AAHL Fish Diseases Laboratory Bacteriology Workshops

FRDC Project Number: 2000/149

Final Report

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1.0 Non-Technical Summary

2000/149 AAHL Fish Diseases Laboratory Bacteriology Workshops

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OBJECTIVES:

1. Introductory Workshop in Fish Bacteriology – to provide training in basic diagnostic techniques for the major diseases of salmonids and other finfish.

2. Bacteriology Workshop - Diagnosis of Vibriosis in aquatic animals.

3. Preparation and submission of a report recommending bacteriological methods for aquatic animals for inclusion in Australian Standard Diagnostic Techniques (SDTs) and agreement on authorship, provided to the Aquatic Animal Health Unit, National Office of Animal and Plant Health.

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED

Two workshops on bacteriology of aquatic animals were held at CSIRO AAHL. The workshops provided veterinary microbiologists from each state and territory with an opportunity to further develop their experience and expertise in the recognition and identification of a comprehensive range of exotic and enzootic bacterial pathogens.

General agreement was reached on an ongoing quality assurance and training scheme for aquatic animal bacteriology.

A report on bacteriological methods for aquatic animals for inclusion in Australian Standard Diagnostic Techniques was prepared for AFFA.

The Sub-Committee of Fish Health first recommended the concept of a series of aquatic animal bacteriology workshops. In December 1998, the Fisheries Resources Research Fund provided funding for a Fish Health Management Committee (FHMC) and Fish Health Coordinating Group (FHCG) meeting on Aquatic Animal Health Technical Issues. Participants at this meeting included representatives from Commonwealth government and all State/Territory governments. At this meeting it was recognised that due to their individual needs and circumstances, diagnostic laboratories had developed their own procedures and reagents for the identification of bacterial pathogens of aquatic animals. As a result there was

little standardisation and considerable variation in the diagnostic capability of laboratories across Australia. There was general agreement that in order to raise the level of technical expertise and work towards a level of standardisation between regional laboratories, a series of workshops to address these issues would be useful. It was on this basis that funding for the bacteriology training workshops was obtained from the FRDC.

The main aim of the workshops was to provide participants with the opportunity to work with a comprehensive range of bacterial pathogens in order to gain experience and to compare and evaluate the various protocols, reagents and methodologies currently available. The workshops also provided an opportunity to consider such issues as quality assurance, training and the need for standard diagnostic techniques for bacterial pathogens of aquatic animals.

Members of VETCOM from each State and Territory were requested to nominate the most experienced veterinary microbiologist with responsibility for laboratory culture, identification and diagnosis of aquatic animal specimens in their respective states or territories to attend.

Two workshops were held at the CSIRO Australian Animal Health Laboratories in Geelong. The first took place from 2-5th October 2000 and the second from 5-9th February 2001. The Australian Animal Health Laboratory is a high security bio-containment facility, designed specifically for work with exotic bacterial and viral animal pathogens. These FRDC funded workshops provided participants with a unique opportunity to make use of these facilities in order to gain hands on experience with a wide range of known exotic and enzootic bacterial pathogens.

The first workshop consisted of a series of integrated lectures, laboratory practicals and discussions on general bacterial disease diagnosis in aquatic animals. The second workshop was slightly longer and while similar to the first, was devoted solely to Vibrio species. Vibrio species produce disease in a wide range of farmed marine species including finfish, crustaceans and shellfish. Vibriosis affects larvae, juveniles as well as adult stages and occurs in temperate through to tropical environments. Vibrio spp. remain the most commonly encountered, poorly characterised and most difficult to identify group of bacteria. As a result a large proportion of the Vibrio species isolated from samples are never identified.

Although attendance at the workshops was confined to experienced veterinary microbiologists. The workshop programs were developed on the understanding that participants would have varying levels of experience and expertise, some with considerable experience in aquatic animal disease diagnosis while others would be more familiar with terrestrial animals. In addition, given the range of climates found across Australia, some participants would be more familiar with tropical species, while others would be more concerned with hosts and pathogens found in cooler temperate areas. In this light, emphasis was given to providing participants with every opportunity to share their ideas and experience, and explore common interests. A further outcome of these workshops is a strengthening of the network of aquatic animal bacteriologists in Australia. The development of professional relationships and individual contacts within this group represents a significant enhancement of the effective diagnostic resources available to aquaculture industries around Australia.

Mr Nicholas Gudkovs (CSIRO), Dr. Jeremy Carson (DPIW&E, Tas.) and Dr. Inger Dalsgaard (Danish Institute of Fisheries Research) provided the majority of lectures at the workshops, with additional lectures by Prof Hisatsugu Wakabayashi (University of Tokyo), Dr. Mark Crane (CSIRO) and Dr. Helen Byers (CSIRO).

KEYWORDS: Training, bacteriology, bacterial disease, diagnosis

2.0 Background

In response to the Report of the National Task Force on Imported Fish and Fish Products [6] and the Nairn Report on quarantine [7], FHMC convened a Strategic Planning Workshop of Government and industry representatives to develop a national five-year plan for aquatic animal health, 28-29 April 1998, Canberra [4]. Participants included representatives of SCARM, SCFA and major aquaculture industry leaders and peak industry bodies.

The outcome of the meeting was an agreed national five-year plan for aquatic animal health in Australia (AQUAPLAN) consisting of eight programs. These programs will be overseen by one or more members of the Fish Health Management Committee. The Quarantine program will be overseen by the Australian Quarantine and Inspection Service (AQIS) which has statutory authority in this area.

The programs and overseers are:

1. International linkages (Gardner Murray, Chief Veterinary Officer; Angus Horwood, RecFish Australia);

2. Quarantine (Paul Hickey, AQIS);

3. Surveillance, Monitoring and Reporting (Gardner Murray; Angus Horwood);

4. Preparedness and Response (John Pollock, SCARM; Angus Horwood);

5. Awareness (Mike Rickard, CSIRO Australian Animal Health Laboratory);

6. Research and development (Rick Scoones, Australian Aquaculture Forum);

7. Legislation, policies and jurisdiction (Alex Schaap, SCFA); and

8. Resources and funding (Brian Jeffries, Australian Seafood Industry Council).

It is significant that, in the period between late 1998 and early 1999, AQUAPLAN was endorsed by key aquaculture and fisheries peak bodies in Australia, and at the 6th Ministerial Council on Forestry, Fisheries and Aquaculture, Sydney, 30 April 1999, Ministers endorsed AQUAPLAN as a strategic framework for the prevention and spread of aquatic animal diseases.

As part of Program 3 of AQUAPLAN, FHMC/FHCG conducted a National Workshop on Aquatic Animal Health: Technical Issues [5] where, amongst other issues, development of Standard Diagnostic Techniques (SDTs) (AQUAPLAN key issue 3.1.4) was discussed. With respect to SDTs for aquatic animal bacteriology, it was recommended that AAHL Fish Diseases Laboratory organise training workshops to facilitate standardisation of diagnostic procedures used at State and Commonwealth diagnostic laboratories, and that funding to support such workshops should be sought from funding bodies such as FRDC.

3.0 Need

It has been recognised for some time that there is kittle or no standardisation amongst laboratories undertaking diagnosis of bacterial diseases in aquatic animals. In part, to address this issue, in December 1998 the Fish Health Management Committee and the Fish Health Coordinating Group recommended the concept a national fish bacteriology training workshop [5]. At this meeting they observed that “Diagnostic laboratories across the country vary in their diagnostic capability for aquatic animal pathogens. Due to their individual needs, each diagnostic laboratory has developed their own reagents/procedures for the identification of bacterial pathogens of aquatic animals.”

They suggested that the outputs of the workshop should include:

- Training of State/Territory bacteriologists in isolation of specific enzootic and exotic aquatic animal pathogens
- Agreement on recommended methods for inclusion in Australian Standard Diagnostic Techniques (SDTs).
- Agreement on authorship of SDTs.
- Agreement on plan to develop and publish SDTs.
- Production of SDTs.

While it was obvious that production of the SDTs was beyond the scope of what could be achieved at a single workshop, it is clear that considerable benefits would accrue from a series of workshops aimed at experienced veterinary microbiologists, from state departments of agriculture or the equivalent.

The workshops could provide participants, especially those new to the area of aquatic animal disease diagnosis, with hands on experience with a range of exotic and enzootic bacterial fish pathogens. For those more experienced in the area of aquatic animal bacteriology, the workshops would provide with an opportunity to evaluate and compare some of the diagnostic protocols and commercial identification systems used in other Australian laboratories.

In addition, all participants would benefit from the opportunity to share their knowledge and experience and strengthen the developing network of people actively involved in fish disease diagnosis. At the conclusion of the workshops participants would be in a good position to recommended what Standard Diagnostic Techniques were required and who could most usefully be approached to prepare these.

4.0 Objectives

The objectives of the project were not modified since the original funding application.

1. Conduct a general introductory workshop in fish bacteriology, providing training and experience with basic diagnostic techniques for the major bacterial pathogens of salmonids and other finfish.

2. Conduct a second workshop focusing on the diagnosis of Vibriosis in aquatic animals.

3. Prepare and submit a report to the National Office of Animal and Plant Health, Aquatic Animal Health Unit, recommending bacteriological methods for aquatic animals for inclusion in Australian Standard Diagnostic Techniques.

5.0 Methods

The principal focus of this project was two workshops, which were held at the CSIRO Australian Animal Health Laboratories, Geelong. The first took place from 2-5th October 2000 and the second from 5-9th February 2001.

The Australian Animal Health Laboratory is a high security bio-containment facility, designed specifically for work with exotic bacterial and viral animal pathogens. These FRDC funded workshops provided participants with a opportunity to make use of these facilities in order to gain further hands on practical experience with a large range of known exotic and enzootic bacterial pathogens (listed in Appendices 3 and 4).

The first workshop consisted of a series of integrated lectures, laboratory practicals and discussions on general bacterial disease diagnosis in aquatic animals. The second workshop was slightly longer and while similar to the first, was devoted solely to Vibrio species. The work programs for both workshops are included in Appendices 3 and 4 of this report.

The majority of lectures where provided by Mr Nicholas Gudkovs (CSIRO) and Dr. Jeremy Carson (DPIW&E, Tas.). However, during the first workshop Dr. Mark Crane (CSIRO) and Dr. Helen Byers (CSIRO) also contributed lectures. In addition, we were fortunate that Professor Hisatsugu Wakabayashi from the Department of Aquatic Bioscience, University of Tokyo, was visiting Australia at the time of the first workshop. Professor Wakabayashi has a long and distinguished career in fish disease microbiology and has published extensively in the field. He has a particular interest in diseases caused by filamentous yellow-pigmented Gram negative bacteria. Professor Wakabayashi kindly offered to talk at the workshop and provided an insight into a range of bacterial diseases currently causing problems in Japanese aquaculture systems.

Dr. Inger Dalsgaard from the Danmarks Fiskeriundersøgelser, Fiskepatologisk Laboratorium (Danish Institute of Fisheries Research, Fish Disease Laboratory) was invited to provide the lectures at the second workshop on Vibrios. Dr. Dalsgaard is an internationally recognised fish bacteriologist with long-standing experience and expertise in disease diagnosis and research. The second workshop provided a unique opportunity for participants to hear Dr. Dalsgaard's views on many diagnostic issues and problems and to talk at length with her. Dr. Carson also gave a lecture reviewing the serology of Vibrios, with emphasis on those species associated with disease in aquatic animals.

6.0 Results / Discussion

Objective 1: General Introductory Bacteriology Workshop

The first workshop held at AAHL between 2-5th October 2000 provided training and experience with a range of diagnostic methods and techniques for the major bacterial pathogens of salmonids and other finfish. While the workshop consisted of a series of integrated lectures, laboratory practicals and general discussions on bacterial disease diagnosis in aquatic animals, the main emphasis was on laboratory-based activities.

The focus of laboratory activities was a large range of clinical and reference cultures (listed in Appendix 3 - Bacterial Species) of both known exotic and enzootic pathogens and other commonly encountered bacterial species. Participants were provided with the opportunity to explore and examine closely this collection based on their own interests, priorities and previous experience. A range of commonly available commercial diagnostic identification systems was made available and compared using a defined sub-set of the cultures provided.

Objective 2: Vibriosis Workshop

The second workshop on Vibriosis was held at AAHL between 5-9th February 2001. As with the first workshop there where lectures, laboratory sessions and discussions on Vibrio identification and diagnosis in aquatic animals. Dr. Inger Dalsgaard was invited to contribute lectures, laboratory support and to participate in discussions because of her long-standing experience and expertise in the area of Vibrio identification and diagnosis. Dr. Dalsgaard's presence meant that participants were able to communicate at length with an internationally recognised expert in the field of fish bacteriology. This was useful both in terms of specific technical issues, but also allowed the microbiologists involved to develop an understanding and perspective on what they were currently able to provide and what might be required in the future as aquaculture in Australia develops and expands and new problems emerge. The workshop also provided an opportunity to evaluate media, reagents and methods used in their own laboratories with the use of the reference cultures provided (Appendix 4 - Bacterial Species).

Detailed workshop notes, including guides and laboratory methods were provided to each participant and are detailed in Appendix 5.

Objective 3: Report on Australian Standard Diagnostic Techniques for the National Office of Animal and Plant Health, Aquatic Animal Health Unit.

In consultation with workshop participants, a report recommending bacteriological methods for aquatic animals for inclusion in Australian Standard Diagnostic Techniques (SDTs) was prepared for the Office of the Chief Veterinary Officer, AFFA.

7.0 Benefits

Representatives from all state and territory government departments responsible for bacterial disease diagnosis in aquatic animals were invited to attend. Participants were able to gain direct experience with living cultures representing the majority of known bacterial fish pathogenic species. The workshops provided an opportunity to discuss, evaluate and compare the different bacterial identification techniques and procedures used for the identification of these pathogens in laboratories currently undertaking this work around Australia.

A range of aquaculture industries, both marine and freshwater and to a lesser extent wild capture fishing industries throughout Australia are the primary beneficiaries of this training project. Additional benefit will flow from laboratories providing better information regarding specific organisms involved with diseased animals. More accurate identification and diagnosis should result in improved animal survival rates as a result of faster response and better disease management. More reliable and accurate information will in turn allow state and federal governments to carry out their local, national and international responsibilities with greater efficiency and confidence.

Further indirect benefits include, the development of professional relationships, consolidation of the network of scientists currently undertaking research and diagnosis in bacterial diseases of aquatic animals, experience with exotic agents, access to established collection of pathogenic bacteria, exposure to new methods and techniques of bacterial analysis.

Bacterial identification systems developed in different laboratories were available for evaluation and provided the focus for discussion. Adoption of these systems, particularly by those laboratories not currently heavy involved in aquatic animal disease diagnosis may provide a measure of efficiency through, avoiding the costs associated with developing new in-house procedures for unfamiliar disease agents, centralised media preparation and standardization of methods.

Thus the benefits and the beneficiaries are the same as those stated in the original application.

8.0 Further Development

During the course of both workshops there was considerable discussion regarding future training needs and also the related issue of quality management in diagnostic laboratories.

There was a view that the comprehensive program developed and presented during these workshops could be adapted to form the basis for an ongoing shorter course for laboratory technicians ensuring Australia's diagnostic capability in this area is developed and maintained at the highest level. This course could be offered biannually or more often as required. There was no clearly identified source of funding for this, although some participants believed that either AFFA or FRDC were best placed to support future undertakings of this sort.

Most laboratories undertaking diagnostic testing have in recent years introduced quality management systems through NATA and ISO accreditation. While these systems are mainly concerned with laboratory processes and procedures, there is also a requirement particularly for NATA accredited laboratories to activity participate in proficiency testing programs. Where these do not exist laboratories should endeavour to establish inter-laboratory testing programs with laboratories undertaking similar work, for this purpose. Currently, in Australia, no such programs exist for infectious disease in aquatic animals. While options to address this issue are currently being explored by AFFA, workshop participants believed there was also a need for an annual meeting of microbiologists undertaking diagnostic work with aquatic animals. The purpose of this meeting would be to provide professional development in this specialised field. The meeting should be conducted in association with the state laboratories activity involved with this work, the venue rotating from state to state. A meeting of 2 to 3 days duration would allow sufficient time for review of the previous years proficiency testing program, with opportunity for seminars on important issues such as the development and implementation of standard diagnostic techniques, laboratory standardisation/harmonisation and laboratory based sessions in order to maintain and develop the technical expertise of those involved.

9.0 Conclusion

The primary objective of the project was to conduct a series of bacteriology training workshops and to make recommendations on standard diagnostic techniques required for aquatic animal bacteriology.

Two workshops were successfully completed in October 2000 and February 2001. The workshops were intended for Australian Veterinary Microbiologists currently undertaking diagnosis of bacterial disease in aquatic animals. A series of lectures on various aspects of disease diagnosis in aquatic animals, but the main focus of the workshops was the large collection of clinical and reference cultures provided. The collection was representative of the majority of known bacterial fish pathogenic species, including both exotic and enzootic pathogens and other commonly encountered bacterial species. Participants were invited to explore and examine this collection based on their own interests, priorities and previous experience.

The microbiologically secure facilities provided by the Australian Animal Health Laboratory, allowed participants to handle a range of cultures that might otherwise be inaccessible, either because they are exotic animal pathogens with restricted access or because they are not routinely identified or encountered in all diagnostic laboratories. Working with these species on the bench provides the experienced microbiologist with a better understanding of what to expect when these agents are encountered in their own laboratories. This experience will result in a higher likelihood that these agents will be recognised and correctly identified when encountered in diagnostic samples.

A range of commonly available commercial diagnostic identification systems was made available and compared using a defined sub-set of the cultures. In addition, bacterial identification systems developed in different laboratories were available. Adoption of these systems, particularly by those laboratories not heavy involved in aquatic animal disease diagnosis may provide a measure of efficiency through, avoiding the costs associated with developing new in-house procedures for unfamiliar disease agents, centralised media preparation and standardization of methods. As a direct outcome of the workshops, 3 laboratories have adopted the Vibrio identification scheme developed by DPIW&E, Tasmania.

A less tangible but nonetheless important outcome of the workshop series was the strengthening of the developing network of scientists currently active in the area of diagnosis of disease in aquatic animals.

10.0 References

1. Fish Health Management Committee (1998). Record of the Second Meeting 29 April 1998, Canberra. Agenda Items 2 and 3.

2. Fish Health Management Committee (1998). Report of the Fish Health Management Committee and the Fish Health Coordinating Group: Workshop on Aquatic Animal Health: Technical Issues, 7-9 December 1998, Melbourne, Victoria.

3. Higgins, R. A. (chair) (1996). Report of The National Task Force on Imported Fish and Fish Products. Department of Primary Industries and Energy, Canberra.

4. Nairn, M. E., Allen, P. G., Inglis, A. R. and Tanner, C. (1996). Australian Quarantine: a shared responsibility. Department of Primary Industries and Energy, Canberra.

11.0 Appendices

Appendix 1: Intellectual Property

No new intellectual property, such as, commercially significant developments, patents or licences, was generated during the course of the project.

Appendix 2: Staff

In addition to the investigators previously identified in this report, the following people made significant contributions to the workshops through the provision of lectures, preparation of materials or assisting with workshop organisation. Their assistance is greatly appreciated.

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Appendix 3: Workshop 1 - General Bacteriology, Tuesday 3rd to Friday 6th October 2000

Participants

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Workshop 1 - General Bacteriology - Program

Day	Time	General Activities	Location
Monday 2nd			
	various	Arrive Melbourne Airport, Tullamarine Transfer via Gull Bus to Geelong Check-In	Mercure Hotel
	7.00	Dinner	
Tuesday 3rd			
	8.15	Pickup at Hotel	NG/LW
	8.30-8.45	Welcome and Introduction to Workshop and AAHL (NG)	Board Room
	8.45-10.00	Local Reviews by Participants (10 min. each)	Board Room
	10.00-10.15	Morning Tea	non-secure canteen
	10.15-10.30	Short tour of library, Level 6 and non-secure SS&S area (NG)	non-secure area
	10.30-11.30	Lecture/Discussion - The Laboratory Program (JC/NG)	Board Room
	11.30-12.00	MSG secure access video Micro-Security Questions Sign Declarations(TB) Enter secure-area via CMS Check Clothes and Shoe sizes (NG/JC/LW)	Board Room
	12.00-12.45	Lunch	secure canteen
	12.45-1.45	Tour of secure area (LW)	secure area
	1.45-2.00	Laboratory orientation - Work Procedures in AAHL secure laboratories (NG)	South Suite Lab. 110
	2.00-3.00	Laboratory Practical	South Suite Lab. 110
	3.00-3.15	Afternoon Tea	secure canteen
	3.15-5.00	Laboratory Practical	South Suite Lab. 110
	5.00-5.30	Shower out	
	5.30	Transfer to Hotel	NG/JC
	6.30	Dinner	
Wednesday 4th			
	8.15	Pickup at Hotel	NG/LW
	8.30-10.00	Lectures/Discussions	Board Room
	10.00-10.15	Morning Tea	non-secure canteen
	10.15-12.15	Lectures/Discussions	Board Room
	12.30-1.00	Lunch	
	1.00-3.00	Laboratory Practical	South Suite Lab. 110
	3.00-3.15	Afternoon Tea	secure canteen
	3.15-5.00	Laboratory Practical	South Suite Lab. 110
	5.00-5.30	Shower out	
	5.30	Transfer to Hotel	NG/JC
	6.30	Dinner	
Thursday 5th			
	8.15	Pickup at Hotel	NG/LW
	8.30-10.00	Lectures/Discussions	Snowdon Room
	10.00-10.15	Morning Tea	non-secure canteen
	10.15-12.15	Lectures/Discussions	Snowdon Room
	12.30-1.00	Lunch	
	1.00-3.00	Laboratory Practical	South Suite Lab. 110
	3.00-3.15	Afternoon Tea	secure canteen
	3.15-5.00	Laboratory Practical	South Suite Lab. 110
	5.00-5.30	Shower out	
	5.30	Transfer to Hotel	NG/JC
	6.30	Dinner	
Friday 6th			
	8.45	Pickup at Hotel	NG/JC
	9.00-10.00	"Disease Problems in Cultured Fish and Shellfish in Japan" Professor Hisatsugu Wakabayashi Department of Aquatic Bioscience, The University of Tokyo	Board Room
	10.00-10.15	Morning Tea	non-secure canteen
	10.15-11.45	QA Program for Australian Laboratories Standard Diagnostic Techniques for Australian Laboratories	Board Room
	11.45-12.00	Conclusion Evaluation Forms	Board Room
	12.00	Workshop Ends	
	12.00-12.30	Lunch	non-secure canteen
	12.30	Participants depart AAHL	NG/LW
	12.45	Airport bus departs Geelong Depot	Gull bus depot

Workshop 1 - General Bacteriology - Lectures and Discussions

<table>
<tr><th></th><th>Tuesday</th><th>Wednesday</th><th>Thursday</th><th>Friday</th></tr>
<tr><td>8.30</td><td>Welcome (MC) 1 min.</td><td rowspan="2">Strategic Context OIE, AQUAPLAN etc (MC)</td><td rowspan="2">Serological Techniques (NG)</td><td rowspan="2">Exotic diseases (NG)</td></tr>
<tr><td>8.45</td><td>Introduction (NG/JC)</td></tr>
<tr><td>9.00</td><td rowspan="4">Overviews by Participants</td><td rowspan="2">Farm Sampling and Data Collection (NG)</td><td>IHC (MC)</td><td rowspan="4">Prof. Wakabayashi
"Disease Problems in Cultured Fish and Shellfish in Japan"</td></tr>
<tr><td>9.15</td><td rowspan="2">Ribosomal RNA (HB)</td></tr>
<tr><td>9.30</td><td rowspan="2">Primary Culture (JC)</td></tr>
<tr><td>9.45</td></tr>
<tr><td>10.00</td><td colspan="4">Morning Tea</td></tr>
<tr><td>10.15</td><td>non-secure tour</td><td rowspan="6">Pathogen ID (Group)</td><td rowspan="2">Molecular Techniques in Bacterial Fish Disease Diagnosis (NG)</td><td rowspan="4">Internet resources
QA Program
Standard Diagnostic Techniques</td></tr>
<tr><td>10.30</td><td rowspan="4">Laboratory Practical Program (JC/NG)</td></tr>
<tr><td>10.45</td></tr>
<tr><td>11.00</td></tr>
<tr><td>11.15</td><td rowspan="3">Fish Sampling (Video)</td></tr>
<tr><td>11.30</td><td rowspan="2">MSG Induction (TB)</td></tr>
<tr><td>11.45</td><td rowspan="2">Draft Workshop Recommendations to AFFA</td></tr>
<tr><td>12.00</td><td>enter secure-area</td><td>Antibiotic testing (JC)</td><td>Fish Vaccination (JC)</td></tr>
<tr><td>12.15</td><td colspan="4">Lunch</td></tr>
<tr><td>PM</td><td colspan="4">Laboratory Activities (see timetable below)</td></tr>
</table>

Workshop 1 - General Bacteriology - Laboratory Activities

Activities	Tuesday	Wednesday	Thursday
Cellular morphology & staining procedures • Gram - All • Ziehl-Neelsen / ZN Mod. - *Mycobacteria, Nocardia* Bacterial Flagella Stain - *Y. ruckeri, V. alginolyticus / parahaemolyticus* broth/plates			
Stained Smears - reading and interpretation			
Examination of colonies			
Biochemical ID - setup			Not available
Biochemical ID - reading and interpretation of results	Not available		
Biochemical ID - comparison of results obtained with different test systems	Not available		
Identification of unknown cultures			Not available
Reading and identification of mixed cultures			
Macroscopic Agglutination Test (Slide Agglutination) • *Y. ruckeri* • *V. anguillarum* • *L. garvieae* • *R. salmoninarum* • *A. salmonicida*			
SDS-PAGE • *A. salmonicida* • *R. salmoninarum* • *Y. ruckeri*			Not available
Western blot analysis • *A. salmonicida* • *R. salmoninarum* • *Y. ruckeri*			Not available
PCR • Comparison of polymerases using serially diluted DNA • Direct PCR of colonies using various systems • Comparison of tissue DNA extraction methods			
Antimicrobial susceptibility Testing - MIC assay			Not available
Fish Dissection and Culture Technique	Not available		

Workshop 1 - General Bacteriology - Bacterial Species

Aeromonas allosaccharophila
Aeromonas bestiarum
Aeromonas caviae
Aeromonas encheleia
Aeromonas eucrenophila
Aeromonas hydrophila
Aeromonas jandaei
Aeromonas salmonicida
Aeromonas schubertii
Aeromonas veronii biovar sobria
Aeromonas veronii biovar veronii
"Heamophilus piscium"
Citrobacter freundii
Edwardsiella ictaluri
Edwardsiella tarda
Enterobacter agglomerans
Escherichia vulneris
Hafnia alvei
Serratia liquefaciens
Yersinia ruckeri
Yersinia intermedia
Flavobacterium columnare
Flavobacterium johnsoniae
Flavobacterium psychrophilum
Flexibacter maritimus
Carnobacterium piscicola
Lactococcus garvieae
Lactococcus piscium
Mycobacterium chelonae ex Snob's Creek
Mycobacterium fortuitum
Mycobacterium marinum
Nocardia sp.
Renibacterium salmoninarum
Streptococcus iniae
Streptococcus anginosus-like
Vagococcus salmoninarum
Plesiomonas shigelloides
Chromobacterium violaceum
Pseudomonas anguilliseptica
Pseudomonas fluorescens
Photobacterium damselae subsp. damselae
Vibrio alginolyticus
Vibrio anguillarum
Vibrio cholerae (non-01)

Appendix 4: Workshop 2 - Vibrios, Monday 5th to Friday 9th February 2001

Participants

Name and Organisation	Contact Details
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Workshop 2 - Vibrios - Program

Day	Time	General Activities	Location
Monday 5th			
	8.30	Meet for pick up and transfer to AAHL	Mercure Hotel Foyer
	9.00 – 9.15	Welcome and Introduction	Non-secure Admin Bldg
	9.15 – 10.15	Dr. Inger Dalsgaard - "Vibrio Isolation and Identification 1"	Non-secure Admin Bldg
	10.15 – 10.45	MORNING TEA	Non-secure canteen
	10.45 – 11.00	OHS	Non-secure Admin Bldg
	11.00 – 11.30	Induction and Declarations	Non-secure Admin Bldg
	11.30 – 12.30	Tour of Secure Area OR Dispensing Media	Secure Area OR South Suite Lab. 110 (secure)
	12.30 – 1.15	LUNCH	Secure Canteen
	1.15 – 3.00	• Vibrio ID - Reference Set	South Suite Lab. 110 (secure)
	3.00 – 3.30	AFTERNOON TEA	Secure Canteen
	3.30 – 4.30	• Vibrio ID - Reference Set	South Suite Lab. 110 (secure)
	4.30 – 5.00	• Review of Laboratory Sessions • Discussion QC Program for Aquatic Animal Bacteriology	Secure area meeting room
	5.00	SHOWER OUT	
	5.30	Meet for pick up and transfer to Mercure Hotel	AAHL Foyer
	6.30	Meet for dinner	Mercure Hotel Foyer
Tuesday 6th			
	8.30	Meet for pick up and transfer to AAHL	Mercure Hotel Foyer
	9.00 – 10.00	Dr. Inger Dalsgaard - "Vibrio Isolation and Identification 2"	Non-secure Admin Bldg
	10.00 – 10.30	MORNING TEA	Secure Canteen
	10.30 – 12.30	• Vibrio ID	South Suite Lab. 110
	12.30 – 1.00	LUNCH	Secure Canteen
	1.00 – 3.00	• Vibrio ID	South Suite Lab. 110
	3.00 – 3.30	AFTERNOON TEA	Secure Canteen
	3.30 – 4.30	• Vibrio ID	South Suite Lab. 110
	4.30 – 5.00	• Review of Laboratory Sessions and Results • Future training • Aquatic Animal Bacteriology Interest Group(s)	Secure area meeting room
	5.00	SHOWER OUT • Inoculate BIOLOG BUGM plates	Non-secure bacteriology laboratory
	5.30	Meet for pick up and transfer to Mercure Hotel	AAHL Foyer
	6.30	Meet for dinner	Mercure Hotel Foyer
Wednesday 7th			
	8.30	Meet for pick up and transfer to AAHL	Mercure Hotel Foyer
	9.00 – 10.00	Dr. Jeremy Carson "Vibrio serology"	Non-secure Admin Bldg
	10.00 – 10.30	MORNING TEA	Secure Canteen
	10.30 – 12.30	• Inoculation of BIOLOG plates • Vibrio ID	• Non-secure bacteriology laboratory • South Suite Lab. 110
	12.30 – 1.00	LUNCH	Secure Canteen
	1.00 – 3.00	• Vibrio ID	South Suite Lab. 110
	3.00 – 3.30	AFTERNOON TEA	Secure Canteen
	3.30 – 4.30	• Vibrio ID	South Suite Lab. 110
	4.30 – 5.00	• Review of Laboratory Sessions and Results	Secure area meeting room
	5.00	SHOWER OUT • Inoculate BIOLOG BUGM plates	Non-secure bacteriology laboratory
	5.30	Meet for pick up and transfer to Mercure Hotel	AAHL Foyer
	6.30	Meet for dinner	Mercure Hotel Foyer
Thursday 8th			
	8.30	Meet for pick up and transfer to AAHL	Mercure Hotel Foyer

	9.00 – 10.00	• Vibrio ID • Inoculation and Reading of BIOLOG plates	• South Suite Lab. 110 (secure) • Non-secure bacteriology laboratory
	10.00 – 10.30	MORNING TEA	Secure Canteen
	10.30 – 12.30	• Vibrio ID	South Suite Lab. 110
	12.30 – 1.00	LUNCH	Secure Canteen
	1.00 – 3.00	• Vibrio ID	South Suite Lab. 110
	3.00 – 3.30	AFTERNOON TEA	Secure Canteen
	3.30 – 4.30	• Vibrio ID	South Suite Lab. 110 (secure)
	4.30 – 5.00	• Review of Laboratory Sessions and Results • Standard Diagnostic Techniques	Secure area meeting room
	5.00	SHOWER OUT	
	5.30	Meet for pick up and transfer to Mercure Hotel	AAHL Foyer
	6.30	Meet for dinner	Mercure Hotel Foyer
Friday 9th			
		Finalise Hotel Accounts	Mercure Hotel
	8.30	Meet for pick up and transfer to AAHL Luggage to be left in AAHL Foyer	Mercure Hotel Foyer
	8.45 – 10.30	• Vibrio ID - Finalisation of tests and data entry in secure-area • Reading of BIOLOG plates	• South Suite Lab. 110 (secure) • Non-secure bacteriology laboratory
	10.30 – 11.00	MORNING TEA	Non-secure canteen
	11.00 – 12.00	Dr. Inger Dalsgaard - "Bacterial Diseases of Fish – A European Perspective"	Non-secure Admin Bldg
	12.00 – 1.00	LUNCH and Farewell	Non-Secure Canteen

Workshop 2 - Vibrios - Laboratory Activities

Activities	Mon	Tue	Wed	Thurs	Fri
Morphologies • Gram • Colonial • TCBS	**Y**	**Y**	**Y**	**Y**	**N**
Biochemical ID - setup	**Y**	**Y**	**Y**	**Y**	**N**
Biochemical ID - reading and interpretation of results	**N**	**Y**	**Y**	**Y**	**Y**
Biochemical ID - comparison of different test systems	**N**	**N**	**N**	**Y**	**Y**
SDS-PAGE • *Vibrio* or *Yersinia* species	**N**	**Y**	**Y**	**Y**	**N**
Western blot analysis • *Vibrio* or *Yersinia* species	**N**	**Y**	**Y**	**Y**	**N**
PCR • Direct PCR of colonies using LifeTech SuperMix®	**N**	**Y**	**Y**	**Y**	**N**
Fish Dissection and Culture Technique	**N**	**Y**	**Y**	**N**	**N**

Workshop 2 - Vibrios - Bacterial Species

Vibrio alginolyticus
Vibrio anguillarum
Vibrio cholerae
Vibrio costicola
Vibrio fischeri
Vibrio fluvialis
Vibrio halioticoli
Vibrio harveyi
Vibrio ichthyoenteri
Vibrio logei
Vibrio mediterranei
Vibrio metschnikovii
Vibrio natriegens
Vibrio navarrensis
Vibrio nereis
Vibrio nigripulchritudo
Vibrio ordalii
Vibrio parahaemolyticus
Vibrio pectenicida
Vibrio pelagius I
Vibrio pelagius II
Vibrio proteolyticus
Vibrio rumoiensis
Vibrio salmonicida
Vibrio splendidus I
Vibrio splendidus II
Vibrio tubiashii
Vibrio vulnificus biotype 1
Vibrio wodanis

Appendix 5: Documents

VETCOM Invitations, June 2000
AAHL Microbiological Security Group - Visitor Information and Form, July 2000
Workshop Questionnaire - Topic Survey, August 2000

Workshop 1

Workshop Guide - General Bacteriology
Emergency Contact Numbers
List of Participants and Contact Details

Program
- Timetable of Lectures and Discussions
- Laboratory Schedule
- Map
- Schedule of Isolate Testing
- Location of Laboratory Equipment, Reagents and Other Materials
- Mistaken Identities
- Clinical Cases
- Bacterial Species

Laboratory Methods:

Acid from carbohydrates - ammonium salts medium
Antibiotics for testing fish pathogens
Bile-aesculin test
Chondroitin AC lyase test
Diagnostic test reagents
Differential tests for *Janthinobacterium, Iodobacter* & *Chromobacterium* species
Differential tests for members of the family *Streptococceae*
Presumptive identification of *Flavobacterium columnare* screening tests
Detection of flexirubin pigment
Flexibacter maritimus
Differential tests for some non-fastidious fish pathogenic gram positive cocci & rods
H_2S medium (Feltham) for Gram positive bacteria
Media for miniaturized tests for the identification of *Aeromonas* species
Media for miniaturized tests for the identification of gram positive cocci and coccobacilli
MIC microbroth dilution – control data
Antibiotic assay - broth microdilution method
Gram positive cocci and rods: miniaturised tests - microtitre tray format
Yersinia ruckeri miniaturised tests - microtitre tray format
Aeromonas sp. Miniaturised tests - microtitre tray format
Miniaturised identification test procedure
Freshwater Ordal's medium
Marine Ordal's medium
Testing for oxidase in pigmented bacteria
Rapid method for determining acetoin production

Medium for rapid VP test for Gram positive bacteria
Shieh's medium
Ward's O-F medium
Ward's O-F test
Determining acrylamide/bis acrylamide concentrations
PCR amplification of the bacterial 16s gene - rDNA primers
Gustafson PCR for *A. salmonicida* (AP1/2)
Preparation of bacteria for SDS-PAGE
Bacterial flagella stain
Conversion of AFDL PCR mixes for use in the PCR suite
Coomassie blue staining of polyacrylamide gels
Estimation of nucleic acids by absorption spectroscopy
Genomic DNA isolation from gram positive bacteria using puregene DNA isolation kit
Fluorescent antibody test (FAT)
SDS-PAGE gels
Silver staining lipopolysaccharide in polyacrylamide gels
Macroscopic slide agglutination test (MAT)
Total RNA isolation from *Renibacterium salmoninarum* or fish tissues
Protein molecular weight determination using page
SDS-PAGE molecular weight standards
Operation of the Genequant II RNA/DNA calculator
Electrophoresis and photography of PCR products
Preparation of SDS-PAGE reducing sample buffer
RNA isolation from tissues using Trizol reagent (Gibco BRL)*
Procedure for western blotting of proteins

Workshop 2

Workshop Guide - Vibriosis
Emergency Contact Numbers
List of Participants and Contact Details
Dr. Inger Dahlsgaard - Workshop overheads

Program
- Timetable of Lectures and Discussions
- Laboratory Schedule
- Schedule of Isolates for Identification
- Location of Laboratory Equipment, Reagents and Other Materials
- Bacterial Species

Laboratory Methods:

FHU Identification of Vibrio and Photobacterium species: Test and Procedures

Appendix 6: Acknowledgements

The contributions of time and salaries by the government departments and private companies with responsibility for fish disease diagnosis in their respective states and territories are gratefully acknowledged. The generous support of the following commercial scientific suppliers Astral Scientific, Promega, Biorad, Invitrogen, Pharmacia Biotech, Metvet Science and in particular Oxoid Australia ensured that participants were provided with a useful range of equipment and reagents for comparison and evaluation.