemicals	Environment treatment chemicals	
	No time limited circulation	6-month limited circulation	No time limited circulation	6-month limited circulation				Total
Domestic products	385	278	440	909	180	560	193	2 945
Imported products	63	151	121	644	34	119	23	1 155
	448	429	561	1 553	214	679	216	4 100

Source : [1]MOFI (2007); [2]MARD (2008); [3]MARD (2009a, 2009b).

PRODUCTION AND SUPPLY OF DRUGS AND CHEMICALS

By 2004, there were 220 domestic companies involved in producing and trading drugs and chemicals used in aquaculture. There were nine countries that had products imported into Viet Nam, with 238 products (61.7 percent) originating from Thailand (Mai, 2004).

A number of products had labels that were not in line with government standards, such as lacking a list of ingredients or only being written in foreign languages, particularly products imported by small-scale border trading. The lack of technical understanding of supplying agents and farmers on the proper use of drugs and chemicals and of awareness of their impacts on the environment was considered a constraint. Almost all agents lacked aquatic animal veterinary or aquaculture certificates.

Currently, there are approximately 230 domestic companies producing products used in aquaculture, of which 136 are producing drugs and/or chemicals. There are more than 15 countries whose products are imported into Viet Nam, the leader being Thailand, with

products also imported from India, China, Indonesia, Taiwan Province of China, United States of America, France and Germany. Antibiotics are mainly produced by domestic companies, while imported products are mainly raw materials used for reprocessing and probiotics. Two companies are currently cooperating on research to develop a vaccine for white liver disease in tra catfish (*Pangasius hypophthalmus*).

DRUGS AND CHEMICALS BANNED FOR USE IN AQUACULTURE

Two lists of banned chemicals and drugs have been issued, one by MOFI in 2005 banning 17 substances and another by MARD in 2009 listing 18 substances (Table 3).

Two lists of drugs and antibiotics with limited use and their associated maximum residue levels (MRLs) have also have been issued with 34 and 33 veterinary medicines listed in 2005 and 2009, respectively (Table 4).

TABLE 3
Lists of drugs and antibiotics banned for use in aquaculture in 2005 and 2009

Name of drug or antibiotic	Name of drug or antibiotic
2005[1]	**2009[2]**
Aristolochia spp. and its products	*Aristolochia* spp. and its products
Chloramphenicol	Chloramphenicol
Chloroform	Chloroform
Chlorpromazine	Chlorpromazine
Colchicine	Colchicine
Dapsone	Dapsone
Dimetridazole	Dimetridazole
Metronidazole	Metronidazole
Nitrofuran	Nitrofuran
Ronidazole	Ronidazole
Malachite green	Malachite green
Ipronidazole	Ipronidazole
Nitroimidazole	Nitroimidazole
Clenbuterol	Clenbuterol
Diethlstilbestrol (DES)	Diethylstilbestrol (DES)
Glycopeptides	Glycopeptides
Trichlorfon (Dipterex)	Gentian violet (Crystal violet)
	Fluoroquinolones (no use for products exported to North America)

Source: [1]MOFI (2005); [2]MARD (2009c).

CONCLUSIONS

There are overlaps within the government management system for the production and use of veterinary medicines in aquaculture. There are a large number of producers with too many commercially registered products available in the market. As the producers and their registered products are regulated by different institutions, there are difficulties in the practical use of veterinary medicines by farmers, their delivery by retailers and their management by government. Measures should be taken to reduce both the number of producers and the number of products.

The pattern of usage of veterinary medicines in Viet Nam has experienced a great change since 2003, with a trend towards a shift away from approaches emphasizing disease treatment and environmental disinfection towards those favouring the improvement of aquatic animal health and the aquaculture environment. Farmers are using less antibiotics and more environmentally friendly products. This trend should be promoted by giving incentives, such as higher prices or certification for products originating from environmentally friendly farming systems as compensation for possible lower production.

The research, monitoring and extension systems play important roles in the practical use of veterinary medicines. There are localized partnerships among the provincial

departments of the DAH, the DOA, the Extension Centre, CEDMA, processing plants and agents/retailers with farming communities and aquaculturists. A large number of small-scale aquaculture producers and their local drug and chemical retailers have critical interlinked roles to play in the practical and prudent use of antimicrobial agents and other substances. There is increasing cooperation among farmers, retailers/agents and local veterinary staff of involved institutions and processing plants. These partnerships and the capacity of research, monitoring and extension systems, as well as the knowledge of farmers and retailers, need to be strengthened.

TABLE 4
Lists of antibiotics and other drugs with limited use and their maximum residue limit (MRL)

20051		**20092**	
Name of drug or antibiotic	**MRL (ppb)**	**Name of drug or antibiotic**	**MRL (ppb)**
Amoxicillin	50	Amoxicillin	50
Ampicillin	50	Ampicillin	50
Benzylpenicillin	50	Benzylpenicillin	50
Cloxacillin	300	Cloxacillin	300
Dicloxacillin	300	Dicloxacillin	300
Oxacillin	300	Oxacillin	300
Danofloxacin	100	Oxolinic acid	100
Difloxacin	300	Colistin	150
Enrofloxacin	100	Cypermethrim	50
Ciprofloxacin	100	Deltamethrin	10
Oxolinic acid	100	Diflubenzuron	1 000
Sarafloxacin	30	Teflubenzuron	500
Flumequine	600	Emamectin	100
Colistin	150	Erythromycin	200
Cypermethrim	50	Tilmicosin	50
Deltamethrin	10	Tylosin	100
Diflubenzuron	1 000	Florfenicol	1 000
Teflubenzuron	500	Lincomycine	100
Emamectin	100	Neomycine	500
Erythromycin	200	Paromomycin	500
Tilmicosin	50	Spectinomycin	300
Tylosin	100	Chlortetracycline	100
Florfenicol	1 000	Oxytetracycline	100
Lincomycine	100	Tetracycline	100
Neomycine	500	Sulfonamide	100
Paromomycin	500	Trimethoprim	50
Spectinomycin	300	Ormetoprim	50
Chlortetracycline	100	Tricainemethanesulfonate	15–330
Oxytetracycline	100	Danofloxacin	100
Tetracycline	100	Difloxacin	300
Sulfonamide	100	Enrofloxacin + Ciprofloxacin	100
Trimethoprim	50	Sarafloxacin	30
Ormetoprim	50	Flumequine	600
Tricaine methanesulfonate	15–330		

Note: ppb = parts per billion.
Source: 1MOFI (2005); 2MARD (2009c).

ACKNOWLEGEMENTS

I would like to express my gratitude to all those who gave me the possibility to complete this report. I want to thank the Research Institute for Aquaculture (RIA1) and the Centre for Environment and Disease Monitoring in Aquaculture (CEDMA), where I am working for their advantages in providing insights into the system. I also thank the Department of Animal Health (DAH) and the Department of Aquaculture (DOA) for providing data and information on state management at the central level. I am grateful to Dr Melba Reantaso and Ms Elena Irde from the Food and Agriculture Organization of

the United Nations (FAO) for their support to participate in the expert workshop held in Bangkok, Thailand, in December 2009. I am deeply indebted to the officers, company staff, farmers, agents and retailers in Ho Chi Minh City, Hai Phong City and Dong Thap Province for providing information on practical use and local practices.

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Antimicrobial resistance: complexities and difficulties of determination

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Smith, P. 2012. Antimicrobial resistance: complexities and difficulties of determination. *In* M.G. Bondad-Reantaso, J.R. Arthur & R.P. Subasinghe, eds. *Improving biosecurity through prudent and responsible use of veterinary medicines in aquatic food production*, pp. 99—118. FAO Fisheries and Aquaculture Technical Paper No. 547. Rome, FAO. 207 pp.

ABSTRACT

The prudent use of antimicrobials in aquaculture requires that these agents be used only when the evidence suggests that they will benefit the health of the treated animals. Thus, prudence can be achieved only when good quality clinical diagnoses are made and when the decisions to treat are based on sound assessments of the susceptibility of the target bacterium. This paper discusses the issues of clinical diagnosis but concentrates on the protocols and interpretive criteria that are necessary for the determination of bacterial susceptibility. It suggests that there are compelling reasons why there should be international harmonization of the susceptibility testing protocols being used and argues that this harmonization should center on the current Clinical and Laboratory Standards Institutes guidelines M42-A and M49-A. The issue of interpretive criteria for the data generated by *in vitro* susceptibility testing is more complex, less resolved, and the extent of interlaboratory variation presents possibly insurmountable difficulties for international harmonization. This paper argues that, at least in the short and medium term, laboratory-specific wild-type cut-off values, estimated using normalized resistance interpretation (NRI), would provide the best way forward. The special situation of susceptibility testing in laboratories with small throughputs is discussed. It is suggested that the Single Plate Internal Control (SPIC) protocol deserves serious consideration as a method of resolving the problems faced by these laboratories.

INTRODUCTION

Antimicrobial agents targeted against bacteria are, by definition, chemicals that exert a biological effect at low concentrations. Even if we possessed no direct evidence of any adverse effects resulting from their use, this observation, on its own, would be sufficient to oblige us to use these agents prudently. The potential adverse effects that might accrue from the use of antimicrobial agents in aquaculture can be divided into those that result directly from the presence of these biologically active agents themselves and those resulting indirectly from their ability to exert a selective pressure for the emergence of resistant variants. The direct effects can occur in the environment or as a result of their presence in human food. The indirect, resistance selection, effects can have an impact on bacteria associated with diseases of aquatic animals or on those associated with human

infections.

This paper will address the prudent use of antimicrobial agents in aquaculture. It will also address the closely related issue of the detection and consequences of resistance in the bacteria that are the target of such use. The decision to concentrate almost exclusively on resistance in target bacteria was made for two reasons. The first is that undetected resistance in the target bacteria is one of the most common reasons for imprudent use of these agents. The second is that increases in the frequency of resistance in these bacteria are the only adverse consequence of aquacultural use of antimicrobial agents for which we have definite evidence (Smith, Hiney and Samuelsen, 1994).

The issue of the adverse consequences of antimicrobial agent use in aquaculture has been addressed by a joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO)/World Organisation for Animal Health (OIE) expert consultation (FAO, 2006). With respect to the evidence for adverse effects, their report was generally in agreement with the conclusions made by Smith, Hiney and Samuelsen, (1994). They did, however, note the conclusions drawn by Angulo *et al.* (2000) that molecular studies provided evidence that resistance genes selected in Japanese aquaculture may have been transferred to human *Salmonella* strains. Subsequent analysis of the primary data has indicated that this is very unlikely to be a valid interpretation (Smith, 2008d, 2009). Equally the claim by Angulo (1999, 2000) that antimicrobial agent use in shrimp farms was causally related to the emergence of multiresistance in *Vibrio cholerae* in Ecuador has not been substantiated by a detailed re-examination of the available evidence (Smith, 2007).

Although we have no direct or compelling evidence of an adverse impact on human health consequent on the use of antimicrobial agents in aquaculture, prudence would require us to make some attempt to estimate the size of the risk this use presents. For this reason, a small section at the end of the paper will discuss the methods appropriate for generating the data that such a risk assessment would require.

TOWARDS PRUDENT USE OF ANTIMICROBIAL AGENTS IN AQUACULTURE

Before discussing the details of prudent use of antimicrobial agents, it is worth considering why any aquacultural enterprise should attempt to be prudent. The need for prudence is not just a requirement of an ethical position, nor is it simply driven by the need to comply with the regulatory requirements of the international market. It has been argued that, for any farmer, the primary need for prudence is rooted in his own economic self-interest (Smith, 2010).

The reason why prudent use of antimicrobial agents is in the economic self-interest of farmers relates to the inexorable negative feedback loop that governs the use of these agents (Smith, Hiney and Samuelsen, 1994). The more an agent is used to control bacterial infections, the more it is likely that the target bacterium will become resistant to that agent. Thus, the more frequently antimicrobial agents are used, the less effective they are likely to be. It follows that farmers can only retain these agents in the range of techniques available to them for limiting losses resulting from bacterial infection of their stock if their use of these agents is prudent. These are not just theoretical considerations. The Scottish Atlantic salmon farming industry indulged in a very widespread use of antimicrobial agents to limit losses during the furunculosis epizootics of the late 1980s. As a result of this extensive use of these agents, strains of *Aeromonas salmonicida* were being isolated in 1988–1990 that were resistant to all the available to the industry (Inglis *et al*., 1991). The observation, that the epizootic was eventually brought under control by improvements in husbandry supported, at a later date, by effective vaccines (Hiney and Smith, 2000), argues that the extensive use of antimicrobial agents must be seen as imprudent. Thus, as a result of their imprudent use of antimicrobial agents, this industry very nearly re-entered the "pre-antibiotic" era. It should also be noted that reports of the extensive use of "drugs" in salmon production also lead to an economically significant reduction in the perception of the product quality in the marketplace.

Factors related to prudent use of antimicrobial agents

Prudent use of antimicrobial agents requires that these agents should be used only when they are likely to have a beneficial impact on the host. Thus, before any aquatic animal health practitioner (AAHP) recommends a treatment with an agent, prudence requires that he should ask two questions:

1. Are there reasonable grounds for presuming that inhibiting a bacterium will have any significant impact on losses?
2. Are there grounds for believing that the proposed administration will be capable of inhibiting the bacterium to the extent that mortality or morbidity in the treated stock will decrease?

The degree of bacterial involvement in the disease process

With respect to question 1, we can state that the potential of antimicrobial agent therapy to have a beneficial effect is related to the degree of bacterial involvement in the disease process.

It should be obvious that, in situations where the primary factor involved in the morbidity or mortality of a stock is a viral infection, antimicrobial agent therapy can have no beneficial effect. In these situations, such therapy must be seen as imprudent and a waste of money. On the other hand, in situations where otherwise healthy animals, being reared in good environmental conditions, are infected by a bacterium of high virulence, it is reasonable to assume that the bacterium plays a dominant role in the disease process. In such situations, we can expect that therapy with an appropriate antimicrobial agent will have a significant beneficial effect and its initiation will be prudent.

Unfortunately, in the majority of disease epizootics, a number of factors contribute to the mortality. We can be reasonably confident in stating that in nearly all bacterial infections, the environment of the host will play some role in the precipitation of disease and extent of any consequent morbidity and mortality. Here we can see a spectrum. At one end of this spectrum, we have situations where we can presume that the bacterium plays a major role and initiating antimicrobial agent therapy would be prudent. At the other end, the environment can be presumed to play a major role and the bacterial involvement would be minor and mediated by opportunistic or secondary pathogens. The administration of antimicrobial therapy in situations where adverse environmental factors play the dominant role is frequently ineffective and, therefore, must be considered as imprudent. The work of Coyne *et al.* (2006) represents one of the few published epizootiological investigations of an inappropriate use of antimicrobial therapy.

Even following detailed examination of all factors, it is often difficult to determine quantitatively the relative importance of environmental and bacterial factors in any specific disease epizootic. In the world of commercial aquaculture, it is rare that the time, money or expertise required to perform such detailed epizootiological examinations is available. In practice, therefore, the assessment of this vitally important issue must be left to the judgement of the AAHP involved. It is important to note the significance of these observations. Clinical diagnosis involves significantly more than laboratory examination of samples and the identification of potential pathogens. Over-reliance on laboratory investigations of epizootics, coupled with inadequate or incomplete field observations, results in too great an emphasis being placed of the bacterial (or viral) role in the aetiology of epizootics. It could even be argued that over-reliance on data from off-site laboratory investigations has been a major factor leading to the excessive and imprudent use of antimicrobial agents.

While considering the relevance of environmental factors prior to initiating any antimicrobial therapy, it must always be remembered that these agents are, at best, capable only of retarding or inhibiting any bacterium involved in the disease process. Antimicrobial agents cannot promote health and they cannot play any role in the recovery of the host. It follows that the overall clinical outcome of any therapeutic intervention will be a function of both the activity associated with the therapeutic agent

and the basic health status of the host. In situations where the underlying health of the host was and is compromised by its environment, we cannot expect to obtain highly beneficial outcomes from any therapeutic intervention. Antimicrobial therapy cannot be used as a replacement for good husbandry – it must always be used together with good husbandry.

Degree of bacterial susceptibility

With respect to question 2, there are two interrelated factors that must be considered. The first is the concentration of the agent that a proposed therapy might achieve in a host (pharmacokinetics [PK]) and the second is the susceptibility of the target bacterium (pharmacodynamics [PD]).

The importance of considering PK parameters in any determination of clinical resistance can be illustrated by the work of O'Grady and Smith (1992) and Darwish, Rawles and Griffin (2002). O'Grady and Smith (1992) studied the outcomes of oxolinic acid therapies of an infection of freshwater Atlantic salmon presmolts with an *Aeromonas salmonicida* that had an *in vitro* minimum inhibitory concentration (MIC) of 0.5–0.75 mg/litre. When the agent was administered by bath, the C_{max} was 36 mg/litre and the relative percent protection was 79 percent. When the agent was administered orally in mediated feed, the C_{max} was 3 mg/litre and no protection was observed. Thus, in this case, the target bacterium would have been correctly classified as clinically sensitive if the proposed treatment was a bath administration but as resistant if it was proposed to administer the treatment orally.

Darwish, Rawles and Griffin (2002) studied orally administered oxytetracycline treatments of infections of blue tilapia with a *Streptococcus iniae* that had an *in vitro* MIC of 0.25–0.5 mg/litre. They demonstrated that, when the dose administered was 25 mg/kg body weight, little or no reduction in mortalities was observed. However, very significant reductions in mortalities were observed when the dose was ≥ 75 mg/kg. Again, these data demonstrate that whether the target bacterium should be considered as clinically sensitive, or resistant, requires that some account be taken of the PK parameters of the proposed therapy.

Smith (2008a) has defined resistance in a clinical context:

> *"A bacterium can be considered as (clinically) resistant if, as a result of its reduced susceptibility to an agent, it can continue to contribute to the morbidity and mortality in a population during and after the administration of a course of therapy with that agent to that population."*

Using this definition resistance is a concept that includes both the susceptibility of the bacterium (PD) and the pharmacokinetics of the therapy (PK). Thus, question 2 can be resolved if we can establish if the bacterium that is the target of the proposed is resistant or not.

Data requirements for a prudent administration

The previous section establishes that, in order to ensure that any decision to initiate antimicrobial agent therapy is prudent, any AAHP must possess information on the relative importance of the target bacterium in the disease process and on whether it is clinically sensitive or resistant in the context of the proposed therapy.

It has to be admitted, however, that it is much easier to state these requirements than to meet them. The relative importance of any bacterium can be established only by a process that requires experience of on-farm clinical diagnosis, and the difficulties in determining clinical resistance will be discussed at length below. One factor that makes decisions with respect to these two issues even more difficult is the need for them to be made rapidly.

The need for rapid initiation of antimicrobial therapy

There is extensive anecdotal information that the efficacy of any antimicrobial agent intervention is very significantly reduced if the commencement of the therapy is delayed.

Elsewhere in this volume (see Zarza, 2012), some quantitative data on the consequence of delay has been presented.

As any delay in recommending the start of therapy has major consequences for the health of the animals, AAHPs must frequently make therapeutic treatment decisions before they have access to all the information they would, in an ideal world, require. In such situations, they must make their decisions on the basis of a number of probabilities. In effect they must bet that:

- Given their understanding of the environmental conditions of a farm, it is probable that bacterial infection plays a significant role in the disease process.
- Given their observations of the health of the animals, it is probable that a particular bacterium will be significantly involved.
- Given their knowledge of previous susceptibility determinations, it is probable that the bacterium that has been presumed to be involved will be clinically sensitive to the proposed antimicrobial therapy.

The first two of these bets can only be based on the experience of the AAHP. In making the third, however, access to historical data on the susceptibility of bacteria previously isolated at that farm, or in that locality or region, can be of great assistance.

Considerations of the health of the animals and the economic well-being of the farm will frequently oblige AAHPs to make treatment decisions before they can be sure that their assumptions are correct. However, considerations of the requirement for prudent use require that they must initiate additional studies to confirm that their decisions are the best they can make. At the same time that they initiate therapy, they should initiate laboratory studies aimed at confirming their diagnosis and at confirming the assumptions they have made about the clinical sensitivity/resistance of the isolated bacterium. If the results of laboratory test confirm that the initial assumptions made were correct, the AAHP could continue with the therapy initially recommended. If, however, the laboratory tests do not confirm these assumptions, there is a clear need and obligation to change the therapy recommendations as quickly as possible.

It should be noted that these arguments indicate that AAHPs need two types of data concerning bacterial susceptibility. To make an initial recommendation, there is a requirement for historical data of the susceptibility of bacteria belonging to a particular group that have been previously isolated within a relevant area. To confirm any recommendation, the requirement is for specific assessments of the susceptibility of the particular bacterium isolated from the epizootic being addressed. This need for two data sets is significant. It is entirely possible that the two types of data may be accessed from different laboratories and that different test protocols may be appropriate for their collection.

ASSESSING BACTERIAL RESISTANCE

In aquaculture, antimicrobial agents are used in an attempt to control loses resulting from bacterial infections of the farmed stock. Any attempt to use antimicrobials in this way will fail if the target bacterium is resistant to the agent. Any such attempt must be seen not only as imprudent but also as poor economics (Smith, 2010). Thus, prudent use of these agents requires that farmers know whether the bacterium that is the target of any proposed therapy is sensitive or resistant to the proposed therapy.

If we are to improve the use of antimicrobial agents in aquaculture, we must increase the frequency with which laboratory-generated susceptibility data is used to inform therapeutic decisions. At the same time, we must also improve the quality of information that susceptibility testing laboratories can provide AAHPs.

Susceptibility testing methods

Susceptibility testing laboratories can perform in vitro tests that allow them to generate a quantitative measure of the susceptibility of any bacterium. It should be noted that as opposed to resistance, which requires a consideration of the PK parameters of a

proposed therapy, susceptibility is a measure of an *in vitro* bacterial phenotype. A quantitative measure of susceptibility can, therefore, be made using the data generated by laboratory tests. The methods used by these testing laboratories fall into one or other of two main classes. One class aims at determining the minimum concentration of an agent required to inhibit the test bacterium in laboratory media (MIC). The other attempts to assess susceptibility by measuring the zone of inhibition produced in a lawn of the test bacterium around a disc containing the agent. Current information suggests that there are no major differences in the precision or accuracy between the two classes of susceptibility test methods. In practice, for reasons of convenience and cost, most front-line laboratories investigating clinical isolates use disc diffusion methods (Smith, 2006). The most common use of MIC tests is in large-scale retrospective studies by research scientists.

Standard susceptibility test protocols

The use of different MIC or disc diffusion protocols (media, temperature, etc.) will generate different results. Thus, the quantitative data generated in susceptibility tests are protocol-specific (Smith, Hiney and Samuelsen, 1994). In the interest of producing data that can be understood in a wide variety of laboratories, there are compelling reasons why all laboratories should strive to use the same susceptibility test protocols. During the last decade, there has been significant progress in harmonizing the test protocols being used (Smith, 2006). There is now substantial international agreement that those initially produced by Alderman and Smith (2001) and subsequently developed, as the M42-A (CLSI, 2006b) and M49-A (CLSI, 2006a) guidelines, by the Clinical and Laboratory Standards Institute (CLSI), are the protocols that should be adopted in all laboratories.

The current situation is such that any laboratory that chooses not to use the CLSI protocols should be obliged to provide detailed arguments as to why they made that decision.

It must be recognized that, as currently developed, these protocols do not provide detailed descriptions of the media and incubation conditions that should be applied to the study of all the bacteria that might be isolated from aquatic animals. In studying bacteria for which the protocols do not provide complete specification of these parameters, laboratories should endeavour to stay as close as possible to the CLSI guidelines.

Interpreting susceptibility test data

Susceptibility testing laboratories can perform *in vitro* tests that allow them to generate a quantitative measure of the susceptibility of a bacterium in terms of either an MIC value or disc-diffusion zone size. However, before they communicate any results to farmers or their AAHP, laboratories must first interpret what the data generated mean in a clinical context.

The AAHP or farmer can make only one of two decisions. They can choose only between initiating a course of therapy and not doing so. Being told that the isolate has a zone size of 22 mm with respect to a particular agent does not, of itself, help them in making the treat/do not treat decision. What the AAHP requires is some interpretation of the zone size (or MIC value) that provides a meaning for that numerical value. In the clinical context, the most important meaning that can be attributed to any susceptibility data is that it does or does not provide reasons why a particular treatment should not be initiated.

In practice, the meaning of quantitative susceptibility data is determined by setting critical values. The use of critical values can be illustrated by an example. Let us assume that, for a particular agent, when a specific test protocol has been used, a critical zone size of 20 mm has been set. If a target bacterium generates a zone size less than 20 mm, the laboratory must report that it has data indicating that the proposed treatment would be imprudent and probably a waste of money.

The use of breakpoints to determine resistance/sensitivity

One set of critical values that can be developed to aid the interpretation of susceptibility data are termed breakpoints, or clinical breakpoints. The aim of breakpoints is to allow the classification of bacteria as clinically sensitive (S) or clinically resistant (R). Frequently, breakpoints are set that also allow the classification of some strains as intermediate (I). This category is used when the testing laboratory is unsure of the true classification of the tested strain or when experience has shown that the bacterium will be clinically sensitive only if higher than recommended doses are used. Smith (2008a) has argued against the use of the intermediate (I) category in aquaculture.

The setting of clinically relevant breakpoints is a complex process that requires the consideration of clinical outcome data together with PK and PD data. The CLSI guideline M23-A3 (CLSI, 2008) provides an introduction to their current thinking on this issue. Readers who wish to know more about current thinking in human medicine are advised to consult Turnidge and Paterson (2007) or Drusano (2004). Smith (2008a) has recently reviewed the application to aquaculture of these very sophisticated PK/PD approaches to setting breakpoints that are being developed in human medicine. He concluded that there are major practical and theoretical problems with the application of these approaches in aquaculture and their application in this context was unlikely to be cost-effective.

An important property of clinical breakpoints is that they are of greatest value when they are established with respect to a standardized dose regimen. In general, there is little standardization of dose regimen in aquaculture. In this hugely diverse industry, the same agent may be administered to a wide variety of species held under a wide variety of environmental conditions. Searching the database generated by Reimschuessel *et al.* (2005) would also suggest that, even with respect to one species of aquatic animal, there are wide variations in the dose and the duration of treatments that have been used. Taken together, these variations have resulted in very significant variations in the PK parameters associated with each agent. The PK properties of oxytetracycline administrations are significantly affected by temperature, and concentrations of this agent achieved in crustaceans are normally much higher than those achieved in finfish. In contrast, the PK parameters of quinolone administrations are less affected by temperature but are strongly affected by salinity (Smith, 2008a). Thus, it is reasonable to suggest that it will always be difficult to set a standard single breakpoint that will relate to all situations where a specific agent can be or will be used.

Standard treatment regimen do exist in aquaculture in situations where Market Authorizations have specified the dose, the species to be treated and the environmental conditions under which treatment is permitted. The diversity of aquaculture, however, makes it almost certain that the variety of conditions in which antimicrobial agents will be used will always exceed the variety that can be included in a Market Authorization. As a consequence, much of the administrations of antimicrobial agents in the industry will have to remain "off-label/extra-label".

The problems in setting breakpoints are not limited to issues arising from the diversity of applications. There is also a very serious shortage of primary data to set such values. The key data are those that relate clinical outcomes to dose regimen PK and the susceptibility of the target bacterium. Examining the scientific literature indicates that there have been very few studies that have reported data of this type. Even with respect to oxytetracycline, the most widely used agent, only a very few (Darwish, Rawles and Griffin, 2002; Bruun, Madsen and Dalsgaard, 2003; Coyne *et al.*, 2004) studies have reported efficacy and PK/PD parameters from treatments of finfish.

The author has been informed that the CLSI, in the next edition of M42-A intend to suggest breakpoints for oxytetracycline and oxolinic acid against *Aeromonas salmonicida*. Even in the case of this much-studied bacterium, it should be noted the breakpoints proposed by CLSI are supported by very limited clinical outcome data. The breakpoints for oxytetracycline are supported by the study of Coyne *et al.* (2004)

and those for oxolinic acid by the studies of O'Grady *et al.* (1987), O'Grady and Smith (1992), Smith and O'Grady (2006) and Hastings and McKay (1987).

In summary, it can be concluded that the most clinically meaningful criteria that could be used to interpret susceptibility data would be clinical breakpoints. There are, however, serious problems with setting these values and, at present, we have very few. The obvious conclusion is that we will, for the foreseeable future, have to operate with some other criteria of interpretation.

The absence of validated clinical breakpoints that can be applied in aquaculture has one major consequence. The lack of breakpoints means that we lack any valid method for classifying any isolate as clinically resistant. It is strongly recommended that the word *"resistance"* should be used only when it refers to clinically relevant resistance as determined by fully validated clinically relevant breakpoints. As, with two exceptions, these breakpoints do not exist for the bacteria encountered in aquaculture, we should be very, very cautious in using the word *resistant* when we are discussing bacteria isolated from aquatic animals.

Major errors in the use of breakpoints

Given the absence of validated breakpoints for aquaculture, there is a temptation to apply agent-specific breakpoints that have been established for human or veterinary applications. The fact that disc manufacturers often provide interpretive charts with their products increases the tendency to surrender to this temptation. There are, however, two major reasons why this temptation should be resisted.

The first is that breakpoints are protocol-specific. Susceptibility test data for bacteria associated with aquaculture are normally performed at temperatures (22 °C or 28 °C) that are lower than those used in human and veterinary studies (35 °C). The lower incubation temperatures and, frequently, the slower growth rates obtained, have a significant effect on the quantitative estimates of susceptibility measures for aquaculture-related bacteria. These factors have a major impact on disc diffusion tests where the zone sizes are growth-rate dependent. Their effect on MIC value determinations, although probably smaller, may still be significant (Martinsen *et al*., 1992). As a consequence, it is only with very, very great caution that breakpoints established with respect to one protocol should be applied to data produced using a different protocol.

The second issue is that breakpoints are also host specific. Human and veterinary clinical breakpoints take into account the PK, PD and clinical outcomes observed in terrestrial animals. There are strong grounds for assuming that there will be important differences between the PK, PD parameters and clinical outcomes associated with treatments of aquatic and terrestrial animals. Thus, to the extent that breakpoints are derived from a consideration of these parameters, it cannot be legitimate to adopt those established for terrestrial animals and to apply them to aquatic animal treatments.

The use of cut-off values to interpret susceptibility data

If it is accepted that, at least in the short to medium term, we will not have valid breakpoints to help us interpret the clinical meaning of susceptibility data in aquaculture, this does not mean that we have to give up any attempt to interpret these data. We can, relatively easily, establish criteria that would allow us to characterize an isolate as fully susceptible or not fully susceptible in comparison to other members of the species or group to which they belong.

In their attempts to harmonize the criteria being used to interpret susceptibility data in various countries, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) developed the concept of epidemiological cut-off values (Kahlmeter *et al*., 2003). These cut-off values can provide a classification of strains as fully susceptible or wild type (WT) or as non-wild type (NWT). CLSI have also adopted a similar parameter that they term a wild type cut-off value (CO_{WT}), as part of the process they recommend for the setting of clinical breakpoints (CLSI, 2008). When the term "cut-off value" is

used in this chapter the term will refer, in all cases, to epidemiological cut-off values.

Epidemiological or wild type cut-off values are set by considering the distribution of susceptibility test data for a number of members of a species or group of related bacteria. They are, therefore, agent-specific, protocol-specific and species- or group-specific. Examples of the setting of cut-off values can be found in Miller and Reimschuessel (2006) and Smith *et al*. (2007), and the principles underlying the process, as they apply to aquaculture, have been discussed by Smith (2008b). Essentially, there have been two methods that have been applied to the setting of CO_{WT} from data that have been generated from bacteria associated with aquatic animals. The first has been called "eyeball technology" where the proposed CO_{WT} is estimated from a visual examination of the distribution of the measures of susceptibility of a number of strains of a species (Miller and Reimschuessel, 2006). The second approach, normalized resistance interpretation (NRI), which was developed by Kronvall and his co-workers (Kronvall, 2003), has a more statistical basis and has been applied to zone size data of *Aeromonas salmonicida* by Smith *et al*. (2007), Ruane *et al*. (2007), Douglas *et al.* (2007) and Smith *et al*. (2009). In applying NRI, it is assumed that the zone sizes for all WT strains will show a normal distribution. NRI analysis involves the calculation of the mean and standard deviation (*sd*) of this normal distribution. The CO_{WT} is the set at the mean minus 2.5 or 3 times the *sd*. A significant advantage of the NRI method over visual examination is that it allows for the fact that strains with a slight but possibly clinically significant, reduction in susceptibility might manifest zones that overlap with the low zone half of the normal distribution of WT strains. To prevent strains manifesting this phenotype biasing any estimate of CO_{WT}, NRI estimates the mean and standard deviation (*sd*) of the WT zone distributions from a consideration of only the high zone half of the normal distribution.

As CO_{WT} values do not take account of the properties of any therapy or the data on the outcomes of similar therapies, they cannot provide a method of assessing clinical resistance or sensitivity. Rather, they provide a critical value of a susceptibility measure that allows isolates to be classified as fully susceptible (WT) or less than fully susceptible (NWT). It has to be noted that the categories WT and NWT have no inherent clinical meaning; however, Smith (2008b) has argued that they can help in reducing the errors associated with the administration of agent to aquatic animals.

If an isolate is classified as WT, it could, in the majority of cases, be assumed that the laboratory testing of its susceptibility has revealed no reasons why a therapeutic administration should not be initiated. There are, of course, exceptions to this general rule. Possibly the most important occur when attempts are being made to control disease losses associated with intracellular bacteria such as *Piscirickettsia salmonis* or *Francisella piscicida*. In these cases, an isolate might be correctly identified as WT and might manifest a high degree of susceptibility in laboratory media. However, as a result of the failure of the agent to achieve sufficient activity within the infected host cells, an epizootic associated with this bacterium might not respond to therapy at all. In this situation, the isolate should be classified as both WT and clinically resistant.

The classification of an isolate as NWT cannot necessarily be taken to indicate that it should be classified as clinically resistant or that an attempt to control it by administering the agent will necessarily fail. All that this classification indicates is that the isolate is less susceptible than other members of the species or group to which it belongs. In such a situation, however, the need for prudent use of antimicrobial agents would require the laboratory to report that there were grounds for not initiating or not continuing with the administration of the agent.

The application dependent validity of the use of cut-off values to interpret susceptibility data

The idea of classifying isolates as WT or NWT by applying (epidemiological) cut-off values to MIC data was originally developed by EUCAST to facilitate the monitoring of

resistance development. They considered this application as valid (Kahlmeter *et al.*, 2003). There would appear to be no reasons why this approach could not be applied to disc diffusion data generated from bacteria associated with aquaculture. The establishment of clinical breakpoints is, therefore, not a prerequisite for the monitoring of resistance development in the aquatic environment or in bacteria associated with aquatic animal disease. For this purpose, cut-off values would be sufficient and their application can be assumed to be valid.

The arguments presented in this section, however, indicate that the classification of isolates as WT or NWT has limited validity if our aim is to assess the clinical significance of susceptibility data. The use of cut-off values to characterize strains does, however, have two major advantages. The first is that the required cut-off values are relatively easy to determine. The second is that their application will almost certainly reduce the frequency of inappropriate administrations. There are reasonable grounds for believing that many laboratories are currently using interpretive criteria that have no empirical basis and are, in all probability, totally invalid. Smith (2006) surveyed the interpretive criteria currently being employed in laboratories involved in susceptibility testing of bacteria associated with diseases of aquatic animals. A comparison (Table 1) of the criteria currently in use with those published in M42/49-S1 (CLSI, 2010) suggests that, at least with respect to some agents, serious errors are being made.

TABLE 1
Comparison of breakpoints currently in use in testing laboratories (Smith, 2006) with the interpretive criteria accepted by the Clinical and Laboratory Standards Institute[1]

	Number of laboratories using breakpoints of various sizes			
Zone size (mm)	Oxolinic acid	Oxytetracycline	Florfenicol	Trimethoprim-sulphamethoxazole
10–11	1			
12–13	2	1		1
14–15	2			
16–17	1	2	1	9
18–19	1	12	5	6
20–21		2	4	1
22–23		1	1	
24–25	1		2	1
26–27	1			
28–29		1		3
30–31		1	1	
32–33		1	1	1
34–35				2
36–37				
38–39		1		
Major error[2]	100%	82%	87%	67%

[1]Shaded areas indicate the interpretive criteria that have been accepted by CLSI (2010). For oxolinic acid and oxytetracycline, these are breakpoints, and for florfenicol and trimethoprim-sulfamethoxazole, they are CO_{WT}.
[2]Major errors occur when the breakpoint being used by a laboratory would classify some isolates as WT or sensitive when the classification based on CLSI criteria would be resistant or NWT.

Addressing the problem of interlaboratory variation in susceptibility determinations

As argued above, the setting of CO_{WT} represent the best option for improving the quality of the information supplied to AAHPs by susceptibility testing laboratories. Of their nature, any CO_{WT} values would, of course, be agent-, species- and protocol-specific. There would, however, be a great advantage if the appropriate CO_{WT} were to prove to be laboratory-independent. If this was the case, a central authority could set protocol-specific CO_{WT} values and all laboratories that used the specific protocol would be able to apply these universal laboratory-independent CO_{WT}. The problem here is

that susceptibility data have been shown to be subject to significant interlaboratory variation. The original moves towards harmonizing test protocols (Alderman and Smith, 2001) were undertaken in an attempt to address this issue of interlaboratory variation. However, even when different laboratories use the same protocol, they do not obtain the same quantitative measure of susceptibility (NicGabhainn *et al.,* 2003). Thus, the introduction of the new standard protocols had only a limited success in reducing interlaboratory variation.

Unless interlaboratory variation can be eliminated or at least significantly reduced, we cannot set universal, laboratory-independent COWT that possess adequate precision. There have been two major approaches to resolving this problem. The first attempts to further reduce interlaboratory variation by introducing rigorous quality control (QC) requirements into the standard test protocols. The second assumes that interlaboratory variation cannot be adequately reduced and, therefore, involves abandoning the search for universal laboratory-independent CO_{WT}.

Reducing interlaboratory variation by improved quality control

The attempt to reduce interlaboratory variation by application of QC requirements is common to all guidelines published by CLSI. This approach is also followed in the guidelines relevant to aquaculture (M42-A, CLSI, 2006b and M49-A, CLSI, 2006a). The CLSI approach to QC involves specification of control strains and the range of acceptable results for determinations of their susceptibility. Laboratories can consider themselves as in compliance with the CLSI test protocols only if their data for the control strains lie within these acceptable ranges. It follows that the breakpoints or CO_{WT} published by CLSI can be applied only by those laboratories that have demonstrated full compliance with the QC requirements of the relevant protocol.

There are a number of problems that are associated with this QC-based approach. The first and most obvious of these is that, so far (early 2010), control strains and their acceptable ranges have been established only for tests performed on unmodified Mueller Hinton agar at 22±2 ºC or 28±2 ºC. At present, there are no QC criteria established for tests performed under any other conditions. This has the consequence, for example, that it is not possible to be in compliance with M42-A or M49-A when using media supplemented with NaCl to perform susceptibility tests on halophilic *Vibrio* spp.

There are, however, two further and possibly more fundamental problems that result from the CLSI approach. The first relates to the width of the acceptable ranges that have been set. The acceptable ranges that it proved possible to set (Miller *et al*., 2003) for the control strains in M42-A (CLSI, 2006b) can be as large as 14 mm. Thus, applying QC requirements based on these ranges will possibly reduce, but cannot eliminate, interlaboratory variation (Smith, 2008c). The residual and, possibly irreducible, variation results in a lack of precision in the application of any CO_{WT} associated with these protocols. It should be noted that the significance of this loss of precision would vary depending on the distribution of the susceptibility measures being analyzed (Smith, 2008b). The data of Miller and Reimschuessel (2006), Smith *et al*. (2007) and Uhland and Higgins (2006) all indicate that the gap between the zone sizes for WT and NWT strains of *Aeromonas salmonicida*, with respect to oxytetracycline, is relatively large. With respect to this agent, therefore, a loss of precision should not necessarily result in an incorrect classification of isolates and could be tolerated. In contrast, the gap between zone size of WT and NWT for oxolinic acid against this species (Miller and Reimschuessel, 2006; Smith *et al*., 2007) is relatively small. Thus, with respect to oxolinic acid, imprecision in the application of CO_{WT} could have clinical significance.

The second problem relates to the difficulty some laboratories may experience in achieving compliance with these acceptable limits (Smith, 2008c). When a laboratory obtains results for control strains that are outside the acceptable range, they are obliged to investigate the causes of this and to develop modifications that bring them back into compliance. This requires time-consuming and reasonably sophisticated experimental work that may present serious problems for some non-research laboratories.

Development of laboratory-specific cut-off values

Having considered the issue of interlaboratory variation in the disc diffusion data sets obtained in laboratories working with human strains, Kronvall and his co-workers (Kronvall, 2003; Kronvall *et al.,* 2003; Joneberg *et al.*, 2003) concluded that it was not possible to specify protocols or QC criteria that would reduce this variation to acceptable levels. They, therefore, proposed that laboratories should develop their own laboratory-specific cut-off values. It was for this purpose that they developed NRI. NRI was designed to provide a standard method by which each laboratory could develop interpretive criteria that were appropriate for the disc diffusion data they generated. If the aim is to produce laboratory-specific CO_{WT}, the issue of interlaboratory variation ceases to be of any significance.

At our present stage of development, there are a number of situations where the setting of laboratory-specific cut-off values represents not only the most reasonable, but possibly the only, approach open to laboratories in attempting to interpret their data. These would include:

- Studies of bacterial groups for which CLSI has yet to establish the appropriate media and incubation conditions.
- Studies using media and incubation conditions for which acceptable control strain ranges have not been set.
- Studies of bacterial groups for which agreed CO_{WT} values have not yet been established.

In effect, at the present stage of our development, this means all studies except those of *Aeromonas salmonicida*.

In theory, once the decision to set laboratory-specific cut-off values has been made, each laboratory could consider itself free to adopt any test protocol it wished. However, the development of susceptibility testing criteria is a developing field, and we would be very unwise to introduce unnecessary complicating factors. It is, therefore, strongly recommended that, even when laboratories are intending to set their own cut-off values, they should, as far as is possible, generate their data using the standard CLSI protocols.

The arguments presented by Smith (2008c), that the QC requirements of the CLSI approach placed a significant burden on laboratories, were mentioned previously. It is equally true that setting laboratory-specific CO_{WT} values would present problems for some laboratories. A prerequisite for the performance of NRI analysis is the availability, within a laboratory, of data sets that include susceptibility measurements made on a number of members of a particular bacterial group by that laboratory. Smith *et al.* (2009) have suggested that, if the NRI CO_{WT} values are to be estimated with adequate precision, such data sets must include at least 20 independent members. It must be noted, that for many laboratories involved in susceptibility testing of bacteria associated with aquatic animals, it might take months or even years to accumulate the necessary data sets.

Are cut-off values species-specific or capable of wider application?

The interpretation of susceptibility test data requires the setting and application of either breakpoints or cut-off values. There is one central problem that has yet to be addressed with respect to the application of these interpretive criteria to bacteria isolated from aquatic animals. This concerns the range of bacteria to which any specific breakpoints or cut-off values can be applied.

If we establish a cut-off value for say *Vibrio harveyi,* can we assume that it can be validly applied to interpreting data for members of all the other *Vibrio* species? Can the cut-off values established for *Aeromonas salmonicida* be applied to data from *Yersinia ruckeri*? If we find that cut-off values have only a species-specific validity, the problem of generating interpretive criteria will be immense and daunting. It should be noted that this issue would be of huge significance independent of the methods used to generate the cut-off values. It would apply equally to laboratory-specific and laboratory-independent

values and to the selection of appropriate control strains in the SPIC approach (see below).

We urgently need data that can help us understand the range of bacteria to which any cut-off value can be applied and the possible loss of precision that may be associated with applying a cut-off value to members of species other than that used to estimate it.

INCREASING THE USE OF SUSCEPTIBILITY DATA

Previously, it was argued that susceptibility testing will or should occur in two contexts. The first is within national or regional surveillance programmes. The data collected in such studies would be used to inform AAHPs as to the probable sensitivity or resistance in any bacterial group prevalent in their area. Here it is possible that the testing will be performed in reasonably large laboratories. These laboratories should be handling sufficient numbers of strains to allow them to apply NRI and to generate appropriate laboratory-specific CO_{WT}. They should also be capable, if they prefer, of meeting the QC requirements of the CLSI protocols, when these are available, and applying the protocol-specific, laboratory-independent CO_{WT} associated with these protocols.

There is, however, a second context where data on susceptibility are required. In this context, the aim is to provide data directly to the AAHP on the susceptibility of a particular isolate made from a particular epizootic. Here the need for speed is critical. If farmers and their AAHPs are to gain rapid access to these susceptibility test data, the testing laboratories must be local. If they are local, they will be small. As argued above, there are reasons for suggesting that small, local laboratories would have difficulties in applying either of the approaches to interpreting susceptibility data outlined previously. Thus, there is a real need to develop and validate test protocols that are suitable for front-line laboratories that test only a limited number (<50) of clinical isolates a year.

A susceptibility test method, originally developed by Stokes (Stokes and Ridgeway, 1980) and that has a long history of use in United Kingdom hospitals (Andrews, Brown and Wise, 1996), has recently been investigated for its potential to meet the need of small laboratories handling aquatic samples (Smith, Fleming and Carroll, 2008). The central elements of this "Stokes" method have been incorporated into a modified version of the Alderman and Smith (2001) tests protocol for disc diffusion. The current version of this protocol, which is termed the Single Plate Internal Control (SPIC) protocol, can be accessed at www.nuigalway.ie/microbiology/prof__peter_smith.html.

In this protocol the zone size of the clinical isolate (test strain) is compared with that of a control WT strain on a single plate. Thus, the SPIC protocol has the potential for a laboratory to generate meaningful interpretations of a susceptibility test after using just one single plate. If this method can be developed and demonstrated to be sufficiently robust, it has a clear potential as a protocol that could resolve the problems of small throughput laboratories. The experimental design incorporated in this protocol includes an internal control in all tests and, therefore, eliminates problems associated with interlaboratory variation.

At present, there are two major issues concerning SPIC that need to be resolved. As mentioned above, the first is the robustness of the protocol in the small laboratories where it is intended that the protocol should be used. Any lack of robustness could be dealt with by increasing the difference (currently ≥ 4 mm) between the zone radii of the test and control strains that can be taken to indicate a NWT phenotype. Any increase in this critical parameter would, however, result in a loss of precision and, therefore, a decreased ability to detect small changes in susceptibility.

The second problem is the identification of the control strains to be used. The essential properties of a control strain in SPIC is that it is freely available, its susceptibility is stable and that it manifests a phenotype that is characteristic of the WT members of the group to which the test strain belongs. It would appear sensible to suggest that the identification, storage and distribution of appropriate control strains should be the responsibility of national or regional laboratories. It is also reasonable to suggest that a

search for appropriate control strains might start in those strains that have already been deposited in international strain collections. These strains are, by definition, available and, as they have often been in storage for many years, may well be stable. As many were originally isolated in the early years of aquaculture, they may also be expected to be fully susceptible to many agents currently in use.

SETTING CUT-OFF VALUES RELEVANT TO RISK ANALYSIS

A joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture, held in 2006, attempted to apply formal risk analysis to assess the risks to humans associated with antimicrobial agent use in aquaculture (WHO, 2006). This consultation concluded that "The greatest potential risk to public health associated with antimicrobial use in aquaculture is thought to be the development of a reservoir of transferable resistance genes in bacteria in aquatic environments from which such genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens."

Smith (2001) addressed the issue of the types of data, and the methods that would be necessary to generate them, that would be required if such a risk analysis were to be performed. He identified methods allowing the determination of the frequency, in the aquatic environment, of bacteria that possess transferable genes that encode resistance, as a primary requirement. It is, therefore, worthwhile examining if interpretive criteria could be developed that could, when applied to the susceptibility test data discussed in this paper, provide this type of information.

In the previous sections, we have discussed the applications for which breakpoints and/or CO_{WT} can provide valid interpretations of susceptibility data. However, for the purpose of identifying bacteria possessing the genes we are interested in, both these criteria have limited validity. Clearly, considerations of treatment PK are not relevant and, therefore, clinical breakpoints would not have direct relevance or *a priori* validity. Wild-type cut-off values would also have limited validity. They are designed to detect all bacteria that manifest less than full susceptibility. Thus, the application of CO_{WT} would lead to the detection of bacteria whose reduced susceptibility was a result of a variety of mechanisms that would include transferable genes but that would also include those that resulted from other, non-transferable mechanisms. These other mechanisms would include membrane alterations (Griffiths and Lynch, 1989; Nikaido, 1989; Barnes *et al.,* 1990), modified efflux systems (Giraud *et al.,* 2004; Poole, 2005) or even phenotypic mechanisms (Balaban *et al.,* 2004). In general, these mechanisms result in a smaller decrease in susceptibility than those that result from the possession of the positive function mechanisms that are encountered in transferable genes. In the context of risk analysis, it would be important to note the existence of these other mechanisms has the result that strains classified as NWT by the application of CO_{WT} would include a number of strains with no relevance to the assessment of the risk to public health. Thus, application of CO_{WT} would lead to an overestimation of the frequency of bacteria possessing the transferable genes of relevance to risk analysis.

One approach can be proposed that might provide a set of interpretive criteria that could have validity in the context of risk assessment. In this approach, known transferable resistance genes of interest could be introduced into bacteria associated with aquatic animals or aquaculture enterprises. Measurement of the zone sizes that are obtained with strains possessing these genes could then be translated, relatively directly, into relevant interpretive criteria that would have increased validity in the context of risk analysis. Alternatively, aquatic bacteria that have already been subject to susceptibility testing could be screened, using DNA probes, for the presence of genes of interest. Although neither of these approaches has yet been reported, both would appear to have potential in setting interpretive criteria that would have validity in the context of risk analysis.

In studies of the risks to humans associated with the use of antimicrobial agents in terrestrial animals, the concept that an intestinal commensal organism could be used

to monitor the development of resistance has been considered. Strictly applied, this concept would appear to have less value in aquatic animals. However, Guardabassi *et al.* (2000) used *Acinetobacter* spp. as indicator of the impact of AA antimicrobial therapy on the bacterial flora of the environment of aquaculture facilities. A combination of this approach with that outlined in the previous paragraph would appear to represent an experimental strategy that should be investigated.

CONCLUSIONS

The prudent use of antimicrobial agents in aquaculture is a complex issue. However, one of the central factors that will facilitate prudent use is the availability of good quality data on the susceptibility of the bacteria that are the targets of any antimicrobial treatment. Good quality data will be available only if appropriate and validated protocols are used to perform susceptibility tests and valid criteria are used to interpret that data.

The arguments in favour of international harmonization of susceptibility test protocols appear compelling, and there would appear to be no reasons why these efforts should not focus on the CLSI guidelines M42-A (CLSI, 2006b) and M49-A (CLSI, 2006a).

Ideally, clinically relevant breakpoints would represent the best way to provide a meaningful interpretation of *in vitro* susceptibility data. Progress towards developing clinical breakpoints that can be applied, with any degree of validity, to the wide variety of treatments that are encountered in aquaculture will, necessarily, be slow.

At the present state of our technical and theoretical development, the best course of action would be to develop wild-type cut-off values (CO_{WT}) and to base clinical advice on the classification of the target bacterium (WT or NWT) that they generate. While far from ideal, this approach would, almost certainly, result in an improvement in the quality of the advice that could be given to AAHPs by testing laboratories.

The advantages that would accrue from harmonization of CO_{WT} are self-evident. However, the extent of interlaboratory variation in susceptibility test data and the difficulties encountered in attempting to resolve them suggest that this may not be practicable. Any laboratory-independent CO_{WT} that could be proposed may be associated with a disturbing and clinically significant loss of precision. In this context, the most effective and valid way forward would be to encourage each laboratory to use NRI analysis to estimate sets of laboratory-specific CO_{WT} for application to their own data.

It is recognized that the adequate monitoring of antimicrobial agent resistance will, in all probability, require the coordinated action of small local laboratories responsible for advising AAHPs and larger regional or national laboratories responsible for overall monitoring. The proposal that an approach involving the adoption of internationally standardized test protocols and NRI-generated, laboratory-specific interpretive criteria is suitable for the larger national laboratories. This approach is, however, unlikely to be realistic for the smaller laboratories. For these laboratories, the biggest gains in the quality of the treatment advice they can offer may be through their adoption of SPIC procedures. The larger national laboratories would have a key role in the development and dissemination of appropriate modifications of SPIC procedures.

The progress of improvements in susceptibility testing for bacteria associated with diseases of aquatic animals has been steady but regrettably slow. If we are to continue to make progress, we need more data. If we are to increase the rate of development, we urgently need to specify and prioritize these research requirements. International agencies and national or regional producer groups will have to act, in conjunction with research scientists, in the setting of and prioritizing of relevant research targets. In planning future work, one central fact must not be forgotten. Central to the making of any progress in susceptibility testing is the provision of adequate funds for research and adequate investment in capacity building within the scientific support structures available to aquaculture.

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Legislation and regulatory efforts in the United States of America relevant to the use of antimicrobials in aquaculture

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ABSTRACT

The legal marketing and use of veterinary drugs in the United States of America is determined by the Federal Food, Drug and Cosmetic Act and its amendments. Approvals are for specific products and include evaluation of effectiveness, animal safety, environmental impact, manufacturing controls and human food safety, including microbial food safety. This is augmented by post-approval monitoring and surveillance. Outreach and international cooperation are also important components of the Food and Drug Administration's efforts to insure the judicious use of antimicrobials in aquatic and terrestrial animals.

INTRODUCTION

The United States of America's Food and Drug Administration's Center for Veterinary Medicine (FDA-CVM) has a critical role in protecting public and animal health. The agency recognizes that antimicrobial resistance is an important public health issue. FDA-CVM currently addresses potential risks associated with the use of antimicrobials in animals through the new animal drug approval process, monitoring and surveillance, research, communication with stakeholders and international efforts. This paper will introduce the reader to the regulatory structure in the United States that applies to veterinary medicines. Additionally, it will discuss some of FDA-CVM's efforts to insure the judicious use of antimicrobials with, true to the scope of the workshop's proceedings, an emphasis on the use of antimicrobials in aquaculture. While this manuscript was written based on a presentation given at the FAO Expert Workshop on Improving Biosecurity through Prudent and Responsible Use of Veterinary Medicines (Antimicrobials) in Aquaculture, the authors have included updated material to reflect some new developments since the workshop.

REGULATION OF DRUGS, BIOLOGICS AND PESTICIDES IN THE UNITED STATES OF AMERICA

Direction for federal regulation in the United States of America comes from laws passed by Congress and signed by the President. The executive branch of the government is responsible for implementing and enforcing the laws and includes the Department of Health and Human Services. The FDA is part of the Department of Health and Human Services. The Federal Food, Drug, and Cosmetic Act (FFDCA) and its amendments provide the legal framework for the FDA, which has authority over drugs and animal feeds.

The FFDCA's definition of a drug includes "articles intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in man or other animals and articles (other than food) intended to affect the structure or any function of the body of man or any animal." The term "new animal drug" distinguishes drugs intended for animals other than humans. Not all animal health products in the United States of America are regulated by the FDA. Under the Federal Insecticide, Fungicide, and Rodenticide Act, the Environmental Protection Agency registers pesticides, such as algicides that may treat fish ponds. Authorized by the Virus-Serum-Toxin Act, the Department of Agriculture (USDA) regulates veterinary biologics (e.g. vaccines, bacterins, antisera, diagnostic kits and other products of biologic origin).

Any new animal drug sale or use is considered to be unsafe and in violation of the law unless the drug has an FDA-approval or conditional approval, is on the Index or has an investigational exemption. Conditional approval and indexing are provisions of the Minor Use and Minor Species Animal Health Act that will be discussed below.

The Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994, an amendment to the FFDCA, allows licensed veterinarians, within a valid veterinarian-client-patient relationship, to prescribe FDA-approved animal drugs or human drugs for extra-label uses. Drugs are approved by the FDA for specific species, dose, route of administration, frequency of administration and conditions of use, including withdrawal time for food-producing species. Any use that deviates from the label instructions is extra-label drug use and requires a valid veterinary-client-patient-relationship. Specific parameters for extra-label use are described in regulations, specifically in Title 21 of the Code of Federal Regulations, Part 530. The following are some of the rules that apply for extra-label use in food-producing animals. If a legally marketed product is labeled for the indication and is effective, it is to be used. Furthermore, extra-label use is only allowed when an animal's health is threatened, or suffering or death may occur if treatment is not administered. Extralabel use is not permitted if it results in a violative food residue or any residue that may present a risk to public health. AMDUCA does not permit the extra-label use of new animal drugs in feed. Recognizing specific needs, FDA provides enforcement discretion for certain extra-label uses in feed for minor species, which include fish as described in Compliance Policy Guide Section 615.115.

The FDA may prohibit extra-label use of certain drugs or classes of drugs that pose a public health concern. The following drugs (both animal and human formulations), families of drugs and substances are prohibited for extra-label uses in food-producing animals:

- chloramphenicol;
- clenbuterol;
- diethylstilbestrol;
- dimetridazole;
- ipronidazole;
- other nitroimidazoles;
- furazolidone, nitrofurazone and other nitrofurans;
- fluoroquinolones;
- glycopeptides;
- phenylbutazone in female dairy cattle 20 months of age or older;

- sulfonamide drugs in lactating dairy cattle (except approved use of sulfadimethoxine, sulfabromomethazine and sulfaethoxypyridazine);
- cephalosporins (not including cephapirin) in cattle, swine, chickens, or turkeys as follows: (1) for disease prevention purposes; (2) at unapproved doses, frequencies, durations, or routes of administration; and (3) if the drug is not approved for that species and production class; and
- adamantanes and neuraminidase inhibitors approved for treating or preventing influenza A – prohibited from extra-label use in chickens, turkeys and ducks.

Provisions of the Animal Drug Availability Act of 1996, another amendment to the FFDCA, included new approaches to the review of new animal drug applications and created a new category of new animal drugs, Veterinary Feed Directive drugs. It was determined that certain new animal drugs, vital to animal health, should be approved for use in animal feed, but only if these medicated feeds are administered under a veterinarian's order and professional supervision. For example, such control of certain antimicrobials is critical to reducing the risk of antimicrobial resistance, and veterinary involvement may be necessary for other drugs that have the potential to be toxic.

As mentioned earlier, the Minor Use and Minor Species Animal Health Act of 2004 introduced two new legal options for marketing of drugs for minor species: conditional approval and indexing. All aquatic animals are minor species under the terms of this law. With a conditional approval (after demonstrating that a drug is safe and there is a reasonable expectation of effectiveness), a sponsor may market the new animal drug for up to five years (with annual renewals) while collecting effectiveness data needed for a full approval. The Index is a list of legally marketed unapproved new animal drugs for non-food-producing minor species and non-food early life stages of food-producing minor species, such as oyster spat. Extra-label use of conditionally approved and indexed products is prohibited.

In 2010, FDA-CVM published draft guidance (#209) on the Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals. FDA-CVM publishes guidance for industry that represents its current thinking, and this guidance outlines potential strategies for assuring that medically important antimicrobial drugs (antimicrobial drugs that are important for therapeutic use in humans) are used judiciously in food-producing animals, including aquatic species, in order to help minimize antimicrobial resistance development. It proposes such measures as 1) limiting medically important antimicrobial drugs to uses in food-producing animals that are considered necessary for assuring animal health; and 2) limiting such drugs to uses in food-producing animals that include veterinary oversight or consultation.

The regulatory authority for evaluating the environmental impact of new animal drugs is contained in the National Environmental Policy Act of 1969. For actions such as granting a marketing authorization for a new animal drug, an environmental assessment is required, unless the action falls into certain categories for which an exclusion to the requirement to prepare an environmental assessment or an environmental impact statement is appropriate.

DRUG APPROVAL PROCESS

The FDA approves specific product formulations; approvals are not generalized to the active ingredient.

FDA-CVM evaluates the following components when considering a new animal drug for approval: effectiveness, target animal safety, human food safety, environmental impact, and chemistry, manufacturing and controls, as well as labelling and all other information, including user safety. The human food safety review includes an evaluation of toxicology, residue chemistry and microbial food safety. The sponsor must provide methods for regulatory testing of residues in food. Guidance documents particularly relevant to microbial food safety include Guidance for Industry (GFI) #152 (Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological

Effects on Bacteria of Human Health Concern)[1] and GFI #159 (Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological Acceptable Daily Intake)[2].

Microbial food safety component of the human food safety evaluation

For a new animal drug approval, the agency must determine that an antimicrobial or veterinary medicine intended for use in food-producing animals is safe with regard to human health. As part of its evaluation of antimicrobials for food-producing animals, the agency considers the potential impact on human health of all proposed uses of all classes of antimicrobial new animal drugs intended for use in food-producing animals. Specifically, the FDA-CVM assesses both the potential for the use of antimicrobial new animal drugs in food-producing animals to result in the emergence or selection of antimicrobial-resistant food-borne bacteria, as well as the effect of drug residues on human intestinal flora.

The agency has published two documents that describe how drug sponsors may address these concerns. GFI #152, "Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern," outlines a risk assessment approach for evaluating the potential for human health to be adversely impacted by the emergence or selection of antimicrobial-resistant food-borne bacteria associated with the use of the drug in food-producing animals. Human health impacts considered include, but are not limited to, increased duration of illness, treatment failure and loss of therapeutic options. The process includes an initial hazard characterization followed by:

- a release assessment that evaluates the probability that the proposed use of the drug will result in the emergence or selection of antimicrobial-resistant bacteria;
- an exposure assessment that describes the likelihood of human exposure to food-borne bacteria of human health concern through animal-derived food products; and
- a consequence assessment that considers the human medical importance of the antimicrobial drug.

The release, exposure and consequence assessments are integrated into an overall risk estimation for the drug under consideration. After review of the assessment and available information, which may include additional review by an advisory committee, the FDA may impose necessary risk management steps, such as requiring prescription only (Rx) or Veterinary Feed Directive marketing status, extra-label use prohibition or use in a limited population of animals. If the proposed use of the drug cannot meet FDA's reasonable certainty of no harm standard, the agency may deny approval.

In VICH GL-36, known as GFI #159 in the United States of America, "Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI," the United States of America, the European Union and Japan provide a harmonized approach for addressing the impact of drug residues on the human intestinal flora. This document describes a pathway that may be followed to determine the effects of antimicrobial residues on the human intestinal flora, particularly in terms of disruption of the colonization barrier and increase in populations of antimicrobial-resistant bacteria in the human intestinal tract. Additionally, it outlines procedures for establishing a microbiological acceptable daily intake (ADI).

POST-APPROVAL MONITORING AND SURVEILLANCE

The FFDCA requires new animal drug sponsors to provide reports on the amount of drug sold or distributed each year to the Department of Health and Human Services. The

1 Available at www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052519.pdf

2 Available at www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM124674.pdf

Animal Drug User Fee Amendments of 2008 further mandate that reports be submitted on a calendar year basis for antimicrobial drugs approved for use in food-producing animals that specify the amounts by container size, strength and dosage form, including, for each dosage form, a listing of the target animals, indications and production classes on the label. The legislation was enacted to address the problem of antimicrobial resistance and to help ensure that FDA has the necessary information to examine safety concerns related to the use of antibiotics in food-producing animals, including aquatic species. Sponsors must report both domestic and export sales for U.S. labeled products.

Additionally, sponsors are required to submit labelling and promotional materials to CVM and to report adverse drug events. Adverse drug events include undesired side effects associated with use of the drug and situations where the drug did not deliver results as expected. FDA-CVM published a brochure directed toward people that treat, work with, or own fish, to encourage the reporting of adverse drug events. If appropriate, modifications to the label or marketing status of the product will be made to insure public and animal safety.

FDA-CVM cooperates with FDA's Center for Food Safety and Applied Nutrition and USDA's Food Safety Inspection Service to insure the safety of the United States food supply. This includes monitoring for unsafe residues in food and taking enforcement action when there is a violation. FDA-CVM researchers develop and validate analytical methods for the detection of drugs and other compounds in fish (and other animal) tissue and feed.

FDA is also part of the National Antimicrobial Resistance Monitoring System (NARMS), which monitors trends in antimicrobial susceptibility among enteric bacteria from humans, food animals and retail meats. Bacterial isolates are collected from human and animal clinical specimens, from healthy farm animals and from raw product from food animals. Retail meats collected from grocery stores were recently added to NARMS sampling. The primary objectives of NARMS include:

- to provide descriptive data on the extent and temporal trends of antimicrobial drug susceptibility in Salmonella and other enteric bacterial organisms from human and animal populations, as well as retail meats;
- to facilitate the identification of antimicrobial drug resistance in humans, animals and retail meats as it arises; and
- to provide timely information to veterinarians and physicians on antimicrobial drug resistance patterns.

Additionally, NARMS provides a national source of enteric bacterial isolates that are invaluable for research, such as diagnostic test development, discovering new genes and molecular mechanisms associated with resistance, studying mobile gene elements, and for virulence and colonization studies. The ultimate goal of these activities is to prolong the lifespan of approved drugs by promoting prudent and judicious use of antimicrobial drugs and to identify areas for more detailed investigation. NARMS is a collaborative effort between the FDA-CVM, USDA, the Centers for Disease Control and Prevention and state partners.

As part of the Clinical and Laboratory Standards Institute Subcommittee on Veterinary Antimicrobial Susceptibility Testing-Aquaculture Working Group, FDA-CVM works to standardize antimicrobial disk and broth dilution susceptibility test methods for bacteria isolated from aquatic species. Use of such standards can assist monitoring efforts by allowing comparison across laboratories, thus informing individuals interpreting the results.

PROMOTING JUDICIOUS USE THROUGH OUTREACH

In January 2006, the FDA-CVM and the American Veterinary Medical Association published a booklet entitled "Judicious Use of Antimicrobials for Aquatic Veterinarians." The booklet was created to help veterinarians treating aquatic animals use antimicrobials judiciously to maintain the effectiveness of these drugs in the treatment and prevention

of bacterial disease of aquatic animals grown for food production, while minimizing the development of resistance in human and animal pathogens. The document includes 15 general principles for veterinary antimicrobial use (Table 1) and specific guidelines for food fish veterinarians (Table 2). While written specifically for food fish veterinarians, the principles are applicable to the treatment of all aquatic animals. The booklet is widely distributed and the text is available online[3].

TABLE 1
Guidelines for judicious therapeutic use of antimicrobial agents

1. Preventive strategies, such as appropriate husbandry and hygiene, routine health monitoring, and immunizations, should be emphasized.
2. Other therapeutic options should be considered prior to antimicrobial therapy.
3. Judicious use of antimicrobials, when under the direction of a veterinarian, should meet all the requirements of a veterinarian-client-patient relationship.
4. Prescription, Veterinary Feed Directive, and extra-label use of antimicrobials must meet all the requirements of a veterinarian-client-patient relationship.
5. Extralabel antimicrobial therapy must be prescribed only in accordance with the AMDUCA amendments to the Food, Drug, and Cosmetic Act and its regulations.
6. Veterinarians should work with those responsible for the care of animals to use antimicrobials judiciously regardless of the distribution system through which the antimicrobial was obtained.
7. Regimens for therapeutic antimicrobial use should be optimized using current pharmacological information and principles.
8. Antimicrobials considered important in treating refractory infections in humans or veterinary medicine should be used in animals only after careful review and reasonable justification. Consider using other antimicrobials for initial therapy.
9. Use narrow spectrum antimicrobials whenever appropriate.
10. Utilize culture and susceptibility results to aid in the selection of antimicrobials when clinically relevant.
11. Therapeutic antimicrobial use should be confined to appropriate clinical indications. Inappropriate uses, such as for uncomplicated viral infections, should be avoided.
12. Therapeutic exposure to antimicrobials should be minimized by treating only for as long as needed for the desired clinical response.
13. Limit therapeutic antimicrobial treatment to ill or at risk animals, treating the fewest animals indicated.
14. Minimize environmental contamination with antimicrobials.
15. Accurate records of treatment and outcome should be used to evaluate therapeutic regimens.

Product labelling, including the package insert, provides critical information regarding directions for use and other considerations for use of a new animal drug. While the label is paramount, additional sources of information can help inform a person with regard to the drug or assist in selecting an appropriate drug. For example, Freedom of Information Summaries that summarize the safety and effectiveness information submitted by a new animal drug sponsor are made publicly available for products that have been approved (including conditional approvals) or indexed. Additionally, FDA-CVM has constructed and made available to the public a searchable database of information on drug residues and pharmacokinetic parameters in fish reported in the literature (Phish-Pharm).[4]

INTERNATIONAL EFFORTS

Food safety and concerns regarding antimicrobial resistance are without borders. FDA's international efforts are critical to meeting its mandates to protect and promote public health.

In an effort to harmonize approval standards to enhance the protection of public health and improve government efficiencies, the FDA participates in international harmonization efforts such as the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). VICH is a trilateral programme involving the United States of America, the European Union and Japan, under the auspices of the World Organisation for Animal Health

3 Available at www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/JudiciousUseofAntimicrobials/ucm095473.htm

4 See www.fda.gov/AnimalVeterinary/ScienceResearch/ToolsResources/Phish-Pharm/default.htm

(OIE). It is aimed at harmonizing technical requirements for veterinary medical product registration. The FDA has also contributed technical expertise to the development of chapters in the OIE's Aquatic Animal Health Code related to antimicrobial resistance. In 2011, the Member Countries of OIE adopted Principles for the Responsible and Prudent Use of Antimicrobial Agents in Aquatic Animals.[5]

TABLE 2
Judicious use of antimicrobial agents for treatment of aquatic animals by veterinarians

1.	Accept responsibility for helping clients design management, immunization, production unit and nutritional programmes that will reduce the incidence of disease, and the need for antimicrobial treatment.
2.	Use antimicrobial drugs only within the confines of a valid veterinarian-client-patient relationship, including both dispensing and issuing of prescriptions and Veterinary Feed Directives. Extralabel usage should be consistent with regulatory agency laws, regulations and policies.
3.	Properly select and use antimicrobial drugs. Veterinarians should participate in continuing education programmes that include therapeutics and emergence and/or development of antimicrobial resistance.
4.	Have strong clinical evidence of the identity of the disease etiology, based on history, clinical signs, necropsy, laboratory data, and/or past experience before recommending antimicrobial drug treatment.
5.	Treat food fish with antimicrobial drugs according to the product label recommendations (including indication, dosage, duration, fish species and environmental conditions).
6.	Choose an antimicrobial drug and treatment regimen based on available laboratory and label (including package insert) information, additional data in the literature, and consideration of the pharmacokinetics, spectrum of activity and pharmacodynamics of the drug.
7.	Use antimicrobial drugs with a specific clinical outcome(s) in mind, including a specific target for population morbidity and/or mortality rate reduction.
8.	Determine production population pathogen susceptibility at the first indication of increasing morbidity or mortality and monitor the therapeutic response to detect changes in microbial susceptibility and to evaluate antimicrobial selections.
9.	Routine necropsy examination of fish populations should be periodically performed, including antimicrobial susceptibility testing, to update historical information for developing treatment and control protocols.
10.	Use products that have the narrowest spectrum of activity and known effectiveness *in vivo* against the pathogen causing a disease problem.
11.	Choose antimicrobial drugs of lesser importance in human medicine, if these receive future food fish use approval, and do not choose an antimicrobial for which emergence of resistance is expected to be in an advanced stage.
12.	Use, whenever possible, an antimicrobial drug labeled to treat the condition diagnosed.
13.	Do not compound antimicrobial drug formulations.
14.	Do not use antimicrobial drugs to treat cases with a poor chance of recovery.
15.	Ensure proper on-farm drug use and protect antimicrobial integrity through proper drug handling, storage and observation of the expiration date.
16.	Prescribe, dispense or write a Veterinary Feed Directive for drug quantities appropriate to the production-unit size and expected need using the approved formulation.
17.	Work with producers and/or facility fish health management personnel to ensure that farm personnel receive adequate training of the use of antimicrobial drugs, including indications, diagnosis, dosages, withdrawal times, route of administration, storage, handling and accurate record-keeping.
18.	Work closely with all other fish health experts involved in fish population health management at the fish production facility.

Similarly, the United States of America is an active member of the Codex Alimentarius Commission established by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization. Codex develops international food standards, such as maximum residue limits for veterinary drugs in food, to protect the health of consumers and ensure fair trade of foods. In 2011, the Codex Alimentarius Comission adopted Guidelines for Risk Analysis of Foodborne Antimicrobial Resistance (CAC/GL 77-2011)[6]. These guidelines provide a structured risk analysis framework to address the risks to human health associated with the presence in food and animal feed, including aquaculture, and the transmission through food and animal feed, of antimicrobial resistant microorganisms or determinants linked to non-human use of antimicrobial agents.

The FDA participates in additional international efforts to advance the responsible

5 www.oie.int/index.php?id=171&L=0&htmfile=chapitre_1.6.3.htm
6 www.codexalimentarius.net/download/standards/11776/CXG_077e.pdf

use of antimicrobials in aquatic species, such as the FAO Expert Workshop on Improving Biosecurity through Prudent and Responsible Use of Veterinary Medicine (Antimicrobials) in Aquaculture; this paper is part of the workshop proceedings.

FDA's Capacity Building Program includes a range of education, outreach and other activities where FDA collaborates with our regulatory counterparts of other countries to improve regulatory infrastructures, preventive controls and production practices to help insure the safety and quality of imported products into the United States of America.

CONCLUSIONS

FDA-CVM is currently addressing potential human health risks associated with the use of antimicrobial drugs in food-producing animals by:

- using risk assessment methodologies (e.g. Guidance 152) during the new animal drug approval process to quantify the human health impact from antimicrobial use in food-producing animals;
- actively conducting research to advance our understanding of antimicrobial resistance mechanisms and to inform our regulatory decisions;
- reaching out to stakeholders, including consumer groups, to strengthen and promote science-based approaches for managing the potential human health risks associated with the use of antimicrobial drugs in food-producing animals;
- assessing relationships between antimicrobial use in agriculture and subsequent human health consequences through NARMS; and
- participating in international initiatives aimed at protecting the safety of the food supply and proactively confronting concerns of antimicrobial resistance.

As science is constantly evolving and the Congress considers legislative proposals to address antimicrobial use in animals, FDA-CVM remains poised to meet its responsibilities for protecting public and animal health.

Oral delivery of veterinary medicines through aquafeed in Mediterranean aquaculture

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Zarza, C. 2012. Oral delivery of veterinary medicines through aquafeed in Mediterranean aquaculture. *In* M.G. Bondad-Reantaso, J.R. Arthur & R.P. Subasinghe, eds. *Improving biosecurity through prudent and responsible use of veterinary medicines in aquatic food production*, pp. 127—140. FAO Fisheries and Aquaculture Technical Paper No. 547. Rome, FAO. 207 pp.

ABSTRACT

Limited availability of licensed veterinary medicines for controlling diseases is one of the major problems in the Mediterranean aquaculture industry. Sanitary risks are increasing and the economic impact of diseases challenges the sustainability of the companies. For this reason, health management programmes should be emphasized in order to minimize the use of antimicrobials in fish farms. Administration of veterinary medicines through medicated feeding stuffs is the preferred method for treating fish in modern aquaculture. In this paper, the process of oral delivery of veterinary medicines is discussed from a practical point of view. The four parts of a prudent and responsible oral treatment are (1) early and accurate diagnosis of the disease; (2) proper selection of premix and dose; (3) good manufacturing of the medicated feedingstuffs; and (4) correct administration of the medicated feedingstuffs. The final success of the treatment will depend on the success of each separate part.

INTRODUCTION

Mediterranean aquaculture

Rainbow trout (*Oncorhynchus mykiss*), gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*) and turbot (*Psetta maxima*) are the main fish species farmed in the European Mediterranean countries. A total of approximately 300 000 tonnes was produced in 2008, the main producer countries being Greece, Spain, Italy and France (FEAP, 2008).

Production systems and techniques are highly diversified (Basurco, 2004). Rainbow trout is mostly farmed in freshwater, basically in intensive culture systems in land-based farms using concrete raceways or earthen ponds. The production of marine fish species, seabream and seabass, occurs mainly in off-shore cages, where there is a high exposure to environmental and thermal influences. There is also still some semi-intensive and extensive production of these species in earthen ponds. Turbot is cultured in intensive land-based farms using concrete tanks and recirculation systems.

Main sanitary risks

During the last five years, the health status of Mediterranean aquaculture has changed remarkably. The economic impacts of diseases are increasing, especially in seabass and seabream farmed in floating sea cages and in turbot culture. The main factors involved in this change are:

- *Growth and intensification of the production*. Farming companies are bigger in size and they work intensively, with more biomass, densities, stress and, consequently, higher sanitary risks. An important issue, especially in off-shore cages, is the concentration of farms in the same zone without minimal safety distances between them, facilitating the horizontal transmission of pathogens.
- *Unfavourable environmental factors*. Several environmental factors increase the susceptibility of cultured fish to pathological problems. These include high and sustained temperatures during summer; stress and mechanical injuries associated with intensive farming; storms and bad weather that affect off-shore cages; and farming systems that favour recirculation of pathogens, such as badly designed land-based farms (e.g. water inlet close to water outlet) and recirculation systems.
- *Deficient health management*. Probably because of the lack of strong regulations in this area, there is not too much awareness of the importance of preventive measures. Class separation, fallowing, effective mortality disposal and escapes control are some of the basic preventive measures that are not well implemented in Mediterranean off-shore cage farming. Other problems we can find are lack of implementation of biosecuriy and sanitary barriers, poor sanitary control of introductions (eggs, fingerlings, broodstock) and deficient early detection of pathologies.
- *Availability of veterinary medicines*. The limited availability of authorized veterinary medicinal products to address health risks remains one of the major problems for the aquaculture industry (COM, 2009). Traditionally, pharmaceutical companies have shown little interest in investing in veterinary medicines for fish, probably because of the low volumes. We have a limited number of antibiotic premixes and vaccines with marketing authorization, but almost no antiparasitic or antifungal drugs or anaesthetics. The few tools available to fight against fish diseases emphasize the importance of applying preventive measures in Mediterranean aquaculture.

Main diseases

Which are the pathological problems observed in the field? Basically, we can put them into four main groups according their origin: increase of prevalence of warmwater pathologies, aggravation of classic bacteriosis, higher impact of external and internal parasitosis and, finally, skin disorders of unknown origin.

Increase of the prevalence of warmwater pathologies

The appearance of these diseases and the difficulties in controlling them are conditioned by adverse environmental conditions present in the farms, such as high and sustained temperatures and poor water quality in recirculation systems.

- Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) is a viral disease affecting larvae and juveniles of many farmed marine fish species around the world (Munday, Kwang and Moody, 2002). During the past few years there have been continuous reports of the disease affecting seabass farmed throughout the Mediterranean, with increased severity owing to mixed infections with bacterial diseases like listonellosis ("vibriosis") and photobacteriosis. Our main concern for the future is the management of this highly contagious viral disease in floating cage areas without a strict legal sanitary policy.
- Streptococcosis is an emerging bacterial disease in freshwater and marine species

throughout the world. The main causal agents are *Lactococcus garvieae* in rainbow trout, *Streptococcus iniae* in seabass and seabream, and *S. parauberis* in turbot (Toranzo, Magariños and Romalde, 2005). Streptococcosis is responsible for severe mortalities during the high temperature season. Because of the lack of effective antibiotics against Gram-positive bacteria, the control method recommended is systematic intraperitoneal vaccination.

- Rainbow trout gastroenteric syndrome, caused by the segmented filamentous bacterium *Candidatus arthromitus*, produces severe catarrhal enteritis in rainbow trout in Europe (Michel *et al*., 2002). It is not possible to work *in vivo* with the bacterium, so the only way to prevent this syndrome is by improving the farming conditions, minimizing all stressors and implementing proper feeding management.
- *Edwardsiella tarda* is the causal agent of edwardsiellosis, an emerging bacterial disease in turbot farming that causes severe pathology and mortalities (Padros *et al.,* 2006). Relapses after antibiotic treatment are frequent if the water conditions are not optimal, so systematic vaccination would be necessary to control the disease in the future.

Aggravation of bacterial "classic" diseases

Well-known classic bacterial diseases, some of them controlled by vaccination, are causing trouble again. Some of the reasons for this recurrence are the appearance of new agents and/or biotypes, increasing resistances to antibiotherapy and mixed infections with other pathogens.

- Rainbow trout fry syndrome, caused by *Flavobacterium psychrophilum*, is an endemic bacteriosis affecting rainbow trout (Nematollahi *et al*., 2003). It can appear at early fry stages in the hatcheries, making it very difficult to effectively apply vaccination protocols. Relapses after oral antibiotherapy are frequently reported, and because of the necessity of continuous treatments, antibiotic resistances are lately observed.
- *Yersinia ruckeri* is the causal agent of enteric redmouth disease, or yersiniosis, a serious bacterial septicaemia affecting rainbow trout. Previously, it was well-controlled with systematic vaccination, but recently an increase of the prevalence of a highly virulent new biotype has made ineffective the licensed vaccines currently available (Fouz, Zarza and Amaro, 2006). Recently, new vaccines including the two biotypes have been developed in some countries.
- Photobacteriosis ("pasteurellosis"), caused by *Photobacterium damselae* subsp. *piscicida*, has lead to high mortalities in several farmed marine fish species all over the world (Toranzo, Magariños and Romalde, 2005). It is still a major problem in marine fish culture in the Mediterranean owing to the poor efficacy of existing vaccine protocols and, more recently, to mixed infections with VNN that cause the failure of oral antibiotherapy.
- Tenacibaculosis, caused by *Tenacibaculum maritimum*, is a common problem in marine fish, especially turbot, producing severe ulcerative skin lesions and high mortalities. In the past, effective vaccines and vaccination protocols were developed, minimizing the impact of the disease. Now existing vaccines are less effective owing to the appearance of new *Tenacibaculum* species antigenically and genetically different from *T. maritimum* (Piñeiro-Vidal *et al*., 2007). New vaccines including new *Tenacibaculum* species are being developed to control the disease.

Parasites without licensed effective treatments

Because of the lack of licensed antiparasitic drugs, external and internal parasites remain one of the main problems in Mediterranean aquaculture.

- Ectoparasitic flukes of the Class Monogenea are a severe problem in marine fish

farmed in floating cages, with *Sparicotyle chrysophrii* being responsible for severe outbreaks of mortality in seabream (Sanz, 1992). This parasite causes severe anaemia owing to its blood feeding and mechanical damage to the gills. Although some experimental trials in fish with oral antiparasitic medicines authorized for non-fish species have shown efficacy (Sitjà-Bobadilla, Conde de Felipe and Álvarez Pellitero, 2008), the lack of maximum residue limits (MRLs) of these medicines for fish prohibits their use in aquaculture.

- External protozoan parasites are a major problem in freshwater and marine land-based farms. The most important are the dinoflagellate *Amyloodinium ocellatum*, responsible for velvet disease in marine fish, and the ciliates *Ichthyophthirius multifiliis* and *Cryptocaryon irritans*, causing freshwater and marine white spot disease, respectively. *Amyllodinium ocellatum* is probably the most harmful; outbreaks can occur extremely rapidly and mortality can reach 100 percent in a few days (Noga and Levy, 2006).
- Scuticociliates are ciliated protozoans causing systemic ciliatosis in cultured turbot (Sterud, Hansen and Mo, 2000). These ciliates are normally free-living saprophytes, but under special circumstances, such as poor water quality or when fish are stressed and immunodepression occurs, they can become especially aggressive pathogens. From an initial external parasitosis, they will spread to almost all the internal organs of the host and can cause very high mortality.
- Enteromyxosis, caused by myxosporeans of the genus *Enteromxyum*, is probably the most serious disease in Mediterranean aquaculture. The main species are *E. leei* in seabream (Diamant, Lom and Dykova, 1994) and *E. scophthalmi* in turbot (Branson, Riaza and Álvarez-Pellitero, 1999). These parasites cause severe haemorrhagic enteritis, with destruction of intestinal mucosa that leads to emaciation and chronic mortality. In seabream, generally reduced growth and poor food conversion is observed. In turbot, several cases with mortality close to 100 percent have been reported.

Skin disorders of unknown origin

In the past few years, skin pathology is becoming more important in farmed rainbow trout and seabream in Europe. Skin disorders are a serious economic threat to the industry because, although they do not cause mortalities, prevalence and morbidity are extremely high, making affected fish unmarketable owing to the presence of extensive lesions on the skin. Although the aetiology remains unknown, the fact that all these problems respond well to antibiotherapy leads to a suspected bacterial involvement.

- Strawberry disease in rainbow trout has been recently subdivided based on its epidemiology (risk temperature) and pathological effects into warm water strawberry disease and cold water strawberry disease, also known as red mark syndrome (Verner-Jeffreys *et al*., 2008)
- Petequial rash skin syndrome is a novel disease affecting the skin and fins of seabream in floating cages, characterized by the presence of haemorrhages and ulcerations on different parts of the body and fins (Padros and Zarza, 2007). The epidemiology and pathology are very similar to cold water strawberry disease. So far the disease has been reported in Greece, Italy and Spain.

PREVENTION IS BETTER THAN CURE: HEALTH MANAGEMENT PROGRAMMES

All these diseases and their causal agents are widespread in all environments and culture systems in the Mediterranean. Considering the high sanitary risks inherent to the systems and explained above, the possibility to have severe disease outbreaks in the future is really high. That is the reason why farmers should act in a preventive way.

Only fish farms with effective health management programmes (HMPs) or good aquaculture practices will be viable in the near future. An effective HMP involves (1) a *horizontal*, holistic or global approach, where all points of the process have the same

level of importance; and (2) a *vertical* approach coordinated at all levels, from the farm to the national authorities. Participation of the governments in the application of the HMP is indispensable for its success. Legislation is the cornerstone in disease prevention (Gudding, 2012).

Which are the main points that should be included in an effective HMP?

- *Suitable farm location and design*. Current cage-farming systems must be reviewed, because the concentration of farms is not sustainable from the sanitary point of view. Recirculation farms should always have a separate quarantine facility to house new fish being introduced into the system.
- *Fish/eggs introduction control*. We must assure the quality and sanitary status of all the inputs to the farms. Pathological analysis by certified laboratories should be performed before fish or eggs enter into the farming systems.
- *Husbandry biosecurity and hygiene*. Optimal environmental conditions and water quality should be provided. Class separation and fallowing are the basic sanitary tools to avoid horizontal transmission of pathogens in floating cages. In land-based farms, sanitary barriers should be also established.
- *Continuous monitoring and early diagnosis*. It is really important to act before the expression of the pathology. We will see that early action is the first step for a successful treatment.
- *Vaccines and vaccination programmes*. Farmers should know the efficacy of the currently licensed vaccines and how to apply them properly in order to protect the fish throughout the production cycle.
- *Environmental and health-associated nutrition*. Diets should be formulated depending on species, size and production objective. Nutritional support with immunostimulants, vitamins and other functional ingredients can be used to enhance the fish immune system during risk periods.

Poor environment, poor water quality and poor on-farm practices mean more chemotherapeutic use, possible abuse and possible additional antibiotic resistances (Koonse, 2010). The final objectives of the HMP are to prevent diseases and to avoid the use of antimicrobial agents. However, if prevention fails and we need to treat with a veterinary medicine, we have to be sure to always treat in a prudent and responsible way. Before starting the treatment, we should know the legislation relating to the use of veterinary medicines in the European Union (EU).

LEGAL FRAMEWORK FOR USE OF VETERINARY MEDICINES IN THE EUROPEAN UNION

These are the most important EU regulations:

- Veterinary medicines in the EU are regulated by the Council Directive 2001/82 (CEC, 2001) amended by the Council Directive 2004/28 (CEC, 2004).
- Veterinary medicines incorporated in medicated feedingstuffs have to be in a specific form, the premix. Premixes follow the regulation of the Council Directive 90/167 (CEC, 1990a).
- Medicated feedingstuffs manufacturing, storage and delivery have to comply with EU legal feed hygiene requirements as laid down in Regulation (EC) No. 183/2005 (CEC, 2005).
- Residue limits of pharmacologically active substances in foodstuffs of animal origin are laid down by Regulation (EC) No. 470/2009 (CEC, 2009). Classification of pharmacologically active substances with regard to MRLs is laid down in Annexes I to IV of Regulation (EEC) No. 2377/90 (CEC, 1990b).

Some important definitions used in EU regulations:

- *Veterinary medicinal product*. (a) Any substance or combination of substances presented as having properties for treating or preventing disease in animals; or (b)

any substance or combination of substances that may be used in or administered to animals with a view either to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.

- *Premix for medicated feedingstuffs*. Any veterinary medicinal product prepared in advance with a view to the subsequent manufacture of medicated feedingstuffs.
- *Medicated feedingstuffs*. Any mixture of a veterinary medicinal product or products and feed or feeds that is ready prepared for marketing and intended to be fed to animals without further processing, because of its curative or preventive properties or other properties as a medicinal product.
- *Withdrawal period*. The period necessary between the last administration of the veterinary medicinal product to animals, under normal conditions of use and in accordance with the provisions of the Council Directive 2001/82, and the production of foodstuffs from such animals, in order to protect public health by ensuring that such foodstuffs do not contain residues in quantities in excess of the MRLs for active substances laid down pursuant to Regulation (EEC) No 2377/90.
- *MRL*. Maximum concentration of a residue of a pharmacologically active substance that may be permitted in food of animal origin. Classification of substances regarding MRL:
  Annex I: MRL established;
  Annex II: no need for MRL;
  Annex III: MRL established provisionally; and
  Annex IV: MRL not established.

Regulation of medicated feedingstuffs:

- A veterinary prescription shall be required for dispensing medicated feedingstuffs for food-producing animals, including farmed fish.
- The quantity prescribed and supplied shall be restricted to the minimum amount required for the treatment or therapy concerned.
- Medicines or pharmacologically active substances contained in the premixes must appear in Annexes I, II or III of Regulation (EEC) No 2377/90. The medicines must have MRL for fish.
- Medicated feedingstuffs for fish must be prepared with a premix licensed for fish. A premix has a licence in a member state for a determined fish species to treat a particular disease at a recommended dose and with a specified withdrawal period.
- If there is no licensed premix for fish containing the antimicrobial substances needed, the veterinarian responsible may, by way of exceptional prescription, under his/her direct personal responsibility and in particular to avoid causing unacceptable suffering, treat the fish species concerned with a premix licensed in the member state for another fish species, or for another food-producing species. In that case, the specified withdrawal period shall not be less than 500 degree-days for fish meat.
- Any other change in the use of the licensed premix (target fish species, particular disease or dose) implies an exceptional use of the premix.
- Medicated feedingstuffs must be prepared only in authorized places. Manufacturing on farms is allowed, but always with the permission from the Government. As the requirements of the facilities are high, few farms in the EU have the legal status to make medicated feedingstuffs. In general, all medicated feedingstuffs are made in feed factories.
- Farmers shall keep records of purchase, possession and administration of medicated feedingstuffs for five years. These records will include disease reports justifying the treatments, copies of veterinary prescriptions, and the lists of treatments including the withdrawal periods.

PRUDENT AND RESPONSIBLE ORAL DELIVERY OF VETERINARY MEDICINES

Treatment of fish diseases via the feed is the preferred administration system in aquaculture (Rodgers and Furones, 2009). The main advantage is the easy management of large volumes of fish without causing excessive stress. The main constraint is the generally compromised appetite of sick fish. Daniel (2009) recently provided a general overview of the current practices in the field of oral treatments.

We have divided the process of the oral delivery of veterinary medicines in four differentiated parts, all of them important for the success of the treatment:

- diagnosis of the pathology;
- selection of premix and dose;
- manufacturing of medicated feedingstuffs; and
- administration of medicated feedingstuffs.

Diagnosis

An early and accurate diagnosis of the disease is the first step to having a successful treatment. In a recent international Food and Agriculture Organization of the United Nations survey on the use of veterinary medicines in aquaculture (Alday-Sanz *et al.* 2012) , it was shown that the first cause of the failure of treatment is a poor/wrong diagnosis.

First of all, we have to be sure that the treatment is needed. Is the pathogen detected the primary cause of the disease? Most of the health problems in aquaculture are multifactorial, with several factors and pathogens involved, and we have to know the real cause, because maybe there is no need for the treatment and we could solve the problem just by reducing stress and changing environmental conditions.

As the main limitation of oral treatment is the poor appetite of sick fish, it is really important to start treatment as soon as possible, and the key for that is an early diagnosis of the disease. As soon as we detect the problem, we can start the treatment. We have to remember that we work with high biomasses of fish, where progression of disease can be very fast and mortality can be very high (e.g. acute bacteriosis in marine fish in floating sea cages). Also we have to keep in mind not only the time for the diagnosis, but also the time for the preparation and delivery of the medicated feedingstuffs to the farm.

We can see some examples of the importance of an early diagnosis in the evolution of the mortality pattern. Below are some field experiences from Spanish fish farms:

- *Pasteurellosis in 5 g seabream*. Mortality starting the treatment the first day of diagnosis was 2.5 percent; after one week it reached as high as 25 percent.
- *Vibriosis in 200 g seabass.* Mortality starting the treatment the first day of diagnosis was 0.7–1.5 percent, after one week it reached as high as 5–16 percent.

Not only is the speed of diagnosis important, but also the accuracy. Our diagnosis determines the veterinary medicine we choose to use for treatment. Empiric administration of medicines should be avoided.

When lots of fish are dying, there is usually high pressure on the aquatic animal health professionals (AAHP) to make a quick diagnosis, and sometimes there is no time to have a definitive diagnosis before we initiate a treatment. For example, we have to send a medicated feed with antibiotic to treat an acute bacterial septicaemia, and we need three or four days to complete the microbiological analysis and sensitivity test (antibiogram). In this case, to avoid great losses, we should initiate the treatment by choosing the antibiotic according to the historical data of the farm and the experience of the AAHP, but we must not forget to continue by finishing the diagnostic protocol. If we made a mistake in the presumptive diagnosis, we should apply corrective measures and, perhaps, change the antibiotic (Smith, 2010).

The following are needed for a quick and accurate diagnosis:

- training of farmers and AAHP in diagnostic protocols, including signal alarms detection and differentiation between normal and sick fish status (this is the most basic requirement);

- application of HMP, including continuous disease monitoring and surveillance programmes; and
- record-keeping, in order to have historical data of the treatments applied on a farm.

Premix and dose selection

Once we know the disease to treat, we first have to choose the antimicrobial agent and the dose. In general, the recommended dose of an antimicrobial for an oral treatment is given in mg of active ingredient per kg of body weight (BW) daily (mg/kg BW/d). Generally, this dose is specific to the antimicrobial agent and is given by the manufacturer of the premix.

Secondly, we have to calculate the desired dosage of the premix in the feed, in general, grams of premix per tonne of feed. Four important factors are involved in this second calculation:

1. *Concentration of active ingredient of the antimicrobial in the premix*. In general, we do not have 100 percent pure premixes, so we have to consider this factor when calculating the dosage in the feed.
2. *Daily feed intake, according to fish species, size and temperature.* It is really important to have real data about how the fish are eating. Trout fingerlings can eat at 2 percent of specific feeding ratio (SFR); in comparison, the SFR of a one kilogram seabream in winter time is really low, around 0.2 percent.
3. *Biomass to treat.* The number and average weight of the fish have to be monitored on a regular basis, because the daily antimicrobial dose depends on the quantity of fish (kg) being treated.
4. *Marine or freshwater species.* Some antimicrobials are less effective in seawater because of reduced bioavailability; for example, oxytetracycline that binds with divalent cations (Smith *et al*., 1996). In general, higher doses are advisable when treating marine fish.

Example: 10 000 seabass of average weight 200 g. Treatment against listonellosis with Flumesyva (Flumequine 10 percent, Syva, S.A.) at 15 °C with a SFR of 0.5 percent.

- Fish biomass to treat: 10 000 fish x 200 g = 2 000 kg
- Flumequine dose: 12 mg/kg BW/d
- Flumequine needed/day: 12 x 2 000 = 24 000 mg = 24 g
- Flumesyva needed/day: 24 x (100/10) = 240 g
- SFR = 0.5% means 0.5 kg feed/100 kg BW
- Total daily feed: 2 000 x (0.5/100) = 10 kg
- Premix final dose: 240 g/10 kg = 24 g/kg = 24 kg/tonne

Depending on the daily feed intake (SFR), we will mix the quantity of premix in a greater or lesser quantity of feed. Feed suppliers should be able to prepare medicated feedingstuffs with different doses of the same premix to give the exact quantity of antimicrobial regardless of SFR variations.

As we saw before, here the problem is that all licensed premixes have specific and strict recommendations from the manufacturer in terms of dose and duration that we should follow. Any variation means exceptional prescription and withdrawal time of no less than 500 degree days. We consider that it is more important to adjust the antimicrobial dose than to have a short withdrawal period.

Manufacturing of medicated feedingstuffs

Medicated feedingstuffs manufacturing, storage and delivery have to comply with EU legal feed hygiene requirements as laid down in Regulation (EC) No. 183/2005. Following this regulation, the European Federation of Feed Manufacturers has published a guide of good practices for the EU industrial compound feed sector, the *European Feed Manufacturers' Guide* (FEFAC, 2009), which includes specific recommendations for medicated feedingstuffs.

Medicines must be incorporated into the feed in the form of licensed premixes, which

are powdered mixtures including the active ingredient and one or several excipients (carriers). The minimum concentration of the premix added into the medicated feed must be 2 kg/tonne. The final dose of the premix can be variable, depending on the feed intake of the sick fish to be treated and the temperature of the water. If the appetite is really compromised and/or the temperature is low, a higher dosage would be advisable.

Example: Our 24 kg/tonne medicated feed with Flumesyva contains 2.4 kg/tonne of Flumequine. If our sick fish are eating at 0.5 percent of SFR, the final dose will be 12 mg/kg BW/d. However, if the temperature drops or the fish were really sick and were eating just 0.25 percent, we will not achieve the necessary therapeutic dose of the antibiotic, so maybe we would need a higher dose of premix.

Medicated feedingstuffs manufacturing systems

a) Pelleted or extruded medicated feedingstuffs

In the past, the medicated feed was made in the same line of manufacturing as the normal feed by adding the premix at the beginning of the process with the rest of the raw materials. This method is now outdated because it caused important problems of contamination and carry-over, and it was also impossible to predict the final concentration of the antimicrobial agent in the feed owing to the great loss resulting from the high temperatures and the pressure used during the manufacturing process.

b) Top coated or surface coated medicated feedingstuffs

Nowadays, the most used manufacturing system is the "surface-coating" system, where the medicine premix is mixed with the base feed in an industrial or pharmaceutical mixer/blender with the help of a binding agent, generally fish or vegetable oil. Generally, the medicated feed is produced in a specific and dedicated line of the feed factory, and the great advantage of this system is the accurate final concentration of medicine achieved in the medicated feed and the easy cleaning of the system. Some of the disadvantages of this system are the frequent problems related to homogeneity, palatability and leaching (Treves-Brown, 2000).

Premixes should be almost 100 percent pure and made of fine-size particles in order to use low doses and to avoid problems related to homogeneity and palatability; however, generally they are not 100 percent pure. The problem is when we have to increase the dose of the premix owing to the low feed intake of the sick fish and/or because the premix is low concentrated and made of rough particles, a situation that is very typical when we have to use by exceptional prescription a non-fish premix. Then we can face serious problems of homogeneity and palatability.

Sometimes good homogeneity of the finished medicated feedingstuffs can be complicated to achieve, depending of the quality and dosage used of the premix. A coefficient of variation not above 10 percent is suggested as quality criterion. Training of the staff responsible for the manufacturing process and continuous monitoring of the antimicrobial level in the finished feed are essential to have a good homogeneity.

Palatability of the medicated feed can be also an issue, causing dramatic reduction of the feed intake because of the bad taste of the premix. Moreover, if fish do not eat the medicated feed quickly, the loss of antimicrobial agent into the water via leaching might be important. In our experience, both problems can be minimized by starting the treatment early, thanks to an efficient and prompt diagnosis. On the other hand, it is possible to increase the palatability of the medicated feed by the addition of attractants such as fish and crustacean oil during the manufacturing process. Careful monitoring when the medicated feedingstuffs is administered would be really important to avoid an excess of pellets in the water.

Quality control and traceability

According to EU regulations, authorized facilities must have a control plan of the

medicated feedingstuffs manufacturing process, which must address the nature, the shelf life and the inclusion rate of the premix for medicated feedingstuffs, as well as the homogeneous dispersion of veterinary medicinal products throughout the finished feed. Particular attention should be paid to the carry-over of veterinary medicinal products into following batches of feed (FEFAC, 2009).

At least every three years, homogeneity, stability and carry-over tests should be conducted at regular intervals at the most relevant stage of the process. Results must be recorded. In practice, feed companies are constantly monitoring the quality of the medicated feed produced.

The manufacturer must also ensure that all information relating to the purchase, manufacture and delivery of medicated feedingstuffs is readily available and can be reconciled to enable traceability. The registers and veterinary prescriptions have to be kept for a period of at least three years.

Use of medicated feedingstuffs on farm

The final part of the process of a successful treatment is the use of the medicated feed on the farm. We have seen previously that starting the treatment too late is a common mistake that leads to high mortality owing to the failure to achieve high therapeutic blood levels because of poor appetite. But what happens in daily practice? From our experience in the field, the main mistakes in the use of medicated feedingstuffs in daily practice are the following:

- *Inadequate medicine selection*. Due to empiric treatments without proper diagnosis and without taking into account the historical data.
- *Low premix dose selection*. Due to (1) failure to accurately estimate the biomass to be treated; (2) lower than expected feed intake; and (3) failure to consider the concentration of antimicrobial agent in the premix. The need to correctly accomplish all these steps seems obvious, but errors still happen frequently.
- *Wrong duration of treatment*. If we start early, mortality is sometimes reduced dramatically after five to six days of treatment, and then frequently the farmer stops the treatment. That is the easiest way to have relapses. We always have to finish the prescribed course of treatment.
- *Use of antimicrobials as prophylactics*. Is it justified to treat with antimicrobials as a preventive because of the high risk of disease? Do we really prevent the outbreaks? We do not recommend this practice because outbreaks are generally not prevented, just delayed. That is the easiest way to create resistances. We see the prophylactic use of antimicrobials as the perfect example of non-implementation of early diagnostic methods on a farm. If you are able to detect the disease and start the treatment the first day, why do you need prophylactic treatments?
- *Use of antibiotics in viral infections (e.g. VNN)*. If we have just viral infections, is it necessary to treat with antibiotics? It can be justified only if there are secondary bacterial infections. We must stop the practice of always using antibiotics, just because "something has to be done".
- *Repeated use of the same antimicrobial agents*. We have a few licensed antimicrobial agents, and this sometimes leads to the use of the same ones continuously. In order to avoid resistance, we have to closely monitor the sensitivity of the isolates we obtained. Resistance does not happen from one day to the next; if we see a slight reduction of sensitivity, it would be time to rotate antibiotics.

These mistakes should be avoided. They can lead not only to treatment failures, but also to the development of resistance in fish pathogens and occurrence of residues in aquaculture products (WHO, 2006).

Best practices

The first thing to do, if possible, is to reduce the daily feed ration. Generally, modern aquaculture is characterized by an intensive feeding. It is advisable to reduce SFR to

between 0.5 and 0.75 percent to ensure that all the population is eating and also to minimize stress during feeding. It is also recommended to maintain the fish in starvation mode for 12 to 24 hours to increase their appetite; this could be coordinated with the time of arrival of the medicated feed to the farm.

In order to maximize the quality of the medicated feed delivered to the sick fish, manual administration of the feed is preferable rather than the use of automatic feeding systems. In giving the feed by hand, we force the feeders to take care of procedures and to monitor closely that sick fish are actually eating the medicated feedingstuffs. When high biomasses have to be fed (e.g. seabass and seabream in floating sea cages), small air cannons can be used, but it is important to do this carefully in order to minimize the loss of pellets outside the cages.

The number of daily feedings will be adapted not only to fish species, size and culture system, but also to the daily logistics of the farm. Small fish in nurseries will have more feedings than adult fish in on-growing, generally several (three to five) feedings for fingerlings and one or two for adult fish.

The duration of treatment is recommended by the premix manufacturer and also prescribed by the veterinarian, and typically will be around 8 to 12 days. Sometimes, in chronic disorders, such as furunculosis in turbot or bacterial kidney disease in trout, it can be longer. From our experience, if after 15 days of treatment, there is no improvement of the situation, it is time to stop the treatment and analyze the fish again; maybe we made a wrong diagnosis at the beginning of the process.

CONCLUSIONS

The lack of pharmacologically active substances with MRL specific for fish is perhaps the main cause of the limited availability of veterinary medicines in European Mediterranean aquaculture. The intention of the new Regulation (EC) No. 470/2009 (CEC, 2009) is to address this problem in the coming years by simplifying the methodology for the establishment of a MRL. In particular, one interesting and quick approach proposed is the extrapolation between food-producing species. This will surely increase the availability of veterinary medicines for treating diseases in farmed fish, but we are sceptical about the suitability of most of the premixes for oral treatments. Probably, pharmaceutical companies will just extend the number of target species of existing premixes to include fish, instead of investing in an expensive development of new premixes designed specifically for fish. As was mentioned previously, non-fish premixes are usually low concentrated and made of rough particles, which sometimes makes their use impossible for manufacturing medicated feedingstuffs for fish owing to the severe problems of homogeneity and palatability.

In the process of the oral delivery of veterinary medicines through aquafeed, all four parts are important individually. An early diagnosis is essential to start the treatment as soon as possible, but also important is the selection of a correct dose of a high-quality concentrated premix to make the most homogenated and palatable medicated feedingstuffs, which will be carefully and responsibly administered. We need to succeed in each individual part in order to have a successful treatment.

The current shortage of veterinary medicines should not discourage fish farmers in their daily fight against diseases. Governments must promote the implementation of holistic and coordinated health management programmes that minimize the need for antimicrobials and contribute to the sanitary sustainability of the companies. Prevention is always better than the cure.

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Disease prevention as basis for sustainable aquaculture

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ABSTRACT

Sustainability should be the basis for aquaculture industry. Disease prevention is fundamental for sustainability. Legislation is the cornerstone in disease prevention. Vaccination is the single most important preventive measure in aquaculture.

INTRODUCTION

The concept of sustainability has been widely used for economic development based on the use of natural resources. The Brundtland Commission defined sustainability as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs". For aquaculture, this means that the fish farming industry should be managed in such a way that negative long-term effects on the environment are acceptable (Håstein, 1995; Roberts and Muir, 1995; Ford and Myers, 2008).

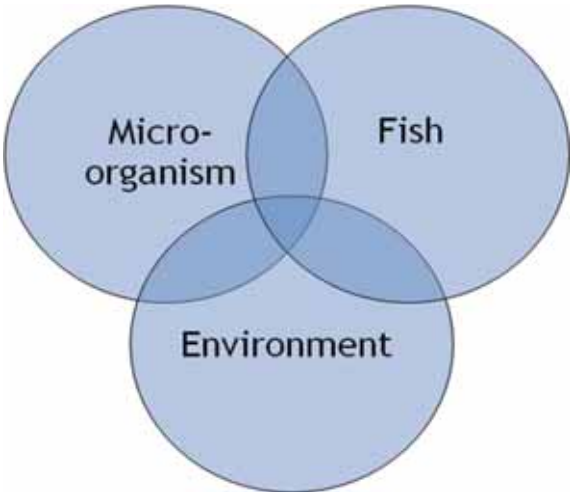
The modern aquaculture industry in Norway is approximately 40 years old. Sustainability without unacceptable impact on environment and populations of wild fish has been an aim during all these years. However, during the last years some scientists and environmentalists have questioned the sustainability of the Norwegian aquaculture industry. Following the recent crisis in the Chilean aquaculture industry, which has collapsed because of lack of sustainability, both industry and authorities are concerned about future development, including sustainability, in the Norwegian aquaculture industry.

There are various indicators of sustainability in aquaculture. Parameters of pollution of water and contamination of products give information about environmental impact. Loss during the production period owing to escapes and diseases is another indicator. Disease prevalence and incidence based on surveillance and monitoring are informative, since the production is based on live animals. Since infectious diseases are treated with antibiotics or chemotherapeutants, the amount of these compounds used during production and the prevalence of residues in aquaculture products are relevant indicators of sustainability. A low prevalence of resistant micro-organisms and parasites might also be indicative of a sustainable bioindustry. Finally, in countries like Norway with wild salmonid fish in marine waters, the prevalence of salmon lice on farmed salmon is a good indicator of sustainability.

Disease prevention and control are, therefore, crucial in order to maintain an efficient

usage of resources and a sustainable aquaculture industry. A good health status is also fundamental for welfare reasons. In order to be efficacious, prophylactic measures should be based on knowledge of the interactions of the health triangle, i.e. the host, the micro-organism and the environment (Figure 1) (Snieszko, 1974).

FIGURE 1
The health triangle: relationship of the micro-organism, the fish and the environment. Disease occurs in the central area where all three circles overlap (from Snieszko, 1974)



ENVIRONMENT

The environmental issues associated with aquaculture have many aspects. In the beginning of the aquaculture era, the use of the sea for fish farming was free. The sea has been regarded as common property, which could be freely used for transport and harvest, i.e. fishing. However, even in Norway, a country with a long coastline, the availability of suitable aquaculture sites is now limited.

The aquaculture industry has high requirements related to water quality. The fish need clean water in order to grow well and to be healthy. At the same time, salmonid production results in some pollution, with consequences for the health and well-being of farmed fish, as well as impacting other activities in rivers, lakes and coastal areas. The fact that farmed fish are living in the same water as wild fish represents another great environmental challenge.

Increasing aquaculture activity in the coastal zone showed that there was a need for regulation by legislation for a variety of reasons, including prevention and control of disease (Håstein and Gudding, 2005). Thus, the first requirement for disease prevention in sustainable aquaculture is a legal framework. In Norway, this is established in the Aquaculture Act. In order to establish a fish-farming operation, a licence is necessary. Before the licence is awarded, all legitimate interests for the particular area of the coastal zone are considered. New operations are only established on sites where the risk for introduction of infectious disease, as well as spread of disease, is low.

Even with regulations for the establishment of fish farms there will always be outbreaks of disease. Consequently, it is crucial to have laws and regulations dealing with prevention, control and eradication of fish diseases. The first law relating to diseases of fish was passed by the Norwegian parliament in 1968, i.e. before the beginning of commercial aquaculture. This law became a useful basis for biosecurity, including disease prevention, in farms with salmonid fish in freshwater as well as in seawater.

Key issues in aquatic animal disease legislation are classification and notification of diseases, approval of establishments, regulations on trade, import and movement of fish, and programmes for science-based surveillance of the population. Furthermore, the

legislation deals with prevention, control and eradication, including contingency plans and vaccination, zoning and fallowing of sites, and disinfection of water and farms.

A licence for a fish farm generally includes more than one site, often three. Two of these may be used at the same time, and one is fallow so that the self-cleaning process of the water and the bottom underneath the cages can take place. The introduction of this system was a consequence of the disease problems in aquaculture, and it is an important measure in the efforts to obtain good management and good health.

FISH FEED

The feed is also a part of the environment. The quality of the feed is important for many reasons. Vertebral malformations, cataract and development of tumours are possible consequences of suboptimal composition or inferior quality of fish feed. These abnormalities are welfare problems and, consequently, not acceptable in sustainable fish farming.

Feedstuffs for the salmonid industry are based on fishmeal and fish oils. With the declining supply from fisheries, plant ingredients are included in increasing amounts as a supplement. One health consequence is the finding of intestinal cancer in Atlantic salmon fed with high amounts of feedstuffs of plant origin (Dale *et al.*, 2009). In sustainable aquaculture, salmonid fish should be allowed to be carnivorous fish species.

The use of genetically modified organisms (GMOs) in fish feed is also a controversial issue when considering sustainability in aquaculture. Feeding fish with feedstuffs containing GMOs does not seem to have negative impact on the health of the fish. However, it may be questioned whether the feeding of fish with feed containing GMOs is in accordance with the aim of a sustainable aquaculture.

There are laws regulating the safety of feed. This is important for preventing diseases and maintaining good welfare and also in securing food safety. The use of GMOs in fish feeds and in the production of human food is also regulated by law. Unauthorized GMOs in feed are forbidden; only GMOs that have been approved based on risk assessment are allowed for use in feedstuffs for animals. Furthermore, the amount of GMOs that can be included in feeds for both terrestrial and aquatic animals is restricted.

MICRO-ORGANISMS AND PARASITES

After 30 to 40 years of intensive fish farming in Norway, infectious diseases remain a threat to sustainability. During the first years, the disease problems were mainly bacterial diseases caused by *Vibrio* spp. The picture today is more complex: viruses, fungi and parasites may also cause disease outbreaks. The disease panorama is multifactorial, and environmental factors are predisposing for clinical signs and mortality. Another challenge is related to the farming of marine fish species. Diseases in new farmed fish species such as cod and halibut are caused by micro-organisms different from those affecting salmonids.

Bacterial diseases caused by the genera *Vibrio* and *Aeromonas* are effectively controlled by vaccination. However, there are still bacterial diseases for which effective preventive measures are lacking. Among these are infections caused by intracellular micro-organisms, such as *Renibacterium salmoninarum* and *Piscirickettsia salmonis* in salmonids and *Francisella philomiragia* in cod.

For many years the most important viral diseases in Norwegian aquaculture were infectious pancreatic necrosis (IPN) and infectious salmon anaemia (ISA). In spite of various prophylactic measures, these two diseases still represent a problem for the industry (Biering *et al.*, 2005).

Other diseases in salmonid fish with a confirmed or suspected viral origin are an increasing challenge to the industry. These include salmonid pancreas disease, heart and skeletal muscle inflammation, and cardiomyopathy syndrome. For the two latter diseases, isolation and propagation of the viruses in the laboratory has so far been unsuccessful. This means that experimental studies of the disease, including the pathogenesis, as well

as research on prophylactic measures like vaccines, are difficult.

Currently, the greatest challenge for the aquaculture industry in Norway is the salmon louse (*Lepeophtheirus salmonis*). Salmon lice may jeopardize sustainability for several reasons, as they have both direct and indirect effects on the salmonid fish that they attack. Furthermore, salmon lice can be transmitted to wild fish. There are pharmaceuticals available for treatment of salmon lice that are fairly effective if used correctly, but the prevalence of resistant salmon lice is increasing. The problems with salmon lice cannot be solved in a sustainable way with chemical treatment by bath or using medicated feed. The solution for sustainable aquaculture should be an integrated approach using different types of prevention such as structural measures, i.e. zoning and fallowing, use of wrasse, treatment with biologically acceptable chemical components and, in the future, possibly even vaccination.

The general lesson learned from the problems with salmon lice in intensive salmon farming is that parasites might be more difficult to control in a sustainable way than micro-organisms such as bacteria, viruses and fungi.

PROPHYLACTIC MEASURES

The emphasis on science-based biosecurity throughout the entire production chain has been fundamental to the progress made in aquaculture, both in developed and developing countries (Corsin, Giorgetti and Mohan, 2007). This includes competence in disease prevention, control and eradication in governmental authorities, as well as in the private sector. Appropriate legislation and enforcement are essential to effective biosecurity. However, this requires an effective organization with clear responsibilities at both the central and local levels. The command principles between the levels of the organization as well as the relationship to institutions providing scientific and technical support should be well defined. Finally, cooperation with the industry and communication to the public are important for the management of any biosecurity programme (Håstein and Gudding, 2005).

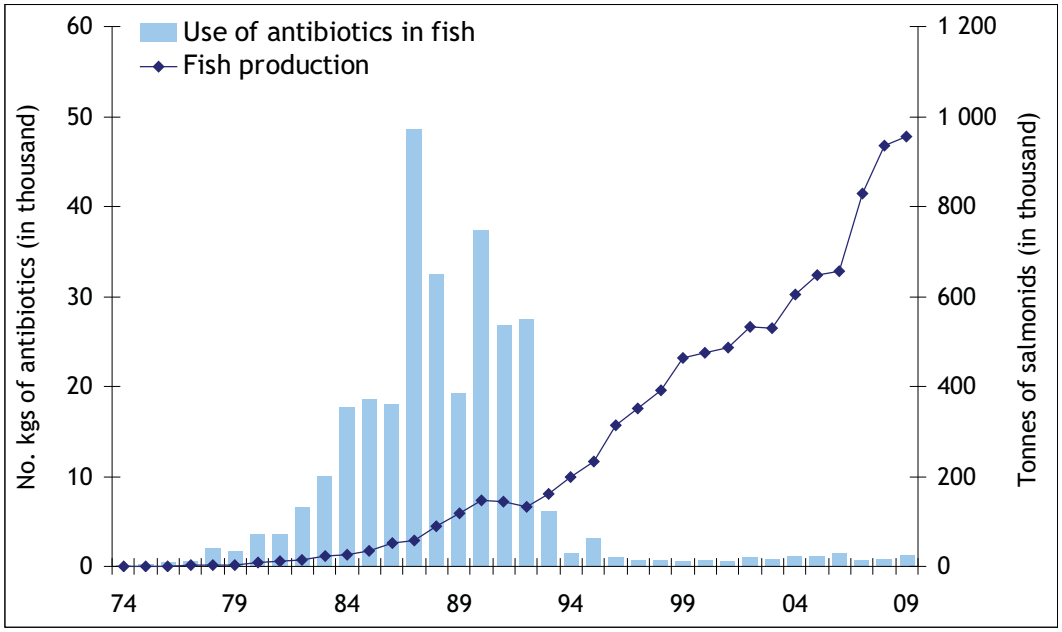
Biosecurity includes effective disease control and management of emergency situations. Implementation of measures such as movement restriction, stamping out, zoning and fallowing, disinfection, health certification, sanitary slaughter, risk analysis and establishment of control and surveillance zones is crucial in order to reduce the economic, social and environmental impacts of diseases (Håstein *et al*., 2008; Subasinghe and Bondad-Reantaso, 2008).

One of the reasons for the successful development of a sustainable fish-farming industry in Norway and other countries is the fact that immunoprophylaxis has been used for disease prevention. The immune system of fish can be stimulated by vaccines and be an effective mechanism preventing development of clinical diseases, as well as reducing spread of infection.

Vaccination with inactivated vaccines has been used for more than 30 years. During the first years, vaccination was performed by immersion. This method was effective against bacteria like *Listonella anguillarum*, *Vibrio salmonicida* and *Yersinia ruckerii*. However, in order to prevent furunculosis caused by *Aeromonas salmonicida* subsp. *salmonicida* adjuvants had to be added to the antigens (Håstein, Gudding and Evensen, 2005). Consequently, the vaccine had to be applied by injection. Today, all salmonid fish in Norway are vaccinated against vibriosis, cold water vibriosis and furunculosis before transfer to seawater. In most cases, antigens from two other micro-organisms are also included in a multivalent vaccine. However, so far the vaccines against winter ulcer (caused by *Moritella viscosae*) and IPN have not given sufficient protection.

The success of vaccination of salmonid fish can be illustrated by figures showing the use of antibiotics in fish farming. In 2009, the production of farmed fish was approximately 954 000 tonnes, with 1 313 kg of antibiotics being used. The data shown below (Figure 2) on production of farmed fish in Norway and use of antibiotics are a documentation of sustainability.

FIGURE 2
Use of antibiotics and production of salmonid fish in Norway from 1974 to 2009



However, there are still challenges, especially related to the relatively small production of cod and other marine fish. In 2009, the amount of antibiotics used was similar for the production of salmonids and marine fish, whereas the production volume of the marine species was less than 2 percent of that for salmonids. The use of antibiotics in the farming of cod is a consequence of the fact that some bacterial diseases affecting this species are difficult to prevent. *Francisella* is an intracellular bacterium, and it is well known that prevention of diseases owing to such intracellular micro-organisms using inactivated vaccines is difficult. In other countries, the use of live attenuated vaccines seems to be a solution leading to the successful prevention of intracellular micro-organisms based on vaccines (Shoemaker *et al.*, 2009). In Canada, a DNA vaccine against infectious haematopoietic necrosis was licensed a few years ago (Salonius *et al.*, 2007). In Norway, the possible risks associated with DNA vaccines have limited the use of such vaccines in aquaculture. However, this issue is now under reconsideration.

Vaccination is the most important preventive measure in order to maintain sustainability in salmonid fish farming. However, there is still a great need for research in this area in order to use the potential of the immune system of fish in a beneficial way. Here the governmental and private sector have to work together. Among the many areas for cooperation are the development of vaccines against intracellular micro-organisms, oral vaccines, live vaccines, adjuvants and improved vaccines based on molecular biology.

CONCLUSIONS

The conclusion based on 30 to 40 years' experience with intensive salmonid fish farming in Norway is that sustainability should be the basis for developing a successful aquaculture industry. The Norwegian experience is that disease prevention is fundamental for sustainability. Legislation is the cornerstone in disease prevention, while vaccination is the single most important preventive measure. Finally, the authorities and the industry must be well organized and have the right competence on all levels.

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Health management tools from a manufacturer's point of view

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ABSTRACT

The objective of this paper is to cover the approach that is taken to developing, implementing and managing the health tools (medicines and diagnostic tools) used in aquaculture. It provides an overview of the development and use of veterinary medicines in aquaculture and highlights some of the major challenges in expanding their availability for aquaculture use. As a manufacturer of medicines and health products for aquatic animals, Intervet Schering-Plough Animal Health, now known as MERCK Animal Health, is fully engaged with producers, veterinarians and feed companies in the actual use of medicines. In the wider context of sustainable medicine use, we also work with the regulatory bodies, quality assurance programmes, best practice schemes and other organizations to ensure that the use of our products is both efficacious, as well as safe for the consumer, the farmer, the fish and the environment. Minimizing the risks from disease and, when required, the treatment of fish with the appropriate medicine, are key parts of sustainability in aquaculture. The paper looks to the future and considers practical solutions to the challenges of providing veterinary medicines for sustainable aquaculture. There is an opportunity to ensure the responsible and sustainable use of medicines in aquaculture worldwide. The knowledge is available and the required products are available or can be developed. With a clear harmonized regulatory environment that will ensure globally accepted standards, the needs and expectations of the producers and the consumer for safe efficacious medicines can be met and sustainable aquaculture can be achieved.

INTRODUCTION

As a specialist aquatic health business within a larger animal and human health company, Intervet Schering-Plough Animal Health shares a similar business environment with that of the aquaculture producers it supplies. The business is influenced by many factors not directly related to producing and selling products. The major components of the environment that we consider are illustrated in Figure 1.

FIGURE 1
Major components of the operating environment of Intervet/Schering-Plough Animal Health

The first circle of interaction is on the farm with veterinary professionals and a focus on delivering health programmes for the fish being produced. This interaction is delivered by our technical representatives and is discussed in more detail later in the paper. However, the use and follow-up on medicines in aquaculture has much wider implications than just "treating" the pond or cage of fish.

As a manufacturer of medicines and health products for aquatic animals, Intervet Schering-Plough Animal Health is fully engaged with producers, veterinarians and feed companies in the actual use of medicines. In the wider context of sustainable medicine use, we also work with the regulatory bodies, quality assurance programmes, best practice schemes and other organizations to ensure that our products are both efficacious, as well as safe for the consumer, the farmer, the fish and the environment. Minimizing the risks from disease and, when required, the treatment of fish with the appropriate medicine, is a key part of sustainability in aquaculture. The efficiency of production is compromised by disease, leading to wasted feed resources, reduced production per area of water or land, and increased risk of having to treat. Effective treatment and disease prevention is one of the main drivers in achieving the required growth of aquaculture production to meet the expanding demand for good quality food for human consumption.

WHAT IS A MEDICINE IN AQUACULTURE?

The following is the European Union (EU) definition of a veterinary medicine that

applies equally to aquaculture:

> *"veterinary medicinal product" means – (a) any substance or combination of substances presented as having properties for treating or preventing disease in animals; or(b) any substance or combination of substances that may be used in, or administered to, animals with a view either to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis.*[1]

If the substance falls under this definition, it must be registered and approved as a medicine. A medicine is not just an active ingredient or chemical entity.

WHAT MEDICINE TYPES EXIST AND ARE REGISTERED FOR AQUATIC SPECIES?

The types of medicine that are registered for use in aquatic species include:

- *Vaccines*. These are products that are directly or indirectly produced from the pathogen and administered to the animal to elicit a specific (lasting) immune response for the prevention of a range of mainly bacterial and viral diseases. Vaccines are widely used in intensive farming conditions worldwide. They are supplied as immersion, oral or injection preparations. Vaccines provide pathogen-specific disease prevention.
- *Antibiotics*. For treatment and cure of bacterial infections in fish.
- *Anti-parasitic products*. Delivered in feed or by bath for the treatment of external parasites such as sea lice and *Benedinia*.
- *Anti-fungal disinfectants.* For eggs and infected fish.
- *Immunostimulants.* These are designed to enhance the natural non-specific immune parameters of fish and shrimp to defend against mild infections and environmental stress that might trigger outbreaks.

AQUACULTURE MEDICINES CYCLE

With the specific definitions of aquaculture medicines in mind, it is possible to consider a cycle for developing and managing an aquaculture medicine (Figure 2).

The cycle starts with the identification of a disease and its underlying cause, normally a pathogen. This requires both a rapid first reaction methodology as well as a more sophisticated scientific investigation system. It is designed to both identify a cause of disease and to implement an immediate remedial reaction, as well as to fully understand a disease aetiology as the basis of developing long-term solutions and prevention methods.

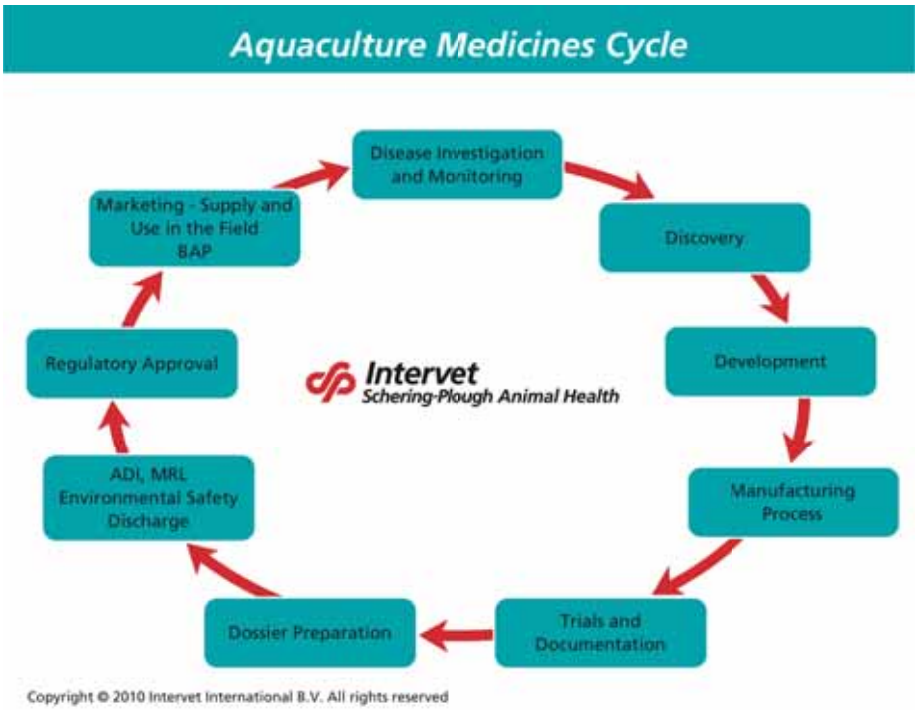
The next steps are to "discover" a cure. A search of the published literature reveals reports of very many substances having efficacy against a very wide range of pathogens. These early discoveries are the first step to developing a medicine. They are a very long way from having an acceptable product that can be safely used to treat or prevent a disease problem in fish or shrimp that are intended for human consumption. Unfortunately, these "published" discoveries" have led to the unregulated use of some very undesirable compounds in aquaculture just because they appear to "work". The introduction of better surveillance and quality programmes, as well as better education, information and pressure from regulatory bodies, have begun to reduce this problem.

The discovery of a compound that is effective against a pathogen leads to the product development phase. This requires a high level of investment and expertise, and a great deal of work is undertaken with the active compound or the vaccine antigen to document its quality, safety and efficacy, addressing the regulatory requirements and above all, ensuring that control systems are in place to guarantee the same product standards throughout. The cost and complexity of the work means that for pharmaceutical products destined for use in aquaculture, the active ingredients will usually be registered for other animal species and/or other markets larger than aquaculture. Vaccines, however, are

[1] *Source:* The Veterinary Medicines Regulations 2009 – United Kingdom, Veterinary Medicines Directorate (www.opsi.gov.uk/si/si2009/uksi_20092297_en_2#pt1-l1g2)

specifically developed and registered for aquaculture. The registration package covers all aspects of the product, and most of the data generated must come from the final product formulation that will be, or is intended to be, placed on the market. The data cannot be extrapolated from other similar formulations or manufacturers.

FIGURE 2
The aquaculture medicines cycle



Development documentation is generated covering the manufacturing processes and procedures, quality control checks and validated pass criteria for each stage of the manufacturing process. Compliance with the process and procedures is key to ensuring the consistency and reliability of the medicine being produced. This is critical for the on-farm performance, but even more importantly, to ensuring that the fish is safe and wholesome for human consumption.

SAFETY AND TOXICITY PROGRAMME: PHARMACEUTICAL PRODUCTS

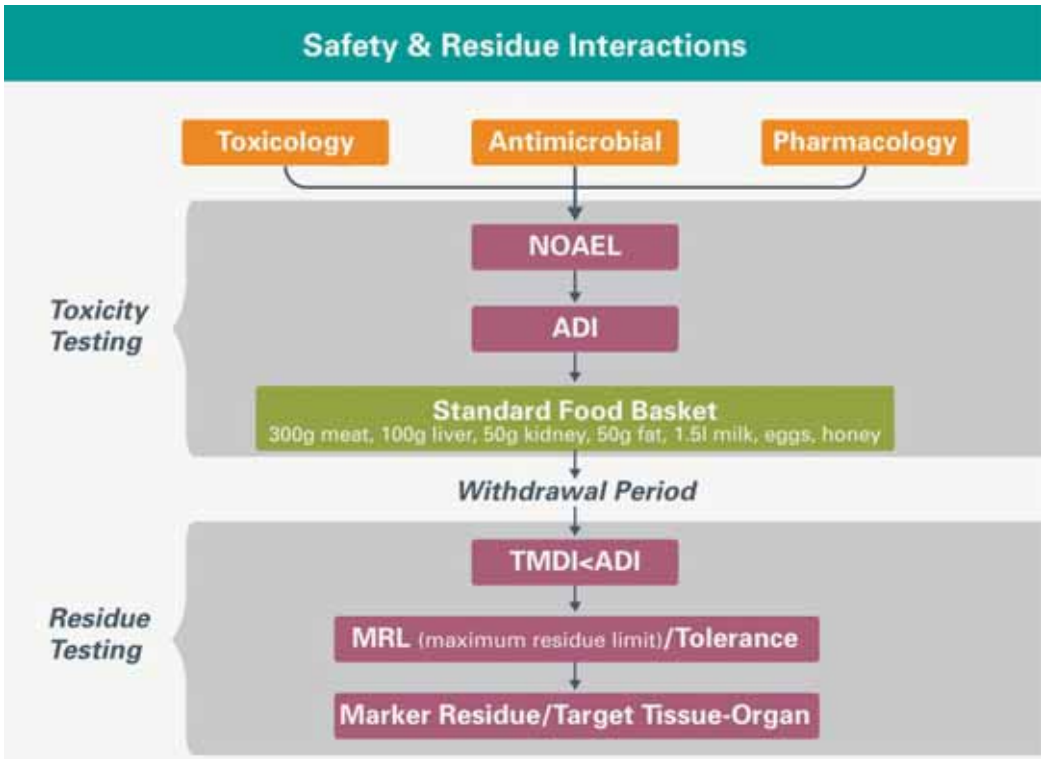
The following list of issues must be evaluated and fully understood, with the active ingredient and safe levels established before an active ingredient can be developed into a medicine:

- pharmacological properties of the active ingredient;
- acute, subchronic and chronic toxicity;
- reproduction and embryo/foetotoxicity;
- mutagenicity;
- carcinogenicity studies;
- immunotoxicity;
- microbial properties of residues;
- target animal safety; and
- environmental issues.

Figure 3 describes the steps and procedures required to establish an acceptable withdrawal time for a pharmaceutical medicine. The toxicological/safety development work allows an acceptable no effect level (NOEL) to be established. The acceptable daily intake (ADI) is then calculated from this level. This establishes how much of the active ingredient or its metabolites can be consumed without posing a risk to the consumer.

The ADI is then compartmentalized between the components of the "standard food basket", with fish being included in the daily meat ration (300 g). This is used to establish the maximum residue limit (MRL) that can be accepted in fish. This is measured in the edible tissues, which are considered to be the fillet, i.e. muscle with normal proportion of skin attached.

FIGURE 3
Safety and residue interactions



Once an MRL is established, the manufacturing company must demonstrate that the formulated product used under the recommended conditions will deplete to ensure that the active compound and/or its metabolites will be at levels lower than the MRL after the defined withdrawal period has elapsed.

The implementation of human food safety procedures is important both in the country where the fish is produced and in the country of destination for exported products. International (Codex Alimentarius) and national requirements have to be strictly followed to ensure that the safety requirements of the importing countries are fully met. These are usually enforced by port of entry inspections. When a farm uses a registered medicine in the correct way and follows the guidelines for withdrawal, it can be confident that the use of the product will not result in a product that contains a harmful residue or cause any disruptions in the trade of foods.

This veterinary medicines approval process ensures that the medication used is safe for the consumers, the environment, the user and, of course, for the fish; that it is efficacious; and that it is produced to an approved quality standard.

Once the medicine has been approved, the manufacturing company continues to bear the responsibility for the marketing and technical support for the product. The pharmaceutical company has to follow specific pharmacovigilance responsibilities to monitor any unexpected problems (adverse reactions) that may arise with the use of the medicine in the field.

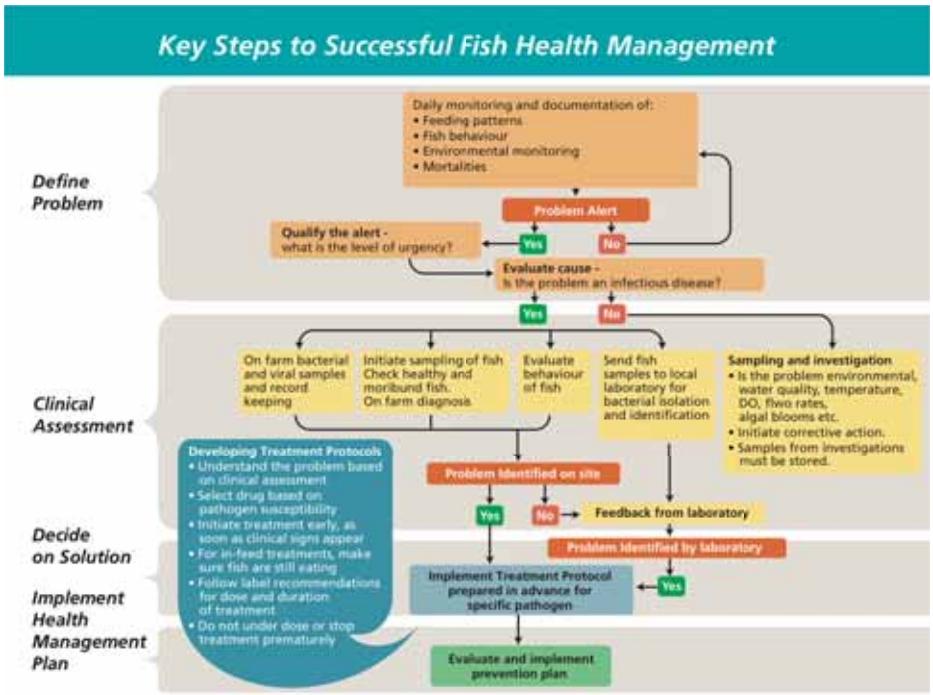
Beyond these responsibilities, Intervet/Schering-Plough Animal Health has a clear role to play in supporting veterinarians and farmers in achieving the best performance from the medicines that they use and rely on to achieve their production goals. The key steps to successful fish health management are shown in Figure 4.

The key steps in this process are:

- Clearly define the health problem or, specifically, the disease that exists on the farm. Solutions are specific to the pathogen and cause of the disease. Therefore, the best way of maintaining the sustainable and responsible use of medicines in aquaculture is the use of the approved medicine for the given pathogenic situation.
- Based upon a correct diagnosis, the choice for the approved medicine and its approved use is mandatory. This will lead to effective treatment and optimal use of therapeutants. This is absolutely key in maintaining the availability and performance of medicines.

FIGURE 4

Key steps to successful fish health management



FUTURE PROSPECTS

In an ideal world, farmers would have a full "tool kit" of medicines and diagnostic services to monitor, control and prevent the diseases that threaten their stock. The tool kit would comprise vaccines for preventing the major endemic diseases, immunostimulants and other such feed additives to enhance the performance of the fish under farming conditions, and a range of treatment products to cure any new or sporadic future infections. All of these products would be fully approved, documenting their quality, efficacy and safety. The farms and industry would have the support of accurate diagnostic services and of veterinarians or health professionals – allowing them to develop and implement effective veterinary health plans and utilize the medicines in compliance with good treatment practices and industry codes of practice.

This is already possible in some parts of the world, and the impact has resulted in great improvements in sustainability and increased productivity, as well as improved farming efficiency. However, there are still challenges to achieving this in Asia, where there are many fish and shrimp species cultured, many diverse pathogens, a diverse environment and variable access to knowledge and information.

SOLUTIONS

From a manufacturer's point of view, solutions to the challenges for the sustainable use of medicines in aquaculture could include international harmonization of regulatory data requirements for approving products in the different regions. Some of the particular challenges that relate to the claims needed to support the use of the products in the variety of species being farmed are:

- the idea of crop grouping, i.e. use of representative species (e.g. Atlantic salmon) of a similar group or production environment to allow use in the whole group (e.g. salmonid fish);
- extrapolation of MRLs from major species to minor species;
- the development of a network of facilities and experts able to disseminate and validate information to support health management in the region; and
- the development and implementation of veterinary health plans so that farmers can treat and sell their produce with confidence.

The provision of these practical solutions needs to be backed up with effective certification and enforcement of the regulation.

CONCLUSIONS

In conclusion, there is an opportunity to ensure the responsible and sustainable use of medicines in aquaculture worldwide. The knowledge is available, and the required products are available or can be developed. With a clear and harmonized regulatory environment that will ensure globally accepted standards and meet the needs and expectations of the producers and the consumer for safe efficacious medicines, sustainable aquaculture can be achieved.

Alternatives to antibiotics in aquaculture

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ABSTRACT

The need to minimize antimicrobial use in aquaculture is widely recognized by academia as well as the industry. Researchers have been investigating different mechanisms by which the impact of diseases in aquaculture can be reduced. Successful health management in aquaculture requires considering all three components of Snieszko's epidemiological triad – the host, the pathogen and the environment. Although vaccination has been very successful in reducing losses owing to some of the major bacterial diseases in salmonid aquaculture, there are very few vaccines available for the cyprinids that remain high in the list of cultured species. There are also few commercial vaccines against viral diseases in salmonids and no vaccines for parasitic diseases. Because the immune system in invertebrates is poorly developed compared with that of vertebrates, vaccination of shrimp and prawn is still a distant goal. Immunostimulants have been widely used in both finfish and crustacean aquaculture to modulate the innate immune response. Strategies such as improving aquaculture practices and using probiotics and bioremediators could play a role by not only improving the host and the environment, but also by suppressing the pathogens. Bacteriophage therapy is now being explored in the medical field for treatment of antibiotic-resistant pathogens, and there are also examples of its application and further potential for use in aquaculture. Thus, there are a number of options available for health management in aquaculture and for minimizing antimicrobial use. A holistic approach emphasizing ecofriendly management is the need of the hour.

INTRODUCTION

Although aquaculture contributes significantly to the economies of many developing countries, the industry has been facing serious problems from the mass mortalities caused by disease. Several countries in Asia and South America have lost millions of dollars owing to the mass mortalities (Bondad-Reantaso *et al.*, 2005), with both finfish and shrimp/prawn aquaculture facing problems due to disease. In this scenario, the aquaculturists tend to fall back on chemotherapeutic agents in an effort to save their stock. The epidemiological triad of Snieszko suggests that disease outbreaks in aquaculture systems are a consequence of the breakdown of a delicate balance between the host, the pathogen and the environment. In finfish, as well as shrimp aquaculture, pathogens are usually present in the environment or even in the host in a dormant state. When there is a shift in the balance between the host, the pathogen and the environment that favours the pathogen, we often see an outbreak of disease. For example, while black

tiger shrimp (*Penaeus monodon*) in aquaculture ponds are often positive for two or three viruses when tested using very sensitive molecular techniques such as polymerase chain reaction, they do not show signs of disease (Umesha *et al.*, 2006). Thus, there can be infection (presence of a pathogen in a host), but no disease (interruption of the normal structure or function of a body part or organ or system characterized by a set of clinical signs). Chemotherapeutic agents or antimicrobials target only the pathogen, and most often do so in a non-selective manner that affects the normal flora as well. They are useful to overcome the acute stage of disease; however, the goal of a health management strategy is to prevent disease. Therefore, a comprehensive health management strategy should involve improving the host conditions and the environment around the host and reducing the pathogens. Some of the health management strategies that have application in aquaculture that are alternatives to the use of antimicrobial agents are discussed in this paper.

IMPROVING THE HOST CONDITIONS AND THE ENVIRONMENT

Good aquaculture practices

Although the use of good aquaculture practices (GAqPs) for health management (also termed "good management practices" (GMPs), "better management practices" (BMPs) or "best management practices") has been advocated by many agencies, there are few studies that attempt to quantify the reduction in disease prevalence achieved by their use. Following the outbreak of disease from the whitespot syndrome virus (WSSV) in India, there were massive crop losses. The Marine Products Export Development Authority of India, in collaboration with the Network of Aquaculture Centres in Asia and the Pacific, initiated a programme to develop BMPs in the State of Andhra Pradesh. The BMPs developed included a comprehensive set of measures such as good pond preparation, water quality management, pond bottom management, biosecurity and avoidance of animals carrying WSSV, good quality seed selection, feed management and waste management (Umesh *et al.*, 2010). As most shrimp farmers in India are small farmers, often with a single pond, a cluster approach was used through which farmers in an area come together and follow the same practices. Over a period of four years, this approach led to a 31 percent reduction in disease prevalence as compared to non-BMP ponds (Umesh *et al.*, 2010).

Vaccination

Vaccination has been very successfully used for disease control in animal husbandry, and early studies done on fish immunology indicated that fish are capable of developing adaptive and specific immunity with a memory component (van Muiswinkel, 2008). In the 1970s, it was observed that immersion of fish in formalin-inactivated broth cultures was effective against *Vibrio* spp. (Evelyn, 1997). Application of vaccination in commercial aquaculture perhaps began in Norway, the major driving force being the huge losses to the salmon aquaculture industry caused by vibriosis in the 1980s. In 1987, nearly 50 000 kg of antibiotics were used for the production of about 5 000 tonnes of salmon; however, the usage dropped dramatically following the development of vaccines (Sommerset *et al.*, 2005). The quantity of antibiotics used by the Norwegian salmon industry in 2003 was only 805 kg active ingredient and the fish production was over 500 000 tonnes (Burridge *et al.*, 2008). However, the use of antibiotics in salmon aquaculture varies depending upon the country; for example, for the production of 280 481 tonnes of salmon in Chile during 2003, 133 800 kg antibiotics were used, while in Canada in 2003, 30 373 kg of antibiotics were used to produce 111 178 tonnes of salmon. Thus, it is evident that apart from availability of commercial vaccines, there are other factors such as regulatory pressure that influence antimicrobial use in the aquaculture industry. Currently, commercial vaccines are available mainly for salmonid species (Table 1). According to the Food and Agriculture Organization of the United Nations (FAO) statistics (FAO, 2007), global aquaculture production in 2004 was dominated by carps and cyprinids (18.3 million tonnes) and shrimps and prawns (2.76

million tonnes), while salmon and trout production was only about 1.9 million tonnes. It is important to take stock of the fact that although carps are high on the aquaculture production list, there are no commercial vaccines available for the carp aquaculture that takes place mostly in Asia.

TABLE 1
Fish pathogens against which commercial vaccines are available for use in aquaculture

Pathogen	Disease	Fish
Aeromonas salmonicida[1]	Furunculosis	Salmonids
Vibrio anguillarum[2]	Vibriosis	Salmonids, cod
Vibrio ordalli	Vibriosis	Salmonids
Vibrio salmonicida	Cold water vibriosis	Salmonids
Edwardsiella ictaluri	Enteric septicaemia	Catfish
Yersenia ruckeri[3]	Enteric redmouth disease	Salmonids
Streptococcus spp.	Streptococcosis	Asian seabass, tilapia
Photobacterium damselae	Pasteurellosis	Seabass, seabream
Moritella viscose	Winter ulcer	Salmonids
Flavobacterium columnnare	Columnaris disease	Salmonids, channel catfish
F. psychrophilum	Flavobacteriosis	Salmonids
Piscirickettsia salmonis	Piscirickettsiosis	Salmonids
Renibacterium salmoninarum	Bacterial kidney disease	Salmonids
Lactococcus garvieae	Lactococcosis	Rainbow trout, Amberjack/ yellowtail
Infectious pancreatic necrosis virus	Infectious pancreatic necrosis	Salmonids
Viral haemorrhagic septicaemia virus	Viral haemorrhagic septicaemia	Trout, flounder
Salmonid pancreas disease virus	Pancreas disease	Salmon
Infectious salmon anaemia virus	Infectious salmon anaemia	Salmon
Infectious haematopoitic necrosis virus	Infectious haematopoietic necrosis	Salmon
Iridovirus	Iridoviral disease	Red seabream, yellowtail

[1]Combined furunculosis-vibriosis vaccine also available.
[2]Combined vibriosis-furunculosis or vibriosis-yersiniosis vaccines marketed.
[3]Combined vibriosis-yersiniosis vaccine also marketed.

There are problems in vaccinating fish related to the route of administration, with immersion and injection being the two commonly practiced modes of delivery. Most vaccines are in injectable form, since they have to be delivered with adjuvants and automated vaccination machines are used (Sommerset *et al*., 2005). A number of factors are known to influence the ability of vaccines to protect fish. These include environmental factors such as temperature and pollutants; host factors such as age and general health; husbandry factors such as handling, stress, diet and antibiotics; and vaccine-related factors such as dose, nature of antigen, route of administration and presence of adjuvants (Karunasagar and Karunasagar, 1999). Most vaccines are against bacterial pathogens, while a few vaccines for viral diseases are available (Table 1). Although parasites cause significant problems in the aquaculture industry, there are no commercial vaccines for parasitic infections.

While teleost fish have a recognizable adaptive immune response, the situation with respect to invertebrates like shrimp and prawn is not clear. Although it is commonly believed that they do not have an adaptive immunity comparable to vertebrates, experimental studies indicate that it is possible to induce protection in shrimp through injection/oral administration of viral proteins (Witteveldt, Vlak and van Hulten, 2004; Witteveldt *et al*., 2004); however, the mechanism of protection is not known.

Use of immunostimulants

Vaccines depend on the acquired immune system. Studies on the ontogenic development of different lymphoid organs (thymus, kidney, spleen), and acquired immune parameters (B and T lymphocytes and expression or secretion of IgM) suggest that this system develops late in salmonid species. Hence, these species depend on innate defence for

the first two to three months after hatching (Magnadottir, 2006) and, most likely, the situation would be similar in freshwater fish such as carps and other cyprinids. Studies done with various fish species show that the innate immune system can be upregulated with the help of various immunostimulants (Sakai, 1999). Many of the immunostimulants reported are molecules derived from microbial cell wall or outer membrane with characteristic patterns consisting of repeating units (e.g. glucans, lipopolysaccharides, peptidoglycans, chitin, chitosan) and have been termed "pathogen associated molecular patterns". These molecules recognize "pattern recognition receptors" or "pattern recognition proteins" of the innate immune system of the host. Stimulation of the innate immune response is indicated by parameters such as phagocytosis, activation of reactive oxygen and microbicidal activity in granulocytes, macrophage migration, complement activation and resistance to challenge by microbial pathogens (Sakai, 1999). There are numerous studies on immunostimulants, and most of them report improved resistance to challenge by various bacterial pathogens; however, some studies indicate that there is no effect (Sakai, 1999). Most commercial immunostimulants are derived from yeast and seaweeds containing β-1, 3 and β-1,6 glucans in the case of the former and alginates and polysaccharides in the case of the latter. Delivery of immunostimulants is generally by bath immersion for larval stages or through feed. Pulse feeding is commonly practiced. In shrimp aquaculture in India, the intervals of delivering immunostimulants through feed ranged from 4–7 days (Karunasagar and Karunasagar, 1999), and in salmonid culture it could range from 4–6 weeks (Bricknell and Dalmo, 2005). In salmonid aquaculture, feeding with diet supplemented with immunostimulants has been demonstrated to reduce sea lice settlement and provide better protection against furunculosis and vibriosis (Bricknell and Dalmo, 2005). Immunostimulants are reported to be widely used in seabass and seabream aquaculture.

Some concerns have also been raised about the use of immunostimulants in larval fish, in which there could be induction of tolerance. Problems also exist with respect to delivery system for fish larvae in flow-through systems and the effect on biological filters in recirculatory systems. Considering the variability in response in different fish species, the interval of feeding in pulse feeding strategy is another issue that needs to be resolved (Bricknell and Dalmo, 2005).

PATHOGEN REDUCTION/ELIMINATION

Probiotics

The term "probiotic" has been traditionally used to refer to live microbial feed supplements that beneficially affect the host by improving its intestinal balance (Fueller, 1989). A Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food recommended the following definition: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). However, the term has been more broadly used in aquaculture to refer to microbial agents that have beneficial effects on cultured animals in a number of ways (Gatesoupe, 1999). Most of the aquaculture probiotics are thought to act by modifying the microbial community around the animals in favour of beneficial micro-organisms that may improve the water or sediment quality, suppress pathogenic bacteria, stimulate the immune system of the host or improve host digestion (Gatesoupe, 1999; Verschuere *et al*., 2000). Suggested mode of action for probiotic bacteria in aquaculture include competitive exclusion (competition for nutrients, iron, adhesion sites), production of compounds inhibitory to fish/shrimp pathogens, enhancement of host immune response and degradation of harmful wastes like ammonia (Verschuere *et al*., 2000)

In contrast to lactic acid bacteria that have been used as probiotics in terrestrial animals, a range of Gram-positive and Gram-negative bacteria have been used in aquaculture (Table 2). *Bacillus* spp. are widely used as probiotics in shrimp aquaculture, but Gram-negative bacteria like *Vibrio alginolyticus* and *Pseudomonas* spp. have been shown to be

effective as well. Probiotic bacteria have been shown to enhance survival, moulting rate and growth of black tiger shrimp (Rengpipat *et al*., 1998) and *Litopenaeus vannamei* (Garriques and Arevalo, 1995). They also reduce populations of pathogenic *Vibrio* spp. (Moriarty 1998; Chythanya, Karunasagar and Karunasagar, 2002; Karunasagar *et al*., 2005), improve digestibility of food (Liu et al., 2009) and stimulate the immune system of *P. monodon* (Rengpipat *et al*., 2000). In various studies, application of probiotics has been either to larval rearing tank water or pond water or by addition to the feed. Addition of probiotic bacteria such as *Lactobacillus* spp., *Bacillus* spp., *Carnobacterium* spp. and *Roseobacter* spp. to larval rearing water has been found to improve survival of turbot larvae, salmonid fingerlings and channel catfish production (Balcazar *et al*., 2006).

TABLE 2
Examples of bacteria reported as probiotics for aquaculture

For finfish	For shrimps and prawns
Aeromonas spp.	*Arthrobacter* spp.
Bacillus spp.	*Bacillus* spp.
Carnobacterium spp.	*Lactobacillus* spp.
Cytophaga spp.	*Pseudomonas* spp.
Lactobacillus spp.	*Thalassobacter* spp.
Pediococcus spp.	*Vibrio* spp.
Pseudomonas spp.	
Roseobacter spp.	
Vibrio spp.	

Feed supplementation has been preferred in grow-out ponds and has been found to be more effective than direct addition to rearing water (Hai, Buller and Fotedar, 2009), but this may depend on the intended purpose in using the probiotic. If the purpose is to improve water quality, then application to rearing water would be more useful. However, although bioremediation potential of probiotic bacteria has been proposed (Gatesoupe, 1999), some studies failed to confirm this in shrimp ponds (Rengpipat *et al*., 1998). In my laboratory, we used a microcosm system (Figure 1) to evaluate the bioremediation ability of probiotic bacteria. In plastic tubs, the pond conditions were simulated using pond sediment, pond water and shrimp feed to create eutrophic conditions.

FIGURE 1
Example of an experimental system for evaluating the bioremediation potential of probiotics (Karunasagar, unpublished)

As shown in Figure 1, in the control tub, the sediment turned black due to hydrogen sulphide production, as happens in a shrimp pond that is poorly maintained. Because the probiotic bacteria had bioremediation potential, in the treated tub we observed reduction in ammonia levels and clearing of the sediment. With funding from the Department of Biotechnology, Government of India, my laboratory has been involved in testing commercial probiotics for shrimp farmers, and some of the common problems noticed have been low counts of bacteria and absence of some of the bacteria claimed to be present in the product label (Karunasagar, unpublished data). Field studies in Indonesia and Thailand show improved shrimp health, better production and increased economic returns to the farmers (Moriarty, 1998). However, there is not much information on the colonization and establishment of probiotic bacteria in the hatchery or pond environment following initial application. In some hatchery studies, daily application of *Bacillus* probiotic was carried out, but the probiotic application resulted in larval survival rates similar to that obtained with antibiotic application (Decamp, Moriarty and Lavens, 2008).

As China is the world's top aquaculture producer, the practices used there would be of interest to other countries. Qi *et al*. (2009) indicated that there are over 100 companies producing over 50 000 tonnes of probiotics for aquaculture in China with a market value of 50 million euros. Interestingly, in China, photosynthetic purple non-sulphur bacteria such as *Rhodopseudomonas palustris*, *Rubrivivax gelatinosa*, *Rhodobacter capsulata*, *R. sphaeroides* and *Phaespirillum fulvum* are used as probiotics in the culture of fish, shrimp and scallops (Qi *et al.,* 2009). Bacteria such as *Bacillus* spp., *Pseudoalteromonas* spp. and *Phaeobacter inhibins* that have antagonistic activity against fish/shrimp pathogens such as *Aeromonas hydrophila*, *Vibrio anguillarum* and *V. harveyi* are also widely used in China. Often, photosynthetic bacteria, *Bacillus* spp., some nitrifiners and denitrifiers are combined and behave as multifunctional probiotics. Another interesting application in China is *Bdellovibrio bacterivorous* in fish, shrimp, crab and sea cucumber aquaculture as a biocontrol agent. Although probiotics for aquaculture had a booming market, in 2008 there was a decline in sales of about 50 percent because of decreased farmer confidence in these products. Qi *et al*. (2009) indicate that quality control of commercial products is an issue. This also seems to be an issue in other countries, as my own experience in India shows that the products often do not contain the range of bacteria claimed in the label and bacterial levels are also below the claimed counts.

Regulatory approval for use of probiotics as feed supplements has been documented in some regions. European Union permits use of authorized probiotics for disease control in organic aquaculture (EC, 2009).

Phage therapy

Bacteriophages were discovered during 1915–1917, and their potential application in treatment of bacterial diseases was recognized soon thereafter. However, the discovery of antibiotics in 1941 and their effectivity in treatment of wounds in soldiers during the Second World War led to a decline in the interest in bacteriophages as therapeutic agents. However, the recent emergence of multi-drug resistance in several bacterial pathogens in human medicine has led to a renewed interest in phage therapy. Bacteriophages are widely distributed in the environment. In the aquatic environment, there are tenfold more phages as compared with bacteria (Skurnik and Strauch, 2006). The life cycle of a bacteriophage may include a lytic stage, and some bacteriophages have their genome inserted into the host chromosome and enter a lysogenic stage. Lysogenic bacteriophages are involved in gene transfer in bacteria, and some of the virulence factors found in bacteria (e.g. ability to produce cholera toxin by *Vibrio cholerae* O1) have been associated with bacteriophages inserted into the bacterial genome. Hence, it would be important to use only lytic bacteria in bacteriophage therapy.

Bacteriophages are host specific; hence, they lyse only the target bacteria, unlike antibiotics, which inhibit the growth of or kill most bacterial groups. Thus, bacteriophage therapy would not suppress useful commensal flora that are required for the health

of the animals. Application of bacteriophages in therapy against fish pathogens was investigated by Nakai and coworkers (Nakai *et al*., 1999; Park *et al*., 2000; Nakai and Park, 2002). They used bacteriophages belonging to the family Siphoviridae that were isolated from the aquaculture environment. Oral administration of bacteriophages against *Lactococcus garvieae* to young yellowtail (*Seriola quinqueradiata*) resulted in 100 percent survival following intraperitoneal challenge with the pathogen as compared with 10 percent mortality in the control (Nakai *et al*., 1999). Oral administration of phage (mixture of two bacteriophages, one belonging to the Myoviridae and another belonging to the Podoviridae) impregnated feed to ayu (*Plecoglossus altivelis*) brought down cumulative mortality to 22.5 percent from 65 percent in the control following oral challenge with *Pseudomonas plecoglossicida* (Park *et al.*, 2000). In both studies, the authors used oral administration, and this would be very convenient in aquaculture. The fish digestive tract has a relatively high pH and, therefore, acid sensitivity of phages would not be an issue in aquaculture (Nakai and Park, 2002).

Bacteriophages against the shrimp pathogen *V. harveyi* may belong to the Siphoviridae or the Myoviridae (Oakey and Owens, 2000; Shivu *et al*., 2007; Crothers-Stomps *et al.,* 2010). Generally, members of the Siphoviridae have been reported to be lytic phages (Vinod *et al.,* 2006; Shivu *et al*., 2007; Karunasagar *et al*., 2007; Crothers-Stomps *et al*., 2010). A *V. harveyi* myovirus-like (VHML) phage has been reported to be temperate and confer virulence to the host strains (Pasharawipas *et al*., 2005). Shivu *et al*. (2007) tested the host range of a collection of *V. harveyi* phages against 180 isolates from different geographical regions. Three strains belonging to the family Siphoviridae were able to lyse 65–70 percent of the strains, indicating broad host range. Vinod *et al.* (2006) tested bacteriophage therapy of larval and postlarval shrimp (*P. monodon*) in both laboratory microcosms and in hatchery during a natural outbreak of luminous bacterial disease. The bacteriophages were added to the larval tanks. In microcosms, larval survival was 25 percent in the control and 85 percent with treatment, while in hatchery trial, the survival was 86 percent with bacteriophage application, 40 percent with antibiotic treatment and 17 percent in the control where there was no application of either phages or antibiotics. Bacteriophage treatment brought down counts of luminous bacteria in the tanks. In another hatchery trial during a natural outbreak of luminous bacterial disease, 86–88 percent survival was obtained with bacteriophage treatment compared to 65–68 percent with antibiotics (Karunasagar *et al*., 2007). These studies show the potential for bacteriophages to be effective alternatives to antibiotics in shrimp larval health management. Bacteriophages used by Vinod *et al.* (2006) and Karunasagar *et al.* (2007) lacked the putative virulence gene carried by VHML and hence the concern regarding carriage of virulence gene could be minimal.

One of the problems in shrimp larval health management is the persistence of *V. harveyi* in the hatchery environment by forming a biofilm that is resistant to antibiotic and sanitizer treatment (Karunasagar, Otta and Karunasagar, 1996). The ability of bacteriophage to bring about a 3-log reduction in biofilm cells of *V. harveyi* on high-density polyethylene surfaces was demonstrated by Karunasagar *et al*. (2007). This provides an additional advantage for use of bacteriophages in shrimp larval health management. However, considering the host range of 65–70 percent for selected phages, phage therapy with a consortium of phages would be necessary to ensure effectivity with unknown strains causing disease outbreaks (Table 3).

Application of bacteriophages in the biocontrol of pathogens has been permitted by regulatory authorities in some situations. Use of the commercial product "AgriPhage" from Omnilytics Inc. against plant-pathogenic bacteria has been permitted by the United States Environmental Protection Agency, and use of Listeria LMP 102 from Intralytix Inc. for control of *Listeria monocytogenes* in ready to eat meats and poultry products has been permitted by the United States Food and Drug Administration (Garcia *et al*., 2008). These show that bacteriophage application in agriculture and aquaculture would be safe from a consumer protection point of view.

TABLE 3
Reported examples of phage therapy in aquaculture

Aquacultured species	Pathogen	Route of administration	Reference
Yellowtail (*Seriola quinqueradiata*)	*Lactococcus garvieae*	Oral	Nakai *et al*., 1999
Ayu (*Plecoglossus altivelis*)	*Pseudomonas plecoglossicida*	Oral	Park *et al*., 2000
Black tiger shrimp (*Penaeus monodon*)	*Vibrio harveyi*	Addition to larval rearing tank water	Vinod *et al*., 2006
Black tiger shrimp (*Penaeus monodon*)	*Vibrio harveyi* biofilm	Addition to water	Karunasagar *et al*., 2007

CONCLUSIONS

Work done in several parts of the world shows that there are a number of technologies available that could contribute to minimizing the use of antimicrobial agents in aquaculture. Good aquaculture practices could contribute to substantial reduction in disease problems. Immunostimulants have potential to improve disease resistance against a wide range of pathogens. Use of other technologies discussed in this paper could be specific to species of fish cultured or to the group of pathogens involved. For example, vaccines are currently available for only a limited number of bacterial and viral pathogens affecting fish cultured in temperate or cold waters. Considering that antimicrobials are primarily used against bacterial diseases, probiotics and bacteriophages are promising alternatives effective against bacterial diseases. The use of bioremediators to manage the environment would help greatly in disease prevention and managing outbreaks.

ACKNOWLEDGEMENTS

Financial support by the Department of Biotechnology, Government of India, through the programme support to aquaculture and marine biotechnology for research on "alternatives to antibiotics in aquaculture" is gratefully acknowledged.

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PART 2

REPORT OF THE FAO/AAHRI EXPERT WORKSHOP ON IMPROVING BIOSECURITY THROUGH PRUDENT AND RESPONSIBLE USE OF VETERINARY MEDICINES IN AQUATIC FOOD PRODUCTION

Bangkok, Thailand, 15–18 December 2009

Report of the FAO/AAHRI Expert Workshop on Improving Biosecurity through Prudent and Responsible Use of Veterinary Medicines in Aquatic Food Production

Bangkok, Thailand, 15–18 December 2009

BACKGROUND

Modern aquaculture, through the intensification of culture systems and the diversification of both the species cultured and the culture methods employed, often creates an ideal environment for disease-causing organisms (pathogens) to flourish. The expanded and occasionally irresponsible global movements of live aquatic animals have been accompanied by the transboundary spread of a wide variety of pathogens that have sometimes caused serious damage to aquatic food productivity and resulted in serious pathogens becoming endemic in culture systems and the natural aquatic environment. One of the most effective management responses to emergencies associated with infectious disease epizootics is the use of appropriate antimicrobial[1] treatments. However, the inappropriate use of antimicrobials can lead to problems related to increased frequency of bacterial resistance, with negative impacts on the efficacy of these agents to control infectious diseases in aquaculture and the potential transfer of resistance genes in bacteria from the aquatic environment to other bacteria and the possibility of resistance extending to human pathogens. Injudicious use of antimicrobials has also resulted in the occurrence of their residues in aquaculture products, resulting in commodity bans by importing countries and associated economic impacts, including market loss. The use of antimicrobials to control infectious diseases in agricultural farming systems, including aquaculture operations, on a routine basis will be difficult to sustain. Since disease emergencies occur even in well-managed aquaculture operations, careful planning on the use antimicrobials is essential in order to maximize their efficacy and minimize the selection pressure for increased frequencies of resistant variants, an automatic consequence of their use.

Traditionally, the threats to aquaculture posed by aquatic pathogens have been addressed through the use of antimicrobials, including chemotherapeutants, disinfectants, antibiotics and vaccines. However, by themselves these agents cannot fully prevent losses due to disease. A holistic approach is required by modern aquaculture, and this can be achieved only through effective biosecurity programmes whereby pathogens are excluded from the culture environment. Biosecurity safeguards animal health, enhances food safety, promotes environmental sustainability and protects biodiversity. It can also stimulate increased market supply and private investments, as it enables farmers to produce healthy products that are highly competitive in the market and also demonstrates that an exporting country is a responsible trading partner. Biosecurity enables developing countries to grow more food efficiently, increase their incomes and thus improve their resilience, reducing their vulnerability and enabling them to respond effectively to the impacts of higher food prices and other food production risks. Effective biosecurity

[1] An antimicrobial agent is any substance of natural, semisynthetic or synthetic origin that at *in vivo* concentrations kills or inhibits the growth of microorganisms by interacting with a specific target.

plays an important role at every stage of the life cycle of an aquatic animal from hatching to harvesting and processing, and is thus essential to ensuring sustainable and healthy aquatic production.

The responsible use of antimicrobials is an important part of farm biosecurity, as this helps ensure that pathogen challenges are minimized, that the natural defence mechanisms of the cultured stocks are maximized, that disease and mortality, including costs of containing, treating and/or eradicating diseases are reduced. The injudicious and/or incorrect use of antimicrobials poses a great concern to successful and sustainable aquaculture. In order to develop appropriate strategies or guidelines that will enable the rational and prudent use of antimicrobials, particularly by small-scale aquaculturists, we need to assess the current situation with regard to the extent of their use and misuse, and to have a good general understanding of how these substances are being applied in aquaculture.

The Food and Agriculture Organization of the United Nations (FAO) is promoting a holistic approach to modern aquaculture through effective biosecurity actions taken at different levels ranging from more responsible international trade in aquatic organisms to better on-farm practices. At the policy level, biosecurity actions may include effective compliance with international standards, national policies and strategies for aquatic animal health and food safety; improved disease diagnosis and extension services; and effective regulations and adequate infrastructure to enforce them. At various levels of the aquaculture production chain (manufacturers, farmers and consumers), biosecurity actions may include capacity building, awareness raising, education and dissemination of extension and other technical materials on good husbandry practices, vaccination and the appropriate use of immunostimulants and probiotics. Prudent and responsible use of antimicrobial therapy (rather than further restrictions), effective enforcement of current regulations and improved access to disease diagnostic services and extension support to farmers will result in better control of antimicrobial usage.

More than ten years ago, FAO, in cooperation with the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC-AQD) and the Canadian International Development Agency (CIDA), organized the Expert Meeting on the Use of Chemicals in Aquaculture in Asia or "Aquachem" from 20–22 May 1996 at the SEAFDEC-AQD headquarters in Tigbauan, Iloilo, the Philippines. Since the 1996 Aquachem expert meeting, a number of other developments and consultations have followed. These include:

- GESAMP Ad-Hoc Meeting of the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection Working Group on Environmental Impacts of Coastal Aquaculture (May 1996);
- Workshop on International Harmonization for Aquaculture Drugs and Biologics (February 1997);
- Workshop and Round Table of the European Association of Fish Pathologists (EAFP) (September 1997);
- World Health Organization (WHO) Consultation (with FAO and the World Organisation for Animal Health [OIE]) on Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food (June 2000);
- First Joint FAO/OIE/WHO Expert Workshop on Nonhuman Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment (December 2003);
- Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL (August 2004); and
- Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance (June 2006).

This project, which builds on these important consultations, is funded under FAO's Regular Programme and the FAO Multi-Partnership Programme (2008 funds) under D.1 Objective under the Aquatic Animal Health and Aquatic Biosecurity Project.

The FAO/AAHRI Expert Workshop on Improving Biosecurity through Prudent

and Responsible Use of Veterinary Medicines in Aquatic Food Production was held in Bangkok, Thailand, from 15 to 18 December 2009.

Purpose

The purpose of the workshop was to understand the current status of the use of antimicrobials in aquaculture, to compare the current usage of veterinary medicines with similar information summarized by FAO in 1996, to identify effective and meaningful alternatives to antimicrobials for aquatic animal diseases as a measure for enhancing biosecurity while conserving biodiversity of the aquatic environment and maintaining safety of aquaculture products, and to prepare a draft outline of technical guidelines on the prudent use of veterinary medicine in aquaculture in support of FAO's Code of Conduct for Responsible Fisheries (CCRF).

Participation

The workshop was attended by a total of 39 experts from 14 countries (Canada, Chile, China, Croatia, India, Indonesia, Ireland, Norway, the Philippines, Spain, Thailand, United States of America, United Kingdom of Great Britain and Northern Ireland and Viet Nam), including additional experts from the Association of Southeast Asian Nations (ASEAN), the European Union (EU), OIE and WHO, as well as experts from the private sector (producers, producer organizations, pharmaceutical and feed companies). The list of experts and their profiles are presented as Annex 1.

Process

Annex 2 provides the programme of work during the workshop. The workshop was informed by 20 presentations on the current situation and the way forward in terms of prudent and responsible use of veterinary medicines in aquaculture.

TECHNICAL WORKSHOP

Opening session

The opening ceremony was graced by the following officials: Dr He Changchui (FAO Assistant Director-General and Regional Representative for Asia and the Pacific), Dr Somying Piumsombun (Director-General Thailand's Department of Fisheries), and Dr Rohana Subasinghe (Senior Fishery Resources Officer, FAO, Rome).

Presentation highlights

Dr Melba Reantaso (FAO) presented "Review of workshop background, goals, process and expected outcomes", a backgrounder on biosecurity and prudent and responsible use of veterinary medicines in aquatic food production. She described the "four Ps": Purpose, Participation, Process and Products. A definition of veterinary medicine (antimicrobials) was presented as substances that kill or inhibit the growth of micro-organisms (bacteria, parasites, fungi, viruses). They include antibiotics, chemotherapeutants, disinfectants and vaccines. She noted that the outputs expected from the workshop were: (i) draft technical guidelines on the prudent and responsible use of veterinary medicines in support of the FAO CCRF; (ii) recommendations on specific actions or follow-up work to be undertaken by FAO and other interested partners and stakeholders; and (iii) an expert workshop report, including contributed technical papers.

Dr Iddya Karunasagar (FAO) spoke on "Public health and trade impacts of antimicrobial use in aquaculture". He noted that this issue was discussed in depth during the FAO/WHO/OIE Joint Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance, which was held in Seoul in 2006. Current and potential future hazards to public health of antimicrobial usage in aquaculture were considered using risk assessment methodology. The hazards identified were: development and spread of

antimicrobial resistant bacteria and resistance genes and occurrence of antimicrobial residues in products of aquaculture. Regarding residues, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has developed risk assessment principles and proposes maximum residue limits (MRLs) for approved veterinary drugs.

Dr Sandra Bravo (Universidad Austral de Chile), in her presentation "Environmental impacts and management of antimicrobials in aquaculture: the case of salmon aquaculture in Chile", explained that salmon are not native to the southern hemisphere, the first stocks being introduced to Chile in 1875 to develop recreational fisheries. Salmon farming activity in Chile started at the end of the 1970s and in 1992, Chile became the second-largest salmon producer after Norway. Since the beginning of the salmon farming industry, diseases have always presented a major threat. This fledgling industry was mainly supported by the importation of salmon eggs from different countries of the northern hemisphere. At the beginning, the three important diseases which affected farmed salmon were: bacterial kidney disease (BKD), salmon rickettsial syndrome (SRS) and *Caligus*. BKD is caused by *Renibacterium salmoninarum*, a pathogen which was introduced to Chile through infected coho salmon eggs imported from the United States of America, as reported in 1970. The main susceptible salmonid species is the chinook salmon, and for this reason this species is not reared in Chile. SRS is caused by *Piscirickettsia salmonis.* This disease was reported in 1989; however, it has previously been recorded as early as the early 1980s. It has been the most serious disease for the salmon industry in Chile because of the high mortality caused in the three salmon species and the large quantities of antibacterial medicines used for its control. *Caligus* is a copepod parasite that is present in wild marine fish from which it is transmitted to farmed salmon reared in cages in Chile. Caligid copepods ectoparasitic on marine fish are commonly known as sea lice. Sea lice have been shown to be the most serious parasitic infestation affecting the Chilean salmon industry. The infestation is controlled with medicines, applied by bath or as feed treatments.

Dr John McEvoy (European Commission Directorate-General for Health and Consumer Protection in the Food and Veterinary Office) made a presentation on "Residue control plans: European Union requirements for third countries". He explained that the aim of EU legislation in respect of production of food is to promote the production of food that is safe and wholesome, thereby helping to protect the health of European consumers. Food which is imported into the EU must also be safe. Third countries exporting food to the EU are thus obliged to have control systems that provide equivalent guarantees on the safety of the food produced. Indeed, food exported to the EU is certified by competent authorities in third countries as having been produced in accordance with EU rules and that the public and animal health standards under which the food has been produced comply with or provide guarantees equivalent to those foreseen by Community rules. Countries use residue control programmes as a means of protecting their own citizens from excessive exposure to potentially harmful residues of, *inter alia*, veterinary medicines, agricultural chemicals and contaminants. Furthermore, the existence of a functioning residue control programme is generally recognized as a prerequisite for the free trade of agricultural commodities between countries. Residue control programmes submitted to the Commission services by third countries are evaluated by the Food and Veterinary Office (FVO), a Directorate within the Directorate General for Health and Consumer Affairs (DG SANCO).

Dr Victoria Alday-Sanz (FAO Consultant) presented "Preliminary Results of the FAO international survey on the use of veterinary medicine in aquaculture". She described the background of the survey and the aim and the structure of the questionnaire. The survey was conducted through e-mail distribution and face-to-face consultation with a wide range of relevant stakeholders (e.g. fish farmers, government staff, feed manufacturers,

feed and drug sellers and extension officers) in selected countries. A total of 196 survey returns were collected from 21 countries (in addition to 3 unknown country sources) and a survey database was created. The methods followed for preliminary analysis were: (i) percentages and medians were used to describe the distribution of categorical (e.g. geographic location of respondents) and continuous variables (e.g. number of substances reported); (ii) species-wide comparisons were conducted using only data from respondents who submitted information by e-mail and for both species being considered in the analysis; (iii) Bartlett's Test for Inequality of Population Variances was used to establish whether an ANOVA (parametric) or Kruskal-Wallis (non-parametric) test was most appropriate to compare continuous variables (e.g. the number of substances reported for different species). All tests were conducted using Epi Info 3.5 and the use of a p-value of 0.05 to identify significant associations.

Dr Carl Uhland (Université de Montréal, Canada) started his presentation entitled "Antimicrobial use and resistance in selected zoonotic bacteria in aquaculture: a survey of aquaculture-allied professionals with various types of expertise – preliminary findings" by explaining that the development of antimicrobial resistance (AMR) among human pathogens has become one of the biggest challenges facing the medical community in the twenty-first century. The limited number of "new" antimicrobials in development emphasizes the need to minimize AMR selection. The use of antimicrobials for growth promotion in animals has been banned in Europe, and therapeutic usage of antimicrobials in animal production (including aquaculture) that are classified as critically important for human use has also been questioned. The magnitude of risk to human health attributable to AMR exposure from aquatic bacteria pathogenic to humans or from the transfer of resistance determinants from aquatic bacteria found on seafood and in the environment to bacteria pathogenic for humans is unknown. He also described the two methods used to identify and evaluate existing information on the prevalence, risk factors and interventions for selected zoonotic bacteria, antimicrobial use (AMU) and AMR in aquaculture (including seafood and ornamental fish).

Dr Puttharat Baoprasertkul (Aquatic Animal Health Research Institute (AAHRI), Thailand) in her presentation entitled "Use of veterinary medicine in Thai aquaculture: current status", presented background information on the use of veterinary medicines and other chemicals in Thai aquaculture, emphasizing that Thailand is one of the world's major producers and exporters. In order to ensure the quality and safety of exported products, the Department of Fisheries (DOF) has continuously controlled and inspected aquatic animals with regard to several aspects, including the use of antimicrobial drugs, chemicals and animal feeds in aquaculture. The Food and Drug Administration (FDA), Ministry of Public Health is in charge of antimicrobial drug control. All drugs must be registered through the FDA. The chemicals used in aquaculture are classified into four types (I, II, III and IV) according to need for control under jurisdiction of DoF. To deal with type II and III chemicals, registration must be approved by DoF Hazardous Substance Committee members. A licence must be obtained through DOF for any production, import, export or possession of type III chemicals. She also clarified that aquatic animal feeds also must be registered and approved by DoF. In addition, the department is responsible for quality control throughout the production process via several approaches, including factory inspection, feed sampling for composition analysis and drug residue examination. She concluded her presentation by disclosing that the DoF intends to improve the knowledge of extension officers and aquaculturists on the appropriate use of antimicrobial drugs and to increase the number of diagnostic service units.

Dr Sonia S. Somga (Bureau of Fisheries and Aquatic Resources [BFAR], the Philippines) presented "Use of veterinary medicine in Philippine aquaculture: current status", in which she provided an overview on the use of veterinary medicines in Philippine aquaculture.

She presented the results of a survey on the use of veterinary medicines and other products in aquaculture in the Philippines. The survey included a total of 69 respondents selected from different key players in aquaculture production (e.g. shrimp, milkfish/marine fish and tilapia hatchery and grow-out operators, government field workers, feed and aquatic products suppliers). Dr Somga explained that most of the veterinary medicines and other products are being applied in shrimp hatchery operations. There are also some that are used in tilapia hatcheries to prevent and treat bacterial, parasitic and fungal infections. Chemotherapeutants are also used for general disinfection. In shrimp grow-out, the use of antibiotics is minimal. Farmers have experienced the short-term benefits of using antibiotics and the development of bacterial resistance. In milkfish grow-out in cages, some operators have opted to use antibiotics at the early stage of culture (just after stocking) for treatment of diseases resulting from transport and environmentally related stress. In tilapia hatcheries, fry are sex-reversed prior to distribution. Some aquaculturists use chemotherapeutants in their operations. In grow-out, the application of probiotics is also practised (several probiotics available in the market). These veterinary drugs and products require registration before they can be placed on the market.

Mr Mai Van Tai (Centre for Environment and Disease Monitoring in Aquaculture, Research Institute for Aquaculture No. 1 [CEDMA–RIA1], Viet Nam) started his presentation on "Use of veterinary medicine in Vietnamese aquaculture: current status" by describing the drug and chemical use before 2004. He explained that by 2004, there were at least 373 kinds of chemicals used in aquaculture systems. By 2002, some of the chemicals had been banned, e.g. chloramphenicol, chloroform, nitrofuran, furazonidone and metronidazone. By 2004, there were 220 domestic companies involved in producing and trading drugs and chemicals used in aquaculture. He then proceeded to describe the drug and chemical use and management in aquaculture from 2007 to 2009. Small-scale or household aquaculture accounts for approximately 70 percent of aquatic production in Viet Nam. Recent field surveys show that farmers have become more prudent in using drugs and chemicals. They just use registered products, and now use less antibiotics and more probiotics, premix, vitamins, minerals, locally mixed herbs (for health improvement) and environmentally friendly chemicals (for enhancing water quality management). However, the availability of too many products can lead to confusion among farmers. Many products have no Vietnamese writing on their labels, and the price of the products is increasingly restricting their proper usage in terms of application of the right product, treatment time and dose.

Dr Yuan Xinhua (Wuxi FFRC, Wuxi, China), in a presentation on "Use of veterinary medicine in Chinese aquaculture: current status", described the status of use of veterinary medicines in Chinese aquaculture, highlighting that fish farming represents about 70 percent of total fishery production. Chinese aquaculture counts more than 40 species cultured and is characterized by polyculture and intensive management. He introduced the survey conducted in November 2009 in Jiangsu and Guangdong provinces. These two areas were selected for the questionnaire because of their importance with respect to Chinese aquaculture production. About 50 questionnaires have been collected from different respondents. After a description of the results, Dr Yuan emphasized the importance of disease prevention instead of treatment and the efforts of farmers, government, media and fishery technical extension in the reformation of the culture model.

Dr Peter Smith (National University of Ireland) started his presentation on "Antimicrobial resistance: complexities and difficulties of determination" by introducing resistance and the prudent use of antimicrobials. He emphasized that when a target bacterium is resistant, the therapeutic administration of antimicrobials is not prudent, not rational and not good economics. In order to ensure prudent use, it is needed to be able to

determine whether the target bacterium is resistant and to access information on its sensitivity or resistance. Then he defined *resistance*, clarifying that a bacterium can be considered as (clinically) resistant if, as a result of its reduced susceptibility to an agent, it can continue to contribute to the morbidity and mortality in a population during and after the administration of a course of therapy with that agent to that population. He strongly suggested that the word *resistance* should be used only when it refers to clinically relevant resistance. Determining clinical resistance requires data on pharmacokinetics and pharmacodynamics integrated with data on clinical efficacy. He explained that in aquaculture it is not possible to determine whether any bacterium is clinically resistant. It is important, indeed, to establish whether a bacterium is fully susceptible or not to the agent in question. He introduced the concept of *breakpoint value*, a critical value of a susceptibility measure that allows isolates to be classified as clinically sensitive or clinically resistant, and *cut-off value*, a critical value of a susceptibility measure that allows isolates to be classified as fully susceptible (wild type) or less than fully susceptible (non-wild type). He concluded by highlighting the urgent need to validate the internal control protocol.

Dr Rohana Subasinghe (FAO) in his presentation on "Nitrofurans in freshwater prawn (*Macrobrachium rosenbergii*) in Bangladesh: what can we do?" explained that the EU forbid the use of nitrofuran drugs in food-producing animals in 1995 based on their carcinogenic and genotoxic effects. In 2008, the Government of Bangladesh approved a ban on the production, trading and use of nitrofurans. However, during the period between September 2008 and June 2009, a total of 62 nitrofuran-related notifications were issued by the EU through the Rapid Alert System for Food and Feed. All of these concerned the detection of semicarbazide, a metabolite of nitrofurazone. In July 2009, FAO fielded a mission to try to identify the sources of nitrofuran residues in *M. rosenbergii* ("golda") from Bangladesh. The conclusion of the mission was that the nitrofurans ban in Bangladesh has not been highly effective and that nitrofurans are used directly and/or indirectly by the *Macrobrachium rosenbergii* industry. Aquaculture products from Bangladesh have been rejected based on the presence of residues of nitrofurans. Most of the nitrofuran-related reports concerned "golda" rather than "bagda" (*Penaeus monodon*). The sector is highly fragmented along the value chain, and the farmers are not aware of the consequences of using banned antimicrobials such as nitrofurans in their culture practices. Diagnostic capabilities and responsible service provision is marginal in Bangladesh, thus resource-poor small-scale farmers are always vulnerable to risks in their culture practices. While enacting new laws to control and monitor the use of antimicrobials is important, the efficiency of enforcement of law is always questionable because of the lack of human and financial resources and capacity. In such a situation, an option for improvement could be to empower small-scale farmers for self-governance, providing them with tools and organizing them into manageable clusters.

Dr Barbara Montwill (Food and Drug Administration [FDA] of the United States of America), in her presentation "Seafood HACCP program and FDA enforcement (inspections, testing, import alerts)" outlined the FDA enforcement strategy for safe aquaculture products. She explained that United States aquaculture production counts 4 309 farms with a total value of USD1 092 386 000 (2005 Census of Aquaculture, USDA). Eighty-four percent of the total food seafood consumed in the United States of America is imported from at least 62 countries, and aquaculture products account for approximately 40 percent of all seafood available on the United States market. In 2008, 60 percent of the seafood was imported from Asia. Sources of contamination are numerous and varied. As the seafood quality and safety controls are performed at every stage from production to processing, storage and transport, export-oriented aquaculture producers must adapt to international sanitary requirements to maintain market access. Traditional methods of preservation, processing and marketing often fail to comply with

standards, leading to bans and/or shipment rejection by importing countries. Consumers demand safe, wholesome and contaminant-free seafood products. She described the FDA Regulatory Authority and HACCP, an inspection approach used by FDA during domestic and foreign inspections of seafood processors to focus attention on the parts of the process that are most likely to affect the safety of the product. She described the potential hazards in fish and fishery and aquaculture products. FDA regulates imported seafood products by conducting inspections of importers and by collecting surveillance samples of imported goods at the time of entry. Ms Montwill concluded her presentation by describing the several FDA surveillance programmes.

Dr Jennifer Matysczak (FDA of the United States of America), in her presentation on "Drug approval process and other regulatory considerations for use of aquaculture drugs in the United States of America", described the regulatory approach to the registration and use of aquaculture drugs in the United States of America and how the FDA is addressing the need for prudent and responsible use of drugs in aquaculture through the drug approval process, research and surveillance, and outreach. Not all animal health products are regulated by the FDA. Under the Federal Insecticide, Fungicide, and Rodenticide Act, the Environmental Protection Agency registers pesticides; and as authorized by the Virus-Serum-Toxin Act, the Department of Agriculture regulates veterinary biologics (e.g. animal vaccines, bacterins). FDA's Center for Veterinary Medicine (CVM) evaluates the following components when considering a new animal drug for approval: (i) effectiveness; (ii) target animal safety; (iii) human food safety; (iv) environmental impact; (v) chemistry; (vi) manufacturing; and (vii) labelling. The human food safety review includes evaluation of toxicology, residue chemistry and microbial food safety.

Dr Carlos Zarza (Skretting, Spain) started his presentation on "Oral delivery of veterinary medicines through aquafeed in Mediterranean aquaculture" by explaining that the main fish species farmed in the Mediterranean countries are rainbow trout (freshwater) and seabream and seabass (marine). The main producer countries are Turkey, Greece, Spain, Italy and France. He described the production chain of the medicated feed and the problems related to its use in cage culture. He highlighted that one of the main limits of this treatment is the lack of appetite of sick fish. He also said that the availability of veterinary medicines for treating non-salmonid fish species is a major problem in Mediterranean aquaculture. There is a limited number of antibiotic premixes and vaccines with marketing authorization, but almost no antiparasitics, antifungals or anaesthetics. He concluded his presentation by considering that inadequate medicine and dosage selection, wrong duration of treatment, use of antibiotics as prophylactics and repeated use of same medicines are some of the main mistakes in the daily practice on fish farms. These mistakes can lead not only to treatment failures, but also to development of resistances in fish pathogens and occurrence of residues in aquaculture products.

Dr Roar Gudding (National Veterinary Institute, Norway) started his presentation on "Disease prevention as basis for sustainable aquaculture" by describing the growth of Norwegian fish production and the use of antibiotics. He defined *sustainable development* as development which meets the needs of the present without compromising the ability of future generations to meet their own needs. He introduced the Norwegian legislation related to aquaculture and the fish disease law. The main diseases are: furunculosis, malformations, infection with *Francisella* spp., infectious salmon anaemia, spring viremia of carp, *Streptococcus* infection, *Saprolegnia* infection and parasites (*Myxobolus* infection and salmon lice). He concluded by saying that sustainability should be the basis for the aquaculture industry. The Norwegian experience is that disease prevention is fundamental for sustainability, legislation is the cornerstone in disease prevention, and vaccination is the single most important preventive measure.

Dr Robin Wardle (Intervet Schering-Plough Animal Health, United Kingdom of Great Britain and Northern Ireland) started his presentation on "Health management tools from a manufacturer's point of view" by introducing the EU definition of a *veterinary medicine*: (i) any substance or combination of substances presented as having properties for treating or preventing disease in animals; or (ii) any substance or combination of substances that may be used in or administered to animals with a view either of restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis. He then gave an overview of the development and use of medicines in aquaculture. The medicine types registered in the EU are vaccines (immersion, oral and injection preparations), antibiotics (in feed), antiparasitic products (for the treatment of external parasites, in feed or bath treatments), antifungal disinfectants and immunostimulants (in feed). He described the main steps involved in the registration of a medicine in the EU. He also identified the challenges for expanding the availability of aquaculture medicines as: (i) the many fish species farmed; (ii) the many pathogens, some shared between species; (iii) the diverse environment; (iv) the feed and feeding systems; and (v) the need for knowledge and information sharing. He concluded by highlighting that there is the opportunity to provide sustainable solutions to health problems in aquaculture worldwide; approved medicines are available or can be developed. Responsible use and uniform standards will drive the investment to meet existing, new and emerging needs for medicines in aquaculture.

Dr Indrani Karunasagar (Department of Microbiology, College of Fisheries, Kartanaka Veterinary, Animal and Fisheries Sciences University, Mangalore, India) presented "Alternatives to antibiotics in aquaculture". She described the problems related to the culture of *Penaeus monodon* and the solutions, pointing out the importance of antibiotics in aquaculture for the treatment of diseases and the prevention of secondary bacterial infections following viral infections and diseases following stress. She emphasized the limitations of vaccines in aquaculture, saying that they are not useful in early larval stages, the immune systems of invertebrates (e.g. shrimps, prawns) are poorly understood and their administration to large numbers of animals in ponds is difficult. She described bacteriophage therapy and the related problems. She explained that it is crucial to look for alternatives to antibiotics for several reasons: (i) the emergence of resistant pathogens; (ii) the build-up of pathogen; (iii) the presence of chemical residues in shrimp meat; (iv) the fact that antibiotics destroy useful micro-organisms and have questionable efficacy in seawater; and (v) the potential for environmental deterioration due to residues. She introduced the concept of *immunostimulant* as a chemical, drug, stressor or action that elevates the non-specific defence mechanisms or specific immune response. She also gave an example of regulatory approval for the use of bacteriophages. Professor Karunasagar concluded by saying that the preventive approach (e.g. good aquaculture practice, vaccines, immunostimulants, probiotics) has potential advantages and that bacteriophage therapy needs further attention as an alternative to antibiotics.

Dr Brett Koonse (FDA of the United States of America), in his presentation "Training and implementation of good aquaculture practices (GAqPs) related to the use, documentation, monitoring of antimicrobials and chemotherapeutants" gave an overview on the importance of GAqPs in minimizing bacterial resistance. GAqPs are preventive measures that will reduce stress and promote animal health and quality, thus reducing the need for chemotherapeutic intervention. In this case, they can be placed in three basic categories: (i) GAqPs for hatcheries and farms (they can be physical, chemical and biological); (ii) GAqPs for regulators (those meant to ensure that only approved chemotherapeutic agents are available and used, that they are used properly, that records are kept and inspections performed); and (iii) GAqPs for academia, health providers and biologists (these include training, certification and licensing of aquaculture professionals; having and using adequate detection and diagnostic tools, programmes and methods;

that only the correct drugs are used and that they are used properly for effectiveness; that the drugs will not leave undesirable residues; and that the health providers or biologists are participating in disinfection and pathogen eradication programmes).

Dr Richard Arthur (FAO Consultant), in his presentation on "Summary of the major issues related to the use of veterinary medicine in aquatic food production and guidance provided by the FAO Code of Conduct for Responsible Fisheries and others", summarized the major issues identified at the 1996 FAO/SEAFDEC/CIDA expert meeting on "Use of Chemicals in Aquaculture in Asia" and the major issues identified during the current workshop. He also provided an overview of how the situation has changed over the past 13 years and introduced the relevant sections of FAO's Code of Conduct for Responsible Fisheries (CCRF). He proposed several topics for further discussion: (i) the need for new medicines; (ii) the potential for alternative approaches to treatment; (iii) capacity building; (iv) surveys of antimicrobial use and how to improve accuracy of data and benchmarking for future assessments; (v) how to implement/enforce existing regulations; (vi) the need for simple and rapid diagnostic techniques and better information on how antimicrobials perform; (vii) how to reduce drug administration failure; (viii) the use of antibiotics essential to human medicine; (ix) avoiding potential resistance of pathogens to antimicrobials other than antibiotics; (x) problems related to "generic" products; and (xi) the need for harmonization and validation of new techniques.

Dr Rohana Subasinghe (FAO), in his presentation on "Aquaculture, new dimensions, markets and trade – status and challenges", reported on the contribution of aquaculture to food-fish supply in a context that is changing. Food safety and health of the consumer, environmental sustainability, social responsibility and economic viability are becoming imperatives in aquaculture. Growth of aquaculture outpaced institutions and governance arrangements increasing emphasis on better governance in trade policy, institutions and legislation. He highlighted that, in line with the growth of aquaculture and trade, there is a growth in certification and an increasing number of public and private standards. He emphasized the importance of small-scale farmers, who are major global producers of aquaculture products, They produce more than 80 percent of aquaculture production in Asia, contributing significantly to global aquaculture production and trade.

Working group sessions

Dr Melba B. Reantaso presented the guidelines for the working group discussion, after which the participants were divided into three working groups that tackled the following themes:

Day 1

- Working Group 1: Discussion of the concerns of irresponsible use of antimicrobials in aquaculture with reference to policy, institutions, law and enforcement, and compliance to relevant international instruments;
- Working Group 2: Analysis of irresponsible use of antimicrobials with reference to knowledge, information, dissemination, communication and capacity building; and
- Working Group 3: Debate on irresponsible use of antimicrobials and research, science, risk assessment, health management and the development of relevant tools.

Day 2

- Working Group 1: Development of ten guiding principles on responsible use of antimicrobials in aquaculture, and the structure and major elements of the proposed CCRF technical guidelines;
- Working Group 2: Development of ten specific action-oriented recommendations targeting the state; and
- Working Group 3: Development of ten action-oriented recommendations targeting

the private sector and non-governmental organizations/intergovernmental organizations (NGOs/IGOs).

Two full days were spent on working group discussions and presentations. The outcomes of the working group discussions and the general and specific recommendations made by the working groups are presented in the section on Working Group Findings.

Closing session

The closing session was held at 14.00 hours on 17 December. The workshop was formally closed by Dr Jirawan Yamprayoon, Deputy Director of Thailand's Department of Fisheries. She congratulated the participants for the success of the meeting and made a synthesis of the previous three sessions. The spirit that pervaded the exercise was marked by the collective desire and a strong commitment to accomplish an important and, it was felt, a challenging task; a large part of the challenge was to frame a practical guide and have its structure and contents approved by the participants.

WORKING GROUP FINDINGS

Day 1

During the first day of the working group sessions, all working groups addressed the following key concerns of irresponsible use of antimicrobials in aquaculture:

- Impacts on aquatic environment
  - biodiversity and ecology
  - resistance
- Impacts on human health
  - residues, food safety
  - resistance
- Impacts on trade
  - compliance to trading standards
  - consumer acceptance

Working Group 1: Discussion of the concerns of irresponsible use of antimicrobials in aquaculture with reference to policy, institutions, law and enforcement, and compliance to relevant international instruments.

Working Group 1 members: Xinhua Yuan, Sonia Somga, Puttharat Baoprasertkul, Jennifer Matysczak, Suriyan Vichitlekarn, Roar Gudding, Iddya Karunasagar, John McEvoy, Pornpun Yutharaksanukul, Richard Arthur, Barbara Montwill.

Working Group 1 pointed out that safe and effective antimicrobials need to be available for efficient aquaculture production, and their use should be in line with established principles on prudent use of antimicrobials to safeguard public and animal health. The use of such medicines should be part of a biosecurity plan and in accordance with an overall national policy for sustainable aquaculture.

Current concerns include perceived widespread use of antimicrobials in aquaculture (worldwide), lack of approved antimicrobials for certain aquaculture species and diseases, and significant variations in regulatory frameworks and enforcement in different countries. These could have implications for the environment, human food safety and the development of antimicrobial resistance; furthermore, these elements have the potential to impact free trade.

The working group identified a number of key elements that should be in place to address these issues, briefly described below:

Regulatory framework for authorization of aquatic veterinary medicines:

- a clear framework should be in place and, where possible, should be in line with international standards to ensure safety and effectiveness;
- regulatory authorities need to be defined with clear roles and responsibilities, and

legal instruments should be developed;
- regulatory authorities need to be adequately resourced and skilled;
- authorization of antimicrobials should be appropriately classified so as to be available upon prescription by veterinarians or suitably trained persons as authorized by national legislation to ensure proper use;
- capacity building needs to be encouraged; and
- enforcement should be part of the framework and should be adequately resourced and effective.

Control on the distribution and use of veterinary medicines:
- inspection and surveillance system for the entire supply chain of veterinary medicines is needed;
- producers and sellers of antimicrobials should be authorized and subject to regular inspection by competent authorities;
- reporting of sale of antimicrobials should be required;
- documentation of use of antimicrobials on the farm should be obligatory and subject to inspection by authorities; and
- risk-based official programmes for monitoring of residues with appropriate follow-up investigations and enforcement are needed.

Technical assistance:
- government should work in partnership with farmers to ensure proper biosecurity and use of antimicrobials; and
- diagnostic capability and capacity should be in place.

International standards:
- international standards should be developed in order to facilitate trade and encourage the judicious use of antimicrobials and alignment with already developed guidelines, including CODEX documents on the prudent use of antimicrobials; and
- appropriate withdrawal periods to ensure compliance with importing country requirements should be established.

Working Group 2: Analysis of irresponsible use of antimicrobials with reference to knowledge, information, dissemination, communication and capacity building.

Working Group 2 members: Qingxiong Zeng, Mukda Uttarapong, Brett L. Koonse, Mai Van Tai, Visanu Boonyawiwat, Carl Uhland, Sandra S. Bravo, Weimin Miao, Victoria Alday-Sanz, Rohana P. Subasinghe, Awa Aidara-Kane.

The Working Group identified a number of concerns, briefly described below:

Husbandry:
- each country needs to generate the information requirements for zoning for aquaculture and sanitary purposes;
- access and ability to disseminate and communicate disease management, prevention and controls are insufficient;
- access to baseline information and knowledge about farm conditions, environment, sanitary conditions, disease occurrence and the treatments used in each country is difficult;
- there is not enough support and education for farmers to reduce the use of antimicrobials;
- more dissemination and capacity building on best management practices (BMPs) and sanitary management (including drug use and within laboratories) are necessary;

- more epidemiological knowledge and information about fish pathogen pathways, transmission and sources are needed; and
- there is a lack of information on how the patterns of use of antimicrobials in aquaculture can impact pathogen resistance.

Aquatic environment:

- knowledge of the sources of entry of antimicrobials into the environment (industrial, aquaculture, human use, etc.) should be improved;
- information about the efficacy of antimicrobial treatments under different environmental conditions should be enhanced;
- there is not enough information about the impacts of antimicrobial treatments upon the environment (e.g. persistence, impact on the resistance of environmental bacteria);
- more standardized methodology to assess the antimicrobial resistance of bacteria in aquaculture is needed; and
- improvement of reliable information on the level of antimicrobial resistance in aquaculture is essential.

Human health:

- information about the risk of aquaculture chemotherapeutic residues to human health is needed; and
- information on the transfer of resistance (bacteria or resistance genes) to human pathogens or microflora because of the use of antimicrobials in aquaculture is needed (it was noted that impacts upon the aquatic environment arising from the use of antimicrobials in human medicine and livestock production could be a problem).

Trade:

- residues in aquaculture are a major trade concern;
- information about some residues that may be appearing from the environment or from other sources and not from aquaculture use should be improved;
- impacts of residues are not disseminated through the value chain (farmers, intermediary, drug retailers, extension agents, processing plants, etc.);
- the presence of antimicrobial residues in aquaculture contributes to a poor perception of the aquaculture industry;
- there is a lack of testing capacity in some countries; and
- the traceability of aquaculture products in order to identify the source of problems should be improved.

Communication:

- there is a lack of an information chain, and the flow of information to farmers is insufficient;
- information on training courses should be provided to farmers, drug providers, extension services and health training of these people (e.g. on good aquaculture practices [GAqPs]); and
- more specific aquaculture training for health professionals, including veterinarians is necessary.

Working Group 3: Debate on irresponsible use of antimicrobials and research, science, risk assessment, health management and the development of relevant tools.

Working Group 3 members: Wen Chen, Varinee Panyawachira, Temdoung Somsiri, Donald Prater, Peter Smith, Snježana Zrnčić, Indrani Karunasagar, Robin Wardle, Carlos Zarza, Jan Koesling, Melba B. Reantaso.

Working Group 3 agreed that diagnosis, prevention and treatment are the three pillars of health management. The members discussed about residues and resistance and the impact on human health. Resistance in aquaculture is mainly an issue of treatment efficacy and preservation of active compounds. The risk of transfer of resistant human bacteria from aquaculture is very low. In general, the risk of impact on human health should be managed and reduced by use of the right product and the recommended therapeutic dose.

Resistance should be minimized by:

- good treatment practice with registered products;
- good therapeutic outcome; and
- methodology for assessing resistance and sensitivity.

The relative contribution of antimicrobial use in aquaculture to environmental reservoirs of resistant human pathogens should be considered together with the antimicrobial use and misuse in human medicine and terrestrial animal agriculture.

With reference to the impact on the aquatic environment, Working Group 3 pointed out that research is needed to generate information on:

- the impacts of veterinary medicines on the environmental biota;
- their effects on biodiversity;
- the development of resistance in non-target microbes;
- the uptake depletion and withdrawal times of veterinary medicines; and
- pathogen source and entry (biosecurity barriers).

Important tasks are to:

- conduct risk assessment for the most significant aquatic pathogens;
- develop methodologies and plans for monitoring sediment, water, animals and other carriers; and
- formulate appropriate aquaculture development plans and health management strategies (e.g. taking into consideration site selection, broodstock source, water quality and treatment, source of seed for stocking, source of feed, quality assessment, traceability issues through record maintenance, etc.).

Regarding the impact on trade, the Working Group agreed that the compliance with trading standards is essential and that the following issues will need to be addressed:

- the need for more globally recognized MRLs for aquaculture drugs;
- the lack of harmonized residue methods for aquaculture;
- the lack of validated laboratories on both trade partner sides;
- the lack of globally recognized requirements for drug prescription standards;
- that the current requirements for seafood health certificates do not adequately address aquatic animal health problems;
- that the requirement of traceability standards favours large companies over small companies; and
- that the lack of traceability system impairs the ability to trace back inappropriate drug use to the farm level.

Consumer acceptance is also important. Consumers are unaware of the lack of food safety and sustainability standards for fisheries versus aquaculture. Importing retailers reflect consumer concerns with internal standards and certification bodies for the value chain. Other aspects such as environmental concerns are incorporated in the list of consumer concerns (e.g. mangroves). Organic certification is a quality and farm management "green card" but is not verifiable.

Day 2

Working Group 1: Development of ten guiding principles on responsible use of antimicrobials in aquaculture, and the structure and major elements of the proposed CCRF technical guidelines.

Working Group 1 members: Donald Prater, John McEvoy, Sandra S. Bravo, Xinhua Yuan, Iddya Karunasagar, Robin Wardle, Peter Smith, Richard Arthur.

Working Group 1 developed the following guiding principles on the responsible use of antimicrobials in aquaculture and the structure and major elements of the proposed CCRF technical guidelines:

- States should promote effective farm and fish health management practices favouring hygienic measures and vaccines. Codes of conduct for GAqPs should be implemented to the fullest extent possible.
- Safe, effective and minimal use of therapeutants, hormones and drugs, antibiotics and other disease control chemicals should be ensured.
- States should regulate the use of chemical inputs in aquaculture that represent a risk to human health and the environment.
- Responsible use of antimicrobials and other veterinary medicines in aquaculture requires a strong commitment to surveillance and research, including monitoring of antimicrobial resistance, tracking the use of veterinary medicines, assessing risk in different settings and evaluating strategies to reduce resistance.
- States should require that the disposal of wastes, such as offal, sludge, dead or diseased fish, excess veterinary drugs and other hazardous chemical inputs, does not constitute a hazard to human health and the environment.
- States should establish effective procedures specific to aquaculture to undertake appropriate environmental assessment and monitoring with the aim of minimizing adverse ecological changes and related economic and social consequences resulting from the use of drugs and chemicals and other aquaculture activities.
- States should increase the use of risk analysis methodologies to understand and reduce the risk associated with the use of antimicrobials in aquaculture.
- Foodborne AMR risk analysis in aquaculture should give consideration to relevant international documents (e.g. recommendations of the joint FAO/WHO/OIE expert meeting on critically important antimicrobials) for setting priorities for risk assessment and/or risk management activities.
- States should have mechanisms in place to ensure that authorized veterinary drugs have been used properly in accordance with label indications. Mechanisms that should be considered include the implementation of appropriately designed monitoring programmes for, inter alia, effectiveness of diagnostics, effectiveness of therapy, residues in food and antimicrobial resistance.
- States should ensure that laboratories used for testing use only appropriately validated methods that are "fit for purpose". Such laboratories do not necessarily need to be located in the country of origin if the technical capability and capacity does not exist in that country.
- The appropriate use of veterinary antimicrobial drugs in aquaculture production is a clinical decision that should be based on the experience and local expertise of the prescribing veterinarian or fish biologist and an accurate diagnosis based on adequate and appropriate diagnostic procedures.
- The use of antimicrobials should be subject to the oversight of either veterinarians or aquatic animal health professionals qualified by training and experience as recognized by national or regional authorities.

Members of Working Group 1 agreed on the five following recommendations:

1. To facilitate trade in safe food, government and food safety standard-setting bodies should urgently consider developing alternative approaches to the elaboration of MRLs for veterinary drugs used in aquaculture species.

2. For example, because the acceptable daily intake (ADI) for many of the drugs that have the potential to be used effectively in the aquaculture sector has already been established following toxicological studies in mammalian species, it may be possible to allocate an unused part of the ADI to aquaculture species. This would allow sponsors to carry out the studies to establish the appropriate withdrawal periods.
3. The development of data to support the registration and recognition of appropriate food safety standards (e.g. MRLs) for veterinary medicines used in aquaculture production should be encouraged.
4. With regard to the use of veterinary medicines and other therapeutic tools, states should promote efforts which improve selection and use of appropriate feeds, feed additives and medicated feedstuffs.
5. States should actively promote the adoption of accreditation systems for diagnosticians.

Working Group 2: Development of ten specific action-oriented recommendations targeting the state.

Working Group 2 members: Roar Gudding, Suriyan Vichitlekarn, Barbara Montwill, Awa Aidara-Kane, Jennifer Matysczak, Robin Wardle, Victoria Alday-Sanz, Varinee Panyawachira, Temdoung Somsiri, Mukda Uttarapong, Puttharat Baoprasertkul, Sonia Somga.

Working Group 2 developed the following ten specific action-oriented recommendations directed to states:

- *Legislation.* Countries should establish a clear regulatory framework for registration of veterinary drugs, inspection and surveillance. Roles and responsibilities of the competent authority should be defined. Country standards should be in line with international standards to ensure safety and effectiveness. Follow-up in enforcement is critical for implementation.
- *Capacity building*. International associations should give more attention to the increasing demands of the aquaculture sector. Governments should work with and train farmers on GAqPs/BMPs, including judicious use of antimicrobials, sanitary management, biosecurity and diagnostics. International associations should also provide technical assistance.
- *Research.* Governments should give resources to research in the aquaculture sector and fish health, with emphasis on disease prevention, efficacy and safety of veterinary medicines in different environmental conditions; environmental impacts; and alternatives to antimicrobials.
- *Laboratory diagnostics.* Governments should ensure a network of national and regional laboratories with equipment and competent staff that can address fish pathology, parasitology, microbiology and water analysis.
- *Communication and information.* States, through the relevant competent authorities, should develop and maintain an information system supporting prudent use of antimicrobials and biosecurity in aquaculture. The information system should include, among others, information on antimicrobials that can be used in aquaculture, guidelines for prudent use of antimicrobials, application of biosecurity in aquaculture and rapid alerts on disease outbreaks. Communication and networking supporting prudent use of antimicrobials and biosecurity in aquaculture should be established and fostered among stakeholders at all levels.
- *International standards and guidelines.* Increased harmonization of standards to facilitate trade and encourage judicious use of antimicrobials should be encouraged. An international system for ring tests (proficiency tests, quality assurance) should be established.
- *Holistic approach.* Governments and international organizations should establish systems for communication and cooperation between the areas of human medicine,

veterinary medicine, and aquaculture, agriculture and other industries.

- *Surveillance.* Countries should establish active and passive surveillance on residues, antimicrobial use and resistance, and diseases in aquaculture; research on methodology should be emphasized.
- *Distribution procedures for antimicrobials.* States should develop a system for the distribution chain of antimicrobials used in aquaculture. Such a system should cover registration, manufacturer inspection, import, retailers and handling/use by farmers. Competent authorities and collaborating mechanisms should be clarified to ensure the effectiveness of the system. Capacity-building activities should be identified and conducted to support relevant stakeholders in the distribution chain, particularly the farmers. Where appropriate, cooperation with international/regional competent organizations and the private sector should be promoted.
- *Biosecurity.* Governments or regional agencies should establish biosecurity systems at several levels (international, national, regional and individual levels) as appropriate for prevention and response to (control and eradication of) disease.

Working Group 3: Development of ten action-oriented recommendations targeting the private sector and NGOs/IGOs.

Working Group 3 members: Indrani Karunasagar (chairperson), Brett L. Koonse, Snježana Zrnčić, Carlos Zarza, Wen Chen.

Working Group 3 formulated the following ten action-oriented recommendations targeting the private sector and NGOs/IGOs.

1. The private sector should follow all the laws and regulations of the government.
2. The private sector should create industry representation from all sectors of the value chain, including producers, processors, exporters, feed manufacturers and hatcheries, including NGOs, and this representation should include small-, medium- and large-scale operators.
3. Private stakeholders and NGOs must be consulted by the government in making laws and regulations in a transparent manner in line with international standards.
4. The private sector value chain should be trained on why veterinary medicines should be used in a prudent and judicious manner, what those practices or techniques are, and how they can be implemented.
5. The private sector should have qualified personnel to prescribe and use veterinary medicines. These qualified personnel must be trained and licensed.
6. Sellers and importers of veterinary medicines should provide data and keep records on the selling of veterinary medicines. Producers should keep records and provide data on what veterinary medicines are used for and should participate in epidemiological investigations.
7. Producers and an industry representative group should establish a consensus on a sustainable production system by working with government, NGOs and other experts. Implementation should be required as the industry standard by producers, processors and exporters. This includes certification by the industry.
8. Small farmers should form associations or clusters in order to pool their resources to learn about the sustainable production system and how it should be implemented. Extension service providers can provide information about the production system, but producers must pay a service fee to them.
9. Incentives for producers to minimize the use of veterinary medicines should be developed by the private sector.
10. A “polluter pay principle” for hatcheries and producers should be developed, accompanied with traceability and a reliable testing programme.

WORKSHOP CONCLUSIONS AND RECOMMENDATIONS

The concluding session highlighted that:

- aquaculture is extremely important;
- improving sustainability is a key feature;
- disease and health are major concerns; and
- aquaculture concerns and the way they are addressed differ from country to country.

The irresponsible use of veterinary drugs is an issue impacting trade, the environment, and human and aquatic health. For this reason, prudent use of antimicrobials is a priority. It is governed by several factors such as knowledge, research, capacity and policy.

Three main problems related to the complexity and diversity of the drug sector were identified: (i) the authorization system for veterinary medicines and related issues; (ii) the insufficient technical assistance (e.g. capacity, environmental and human impact evaluation capacity; trading compliance); and (iii) the lack of harmonized international standards.

The responsible use of veterinary medicines in aquatic food production offers numerous opportunities in terms of improvement of policy on matters of veterinary medicines; better drug control; development of useful standards; harmonization of trading standards; improvement of hygiene and health management; reduction of use of veterinary medicines (develop incentives to reduce reliance on antimicrobials and other veterinary medicines); enhancement of services such as laboratories and levels of competence (especially at the field observation level); development of a responsible service by the private sector and responsible production practices; improving biosecurity (keep diseases out to have better control and a better product); and the requirement of GAqPs at the production level and throughout the production chain.

The workshop pointed out the challenges to fish farming:

- Aquaculture is one of the most complex food production sectors in the world. In contrast to traditional agriculture, it is not possible to isolate the fish completely, and it is extremely difficult to restrict contact with the natural environment.
- Developing countries should pay attention to the responsible use of drugs, and to human health and the environment.
- Although national economic systems differ significantly between countries, small-scale farmers play an important role in all countries. Thus, it is important to improve their capacity in management and organization.
- Improved legal frameworks, public-private partnerships and the accreditation of diagnosticians are important for further development of the aquaculture sector.

ANNEXES

Annex 1

EXPERTS AND EXPERT PROFILES

Name and contact details	Expert profile
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Victoria Alday-Sanz FAO Consultant Gran Via 658,4-1 Barcelona 08010 Spain Tel.: 3461555844 E-mail: victoria_alday@yahoo.com	DVM from University of Zaragoza Veterinary School (1989); M.Sc. (1991) and Ph.D. (1994) from University of Stirling. 20 years experience working on aquatic animal health (diseases, diagnostics, health management, sanitary legislation and biosecurity). Expert in EU, FAO, EFSA, OIE and ADB projects and collaborates with private sector. Has 28 refereed publications; over 50 articles in industry magazines; one of the co-authors of the CD-ROM Diagnosis of Shrimp Diseases with Emphasis on the Black Tiger Shrimp, sponsored by FAO, NACA, Biotec and WAS; editor of *The Shrimp Book, Theory and Practice of Penaeid Aquaculture*.

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Private consultant in international aquatic animal health issues. Career includes periods in Asia with IDRC (Fish Health Network Coordinator and as Fisheries Program Officer for Asia and Pacific); in Canada, as research scientist in aquatic parasitology with the Canadian Department of Fisheries and Oceans (DFO). Over the past 25 years, international experience primarily in Asia, but also in projects in Africa, Latin America, Eastern Europe, the Middle East and the South Pacific. Recent work includes FAO projects (West Balkans, southern Africa); with ADB as expert on fisheries quarantine as part of the BIMP-EAGA Task Force on Customs, Immigration Quarantine and Security; keynote speaker at the recent APEC Symposium on Meeting OIE Standards for Aquatic Animal Health; frequent editor of scientific and technical publications on aquatic animal health, aquaculture and fisheries for FAO, including *Use of Chemicals in Aquaculture in Asia,* the proceedings of the 1996 workshop held in Iloilo, the Philippines.

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Fisheries Biologist at AAHRI, Department of Fisheries, Thailand. Ph.D. from the Department of Fisheries and Allied Aquaculture, Auburn University (2006). Started career as Fisheries Biologist at National Aquaculture Genetic Research Institute (1992); moved to work at Drug and Chemical Control Group, AAHRI in 2007. Research mainly on molecular genetics, expressions of immune-related genes, giant freshwater prawn virus, hormone residues in fish and drug resistant genes. Published more than 15 papers in peer-reviewed journals.

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DVM from Veterinary School, Kasetsart University (1994); Ph.D. (Agricultural Biotechnology) from Center of Agricultural Biotechnology, same university (2009). Professional career started at the technical service of chemical supply company in field of shrimp culture (two years). Joined the Faculty of Veterinary Medicine, Kasetsart University in 1996 with an academic position. Currently, Assistant Professor of Department of Farm Resources and Production Medicine and Head of the Aquatic Diseases Diagnostic Laboratory, Diagnostic Center at the Faculty of Veterinary Medicine, Kasetsart University. 15 years experience working on aquatic animal health.

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Associate Professor at the Aquaculture Institute, Universidad Austral de Chile (UAC) (1998 to present). Fishery Engineer, the Universidad Católica de Valparaíso (1982); M.Sc. in Aquaculture and Environment from the University of Genova (2005); currently a Ph.D. candidate from the Norwegian School of Veterinary Science (NSVS). Professional career started at the salmon company Mares Australes (1982); Technical Manager (1987 and 1997); General Manager of Diagnostic Laboratory for Fish Health, Salmolab S.A. Was part-time Professor in fish health since 1982 at UAC. Since 1987, recognized as a fish health inspector by both the United States of America and Canada. 27 years experience in fish health and author of several papers reporting the findings of pathogens affecting wild and farmed fish in Chile. Led the first survey of chemicals used in the aquaculture in Chile (1999–2003); international experience includes work as a consultant for FAO (1998) and as chair of the 7th Sea Lice Conference (2008).

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B.Sc. in Fisheries (Kasetsart University); M.Sc. in Fisheries Parasitology (Auburn University); Ph.D. in Fish Pathology (University of Stirling). Over 40 years experience in research, diagnosis and teaching (both national and international) in aquatic animal health. Author or co-author of >100 technical publications and >60 general fisheries articles. Chairperson, Fish Health Section/Asian Fisheries Society (2001–2003). Presently a Senior Advisor on Fish Diseases of the Department of Fisheries, Thailand and a member of the Advisory Group for NACA.

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DVM from the Norwegian School of Veterinary Science (1968); Dr. Scient. degree and Dr. Med. Vet. degree from the same school in 1974 and 1980, respectively. Professional career began at NSVS; worked for six years in the field of food microbiology and hygiene. Joined the National Veterinary Institute in Norway in 1975 – last position as Director General from 2003–2009. Until 2003, also part-time professor in immunoprophylaxis at NSVS. Research mainly on disease prevention in terrestrial and aquatic animals, with special emphasis on vaccinology. >60 papers published in peer-reviewed journals. Vaccination of salmonid fish has been crucial for the development of a sustainable aquaculture industry in Norway; has had a central role in the development of fish vaccinology in the country for more than 20 years. Published review papers in fish vaccinology and author of the textbook *Immunoprophylaxis in Veterinary Medicine* (in Norwegian).

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Son of the owner of Sureerath Prawns, one of the largest shrimp farms and the first organic black tiger prawn producer in Thailand. M.Sc. in Environmental Management from the National Institute Development Administration. Helped develop to meet organic standards. Farm operations have implemented innovative environmental production technologies such as pond-based full recirculation systems. Farm operation does not discharge any waste material to the surrounding ecological system, a policy since the inception of Sureerath Farm in 1985, the first such system used in Thailand. Sureerath Prawns converted to organic farming in 2004 and was certified by Naturland Germany (2007), BioSuisse Switzerland (2008) and Organic Thailand (2009). Today, entire production of organic shrimp is facilitated by Sureerath, with its own hatchery, feed mill and farms; processing is in alliance with frozen food processing partners.

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M.Sc. in Environmental Sciences (University of Tuscia, 2004); worked with private sector as environmental analyst editing studies on environmental impacts of proposed infrastructure projects, primarily railways and highways. Started at FAO in 2006 under the volunteer programme; assisted and contributed to the completion of outputs in the areas of information systems, GIS and environmental studies. In 2007, recruited as FAO consultant, assisting in implementation of the fisheries gateway page and in editing technical publications.

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M.Sc. and Ph.D. in Microbiology from Mysore University (1979); postdoctoral training at University of Maryland and University of Wuerzburg (as Alexandervon Humboldt Fellow). Full Professor (1992) and Director of Research (2005) at the University of Agricultural Sciences, College of Fisheries, Mangalore (1992); appointed by the Ministry of Agriculture in India as a National Professor (2006). Research thrust on pathogens associated with aquatic animals with experience on pathogens affecting fish safety and those causing diseases in aquaculture animals. Participated in several meetings of the FAO/WHO Joint Ad-hoc Expert Committee on Microbiological Risk Assessment and member of the drafting group for FAO/WHO risk assessment for pathogenic *Vibrio* spp. in seafoods. Joined FAO as Senior Fishery Industry Officer (Quality Assurance) in 2007, working closely with the Codex Committee on Food Hygiene and Codex Committee on Fish and Fishery Products.

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DVM with practical clinical courses overseas in the Netherlands, United Kingdom, Canada and the United States of America; Ph.D. from the Tieraerztliche Hochschule, Germany specializing in Veterinary Immunology. >4 years of successful experience as post-doctoral researcher in Vaccinology at Max Planch Institute of Infection Biology, Berlin. From 2001 to 2007, Head of Technical and Marketing Department of Bayer Vietnam Ltd. Animal Health Division. His team developed benchmarking aquaculture pharmaceutical product ranges for shrimp and freshwater fish. In 2008, transferred to Bayer Thai Co., Ltd. for Bayer Health Care Animal Health; presently working as a Regional Business Development Manager Aquaculture and Nutrition in Asia Pacific. Responsible for extending Bayer's products in the feed additive and the aquaculture pharmaceutical segment.

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Worked in fisheries and seafood safety for 30 years; currently an aquaculture food safety expert with the United States Food and Drug Administration (FDA). Grew up fishing, occasionally selling his daily catch along the Gulf of Mexico and Pacific Ocean. Marine Biology graduate from the University of California; worked ten years for the State of Texas as a Marine Environmental Specialist. Joined the United States FDA (1990), eventually becoming the Lead Shellfish Inspector. After a *Salmonella* outbreak traced back to aquacultured shrimp, led a collaborative research study in 12 countries on the source, prevalence and occurrence of *Salmonella* in aquaculture. Has written extensively about aquaculture food safety. In 2007, collaborated with the Joint Institute of Food Safety and Applied Nutrition; helped develop a preventive food safety program called Good Aquaculture Practices (GAqP); Team Leader for GAqP training around the world; convener of an ISO/TC 234 Work Group developing ISO standards for aquaculture farm food safety.

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Leader of the Aquaculture Drug Team in the Office of New Animal Drug Evaluation in the United States Food and Drug Administration's Center for Veterinary Medicine (US FDA/CVM). Received a VMD from the University of Pennsylvania's School of Veterinary Medicine with elective training in aquatic animal medicine. Before joining the FDA, completed a two-year postgraduate veterinary internship with the University of Florida's Tropical Aquaculture Laboratory, providing extension and diagnostic services to the ornamental fish industry and the Florida Aquarium. Joined the US FDA/CVM as a reviewer on the Aquaculture Drugs Team, and currently serves as the leader of this team and CVM's representative to the United States Joint Subcommittee on Aquaculture, an interagency (federal government) coordinating group.

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Qualified as a veterinary surgeon from University College Dublin (1985); Ph.D from Queen's University Belfast (1997) for studies on anabolic steroid metabolism in cattle. Following five years spent in mixed veterinary practice in Ireland and Australia; joined the laboratory service of the Department of Agriculture and Rural Development for Northern Ireland in 1990. Over an 11-year period, worked in the area of veterinary drug residue analysis in the National Reference Laboratory for drug residues and occupied several senior management positions in that period. Published over 50 peer-reviewed scientific papers. In 2003, took up a post with the European Commission's Directorate-General for Health and Consumer Protection in the Food and Veterinary Office (FVO). Currently Head of Sector for Residues, leading the FVO team responsible for auditing residue control systems in Member States and third countries and the evaluation of third country residue control plans for food of animal origin.

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B.Sc. in Inland Fisheries. Shanghai Fisheries University (1982); M.Sc. in Aquaculture, University of the Philippines (1986). Joined Freshwater Fisheries Research Centre (FFRC) of Chinese Academy of Fishery Sciences (CAFS) in 1982. Lecturer and later Chief of the Training Division of FFRC. Appointed by CAFS as Deputy Director of FFRC and Director of Asia-Pacific Regional Research and Training Centre for Integrated Fish Farming. Coordinated international training and local higher education and international cooperation activities of FFRC for 15 years. Led and participated in >12 research projects sponsored by the Chinese Government and international donor agencies on carp genetic improvement, fisheries/aquaculture socio-economics and policy, aquaculture environment and integrated aquaculture systems. Since 2008, as Aquaculture Officer for the FAO Regional Office for Asia and the Pacific, responsible for coordinating FAO aquaculture activities, developing and implementing FAO technical cooperation programmes in the region and providing technical advice for the member governments in the area of aquaculture and inland fisheries and representing FAO in relevant regional fora.

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Aquaculture specialist with the United States Food and Drug Administration (FDA) since 2000. M.Sc. in Marine Biology, University of Gdansk. Professional career started at the Institute of Oceanology, Polish Academy of Science, conducting studies of the marine ecosystem in the areas of environmental biology, ecotoxicology and biogeochemical cycles. From 1993 to 2000, worked for a pharmaceutical company on R&D. Current work involves the public health issues pertaining to aquaculture, particularly chemical contaminants and animal drug residues in seafood. Engaged in the audit programs. Provides technical input to Codex and WTO. Member of the United States delegation to the 4th Session of the FAO Subcommittee on Aquaculture and participated in work on aquaculture certification guidelines. Represents FDA on policies relating to aquaculture and seafood safety issues at national and international professional meetings and conferences. Published papers on impact of environmental contaminants on marine organisms and exposure to methylmercury from seafood consumption.

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Fisheries Biologist at the Inland Aquatic Animal Health Research Institute (AAHRI), Department of Fisheries, Thailand. M.Sc., Faculty of Fisheries, Kasetsart University (2004). PCR technician at black tiger shrimp culture farm in the south of Thailand. Joined AAHRI; moved to Bangkok aquarium for six months where she was involved in disease prevention and treatment for all aquarium animals; returned to AAHRI where she has served in her current position as a head of histopathology and mycology laboratory since 2004. Involved in disease diagnosis, farm monitoring and salinity for both establishment and exportation. Current research is mainly on surveillance and monitoring for controlling diseases in aquatic animals.

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Director of the Division of Scientific Support at the United States Food and Drug Administration's Center for Veterinary Medicine. DVM degree (1996) and residency training in anatomic pathology (1999) at the Virginia-Maryland Regional College of Veterinary Medicine. During his veterinary training, investigated the pharmacokinetics of antimicrobials in tilapia. Joined FDA/CVM in1999 as a scientific reviewer and then led the Aquaculture Drugs Team from 2003–2008. Co-authored the Judicious Use of Antimicrobials for Aquatic Veterinarians, participated in the Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance (Seoul, 2006), and collaborated on the development of the public data generating partnership for aquaculture drugs in the United States. Member of the United States Delegation to the Codex Alimentarius ad hoc Intergovernmental Task Force on Antimicrobial Resistance. Currently oversees a group of interdisciplinary scientists working in the areas of biostatistics, environmental science, risk analysis and pathology.

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Diploma on fish disease examination and diagnosis, currently working on his bachelor degree in aquaculture at Shanghai Ocean University. Joined the Guangdong Provincial Aquatic Animal Epidemic Disease Prevention and Control Center in1997, mainly in charge of aquaculture technology extension, aquatic disease prevention and control, remote diagnosis system for aquatic animals and epidemic monitoring in Guangdong Province. Author of several articles on the rapid diagnosis of marine fish diseases and scientific application of fish drugs.

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Senior Aquaculturist, Bureau of Fisheries and Aquatic Resources (BFAR), Philippines (1981–2002); NACA Regional Aquatic Animal Health Specialist (1999–2002); Research Pathologist at the Cooperative Oxford Laboratory (fish disease research facility of Maryland Department of Natural Resources (DNR) and National Oceanographic and Atmospheric Administration (NOAA) National Marine Fisheries Service) (2002–2004); FAO Fishery Resources Officer (2004). Ph.D. (Monbusho scholar, University of Tokyo, 1995) and post-doctoral fellow (JSPS fellow), Nippon Veterinary and Animal Science University (1998). >25 years of academic and professional work in aquaculture and fish health management and extensive travel to >45 countries. Led two international emergency disease investigation task forces: KHV in Indonesia (2002) and EUS in southern Africa (2007); introduced the concept of applying risk analysis in aquaculture to NACA/FAO members in 2002. Editorial responsibilities include FAO Aquaculture Newsletter and other publications, Diseases in Asian Aquaculture series; >80 FAO technical papers, peer-reviewed articles and other publications. Currently involved in FAO's normative and field programmes on small-scale aquaculture, gender, aquatic biosecurity (field projects in southern Africa region, Caribbean, Gulf region, Pacific Islands, Western Balkan region).

Peter Smith
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Emeritus Professorship at the Department of Microbiology, National University of Ireland Galway. Editor of the disease section of the journal *Aquaculture*. Interests have always been in applied research and for the last 30 years, research focused on providing the scientific research that would help fish farmers deal effectively with the problem of infectious diseases of their fish. Had major interest in the use of chemotherapy in aquaculture and published approximately 90 scientific papers on this topic. Initial interests focused on the administration, efficacy and environmental impacts of these agents. In the last decade, had a major interest in promoting the rational and prudent use of antimicrobial agents in aquaculture. Currently involved in developing standard susceptibility test protocols and the interpretive criteria that can provide meaning for the data they generate. Member of the Aquaculture Working Group of the Veterinary Sub-Committee of the Clinical and Laboratory Science Institute, co-author of the standard susceptibility test protocols produced by this organization. Incoming chair of the future OIE ad hoc group on antimicrobial resistance in aquatic animals.

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Senior Aquaculturist at BFAR (Philippines) Fish Health Management and Quality Assurance Section (FHMQAS). Specializes in fish health management; currently involved in BFAR's aquaculture food safety and quality programmes, particularly as the coordinator for the implementation of the national residues monitoring and control programme. Designated as Quality Assurance Manager of the residue laboratory of FHMQAS. M.Sc. in Aquaculture, Universiti Putra Malaysia (1997), with research on fish physiology. DVSM from Central Luzon State University (1990). 12 years in government service working on various aspects of fish health, including research, diagnostics, laboratory, training and technical assistance, and aquaculture food safety and quality, particularly veterinary drug residues. Involved in several regional and international collaborative projects on fish health and food safety.

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Head of the aquatic animal health research section of AAHRI. Expertise in fish and shellfish microbiology. 20 years' experience; involved in disease diagnosis and disease control regimes for both local consumption and exportation; involved with governmental aquaculture policy and the registration of chemicals and micro-organisms used in aquaculture. Most recent research concerns EU-funded Asia resist project focused on three major subjects: assessment of the potential for antibiotic resistance in aquaculture, assessment of the potential for antibiotic resistance transferring in aquaculture, and identification of critical control points to eliminate antibiotic resistance, especially chloramphenicol resistance in the Southeast Asian aquaculture environment. Supervised M.Sc. and Ph.D. students of Kasetsart University since 1985.

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Senior Fishery Resources Officer of the Fisheries and Aquaculture Department of FAO. Specialized in aquaculture, disease control and health management (with particular reference to microbiology and immunology). Worked in all parts of the world, with most experience in Asia and recently Africa. Responsible for many projects on aquaculture and aquatic animal health at national, regional and international levels. A former teacher at the University of Colombo and the Universiti Putra Malaysia. Ph.D. from University of Stirling. Responsible for initiating major policy changes in aquatic animal health in relation to aquaculture at global level. Currently Technical Secretary to the Sub-Committee on Aquaculture of the Committee on Fisheries of the FAO. Continuously served as FAO representative to the OIE Aquatic Animal Health Standards Commission during the last ten years. Founding member of Asian Fisheries Society (AFS) and served as Chairperson and Vice-Chairperson of the Fish Health Section of the AFS.

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Director of AAHRI. Background in fishery biology. Head of the Antibiotic Residue Inspection Unit at Phuket Coastal Fisheries and Development Center (1990–2003); at which time involved in a number of research studies on shrimp diseases, especially parasitic and viral diseases. Currently involved with the national aquatic animal disease control policy under the Animal Epidemic Act. Also appointed as member of the National Fish Disease Committee as well as the Committee of the Antibiotic Control Plan.

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Veterinary microbiologist. Veterinary diploma from the University of Illinois in Champaign-Urbana (1990); worked in the private sector in the following five years. From 1994–1995, participated in a fish health/pathology internship at the Atlantic Veterinary College, Prince Edward Island, and subsequently established a private veterinary clinic specializing in fish medicine in Quebec. From 1998–2008, employed by the Université de Montréal, Faculté Medicine Vétérinaire (UdeM, FMV) offering fish diagnostic services; concurrently participated in a microbiology residency programme which examined antimicrobial resistance (AMR) in *Aeromonas salmonicida*. Following the residency, successfully completed the board examination of the American College of Veterinary Microbiologists (2000). Acts as an expert adviser on projects dealing with AMR with the Public Health Agency of Canada and the University of Guelph. Currently participating in an M.Sc. degree project supported by the Public Health Agency of Canada at the UdeM, FMV, studying molecular microbiology and AMR in *Vibrio* and *Aeromonas* species isolated from retail seafood.

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Senior Fisheries Biologist at the Inland Fisheries Research and Development Bureau. M.Sc. Biology from Kasetsart University (1980). Worked with the Department of Fisheries (1981 to present) conducting research on pesticide residues in the aquatic environment. Aside from research, her responsibility also concerns chemical registration and chemicals allowed for use in aquaculture. Member of the Hazardous Substance Committee (HSC) in Thailand and subcommittees under HSC from 1981 to present.

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Marine science graduate from Kasetsart University (1990). Continued his study of management of agricultural knowledge systems at Wagenigen Agricultural University, Netherlands (1994–1996). Worked in various positions with SEAFDEC for 18 years, last 4 years as Policy and Program Coordinator, overseeing all fisheries and aquaculture disciplines of the organization. Joined the ASEAN Secretariat (2008) as Senior Officer in Agriculture, responsible for ASEAN policy and cooperation on agriculture, covering crops, livestock, fisheries and aquaculture, agricultural cooperatives, etc. Coordinates SPS issues as part of ASEAN trade promotion and facilitation. Currently a steering committee member of international/regional bodies/initiatives, including the World Bank's Global Program on Fisheries and FAO/OIE Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs). As ASEAN flagship initiatives, currently promoting the development of ASEAN Good Agricultural Practices (GAPs), covering fruits and vegetables, aquaculture and animal husbandry, as well as responsible use of veterinary drugs in aquaculture.

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Director of Technical Services and Market support in Intervet Schering-Plough's Aquatic Animal Health Business unit. B.Sc. in Zoology, University of Aberdeen (1993). Spent several years working in the fish farming industry with experience in trout, salmon and the then-emerging seabass industry before taking a post at Kingston University to develop vaccines for fish. In 1989, joined Aquaculture Vaccines Ltd., which is now part of Intervet Schering-Plough. His roles have covered all aspects of developing and using vaccines and medicines in aquaculture, including the development and registration of the first orally applied vaccines for fish. Current role is directing the company's technical team to develop and implement health control programmes for the major production species around the world. Intervet Schering-Plough is the leading manufacturer and supplier of vaccines and pharmaceuticals for the aquaculture industry.

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Social economist and aquaculturist from the FFRC; M.Sc. in Aquaculture from Huazhong Agricultural University (1996) and Ph.D. in Agriculture Economics and Management from Nanjing Agricultural University (2008). Member of Technical Advisory Committee of the Network of Aquaculture Centres in Asia-Pacific. Involved with environmentally friendly aquaculture research and social-economic analysis on fishery and aquaculture since 1997. Was a key member of the research team on carp genetic improvement and the use of aquaculture for poverty alleviation in flood areas in China. Field survey and participatory approach were applied in his research on economic and efficiency analysis. FAO consultant on economical analysis on feed application in Chinese carp farming system. More than 20 scientific papers published in national and international journals. One of the authors of the FAO Fisheries Technical Paper *Economics of Aquaculture Feeding Practices in Selected Asian Countries*.

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President of Thai Aquaculture Business Association (TABA) and business development manager for Alltech Biotechnology Corporation. M.Sc. in Fisheries Science (1995); B.Sc. in Aquaculture from Kasetsart University (1989). Professional career started at the companies where he worked for 20 years in the field of shrimp and fish farm and hatchery. Joined the TABA in 2002 where he has held various positions, most recently President (2006–2009). Until 2000, was also part-time lecturer in environment for aquatic animal diseases at the veterinary faculties of Kasetsart University and Mahidol University. Work has mainly been on disease prevention in aquatic animals, with special emphasis on aquatic animal heath products. Has been crucial in the development of a sustainable aquaculture industry in Thailand.

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DVM (1994) from the Faculty of Veterinary Medicine, Universitat Autónoma de Barcelona (UAB). Started career as fish pathologist at the Fish Diagnostic Pathological Service of the UAB, worked for seven years in the field of pathological diagnosis, particularly in histopathology. In 2001, joined Skretting Spain as manager of the Fish Health Service, providing technical support and health advice to more than 100 fish farms in the Mediterranean basin. During this period, focused on several fish species, mainly rainbow trout, seabream, sea bass and turbot. Has worked in the areas of clinical and pathological diagnosis, development of preventive and control programmes, biosecurity, sanitary legislation and fish health education of farmers. Continued collaborating with research centers and universities in Europe on several aspects of fish pathology, mainly etiopathogenesis of emerging diseases and the development of diagnostic tools, vaccines and vaccination programmes. Since January 2009, a Speciality Feeds Product Manager in Skretting Spain, responsible for health-promoting diets and medicated feeds.

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DVM (1985), M.Sc. (1990) and Ph.D. (1999) from the Veterinary Faculty, University of Zagreb with theses "Spreading of swim bladder inflammation in carp" and "Epizootiology, patholomorphology and bacteriology of vibriosis in sea bass cultivated along eastern Adriatic coast". Professional career started at the veterinary university at the Department of Fish Biology and Pathology in 1986, where she worked mainly in the field of cyprinid pathology. In1993, started to be involved in studies on pathology of Mediterranean cultivated species (sea bass and seabream), working both in the field and laboratory and doing consulting. Joined the Croatian Veterinary Institute (1995) and participated in the establishment of the Laboratory for Pathology of Aquatic Animals, now designated as the National Reference Laboratory for fish and molluscan diseases. Took part in preparing national legislation and educating field veterinarians on aquatic animal health. Research has mainly been on fish and molluscan pathology in farms and open waters; published >40 peer-reviewed papers in national and international journals.

Annex 2

EXPERT WORKSHOP PROGRAMME

Date and Time	Activity
14 December (Mon)	Arrival of participants
15 December (Tues)	Opening session
08:30-08:45	Registration and distribution of information package
08:45-09:30	Welcome remarks and self-introduction by participants Dr He Changchui, FAO Assistant Director General and Regional Representative for Asia and the Pacific (to be confirmed) Dr Somying Piumsombun, Director-General, Thailand's Department of Fisheries (to be confirmed)
	Setting the scene
09:30-09:45	Review of workshop background, goals, process and expected outcomes *(Dr Melba B. Reantaso, FAO)*
09:45-10:15	Coffee break (picture taking will be done before coffee break)
	Session I: The current situation Session I will be informed by 14 thematic papers
Chairperson:	**Rapporteur:**
10:15-10:45	**Presentation 1:** Public health and trade impact of antimicrobial use in aquaculture *(Dr Iddya Karunasagar, FAO)*
10:45-11:15	**Presentation 2:** Environmental impacts and management of veterinary medicines in aquaculture: the case of salmon aquaculture in Chile *(Dr Sandra Bravo, Chile)*
11:15-11:45	**Presentation 3:** Residue control plans: European Union requirements for third countries *(Dr John McEnvoy, European Commission)*
11:45-12:15	**Presentation 4:** Results of the FAO international survey on the use of veterinary medicine in aquaculture *(Dr Victoria Alday, FAO Consultant)*
12:15-12:45	**Presentation 5:** Results of the international survey on antimicrobial resistance in aquaculture *(Dr Carl Uhland, Canada)*
12:45-14:15	Lunch Break
14:15-14:45	**Presentation 6:** Use of veterinary medicines in Thai aquaculture: current status *(Dr Puttharat Baoprasertkul, Thailand)*
14:45-15:15	**Presentation 7:** Use of veterinary medicines in Vietnamese aquaculture: current status *(Mr Mai Van Tai and Ms Phan Thi Van, Viet Nam)*
15:15-15:45	**Presentation 8:** Use of veterinary medicines in Philippine aquaculture: current status *(Dr Sonia Somga, the Philippines)*
15:45-16:15	Coffee break
16:15-16:45	**Presentation 9:** Use of veterinary medicines in Chinese aquaculture: current status *(Dr Yuan Xinhua and Dr Wen Chen, China)*
16:45-17:15	**Presentation 10:** Antimicrobial resistance: complexities and difficulties of determination *(Prof. Peter Smith, Ireland)*
17:15-17:45	Wrap-up of day session (Chairperson) Announcement and schedule for Day 2
16 December (Wed)	**Session I: The current situation (continued)**
08:30-08:40	Introduction to Day 2 (Chairperson)
08:40-09:10	**Presentation 11:** Antibiotic contamination pathways in shrimp aquaculture: the case of Bangladesh *(Dr Rohana Subasinghe, FAO)*
09:10-09:40	**Presentation 12:** Seafood HACCP program and FDA enforcement (inspections, testing, import alerts) *(Dr Barbara Montwill, USA)*
09:40-10:10	**Presentation 13:** Drug approval process and other regulatory considerations for use of aquaculture drugs in the United States of America *(Dr Jennifer Matysczak, USA)*

10:10-10:40	Coffee break
10:40-11:10	**Presentation 14:** Oral delivery of veterinary medicines through aquafeed in Mediterranean aquaculture *(Dr Carlos Zarza, Skretting)*
11:10-11:50	General discussion
	Session II: The way forward Session II will be informed by 5 thematic papers and 1 summary paper
Chairperson:	**Rapporteur:**
11:50-12:00	Introduction to Session II
12:00-12:30	**Presentation 15:** Disease prevention as basis for sustainable aquaculture *(Dr Roar Gudding, Norway)*
12:30-14:00	Lunch break
14:00-14:30	Presentation 16: Health management tools from a manufacturing point of view *(Dr Robin Wardle, Intervet-Scherring Plough)*
14:30-15:00	**Presentation 17:** Alternatives to antibiotics in aquaculture *(Dr Indrani Karunasagar, India)*
15:00-15:30	**Presentation 18:** Training and implementation of good aquaculture practices (GAqPs) related to the use, documentation, monitoring of antimicrobials and chemotherapeutants *(Dr Brett Koonse, USA)*
15:30-16:00	Coffee break
16:00-16:30	**Presentation 19:** Best practices in the use or avoidance of chemo-therapeutants in aquaculture: Indian experience *(Dr CV Mohan, NACA)*
16:30-17:00	**Presentation 20:** Summary of the major issues related to the use of veterinary medicine in aquatic food production and guidance provided by the FAO Code of Conduct for Responsible Fisheries and others *(Dr J Richard Arthur, FAO Consultant)*
17:00-17:30	General discussion and wrap-up of Day 2 (Chairperson) Announcement and schedule for Day 3
17 December (Thurs)	**Session III: Working Group exercise**
Chairperson:	**Rapporteur:**
08:30-08:40	Introduction to Session III Working Group guidelines
08:40-11:30	Working Group exercise 1
09:30-10.00	Coffee break
11:30-12:30	Working Group 1 presentation and discussion
12:30-14:00	Lunch break
14:00-16:00	Working Group exercise 2
16:00-16:30	Coffee break
16:30-17:30	Working Group 2 presentation and discussion
17:30-17:40	Announcement and schedule for Day 3
18:00 -	Dinner
18 December (Fri)	**Session III: Working Group exercise (continued)**
08:30-10:00	Working Group exercise 3
10:00-10:30	Coffee break
10:30-11:30	Working Group exercise 3 (continued)
11:30-12:30	Working Group 3 presentation and discussion
12:30-14:00	Lunch break
	Session IV: Conclusions and the way forward
Chairperson:	**Rapporteur:**
14:00-15:30	Discussion on recommendations and conclusions *(Dr Rohana Subasinghe)*
15:30-16:00	Coffee break
16:00-17:00	The way forward and closing
19 December (Sat)	Departure of participants

Annex 3

EXPERT WORKSHOP GROUP PHOTO

Experts participating in the FAO/AAHRI Expert Workshop on Improving Biosecurity through Prudent and Responsible Use of Veterinary Medicines in Aquatic Food Production, held in Bangkok, Thailand, from 15–18 December 2009. Seated left to right: Dr He Changchui (FAO Assistant Director-General and Regional Representative for Asia and the Pacific, now retired), Dr Somying Piumsombun (Director-General Thailand's Department of Fisheries), and Dr Rohana Subasinghe (Senior Aquaculture Officer, FAO, Rome).

Glossary[1]

Anthelminthics Veterinary medicines effective in treating diseases caused by parasitic helminths.

Antibiotic A drug of natural or synthetic origin with the capacity to inhibit the growth of or to kill microorganisms. Antibiotics that are sufficiently non-toxic to the host are used as chemotherapeutic agents in the treatment of infectious diseases of man, animals and plants.

Antimicrobial agent Any substance of natural, semisynthetic, or synthetic origin that at in vivo concentrations kills or inhibits the growth of microorganisms by interacting with a specific target.

Antiparasitics Veterinary medicines effective in treating diseases caused by parasites.

Aquatic animals All life stages (including eggs and gametes) of fish, molluscs, crustaceans and amphibians originating from aquaculture establishments or removed from the wild, for farming purposes, for release into the aquatic environment, for human consumption or for ornamental purposes.

Aquatic animal health practitioners Veterinarians working in the field of aquatic animal health and non-veterinary aquatic animal health experts trained and authorized to prescribe and/or supervise the use of veterinary medicines in aquaculture production facilities.

Bacterium A unicellular prokaryotic microorganism that multiplies by cell division, typically has a cell wall and may be aerobic or anaerobic, motile or non-motile, free-living, saprophytic or pathogenic.

Biosecurity The sum total of the activities and measures taken by a region, country, group of aquaculture producers or single aquaculture production facility to protect its natural aquatic resources, capture fisheries, aquaculture, biodiversity and/or cultured stocks and the people who depend on them from the possible negative impacts resulting from the introduction and spread of serious aquatic animal diseases.

Chemotherapeutants Chemicals used to treat infections or non-infectious disorders.

Competent authority The veterinary services, or other authority of an OIE Member Country, having the responsibility and competence for ensuring or supervising the implementation of the aquatic animal health measures or other standards in the World Organisation for Animal Health's (OIE) Aquatic Animal Health Code.

Disease Clinical or non-clinical infection with an etiological agent.

1 The following definitions were adopted by the workshop participants for use in the FAO Technical Guidelines for Responsible Fisheries, Prudent and Responsible Use of Veterinary Medicines in Aquaculture (FAO, in preparation), and are used in this volume.

Disinfectants	Chemical compounds capable of destroying pathogenic microorganisms or inhibiting their growth or survival ability.
Extra-label/Off-label Use	The use of an antimicrobial agent that is not in accordance with the approved product labelling. Such uses may be allowed under certain national regulations.
Fungus	Any member of the Kingdom Fungi, comprising single-celled or multinucleate organisms that live by decomposing and absorbing the organic material in which they grow.
Health certificate	A certificate issued by an exporting country's competent authority attesting to the health status of a shipment of aquatic animals (also see International aquatic animal health certificate, as defined by the OIE).
High health	Aquatic animals originating from a production facility having specific pathogen free (SPF) status, but which are now held in commercial facilities under less rigorous biosecurity conditions and thus a lower guarantee of health status. Once animals leave a high health production facility, they are no longer considered to have high health status.
Microorganisms	Principally viruses, bacteria and fungi (microscopic species, and taxonomically related macroscopic species). Microscopic protistans (protozoa) and algae may also be referred to as microorganisms.
Monitoring	The intermittent performance and analysis of routine measurements and observations, aimed at detecting changes in the environment or health status of a population.
Parasite	An organism that lives upon or within another living organism (host) at whose expense it obtains some advantage, generally nourishment.
Pathogen	An infectious agent capable of causing disease.
Quarantine	Maintaining a group of aquatic animals in isolation with no direct or indirect contact with other aquatic animals, in order to undergo observation for a specified length of time and, if appropriate, testing and treatment, including proper treatment of the effluent waters.
Interlaboratory comparison (ring test)	Any evaluation of assay performance and/or laboratory competence in the testing of defined samples by two or more laboratories; one laboratory may act as the reference in defining test sample attributes.
Risk	The likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal or human health.
Specific pathogen free (SPF)	Aquatic animals that have been produced and are tested and held under rigorous conditions of biosecurity that provide assurances that they are free of certain specified pathogens. Once animals leave an SPF facility, they are no longer considered to have SPF status.

Specific pathogen resistant (SPR)	A stock of aquatic animals that has been bred to have genetic resistance to or improved tolerance of infection by a specific pathogen.
Surveillance	A systematic series of investigations of a given population of aquatic animals to detect the occurrence of disease for control purposes, and which may involve testing samples of a population.
Transboundary aquatic animal diseases (TAADs)	Aquatic animal diseases that are highly contagious or transmissible, with the potential for very rapid spread irrespective of national borders that cause serious socio-economic and possibly public health consequences.
Vaccines	Antigen preparation from whole or extracted parts of an infectious organism, which is used to enhance the specific immune response of a susceptible host.
Veterinary medicines	Any substance or combination of substances presented for treating or preventing disease in animals or which may be administered to animals with a view to making a medical diagnosis or to restoring, correcting or modifying physiological functions in animals.
Virus	One of a group of minute infectious agents, characterized by a lack of independent metabolism and by the ability to replicate only within living host cells.

Traditionally, the threats to aquaculture posed by aquatic pathogens have been addressed through the use of antimicrobials, including chemotherapeutants, disinfectants, antibiotics and vaccines. However, the inappropriate use of antimicrobials can lead to problems related to increased frequency of bacterial resistance, with negative impacts on the efficacy of these agents to control infectious diseases in aquaculture and the potential transfer of resistance genes in bacteria from the aquatic environment to other bacteria and the possibility of resistance extending to human pathogens. Injudicious use of antimicrobials has also resulted in the occurrence of their residues in aquaculture products, resulting in commodity bans by importing countries and associated economic impacts. The FAO/AAHRI Expert Workshop on Improving Biosecurity through Prudent and Responsible Use of Veterinary Medicines in Aquatic Food Production was convened in Bangkok, Thailand, from 15 to 18 December 2009, in order to understand the current status of the use of antimicrobials in aquaculture and to discuss the concerns and impacts of their irresponsible use on human health, the aquatic environment and trade. Such discussions became the basis for drafting recommendations targeted for both government and private sectors and for developing guiding principles on the responsible use of antimicrobials in aquaculture to be considered as part of future FAO CCRF Technical Guidelines on Prudent and Responsible Use of Veterinary Medicines in Aquaculture. Safe and effective veterinary medicines need to be available for efficient aquaculture production, and their use should be in line with established principles on prudent use to safeguard public and animal health. The use of such medicines should be part of national and on-farm biosecurity plans and in accordance with an overall national policy for sustainable aquaculture.

ISBN 978-92-5-106975-2 ISSN 2070-7010

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BA0056E/1/01.12