Annex 1

COMPOSITION OF THE INTERNATIONAL EMERGENCY DISEASE INVESTIGATION TASK FORCE ON A SERIOUS FISH DISEASE OUTBREAK IN THE CHOBE/ZAMBEZI RIVER SYSTEM

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Annex 2

PROCEDURES FOR INVESTIGATING A DISEASE OUTBREAK

There are 9 basic steps[5] for investigating an outbreak of a disease, however, not all steps are necessarily included in every investigation nor do they follow the same sequence, and several steps may be taken simultaneously:

<table>
<tr><th colspan="2">9 Basic steps</th><th colspan="3">Information/Action required</th></tr>
<tr><td>1</td><td>Establish a diagnosis</td><td colspan="3">Provisional diagnosis based on:
species of fish affected
clinical signs
gross pathology
seasonality (if applicable)
Verification of provisional diagnosis based on laboratory results</td></tr>
<tr><td>2</td><td>Define a “case”</td><td colspan="3">A ‘case definition’ is simply an agreed set of rules which permits investigators to uniformly decide that a particular individual has or does nor have a particular disease as defined; it is important to develop a set of rules that will define both suspect and confirmed cases.
By definition, a case definition is a set of standard criteria for deciding whether an individual study unit of interest has a particular disease or other outcome of interest. The study unit may be an individual animal or group of animals such as a pond of shrimp, a cage of fish, an entire farm or a village, an entire river system.</td></tr>
<tr><td>3</td><td>Confirm that an outbreak is actually occurring</td><td colspan="3">It is important to know the normal percentage of a mortality event versus an outbreak caused by a disease, for example. Confirmation that an outbreak is actually occurring is particularly required in cases where a disease is endemic or prevalent.</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">Characterise the outbreak in terms of time, afected/ unaffected fish, and place</td><td>Time:
• What is the exact period of the outbreak?
• Given the diagnosis, what is the probably period of exposure?
• Is the outbreak most likely a common source (e.g. intoxication, contaminated water or equipment), propagated (e.g. animal to animal transmission as in infectious agents) or both?</td><td>Fish:
• Any characteristic about the fish for which specific attack rates vary?
• Which groups have the highest and which have the lowest attack rates?
Example of computation for Attack Rate (AR):
AR_1 = Number with Disease/Total # of fish in a sample</td><td>Place:
• Significant features of the geographical distribution of cases?
• Relevant attack rates?</td></tr>
<tr><td colspan="3">Time: Duration of an outbreak is influenced by: the # of susceptible animals exposed to a source of infection which become infected; the period of time over which susceptible animals are exposed to the infection source; the minimum and maximum incubation period of the disease.
Fish: species, age, sex and geographical origin
Place: for example in farmed fish, looking at patterns in different ponds, making diagram are useful; type of fishery ital information about the Chobe-Zambezi River system, e.g. fish species, fish stocking activities, water quality and other environmental data, etc.</td></tr>
</table>

[5] From Lilley, J.H., Callinan, R.B., Chinabut, S., Kanchanakhan, S., MacRae, I.H. and Phillips, M.J. 1998. EUS Technical Handbook. AAHRI, Bangkok. 88 p.

9 Basic steps (Continued)		Information/Action required
5	Analysing the data	Include specific factors such as species, age, sex, etc. Analysis of time, place and fish data
6	Working hypothesis	Based on outcomes of 5: Whether an outbreak is common source or propagating? If common source, whether it is point or multiple exposure? Mode of transmission – contact, vehicle or vector? Any hypothesis should be compatible with facts.
7	Intensive follow-up	Clinical, pathological and microbiological examinations; water quality data analysis; relevance of recent meteorological data Epidemiological follow-up – search for additional cases Flow charts of management and movements of fish, water and equipment Transmission trials
8	Control and prevention	Recommendations and advice to terminate the outbreak (if possible) and reduce the risk of similar or future outbreaks
9	Reporting	Written report to serve a permanent record as reference for future outbreaks. Background, methods, results, case definition, hypothesis, financial impacts, recommendations, appendices containing laboratory reports, etc. All other relevant information, for e.g.: Any human health implications Analogy to other disease outbreaks Marketing of fish Local fish disease diagnostic capacities (fisheries, veterinary and/or other relevant departments/universities)

Annex 3

STANDARDIZED PROCEDURES FOR PARASITOLOGY, BACTERIOLOGY, VIROLOGY AND HISTOPATHOLOGY

Bacteriology examination

Only clinically diseased specimens were subjected to bacterial isolation using tryptone soya agar (TSA) or cytophaga agar (CA). Fish with white patches on the body were subjected to flexibacterial isolation using CA while fish with haemorrhagic lesion on the body or showing abdominal swelling were used for bacterial isolation in TSA.

Fish were sacrificed by a pit in the brain or a cut in the notochord. For external surface, the wound surfaces were cleaned with a tissue paper or cotton. External contamination were disinfected using hot spatula or wiped with 75 percent alcohol. Using a sterile scalpel blade, a cut was made through the wound surface and a sterile bacterial loop was used to isolate bacteria in the muscle tissue beneath the wound. For internal organs, fish abdomen was aseptically opened with a sterile pair of scissors or a scalpel blade. A small cut in the liver, kidney or spleen was made using a sterile scalpel blade and bacteria was isolated using a sterile bacterial loop and placed in TSA medium. Agar plates are incubated at room temperature under moisture container. Isolated bacteria may need to be sub-cultured before transferring into transporting medium containing TSA.

Mycology examination

Only clinically diseased specimens with visible oomycete infection were subjected to oomycete isolation using glucose-peptone agar (GP).

For large fish, those showing moderate, pale, raised, dermal lesions are most suitable for oomycete isolation attempts. Scales around the periphery of the lesion were removed and the underlying skin was seared with a red-hot spatula to sterilise the surface. Using a sterile scalpel blade and a sterile fine-pointed forceps, a cut was made through the stratum compactum underlying the seared area and the underlying muscle was exposed by cutting horizontally and reflecting superficial tissues. In order to prevent contamination, instruments should not make contact with external surface. Using aseptic techniques, affected muscle, approximately 2 mm^3, were carefully excised and placed in a petri dish containing GP agar with penicillin G (100 units/ml) and oxolinic acid (100 µg/ml).

Specimens smaller than <20 cm in length can be sampled by cutting the fish into two using a sterile scalpel and slicing a cross-section through the fish at the edge of the lesion. Flame the scalpel until red-hot and use this to sterilise the exposed surface of the muscle. Use a small sterile scalpel blade to cut out a circular block of

muscle (2-4 mm^3) from beneath the lesion and place it in GP agar plate. Seal plates, incubate at room temperature or at 22 °C – 25 °C and examine daily. Emerging hyphal tips should be repeatedly transferred on to fresh plates of GP agar until cultures are free of contamination.

During the field visit, the GP plates were incubated or kept on top of small refrigerator in the hotel room to keep the plate warm at 22 °C–25 °C. As the hotel's room temperatures were around 15 °C–20 °C, the oomycete hyphae were transferred from GP plates to GP tubes before transport to AAHRI.

Virology examination

Only clinically diseased specimens were subjected to virus isolation. One gram of pooled organs was placed in a vial containing transporting medium, Hanks' balanced salt solution (HBSS) containing penicillin (800 IU/ml), streptomycin (800 µg/ml) and 2 percent serum (one volume of organs in nine volumes of transportation fluid). The specimens were kept in HBSS vials and stored in a cool box until virus extraction.

Virus extraction had been carried out within 10 hrs after fish sampling using the following procedures. Decant transporting medium from organ sample, homogenize organ pools using a mortar and pestle until a paste is obtained followed by dilution in fresh transport medium at a dilution rate of 1/10. Sterile fine sand was added to facilitate grinding. Tissue debris and sand were separated using a hand centrifuge to obtain clear tissue extract. Extracts were diluted using HBSS (1:50 final dilution) and filtered through 0.45 micron syringe-attached disposable filter units. Extracts were kept in cool box prior to transport to AAHRI in Bangkok.

Simultaneous cell culture and extract inoculations were carried out using 2 different fish cell lines, EPC and BF2. Viral isolation was conducted in 24-well plates. The following steps are general procedures practised at AAHRI:

1. The 24-well plate is first seeded with a single cell suspension of the fish cell line in maintenance medium (L-15 medium containing 2 percent fetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin).
2. Each well receives 1.3-1.4 ml of cell suspension. Cells with complete monolayer in 25 cm^2 tissue culture flask is sufficient to produce 80 to 90 percent confluent monolayer in 1 day after seeding in one 24 well tissue culture plate.
3. One tissue extract (1:50 dilution) is immediately inoculated into 2 wells. First well receives 200 µl inoculum; while the second well receives 50 µl inoculum. The same numbers of replicate wells are used as negative controls for each plate.
4. The tissue extract-inoculated cells are incubated at 22 °C and observed daily for cytopathic effect or CPE for at least 14 days.
5. A first blind passage of culture fluids is performed on days 7 to 10. Viral passage or subculture is done by transferring 200 µl of supernatant from each well to fresh culture 24-wells plate. CPE observation is still continuing

in the old plates for a further 5 to 7 days. Second blind passage was also carried out.

6. Samples showing CPE in which the cell monolayer changed, disintegrated, sloughed off the surface of the tissue-culture wells or ended with cell lysis, will be passaged to provide larger quantities of suspect virus. If viruses are isolated, the supernatants will be collected, aliquoted in tubes with 1 ml quantities and stored, some tubes at 4 °C and some tubes at –20 °C or –80 °C, for further characterisation.

Histology examination

Procedures for collecting samples for histology follow the steps below:

1. Sample only live or moribund specimens of fish with clinical lesions.
2. Take samples of skin/muscle (<1 cm^3), including the leading edge of the lesion and the surrounding tissue. Parts of internal organs may also be collected. For small fish, the fish operculum and abdomen were cut and opened.
3. Fix the tissues or fish specimens immediately in 10 percent formalin. The amount of formalin shall be 10 times the volume of the tissue to be fixed. The tissues were properly fixed for at least 24 hour.
4. Transfer the fixed tissue into small bags with formalin-moistened tissue paper them wrap properly to prevent leakage or smell.
5. Transport the bags of fish tissue in a cool box to AAHRI for analysis.

Histological procedures include processing of the fixed tissue involves dehydration through ascending alcohol grades, clearing in a wax-miscible agent and impregnation with wax in an automate tissue processor. The blocks of fish tissue are cut at about 5 µm and mounted on a glass slide. Before staining, the section must be completely de-waxed and stained in haematoxylin and eosin (H&E). H&E and general fungus stains (e.g. Grocott's stain) will demonstrate typical granulomas and invasive hyphae.

Annex 4

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Annex 5

RECOMMENDATIONS OF THE FAO LILONGWE WORKSHOP

Recommendations, outputs and agreed follow-ups of the Lilongwe Workshop

In order to improve aquatic biosecurity in Southern Africa, the participants at the FAO Workshop on Development of an Aquatic Biosecurity Framework for Southern Africa held at the Sunbird Hotel in Lilongwe, Malawi from 22-24 April 2008 made the following recommendations:

1. Participants strongly recommended that the countries in the region should work closely in collaboration with the Food and Agriculture Organization and the World Organisation for Animal Health in addressing matters pertaining to aquatic animal health and biosecurity.
2. FAO should write to participating governments participating in the Regional TCP Project to highlight the importance of establishing formal focal points (akin to those established under an OIE initiative), asking for nominations. It was suggested that workshop attendees be the FAO focal point for aquatic biosecurity issues. It would be necessary to develop terms of reference for the focal points, including responsibilities and accountability, including raising awareness.
3. FAO should develop a follow-up project aimed at aquatic biosecurity capacity building in Southern Africa. There is also an urgent need for a regional project for evaluating legal frameworks for aquatic biosecurity (with the need to link biodiversity, production and trade). Several countries advised of their intention to write a letter of support/request to the FAO for a regional project addressing both legal and capacity building issues.
4. The University of Zambia's School of Veterinary Medicine, through Dr Hang'ombe Bernard Mudenda, was identified as potential regional diagnostic centre and Uganda as a regional coordination centre.
5. FAO should develop a Southern Africa regional model on import risk assessment for introductions and transfers of live aquatic animals.
6. Ministerial level meeting for Southern African countries should be held to discuss aquatic animal biosecurity needs.
7. There is a need for a Web site on aquatic biosecurity to assist the Southern African region on aquatic biosecurity issues. Participants recommended the establishment of a regional aquatic biosecurity information network including a dedicated website. As the first step, Mr Wilson Waiswa Mwanja

of Uganda would coordinate establishment of an email group for networking on aquatic biosecurity issues.

8. The participants identified the need for a joint FAO/OIE/Workshop statement as an outcome of the workshop.

Follow-up activities

The follow-up activities listed below are being initiated/completed.

- The Aquatic Biosecurity Framework which will contain the broad development needs and recommendations for projects and activities with associated timelines aimed at enhancing southern African region's (as well as individual participating countries) capacity to effectively manage aquatic biosecurity risks and the Workshop Report are being finalized.
- Correspondence concerning establishing a communication platform on aquatic biosecurity among fisheries and focal points (FAO and OIE) in southern Africa had been initiated. The representative from Uganda volunteered to take a lead on this.
- At the recommendation of this regional workshop, a number of FAO focal points participated in the OIE seminar on "OIE international standards, a lever for growth in the fisheries and aquaculture sector in Southern Africa" organized by the OIE Sub-Regional Representative, held in Maputo, Mozambique, from June 10-12, 2008.
- Discussion are being made to include the southern Africa aquatic biosecurity framework in the broad FAO programme of work on SPADA (Special Programme for Aquaculture Development in Africa) and the aquatic biosecurity workshop participants to be included in the newly established Aquaculture Network for Africa (ANAF).

Annex 6

RECOMMENDATIONS OF THE OIE MAPUTO WORKSHOP

***"OIE international standards, a lever for growth in the fisheries and aquaculture sector in southern Africa"* Maputo, Mozambique, 10–12 June 2008**

Recommendations

Considering

- OIE's mandate and responsibilities to promote aquatic animal health; and
- the international resolve and numerous instruments on fisheries and aquaculture in relation to food security, trade, environmental concerns, income generation and achievement of the *Millennium Development Goals;* and
- the potential benefits from sustainable fisheries and aquaculture and the opportunities to meet increasing demand for food from fish and other aquatic animals, as well the enhancement of natural resources; and
- the need to improve skills, knowledge and information exchange on aquatic animal diseases in the OIE Members in the SADC region; and
- the crucial role played by veterinary and other aquatic animal health professionals in the development and sustainability of the fisheries and aquaculture sector in the OIE Members in the SADC region; and
- the need for harmonised development of the fisheries and aquaculture sector across the SADC region, both at private and public levels; and
- the international obligations of the countries in the region as Members of both the OIE and the *World Trade Organisation* (WTO); and
- the recent *epizootic ulcerative syndrome* (EUS) outbreak in the Chobe-Zambezi river catchment and the questions it raises with regard to preparedness and disease intelligence at national and regional levels;

the OIE seminar on International Standards : a level for growth in the fisheries and aquaculture sector in Southern Africa, recommends:

To the OIE Members in southern Africa :

1. To ensure that OIE Delegates appoint the aquatic animal health focal points and that these appointees be officially communicated and regularly updated to the OIE Central Bureau.
2. To provide national focal points with adequate resources in order to fulfill their terms of reference.

3. To ensure that the OIE Delegates provide the nominated national OIE focal points with the reports from the *Aquatic Animal Health Standards Commission* and that the focal points coordinate the in-country consultation to provide a consolidated national response for submission to the OIE through the OIE Delegate and hence take an active part in the OIE standard setting process.
4. To ensure that national OIE focal points assist the OIE Delegate so as to comply with reporting requirements to the OIE through the WAHIS reporting system.
5. To encourage twinning between national diagnostic laboratories and with OIE Reference Laboratories. To encourage similar agreements with OIE Collaborating Centers.
6. To encourage the inclusion of aquatic animal health issues into the veterinary, fisheries and aquaculture curricula and provide opportunities for continuous education.
7. To promote dialogue between veterinary authorities or other relevant competent authorities, as well as the private sector, to identify their respective roles and responsibilities in aquatic animal health matters.
8. To review the national legislative framework for allowing the development of the fisheries and aquaculture sector.
9. To prioritise aquatic animal diseases of concern and fast track implementation of surveillance programmes in line with art. 13.9 of the *SADC Protocol on Fisheries* (2001) and OIE guidelines. To enhance cross-border cooperation between competent authorities to control aquatic animal diseases.

To the OIE Central Bureau and the Sub-Regional Representation for Southern Africa:

10. To facilitate OIE Members in the surveillance and notification of aquatic animal diseases by supporting training on the use of WAHIS.
11. To coordinate and support the establishment of a regional aquatic animal health network for fisheries and aquaculture in southern Africa in close collaboration with relevant bodies at national, regional and international level.
12. To promote the inclusion of aquatic animal health training into the ongoing process of harmonisation of the veterinary curriculum.

Endorsed by all participants on 12 June 2008 in Maputo, Mozambique.

Source:

www.rr-africa.oie.int/docspdf/en/Mozambique%202008%20Recommendations.pdf

This document is the final report of the work carried out by the International Emergency Disease Investigation Task Force on a Serious Finfish Disease in Southern Africa, a joint undertaking by the Food and Agriculture Organization of the United Nations (FAO), Botswana's Department of Wildlife and National Parks (DWNP) and Department of Animal Health and Production (DAPH), the Aquatic Animal Health Research Institute (AAHRI) of Thailand's Department of Fisheries and the Network of Aquaculture Centres in Asia and the Pacific (NACA), as a result of a technical mission to Botswana undertaken from 18 to 26 May 2007 and the subsequent outcomes of laboratory analysis of field samples conducted by AAHRI. This report provides comprehensive information on the outcomes of the 2007 Task Force investigation, building on earlier reports, and including further updates on epizootic ulcerative syndrome (EUS) occurrence in southern Africa based on an active surveillance programme that was implemented by FAO and partners in late 2007 until 2008. It also includes other ongoing activities and developments aimed at further enhancing aquatic biosecurity in southern Africa.