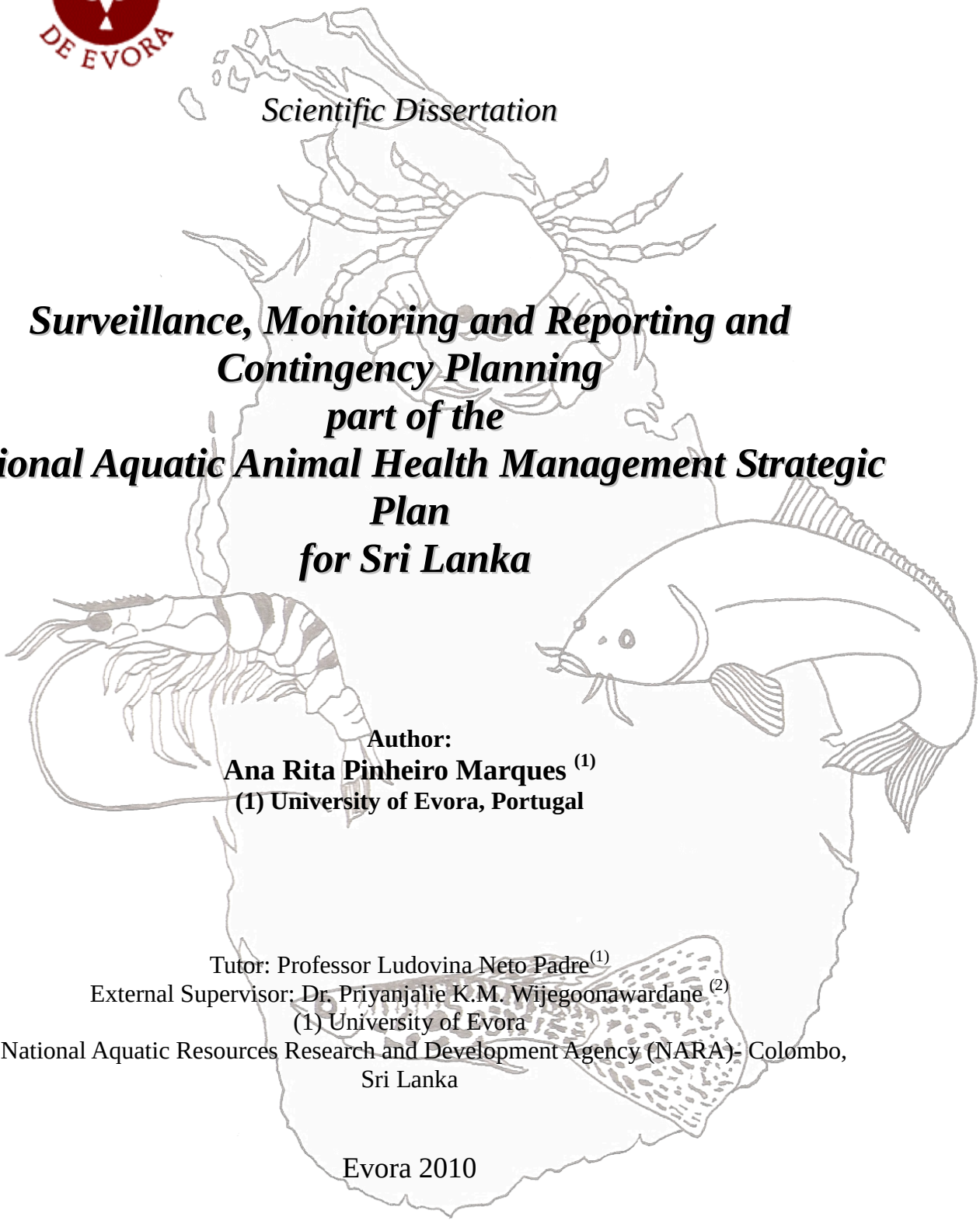
UNIVERSITY OF EVORA
Integrated Masters in Veterinary Medicine

Scientific Dissertation

Surveillance, Monitoring and Reporting and Contingency Planning part of the National Aquatic Animal Health Management Strategic Plan for Sri Lanka



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Abstract

Implementing Aquatic Animal Health Management programs is of crucial importance to any country meaning to achieve and maintain the development and sustainability of their aquaculture sector. The initial approach to implementing an efficient Aquatic Animal Health Management program in Sri Lanka will rely on the development of two of its key components, namely aquatic animal disease surveillance, monitoring and reporting and the enhanced coordinated response to aquatic animal disease emergencies. The drafted framework for these two components was built upon the results from surveyed and consulted laboratories and institutions involved in aquatic animal health diagnostics and through two stakeholder meetings in order to achieve general consensus for the proposed components, focusing on maximizing the existing resources for cost efficiency. Initial drafting of a list of diseases of concern to Sri Lanka, concentrating on the diseases affecting major finfish and crustacean cultured species, and the proposed framework for a coordinated, joint approach to aquatic animal disease surveillance, monitoring and reporting will help to describe the aquatic animal disease profile in Sri Lanka, fulfilling international OIE reporting obligations while attempting to determine disease free status and the defining disease free zones, and inclusively ensure the early detection of new exotic diseases. The information generated through the surveillance program will also support applied risk analysis and improved quarantine for imported aquatic animals. Establishing a database for reporting diagnostic findings from surveillance and monitoring, along with the reporting of other routine and quarantine diagnostic findings, is crucial for up-to-date description of the countries aquatic animal disease profile and for the early detection of an aquatic animal disease emergency. Improving the existing arrangements for intervening in an aquatic animal disease emergency is fundamental for successful disease control or eradication, achieved through a much needed here proposed contingency plan for a coordinated response on behalf of the countries institutions and laboratories managing and diagnosing aquatic animal diseases, supervised by a Ministry of Fisheries and Aquatic Resources appointed committee of aquatic animal health specialists.

Key words: Aquatic Animal Health Management, surveillance, monitoring, reporting, contingency plan.

Resumo

A implementação de programas de gestão de sanidade de animais aquáticos é fundamental para qualquer pais que queira atingir e manter o desenvolvimento sustentável do sector da produção aquícola de animais aquáticos. A implementação de um programa de gestão de sanidade de animais aquáticos no Sri Lanka deve se apoiar no desenvolvimento inicial de dois componentes fundamentais do mesmo, nomeadamente a "Vigilância, Monitorização e Notificação" de doenças em animais aquáticos e a resposta optimizada a situações urgentes de doença nestas populações seguindo as directrizes estabelecidas num "Plano de Contingência". A estrutura geral para a implementação destes dois componentes é aqui apresentada nesta dissertação de natureza científica, baseada na informação recolhida através da consulta e avaliação dos laboratórios e instituições envolvidas no diagnóstico de patologias afectando os animais aquáticos e através da realização de duas reuniões com os seus membros mais relevantes. Desta forma pretende-se maximizar os recursos existentes para a implementação destes componentes com o consenso de todos os futuros participantes.

O programa de vigilância de sanidade em animais aquáticos terá como objectivo declarar a ausência de doença a nível nacional para um grupo de doenças e, deste modo motivar a exportação de animais aquáticos e seus produtos ou, permitir o identificar de infecções outrora desconhecidas. Desta forma, consegue-se uma descrição mais precisa do estado de saúde das populações de animais aquáticos no país sendo possível declarar a presença ou ausência de doença às autoridades internacionais da Organização Internacional de Saúde Animal- OIE. Para as doenças já estabelecidas nas populações de animais aquáticos, o componente "Vigilância, Monitorização e Notificação" determina que sejam reconhecidas zonas livres de doença para promover a translocação segura de animais a nível nacional e internacional. Inicialmente, redigiu-se uma lista de doenças importantes para o país, passíveis de afectar as espécies de crustáceos e peixes, de acordo com os registos de notificações submetidos à OIE e através da informação recolhida pela consulta local de peritos em sanidade de animais aquáticos. Desta lista foram seleccionadas as doenças para intervenção inicial, com base no potencial impacto da sua incursão nas populações produzidas em aquacultura de crustáceos e peixes, ou que, no caso de estabelecidas no país, possam causar surtos de doença e graves perdas de produção. De seguida,

atribuíram-se áreas geográficas a cada laboratório que participará no programa de vigilância, por província e de acordo com a sua proximidade geográfica.

A informação gerada pelo programa de vigilância deve ser reportada numa base de dados que servirá igualmente para a notificação de achados de diagnósticos rotineiros ou resultantes das actividades de quarentena. Através desta base de dados é mantido um perfil actualizado do estado de saúde das populações de animais aquáticos, sendo também possível por este meio a identificação e intervenção atempada de situações de doença que requeiram intervenção urgente. A informação gerada pelo programa de vigilância servirá também para a implementação de um programa de análise de risco para as importações de animais aquáticos e seus produtos e o melhoramento das práticas de quarentena.

É fundamental para o Sri Lanka melhorar a resposta actual, face à ocorrência de surtos de doença nas populações de animais aquáticos, para garantir o sucesso destas intervenções. Tal pode ser realizado através da formulação de um plano de contingência que, baseado nos procedimentos actualmente legislados, venha a assegurar uma resposta coordenada entre os diferentes laboratórios, com base em metodologias determinadas no programa de vigilância e a assistência de uma Comissão de Consulta para a Gestão da Sanidade em Animais Aquáticos.

Palavras-chave: Gestão de Sanidade em Animais Aquáticos, vigilância, monitorização, notificação, plano de contingência.

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List of Abbreviations

ALOP- appropriate level of protection

AAPQIS- Aquatic Animal Pathogen and Quarantine Information System

APHIS- Animal and Plant Health Inspection Service

CAADR- Center for Aquatic Animal Disease Diagnostics and Research

CCAAHM- Consultative Committee for Aquatic Animal Health Management

CPE- cytopathogenic effect

DAPH- Department of Animal Production and Health

DNA-deoxyribonucleic acid

dsDNA- double-stranded deoxyribonucleic acid

EHN- Epizootic Haematopoietic Necrosis

EHNV- Epizootic Infectious Haematopoietic Necrosis Virus

ELISA- enzyme-linked immunosorbent assay

EUS- Epizootic Ulcerative Syndrome

FAO- Food and Agriculture Organization of the United Nations

GIS- geographical information system

GIV- Grouper Iridovirus

GIVD- Grouper Iridoviral Disease

IFAT- fluorescent antibody test

IHHN- Infectious Hypodermal and Haematopoietic Necrosis

IHHNV- Infections Hypodermal and Haematopoietic Necrosis Virus

IHN- Infectious Haematopoietic Necrosis

IHNV-, Infectious Haematopoietic Necrosis Virus

IMN- Infectious Myonecrosis

IMNV- Infectious Myonecrosis Virus

IRA- import risk analysis

ISKNV- Infectious Spleen and Kidney Necrosis Virus

KHV- Koi Herpes Virus

KHVD- Koi Herpes Virus Disease

LSV- Laem-Singh Virus

MHD-SL- Milky Haemolymph Disease of Spiny Lobster

Mn- million

MrNV- *Macrobrachium ronsenbergii* Nodavirus

MSGS- *Monodon* Slow-Growth

MSGSV- *Monodon* Slow-Growth Virus

NACA- Network of Agriculture Centres in Asia-Pacific

NAQDA- National Authority for Aquaculture Development

NARA- National Aquatic Resources Research and Development Agency

NHP- Necrotizing Hepatopancreatitis

NHPB- Necrotizing Hepatopancreatitis bacteria

nRT-PCR- nested reverse-transcription polymerase chain reaction

OIE- World Organization for Animal Health

PCR- polymerase chain reaction

QAADR- Quarterly Aquatic Animal Disease Report

RNA- ribonucleic acid

Rs- rupees

RSIV- Red Sea Bream Iridovirus

RSIVD- Red Sea Bream Iridovirus Disease

RT-PCR- reverse-transcription polymerase chain reaction

SAARC- South Asian Association for Regional Cooperation

Se- sensitivity

Sp-specificity

SPS Agreement- Agreement on the Application of Sanitary and Phytosanitary Measures

ssDNA- single-stranded deoxyribonucleic acid

ssRNA- single-stranded ribonucleic acid

SVC- Spring Viraemia of Carp

SVCV- Spring Viraemia of Carp Virus

TS- Taura Syndrome

TSV- Taura Syndrome Virus

VER- Viral Encephalopathy and Retinopathy

VERV- Viral Encephalopathy and Retinopathy Virus

VHS- Hemorrhagic Septicaemia

VHSV- Hemorrhagic Septicaemia Virus

VIC- Veterinary Investigation Center

VRI- Veterinary Research Institute

WAHIS- World Animal Health Information System

WSD- White Spot Disease

WSDV- White Spot Disease Virus

WTD- White Tail Disease

XSV- Extra Small Virus

YHD- Yellowhead Disease

YHV- Yellowhead Disease Virus

Chapter 1

I. Literature Review

1. The Increasing Global Concern for Aquatic Animal Health

Globalization and the associated intensification of the aquaculture industry has brought about increased international concern of disease outbreaks that will come to compromise the aquaculture sectors further development and sustainability. Increased disease occurrence and broadened pathogen geographic range will be mostly associated to the increase of national and international translocation of live aquatic animals, especially when considering the ornamental finfish trade (FAO, 2007). As aquaculture activities intensify ill-management will also bring on disease, as factors such as high stocking densities, stocking hatchery raised aquatic animals, misunderstood use of specific pathogen free stocks, unanticipated negative interactions between cultured and wild fish populations, absent or ineffective biosecurtiy, climate change, basic general unawareness of emerging diseases and increased human travel take their toll (Bondad-Reantaso, 2005; FAO, 2007). Also contributing to increase the frequency and diversity of disease outbreaks is the ongoing microbial adaptation, both in the form of environmental adaptation, antibiotic resistance and adaptation to new hosts (FAO, 2007). The circumstances for pathogen diversification and spread are great, disease patterns changing in unpredictable ways, constraining and threatening the development of the aquaculture sector through increased operation costs, reduced production and restrictions on trade (Bondad-Reantaso, 2005).

Countries in an attempt to minimize disease introduction through transboundary movement of aquatic animals and aquatic animal products will come to develop non-lethal and rapid diagnostic methods capable of identifying infected aquatic animals, imposing tougher regulations for approving the entry of these into their territory. Following the same trend, international legislation in general will tighten, imposing higher standards for health certification. The importing countries will invariably evaluate Sri Lanka´s capabilities and effectiveness in disease surveillance, monitoring, reporting and contingency planning and so Sri Lankan Government must be the first to recognize the importance of supporting such programs to keep up with the increasing standards for international aquatic animal health certification while promoting the industry and attracting new international trade partners (FAO, 2007).

2. The Importance of the Aquaculture Sector in Sri Lanka

The aquaculture sector in Sri Lanka is an important source of foreign income, providing directly and indirectly with employment and contributing to poverty alleviation. Furthermore, religious and cultural biases favor the consumption of fish and so fish have high local demand.

Fresh water finfish aquaculture, based on seasonal village tank and pond culturing, produces mainly tilapia and Indian and Chinese carp varieties that serve as an important source of animal-base protein and subsistence for the rural poor (Kasagala, 2008, FAO 2010). Commercial aquaculture activities in Sri Lanka are focused on marine coastal production of black tiger prawn and fresh water ornamental finfish breeding. Both inland aquaculture as a means of poverty alleviation and coastal shrimp production and ornamental finfish breeding for commercial purposes have the potential for further expansion in Sri Lanka(FAO, 2010).

Commercially reared shrimp and ornamental finfish, mostly destined for foreign markets, have increased in production in the past years. Statistics show shrimp farm production has risen, from 2,220 tons in 2008 to 3,500 tons in 2009 with the associated rise in exported quantity from 12,6% to 14,6% (MOFAR, 2009b). The income generated through ornamental fish breeding rose from in 2,6 Mn Rs. in 2008 to 3,4 Mn Rs. in 2009, generating 979 Mn Rs representing 4,66% of total export value for fish and fisheries products (MOFAR, 2009a).

3. International Disease Reporting

Sri Lanka presently reports aquatic animal pathogen findings to the World Organization for Animal Health (OIE) Quarterly Aquatic Animal Disease Report (QAADR) (Annexure I) in cooperation with the Food and Agriculture Organization of the United Nations (FAO) and the Network of Agriculture Centres in Asia-Pacific (NACA). The OIE uses the information gathered through the reports of the member countries to make valuable information on disease occurrence available to assist the responsible movement of aquatic animals at an international level, minimizing the probability of disease spread and occurrence.

The OIE defines a list of notifiable diseases, along with other non listed diseases of concern to the region, for quarterly disease notification in the QAADR. Prioritizing which of these diseases to be subject to initial surveillance efforts is done after considering if disease is known to be present or thought to be abscent in the country. If disease is present, initial efforts should concentrate on the diseases affecting the populations of aquatic animals of greatest economic significance, for investing and defining disease free zones. For diseases unaccounted for, initial

surveillance efforts should focus on the diseases susceptible of infecting the main cultured species in Sri Lanka. Other diseases can be suggested to incorporate the diseases listed in the QAADR, if such is determined of National interest.

At the moment limited resources and lack of cooperation and organization among the countries institutions diagnosing aquatic animal pathogens makes disease reporting in Sri Lanka inefficient, limited to a few diseases. Implementing a Health Management Program will help Sri Lanka to fulfill disease reporting obligations for the listed diseases of concern to the country, generating information on the aquatic animal health profile through "Surveillance, Monitoring and Reporting" (Aquaplan, 1999). Implementing the Aquatic Animal Health Management Program will assure trade partners that all efforts are made to prevent introduction of disease and determine the health status of the exported live aquatic animals and their products.

4. Aquatic Animal Health Management

Aquatic Animal Health Management programs have been taken on by many countries around the globe for the protection of the aquatic animal resources and the development of the aquaculture sector. The eight key components that define the Australian example of a successful Health Management Program, "National Strategic Plan for Aquatic Animal Health- Aquaplan" are (Aquaplan, 1999):

- Component 1: International Linkages;
- Component 2: Import Risk Analysis and Quarantine;
- Component 3: Surveillance, Monitoring and Reporting;
- Component 4: Contingency Planning for Aquatic Animal Disease Emergencies;
- Component 5: Awareness;
- Component 6: Research and Development;
- Component 7: Legislation and Policies;
- Component 8: Resources and Funding.

Given their importance, focus will be given for developing the necessary framework for the components "Surveillance, Monitoring and Reporting" and "Contingency Planning for Aquatic Animal Disease Emergencies". The component "Import Risk Analysis and Quarantine" will also be subject to some appreciation, as it stands as the main measure to prevent the introduction of transboundary disease to Sri Lanka.

4.1. Surveillance for Import Risk Analysis and Quarantine

As a member of the World Trade Organization (WTO) Sri Lanka needs to satisfy trading obligations set by the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) (FAO, 2007). The SPS Agreement states, among other rights and obligations, the right for each country to determine its appropriate level of protection (ALOP) and to take the necessary measures to achieve what it considers to be an ALOP, while as an importing country avoiding arbitrary or unjustified levels of protection that could be regarded as disguised restrictions. The least restrictive and most feasible measures to reduce the risk posed by the import, are identified and selected. If the ALOP is met, an import can be approved (FAO, 2007). Determining and achieving an ALOP is only possible through applied import risk analysis and efficient aquatic animal quarantine. The information generated through an implemented Aquatic Animal Health Management Program, particularly through "Surveillance, Monitoring and Reporting," capable of describing the countries aquatic animal health status, will support systematic application of risk analysis for enforced quarantine, minimizing the risk of introducing transboundary diseases to Sri Lanka (Bondad-Reantaso, 2005).

4.1.1. Import Risk Analysis

Evaluating the risk associated with trade of aquatic animals and aquatic animal products is known as import risk analysis (IRA) (Peeler, 2005). IRA works to identify the hazards associated with imported aquatic animals and aquatic animal products, allowing for the application of the most appropriate measure to reduce the level of risk of introducing disease to an acceptable level, enforcing quarantine or alternatively rejecting the import (Bondad-Reantaso, 2005). A pathogen regarded as a hazard is one that is present in the exporting country and capable of producing adverse consequences in the importing country where disease is exotic or subject to control or eradication efforts (Kleeman, 2005). To date there is no import risk analysis program for aquatic animal diseases applied in Sri Lanka.

The competent authority responsible for risk analysis, most often and appropriately a Veterinary Quarantine Officer, will identify the potential pathogens that could be introduced through the import. Import risk analysis continues with the risk assessment stage where the risk associated with the hazard is quantified or qualified according to the likelihood of its occurrence (Peeler, 2005; Bondad-Reantaso, 2005). The resulting information serves the purpose of risk management, for determining which measures to apply to achieve a National ALOP (FAO, 1998).

The OIE supplies the international risk analysis guidelines in the Aquatic Animal Health Code (OIE, 2009d). Information on international aquatic animal diseases, to assist risk analysis and aquatic animal health management in general, is available at the FAO Aquatic Animal Pathogen and Quarantine Information System (AAPQIS), the OIE- World Animal Health Information System (WAHIS) and the OIE Collaborating Center for Aquatic Animal Diseases, an international database on aquatic animal diseases.

4.1.2. Aquatic Animal Quarantine Overview for Sri Lanka

Sri Lanka is an island nation with no common waterways or boundaring countries. Aquatic animal quarantine is presently the most effective measure to prevent the introduction of transboundary disease. The Health Management program will enhance aquatic animal quarantine by reviewing quarantine procedures, providing specific training to quarantine officers and implementing a reporting system for pathogen findings resulting from the quarantine activities (Aquaplan, 1999). As a result aquatic animal quarantine could receive quality certification and international accreditation. So far no OIE notifiable diseases have been reported from the imported aquatic animals to Sri Lanka and this can be due mostly to under-diagnosing or inefficient diagnostic procedures at the countrie´s different assigned laboratories.

Reviewing the present quarantine arrangements for imported aquatic animals was done through consultancy of the Chief Veterinary Quarantine Officer at Colombo´s Animal Quarantine Station. Sri Lanka´s Department of Animal Production and Health (DAPH) is in charge of coordinating and supervising the countries aquatic animal quarantine for imported aquatic animals, assuring that the necessary conditions for receiving aquatic animal imports are met. The DAPH Director General currently issues an import permit after considering the exporting countries aquatic animal health status. A health certificate is required from the exporting country that will have to state that the aquatic animals arrive from a disease free zone for OIE notifiable aquatic animal diseases. Whoever the same guarantees cannot be applied to the aquatic animals exported from Sri Lanka, making for unfair trade criteria. Therefore, to justify this ALOP determined for imported aquatic animals, Sri Lanka should supply its trade partners with the same guarantees required from them.

Once the aquatic animals arrive to Sri Lanka the quarantine facilities are provided for at the receiving farm, and their design, facilities and biosecurity inspected on arrival of the imported aquatic animals by an appointed Veterinary Quarantine Officer. Samples are randomly collected at the receiving farm on arrival for the fish showing clinical or behavioral signs of disease. The imported animals are then subject to a quarantine period of two weeks at the receiving farm. For the fish showing clinical or behavioral signs of disease, samples are also collected and

analyzed during the quarantine period. The samples collected by the Veterinary Quarantine Officer are sent to be analyzed at the Veterinary Investigation Center (VIC). When required, diagnostic support is also provided by the Center for Aquatic Animal Disease Diagnostics and Research (CAADDR) and the Veterinary Research Institute (VRI).

The water that was used for shipping is chlorinated and disposed of in a dug hole at the receiving facilities, under the Veterinary Quarantine Officers supervision. All stocks of aquatic animals diagnosed positive for OIE notifiable diseases causing pathogen are destroyed, also under a Veterinary Quarantine Officer´s supervision.

4.2. Surveillance, Monitoring and Reporting

Surveillance is a means of data collecting and interpreting to detect previously unknown and exotic pathogens, done through systematic investigation and sample collecting of the aquatic animal populations of concern and supported by the necessary diagnostic and reporting arrangements (Subasinghe, 2004; FAO, 2007). Surveillance to declare freedom from disease will support disease reporting obligations by detecting previously unknown infections, helping to build an accurate description of the status of the countries aquatic animals for a list of diseases of concern. On the other hand, disease monitoring, also known as surveillance for distribution and occurrence of disease, serves the purpose of identifying changes in the aquatic animal endemic disease profile, for example, to detect changes in infection incidence or prevalence in cultured or wild animal populations (Cameron, 2004; OIE, 2009b). When disease is known to be present disease monitoring can attempt to define disease free zones, supporting the responsible movement of aquatic animals and aquatic animal products at a national and international level. Surveillance and monitoring activities are also particularly useful for the early detection of a disease emergency (Subasinghe, 2004). When referred to on its own, the term surveillance program will incorporate both surveillance and monitoring activities (FAO, 2007).

Sri Lanka is presently without an implemented surveillance, monitoring and reporting system for aquatic animal diseases. Surveillance of the aquatic animal populations is scarce, unorganized, without the support of a structured plan for cooperation among institutions and laboratories to maximize the existing resources and share knowledge of the diseases affecting the countries aquatic animal species. Cost effective surveillance is a priority for Sri Lanka who should initiate its surveillance program by prioritizing diseases for initial action, taking into account the existing resources and what are the achievable surveillance objectives (East, 2005; Bondad-Reantaso, 2005).

4.2.1. Surveillance to Declare National Freedom from Disease

Declaring national freedom from disease means confirming the absence of infection in the countries populations of susceptible aquatic animal species (OIE, 2009b). In practice every member of the population would have to be tested using perfect test methods, providing 100% specificity and sensitivity, in order to declare total freedom from disease (Cameron, 2004). As this would prove impracticable, declaring freedom means that a country is able to provide evidence that, if disease is present, that it is present at levels bellow a specified proportion in the population, with an acceptable level of confidence (OIE, 2009b). Therefore, rather than a statement of freedom, what surveillance to declare freedom provides is a probabilistic approach to disease free status, making use of OIE protocols in the OIE Manual of Diagnostics for Aquatic Animal Diseases and built on the internationally accredited standards set in the OIE Aquatic Animal Health Code (Cameron, 2004).

The OIE Aquatic Animal Health Code states that where disease status is unknown, two yearly pathogen-specific surveys held for a minimum of two consecutive years and at least three months apart, are needed to claim disease free status, providing that for the past ten years no prior vaccinations were undergone and the basic biosecurity measures were in place and enforced. Surveys should inclusively be held during life cycle and season that offers greatest probability of detecting disease and include specific surveillance of both cultured and wild susceptible species (OIE, 2009b).

Given the high risk of prior introduced aquatic animal diseases through imported aquatic animals, the questionable efficiency of aquatic animal quarantine and the absence of documented and consistent records of species introductions, along with low standard biosecurity measures applied at production level and in general, capable of minimizing disease incursion, disease free status cannot be claimed without the necessary pathogen specific surveillance (OIE, 2009b).

The key concepts taken into consideration to demonstrate disease free status are (Cameron, 2004):

- Population;
- Statistical Methodology, Survey Design and Analysis;
- Design Prevalence;
- Clustering;

- Test Characteristics;
- Sampling;
- Quality Assurance.

4.2.1.1. Population

For declaring national freedom from disease the epidemiological unit studied for surveillance must be clearly defined and surveillance focused on the population at risk, the target population (OIE, 2009b). The target population is defined as all individuals of susceptible species within a country, zone or compartment. Most frequently pathogens will affect preferably a group within the same species from the target population, that is disease will tend to cluster at different levels, in many cases during a particular time of the year or life cycle stage. These populations are subpopulations of the targeted population called the study population, and as they increase the probability of detecting disease, the study population is favored for sampling to declare national freedom from disease (Cameron, 2004).

4.2.1.2. Statistical Methodology, Survey Design and Analysis

A convenient approach for gathering evidence of disease free status is through a structured population-based survey, sampling and then using one or more tests to detect an infection or declare freedom from the diseases of concern to the country. Surveillance for homogenous populations, such as farm raised finfish and hatchery raised shrimp larvae and postlarvae, can be done through a single-stage survey, while larger populations where disease clusters a multi-stage sampling survey is required. Whatever may be the case, the approach must be fully described and documented, including a description of sampling process and any biases taken into account (OIE, 2009b).

The surveillance data generated through population based surveys to declare disease free status is based on hypothesis testing . Declaring freedom from disease analytically means estimating the probability of rejecting a null hypothesis. The null hypothesis, that disease is present, can be either supported or rejected by the surveillance data (Cameron, 2004). The confidence required for the surveillance system, representing the systems sensitivity, is 1-α, is the measure for supporting the null hypothesis (OIE, 2009b). If α is large then evidence for supporting the null is low (Cameron, 2004). When we reject the null we accept that if disease is present it is present bellow a predetermined design prevalence.

For declaring freedom from disease a level of confidence of 95% or higher is required. This value represents the probability that the system will detect infection at a level equal or greater to

95%, if disease is present for a certain design prevalence (OIE, 2009b). This high level of confidence ensures the small probability of a country declaring freedom from a disease that in fact is present (Cameron, 2004). Calculating the system confidence should be scientifically based and well documented. Final data analysis must take into consideration the surveys design, test sensitivity and specificity and design prevalence (OIE, 2009b).

Non-random data sources can also be used to demonstrate disease free status, such as routine diagnostic findings, relevant aquatic animal disease related studies, history of imports or knowledge of the biology of the infectious agent. These data sources can be used alone or together with the population-based surveys, as long as any potential biases are referred to and taken into consideration for data analysis (OIE, 2009b).

4.2.1.3. Design Prevalence

The value for design prevalence is the value for which infection may exist undetected when declaring freedom and will depend on the biology of the infection in the study population. The design prevalence values are used to satisfy claims of freedom, as we cannot prove with 100% confidence that a country is free from disease. However, if a single animal is diagnosed as infected, then it is not possible to claim freedom, given that freedom means zero prevalence and not low prevalence (Cameron, 2004).

The null hypothesis states that disease is present, at a level equal or greater than the design prevalence. The design prevalence, or minimum expected prevalence in the presence of disease, is therefore part of the null hypothesis. For declaring freedom the suggested ranges for design prevalence at the animal level are 1% to 5%, for slowly transmitted diseases, and for more contagious diseases, values above 5% may be suitable. By sampling animals from the study population as an indicator for design prevalence it is easier to provide evidence of freedom since higher values of design prevalence can be used. Other values for design prevalence are available in the OIE Aquatic Animal Health Code (Cameron, 2004).

4.2.1.4. Clustering

Frequently populations have a complex structure and disease tends to cluster at many levels, increasing in frequency within certain age groups or life cycle stage. In these cases the value for design prevalence must be determined for each clustering level (OIE, 2009b). It is often recommended to consider disease clustering at one major level so as to not complicate survey design and analysis (Cameron, 2004).

4.2.1.5. Test Characteristics

There are many types of tests that can be done to determine infection in a population. Tests can range from farmer observation to detailed laboratory diagnosis (Cameron, 2004). Whatever test or tests chosen their performance must be described in terms of the tests sensitivity and specificity, where imperfect values should be taken into consideration when choosing sample size and interpreting data (OIE, 2009b). According to guidelines provided by the OIE Aquatic Animal Code any test can be used as long as the values for sensitivity and specificity are determined (Cameron, 2004).

Test sensitivity (Se) and specificity (Sp) quantify the diagnostic ability of a diagnostic test. Test sensitivity is the proportion of infected animals that yield positive test results within the aquatic animals with infection. Test specificity is the proportion of uninfected and diagnosed as negative the aquatic animals within the aquatic animals without infection. A screening test is used to determine the presence of infections previously unaccounted for. Highly sensitive tests should be preferred for screening purposes to ensure that no case of infection goes undetected, and confirmatory diagnosis should be done afterwards, through highly specific tests, that will help confirm negative test results (OIE, 2009b).

To provide evidence of freedom from disease, surveillance activities will require performing one or more diagnostic tests. If more than one test is being used, overall sensitivity and specificity must be calculated. Formulas for calculating combined sensitivity and specificity are complex and using simple formulas will consider tests as independent (Cameron, 2004). Furthermore, a test done for confirmatory diagnosis should have a different biological basis than the test performed for presumptive diagnosis (OIE, 2009b).

Test sensitivity and specificity vary for different population and test scenarios and should be determined for each individual circumstance. Standard values for test sensitivity and specificity are referred to in the OIE Aquatic Animal Code and should be taken into consideration with some level of uncertainty (OIE, 2009b). Furthermore, test sensitivity and specificity can be inferred using data from similar studies performed in other countries or provided through expert opinion (Cameron, 2004).

Pooling samples from multiple individuals for single test purposes is also acceptable and test sensitivity and specificity should also be determined or estimated in such cases (OIE, 2009b).

4.2.1.6. Sampling

The diseases chosen for the surveillance program are infectious aquatic animal diseases. An infected animal may or may not present clinical signs of disease, especially when recently exposed (Whitman, 2004). For this reason surveillance programs have to sample from a wide range of clinically healthy host species, both wild and cultured species, increasing the surveillance efforts.

Probability sampling provides the most population-representative samples and requires a sampling frame, where every member of the population is listed, or in alternative a sampling frame of epidemiological units such as ponds, farms or village tanks (Cameron, 2004). Non-probability based sampling can also be used when biases are considered. This biased sampling, also known as targeted sampling, maximizes the probability of detecting an infection, as the sampled animals are selected from the study population where it is most probable to detect infection. Biased sampling should also generate a representative sample of the study population (OIE, 2009b).

Determining the correct sample size for claiming disease free status is of great importance. Sample size should be determined through a statistically valid technique and take into consideration the survey test or tests sensitivity and specificity, design prevalence and desired confidence in addition to population size and desired survey power. It is often required to make presumptive estimations of certain population parameters and test characteristics, therefore uncertainties must be considered for data analysis. Sample size can be calculated through available free software such as Freecalc or Survey Toolbox for Aquatic Animal Diseases developed by the Australian Centre for International Agricultural Research (ACIAR) or using tables provided in the OIE Aquatic Animal Health Code 2009 (OIE, 2009b).

4.2.1.7. Quality assurance

Surveillance programs should include a documented quality assurance system, based mostly on efficient record keeping, which ensures that all procedures are being carried out according to survey design and stipulated protocols (Cameron, 2004).

4.2.2. Zoning

Aquatic animal populations are often organized into subpopulations, by natural or artificial barriers, defining compartments or zones. Compartments are defined by management practices related to biosecurity while zones are defined geographically (OIE, 2009e).

Disease zoning, as the process of delineating geographical areas according to the presence of infection, is a tool for facilitating animal movement at a national and international level, while conserving valuable disease free zones (OIE, 2009e). When a country suffers a disease incursion rather than defining the entire country as infected, zoning allows the establishing of infected zones and disease free zones within country borders (Cameron, 2004).

In order to maintain disease free zones animal movement between zones must follow the disease zoning principle that states that live aquatic animals may be moved between zones of equal status, or from higher to lower status, but not otherwise (Subasinghe, 2004). Higher zones are the disease free zones that are high priority zones for preventing incursion of new exotic disease, while lower status zones are the infected zones where disease is present. Figure 1 represents the movement of live aquatic animals according to the zoning principles, where full lines with arrows indicate low risk transfers while broken lines indicate movements that should be subject to control (Cameron, 2004).

The surveillance program for declaring national freedom from disease can be applied to define disease free zones. The OIE sets the principles for defining a zone or compartments in the zoning and compartmentalization chapter of the Aquatic Animal Health Code (OIE, 2009e).

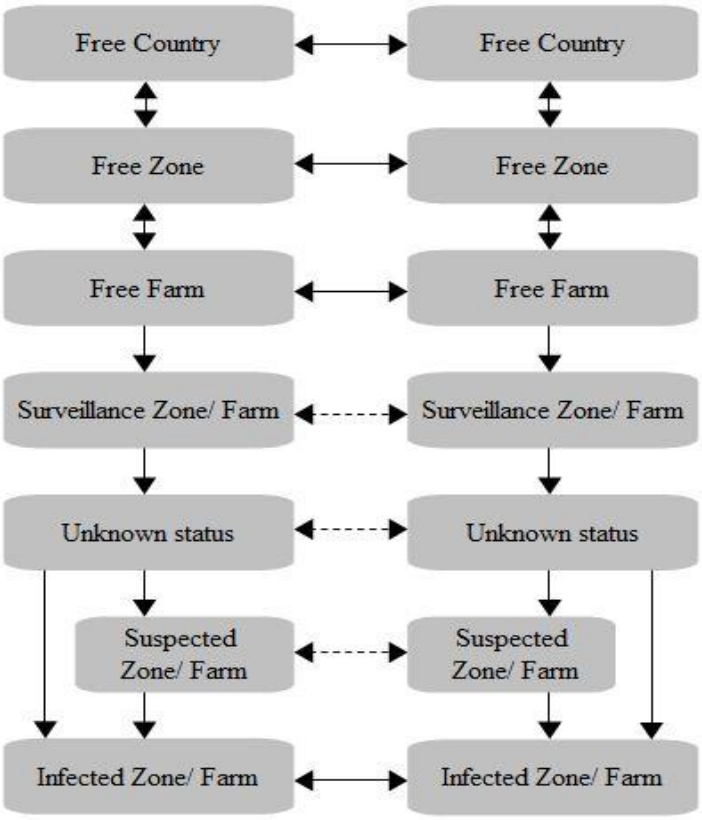

Figure 1: Movement of live aquatic animals between zones. Full lines represent low risk movement of animals between zones while broken lines illustrate movement subject to control.
(Adopted from Subasinghe, 2004)

As surveillance programs require significant investment, Sri Lanka´s surveillance program used to help define and maintain disease free zones, should apply to the endemic diseases that pose highest risk to valuable cultured species (Subasinghe, 2004).

4.2.3. Listing Diseases of Concern to Sri Lanka

Finfish and crustacean aquaculture plays a vital role in Sri Lanka´s agricultural economy with special significance in poverty alleviation for the rural communities (Kasagala, 2008). Many brackish water (black tiger prawn and tilapia), fresh water (various ornamental finfish and carp varieties) and marine species (Sea bass) are cultured in Sri Lanka, with further capacity and potential for diversification, considering the country's vast aquatic resources (FAO, 2010). Therefore understanding the potential risks associated with disease occurrence in these cultured species is of great importance.

It is necessary to establish a list of the diseases of concern to Sri Lanka to be considered for the surveillance program. This list should be based on the OIE list of notifiable diseases of concern to the Asia-Pacific region (Table 1) and other diseases known to frequently affect Sri Lanka´s major cultured species (FAO, 2007). The criteria for listing diseases of concern can be found in the OIE Aquatic Animal Code. This criteria focuses on the consequence of disease incursion: high mortality or morbidity, susceptibility of wild and cultured populations, if the disease agent is of public health concern or if the agent is potentially infectious or suspected to be infectious and can spread through the movement of live aquatic animals and aquatic animal products (OIE, 2009c; OIE, 2009d).

OIE LISTED DISEASES PREVALENT TO THE ASIA-PACIFIC REGION
FINFISH DISEASES
OIE-listed diseases
1. Epizootic haematopoietic necrosis
2. Infectious haematopoietic necrosis
3. Spring viraemia of carp
4. Viral haemorrhagic septicaemia
5. Epizootic ulcerative syndrome
6. Red seabream iridoviral disease
7. Koi herpesvirus disease
Non OIE-listed diseases
8. Grouper iridoviral disease
9. Viral encephalopathy and retinopathy
10. Enteric septicaemia of catfish
CRUSTACEAN DISEASES
OIE-listed diseases
1. Taura syndrome
2. White spot disease
3. Yellowhead disease
(Continued)

(Continuation)
4. Infectious hypodermal and haematopoietic necrosis
5. Infectious myonecrosis
6. White tail disease (MrNV)
Non OIE-listed diseases
7. Necrotising hepatopancreatitis
8. Milky haemolymph disease of spiny lobster (*Panulirus sp.*)
9. Monodon slow growth syndrome

Table 1: List of OIE diseases of concern in the QAADR for Sri Lanka divided by OIE listed diseases for international disease reporting and non-listed diseases (QAADR, 2010).

It is important to understand the nature of the diseases suggested for listing as diseases of concern to Sri Lanka in order to apply the surveillance strategy for each one. The OIE diseases of concern to the region and other diseases of concern to the country are here described for beter understanding of their nature and threat to the countries cultured finfish and crustacean species. Description of the causative agent, their known distribution in the Asia-Pacific region, epizootology and the recommended diagnostics are here summarized. In many cases the OIE has no recommended screening tests for targeted surveillance to declare national freedom from disease. In these cases and when such tests are not mentioned, molecular based techniques such as polymerase chain reaction (PCR) are frequently applied.

4.2.3.1. Epizootic Haematopoietic Necrosis

i) Causative agent and geographic distribution

Epizootic Haematopoietic Necrosis (EHN) is an infectious necrotizing disease caused by Epizootic Infectious Haematopoietic Necrosis Virus (EHNV), a double-stranded DNA (dsDNA), virus of the family *Iridoviridae*, genus *Ranavirus* (OIE, 2009f). The virus was first identified in Australia in 1986 and since then is suspected to have caused outbreaks of disease in Kuwait and Pakistan (Figure 2) (Bondad-Reantaso, 2001; Iowa State University, 20010a).

Figure 2: Countries where EHN is known to occur in the Asia-Pacific.

ii) Susceptible host species

EHNV infects all life stages of redfin perch and rainbow trout farmed and wild populations (Iowa State University, 2010a). The susceptible host species for EHNV infections are described in Annexure II (OIE, 2009f; Fishbase, 2010). From the list of susceptible species, mosquito fish and rainbow trout are the two uncultured susceptible species present in Sri Lanka (OIE, 2009f; Fishbase, 2010).

iii) Epizootology

Outbreaks of disease among rainbow trout are related to bad management practices and poor water quality, occurring between 11ºC and 20ºC. Cumulative mortalities are low for this species, ranging from 0,2-4% in fingerlings (OIE, 2009f). The absence of clinical signs and high mortality may not cause suspicion of disease occurrence, making disease hard to detect (OIE, 2009f; Iowa State University, 2010a).

The route of transmission for EHNV is incompletely understood, thought to be horizontal through water shed of disintegrated infected tissue, transmitted by bath or orally (Iowa State University, 2010a; OIE, 2009f). The virus is highly resistant and can spread through fomites and also by birds who can regurgitate infected material. Otherwise bird body temperature, from 40ºC to 44ºC, seems to inactivate the virus (OIE, 2009f). EHNV can also be transferred through frozen fish products (Plumb, 1999).

iv) Diagnosis

Initial presumptive diagnosis of EHN should be done through histopathology followed by cell culture observation of cytopathogenic effect (CPE) and virus identification using biomolecular techniques such as PCR or antigen-capture enzyme-linked immunosorbent assay (ELISA). The OIE recommends identifying infection through routine sample collection whenever mortalities are observed in susceptible host species to be subject to antigen-capture ELISA and cell culture

to determine EHNV as the causative agent (OIE, 2009f).

4.2.3.2. Infectious Haematopoietic Necrosis

i) Causative agent and geographic distribution

Infectious Haematopoietic Necrosis (IHN) is a viral disease of salmonid fish caused by a single stranded RNA (ssRNA) virus with the same name, Infectious Haematopoietic Necrosis Virus (IHNV), member of the family *Rhabdoviridae* and genus *Novirhabdovirus,* for which various strains are known with varying pathogenicity (Bondad-Reantaso, 2001; Iowa State University, 2010b). Outbreaks of IHN were first accounted for during the 1950´s in the Pacific Northwest regions of North America (Iowa State University, 2010b). In the past India reported occurrence of IHN for the last quarter of 1999 (Bondad-Reantaso, 2001). Disease is now endemic to Europe and Japan and outbreaks of disease have also been reported in South Korea, Iran, China and Pakistan (Figure 3) (Iowa State University, 2010b; OIE, 2010a).

Figure 3: Countries where IHN is known to occur in the Asia-Pacific.

ii) Susceptible host species

IHNV primarily infects juvenile rainbow trout and several species of Pacific salmon, but can also infect and cause disease in other susceptible host species such as sockeye, chum, pink, angoo, masou, coho, Atlantic salmon, brown trout and cutthroat trout, among other species (Plumb, 1999; OIE, 2009g; NACA, 2010a). Annexure III lists the susceptible host species, being that only rainbow trout and brown trout are present to Sri Lanka, where neither are cultured species (Fishbase, 2010).

iii) Epizootology

The IHNV is considered highly virulent to young fish, mortalities depending on species, age and size, but mostly on water temperature and virus strain (Plumb, 1999). IHN is a cold, fresh and saltwater disease affecting fry in particular at 8ºC-15ºC. Fish at spawning also seem to be more susceptible. Outbreaks also tend to occur when fish are unhealthy given their impaired

immunity. Accumulated mortalities can range from 90-95%, registering highest mortalities for water temperature in the range of 5ºC-18ºC (OIE, 2009g). Outbreaks are rare above 15ºC (Bondad-Reantaso, 2001).

Transmission is horizontal and occurs through direct exposure to contaminated faeces, urine, mucus and spawning fluids, entering the body through digestion, gills and fin base (NACA, 2010a; OIE, 2009g). Vertical egg-associated transmission has also been recorded (OIE, 2009g). Contaminated feed has also contributed to disease spread (Iowa State University, 2010b). Chronically infected and asymptomatic carriers may infect other healthy animals (OIE, 2009g).

iv) Diagnosis

Presumptive diagnosis can be obtained by observation of clinical signs, CPE in histopathology or single positive results in virus isolation, antibody-based or molecular based assays. Confirmatory diagnosis is obtained after virus isolation and identification using molecular or antibody-based assays. For the purpose of declaring freedom, the OIE recommends virus isolation in cell culture (OIE, 2009g).

4.2.3.3. Spring Viraemia of Carp

i) Causative agent and geographic distribution

Spring Viraemia of Carp (SVC) results from the infection with Spring Viraemia of Carp Virus (SVCV), an ssRNA virus of the family *Rhabdoviridae* genus *Vesiculovirus*. SVC was first described in 1971 in Europe, having spread to establish global distribution (OIE, 2009h). In the Asia-Pacific region disease has been reported in China and Iran (Figure 4) (NACA, 2010b). Disease was reported as suspected in Sri Lanka for the year of 2007, 2008 and 2009 in the OIE QAADR and was last reported as suspected to Sri Lanka in the QAADR for January to March 2010 (OIE, 2010a).

Figure 4: Countries where SVC is known to occur in the Asia-Pacific.

ii) Susceptible host species

Common carp are susceptible to disease that occurs primarily in ponds with fish population one year and older (Plumb, 1999; NACA, 2010b). Other carp species follow in host susceptibility as do carp hybrids (OIE, 2009h). Non cyprinids are least vulnerable and most cyprinids can be considered susceptible to infection (OIE, 2009h). A list of susceptible host species is suggested by the OIE and their distribution and habitat considered in Annexure IV (OIE, 2009h; Fishbase, 2010). Susceptible species present and cultured in Sri Lanka are common carp and carp varieties, crucian carp, silver carp, big head carp, grass carp, goldfish, zebra fish and guppy (NACA, 2010b; Fishbase, 2010).

iii) Epizootology

Pathogen transmission can be vertical, from parent to progeny, or horizontal through water shed skin, faeces, urine, mucus and spawning fluids or contact with infected objects or vectors, such as haematophague parasites, namely leaches and anchor worms (OIE, 2009h; NACA 2010b). Mechanical transmission can occur after pond substrate has dried (APHIS, 2010). Virus enters host through gills where it replicates and spreads through the blood stream. Contaminated fish feed can also serve as a source of viral infection (Plumb, 1999). Sick fish and survivors can serve as reservoirs contributing to disease spread (APHIS, 2010). SVCV resistance, wide range of direct, indirect mechanisms for transmission and capacity to maintain reservoirs make this disease difficult to control or eradicate without total destruction of production (Bondad-Reantaso, 2001; OIE, 2009h).

Young fish are most susceptible to disease outbreaks. Fry can be infected at temperatures as high as 23ºC (NACA, 2010b). Frequently, carp are cultured in polyculture systems, accounting for high transmission rates (OIE, 2009h). Outbreaks tend to occur for over-wintered animals at 11-17ºC or subject to stress, overcrowding and impaired immune status (NACA, 2010b; OIE, 2010h). Cumulative mortalities can reach 70% in young carp but tend to range around 1% to 40% (OIE, 2010h). Tropical and subtropical climates are unfavorable for disease outbreaks (APHIS, 2010).

iv) Diagnosis

Rapid mortalities and clinical signs, histopathology or CPE observation of infected cells or single positive results to molecular or antibody-based assays can define a suspected case while confirmation can be achieved through one of the following methodologies:

- CPE of cell culture and serological identification of agent or;
- PCR and sequencing or;
- molecular or antibody-based assays identifying causative agent after a separate method

is used for presumptive diagnosing.

For targeted surveillance to declare disease free status the OIE recommends inoculation of cell culture with tissue extracts (OIE, 2009h).

4.2.3.4. Viral Hemorrhagic Septicaemia

i) Causative agent and geographic distribution

Viral Hemorrhagic Septicaemia (VHS) is a viral disease caused by infection with Viral Hemorrhagic Septicaemia Virus (VHSV), an ssRNA virus member of the family *Rhabdoviridae and* genus *Novirrhabdovirus*. (Iowa State University, 2010c; Bondad-Reantaso, 2001). The virus has both marine and fresh water isolates, infecting a large range of host species (Iowa State University, 2010c; OIE, 2009i). The virus has infected species in Asia, Europe and North America, having been reported in Iran, Japan and North Korea (Figure 5) (NACA, 2010c).

Figure 5: Countries where VHS is known to occur in the Asia-Pacific.

ii) Susceptible host species

The OIE proposes an extensive list of known susceptible host species (OIE, 2009i). The species most susceptible to VHSV infection are rainbow trout and Japanese flounder while turbut are also severely affected (Iowa State University, 2010c). Annexure V indicates susceptible host species habitat and distribution for a selected group of susceptible host species present in the region from which zebra fish is the susceptible species known to Sri Lanka, also an aquacultured species (NACA, 2010c; Fishbase, 2010)

iii) Epizootology

VHSV transmission occurs horizontally through water shed skin, reproductive fluids, urine and feaces (Iowa State University, 2010c; Plumb, 1999). Survival outside host and above 20ºC is short (Iowa State University, 2010c). All life stages of susceptible finfish are known to be affected (OIE, 2009i). Whoever younger fish are most susceptible (Plumb, 1999). Outbreaks

generally occur at 14-18ºC (NACA, 2010c). Above 15ºC mortality and morbidity are low and the virus has rarely been isolated for species living were temperature rises above 15ºC (Aquavet, 2005a). Temperature fluctuation seems to be the main dictator of disease outbreaks, being that disease transmission is optimal at 9-12ºC, with mortalities ranging from 5-90%, though transmission can also occur from 2-20ºC (OIE, 2009i). Outbreaks have also been associated to overfeeding (NACA, 2010c).

iv) Diagnosis

Presumptive diagnosis can be obtained after detecting mortalities associated with clinical signs of disease, observing CPE in cell culture or through antibody detection. Confirmatory diagnosis can be obtained through cell culture isolation followed by antibody-based test or PCR, or in alternative PCR and sequencing. The OIE recommends targeted surveillance for disease free status through culture of fish tissue specimens on BF-2 cells with subsequent virus identification by immunochemical or nucleic-acid-based tests (OIE, 2009i).

4.2.3.5. Epizootic Ulcerative Syndrome

i) Causative agent and geographic distribution

Epizootic Ulcerative Syndrome (EUS), also known as Red-Spot Disease, is caused by infection with water mold oomycete A*phanomyces invadans*, (OIE, 2009j; Bondad-Reantaso, 2001). Often associated with disease outbreaks are secondary infection with bacteria such as *Aeromonas hydrophila*, *Aeromonas sorbia* and *Vibrio angullarium* (Ahmed, 2005).

EUS has spread rapidly in South Asia (Ahmed, 2005). EUS is known to Australia, Bangladesh, Myanmar, Cambodia, India, Indonesia, Japan, Laos, Nepal, Pakistan, Philippines, Thailand, Vietnam and Sri Lanka (Figure 6) (NACA, 2010d; OIE 2010a). In Sri Lanka EUS has been reported as present in the QAADR since the year 2008 (OIE, 2010a).

Figure 6: Countries where EUS is known to occur in the Asia-Pacific.

ii) Susceptible host species

More than one hundred estuarine and fresh water species have been reported susceptible hosts to EUS infection around the world. Common carp, Nile tilapia and milk fish are considered resistant to infection. Indian carp, rohu, catla, migral and goldfish are considered susceptible species (OIE, 2009j). All the previously mentioned species are present and cultured in Sri Lanka. Recently introduced sea bass (*Lates calcalifer*) culture to Negombo Lagoon alerts to possibility of introduction of disease through this susceptible species (NACA, 2010d). Susceptible species known to Sri Lanka also include catfishes (*Bagridae* sp.), climbing perch (*Anabas testudineus*), fresh water eels (*Anguillidae* sp.), jacks (*Caranx* sp.), striped snakehead (*Channa striatus*), cichlids (*Cichlidae* sp.), torpedo shaped catfishes (*Clarius* sp.), *Cyprinidae* sp., flying fishes (*Exocotidae*), gobies (*Gobidae* sp.), rhinofishes (*Labeo* sp.), mullets (*Mugilidae* sp.) and wells catfishes (*Siluridae* sp.) (NACA, 2010d; OIE, 2009j; Fishbase, 2010).

iii) Epizootology

Transmission occurs by means of motile zoospore penetration in skin lesions, progressing towards muscle and internal organs. Disease outbreaks occur after prolonged winters or heavy rainfall when temperatures are low (18-22ºC) allowing for best sporulation and lowest immune response. A*phanomyces invadans* grows best at 20-30ºC but salinities above 2 ppt will difficult agent spread (OIE, 2009j). Outbreaks are also referred to be associated to acid water run-off and bacterial and viral infections, and are associated with mass mortalities (NACA, 2010d).

iv) Diagnosis

Typical signs of disease, such as one or more red spots during the seasons of expected water temperature range for disease occurrence, observation of branching non-septate oomycete hyphae in muscle squash or agent isolation without identification, can stand as presumptive diagnosis for EUS infection. Confirmatory diagnosis can be obtained through histopathology of typical signs of infection in tissue sections, positive PCR, fluorescent peptide nucleic acid *in-situ* hybridisation (FISH), sequencing or bioassay identification of isolated agent (OIE, 2009j).

4.2.3.6. Red Sea Bream Iridovirus Disease

i) Causative agent and geographic distribution

Red Sea Bream Iridovirus Disease (RSIVD) is caused by two viruses from the *Iridoviridae* family, the virus with the same name, Red Sea Bream Iridovirus (RSIV) a DNA virus, genus *Magalocystivirus*, among other genotypes of RSIV, associated with a second virus, the Infectious Spleen and Kidney Necrosis Virus (ISKNV) (OIE, 2009k; Shinmoto, 2009). Various other iridoviruses are known to cause disease in fresh water ornamental fish, difficult to

distinguish from the previous two iridoviruses. Outbreaks of RSIVD were first reported in Japan in 1990 and since then have been responsible for mass mortalities to cultured fish, in particular juvenile red sea bream (OIE, 2009k). In the Asia-Pacific disease is known to occur in the Chinese Taipei, Peoples Republic of China, Japan, Korea, Hong Kong, Malaysia, Philippines, Singapore and Thailand (Figure 7) (OIE, 2009k; OIE, 2010a). RSIV was reported as suspected in Sri Lanka in the QAADR for Asia-Pacific for the period from July to September 2008 (OIE, 2010a).

Figure 7: Countries where RSIVD is known to occur in the Asia-Pacific.

ii) Susceptible host species

The only known susceptible host species for infection with RSIV in Sri Lanka are sea bass (*Lates calcalifer*), also a cultured species in the country (NACA, 2010e; Fishbase, 2010). Other susceptible species listed by OIE are included in Annexure VI (OIE, 2009k).

iii) Epizootology

Pathogen transmission seems to be horizontal, through water shed virus from infected fish, with outbreaks occurring at water temperatures equal or above 25ºC with wide ranging mortality of 0% to 100% (OIE, 2009k).

iv) Diagnosis

Presumptive diagnosis can be based on microscopic observation of typical affected cells in tissue stamp-smear or histopathology along with the presence of clinical signs of disease, virus isolation and CPE effect or fluorescent antibody test (IFAT) positive stamp-smear. For confirmatory diagnosis, CPE effect followed by positive IFAT or PCR can be used. There are no recommended tests for targeted surveillance to declare disease free status, though tentative methods could include virus isolation followed by IFAT or nested PCR (OIE, 2009k).

4.2.3.7. Koi Herpes Virus Disease

i) Causative agent and geographic distribution

Koi Herpes Virus Disease (KHVD) is caused by the cyprinid herpes virus Koi Herpes Virus (KHV) of the *Herpesviridae* family (OIE, 2009m). Disease is known to occur in Germany, Israel, the United States and South Africa and in Asia disease is known to Hong Kong, Indonesia, Japan, Republic of Korea, Taipei, Malaysia, Singapore, Thailand and the Philippines (Figure 8) (OIE, 2009m; OIE, 2010a). KHV has been reported as suspected but not confirmed for Sri Lanka in the QAADR from 2007 to 2009 (OIE, 2010a).

Figure 8: Countries where KHV disease is known to occur in the Asia-Pacific.

ii) Susceptible host species

KHV is known to infect all age groups of common carp, its varieties of ghost and koi carp, carp hybrids and carp and goldfish hybrids (OIE, 2009m). The following susceptible host species are known and cultured in Sri Lanka: common carp, koy, grass and ghost carp and goldfish (NACA, 2010f; Fishbase, 2010). Outbreak investigations in Germany suggest that goldfish and grass carp are susceptible under experimental conditions and conclude that if the study is confirmed all cyprinid species are susceptible to infection with KHV (OIE, 2009m). Annexure VII lists the susceptible host species, their habitat and distribution (NACA, 2010f; Fishbase, 2010).

iii) Epizootology

KHV transmission is mostly horizontal, being that gills are suggested as the major portals of entry of water shed virus from infected and carrier fish skin, gills, feaces, urine and hyper-secreted mucus. Disease outbreaks are associated with high mortalities and stand as a major concern to the ornamental fish industry worldwide (NACA, 2010f). Mortality rates are very high, up to 100%, highest for common carp, followed by the koi and ghost carp varieties of

common carp and finally by their hybrids and goldfish x carp hybrids. Highest transmission rates occur at 16ºC for common carp. Optimal water temperature for disease outbreaks ranges from 23ºC to 25ºC, declining below 23ºC. Virus can survive experimentally at 28ºC (OIE, 2009m).

iv) Diagnosis

Presumptive diagnosis can be achieved after observing clinical signs of disease, through typical histopathology, CPE on cell culture, single positive PCR methods or IFAT assay, traced epidemiological links or antibody detection. When mortalities are high, specific antibody detection, isolation in cell culture and identification or PCR methods can be applied to achieve confirmatory diagnosis. In the event of low mortalities, confirmation may be accomplished through PCR detection of the infectious agent. Carrier species are best diagnosed for viral infection using PCR rather than through virus isolation (Crane, 2010). The OIE has no recommended diagnostic for surveillance to determine disease free status, though it mentions PCR techniques applied in areas where disease may affect susceptible species (OIE, 2009m).

4.2.3.8. Grouper Iridoviral Disease

i) Causative agent and geographic distribution

Grouper Iridoviral Disease (GIVD) is caused by a dsDNA iridovirus, Grouper Iridovirus (GIV), family *Iridoviridae*, and genus *Ranavirus* (Yeh, 2008). Disease is known to occur in Hong Kong, Indonesia, Republic of Korea, Malaysia, the Philippines, Singapore, Thailand and Vietnam (Figure 9) (NACA, 2010g).

Figure 9: Countries where GIV disease is known to occur in the Asia-Pacific.

ii) Susceptible host species

Typically associated to high morbidity and mortalities, GIVD affects all life stages of many highly priced tropical mariculture food fish. Host species known susceptible to GIV infection

are brown spotted grouper (*Epinephelus tauvina*), yellow grouper (*Epinephelus awoara*) and nursing grouper (*Epinephelus malabaricus*) whose distribution is analyzed in Annexure VIII (NACA, 2010g). In Sri Lanka the only known susceptible host species present are nursing grouper (NACA 2010g; Fishbase, 2010).

iii) Epizootology

GIV is associated with high mortalities. GIV transmission is poorly understood but thought to be established through the water column, through virus shed from infected to non-infected fish (NACA, 2010g).

iv) Diagnosis

Presumptive diagnosis can be made through observation of clinical signs of disease, histopathology or virus isolation in cell culture. Confirmatory diagnosis can be established through PCR testing (NACA, 2010g).

4.2.3.9. Viral Encephalopathy and Retinopathy

i) Causative agent and geographic distribution

Viral Encephalopathy and Retinopathy (VER) disease is caused by Viral Encephalopathy and Retinopathy Virus (VERV), an RNA virus of the *Nodaviridae* family and genus *Betanodavirus*, affecting mostly larval and juvenile stages of many marine fish species worldwide (OIE, 2003a). Disease is known to occur in Australia, Hong Kong, Indonesia, Malaysia, the Philippines, Republic of Korea, Japan, Thailand, Taiwan, India, Iran and Vietnam (Figure 10) (NACA, 2010h; OIE, 2010a).

Figure 10: Countries where VER is known to occur in the Asia-Pacific.

ii) Susceptible host species

More than 30 fish species are susceptible hosts to infection with VERV, with disease causing greatest impact to sea bass (*Lates calcalifer*), groupers, silver trevally, parrot fish, puffer and

flatfish (NACA, 2010h; OIE, 2003a; OIE, 2010a). Susceptible host species and their distribution is analyzed in Annexure IX (NACA, 2010h; Fishbase, 2010). Susceptible species known to Sri Lanka are nursing grouper and sea bass, the later a cultured species in Sri Lanka (Fishbase, 2010).

iii) Epizootology

VERV transmission in sea bass is horizontal, through water shed virus entering host through mouth, gills and skin. Silver trevally are inclusively susceptible to vertical transmission through eggs and sperm. Egg disinfection with ozone seems to be effective in controlling disease (OIE, 2003a). Mortality ranges from 50% to 100% over several weeks (NACA, 2010h). The earlier the onset of clinical signs of disease, the greater the associated mortality (OIE, 2003a). The incubation period varies among species ranging from 9 to 28 days after hatching (NACA, 2010h).

iv) Diagnosis

Presumptive diagnosis for VERV infection is done through histopathology or microscopic examination. Immunohistochemistry, PCR methods or cell culture with agent identification can be used to obtain confirmatory diagnosis to declare disease free status (OIE, 2003a).

4.2.3.10. Enteric Septicaemia of Catfish

i) Causative agent and geographic distribution

Enteric Septicaemia of Catfish (ESC) is caused by *Edwardsiella ictalluri* bacterium, family *Enterobacteriaceae* (OIE, 2003b). ESC is known to Australia, Japan, Singapore, Indonesia, Thailand, China and Vietnam (Figure 11) (NACA, 2010i; OIE, 2010a).

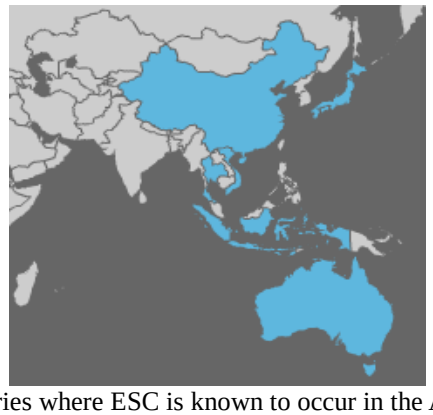

Figure 11: Countries where ESC is known to occur in the Asia-Pacific.

ii) Susceptible host species

Many species have been determined as susceptible hosts to infection with *Edwardsiella ictalluri*. Susceptible species and their distribution are mentioned in Annexure X (NACA, 2010i; Fishbase, 2010). Susceptible host species known to Sri Lanka are rainbow trout the cultured ornamental zebra danio (Fishbase, 2010).

iii) Epizootology

Transmission occurs through faecal-oral route, cannibalism, contaminated water or through handling and contaminated equipment. The intestinal tract is the main site of infection and faeces the main source of bacteria (NACA, 2010i). Fish surviving infection can be carriers and may die under stressful conditions, in spite of acquired immunity (OIE, 2003b). The pathogen is highly resistant, remaining viable in fish gut and surviving 3 to 4 months in water, mud or vegetation (OIE, 2003b; NACA, 2010i).

Outbreaks of disease occur in the temperature range of 18ºC to 28ºC but mortalities can also occur outside this range, varying from 10% to 50% (Plumb, 1999; OIE, 2003b).

iv) Diagnosis

Edwardsiella ictalluri can be detected through isolation followed by identification using biochemical or serological techniques. Confirmation of disease can be made through PCR amplification and sequencing, fluorescent antibody technique or ELISA (OIE, 2003b).

4.2.3.11. *Tetrahymena* spp. Infection in Guppies

i) Causative agent and geographic distribution

*Tetrahymen*a spp. is a highly adaptable, invasive ciliated protozoan parasite (Rodgers, 2010). *Tetrahymena* spp. has become a major parasitic disease to guppy species in South-East Asia, associated with high mortalities and frequent secondary fungal infections (Lawhavinit, 2002).

ii) Susceptible host species

*Tetrahymen*a spp. preferably infects guppy ornamental fish *Poecilia reticulate*, also infecting many other tropical and coldwater fish species. *Poecilia reticulata* accounts for 75% of the ornamental fish exported species from Sri Lanka. Studies in Sri Lanka have also isolated *Tetrahymena* spp. from skin lesions of cultured angelfish (*Pterophyllum scalare*), platyfish (*Xiphrophorus maculates*), tetras (*Hyphessobrycon sp.*), goldfish (*Carassius auratus*), molly (*Poecilia sphenops*), barbs (*Capeota sp.* and *Puntius* sp.), fighters (*Betta splendens)*, gourami (*Colisa* sp.) and carp (*Cyrpinus carpio*). Studies to determine *Tetrahymena* spp. prevalence in

Sri Lanka showed that infection with *Tetrahymena corlossi* affected preferably guppies, while *Tetrahymena pyriformis* equally infected guppies, goldfish, molly, carp, angelfish and barbs (Thilakaratne, 2003).

iii) Epizootology

Tetrahymena spp. is a highly adaptive parasite. The free living parasite is capable of living off organic matter and bacteria. While associated to its host, *Tetrahymena* spp. can assume commensal and parasitic modes of infection. As a commensal *Tetrahymena* spp. grazes on skin cells, bacteria, organic matter and mucus of infected fish without causing harm. When lesions to the skin allow for parasite penetration into the hosts body, *Tetrahymena* spp. will borrow deep within skin and musculature, entering the bloodstream resulting in systemic infection. As a result, *Tetrahymena* spp. will remain protected from external the chemoterapics frequently used in Sri Lanka for treating external parasites before exporting the ornamental fish. Mortalities associated with systemic infection are high, in particular for young fry (Rodgers, 2010).

Fish subject to high stress due to high density production, mal-nourishment, water temperature fluctuations or poor water quality, are most susceptible to infection (Thilakaratne, 2003). Outbreaks of disease will occur at optimum water temperature for parasitic reproduction rate at 28ºC (Lewbart, 2010). *Tetrahymena pyriformis* whoever, only multiplies in the temperature range of 7,5ºC to 32,5ºC. Live fish-feed and water plants can introduce *Tetrahymena* spp. into a pond or tank (Rodgers, 2010).

iv) Diagnosis

Tetrahymena spp. can be identified under a low power microscope using 10x and 40x amplification of mucus scrapings of recently dead fish. Small dead fish can be mounted directly for microscopic observation. Massive infections can produce observation of swarms of *Tetrahymena* spp. (Rodgers, 2010). Wet mounts and histological exams reveal parasites in skin, gills and caudal fin blood vessels and surrounding internal organs and peri-orbital eye region of fish embryo (Leibowitz, 2009).

4.2.3.12. Taura Syndrome

i) Causative agent and geographic distribution

Taura Syndrome (TS) is caused by Taura Syndrome Virus (TSV), an ssRNA virus of the family *Dicistroviridae*, that affects penaied shrimp (Bondad-Reantaso, 2001; OIE, 2009n). Disease is thought to have been introduced to the Chinese Taipei in 1999 with imported *Penaeus vannamei* from Central and South America, spreading through broodstock movement to the other

countries causing major mortalities in this host species (OIE, 2009n). In the Asia-Pacific region TS is known to occur in Myanmar, China, South Korea, Taiwan, Indonesia, Thailand, Malaysia and Vietnam (Figure 12) (NACA, 2010j; OIE, 2010a).

Figure 12: Countries where TS is known to occur in the Asia-Pacific.

ii) Susceptible host species

Species so far determined as susceptible hosts are the Pacific white shrimp (*P. vannamei*) followed in susceptibility by the Pacific blue shrimp (*P. stylirostiris*) (European Community Reference Laboratory, 2008a). Other susceptible species where signs of disease do not develop include *P. setiferus*, *P. shmitti*, *P. monodon*, *P. chinensis*, *P. japonicus*, *P. aztecus*, *P. duorarum* and *Metapenaeus ensis*. (OIE, 2009n; Holthuis, 1998). From the referred to species, black tiger prawn (*P. monodon),* is the main cultured species in Sri Lanka. *Metapenaeus ensis* is also present from the list of susceptible host species though not cultured in Sri Lanka (Holthuis, 1998). Other crustaceans susceptible to TS are crabs, crayfish, spiny lobster and fresh water prawns (European Community Reference Laboratory, 2008a).

iii) Epizootology

TSV transmission is horizontal, through cannibalism or contaminated water (OIE, 2009n). Survivors are known to carry virus within the lymphoid organ for the remainder of their lives (Bondad-Reantaso, 2001). Seabirds are important vectors, carrying viable virus after ingesting infected shrimp carcasses. Frozen commodity products can carry virus, not possing risk of introduction to Sri Lanka as shrimp are most frequently export products. Waste from value-adding processing, emptied into the sea or other water bodies can promote disease spread (OIE, 2009n). Humans are likely mechanical vectors (NACA, 2010j).

Disease is widely distributed to shrimp farms in the regions affected with TSV, with prevalence varying from 0-100%. Outbreaks are most frequent at salinities below 30 ppt. Mortalities range from 40- 90% for all life stages, but TS is best known as a nursery or grow-out phase disease, occurring frequently within 14 to 40 days of postlarvae stocking (OIE, 2009n).

iv) Diagnosis

Tests recommended for targeted surveillance to declare freedom from TSV are RT-PCR confirmed through histology, DNA probes *in-situ*, or sequencing of the RT-PCR product (European Community Reference Laboratory, 2008a).

4.2.3.13. White Spot Disease

i) Causative agent and geographic distribution

White Spot Disease (WSD) is caused by White Spot Disease Virus (WSDV), family *Nimaviridae*, genus *Whispavirus* , a dsDNA virus known to be highly contagious, causing mass mortality to farmed penaeid shrimp worldwide (Bondad-Reantaso, 2001; OIE, 2009o; NACA, 2010k). WSD was first reported in Taiwan in 1992, spreading rapidly to other Asian countries such as the Peoples Republic of China, Japan, Republic of Korea, Indonesia, Hong Kong, Iran, Bangladesh, Taiwan, the Philippines, Myanmar, Malaysia, Vietnam, Thailand, Singapore, India and Sri Lanka. (Figure 13) (OIE, 2009o; OIE, 2010a).

Figure 13: Countries where WSD is known to occur in the Asia-Pacific.

ii) Susceptible host species

A wide range of aquatic crustaceans are susceptible hosts to WSD, with all life stages of marine, fresh water and brackish water decapods susceptible to infection (OIE, 2009o). In Sri Lanka the species known susceptible to WSD are the countries major cultured black tiger prawn (*Penaeus monodon*), along with the also present gulf banana prawn (*P. merguiensis*), Indian banana prawn (*P. indicus*), green tiger prawn (*P. semisulcatus*), sand shrimp (*Metapenaeus ensis*), mud crab (*Scylla serrata*), blue swimming crab (*Portunus pelagicus)* among other *Portunus sp.* species (Aquavet 2005b; OIE, 2009o).

iii) Epizootology

WSV transmission can be both vertical from parent to progeny, and horizontal through the consumption of infected tissue or water-born routes. The occurrence of WSD is increased under stress, such as eye-ablation, at spawning or moulting, changes in water parameters such as salinity, pH and temperature and also during plankton blooms (OIE, 2009o). In Sri Lanka the erratic installation of shrimp farms has contributied to disease spread. Sri Lanka is home to a rich variety of avifauna, contributing to viral agent spread among farms and from pond to pond as birds release infected prawns from one place to the next (NACA, 2010k). Rapid disease spread can also be attributed to the high levels of virus that can be present in a wide range of vectors such as rotifers, marine mollusks, polychaet worms and non-decapod crustaceans (OIE, 2009o). There is no evidence to support that commercial *Artemia* sp. are infected and can transmit WSDV to prawns. WSDV spread has also been associated with imported live and uncooked harvested aquatic animals (Aquavet, 2005b).

WSD is endemic to Sri Lankan cultured black tiger prawn in the Northwestern Province Dutch Canal and Me-oya Estuary in the Puttlam Lagoon brackish water system. The first outbreak of disease was registered during May 1996, having spread rapidly to affect 80% of farmed area in only three months. Farmers, after restarting culture activities during the third quarter of the year, were struck by a second disease outbreak in December, causing many to abandon the shrimp culturing activities (Jayasinghe, 1997). Disease outbreaks were associated to stress resulting from suboptimal water parameters of the Dutch Canal water system, the high stocking densities and the overcrowding farms with close water inlets and outlets contributing to disease spread (Jayasinghe, 1993; Wijegoonawardane, 1996).

Mortality and morbidity will vary among species, being that penaeid shrimp are considered susceptible to highest mortality while infection levels are considered highest in crab species (OIE, 2009o). In Sri Lanka disease outbreaks caused up to 100% cumulative mortalities within a few days of onset of signs of disease (Jayasinghe, 1997). Disease prevalence can range from less than 1% in wild populations to 100% in captive populations (OIE, 2009o). The OIE QAADR for the first quarter of 2010 determined, through a two-step PCR protocol part of a screening program for WSDV infection at NARA Colombo, a prevalence of 35% WSDV infection in wild black tiger prawn broodstock in Sri Lanka. Infection prevalence for postlarvae collected from sampled hatcheries standed at 10% (QAADR, 2010).

iv) Diagnosis

Presumptive diagnosis for WSD is done through PCR testing, a first-step positive result indicating infection while only positive second-step results can indicate either carrier species or

latent infection. Confirmatory diagnosis is established through histology, DNA probes for *in situ* hybridization assays or PCR and sequencing of amplified 2-step nested PCR or direct PCR amplicon sequencing with phylogenetic affiliation (European Community Reference Laboratory, 2008b). The OIE recommends targeted surveillance to determine disease free status through two-step PCR followed by sequencing (OIE, 2009o).

4.2.3.14. Yellowhead Disease

i) Causative agent and geographic distribution

Yellowhead Disease (YHD) is caused by infection with Yellowhead Disease Virus (YHV), genotype 1 of 6 known genotypes of the genus *Okavirus*, family *Roniviridae*, order *Nidovirales*. YHV is an ssRNA corona-like virus, known to infect primarily the lymphoid organs of healthy *P. monodon*, being that infection is rarely associated with disease. (Bondad-Reantaso, 2001; NACA, 2010l; European Community Reference Laboratory, 2008c; OIE, 2009p)

YHD is known to China, India, Bangladesh, Australia, Indonesia, Myanmar, Malaysia, the Philippines, Thailand and Vietnam (Figure 14) (OIE, 2009p; OIE, 2010a).YHD has been reported as suspected but not confirmed to Sri Lanka in the QAADR since the year 2000 and was recently confirmed through a screening program at NARA Colombo PCR laboratory (OIE, 2010a).

Figure 14: Countries where YHD is known to occur in the Asia-Pacific.

ii) Susceptible host species

YHD has been reported most frequently in black tiger prawn (*P. monodon*) and Pacific white shrimp (*P. vannamei***)** (OIE, 2009p). Apart from the black tiger prawn other known susceptible species present in Sri Lanka include gulf banana prawn (*P. merguiensis*), greentail shrimp (*Metapenaeus bennettae*), sand shrimp (*Metapenaeus ensis*), tropical krill (*Acetes sp.)* and kiddy shrimp (*Penaeus styliferus*)(OIE, 2009p; NACA, 2010l; Holthuis, 1998).

iii) Epizootology

YHV transmission is horizontal, through ingestion of infected tissue and water-born routes. *P. monodon* are suggested life-long carriers and many other penaeid and palemonid shrimp can act as carrier species (OIE, 2009p). Coexisting invertebrates may act as mechanical vectors (European Community Reference Laboratory, 2008c).

Life stages of *P. monodon* beyond the day-old postlarvae are susceptible to infection and disease (OIE, 2009p). YHV will replicate in *P. monodon* within 7 to 10 days of exposure and in the event of virus amplification and associated of physiological stress, disease outbreak can cause total crop loss within a matter of days (European Community Reference Laboratory, 2008c; OIE, 2009p).

YHD prevalence can range from less than 1% in healthy shrimp to up to 100% during disease outbreaks (European Community Reference Laboratory, 2008c). Mortalities may reach 100% within the first 3 to 5 days of onset of clinical signs of disease (OIE, 2009p). Secondary bacterial infection is frequent and may support the high mortality rate (European Community Reference Laboratory, 2008c).

iv) Diagnosis

Confirmatory diagnosis may be obtained through PCR or RT-PCR and sequencing of the PCR products (European Community Reference Laboratory, 2008c; OIE, 2009p). The OIE recommends RT-PCR for screening or sensitive multiplex RT-nested PCR (OIE, 2009p). Alternatively, two-step PCR and sequencing is recommended for screening to state freedom from disease (European Community Reference Laboratory, 2008c).

4.2.3.15. White Tail Disease

i) Causative agent and geographic distribution

White Tail Disease (WTD) is caused by the associated infection of two viruses, *Macrobrachium ronsenbergii* Nodavirus (MrNV) and gill associated Extra Small Virus (XSV), MrNV being the primary agent for WTD. The role of XSV is not yet fully understood (OIE, 2009q).

The news of WTD to giant fresh water prawn, formerly considered disease resistant, sent the fresh water prawn aquaculture industry into alarm (NACA, 2006). Countries in the Asia-Pacific where disease has been reported are Thailand, Australia, Taiwan, Vietnam and India (Figure 15) (NACA, 2010m; OIE, 2010a; NACA, 2006). Disease was reported as suspected but not confirmed to Sri Lanka in the QAADR for September to December of 2007 (OIE, 2010a).

Figure 15: Countries where WTD is known to occur in the Asia-Pacific.

ii) Susceptible host species

WTD is known affects giant fresh water prawn *Macrobrachium rosenbergii*, a recently cultured species in Sri Lanka (OIE, 2009q).

iii) Epizootology

WTD can be transmited both vertically, from infected broodstock to larvae and postlarvae, and horizontally. Penaeid shrimp such as *P. monodon* and *Artemia* sp. are believed to be vectors for WTD. Rapid changes in environmental parameters are believed to trigger disease outbreaks. *Macrobrachium ronsenbergii* adults are resistant to disease but can act as lifelong carriers (OIE, 2009q).

iv) Diagnosis

Suspicion of disease after observing gross signs, histopathology or PCR detection should be confirmed through RT-PCR or nRT-PCR, followed by product sequencing. Targeted surveillance to declare freedom from WTD is recommended through nRT-PCR (OIE, 2009q).

4.2.3.16. Infectious Hypodermal and Haematopoietic Necrosis

i) Causative agent and geographic distribution

Infectious Hypodermal and Haematopoietic Necrosis (IHHN) is caused by infection with Infections Hypodermal and Haematopoietic Necrosis Virus (IHHNV), genus *Brevidensovirus*, family *Parvoviridae*, a ssDNA virus for which account three genotypes, genotype 1, 2 and 3, being that type 1 and 2 is infectious to *P. monodon* and other highly valued cultured and wild penaeid shrimp species (OIE, 2009r; Bondad-Reantaso, 2001).

IHHN is wide-spread in Asia and known to occur in Australia, Myanmar, Iran, India, China, South Korea, Malaysia, Indonesia, Japan, China, South Korea, the Philippines, Vietnam and Thailand (Figure 16). (NACA, 2010n; OIE, 2010a). In Sri Lanka IHHN was reported as suspected but not confirmed for 2007, 2008 and 2009 having been confirmed later on in the QAADR for the year of 2010 (OIE, 2010a).

Figure 16: Countries where IHHNV infection is known to occur in the Asia-Pacific.

ii) Susceptible host species

Sri Lankan cultured black tiger prawn (*P. monodon)* is usually subject to subclinical infection with IHHNV (NACA, 2010n). Other shrimp host species present in Sri Lanka susceptible to infection are green tiger prawn (*P. semisulcatus*), gulf banana prawn (*P. merguiensis*) and Indian banana prawn (*P. indicus*) (NACA, 2010n; Holthuis, 1998). Other decapod crustaceans are not known to be susceptible to IHHNV (Bondad-Reantaso, 2001).

iii) Epizootology

IHHNV transmission can occur both vertically, from parent to progeny, or horizontally through water-born route and ingestion or cannibalism (OIE, 2009r; NACA, 2010n). IHHNV is a very stable virus, remaining ineffective after subject to extreme temperatures ranging from -20-80ºC (NACA, 2010n). In Sri Lanka the ongoing NARA screening program for IHHNV, during the first quarter of 2010, detected a 70% prevalence of infection for *P. monodon* postlarvae, 58% prevalence for *P. monodon* brooders from a sample of 45 brooders and 45% prevalence for sub-adults collected from grow out ponds (OIE, 2010a).

iv) Diagnosis

Whenever *P. monodon* show slow growth and deformities, histopathology should initially be done followed by PCR or *in situ* hybridization. Confirmatory diagnosis can be obtained in such a way or through PCR and product sequencing. Screening for the purpose of declaring disease free status is recommended through PCR (OIE, 2009r).

4.2.3.17. Infectious Myonecris

i) Causative agent and geographic distribution

Infectious Myonecrosis (IMN) is caused by Infectious Myonecrosis Virus (IMNV), a dsRNA virus classified in the family *Totiviridae*, recently identified in 2004 in Brazil cultured Pacific white shrimp (OIE, 2009l; NACA, 2010o). IMNV infects penaeid shrimp being the principal host species, the Pacific white shrimp (*P. vannamei)* (OIE, 2009l).

For the Asia-Pacific region, IMN has been reported in Indonesia (Figure 17) (NACA, 2010o). Disease has been reported as suspected to Sri Lanka in the past QAADR for the period from October to December 2007 (OIE, 2010a).

Figure 17: Countries where IMN is known to occur in the Asia-Pacific.

ii) Susceptible host species

The IMNV is known to infect preferably juvenile and subadult Pacific white shrimp, which are not present to Sri Lanka. The Sri Lankan cultured prawn, *P. monodon* was demonstrated experimentally susceptible to disease alerting for the need for adapting a precautionary approach and consider IMN for surveillance efforts (OIE, 2009l).

iii) Epizootology

IMN results in high mortalities and morbidity during acute infection, producing extensive focal lesions corresponding to tissue necrosis in the abdominal and tail skeletal muscle, reddened in some animals. Survivors remain as lifelong carriers of IMNV (OIE, 2009l).

iv) Diagnosis

General signs of disease and mass mortalities can serve as a presumptive diagnosis, while a combination of two techniques from histology, *in situ* hybridization or positive RT-PCR will serve for confirmatory diagnosis. RT-PCR is recommended for targeted surveillance to declare

disease free status (OIE, 2009l).

4.2.3.18. Necrotizing Hepatopancreatitis

i) Causative agent and geographic distribution

Necrotizing Hepatopancreatitis (NHP) is caused by a proteobacterium, intracellular, ricketsial-like and gram negative bacteria, Necrotizing Hepatopancreatitis Bacteria (NHPB) (OIE, 2010b; NACA, 2010p). NHP has been officially reported in Vietnam (Figure 18) (NACA, 2010p).

Figure 18: Countries where NHP is known to occur in the Asia-Pacific.

ii) Susceptible host species

Susceptible host species for NHPB infection are *P. stylirostiris*, *P. setiferus*, *P. aztecus*, *P. californiensis* with highest mortality to *P. vannamei*, none of which known to Sri Lanka (NACA, 2010p; Holthuis, 1998).

iii) Epizootology

High mortalities are known to occur during periods of high air temperature from 29ºC to 30ºC, and salinities from 20 to 40 ppt (NACA, 2010p).

iv) Diagnosis

Clinical signs of disease are not specific to NHP and so diagnostic methods should ally with other methods for presumptive diagnosis, such as histopathology. Infection with NHPB is confirmed through PCR techniques or *in situ* hybridization (OIE, 2010b).

4.2.3.19. *Monodon* Slow-Growth Syndrome

i) Causative agent and geographic distribution

Monodon Slow-Growth Syndrome (MSGS) is caused by the associated infection of two viruses, RNA *Monodon* Slow-Growth Virus (MSGV) and Laem-Singh Virus (LSV), the later virus necessary but not causative of MSGS. *P. vannamei* was brought from the Americas for culturing in Thailand and bred together with *P. monodon* providing the opportunity for virus transfer between species and resulting in this syndrome. Disease is presently known to Thailand, India and Vietnam (Figure 19) (Flegel, 2010).

Figure 19: Countries where MSGS is known to occur in the Asia-Pacific.

ii) Susceptible host species

Black tiger prawn (*P. monodon*) is the susceptible host species for MSGS (Flegel, 2010).

iii) Diagnosis

RT-PCR is recommended for screening purposes (Flegel, 2010).

4.2.3.20. Milky Haemolymph Disease of Spiny Lobster

i) Causative agent and geographic distribution

Milky Haemolymph Disease of Spiny Lobster (*Panulirus* sp.) (MHD-SL) is caused by a ricketsia-like, gram negative bacteria (OIE, 2010c). Disease first appeared in Vietnam in 2006 causing severe production loss (Williams, 2009). Disease is known only to Vietnam (Figure 20) (OIE, 2010c).

Figure 20: Countries where MHD-SL is known to occur in the Asia-Pacific.

ii) Susceptible host species

In Sri Lanka the spiny lobster susceptible host species are wild caught species: the scalloped spiny lobster (*Panurilus homorus*), ornate spiny lobster (*P. ornatus*), pronghorn spiny lobster (*P. penicillatus*), painted spiny lobster (*P. versicolar*) and slipper lobster (*P. polyphagus*) (MOFAR, 2009a).

iii) Epizootology and clinical signs

MHD-SL transmission is horizontal through direct contact with contaminated water or equipment (OIE, 2010c). Outbreaks of disease seem to be associated with deteriorated water quality, diminishing the lobster´s capacity to fight the infection (Williams, 2009).

iv) Diagnosis

Microscopic observation of connective tissue squash and histology can serve as preliminary diagnosis. PCR and sequencing can serve to confirm infection with MHD-SL (OIE, 2010c).

4.3. Contingency Planning for Aquatic Animal Disease Emergencies: present arrangements

Contingency plans set the operational framework to be adopted in the event of a disease emergency (Mohan, 2009). Such framework is developed with the cooperation of all stakeholders capable of identifying and intervening in such an event and providing crucial, rapid identification of the underlying disease, taking on the necessary actions for disease control or eradication. Built on the improved framework plans will need to be drafted for specific high priority diseases individually.

Sri Lanka´s Animal Disease Act No. 59 of 1992 sets the guidelines for the prevention and control of contagious diseases to be carried out in the event of disease occurrences affecting the animal populations, the term “animal” including fish or any aquatic animal, wild or

domesticated, cooked, canned, dried or smoked. The DAPH Director General holds the authority to take the necessary measures to eradicate or limit disease spread for the diseases stated in the First Schedule, which extend to the diseases stated in the Aquaculture Management Disease Control Regulations 2000 of the Fisheries and Aquatic Resources Act No. 2 of 1996 (The Gazette, 2000; The Gazette, 1992). The diseases for which corrective measures should apply are also referred to in the Fisheries and Aquatic Resources Act No. 2 of 1996 and are listed in Annexure XI, with reference to which diseases are OIE diseases of concern to the region. Re-evaluating this schedule is suggested to include the diseases proposed for the surveillance program.

The Aquaculture Management Disease Control Regulations, 2000 regulates aquaculture enterprise activities such as routine equipment and facilities disinfection and other sanitary measures, as well as an outline for the procedures taken in the event of suspicion of a disease occurrence. Whenever a disease emergency is suspected, it is the enterprise licensee's duty to inform the DAPH Director General who takes the necessary steps to immediately carry out an investigation and appoint a Veterinary Quarantine Officer to obtain the necessary diseased fish samples. This principle applies well to the aquaculturists who are motivated in informing the aquatic animal health authorities but does not apply to many others who will preferably conceal or overlook mortalities and suspicions of disease occurrence in fear of the mitigative measures or market depreciation (The Gazette, 2000). Regular surveillance and monitoring will help minimize this situation as will campaigns to alert aquaculturists to the benefits of early response to a disease emergency.

The Aquaculture Management Disease Control Regulations, 2000 continues to state that the Veterinary Quarantine Officer communicates the investigations findings back to the DAPH Director General, who temporarily restricts animal movement from within the enterprise. After receiving the laboratories report on the pathogen findings, DAPH Director General will receive advice from NARA as whether to extend imposed suspensions and which corrective measures to apply. The corrective measures are then applied under the supervision of the appointed Veterinary Quarantine Officer. In the event of an outbreak the DAPH Director General reports to a Magistrate for approval on sealing and limiting access to the infected area and for taking any other actions to limit the disease spread. Whenever faced with the decision to seize or destroy animals the DAPH Director General will report to the Magistrate who may approve the preventive measure. The DAPH Director General appoints a Veterinary Quarantine Officer to manage locally the outbreak for its control or eradication. Infected animals are to be destroyed and disposed under the authorized Veterinary Quarantine Officer supervision. Infected materials should be destroyed, and it is the owner of the aquaculture establishment to provide for the

disinfection of all possibly contaminated equipment, premises and other materials according to Veterinary Quarantine Officers instructions. Furthermore, a notice is issued to all holders of animals susceptible to infection within the suspected area. The Director General can revoke proclamation of the infected area when satisfied that the measures assure no further spread of disease (The Gazette, 2000).

4.4. Awareness

Ongoing training for scientists, field officers, policy makers and all stakeholders in general along with implementing of awareness campaigns on the individual roles and responsibilities for participating in surveillance, monitoring and reporting activities and contingency plans, are some of the objectives with regards to this component. Supporting documentation for improved identification of a disease emergency, such as field guides for major aquatic animal diseases, should inclusively be drafted, edited and made available (Aquaplan, 1999).

4.5. Research and Development

Aquatic Animal Health Management will bring on the need for developing supporting research programs to assist in many situations such as when researchers and laboratory personnel are faced with the need to enhance pathogen identification or to monitor environmental parameters to help determine the cause of increased mortalities or morbidity. A variety of other supporting programs should also be implemented for continuously enforced biosecurity and improved management practices (Aquaplan, 1999).

4.6. Legislation and Policies

The official authorities must assist in developing and implementing the necessary legislation and policies and the continuous application of the Aquatic Animal Health Management program and its key components (Aquaplan, 1999). This must be done through the assistance of the envolved stakeholders and aquatic animal health expert consultation.

4.7. Resources and Funding

Identifying and attempting to declare disease free status and disease free zones will require some degree of investment in equipment, manpower and continuous training opportunities. The different institutions and stakeholders should be motivated to initiate the program making best use of the available resources. International agencies for the promotion of aquatic animal health such as the OIE and NACA along with the countries aquaculture private sector industry should assist and support Sri Lanka public institutes in their efforts to implement and enhance the Health Management Program.

Chapter 2

I. Introduction

1. Implementing Aquatic Animal Health Management in Sri Lanka

The increased global demand for food fish and ornamental fish has lead to the mass intensification of the aquaculture industry. Intensive aquaculture of aquatic animals under suboptimal conditions has increased the frequency of disease incidence affecting cultured aquatic animals worldwide. The increased market demand for fish and their products has been responsible for the increased translocation of aquatic animals and as such the increase in geographic distribution of aquatic animal diseases on a global scale.

The economic impact of disease to the aquaculture sector is a major constraint for its further development. Disease outbreaks are responsible for decreased production, decreased market appreciation and increased investment in disease research, disease control and health management. As a result many countries are developing or implementing aquatic animal health management programs for the protection of the aquaculture sector and the aquatic animal resources, as applied prevention is always better than treatment. Aquatic animal health management programs such as Thailand's “Thailand National Strategy for Aquatic Animal Diseases” and Australia's “National Strategic Plan for Aquatic Animal Health- Aquaplan” are examples of high-investment programs especially designed and implemented to ensure the sustainable development of their national aquaculture industry. Many other countries around the globe, such as Norway, the United States and Canada, have destined a large budget to support aquatic animal health management programs (Bondad-Reantaso, 2010). This cannot be the case for Sri Lanka where initial implementing of the two key components of a Health Management Program, namely “Surveillance, Monitoring and Reporting” and enhanced “Contingency Planning”, should be phased and focused on maximizing the existing resources in a cost effective manner, through the joint efforts of all concerned stakeholders and government and private sector support.

1.1 Surveillance, Monitoring and Reporting

Implementing a "Surveillance, Monitoring and Reporting" program is of great importance to Sri Lanka, currently without and organized approach for surveillance and monitoring activities or system for disease reporting capable of providing updated knowledge on the aquatic animal pathogen findings in the country.

In order to implement the key component "Surveillance, Monitoring and Reporting" a list of diseases of concern to the country must be drafted, based on OIE listed diseases prevalent to the region and diseases affecting the countries major cultured species. After defining a list of diseases of concern to the country, the diseases capable of infecting important cultured species or known to infect these species were chosen for initial surveillance efforts. The proposed surveillance program continues by chosing the surveillance strategy to apply to each disease: declaring National freedom from infection or defining disease free zones. The surveillance strategy applied for diseases unaccounted for in Sri Lanka is surveillance to declare national freedom from disease. This strategy will allow for establishing disease free status for enhanced international market appreciation of the countries aquatic animals and their products and applied risk analysis to minimize the risk of introducing disease through imports to the country. This surveillance strategy will also identify previously unaccounted infections while attempting to establish disease free status and allow for the fulfilling of international OIE reporting obligations. For the diseases known to occur in the country defining disease free zones should be a priority to conserve such status and limit pathogen spread.

Laboratories diagnosing aquatic animal pathogens are assigned the field work, sampling and laboratory diagnostic surveillance duties by province, in accordance to geographic proximity, for national coverage of the susceptible aquatic animal host populations.

1.2. Contingency Planning for Aquatic Animal Disease Emergencies

The present arrangements for emergency intervention for aquatic animal diseases occurring in Sri Lanka could be benefited through enhanced framework. In the event of such disease emergencies the various agencies and organizations involved in aquatic animal health will frequently gather at the emergency disease site and carry on separate investigations, duplicating time, efforts and public resources. A joint approach to aquatic animal disease emergencies is also fundamental as expert opinion and resources are scarce and scattered among the different institutions working in aquatic animal health.

Enhancing contingency planning for aquatic animal disease occurrences should be based on the present arrangements for responding to a disease emergency, for reasons of acquaintance and agreement with the present legislation. To achieve such results, it is necessary to maximize the existing resources and organize the laboratories appointed to investigate such occurrences for a systematic approach. The provinces assigned to the different laboratories for the surveillance program will also be the areas assigned for disease occurrence investigation, linking the two components for efficient intervention and an overall harmonized Aquatic Animal Health Management Program.

Actions must be taken quickly as time dictates the success of the disease control or eradication efforts and so specific contingency plans for the diseases of concern to the country need to be drafted individually, based on the general contingency plan framework here proposed.

Government should prepare for aquatic animal emergency situations through a strong national approach, drafting and testing contingency plans and their supporting legislation. Such plans should take into account the needs of the aquaculturists so as to avoid situations where legislation is not complied with (Arthur, 2005).

II. Objectives

The proposed plans intend to provide the necessary framework and guidelines to initiate a much needed National Aquatic Animal Health Management Program for Sri Lanka. As a National plan consists of many important components the "Surveillance, Monitoring and Reporting" and "Contingency Planning for Aquatic Animal Disease Emergencies" will be the components delt in this thesis, as I felt it is the most timely needed requirement to be organized for Sri Lanka and to work on during my short period of study, from January to mid July, 2010.

The component "Surveillance, Monitoring and Reporting" will set the needed framework for determining the present status for aquatic animal health, based on OIE standards, to identify the presence of infections for OIE reporting purposes and also to benefit the fisheries and aquaculture sector with the advantages of recognizing the presence or freedom from certain pathogens. This first component will include the establishing of a list of diseases of national concern that will include OIE listed diseases of concern to the region, along with the surveillance strategy to be applied for each disease. The program will also include *Tetrahymena* spp. Infection affecting the highly- valuable ornamental guppy, not listed by the OIE but of national concern.

Enhancing the present arrangements in intervening to aquatic animal disease emergency situations, by maximizing the existing resources and establishing the needed cooperation among institutions and laboratories, is essential at the moment. It is important to propose the necessary framework for this purpose, built upon the existing legislation and with stakeholder feedback for improvement

As there are many aquatic animal species to consider, these plans will focus on the two major groups of cultured species in Sri Lanka, crustaceans and finfish, though the guidelines here suggested can easily be applied to the remaining groups of aquatic animals, namely mollusks and amphibians, when required.

III. Methodology

Developing a strategy to implement the proposed key components in the National Aquatic Animal Health Management Program requires background knowledge of the countries past and present disease profile and the consideration of the existing resources, evaluating diagnostic capabilities and there by identifying gaps and needs. The information needed to develop the strategy should focus on the existing infrastructure, facilities and the diagnostic skills of the personnel, as well as the examining of the present reporting arrangements and the approach to aquatic animal health management.

Knowledge of past and present diseases affecting the countries aquatic animals was determined by consulting the local professionals relevant to aquatic animal health and through a comprehensive literature review. In order to identify the gaps and needs for monitoring and surveillance, Sri Lankan public and private sector laboratories and organizations engaged in relevant work were visited and surveyed using a prepared questionnaire (Annexure XII) and also through personal communication. The information provided was also used to construct methodologies to carry out the proposed key components in the intended Aquatic Animal Health Management Program for Sri Lanka.

The location for the agencies and laboratories consulted for the present study is illustrated in Annexure XIII and they are:

- The National Aquatic Resources Research and Development Agency (NARA) Head Office, Colombo;
- The Veterinary Investigation Center (NAQDA) Head Office, Colombo;
- The National Authority for Aquaculture Development (NAQDA), Shrimp Farm Monitoring and Extension Unit, Chillaw;
- The National Authority for Aquaculture Development (NAQDA) Ornamental Fish Breeding and Training Center, Rambodagalla;
- The Center for Aquatic Animal Disease Diagnostics and Research (CAADR), University of Peradeniya, Peradeniya;
- The Veterinary Research Institute (VRI), Peradeniya;
- The Veterinary Investigation Center (VIC), Welisara;
- The Veterinary Quarantine Station, Colombo.

A stakeholders meeting was held on the 19th May, 2010 at NARA Colombo auditorium for introduction to the project, to receive suggestions and overall project appreciation (Annexure

XIV). The feedback received from those attending this meeting was used to enhance the drafted plan. A second meeting for final plan appreciation was held on the 16th July, 2010 at NARA Colombo with key staff from the previously refered to diagnostic laboratories and institutions for further plan improvement.

IV. Results and Discussion

1. Implementing a Surveillance Program

When implementing a surveillance program many specific requirements must be satisfied. In Sri Lanka initiating the surveillance program will mean having to satisfy the following needs:

- Establishing a list of diseases of concern to the country and the associated strategy applied to applied to each one, considering the initial priorities of the surveillance program;
- Determining what are the existing resources laboratories have to offer in terms of personnel, equipment and facilities, identifying gaps and needs;
- Assigning laboratories for the surveillance program and their areas of intervention;
- Establishing case definitions through careful analysis;
- Establishing a reporting system.

1.1 A National List of Diseases for the Surveillance Program

Initially, drafting a list of diseases of concern to the country is of great importance to implement the surveillance program. The strategy applied to each disease was based on OIE criteria for listing diseases, concentrating on present reporting status and the susceptibility of cultured species, as summarized in Table 2.

	Disease	Known present to Sri Lanka	Previously Reported in the QAADR as suspected	Presence of Susceptible Cultured Species in Sri Lanka
FINFISH	EHN			
	IHN			
	SVC		√	√
	VHS			√
	EUS	√		√
	RSIVD		√	√
	KHVD		√	√
	GIVD			
	VER			√
	(Continued)			

	(Continuation)			
	Tetrahymena spp. infection	√		√
	ESC			√
CRUSTACEANS	TS			√
	WSD	√		√
	YHD	√		√
	IHHN	√		√
	IMN		√	√
	WTD		√	√
	NHP			
	MSGS			√
	MHD-SL			

Table 2: Diseases determined for the surveillance program, susceptible cultured species and reported status for prioritized surveillance efforts.

In accordance to the previous analysis, indicated in Table 3 are the diseases determined of concern to Sri Lanka for initial surveillance efforts and the strategy applied to each one. Diseases with susceptible host species known to Sri Lanka, including species known experimentally susceptible to infection for a precautionary approach, were considered for initial surveillance. From these diseases, those known to Sri Lanka were considered for monitoring and zoning efforts while diseases not yet identified in the country were considered for surveillance to declare national freedom from disease. The diseases not chosen for initial surveillance efforts should be considered for the program in the near future as further resources are made available.

		OIE Diseases Prevalent in the Region		For Evidence of Freedom	For Monitoring and Zoning	For Initial Action
		OIE Listed	Non OIE Listed			
FINFISH	1. EHN	✓		✓		
	2. IHN	✓		✓		
	3. SVC	✓		✓		✓
	(Continued)					

	(Continuation)					
	4. VHS	✓		✓		✓
	5. EUS	✓			✓	✓
	6. RSIVD	✓		✓		✓
	7. KHV	✓		✓		✓
	8. GIVD		✓	✓		
	9. VER		✓	✓		✓
	10. ESC		✓	✓		✓
	11. *Tetrahymena spp*. infection in guppy				✓	✓
CRUSTACEANS	1. TS	✓		✓		✓
	2. WSD	✓			✓	✓
	3. YHD	✓			✓	✓
	4. IHHNV	✓			✓	✓
	5. IMN	✓		✓		✓
	6. WTD	✓		✓		✓
	7. NHP		✓	✓		
	8. MSGS		✓	✓		✓
	9. MHD-SL		✓	✓		

Tabel 3: List of diseases of concern to the country and strategy.

1.2 Adequate Resources for Implementing the Surveillance Program: Personnel, Equipment and Facilities

Trained, dedicated personnel make for accurate identification of disease occurrences and sample analysis and as such assure a quality surveillance program is operated in the country.

Laboratories should be prepared in terms of facilities and equipment to follow the OIE recommended protocols for diagnosing samples submitted for the surveillance program. In international OIE disease reporting there are three levels of diagnostics. Each level includes certain diagnostic techniques requiring increased expertise. These levels are used to describe the tests performed when notifying international authorities of the countries aquatic animal health status for notifiable diseases and other diseases of concern (Subasinghe, 2004). Table 4 illustrates the requirements and responsibilities for these three levels of diagnostics and their availability at the laboratories carrying out the surveillance activities.

Level I to III Activities	Skills and Equipment	Responsibilities	Requirements	Diagnostic availability (total or parcial) at:
Level I – Activities Observation of animal and the environment; Clinical examination; Gross pathology	Knowledge of normal (feeding, behavior, growth of stock, etc); Frequent/regular observation of stock; Regular, consistent record-keeping and maintenance of records - including fundamental environmental information; Knowledge and contacts for health diagnostic assistance; Ability to submit and/or preserve representative specimens.	Farm Workers/Managers ; Fisheries Extension Officers; Field Veterinarians; Local Fisheries Biologists.	Field keys; Farm record keeping formats;Equipment list; Model clinical data sheets; Pond-side check list; Protocols for preservation and transport of samples.	NARA – Colombo NAQDA-Chilaw and Rambodagala CAADR VRI VIC Chilaw private laboratories Veterinary Quarantine Office DAPH

(Continued)

(Continuation)				
Level II – Activities Parasitology Bacteriology Mycology Histopathology	Laboratories with basic equipment and personnel trained/experienced in aquatic animal pathology; keep and maintain accurate diagnostic records; Preserve and store specimens; knowledge of/contact with different areas of specialization within Level II Knowledge of who to contact for Level III diagnostic assistance.	Fish biologists; Aquatic Animal Veterinarians; Parasitologists; Mycologists; Bacteriologist; Histopathologists; Technicians.	Model laboratory recordkeeping system; Protocols for preservation/transport of samples to Level III; Model laboratory requirements equipment and consumable lists; Contact information for accessing Level II and Level III specialist expertise; Asia Diagnostic Guide to Aquatic Animal Diseases; OIE Manual of Diagnostic Tests for Aquatic Animals; Regional General Diagnostic Manuals.	NARA-Colombo NAQDA-Chilaw and Rambodagala CAADR VRI VIC (limited) Chilaw private laboratories
Level III – Activities Virology Electron Microscopy Molecular Biology Immunology	Well equipped laboratory with highly specialized and trained personnel; Keep and maintain accurate diagnostic records; Preserve and store specimens; Maintenance of contact with people responsible for sample submission.	Virologists; Ultrastructural histopathologists; Molecular biologists; Technicians.	Model Laboratory requirements, equipment, consumable lists; Model job descriptions skills for requirements; Contact information for reference laboratories; Protocols for preservation of samples for consultation and validation;OIE Manual of Diagnostic Tests for Aquatic Animals; General molecular and microbiology diagnostic references; Asia Diagnostic Guide to Aquatic Animal Diseases.	NARA-Colombo CAADR VRI

Table 4: Required skills and responsibilities for the three levels of disease diagnostics and the laboratories capable of providing the diagnostics needed for the surveillance program.

(Adapted from Subasinghe, 2004).

In Sri Lanka most laboratories are capable of performing the OIE recommended protocols though specific methodologies, such as immunodetection and electron microscopy, are not yet

being used for diagnosing aquatic animal pathogens. At the present, most laboratories chosen for the surveillance program activities are capable of performing PCR testing, making use of commercial testing kits, bacteriology, parasitology and histopathology

1.2.1. Laboratory Activities and the Identifying of Gaps and Needs

The consulted and surveyed laboratories are under the private sector or public sector agencies and play an active role in diagnosing for infectious pathogens affecting finfish and crustaceans. For the purpose of participating in the surveillance program, their present activities, facilities, equipment and skills were considered.

i) National Aquatic Resources Research and Development Agency (NARA)

The National Aquatic Resources Research and Development Agency (NARA), Colombo, stands as the research arm of the Ministry of Fisheries and Aquatic Resources (MOFAR) in Sri Lanka. NARA is mandated to undertake the research activities in various disciplines of the fisheries sector, and aquatic animal health is one of them. The laboratories at NARA carry out aquatic animal health related research while providing services to both government and private sector organizations on request.

The research work undertaken at NARA develops the countries knowledge of the aquatic animal health status, carried out at its laboratories for PCR technology, bacteriology, parasitology and histology. NARA provides expertise in the area of PCR testing, dedicated to developing projects that survey and monitor Sri Lanka´s major shrimp viral diseases and fish diseases through an effective approach to disease diagnosis, under the supervision of Dr. P. K. M. Wijegoonawardane, who is also the OIE focal point for reporting the status of aquatic animal diseases in the country. At present, screening programs are under way for IHHNV, YHV and WSDV at NARA´s PCR laboratory. Shrimp and fish bacterial pathogens are also studied at the NARA bacteriology laboratory and parasite identification and histology done at NARAs histology laboratory. The NARA extension office at Rekawa, Southern Province, also accounts for personnel specialized in fish health, particularly in bacterial diseases. In addition, NARA works in collaboration with national and international agencies and student interns, supervising undergraduate research programs and conducting farmer training programs in aquatic animal health and diagnostics.

Though such responsibilities lie in the hands of NARA the current infrastructure is in need of expansion and improvement and resources are scarce for the performing of field diagnostics efficiently, to the expectations of the country. The molecular diagnostic laboratory at NARA has

limited work space and inadequate location, which may lead to possible cross contamination between samples and as such expanding the facilities would prove valuable. In terms of equipment, major diagnostic limitations are found at the histology laboratory where a much needed tissue processor, fume hood and tissue embedding unit are not present and as such very laborious work is carried out manually, which may not produce high quality performance. The tissues fixed could also be analyzed using immune-histochemistry and other immunological methods which are not performed at NARA due to lack of facilities.

ii) National Aquaculture Development Authority (NAQDA)

NAQDA is the main state sponsored organization for the conservation, development and sustainable management of aquaculture and inland aquatic resources in Sri Lanka. NAQDA provides services and formulates health management practices and policies for the aquaculture industry.

The NAQDA laboratory at Chillaw provides basic bacteriology and PCR testing for cultured shrimp through the use of commercial testing kits for WSDV, YHV and TSV. Screening postlarvae is also performed for *Penaeus monodon*-type baculovirus (MBV) using the wet mount method. This laboratory, recently built and equipped, offers good quality equipment, the major limitation being the absence of specific equipment and working area for diagnostics through histology and the absence of internet facilities.

The Ornamental Fish Breeding and Training Center at Rambodagalla is a second laboratory working under NAQDA for public and private sector ornamental fish breeders and exporters and diagnosing in bacterial pathogens and parasites. This laboratory has adequate facilities and equipment for carrying out the present work in ornamental finfish diagnostics and could participate in the surveillance program activities.

iii) Veterinary Investigation Center (VIC)

The Veterinary Investigation Center at Welisara is a public sector laboratory that provides basic parasitology and bacteriology for finfish samples, the majority supplied by the Quarantine Office and at the request of ornamental finfish breeders.

Currently the facilities and equipment at the VIC are inadequate for diagnosing aquatic animal diseases. Improving work conditions at the VIC could enable its participation in the surveillance program and also improve the countries quarantine capabilities as is their mandate.

iv) Veterinary Research Institute (VRI)

The Veterinary Research Institute (VRI) at Peredeniya is a public sector laboratory whose work comes from public sector agencies, such as the Veterinary Quarantine Office and the CAADDR laboratory.

The VRI has excellent equipment for the purpose of molecular diagnostics, allowing for large scale sample testing. The VRI offers standard PCR, two real time PCR machines and automatic sequencing for rapid and efficient pathogen identification, making this an ideal laboratory for confirmatory diagnostics. It can inclusively develop serological testing for aquatic animal diseases as it presently does for terrestrial animals. The major limitation at the VRI laboratory is its facilities, in need of upgrading and spatial organization, which is foreseen for the near future.

v) Centre for Aquatic Animal Disease Diagnosis and Research (CAADDR)

The University of Peredeniya Centre for Aquatic Animal Disease Diagnosis and Research (CAADDR) provides diagnostics for freshwater aquatic animal disease diagnostics. The CAADDR laboratory offers modern facilities and equipment. Many undergraduate students participate in the centre´s daily activities and the skills of its staff are adequate for consultation and participating in the surveillance program.

The CAADDR building accounts for spacious and well equipped laboratories for histology, molecular biology, bacteriology and parasitology all of which are capable of incrementing their work load. The advanced molecular laboratory offers spacious yet unorganized facilities that could benefit from compartmentalization for improved PCR testing and decreased cross-contaminations. Protocols for PCR testing at CAADDR conform to OIE protocols, though reference was made to how at the present moment reagents for this purpose were not easily accessible.

vi) Private Small Scale Laboratories at Chillaw

There are various small scale laboratories diagnosing for the shrimp diseases affecting the hatcheries and farms in Sri Lanka´s North-Western Province and also working in cooperation with public research laboratories such as NARA, NAQDA and CAADDR. These laboratories offer basic microscopy and PCR testing. Among the consulted laboratories, reference was made for the need for better equipment and working materials and that the diagnostic capabilities of their staff would benefit from staff training in aquatic animal disease diagnostics. These laboratories can eventually participate in the surveillance program as many can provide for diagnostics using OIE approved protocols.

In general, the surveyed laboratories indicated sufficient staff to carry out the day to day activities and their satisfaction in regards to the present performing of diagnostic procedures. Knowledge in aquatic animal diagnostics seems to be sufficient to initiate the surveillance program and carry out the activities to a certain extent. The major limitation seems to reside in the fact that skilled personnel, though existent in the country, are scattered among institutions and laboratories. There is some variability in the quality of the equipment and laboratory facilities, though the existing resources can be put to use and allow for the surveillance program´s initial implementation.

1.2.2. The Laboratories and their Participation in the Surveillance Program and Contingency Plan

The laboratories participating in the surveillance program are chosen in accordance with the available equipment, facilities and overall diagnostic capability. The following selection of laboratories and institutions have been chosen to participate in the surveillance program and contingency plan field activities and diagnostics, according to their resources evaluation, as illustrated in the Table 5.

Laboratories	Staff skills & knowledge of aquatic animal health	Equipment	Facilities	Considered for Surveillance activities	Role in an Aquatic Animal Disease Emergency			
					CRUSTACEANS		FINFISH	
					Counseling	Diagnosing	Counseling	Diagnosing
NARA Colombo								
PCR	A	B	B	✓	✓	✓	✓	✓
Bacteriology	B	B	B			✓		✓
Histopathology and Parasitology	A	B	B			✓		✓
NAQDA								
Chilaw- PCR	B	A	A	✓	✓	✓	✓	
Chilaw- Bacteriology	B	A	A			✓		
Rambodagala- Bacteriology and Parasitlogy	B	B	B					✓
VIC Welisara								
Bacteriology and Parasitology	B	C	C				✓	
VRI Peredeniya								
Parasitology	B	B	B	✓	✓		✓	
PCR	A	A	B			✓		✓
Histopathology	B	B	B					
Bacteriology	B	B	B					
CAADDR Peredeniya								
Parasitology	A	A	A	✓	✓	✓	✓	✓
Histopathology	A	A	A			✓		✓
PCR	A	B	B			✓		✓
Bacteriology	A	A	A			✓		✓
Chilaw's Private Laboratories								
PCR	B	B	B	✓	✓	✓		

Table 5: General appreciation of the laboratories for surveillance and contingency plan for disease emergency intervention, where:

- (A) high ranking for the purpose of intervention in aquatic animal disease diagnostics;
- (B) standard ranking for the purpose of intervention in aquatic animal disease diagnostics;
- (C) inadequate for the purpose of intervention in aquatic animal disease diagnostics.

1.2.3. Assigning Laboratories Specific Provinces for the Surveillance Program

The main criteria for assigning each one of the chosen laboratories specific provinces for the proposed surveillance program relied on the proximity to the sampling sites. In order to obtain specific demarcations in assigning responsibilities to each laboratory, these were assigned areas by provinces. This way unnecessary costs with transportation, sample conservation and duplicated efforts are prevented as each province has its own laboratory assigned for the surveillance program. These provinces are also assigned for the contingency plan intervention and diagnostics. In the event of a disease occurrence the time spent travelling is shortened when the field worker is close to the site, allowing for early intervention and the obtaining of moribund specimens, valued for diagnostics. As there are no laboratories located in each province, laboratories were assigned for more than one province.

1.2.3.1. Surveillance of the Crustacean Populations

Surveillance of the brackish water crustacean populations should be appointed to NARA and NAQDA, who have so far carried out the countries major diagnostics for these populations. Chillaw private laboratories can provide diagnostic support when equally following recommended OIE recommended protocols. NARA and NAQDA should perform joint field work and diagnostics for surveying the shrimp hatcheries and farms located in the Dutch Canal interconnected water bodies in Sri Lanka´s North-Western Province and their associated broodstock collection sites. Given NAQDA efforts in establishing shrimp culturing systems for the Eastern Province at the Batticalloa Lagoon, sampling and diagnostics for surveillance is therefore assigned to NAQDA for this province to include shrimp hatcheries, farms and associated broodstock collection sites. The remaining Northern Province, North-Central Province, Central Province, Uva Province, Western, Southern and Sabaragamuwa Province should also be subject to sampling for wild host species, with the field-work performed by a regional DAPH Veterinary Surgeon with the guidance and the diagnostic support of NARA and NAQDA (Figure 21).

Surveillance related diagnostics for diseases affecting freshwater crustaceans should be assigned to the CAADR laboratory with field activities supported by a regional DAPH Veterinary Surgeon at a National level.

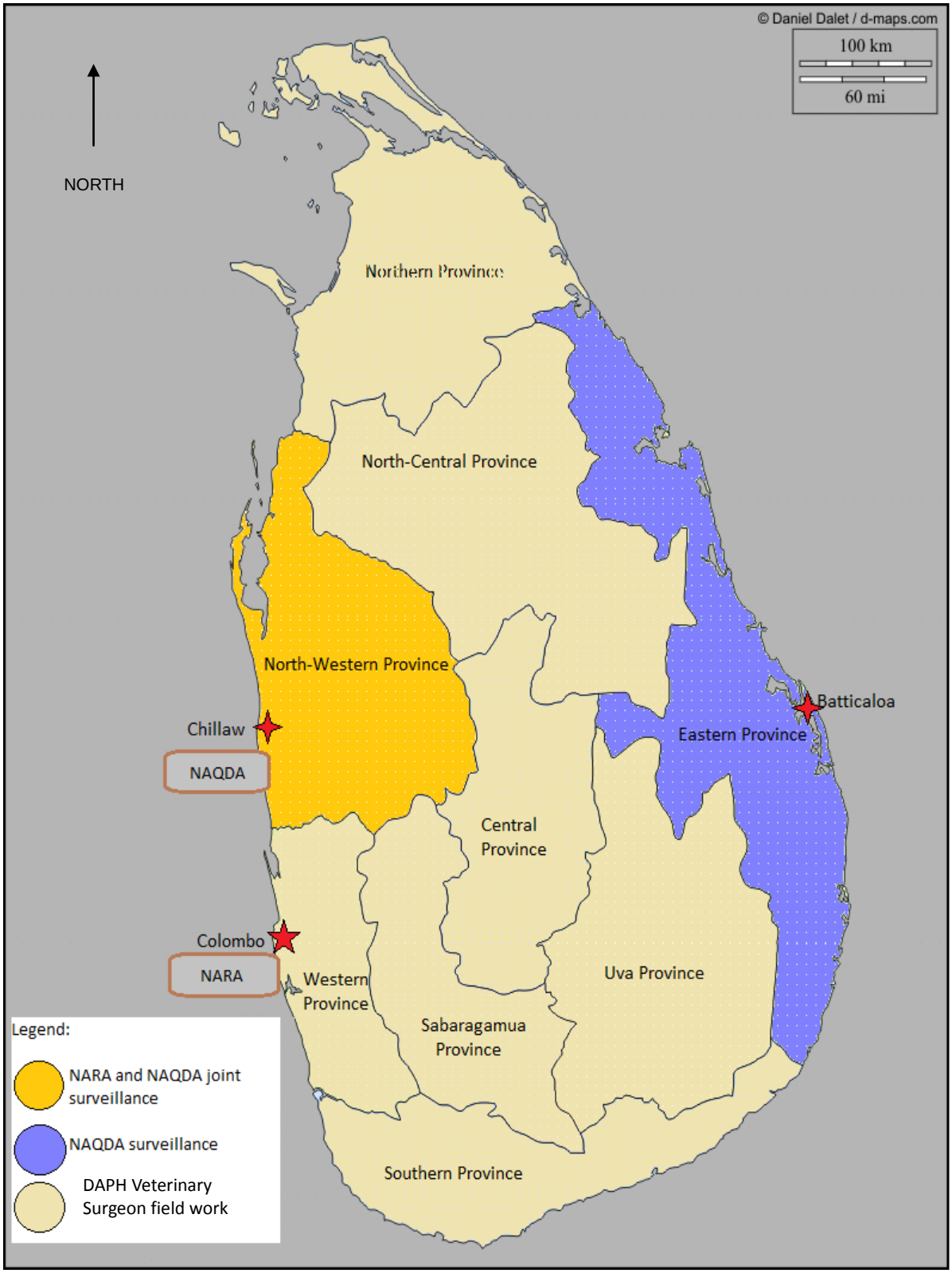

Figure 21: Provinces suggested for brackish water crustaceans surveillance activities.

1.2.3.2. Surveillance of the Finfish Populations

Surveillance for finfish water bodies and aquaculture sites is assigned to NARA, NAQDA, CAADDR, and the VRI. Surveillance field activities and diagnostics are appointed for NARA for the finfish water bodies and culturing facilities in the North-Western, Western, Southern and Sabaragamuwa Provinces. The NARA extension office in Rekawa in Sri Lanka´s Southern Province, should participate in field activities for the Southern and Sabaragamuwa Provinces given its location, while for the Western and North-Western Provinces cooperation can be held with NAQDA Rambodagalla who is able to assist mainly in field activities, supporting bacteriology and parasitology if needed. The suggested provinces for CAADDR laboratory participation in field and diagnostic activities are Central, North-Central, Eastern, Northern and Uva Province, with cooperation from the VRI for PCR diagnostics. Support for field activities for these provinces can be provided by a regional DAPH Veterinary Surgeon. Figure 22 illustrates geographically the areas assigned to each laboratory considered for finfish population surveillance.

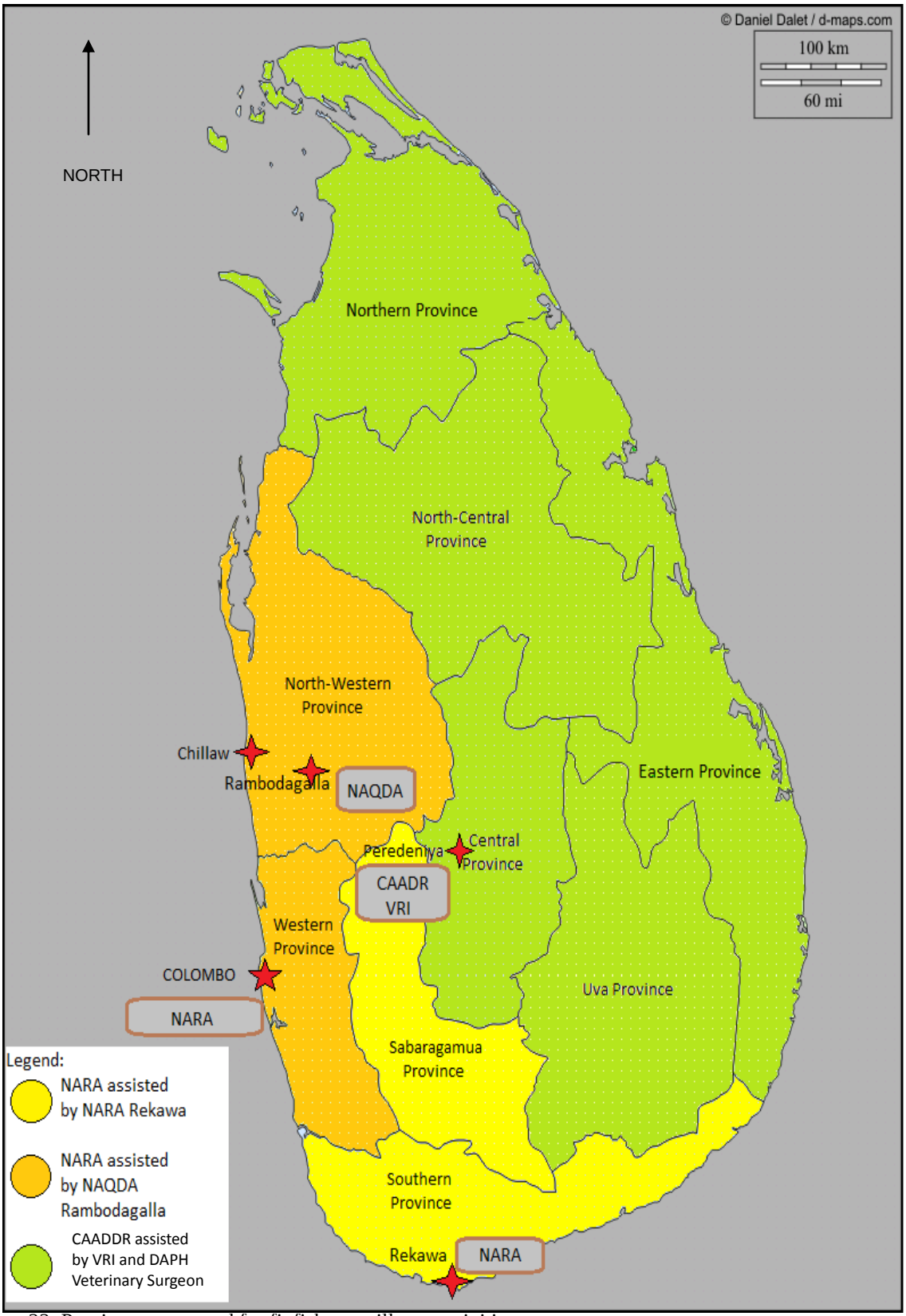

Figure 22: Provinces suggested for finfish surveillance activities.

1.3. Case Definitions

A surveillance program requires that case definitions be chosen for consistent identification of disease occurrences, according to the surveillance programs objectives for each of the listed diseases (Subasinghe, 2004).

Case definitions can span from most pathogen specific and least sensitive, to less pathogen specific but most sensitive, broad-scope definitions. A case definition with low sensitivity and most pathogen specific may lead to higher false positive detection while a most sensitive and less specific case definition would result in higher false negative identifications. For exotic suspected diseases a broad case definition should be considered, that is a more sensitive case definition and protocol, to identify all cases of disease (Subasinghe, 2004).

It is hard to provide a case definition that includes all true cases of disease excluding all unrelated cases, especially for aquatic animal diseases where signs of disease are rarely pathognomonic. It is important to note that diseased fish may not present all of the signs of disease in a case definition and in many cases no signs of disease will be present, adding to the challenge of disease detection. Furthermore, in many cases disease etiology is incompletely understood being that there are often no clear case definitions for many diseases (Subasinghe, 2004).

At the present moment many countries are implementing health management programs and developing National case definitions for the diseases of concern to their countries. Sri Lanka should develop national standardized case definitions for the listed diseases of concern, drafted by NARA Research Officers and DAPH Veterinary Surgeons with the input of fisheries and aquaculture sector stakeholders.

1.4. Reporting System- Implementing a Database

The data collected through surveillance and monitoring will help characterize the countries disease profile for the listed diseases of concern to Sri Lanka. It is imperative for this purpose that the surveillance programs diagnostic findings, along with other routine diagnostic findings, be reported in an Aquatic Animal Health Management Database, for accurate characterization of the aquatic animal health profile. The proposed database would be updated as information is made available and be accessible to all stakeholders contributing to the database. As the database would allow for written record keeping it would serve as the base for the surveillance programs quality assurance (Cameron, 2004).)

The database can also support preparedness in the event of an aquatic animal disease occurrence by providing on-line information on:

- Contingency plan framework;
- Stakeholders roles and responsibilities;
- Recommended diagnostic protocols;
- The diagnostic findings, nature of the suspected disease and modes of transmission;
- The range of susceptible host species and their geographic distribution;
- The boundaries for the areas investigated and determined contaminated.

Such information would contribute to disease control or eradication while keeping aquatic animal health specialists and institutions updated on the investigations findings and progress.

2. Contingency Planning for Aquatic Animal Disease Emergencies in Sri Lanka

2.1 Proposed Contingency Plan for Aquatic Animal Disease Emergencies in Sri Lanka

The following recommendations are based on Sri Lanka´s current emergency disease intervention plan in the Animal Disease Act Nº 59 of 1992 and Aquaculture Management Disease Control Regulations 2000 in the Fisheries and Aquatic Resources Act, No. 2 of 1996. The framework was developed after considering the present need for joint institutional cooperation for improved intervention in an aquatic animal disease emergency. The framework here proposed outlines the general procedure for responding to crustacean and finfish disease occurrences. In some instances the proposed procedures are particularized for each group of species.

Three phases can be activated in an aquatic animal disease emergency (Figure 23). Different procedures will apply to different phases, helping to determine what efforts have been adopted and the campaigns overall progress.

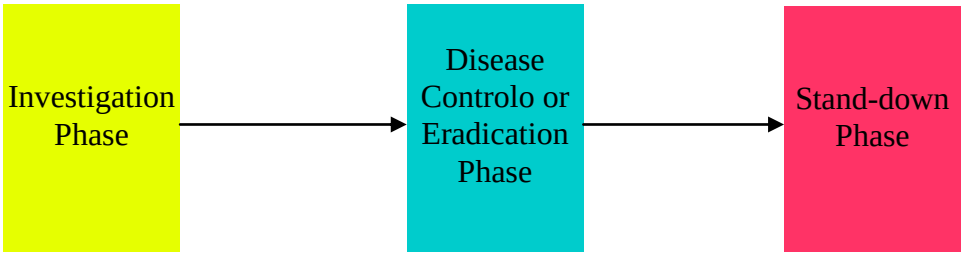

Figure 23: Phases activated in an aquatic animal disease emergency.

2.1.1. Investigation Phase Outline

The investigation phase includes the activities carried out from when disease suspicion is first reported to its initial investigation. During this phase information is gathered on the disease occurrence, samples are collected and the first measures to control disease spread are carried out.

Early observation of a disease occurrence may come from various sources such as:

- Villagers, fisherman, aquaculturists;
- Public or private sector laboratories;
- Quarantine officers;

- Veterinary officers;
- Field workers carrying out surveillance and monitoring activities for the surveillance program.

Considering those involved in specific surveillance and monitoring it is easily understood that the wider the range of personnel and the frequency of surveillance program activities the greater the chance of early detection of aquatic animal diseases. The Veterinary Quarantine Officers, supervising quarantine activities at ornamental finfish farms and sampling lots of ornamental fish destined for international trade, can also come across what they suspect to be a disease emergency and should alert the DAPH Director General and be ready to provide information such as from where in the country the animals originate from and their behavioral changes and clinical signs of disease.

When alerted to the possibility of a disease emergency, the stakeholders should proceed as currently planned for aquatic animal disease occurrences and inform the DAPH Director General, through phone or on-line reporting of the disease suspicion. It is important that awareness campaigns inform all stakeholders of this procedure and when and how to report their suspicion of a disease occurrence.

The DAPH Director General will now communicate the suspicion of an aquatic animal disease occurrence to a predetermined officer at the MOFAR. The aquaculturists in the area should also be alerted by the DAPH to the possible disease occurrence, providing them the assurance that all measures are in place to determine cause and origin of the suspected disease.

The DAPH Director General will inform the laboratory in charge of disease surveillance for the province in which the suspected disease occurrence is located to dispatch a field worker to carry out the initial investigation. The DAPH Director General will then inform the contingency plans Senior Manager at NARA of the event and of the arrangements taken to dispatch the field worker for initial investigation.

The field worker dispatched to the site should initiate the necessary steps to contain the spread of disease as soon as possible. These actions require these professionals to poses sufficient skills in aquatic animal health. Investigation findings should be reported back to the contingency plans Senior Manager at NARA.

The field worker should gather the greatest amount of information on the disease occurrence.

This information, to be immediately handed to the Senior Manager in the form of a report, should include pictures taken at the site of the disease occurrence and of the diseased animals, and should answer the following questions:

- Where is the infected area and its characteristics?
- What numbers/percentage of animals/ponds are affected?
- What are the clinical signs and behavioral signs of disease?
- What decontamination methods might be needed?
- What are the urgent tracings to be determined, downstream or upstream?
- Have there been any specific weather conditions or climate changes prior and during the disease ocurrance?
- Have there been changes in management practices?

The field worker should also carry out the necessary aquatic animal sampling, gathering specimens of moribund fish or fish with clinical or behavioral signs of disease. Moribund fish are preferred for diagnostic purposes. Water quality parameters should also be analyzed and as such a sample of water should be taken from the site of suspected disease occurrence.

The samples should be immediately dispatched to the laboratory appointed for surveillance activities corresponding to the province where the site of disease occurrence is located. The Senior Manager at NARA maintains regular feedback with the appointed laboratory´s chief officer and assures that the diagnostics are being carried out according to approved protocols. Laboratories should privilege the diagnostics for the samples received and as such diagnostic results should be submitted no longer than 4 days from the date of sample submission. The diagnostic results should be reported immediately by the laboratory chief supervisor to the contingency plans Senior Manager at NARA.

From the moment of suspicion of a disease occurrence all communications should be recorded in writing, therefore all phone communications should be registered and accompanied by fax or e-mail. An Aquatic Animal Health Management Database should be implemented to serve this purpose, functioning as an on-line reporting system. This on-line network of communications would be valued through-out the entire intervention, recording communications and providing the necessary information for the approach and the individual roles and responsibilities in the contingency plan. For the upcoming phases this network can also provide progress reports on the different disease control or eradication efforts, host distribution and other useful information.

The chain of communications for the investigation phase is illustrated in Figure 24.

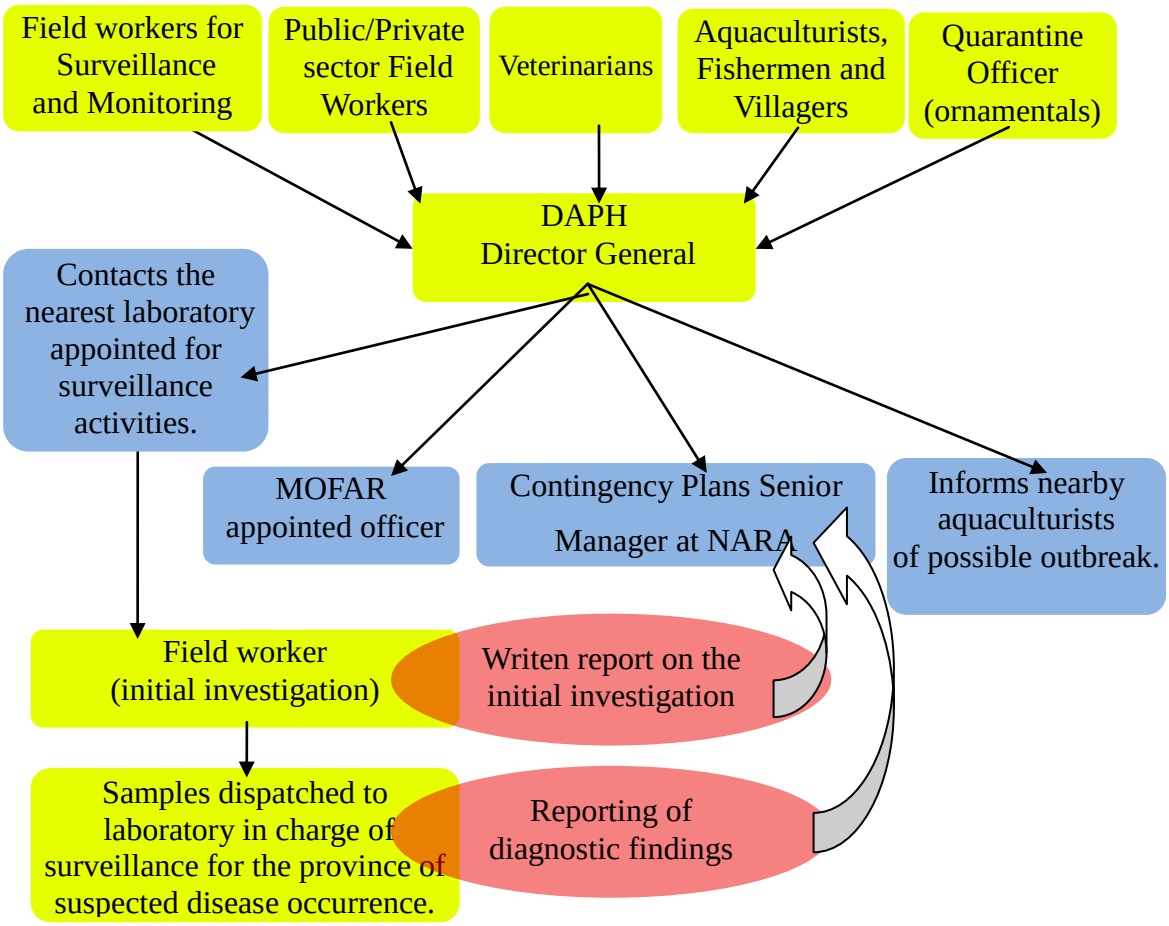

Figure 24: Chain of communications for the Investigation Phase.

2.1.2. Disease Control or Eradication Phase

The disease control or eradication phase is activated the moment the diagnostic results are reported to the Senior Manager at NARA and includes the actions taken to bring the disease occurrence into control or eradicate the pathogen from the affected area.

The Senior Manager informs the DAPH Director General of the diagnostic results, confirming the disease emergency. For those cases where the investigations failed to confirm disease, the NARA Senior Manager will inform the DAPH Director General who will then inform the stakeholders, formerly alerted of the possibility of a disease emergency, that such an event was not confirmed.

2.1.2.1. A Consultative Committee for Aquatic Animal Health Management (CCAAHM)

The DAPH Director General should schedule for the meeting of the Consultative Committee for Aquatic Animal Health Management (CCAAHM) once informed by the Senior Manager at NARA of the confirmed disease occurrence. This committee will include the countries major aquatic animal disease experts with the DAPH Director General as the committee chairperson. A MOFAR appointed officer should also integrate this committee, taking part in general decision making. A third key member of the CCAAHM is the Senior Manager at NARA, presently mandated by legislation to provide counseling in emergency aquatic animal disease occurrences.

The CCAAHM would be made up of two separate groups, one called upon for the disease emergencies affecting crustaceans and another for the diseases affecting the finfish populations. Some members will hold participation in both committees while others will be considered for one committee according to their area of expertise. Annexure XV illustrates the suggested members for the CCAAHM.

The CCAAHM will be responsible for preparing contingency plans for individual diseases of highest priority, implementing simulation exercises and maintaining the preparedness of personnel and equipment for real-time response to a confirmed disease occurrence (Arthur, 2005). Furthermore, the CCAAHM should continuously enhance the Health Management Program, reviewing surveillance, monitoring and reporting strategies and joining efforts to overcome any challenges that arise.

The existence of the CCAAHM allows for certain flexibility when appointing personnel and facilities as the committee holds the power to apply any needed changes, as long as they comply with the existing legislation and are determined under general consensus. The approval of any needed measures that the committee might decide to apply, such as the destruction of animals, the sealing of infected premises and limiting aquatic animal movement, should be reported by the DAPH Director General to a Magistrate, according to the existing legislation.

2.1.2.2. Disease Control or Eradication Phase Outline

Once the Senior Manager confirms the disease outbreak the CCAAHM will meet and recommend the control and eradication efforts to be supervised by a DAPH appointed Veterinary Quarantine Officer.

At the site a Veterinary Quarantine Officer coordinates and supervises disease control or

eradication efforts for a team of field workers, the infected area operations team. This team should enforce quarantine and biosecurity, conduct animal slaughter and disposal, cleaning and disinfection of the premises, equipment or other infected materials and continue sampling aquatic animals to help define the extent of disease spread and the effectiveness of the disease control and eradication efforts. The Veterinary Quarantine Officer should also trace and follow up on any possible movements of suspected animals, vehicles or persons and submit regular reports to the contingency plans Senior Manager and CCAAHM chairperson, the DAPH Director General.

Sampling should be a time to gather information on environmental conditions in general and on water quality in particular. This type of information could be applied for better management practices and concentrated monitoring efforts and so laboratories should also be prepared to gather such information. Not all institutions are equipped and possess the necessary knowledge to perform water quality testing and characterizing of the environmental parameters. Presently NARA Colombo and CAADDR laboratories are familiar with such procedures.

Movement controls are not specialized work and should be done by a contracted security team or by the local authorities who should inclusively record all the movements of people, aquatic animals or aquatic animal products from within and into the infected areas. These workers will require initial briefing on the nature of the disease focusing on the means by which it may spread outside of the infected area.

Support from on site facilities may be necessary, usually in the form of campaign tents and sanitary equipment, and should be discussed and determined by the CCAAHM. Alternatively any one of the nearby institutions should provide this support.

The chain of communication for the disease control or eradication phase is illustrated in Figure 25.

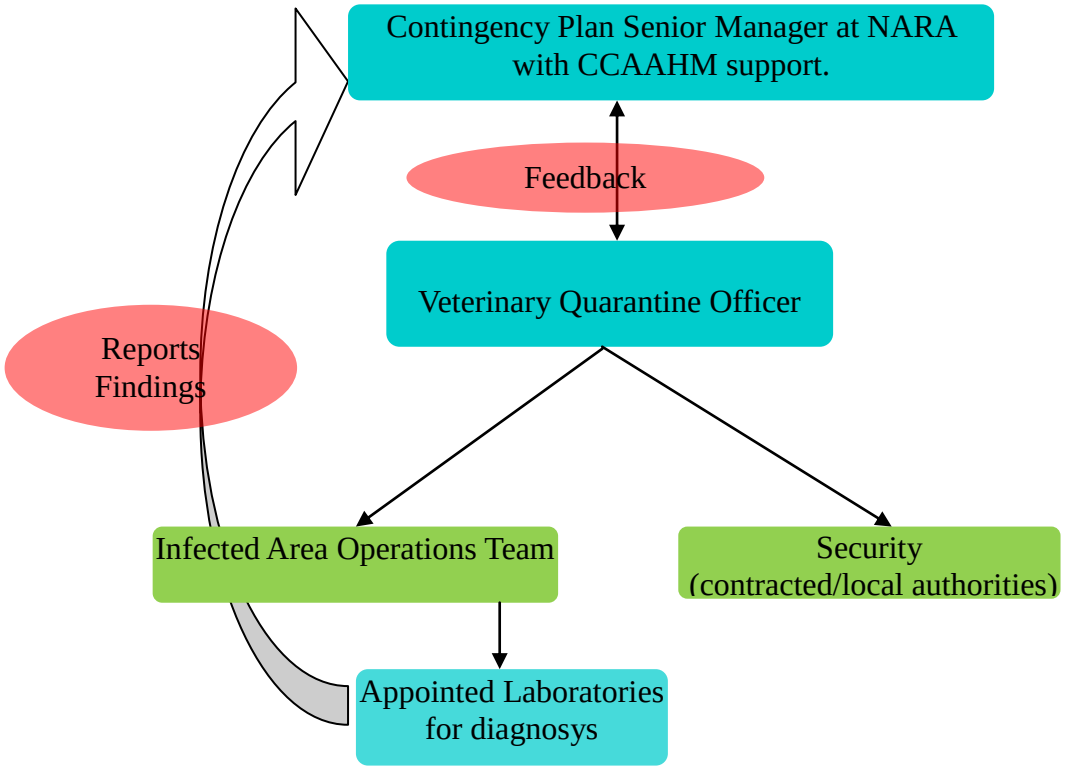

Figure 25: Chain of communication for the Disease Control or Eradication Phase.

2.1.3.) Stand-Down Phase

When the Veterinary Quarantine Officer and the Senior Manager at NARA consider that the contingency plan objectives are achieved and the disease is controlled or eradicated a final reunion of the CCAAHM and all of the involved participants will discuss the results of the campaign and how to improve future approaches to aquatic animal disease occurrences. The procedures and overall evaluation of the campaign should be drafted by the CCAAHM and recorded in the form of a document for future consultation.

V. Conclusion

1. Surveillance, Monitoring and Reporting

The absence of a structured surveillance and monitoring program in Sri Lanka, supported through disease reporting for updated knowledge of the health status of the countries finfish and crustacean populations, needs to be recognized and resolved. Implementing the framework developed in this program, through the consultancy of the different institutions and laboratories engaged in aquatic animal health management and diagnosing, is vital for fulfilling OIE disease reporting obligations and acquiring the knowledge of the presence or absence of the listed diseases of concern to the country.

The surveillance program will generate information to assist the responsible movement of aquatic animals, both inside National territory and at an international level. The information generated through the surveillance program can assist the implementing of a structured system for import risk analysis and improved quarantine, lowering the risk of introducing transboundary disease. The international community will recognize the efforts to control introduction of transboundary disease to the country and to generate an accurate description of the aquatic animal health profile in Sri Lanka, motivating exports and attracting new trade partners.

The path to implementing the proposed framework for the surveillance program in Sri Lanka is through the collaboration among institutions, working together to achieve the surveillance programs objectives. These institutions and laboratories should join efforts to guarantee the nation-wide coverage of sample collecting and diagnosing according to OIE recommended protocols. As resources are scarce this should be done initially for the diseases defined as a priority, to define disease free status or disease free zones. In time, as resources are made available, the surveillance program could extend to include all the listed diseases of concern to the country.

Government and private sector should recognize the need for implementing and supporting this program, as it is vital for the aquaculture sectors productivity and sustainability.

2. Contingency Planning for Aquatic Animal Disease Emergencies

Implementing the suggested framework, established through stakeholder consultancy, will assure a much needed organizational approach in the event of a disease emergency affecting crustaceans and finfish. Once more, the proposed surveillance program and reporting system are

indispensable for early detection of a disease emergency, and will also serve to support the actions to be taken in the event of an aquatic animal disease occurrence.

A Consultative Committee on Aquatic Animal Health Management will help in directing and assuring the efficiency of the actions taken to manage the disease outbreak and should also be involved in maintaining and enhancing the broader Aquatic Animal Health Management program.

The general outline of an intervention in the event of a disease occurrence affecting finfish and crustaceans is illustrated in Figure 26.

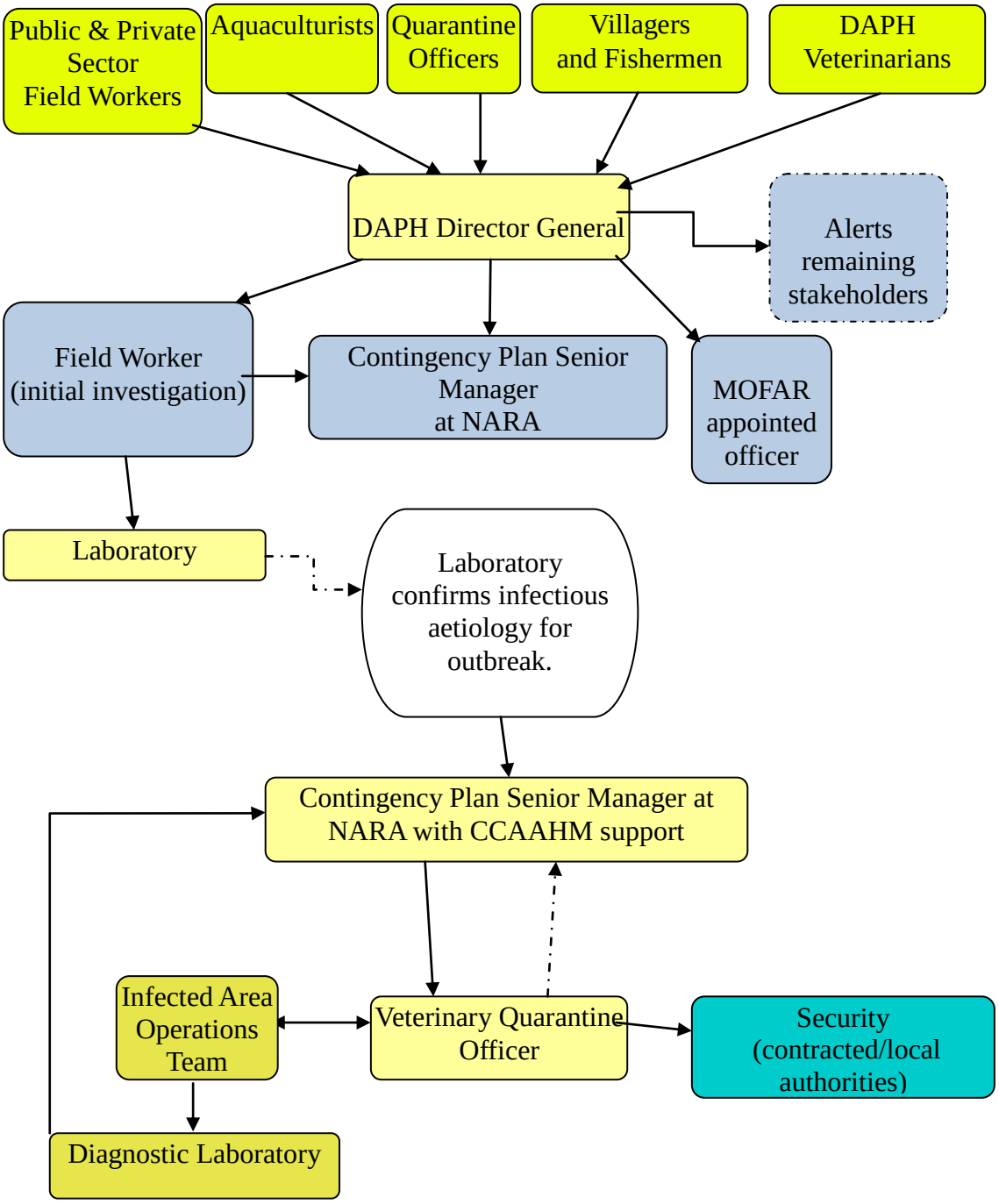

Figure 26: Chain of communication for the proposed National Contingency Plan for Aquatic Animal Disease Emergencies in Sri Lanka.

VI. Recommendations

1. Recommendations for Implementing Surveillance, Monitoring and Reporting

1.1. General Recommendations

Implementing a structured "Surveillance, Monitoring and Reporting System" is vital to Sri Lanka and should begin with the recognition of Government, public research and development agencies and laboratories, along with private sector enterprises, that only a joint approach can make it possible for this program to succeed both in implementation and in duration.

At the moment, the existing resources make it possible to initiate the surveillance program though in the long run it is vital to assure that diagnostic facilities and equipment continue to improve.

A National Health Management Database for reporting diagnostic findings needs to be implemented to assist aquatic animal health management in general and assist the National OIE focal point when fulfilling disease reporting obligations to the FAO/OIE/NACA QAADR.

Funding for implementing and maintaining the surveillance program can arrive from international agencies for the promotion of aquatic animal health such as the OIE, NACA, the South Asian Association for Regional Cooperation (SAARC), and from Sri Lankan Government patronage. Motivating the countries private sector to invest in the aquatic animal health program is also of great importance. Furthermore, international cooperation with other countries, developing and implementing surveillance programs, will prove most valuable. International cooperation can provide training opportunities and the exchange of information that will enhance the surveillance program.

1.2 Recommendations for Improved Quarantine and Import Risk Analysis

Future re-structuring of quarantine procedures is needed to minimize the risk of introducing transboundary diseases through aquatic animal movement. Improving the diagnostic capacity for aquatic animal surveillance and monitoring activities will allow for improved quarantine related diagnostics. Furthermore, the information generated through surveillance and monitoring will support applied import risk analysis and improved quarantine.

An evident flaw in the present quarantine procedures is the absence of a government quarantine facility for imported aquatic animals, making the entire application of the quarantine process doubtful as there is no reassurance as to the isolation and completion of the quarantine process at the receiving farms.

Although Veterinary Quarantine Officers are familiar with the epidemiological investigations and sampling procedures for terrestrial animals, training is suggested for these professionals in aquatic animal health so as to enhance their capacity to correctly identify and sample for diseased animals.

There is no efficient and documented information system to register quarantine diagnostic findings. This information system should be built into the proposed Aquatic Animal Health Management Database.

1.3. Specific Recommendations for the Laboratories

Given its present work load, existing expertise and proposed activities in not only surveillance and monitoring but also in emergency preparedness, the NARA laboratories for PCR, bacteriology, histopathology and parasitology should be subject to improvement, expanding and generally upgrading the present facilities to establish NARA as a reference laboratory for aquatic animal disease diagnostics.

The NAQDA facilities and equipment at Chillaw are recent but the do not account for histopathology which should be implemented in the future to support diagnostic needs and surveillance program requirements. At the present moment internet facilities are inexistent at NAQDA Chillaw and considered a priority easily accomplished. Consideration should be made as to implement PCR diagnostic facilities at NAQDA Rambodagalla for the long term, given the fact that it stands as a training center and its role in diagnosing ornamental finfish diseases for the industry.

The VIC laboratory is clearly inadequate for diagnosing aquatic animal diseases as it has presently very limited equipment and inadequate facilities. Benifiting the VIC would not only allow for its participation in the surveillance program but would also provide quality diagnostics and improved capacity for the quarantine diagnostics there performed.

The CAADDR facilities for PCR diagnostics could benefit from spatial compartmentalization to minimize contamination.

It is important to stress the fact that the existing laboratory personnel for all of the considered laboratories carry out routine diagnostics and research, and as such serious consideration should be made to appoint teams for the sole purpose of carrying out the surveillance program field work and diagnostic activities. Investing in local training programs in aquatic animal health and diagnostics will also prove valuable for assuring the effectiveness and quality of the surveillance program

2. Recommendations for Implementing the Proposed National Contingency Plan for Aquatic Animal Disease Emergencies in Sri Lanka

Government should initially recognize the need to enhance the existing approach for responding to an aquatic animal disease emergency and implement the necessary legislation to support the contingency planning, as suggested in this thesis.

For timely communication of a disease occurrence, developing a National Health Management Database is also a priority. Contingency plans will also require telephone communication of suspected disease emergencies and so implementing a National hotline for aquatic animal disease reporting should be considered.

Assuring resources are available for immediate intervention and the compliance with the contingency plan set framework can be achieved through the coordinated supervision of the CCAAHM. This committee will also help improve and assure continuous enhancing of the surveillance program.

Spatial distribution using a geographical information system (GIS) has been developed in other countries for early detection of disease emergencies, based on production surveillance, serving as an important tool for the aquaculture industry. Normal conditions of production could indicate absence of disease while suboptimal production levels will alert to possible disease occurrences. Authorities can investigate the reason behind suboptimal levels of production and apply control measures to reduce spread or eradicate disease (Bayot, 2008). This information system will rely on the information supplied by the aquaculture industry for which the benefits of such a system would need to be previously demonstrated in order to encourage their cooperation.

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ANNEXURES

Annexure I: The OIE QAADR form for Sri Lanka.

Quarterly Aquatic Animal Disease Report (Asia-Pacific Region) – 2009/3

Country: SRI LANKA **Period: July-September 2009**

Item	Disease status [a/]			Level of diagnosis	Epidemiological comment numbers
DISEASES PREVALENT IN THE REGION	Month				
FINFISH DISEASES	July	August	September		
OIE-listed diseases					
1. Epizootic haematopoietic necrosis	***	***	***		
2. Infectious haematopoietic necrosis	***	***	***		
3. Spring viraemia of carp	?	?	?		
4. Viral haemorrhagic septicaemia	0000	0000	0000		
5. Epizootic ulcerative syndrome	-	-	-		
6. Red seabream iridoviral disease	0000	0000	0000		
7. Koi herpesvirus disease	?	?	?		
Non OIE-listed diseases					
8.Grouper iridoviral disease	***	***	***		
9.Viral encephalopathy and retinopathy	***	***	***		
10.Enteric septicaemia of catfish	***	***	***		
MOLLUSC DISEASES					
OIE-listed diseases					
1. Infection with *Bonamia exitiosa*	***	***	***		
2. Infection with *Perkinsus olseni*	***	***	***		
3. Abalone viral mortality	0000	0000	0000		
Non OIE-listed diseases					
4. Infection with *Marteilioides chungmuensis*	***	***	***		
5. Acute viral necrosis (in scallops)	***	***	***		
6.Akoya oyster disease	0000	0000	0000		
CRUSTACEAN DISEASES					
OIE-listed diseases					
1. Taura syndrome	0000	0000	0000		
2. White spot disease	+	+	+	III	1
3. Yellowhead disease	***	***	***	III	4
4. Spherical baculovirosis (*Penaeus monodon*-type baculovirus)	+	+	+	I and III	2
5. Infectious hypodermal and haematopoietic necrosis	?	?	?	III	3
6. Tetrahedral baculovirosis (*Baculovirus penaei*)	***	***	***		
7. Infectious myonecrosis	***	***	***		
8.White tail disease (MrNV)	***	***	***		
9. Necrotising hepatopancreatitis	***	***	***		
10. Hepatopancreatic parvo virus disease	***	***	***		
11. Mourilyan disease	***	***	***		
Non OIE-listed diseases					
12. *Monodon* slow growth syndrome	***	***	***		
13. Milky lobster disease	***	***	***		
AMPHIBIAN DISEASES					
OIE-listed diseases					
1. Infection with Ranavirus	***	***	***		
2. Infection with *Batrachochytrium dendrobatidis*	***	***	***		

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ANY OTHER DISEASES OF IMPORTANCE					
1.					
2.					

DISEASES PRESUMED EXOTIC TO THE REGION[b]
LISTED BY THE OIE
Finfish: Infectious salmon anaemia; Gyrodactylosis (*Gyrodactylus salaris*).
Molluscs: Infection with *Bonamia ostreae*; *Marteilia refringens*; *Perkinsus marinus*; *Xenohaliotis californiensis*.
Crustaceans: Crayfish plague (*Aphanomyces astaci*).
NOT LISTED BY THE OIE
Finfish: Channel catfish virus disease

a/ Please use the following symbols:

+	Disease reported or known to be present	+( )	Occurrence limited to certain zones
+?	Serological evidence and/or isolation of causative agent but no clinical diseases	***	No information available
		0000	Never reported
?	Suspected by reporting officer but presence not confirmed	-	Not reported (but disease is known to occur)
		(year)	Year of last occurrence

b/ If there is suspicion or confirmation of any of these diseases, they must be reported immediately, because the region is considered free of these diseases

1. Epidemiological comments:

(Comments should include: 1) Origin of the disease or pathogen (history of the disease); 2) Species affected; 3) Disease characteristics (unusual clinical signs or lesions); 4) Pathogen (isolated/sero-typed); 5) Mortality rate (high/low; decreasing/increasing); 6) Death toll (economic loss, etc); 7) Size of infected areas or names of infected areas; 8) Preventive/control measures taken; 9) Samples sent to national or international laboratories for confirmation (indicate the names of laboratories); 10) Published paper (articles in journals/website, etc). and 11) Unknown diseases: describe details as much as possible.)

Comment No.	
1	A total of 31 samples of P.monodon have been tested at PCR lab of Department of Fisheries (DOF), of which 4 samples (12.9%) were recorded as TSV positive
2	A total of 31 samples of P.monodon have been tested at PCR lab of Department of Fisheries (DOF), of which 5 samples (16.139%) were recorded as WSSV positive
3	A total of 31 samples of P.monodon have been tested at PCR lab of Department of Fisheries (DOF), of which 1 samples (3.23%) were recorded as IHHNV positive

2. New aquatic animal health regulations introduced within past six months (with effective date): None

New Instructions on how to fill in the
QUARTERLY AQUATIC ANIMAL DISEASE REPORT
(Revised during the Provisional Meeting of the AG[1], Bangkok, Thailand, November 7-9, 2001)

Symbols used in the report are similar to those used by FAO, OIE and WHO for the *Animal Health Yearbook*. Please read these instructions carefully before you fill in the forms.

Under the heading 'Country', please enter your country.

Under the heading 'Period', please enter the reporting quarter (months) and year, e.g. January to March 2002.

Under the heading "Month", please enter months of a quarter in question, e.g. January, February, March.

In "Level of Diagnosis", please enter the Level of Diagnosis used, e.g., I, II, or III. See Section C below.

In "Epidemiological Comment Numbers", please enter the serial numbers, and write your corresponding epidemiological comments on page 2. See Section D below for guidance on the subjects to be covered under Epidemiological Comments.

If an unknown disease of serious nature appears, please fill in the last line of the form, with additional information on "Level of Diagnosis" and "Epidemiological Comment Numbers" as above.

Please do not fail to enter "***" or "-" as appropriate against each disease, which is essential to incorporate your information on the *Quarterly Aquatic Animal Disease Report (Asia and Pacific Region.)*

If you have new aquatic animal health regulations introduced within the past six months, please describe them under Section 2 on page 2.

Please use the following symbols to fill in the forms.

A. Symbols used for negative occurrence are as follows:

*** This symbol means that no information on a disease in question is available due to reasons such as lack of surveillance systems or expertise.

- This symbol is used when a disease is not reported during a reporting period. However the disease is known to be present in the country (date of last outbreak is not always known).

0000 This symbol is used when disease surveillance is in place and a disease has never been reported.

(year) Year of last occurrence (a disease has been absent since then).

B. Symbols used for positive occurrence are shown below.

+ This symbol means that the disease in question is reported or known to be present.

+? This symbol is used when the presence of a disease is suspected but there is no recognised occurrence of clinical signs of the disease in the country. Serological evidence and isolation of the causal agent may indicate the presence of the disease, but no confirmed report is available. **It is important that the species of animals to which it applies is indicated in the "Comments" on page 2 of the form if you use this symbol**.

+( ) These symbols mean that a disease is present in a very limited zone or zones as exceptional cases. It may also include the occurrence of a disease in a quarantine area.

? This symbol is used only when a disease is suspected by the reporting officer, but the presence of the disease has not been confirmed.

[1] Regional Advisory Group on Aquatic Animal Health (AG)

C. Levels of Diagnosis

LEVEL	SITE	ACTIVITY
I	Field	Observation of animal and the environment Clinical examination
II	Laboratory	Parasitology Bacteriology Mycology Histopathology
III	Laboratory	Virology Electron microscopy Molecular biology Immunology

D. Subjects to be covered in the Epidemiological Comments

1. Origin of the disease or pathogen (history of the disease);
2. Mortality rate (high/low or decreasing/increasing);
3. Size of infected areas or names of infected areas;
4. Death toll (economic loss, etc.);
5. Preventive/control measures taken;
6. Disease characteristics (unusual clinical signs or lesions);
7. Pathogen (isolated/sero-typed);
8. Unknown diseases (describe details as much as possible);
9. Samples sent to national or international laboratories for confirmation (indicate the names of laboratories); and
10. Published paper (articles in journals)/web site, etc.

IMPORTANT

Please send the **original report** or the best photocopy thereof to the OIE and/or NACA **by fax** and **registered airmail**. Faxed reports are needed to check whether or not the reports are all right. The deadline for submission of the reports is **two and a half months (75 days)** after the end of the quarterly period.

If you require further explanation, please write to the OIE (Tokyo), NACA (Bangkok) or FAO (Rome) at the following addresses, respectively:

OIE Regional Representation for Asia and the Pacific
Sanseido Building, 4F
2-4-10 Kojimach, Chyoda-Ka
Tokyo 102-0083, Japan
Tel: 81-3-5212-3191, Fax: 81-3-5212-3194
E-Mail: rr.asiapacific@oie.int

NACA
P. O. Box 1040, Kasetsart Post Office, Bangkok 10903, Thailand
Tel: 66-2-561-1728/9 (ext. 117); Fax: 66-2-561-1727
Dr. C.V. Mohan
E-mail: mohan@enaca.org

FAO
Fishery Resources Division, Fisheries Department
FAO of the United Nations
Viale delle Terme di Caracalla, 00100 Rome
Tel. +39 06 570 56473; Fax + 39 06 570 530 20
E-mail: Rohana.Subasinghe@fao.org

Annexure II: Susceptible species for EHNV infection, their habitat and distribution (Fishbase, 2010; OIE 2009f).

Species Name	Habitat	Distribution
Silver perch- *Bidyanus bidyanus*	Fresh water	Only from the Murray-Darling River System in Australia
Macquire perch- *Macquoria australasica*	Fresh water	Only from the Murray-Darling River System in Australia
Mosquito fish- *Gambusia affinis*	Fresh water	North and Central America. Wide range of introduction and so considered to have a pan-global distribution. Introduced to Sri Lanka.
Mountain galaxias- *Galaxias olidus*	Fresh water	South-East to Eastern South Australia
Murray cod- *Maccullochella peelii peelii*	Fresh water	Australia
Atlantic salmon- *Salmo salar*	Fresh water Brackish water Marine	Atlantic Ocean
Rainbow trout- *Oncorhynchus mykiss*	Fresh water Brackish water Marine	Southwest Atlantic and East Pacific. Widely introduced and so can be regarded to have a global distribution. Introduced to Sri Lanka.
Redfin perch- *Perca fluviatilis*	Fresh water Brackish water Marine	Australia, Europe and Siberia.

Annexure III: Susceptible species for IHNV infection, their habitat and distribution. (Fishbase, 2010; NACA, 2010a; OIE, 2009r).

Species Name	Habitat	Distribution
Rainbow trout- *Oncorhynchus mykiss*	Fresh water Brackish water Marine	Southwest Atlantic and East Pacific. Widely introduced and so can be regarded to have a global distribution. Introduced to Sri Lanka.
Pacific Salmon- *Oncorhynchus tshauytscha*	Fresh water Brackish water Marine	Artic and North-West to North-East Pacific
Sockeye- *Oncorhynchus nerka*	Fresh water Brackish water Marine	North Pacific: North Japan to Baring Sea and California
Chum- *Oncorhynchus Keta*	Fresh water Brackish water Marine	North Pacific: Korea and Japan to Baring Sea and California Asia: Iran
Pink- *Oncorhynchus gorbuscha*	Fresh water Brackish water Marine	West, North and East-Central Pacific
Amago- *Oncorhynchus rhondurus*	Fresh water Brackish water Marine	Endemic to Japan
Masou- *Oncorhynchus masou*	Fresh water Brackish water Marine	North Japan and Korea Peninsula
Coho- *Oncorhynchus kisutch*	Fresh water Brackish water Marine	Russia to Southern Japan, Alaska to California
Brown Trout- *Salmo trutta*	Fresh water Brackish water Marine	Europe and Asia Introduced to Australia, Americas and Sri Lanka.
Cutthroat trout- *Oncorhynchus*	Fresh water	East Pacific and its emptying

clarki	Brackish water Marine	streams.
Salvelinus namayoush	Fresh water	North Korea to Alaska. Great lakes and New England
Salvelinus alpinus	Fresh water Brackish water Marine	North Atlantic Europe and land-locked in North America.
Salvelinus fontinalis	Fresh water Brackish water Marine	East Canada and Great Lakes, Mississippi and Georgia USA, Argentina and widely introduced to other countries.
Salvelinus leucomaenis	Fresh water Brackish water Marine	North-West Pacific from Japan to Korea and Bering and Okhutsk Seas.
Grayling- *Thymallus articus*	Fresh water	North America Artic to Hudson Bay and Pacific Canada and Great Lakes. Asia: Russia and Siberia
Ayu- *Plecoglossus altivelis*	Fresh water Brackish water Marine	West Japan to Korea Peninsula, Taiwan and China.
Clupea pallasi	Fresh water Brackish water Marine	Artic, West and East Pacific.
Gadus morhua	Brackish water Marine	North-West and North-East Atlantic, Coast of Europe Biscay and Barents Sea.
Acipenser Transmontanus	Fresh water Brackish water Marine	East Pacific Alaska to California.
Esox lucius	Fresh water Brackish water	North America and Europe. Introduced to other countries.

Cymantogaster aggregata	Fresh water Brackish water Marine	East Pacific to North California.
Aulorhynchus flavidus	Marine	Alaska to North California.

Annexure IV: Susceptible species for SVC infection, their habitat and distribution. (Fishbase, 2010; NACA, 2010b; OIE, 2009h).

Species Name	Habitat	Distribution
Common carp & Koi carp- *Cyprinus carpio carpio* & *Cyprinus carpio koi*	Fresh water Brackish water	Global distribution. Cultured in Sri Lanka.
Crucian carp- *Carassius carassius*	Fresh water Brackish water	Spain, across Europe and North-Central Asia to North China. Cultured in Sri Lanka.
Silver carp- *Hypophthalmichthys molitrix*	Fresh water	Worldwide introduction, including Sri Lanka.
Bighead carp- *Aristichtys nobilis*	Fresh water	Near global distribution, including Sri Lanka.
Grass carp- *Ctenophoryngodon idella*	Fresh water	Widely distributed, including Sri Lanka.
Goldfish- *Carassius aureatus*	Fresh water	Widely distributed, including Sri Lanka. Culturd in Sri Lanka.
Orfe- *Leuciscus idus*	Fresh water Brackish water	Rhine basin to the west of the Yakutsk-Sakha Republic Ornamental in America and Europe.
Tench- *Tinca tinca*	Fresh water Brackish water	All Europe Artic river drainage.
Wells catfish- *Silurus glanis*	Fresh water Brackish water	Europe and Asia.
Rainbow trout- *Oncorhynchus mykiss*	Fresh water Brackish water Marine	Southwest Atlantic and East Pacific. Widely introduced and so can be regarded to have a global distribution. Introduced to Sri Lanka.
Roach- *Rutilus rutilus*	Fresh water Brackish water	Europe to Caspian sea and Turkmenistan.
Zebra fish- *Danio rerio*	Fresh water	Pakistan, India, Bangladesh, Nepal and Myanmar , Bhutan and Sri

		Lanka. Culturd in Sri Lanka.
Guppy- *Poecilia reticulate*	Fresh water Brackish water	South America, Africa and widely distributed including Sri Lanka. Culturd in Sri Lanka.
Pumpkinseed- *Lepomis gibossus*	Fresh water	North America and widely introduced.

Annexure V: Susceptible species for VHS infection, their habitat and distribution. (Fishbase;2010; NACA , 2010c; OIE, 2009i)

Species Name	Habitat	Distributio
Rainbow trout- *Oncorhynchus mykiss*	Fresh water Brackish water Marine	Southwest Atlantic and East Pacific. Widely introduced and so can be regarded to have a global distribution. Introduced to Sri Lanka.
Japanese Flounder- *Paralichthys olivaceus*	Marine	Western Pacific: Japan, Sakhalin, Kuril Islands, Korean Peninsula to the South China Sea.
Zebra fish- *Danio rerio*	Fresh water	Pakistan, India, Bangladesh, Nepal and Myanmar , Bhutan and Sri Lanka
European Sea bass- *Dicentrarchus labrax*	Fresh water Brackish water Marine	Eastern Atlantic and , Mediterranean to Black Sea.
Indian Turbot / Indian Spiny turbot- *Psettodes erumei*	Marine	Indo-West Pacific: Red Sea and East Africa to Japan and Australia.
Pacific herring- *Clupea pallasii*	Fresh water Brackish water Marine	Artic, Western Pacific: Anadyr Bay, eastern coasts of Kamchatka, possibly the Aleutian Islands southward to Japan and west coast of Korea. Eastern Pacific.

Annexure VI: Susceptible species for RSIV infection, their habitat and distribution. (Fishbase,2010; NACA, 2010e)

Species name	Habitat	Distribution
Sea Bass- *Lates calcarifer*	Fresh water Estuarine Marine	Indo-West Pacific: eastern edge of the Persian Gulf to China, Taiwan and southern Japan, southward to southern Papua New Guinea and northern Australia
Black sea bream- *Acanthopargus schlegeli*	Estuarine Marine	North-West Pacific
Chicken grunt- *Parapristipama trilineatum*	Marine	Indo-West Pacific: southern Japan, East China Sea and Taiwan
Crimson seabream- *Evynnis japónico*	Marine	Western Pacific: southern Japan, Korea to East China Sea
Estuarine Rockhead- *Epinephelus tauvina*	Marine	Indo-Pacific
Girella- *Girella punctata*	Marine	Northwest Pacific
Japanese Amberjack- *Seriola quinqueradiata*	Marine	Northwest Pacific
Japanese flounder- *Paralichthys olivaceus*	Marine	Western Pacific: Japan, Sakhalin, Kuril Islands, Korean Peninsula to the South China Sea.
Japanese Horse Mackeral- *Trachurus japonicus*	Marine	North-West Pacific and Pacific coast of South-East Asia.
Japanese Parrot fish- *Oplegnathus fasciatus*	Marine	North-West and East-Central Pacific.
Japanese sea bass- *Lateolabrax japonicus*	Marine Fresh water Brackish	Western Pacific: Japan to the South China Sea
Large mouth bass- *Micropretus salmoides*	Freshwaater	Introduced widely (Asia, Europe, Africa, North America, Central America).
Malabar rockcod- *Epinephelus malabaricus*	Brackish Marine	Indo-Pacific to South Australia and Persian Golf.
Northern Bluefin Tuna- *Thunnus thynnus*	Brackish Marine	Atlantic Ocean and Mediterranean.
Red Seabream- *Pagrus major*	Marine	Distribution Northwest Pacific: northeastern part of South China Sea northward to

		Japan.
Red Spotted Grouper- *Epinephelus akaara*	Marine	Northwest Pacific: southern China, Taiwan, East China Sea, Korea, and southern Japan. Philippines and India are unsubstantiated.
Samsonfish- *Seriola hippos*	Marine Brackish	Indo-Pacific: endemic to Australia, Norfolk Island and New Zealand.
Sea bass- *Lateolabrax* sp.	Fresh water Marine Brackish	L. japonicus: Western Pacific: Japan to the South China Sea. L. latus: (marine) Japan and South Korea.
Seven band grouper- *Epinephelus septemfasciatus*	Marine	North-West Pacific
Silver trevally- *Pseudocaranx dentex*	Marine Brackish	West Atlantic, Mediterranean, Indo-Pacific: South Africa, Japan, Hawaii, Australia, Lord Howe and Norfolk islands, New Zealand. Reported from New Caledonia.
Sixbar grouper- *Epinephelus sexfasciatus*	Marine	Western Central Pacific: known only from tropical waters, from Thailand and the Philippines to northern Australia.
Snapper- *Pagrus auratus*	Brackish water Marine	Indo-Pacific: widely occurring off New Zealand, Australia, Philippines, Indonesia, China, Taiwan, and Japan.
Snubnose dart- *Trachinotus blochii*	Brackish water Marine	Indo-Pacific: Red Sea and East Africa to the Marshall Islands and Samoa, north to southern Japan, south to Australia.
Spotted Knifefish- *Oplegnathus punctatus*	Marine	Pacific Ocean: Central Honshu, Japan to the South China Sea.
Tiger puffer- *Takifugu rubripes*	Brackish water Marine Freshwaater	Northwest Pacific
Grouper- *Epinephelus awoara*	Marine	Northwest Pacific
Golden striped amberjack- *Seriola lalandi*	Marine Brackish water	Circumglobal, in subtropical waters, and with a series of

		disjunctive populations. Indo-Pacific: South Africa, Walter Shoals, Amsterdam Island, Japan, Australia, New Zealand, New Caledonia, Hawaii, Rapa, Pitcairn Island, and Easter Island.

Annexure VII: Susceptible species for KHV infection, their habitat and distribution. (Fishbase,2010; NACA, 2010f; OIE, 2009m)

Species Name	Habitat	Distribution
Common carp- *Cyprinus carpio*	Fresh water Brackish	Global (present in Sri Lanka)
Koy carp (Common carp variety)	Fresh water Brackish	Global (present in Sri Lanka)
Ghost carp (Common carp variety)	Fresh water Brackish	Global (present in Sri Lanka)
Goldfish- *Carassius aureatus*	Fresh water	Global (present in Sri Lanka)
Grass carp- *Ctenopharyngodon idella*	Fresh water	Global (present in Sri Lanka)

Annexure VIII: Susceptible species for GIV infection, their habitat and distribution. (Fishbase, 2010; NACA, 2010g)

Species Name	Habitat	Distribution
Brown spotted grouper- *Epinephelus tauvina*	Marine	Indo-Pacific
Yellow gruper- *Epinephelus awoara*	Marine	North-West Pacific
Nursing gruper- *Epinephelus malabaricus*	Marine Brackish water	Indian and East Pacific Ocean (including Sri Lanka)

Annexure IX: Susceptible species for VER, their habitat and distribution. (Fishbase, 2010; NACA, 2010h)

Species Name	Habitat	Distribution
Atlantic cod- *Gadus morhua*	Brackish water Marine	North Atlantic
Atlantic halibut- *Hippoglossus hippoglossus*	Marine	East and West Atlantic
Australian bass- *Macquaria novemaculeata*	Fresh water Brackish	Oceania
Barfin flounder- *Verasper moseri*	Marine	North-West Pacific
Sea bass- *Lates calcalifer*	Fresh water Brackiswater Marine	Indo-West Pacific including Sri Lanka.
Brown marbled grouper- *Epinephelus fuscoguttatus*	Marine	Indian and Pacific Oceans (including Sri Lanka).
Cobia- *Rachycentron canadum*	Marine Brackish water	Worldwide in tropical and subtropical waters.
Comon sole- S*olea solea*	Marine Brackish water	East Atlantic and Mediterranean Ocean
Estuary cod- *Epinephelus tauvina*	Marine	Indo-Pacific
Eusopean eel- *Anguilla anguilla*	Marine Brackish water Fresh water	East Atlantic and Mediterranean Ocean.
Seabass- Dicentrarchus labrax	Brackish water Fresh water	Recently introduced cultured species in Sri Lanka.
Flounders- *Paralichtydae*		Present in Sri Lanka (marine)
Gilt-head sea bream- *Sparus aurata*	Marine Brackish water	East Atlantic and Mediterranean Ocean.
Greater amberjack- *Seriola dumerili*	Marine	Indo-West Pacific Ocean
Grouper *Epinephelus sp.* and	Marine	Some species known to the region

estuary cod or *E. malabaricus*		such as *E. malabaricus.*
Humpback gruper- *Cromileptes altivelis*	Marine	Western Pacific, from Japan to Australia.
Japanese flounder- *Paralichtys olivaceus*	Marine	Western Pacific: Japan, Sakhalin, Kuril Islands, Korean Peninsula to the South China Sea.
Japanese parrotfish- *Oplegnatus fasciatus*	Marine	North-West and East-Central Pacific.
Japanerse puffer- *Takifugu rubripes*	Marine	Western Pacific
Japanese seabass- *Lateolabrax japonicus*	Marine Brackish water Fresh water	Western Pacific: Japan to the South China Sea
Longtooth grouper- *Epinephelus moara*	Marine	North-West Pacific
Malabar grouper- *Epinephelus malabaricus*	Marine Brackish water	Indo-Pacific to South Australia and Persian Golf.
Orange-spotted grouper- *Epinephelus coioides*	Marine Brackish water	Indo-West Pacific
Red drum- *Sciaenops ocellatus*	Marine Brackish water	West Atlantic
Red snapper- *Lutjanus erythropterus*	Marine	South-East Asia to Australia.
Red-spotted grouper- *Epinephelus akaara*	Marine	Northwest Pacific: southern China, Taiwan, East China Sea, Korea, and southern Japan. Philippines and India are unsubstantiated.
Seven-band grouper- *Epinephelus septemfasciatus*	Marine	North-West Pacific

Shi drum- *Umbrina cirrosa*	Marine Brackish water	East Atlantic and Mediterranean Ocean.
Silver trevally- *Pseudocaranx dentex*	Marine Brackish water	West Atlantic, Mediterranean, Indo-Pacific: South Africa, Japan, Hawaii, Australia, Lord Howe and Norfolk islands, New Zealand. Reported from New Caledonia.
Striped trumpeter- *Latris lineata*	Marine	Atlantic, Pacific and Indian Ocean.
Turbot- *Psetta maxima*	Marine Brackish water	North-East Atlantic and Mediterranean Ocean.
White seabass- *Atractoscion nobilis*	Fresh water	Near global distribution, including Sri Lanka.
Winter flounder- *Pseudopleuronectes americanus*	Marine	West Atlantic
Yellow-wax pompano- *Trachinotus falcatus*	Brackish water Marine	Indo-Pacific: Red Sea and East Africa to the Marshall Islands and Samoa, north to southern Japan, south to Australia.
Australian catfish- *Cnidoglanis macrocephalus*	Fresh water Brackish	Indo-Pacific: endemic to Australia.
Barcoo grunter- *Scortum barcoo*	Fresh water	Oceania
Golden perch- *Macquaria ambígua*	Fresh water	Oceania
Macquarie perch- *Macquaria australasica*	Fresh water	Oceania
Murray cod- *Maccullochella peelii peelii*	Fresh water	Australia
Samson fish- *Seriola hippos*	Marine Brackish water	Indo-Pacific: endemic to Australia, Norfolk Island and New Zealand.
Silver perch- *Bidyanus bidyanus*	Fresh water	Only from the Murray-Darling

		River System in Australia.
Sleepy cod- *Oxyeleotris lineolatus*	Fresh water	Oceania

Annexure X: Susceptible species for ESC, their habitat and distribution. (Fishbase, 2010; NACA, 2010i).

Species Name	Habitat	Distribution
Black bullhead- *Ictalurus melas*	Fresh water	Europe and North America
Blue catfish- *Ictalurus furcatus*	Fresh water Brackish water	North and Central America.
Brown bullhead- *Ictalurus nebulosus*	Fresh water	North America. Introduced to many countries in Europe and Asia (China, Iran).
Channel catfish- *Ictalurus punctatus*	Fresh water	North America
Rosy barb- *Puntius conchonus*	Fresh water	Asia: Afghanistan, Pakistan, India, Nepal, and Bangladesh. Worldwide introductions.
Sind danio- *Puntius devario*	Fresh water	Asia: Pakistan, India, Nepal and Bangladesh
Sutchi catfish- *Pangasius hypophthalmus*	Fresh water	Asia: Thailand, Bangladesh, Myanmar, Laos, Philippines, Singapore, Taiwan, Viet Nam.
Walking catfish- *Clarias batrachus*	Fresh water Brackish water	Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra, Java, Borneo. Reported from Sri Lanka; popular for aquaculture in its native range but not regarded as such in other Southeast Asian countries.
White catfish- *Ictalurus catus*	Fresh water	North America
Yellow bullhead- *Ictalurius natalis*	Fresh water	North America
Zebra fish- *Danio rario*	Fresh water	Pakistan, India, Bangladesh, Nepal and Myanmar, Bhutan and Sri Lanka.
Chinook salmon- *Oncorhynchus tshawytscha*	Brackish water Marine Freshwaater	North Pacific, Japan to USA.
Rainbow trout- *Oncorhynchus mykiss*	Brackish water Marine Freshwaater	Southwest Atlantic and East Pacific. Widely introduced and so can be regarded to have a global distribution. Introduced to Sri Lanka.

Annexure XI: Diseases presently included in the Schedule for emergency intervention in the event of an aquatic animal disease emergency in the Aquaculture Management Disease Control Regulations 2000 in the Fisheries and Aquatic Resources Act No.2 of 1996 with reference to their status as OIE listed diseases of concern to the region.

Finfish Diseases	Of which are OIE diseases of concern to the region
Epizootic haematopoietic necrosis (EHN)	X
Infectious haematopoietic necrosis (IHN)	X
Oncorhyncus masou virus disease	
Infectious pancreatic necrosis (IPN)	
Viral encephalopathy and retinopathy	X
Epizootic ulcerative syndrome (EUS)	X
Bacterial kidney disease (BKD)	
Spring viraemia of carp (SVC)	X
ViraI haemorrhagic septicaemia (VHS)	X
Mollusc Diseases	Of which OIE Listed Diseases
Bonamiosis *(Bonamia ostreae)*	X
Marteiliosis : *Martelin refringens*	X
Marteiliosis : *M. S ydneyi*	
Microcytosis *(Microcytos mackini, M. roughelyi)*	
Perkinosis : *Perkinsus marinuns & P. olseni*	X
Haplosporidiosis: *Haplosporidium costale & H.* n*elsoni*	
Crustacean Diseases	Of which OIE Listed Diseases
Yellow head disease	X
Infectious hypodermal and haematopoietic necrosis	X
White-spot disease	X
Baculoviral midgut gland necrosis	
GiIl associated virus disease	
Spawner mortality syndrome (Midcrop mortality syndrome)	

Annexure XII: Questionnaire submitted to the different laboratories.

Questionnaire for Aquatic Animal Health Surveillance and Reporting: current situation analyses

Laboratory designation:
_____
Date: _____

Please read through this questionnaire before answering.

Sector and policies:

1. To what sector does your laboratory work under:

☐ Private sector industry
☐ Public sector agency

2. Does your laboratory work solely for the purpose of satisfying specific needs for a farm or business venture?

☐ Yes
☐ No

3. Who supplies your laboratory with the most work-load?

☐ Private sector industries
☐ Public sector agency

4. Does your laboratory hold cooperation with any other agency or laboratory?

☐ Yes
☐ No

5. If so, please state its designation.
_____

6. Is your laboratory currently integrated in a reporting system?

☐ Yes
☐ No

7. Do you report your findings?

☐ Yes
☐ No

8. If so, who do you normally report your findings to:

☐ Private sector
☐ Government official

9. On what basis?

☐ Monthly
☐ Quarterly
☐ Every Semester
☐ Other:

_____

10. Does your laboratory receive advisory/counceling/cooperation from any foreign agency/laboratory?

☐ Yes
☐ No

11. If so, what agency?

_____

Facilities:

12. Do the current laboratory facilities respond to your needs?

☐ Yes
☐ No

13. If not, what is the major limitation:

☐ Insufficient personnel
☐ Insufficient work space
☐ Insufficient working material
☐ Insufficient equipment
☐ Other:

_____

Personnel:

14. How many technicians are currently working permanently at the diagnostic laboratory?

- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ >5

15. What are their qualifications?

Number of Technicians	Degree, Diploma or other	Masters	PhD
1			
2			
3			
4			
>5			

16. Can technicians manipulate existing laboratory protocols or develop new protocols for diagnosing new and emerging pathogens?

- ☐ Yes
- ☐ No

Laboratory diagnostics:

17. On average, how many samples are successfully processed on a daily basis?

- ☐ <30
- ☐ 31-60
- ☐ 61-90
- ☐ 91-120
- ☐ >120

18. Have you ever had to deny sample analyses?

- ☐ Yes
- ☐ No

19. For what reason?

☐ Insufficient equipment
☐ Insufficient diagnostic material
☐ Insufficient staff
☐ Insufficient staff training
☐ Other:

_____

20. Can your laboratory successfully carry out the whole of the diagnosing procedure, from sample collecting to asseptical examination and diagnostic?

☐ Yes
☐ No

21. Does the work rate conveniently satisfy timely result delivery?

☐ Yes
☐ No

22. If not, which of the following would stand as most relevant in order to achieve a satisfactory work rate?

☐ More personnel
☐ Better equipment
☐ Better working materials
☐ Applying other technical procedures
☐ Other:

_____

23. Which of the following diagnostic methods are you capable of providing?

☐ Microscopic examination
☐ Histological examination

-Isolation and identification:

☐ through artificial culture media
☐ through staining methods
☐ through bacteria biochemical activity
☐ through serology
☐ through commercial phenotypic tests
☐ through antibiotic testing

☐ Polyclonal and monoclonal antibodies
☐ Latex Coagulation test
☐

Coaggulatination test
☐ Agglutination test
☐ Fluorescent antibody technique
☐ Enzyme Immunoassay
☐ Immunohistochemical technique
☐ Immunomagnetic seperation of antigens
☐ Immunodiffusion
☐ Immunoelectrophoresis
☐ Western Blot Analises
☐ Polymerase Chain Reaction
☐ ELISA test
☐ Trypsin digest test
☐ Antibiotic sensitivity testing E-test

☐ Other:

_____

24. Which of the following diagnostic methods would immediately and most significantly enhance your response capacity?

☐ Microscopic examination
☐ Histological examination

-Isolation and identification:

☐ through artificial culture media
☐ through staining methods
☐ through bacteria biochemical activity
☐ through serology
☐ through commercial phenotypic tests
☐ through antibiotic testing
☐ Polyclonal and monoclonal antibodies
☐ Latex Coagulation test
☐ Coaggulatination test
☐ Fluorescent antibody technique
☐ Enzyme Immunoassay
☐ Immunohistochemical technique
☐ Immunomagnetic seperation of antigens
☐ Immunodiffusion
☐ Immunoelectrophoresis
☐ Western Blot Analises
☐ Polymerase Chain Reaction
☐ ELISA test
☐ Trypsin digest test
☐ Antibiotic sensitivity testing E-test

☐ Other:

_____

25. Please state why the above techniques would enhance your diagnosing capacity.

_____
_____
_____

Current pathogen findings:

26. From the following list of OIE notifiable diseases, which have been identified in your laboratory?

Finfish:

- ☐ Epizootic haematopoietic necrosis
- ☐ Infectious haematopoietic necrosis
- ☐ Spring viraemia of carp
- ☐ Viral haemorrhagic septicaemia
- ☐ Infectious salmon anaemia
- ☐ Epizootic ulcerative syndrome
- ☐ Gyrodactylosis (*Gyrodactylus salaris*)
- ☐ Red sea bream iridoviral disease
- ☐ Koi herpesvirus disease

Molluscs:

- ☐ Infection with *Bonamia ostreae*
- ☐ Infection with *Bonamia exitiosa*
- ☐ Infection with *Marteilia refringens*
- ☐ Infection with *Perkinsus marinus*
- ☐ Infection with *Perkinsus olseni*
- ☐ Infection with *Xenohaliotis californiensis*
- ☐ Infection with abalone herpes-like virus.

Crustaceans:

- ☐ Taura syndrome
- ☐ White spot disease
- ☐ Yellow head disease
- ☐ Tetrahedral baculovirosis (*Baculovirus penaei*)
- ☐ Infectious hypodermal and haematopoietic necrosis
- ☐ Spherical baculovirosis (*Penaeus monodon*-type baculovirus
- ☐ Crayfish plague (*Aphanomyces astaci*)
- ☐ Infectious myonecrosis
- ☐ White tail disease

Amphibians:

- ☐ Infection with *Batrachochytrium dendrobatidis*
- ☐ Infection with ranavirus

27. What other pathogens have you recently identified with claims of serious mortality/morbidity/zoonotic character?

_____

_____

28. In what order have species been subject to most testing in your laboratory?

	Aquaculture		Wild Capture	
	Brackish water	Fresh water	Brackish water	Fresh water
Finfish				
Molluscs				
Crustaceans				
Amphibians				

29. What region of the country do most of your samples come from?

- ☐ North Province
- ☐ Central Province
- ☐ Eastern Province
- ☐ Western Province
- ☐ Southern Province
- ☐ Sabaragamua Province
- ☐ North-Central Province
- ☐ North-Western Province
- ☐ Uva Province

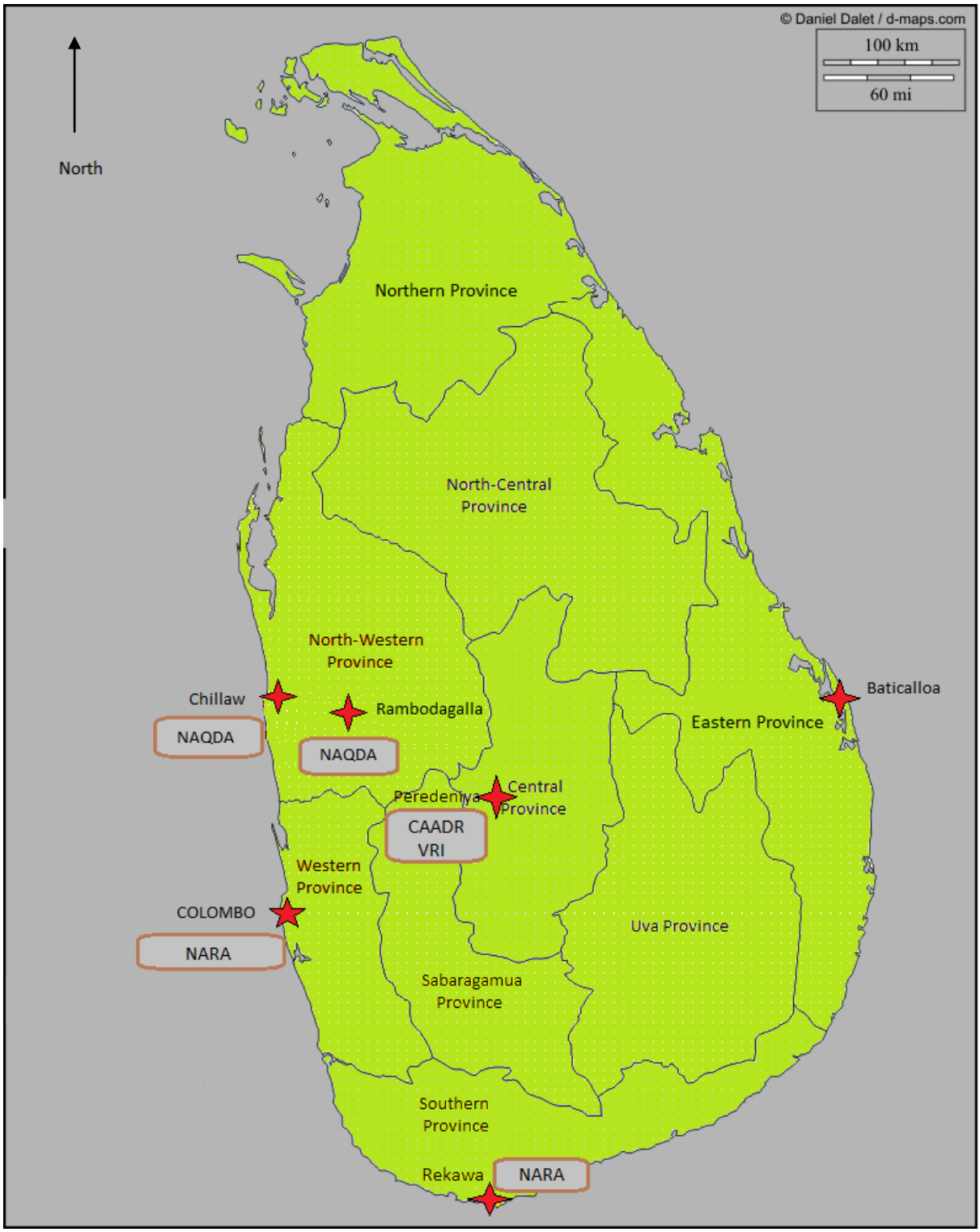
30. To what region do most pathogen findings correspond to?

- ☐ North Province
- ☐ Central Province
- ☐ Eastern Province
- ☐ Western Province
- ☐ Southern Province
- ☐ Sabaragamua Province
- ☐ North-Central Province
- ☐ North-Western Province
- ☐ Uva Province

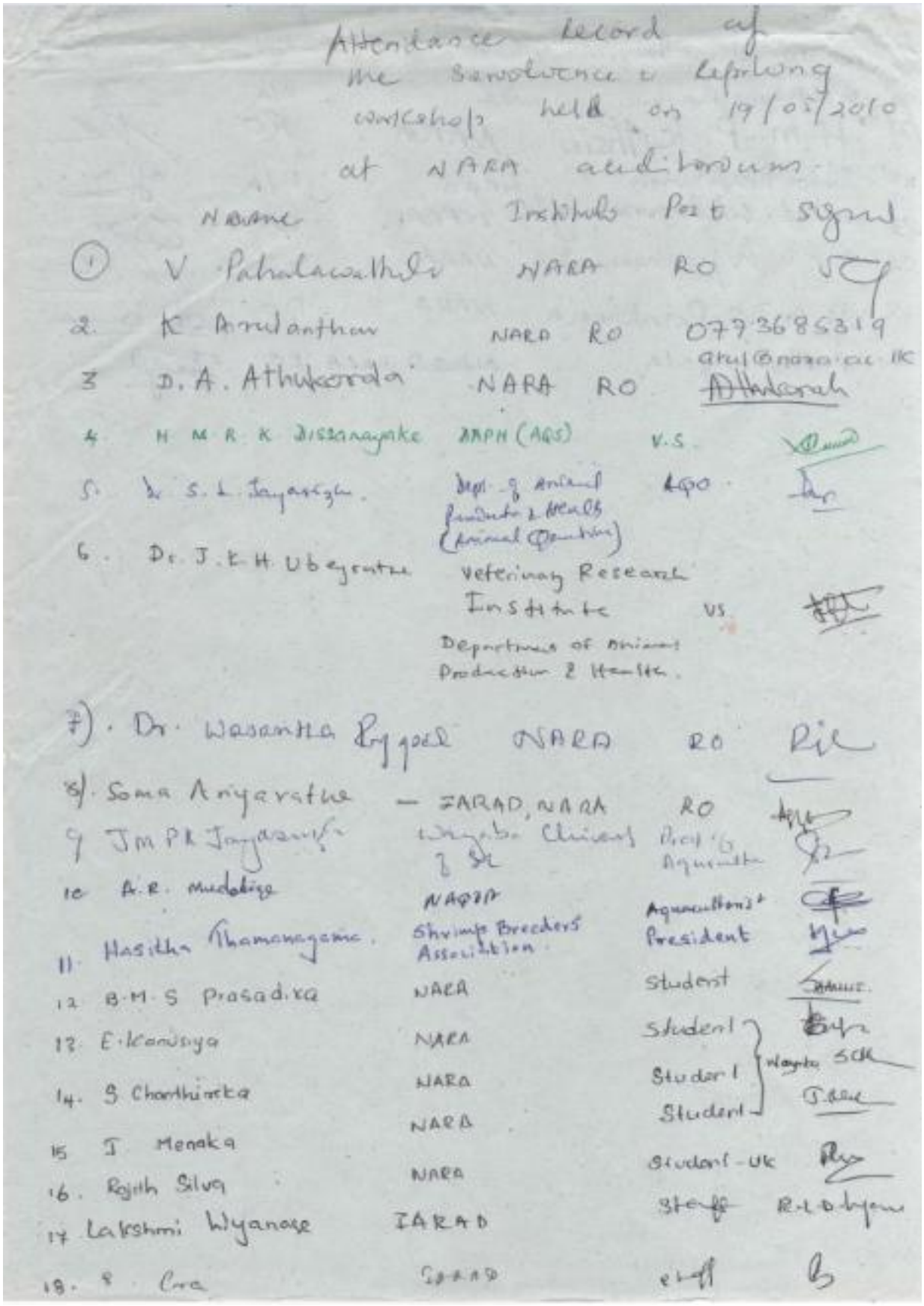
31. To what species do most pathogen findings correspond to?

	Aquaculture		Wild Capture	
	Brackish water	Fresh water	Brackish water	Fresh water
Finfish				
Molluscs				
Crustaceans				
Amphibians				

Annexure XIII: Location of the main laboratories diagnosing for aquatic animal pathogens.

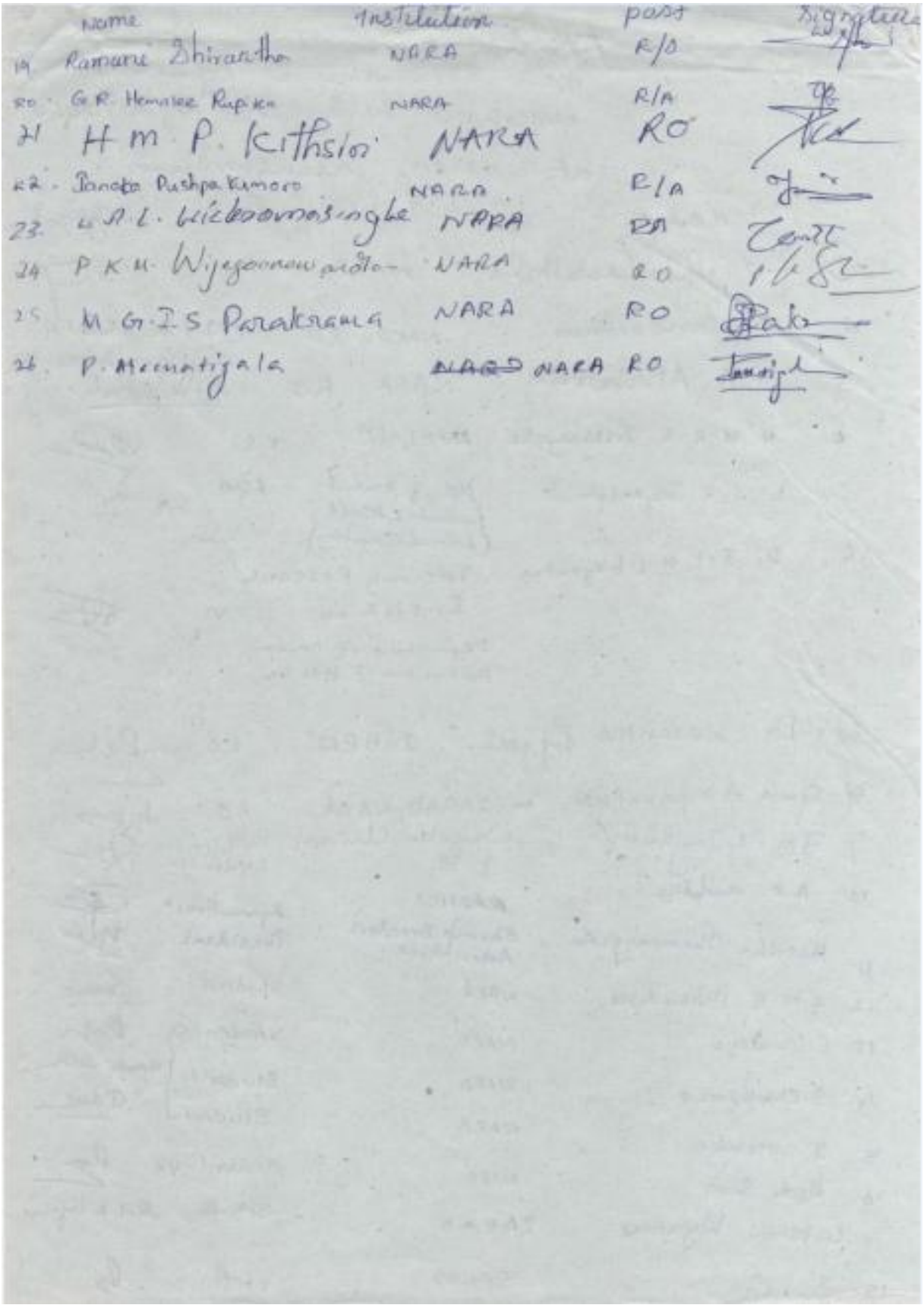

© Daniel Dalet / d-maps.com
100 km
60 mi
North
Northern Province
North-Central Province
North-Western Province
Chillaw
NAQDA
Rambodagalla
NAQDA
Baticalloa
Eastern Province
Peredeniya
Central Province
CAADR
VRI
Western Province
COLOMBO
NARA
Uva Province
Sabaragamua Province
Southern Province
Rekawa
NARA

Annexure XIV: Attendance sheet for the first stakeholders meeting.



Attendance record of the Surveillance & Reporting workshop held on 19/01/2010 at NARA auditorium.

	Name	Institute	Post	Signature
1	V. Pahalawaththa	NARA	RO	
2.	K. Arulanthan	NARA	RO	0773685319 arul@nara.ac.lk
3	D. A. Athukorala	NARA	RO	
4	H M R K Dissanayake	DAPH (AQS)	V.S	
5.	Dr S. L. Jayasinghe.	Dept. of Animal Production & Health (Animal Quarantine)	AQO	
6.	Dr. J. K. H. Ubeyaratne	Veterinary Research Institute / Department of Animal Production & Health.	VS	
7).	Dr. Wasantha Rupasinghe	NARA	RO	
8).	Soma Ariyaratne	IARAD, NARA	Dep. Director & Dr	RO
9	JMPK Jayasinghe		Prof. & Aquatic	
10	A.R. Mudalige	NAQDA		
11.	Hasitha Ranamagamage.	Shrimp Breeders Association	Aquaculturist / President	
12	B.M.S Prasadika	NARA	Student	
13	E. Keerthiya	NARA	Student	
14.	S Chathurika	NARA	Student	
15	I Menaka	NARA	Student	
16.	Rajith Silva	NARA	Student - UK	
17	Lakshmi Wijayarathne	IARAD	Staff	R.L.D.Wijesinghe
18.	S Cooray	IARAD	Staff	



	Name	Institution	post	Signature
19.	Ramani Dhirantha	NARA	R/O	
20.	G.R. Hemalee Rupika	NARA	R/A	
21	H m P. Kithsiri	NARA	RO	
22.	Pancha Pushpa Kumara	NARA	R/A	
23.	W.A.L. Wickramasinghe	NARA	RA	
24	P K H. Wijegunawardana	NARA	RO	
25	M G.I.S Parakrama	NARA	RO	
26.	P. Marmatigala	~~NARA~~ NARA	RO	

Annexure XV: Suggested members for the CCAAHM.

Institutions	Persons	CCAAHM	
		Crustaceans	Finfish
NARA: Slave Island, Colombo 15, Sri Lanka Tel.: 94 11 2521000/2521006 Fax: 94 11 2521932 www.nara.ac.lk	Dr. P. Wijegoonawardane Dr. Wasantha Rajapaksha	✓	✓
NAQDA: Nº41/1 New Parliament Rd., Pelawatte, Battaramulla, Sri Lanka. Tel.: 94 11 2786495/2786677 Fax: 94 11 2786493 www.naqda.gov.lk	- Director General Professor E. I. L. de Silva	✓	✓
	-Director Fresh water Aquaculture Development Mr. R. A. Ranasinghe		✓
	- Director Coastal Aquaculture Mr P. N. Chandraratne	✓	
	Mr. J.A. Athula	✓	
	Mr. Mudalige		✓
	Mr. Danwatta		✓
	Mr. Ranathunga		✓
VIC: Welisara, Ragama, Sri Lanka Tel.: 00 94 11-2958213	Dr. (Mrs.) G. R. Rajapaksha		✓
CAADDR University of Peradeniya, Peradeniya, Sri Lanka Tel.: 94 060 2804882 Fax: 081-2392186 www.pdn.ac.lk/vet/outreach_caaddr.php e-mail: caaddr@pdn.ac.lk	- Dr. A. Arulkanthan - Dr. Samanthica - Dr. M.N.M. Fouzi	✓	✓
VRI: P.O. Box 28, Gannoruwa	Director of Veterinary Research Dr. J. Dharmawardane	✓	✓

Peredeniya Sri Lanka Tel.: 94 81 2388312 Fax: 94 81 2387379 www.vri.lk			
DAPH P.O. Box 13 Peradeniya Sri Lanka Tel.:81 2388184/2388189/2388337 www.daph.gov.lk	-Director General Dr. Nimal Chandrasiri -Director Animal Health Dr. H. M. A. Chandrasoma	✓	✓
Veterinary Quarantine Station 42 Morgan Rd. Colombo 2, Sri Lanka Tel./Fax: 94 11 2448683	Dr. Hewa Kopparage (Chief Quarantine Officer)	✓	✓
MOFAR New Secretariat, Maligawatta, Colombo 10, Sri Lanka Tel.: 94 11 2446183 Fax: 94 11 2541184 www.fisheries.gov.lk	-Director of Planning and Monitoring Mr. N. Abeywickrama -Director General Development Mr. Indra Ranasinghe	✓	✓
Ornamental Fish Breeders Association			✓
Ornamental Fish Exporters Association			✓
Shrimp Breeders Association		✓	
Sri Lanka Aquaculture Development Alliance (SLADA)		✓	✓
Academic aquatic animal health experts	Professor J. P. K. M. Jayasinghe (Jaffna University, Sri Lanka)	✓	✓