Report of the Emergency Disease Control Task Force on a Serious Disease of Koi and Common Carps in Indonesia, June 2002

Network of Aquaculture Centres in Asia-Pacific

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Acknowledgements

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Executive Summary

An Emergency Disease Control Task Force on a Serious Disease of Koi and Common Carps in Indonesia was organised by NACA in June 2002 at the request of the Government of Indonesia. The Task Force (consisted of international and local experts and a number of participating laboratories and organizations) conducted an emergency assessment of the disease situation in July through field observations (*i.e.*, field visits, local/district officials and farmer interviews) and laboratory examinations (*e.g.*, histopathology, virology, PCR, and EM) of collected samples.

The Task Force collected vital information about the disease occurrence, but could not make a confirmatory diagnosis of the aetiological agent/s involved in the disease under consideration.

The Task Force findings revealed that an infectious agent/s is involved in the current outbreak (based on epidemiological observations such as sudden onset, rapid spread, specificity to koi and common carp, analogy with Koi herpes virus (KHV) outbreaks), that the disease was introduced to Indonesia through fish importation and spread into other areas through fish movements. The observed pathology did not reveal a diagnosis of the earlier suspected KHV, no virus could be isolated nor virions observed through electron microscopy. However, KHV was detected from all case samples which indicates that KHV might have played a role in the observed mortalities.

This serious disease outbreak in Indonesia will have a huge trade implication that will affect the high value koi carp and the regionally important food fish common carp. A careful diagnosis is essential and this will be the subject of future follow-up work as a matter of priority. The Task Force findings recommends that this serious outbreak of koi and common carp be called ***'Mass mortality of koi and common carp'*** until a clear association with KHV or any other specific disease can be established.

Follow-up actions are on-going to further assist the Government of Indonesia in identifying measures to reduce the risks of the disease and its potential spread to neighbouring countries.

I. Background

In June 2002, Indonesia was faced with a serious disease outbreak among koi and common carp populations. In view of the epizootic nature of the disease and potential spread to neighbouring Asian countries, the current economic impact (losses are currently estimated at 50 Billion Indonesian rupiah (1 US$ = 9 000 Indonesia rupiah) and the disease is still spreading). The potential threat to the production of this important food fish group (common carp), the trade implications of these high value species (koi carp) as well as the threats to livelihoods of small farmers and other groups involved in the chain, from seed production, nursing, grow-out and marketing were highlighted in a letter from the Government of Indonesia (dated 20 June 2002 from Dr. Fatuchri Sukadi, Director General of Aquaculture, Ministry of Marine Affairs and Fisheries) requesting NACA's assistance. NACA's response was to organize the Emergency Disease Control Task Force on a Serious Disease of Koi and Common Carps in Indonesia, subsequently referred to as 'Task Force' in this document1 in cooperation with ACIAR2 and AAHRI3. FAO4 and the OIE5 Regional Representation for Asia-Pacific provided valuable advice to the organization and implementation of the terms of reference of the Task Force. They also pledged support to follow up actions.

Preliminary investigations conducted by Indonesian fish health officers with technical assistance from NACA, in June 2002, indicated that the disease was possibly of viral origin based on the pattern of spread of mortality, specificity to koi and common carp and clinical signs which were characteristics of the outbreaks of koi herpes virus (KHV) reported in 1999 in mid-Atlantic USA and in 1998 and 1999 in Israel (Hedrick *et al.* 2000). A comprehensive information packet about KHV was published by OATA6 (2001) and can be found at http://www.ornamentalfish.org; http://www.aquaticsworldwide.org or http://www.enaca.org. Clinical signs from the current Indonesian outbreaks were characterized by severe branchial hemorrhage and necrosis, and hemorrhages on the body surface. The affected populations, which were limited to koi and common carp, were also suffering from non-specific secondary infections of bacterial, parasitic and fungal origin. Internally, the kidney and liver consistently showed abnormal conditions and extensive abdominal adhesions. The disease was reportedly first observed in April 2002 affecting East Java and subsequently spreading to West and Central Java. Almost all koi and common carp farms in Java Island were affected. A diagnosis of the causative agent/s and determination of other possible contributing risk factors were required at the first instance.

This Summary Report contains the major highlights of the findings of the Task Force. A comprehensive report to include detailed annexures of the various reports received and photographs will follow.

1 Network of Aquaculture Centres in Asia-Pacific (NACA)
2 Australian Centre for International Agricultural Research (ACIAR)
3 Aquatic Animal Health Research Institute of Department of Fisheries of Thailand (AAHRI-DOF)
4 Food and Agriculture Organization of the United Nations (FAO)
5 Office International des Epizooties (OIE)
6 Ornamental Aquatic Trade Association (OATA)

II. Terms of Reference (TOR)

The Task Force, composed of a team of experts in aquatic animal health management, pathology, virology and epidemiology, had the following TOR:

- Travel to Indonesia to investigate the disease situation during July 2002;
- Collect relevant information and samples for processing on site as applicable, according to their areas of expertise;
- Work together as a Team and together with local Fish Health team make a confirmatory diagnosis, as far as possible, of the disease;
- Write a comprehensive report of the findings which will include the diagnosis of the disease, and make specific recommendations, as far as possible, on the following:

    - National policy measures to prevent the further spread of disease to uninfected farms and areas;
    - Bio-security and husbandry management measures at farm level;
    - Epidemiological analysis of the outbreak (origin and source, mode of spread, other risk factors) and recommendations on the use of epidemiological surveillance techniques (*e.g.*, data sampling and collection, quantifying the level of infection and disease distribution, determining the origin and source of infection and problem analysis) using the emergency disease situation in Indonesia;
    - Surveillance, monitoring and reporting systems for the disease at national and regional levels through the OIE/NACA reporting system;
    - Health certification requirements for future imports and exports;
    - Isolation procedures for new stocks;
    - General recommendations for retailers and wholesalers;
    - Contingency plan for future outbreaks;
    - Appropriate disposal of fish from affected premises;
    - Feasibility of eradication;
    - Future research and training needs;
    - Public awareness and farmer information support programmes;
    - Other practical preventive measures.

Additionally, the Task Force will also develop recommendations on Asian regional measures that should be taken to reduce risks of trans-boundary spread of the disease, for consideration by the Asia Regional Aquatic Animal Health Advisory Group, and for OIE and FAO.

The financial support to the Task Force was on a cost-sharing arrangement between NACA, ACIAR and the Government of Indonesia.

NACA was responsible for overall coordination, identification and selection of external experts for the Task Force, organization of the trip, provision of a complete briefing to the Task Force, and provision of a package of relevant available technical information . NACA was also responsible for assuring that the terms of reference of the Task Force, including finalisation of the full report, were satisfactorily completed. NACA supported the aquatic animal health specialist and virologist (AAHRI) as members of the Task Force. NACA also coordinated the involvement of other partners in the work (please refer to section on Task Force Members and Partners).

The Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries (MMAF), Government of Indonesia was responsible for providing the local logistical requirements, including arrangements for domestic travel, field visits *etc.*, and provided a Team of local

technical specialist(s) to join the Task Force. MMAF was also responsible for the technical and staff assistance needed to support the work of the Task Force Members, including technical support for storage and processing of samples in local laboratories (as necessary) and providing requested information valuable for the diagnosis and other requirements under the TOR.

ACIAR provided support for the epidemiologist in the Task Force.

NACA maintained contact with FAO throughout the process.

III. Members of Task Force and Participating Scientists and Institutions

The members of the international 'Task Force' were Dr. Angus Cameron of AusVet (Australia), Dr. Somkiat Kanchanakhan of AAHRI, DoF (Thailand) and Dr. Melba B. Reantaso of NACA (Thailand).

The local 'Task Force' consisted of officers and staff of the Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries (MMAF) and officers and staff of the Institute Pertanian Bogor (IPB). The lead scientists ere Dr Achmad Rukyani, Head of the Fish Health Division of the Directorate of Aquaculture and of IPB.

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IV. Activities

International Task Force
The international emergency Task Force visited Indonesia in July 2002 to undertake an emergency assessment of the disease situation and collect samples for laboratory analysis.

Local Task Force
A local task force was formed by the Government of Indonesia to assist the International Task Force and implement the preliminary and immediate control measures recommended at the time of the outbreak (*e.g.*, restriction of movement, information dissemination, *etc.*).

Laboratories
The different laboratories listed in participating institutions conducted the required laboratory examinations (pathology, virology, and immunoassays) on the samples collected by the local and international Task Force members.

V. Highlights of Findings

The highlights of findings included in this summary report are those that resulted from samples collected which satisfied the case definition for the disease outbreak.

A. Case Definition

Establishing a case definition was an important step used in the investigation, as there are many different diseases currently affecting koi and common carp, some of which present similar clinical signs to the disease in question. Case definition establishes whether an individual fish, pond, or tank is suffering from a disease or not. It has therefore become essential to determine which of the various samples collected fit the **case** (a fish or farm that is affected by the disease under consideration) or **non-case** (one which is not affected by this particular disease, although it may currently be suffering from another disease) category. The case definition is used to minimise the expected mis-classification and was strived to be consistently applied in the various examinations and observations used by the Task Force.

The case definitions used for the disease under consideration are:

Outbreak of disease in a pond / tank / cage
An outbreak of disease amongst common carp or koi starting after March 2002 causing >25% mortality of fish within one week of the start of symptoms. All affected fish show gill damage, while some may show no other signs, and some show skin damage.

Case of disease in an individual fish
A koi or common carp, from a pond meeting the outbreak case definition, that shows gill damage, with or without other skin lesions.

B. Description of Samples for Laboratory Examination

A description of the samples which satisfied the above case definitions are given below. Results of laboratory examination for non-case samples were not included in this report.

A. Tapos Village (common carp)

The farm was located mid-stream, water being sourced from the Chinangneng River. In this area, the first farm which experienced disease was located downstream, beginning April. Farmer Joko appeared to be the last farm which experienced the disease outbreak.

Unusual events before the outbreak in the area
Two to three weeks before the outbreak in the area, there was large water supply and low water temperature (approximately 19-20 °C), following a long dry season. Normal temperature was about 26 C. Temperature at the time of sampling was about 23 °C.

Type of farm
Concrete tanks (n=20, tanks are numbered A1-10 and B1-10), running water system, common carp only.

First sign of abnormality
Observed on 7 July afternoon (approximately 2 pm) where fish were observed swimming near screen of water outlet, gasping for air; some fish already dying. The following day, Monday, dead fish (1-2/pond) were observed. By Tuesday, dying fish were observed every minute in all tanks. At this point farmer, decided to make an emergency harvest.

Mortality/Losses
Farmer cannot make an accurate estimate of mortality as different tanks have different mortality rates. In one tank, which normally harvests 1000 kg, only 700 kg were emergency harvested, with loss of about 30% within 3 days. In terms of value, he estimated that the loss in his farm of about 2.5 Million Rupiah (1 US$ = 9 000 Indonesia rupiah). All sizes of fish in his farm were affected. There was no record of introduction of new fish in the farm; last introduction of new fish for stocking was 2-3 months ago.

Marketing of fish
Normally all the harvest was sold to Jakarta directly, where traders will come directly to farms using a packaging of 12.5 kg/package. During the disease outbreak, fish were being sold to local people in the area. Before disease outbreak, the price of fish was 7000 rupiah/kg; during the outbreak, the price was 3000 rupiah/kg; dead fish were given out free.

B. Cirata Reservoir (common carp)

Citarum River System
Cirata Reservoir is one of three reservoirs along the Citarum River – composed of Saguling Reservoir in upper stream, Cirata Reservoir in middle stream and Jatinluhur Reservoir in the downstream. Cirata Reservoir was built in 1986 and presently there are about 33 000 cages in operation. There was no history of a disease outbreak of this nature but there are cases of mortality due to upwelling that occurs every year, particularly during the beginning of the rainy season (October, November, December).

Culture system
Double cages; first layer cage measures 7 x 7 m, 2.5 m water depth for common carp; second layer for tilapia; each cage stocked with 50 kg of fry measuring 100-150 fishes/kg; estimated harvest – 2 tons/cage; discontinuous harvest.

First recorded outbreak of disease under investigation on common carp
Occurred in early July 2002. Clinical signs include reduced feed consumption, lethargy, inconsistent clinical abnormalities except for the characteristic gill necrosis/rot.

Affected species
Mortalities occur only among common carp but not tilapia despite the double cage system. Mostly big fish are affected with characteristic gill rot; small fish in separate cages looked healthy; but cages with small and big fish – both sizes of fish were affected.

Estimates of mortality/losses/emergency harvest
15 kg/cage/day; in the farm visited 4 cages were affected with loss of about 60 kg/day. Farmers harvest fish when they find 4-5 fish dying in the cage.

Other relevant information:
Saguling Reservoir: 4 425 cages; first outbreak with common carp in end of May to early June 2002, no accurate estimate of losses, Farmer Edy estimated about 400 tons

Jatinluhur Reservoir: 2 100 cages; no disease outbreak reported yet
Water flow: it takes about one month for the water from Saguiling Reservoir to reach Cirata Reservoir

C. Ciampea, Udik, Bogor (common carp)

System
Hatchery, grow-out

Species
Common carp

Gross clinical observations
1 kg size fish samples with haemorrhages on surface of abdomen, severe gill necrosis, tail and fin haemorrhages, heavy infection with *Argulus*

Mortality
2 fish/day among big fish; 10 fish/day among small fish

D. Lembah Koi Farm (koi carp)

An area where disease were reported to occur.

E. Risma Koi Farm (koi carp)

An area where diseases were reported to occur.

F. Normal Samples (common carp)

Samples of common carp from a station in Bogor selling fish. According to the vendor, fish samples being sold from an area where there was no history of disease outbreak

C. Epidemiology

A comprehensive epidemiology report was prepared by Dr. Angus Cameron of AusVet and the following are the important highlights of the findings:

1. Confirmation of a disease outbreak
Although it was not possible to accurately estimate the current level of morbidity and mortality, there is good indication to suggest that the 50-100% mortality rates reported by farmers far exceeds what is considered as normal, and therefore a disease outbreak is occurring.

2. Pattern of spread of disease
The pattern is typical of a propagating infectious disease; starting in one location, and subsequently spreading both locally and over long distances. From each new disease location, further local spread has occurred. The evidence of spread of disease within farms after the introduction of fish is also consistent with the disease being caused by an infectious agent.

3. Host range
The disease clearly affects both koi and common carp. There is some evidence that the signs observed in the two species may be somewhat different (based on a very limited number of observations) but in general the pattern of mortality, principal signs and rate of spread of the disease is similar in both species.

4. Nature of the pathogen
Based on the available epidemiological evidence, it can be reported with **extreme confidence** that the disease is caused by a directly transmitted infectious agent. Infectious agents may be directly transmitted from one fish to another, or may require intermediate hosts to complete their life cycle. Pathogens with complex life cycles require more time to pass through the different stages and hosts before they can infect another fish. They are also dependent on the presence of appropriate intermediate hosts, which may limit their range to places where such hosts are available. The rapid transmission of the disease (about 7 days between introduction of infected fish and the onset of clinical signs in in-contact fish), and the observation that transmission has occurred in clean cement tanks with very few potential intermediate hosts present both strongly indicate that the disease is directly transmitted.

5. Cause of the current outbreak
Variation in the behaviour of the disease (*e.g.,* mortality) indicates that there are a number of different causes of the disease (*i.e.,* it is multifactorial). It is clearly possible that a number of the pathogens under consideration may play a role, along with environmental and other factors. However, it is also clear, based on the sudden start, and rapid spread of the outbreak, that there is likely to be (probably a single) **necessary cause**, which is a directly transmitted pathogen. Using this understanding of disease causation, it is possible to eliminate as a possible necessary cause any pathogen that was present in Java prior to the outbreak. This is one aspect of the time sequence criteria for causation. The outbreak is only possible in the presence of the agent. If it was present prior to the commencement of the outbreak, there can be no explanation for the sudden commencement and spread of the disease. There are a number of pieces of evidence that strongly suggest that the cause is viral, and may be Koi Herpes Virus. The host specificity and pattern of mortality is the same as that described for KHV in earlier outbreaks (*analogy)*. The observation in one experiment that the disease recurred in recovered fish once they were stressed is typical of latent herpes virus behaviour, and unlikely in any of the other pathogens under consideration (*experimental)*. The failure of response to treatment with antibiotics reported in a number of cases probably provides a weak evidence that the cause may not be bacterial (*response to treatment)*.

6. Source of the pathogen
Existing evidence indicates that the most likely source is from an exporter based in Hong Kong. Two hypotheses are being considered. First, at least one of the batches of imported fish was latently infected before exportation from Hong Kong where fish showed no signs of disease for a number of weeks after the quarantine period; and after removal from quarantine and mixing with other fish at the importer's premises, the disease recurred, and spread to the other fish. The importer, in order to prevent the disease from spreading to other fish (non-imported fish) transported the larger fish to Blitar farm, a facility used as holding place for stocks. The disease outbreak commenced at the importer's place shortly before 14th

March. The fish sent to Blitar farm were infected 2 or 3 days before transport; they showed signs 4 days after arrival and spread the disease to other fish in the same pond. The second hypothesis is that an illegal import was made by Surya Koi sometime between 17th January and 14th March, from an unknown source, and this was the origin of the pathogen. The presumed index farm in Blitar was not the index farm but was infected through local spread from another nearby farm. The other farms had an unknown source of infection.

7. Distribution of disease
Based on the information available at the time of the mission, the distribution of the disease is on the island of Java. The disease is not yet known to have spread to any other parts of the country. A shipment of 5 Koi imported into Denpasar in Bali showed signs of disease that may have been similar to the current outbreak, around the same time as the initial outbreak in Blitar. The disease was detected in the quarantine station, and no spread has been recorded from there. A report has been received from Germany of mass mortality in a batch of 25 boxes of Koi imported from Indonesia on the 4th and 15th of June. The fish showed signs of disease 7 days later and suffered 80% to 90% mortality. Again, the disease was detected during quarantine, and no spread is thought to have occurred.

8. Portal of entry
The gills are the **most likely** portal of entry, as they filter with large volumes of water and have the most opportunity to come into contact with the pathogen.

It is also possible that the pathogen enters the fish through another route, is circulated through the body (for instance, a viraemia) and then localises in the target organs including the gills. If an intermediate host or mechanical host is involved (*e.g.,* an external parasite), that host may facilitate introduction of the pathogen through the integument.

9. Incubation Period
The incubation period is closer to 7 days than 2 days, and the contact rate is very high, allowing a large number of fish to become infected from a single shedding fish.

10. Risk factors
A number of risk factors have been identified which may play a role in disease development. These include the following: size of fish, oxygen, water flow, rapid collection of affected fish, temperature, water quality, management factors, feed and treatments.

11. Spread of the disease
(a) Spread between enclosures in a farm through fish to fish contact, through water and possibly through movement of dead fish or tissues; (b) Local spread between farms through water and possibly non-water mechanisms such as transport of live fish, or the transport of the pathogen via humans, other animals or inanimate objects (fomites) such as aquaculture equipment, and feed; (c) long distance spread – mainly through fish movements.

12. Trade Implications
It can be expected that most countries importing koi or common carp from Indonesia will suspend this trade until the situation becomes clearer. The ban, which will likely be temporary, may include all of Indonesia in the first instance, until evidence that the disease is confined to Java can be provided. This will require a survey to demonstrate the absence of infection from other islands. The relative importance of this survey depends on the value of the export trade from the rest of Indonesia, and it is suspected that control activities on Java should take higher priority in the short term.

13. Human Health Implications
Available evidence indicates that the disease poses no significant health risk. Some parasites of fish are zoonotic, as are a very limited number of bacteria, but there have been

no known reports of zoonotic viruses of fish. The pathogen is clearly very host specific, not affecting even closely related fish species, which makes it much less likely that it would affect humans or other mammals. If the pathogen is Koi Herpes Virus, it is reportedly very sensitive to temperature, and is inactive or killed at temperatures greater than 30 or 32°C. Few of the herpes viruses are zoonoses, although Herpes B in simians will infect humans. Furthermore, in some outbreaks, large volumes of affected fish were sold or given away to people living in the local area for consumption, and there have been no reports of ill effects as a result of this.

D. Pathology

IA, Stirling University
Described below are the major pathological findings found from samples processed for histopathology:

Tapos Village
Severe necrotizing branchitis (1 of 2 fish) associated with the presence of large numbers of filamentous bacteria typical of columnaris disease. In addition, there were multi-focal myxosporea in gills, plus low numbers of monogeneans, embryonated digenean cercaria and *Icthyopthirius* parasites. Tissue inflammatory response to the parasite was moderate.

Cirata Reservoir
Findings were very similar to Tapos Village samples, with branchial columnaris disease in ½ fish, plus a similar range of other gill parasites, plus trichodinids.

Risma Koi Farm
Severe branchial costiasis in the tissues, with massive numbers of parasites accompanying marked loss of lamellar structure, fusion and inflammation. *Icthyopthirius* and monogenean parasites were also present.

Ciampea, Udik, Bogor
Major findings in all gills included diffuse lamellar epithelial degeneration and exfoliation. Only a few monogenean parasites were present in terms of an obvious pathology. The changes present are typical of those seen in a water-quality/toxicological type of disease problem.

Normal samples of both species revealed no significant abnormalities histopathologically.

UC Davis
The pathology report indicated no microscopic signs of KHV infection in two samples sites (Lembah Koi Farm and Cirata Reservoir); and one sample site (Tapos Village) indicated severe to moderate branchitis and focal areas of severe necrosis. Hypertrophied nuclei with marginated chromatic and flocculent eosinophilic inclusions that are typical for KHV infections were not detected in the gill, kidney or spleen.

AAHRI

Pathology report from AAHRI and Centex Thailand revealed various pathological observations as follows:

Tapos Village
Areas of hyperplasia and fusion of some adjacent secondary lamellae with multifocal necrosis; nuclear hypertrophy, chromatin margination and eosinophilic intranuclear inclusions in gill epithelium. Hepatopancreas with areas of hepatocellular necrosis, inflammation (and possible inclusions (few) foci of periportal inflammation, sometimes with melanomacrophage

center (MMC) formation. Pancreatic tissues (also in spleen and kidney) inflammation and MMCs necrosis, acinar cells with decreased or lack of zymogen granules. Kidney showed proliferation of haematopoietic cells especially macrophages.

Cirata Reservoir
Slight inflammation of pancreatic acinar tissues and mesenteries of hepatopancreas; liver with periportal and bile duct inflammation and focal necrosis; MMC formation. Intestine showed inflammation, multifocal areas of necrosis and possibly coccidian parasites. Kidney showed proliferation of haematopoietic cells especially macrophages.

Normal samples: Sample 1 – gills showed areas of epithelial hyperplasia and fusion of adjacent second lamella (possibly inclusions?). Spleen congested and MMC formation on pancreatic areas and elsewhere in parenchyma. Intestine showed slight to moderate inflammation of the lamina propria including MMCs. Sample 2 - – Kidney showed haematopoetic tissue very cellular, possibly proliferation (?); inflammation of lamina propria of intestine; skin showed inclusions (?) in epithelial cell and connective tissue. Gills hyperplastic, secondary lamella fusion with inclusions (?). Sample 3 – some areas of the gills showed hyperplasia and fusion of adjacent secondary lamellae and presence of trematode parasites (?). Skin showed inclusions (?) in epithelial cells, focal blood cell necrosis in connective tissues.

E. Virology

UC Davis
Tissue extracts from Cirata Reservoir, Lembah and Risma Koi Farms, and Tapos Village were examined. All 11 tissue extracts inoculated onto the KF-1 cell line, the cell line most appropriate for the isolation of KHV, did not result in any observable cytopathic effects (CPE). Since these extracts had arrived in the laboratory after several days of shipping and no refrigeration, it would not be surprising that no viral agents could be isolated.

AAHRI
No viruses could be isolated from 17 tissue extract samples using 5 fish cell lines (EPC, BB, BF-2, DT and SSN-1).

Intervet Norbio Singapore
Virus isolation was attempted on two cell lines, namely GP (a grouper fry cell line obtained from the AVA, Singapore) and the EPC cell line. One blind passage was carried out with PCR positive material, from Tapos Village (C3, C4), Lembah Koi Farm (K7) and Risma Koi Farm (K8). No CPE is evident for both passages. PCR tests on GP-inoculated cells were negative (day 11, second passage). Results for EPC-inoculated cells at the second passage were also negative.

F. Polymerase Chain Reaction (PCR)

UC Davis
PCR analyses using single round PCR for KHV as described by Gilad *et al.* (2002) revealed samples from Cirata Reservoir - 4 samples to be strongly positive for KHV; and three samples were weakly positive.

Intervet Norbio Singapore
DNA was extracted from all the samples using a commercial kit (Qiagen Mini DNA kit). PCR tests were carried out using two pairs of primer sets as described in Gray *et al.* (2002). The expected sizes of the amplified fragments using primer pair KHVF1/R1 and KHVF2/R2 are 365bp and 290bp, respectively. Four of the samples from Lemah Koi and Risma Koi Farms

(K7, K8) and Tapos Village (C3 and C4) were found to be positive for KHV with both pairs of primers. C3 was weakly positive. The rest of the samples were negative for KHV by this method.

In vivo experiments

Some healthy koi fish (<10g) were obtained locally and kept at our facilities. Two were initially injected intra-peritoneally with C2 (Ciampea) and another two with K6 (Lembah Koi Farm). The injection volume was 0.1ml. The injected fish were kept in separate tanks with uninfected healthy koi fish of similar size. All fish remained healthy with no signs of KHV. On day 17, the injected fish were sacrificed and PCR tested. The results were negative. Based on the PCR results, one koi from each tank was later injected intra-muscularly with 0.1ml of K8 and C4. Both fish remained healthy and they were sacrificed on day 14 for PCR testing. The results were negative.

AAHRI
Extracts C1 (Ciampea), C3 and C4 (Cirrata Reservoir), K7 (Lembak Koi Farm) and K8 (Risma Koi Farm) gave positive PCR. As AAHRI did not have positive KHV gene, a positive control was not included in the PCR test.

Local Task Force
Using KHV primer provided by AAHRI, samples from Ciampea and additional samples from Cirata Reservoir collected after the Task Force visit, were positive against KHV. Using HBSS from AAHRI, pooled samples of gill, kidney and spleen of diseased fish were extracted, centrifuged at 2000 rpm for 10 min and then filtered (0.45 *um)*. The filtrate was then used to inject healthy fish. The fish died up to 70% within a 10 days period, while other healthy fish cohabitated with diseased fish died within an 8 day period.

G. Electron Microscopy

UC Davis
No evidence of viral particles found in gill sample received. No microscopic signs of KHV infection.

Intervet Norbio Singapore
Samples for EM were sent to Intervet International, Netherlands for processing and diagnosis. There is quite a bit of distortion in the sections. This could be due to the fixation method employed. Huge crystalline arrays were seen in Ciampea samples (C2) which could be viral in origin. Virions of irregular shape and certainly much smaller (about 100 *nm* to 170 *nm*) than that described by Hedrick *et al. (*2000) could be seen in samples from Lembah Koi (K7) and Ciampea (C1). The virions were not present in large quantities. While it cannot be conclusively identified that the virions seen are indeed herpes viruses by EM, this is not ruled out. The size range of the virions seen are close to that described in Neukirch and Kunz (2001).

VI. Summary of Diagnosis from IA, Stirling University and UC Davis

IA, Stirling University

Diagnosis: 1. Most obviously moderate parasitic and necrotizing branchitis, typical of columnaris disease. 2. On one case, severe branchial costiasis. Pathology report of Prof. Hugh Ferguson had an overall assessment, that the 'sick' fish were indeed suffering from gill disease, severe enough to explain the clinical signs of respiratory disease and mortality observed. There was little evidence in the tissues examined to support the notion that the lesions were due to KHV, although KHV cannot be ruled out, as absence of evidence should never be misconstrued as evidence of absence. There is good evidence to support a diagnosis of columnaris disease in most 'sick' fish, with costiasis as the major problem in one site. A range of other parasites was seen in the gills of most fish, while internal lesions were minimal, suggesting little in the way of systemic involvement. There is nothing in the observed pathology to support a diagnosis of KHV, but non-observation of typical KHV lesions or inclusions in tissues examined does not preclude KHV involvement, and this should be sought using molecular approaches. There is no doubt, however, that the lesions seen would have easily explained the mortality, and although they were typical for the pathogens involved, there could still have been a primary insult by something like a virus.

UC Davis

The causes for the unusual mortality of koi and common carp in Indonesia could not be determined by the analyses that focused on the presence of KHV as the potential pathogen. It was clear that other bacterial, protozoan and metazoan parasites were present in several of the samples examined for microscopic pathology and these could have easily contributed to the losses observed. That KHV was detected by PCR, the most sensitive procedure for determining presence of the virus, suggests that the agent may have had a role in the observed mortality. UC Davis experience with KHV from outbreaks in the USA and Israel indicate that virus isolation and typical microscopic lesions may be found optimally during peak mortality periods but less often after this period. Also, changing water temperatures can greatly influence the progression of KHV infections, sometimes complicating diagnoses. Fish with KHV infections can suffer from secondary infections with numerous other bacterial and protozoan parasites, so finding additional pathogens would not be unusual. Columnaris disease, which can induce similar branchial changes, tends to respond to antibiotic treatments while disease due to KHV does not. KHV involvement would have been confidently associated with the observed losses with observations of typical KHV microscopic changes, particularly nuclear changes, in gills and internal tissues (kidney and spleen) and typical virions directly from tissues processed for electron microscopy observed.

VII. General Conclusions

The general direction of the Task Force investigation was to determine the possible involvement of Koi herpes virus (KHV) as this was the disease whose characteristics were found to be similar to that of the mass mortality of koi and common carp in Indonesia, based on preliminary observations available at that time.

The Task Force investigations revealed that there are supporting evidence based on epidemiological observations (*e.g.,* sudden onset, rapid spread, specificity to koi and common carp, analogy with KHV outbreaks) that an infectious agent/s played a role in the observed mortality and that it has been introduced to Indonesia through fish importation and spread into other areas through fish movements. The epidemiological observation which pointed to Hong Kong as a possible origin of the suspected infected imported stocks needs to be verified with Hong Kong authorities. Preliminary correspondence from Hong Kong official (Dr Trevor Ellis, Senior Veterinary Officer, AFCD) indicated that the only official endorsed export fish shipment to Indonesia during the past 12 months were some goldfish from one of the health certified ornamental fish farm on 24 July 2002. In addition, Hong Kong surveillance programme for local ornamental fish farms did not lead to any positive viral isolations (which includes specific testing for Spring Viremia of Carp Virus). This could very well be a case of illegal importation.

It is difficult at this stage to confirm the identity of the aetiological agent/s involved in the disease outbreak under consideration. As indicated in the pathology reports, there was nothing in the observed pathology that supports the diagnosis of KHV, as the typical KHV pathology (*i.e.*, nuclear changes in gills and internal tissues such as kidney and spleen) were not observed both in tissues for histopathology and typical virions from electron microscopy examination. Although one laboratory result showed the presence of huge crystalline arrays which could be of viral origin, it was later confirmed, after further consultation, that these were believed to be endoplasmic reticulum and that there were no clear herpes-like virus in the samples. Other agents may also be well involved such as parasites, bacteria (based on pathology report) and other environmental factors.

The absence of typical KHV pathology, failed viral isolation, and non-observance of typical virions through electron microscopy should not rule out KHV involvement, and as earlier indicated ‘absence of evidence should not be misinterpreted as evidence of absence’. The detection of KHV by PCR from all case samples indicates that KHV might have played a role in the observed mortalities.

In view of the potential trade implication of this serious disease outbreak affecting the high value koi carp and a regionally important food fish common carp, a careful diagnosis is essential and this should be the subject of future follow-up work as a matter of priority. It is therefore concluded that this serious outbreak of koi and common carp be called ***‘Mass mortality of koi and common carp’*** until a clear association with KHV or any other specific disease can be established.

The Government of Indonesia has been advised to temporarily restrict the movement of koi and common carp and a Ministerial Circular took effect in July 2002. An intensive information dissemination was also undertaken to raise awareness and inform the public sector about relevant information including risks to human health available at that time. The Government of Indonesia was also advised to report the matter to OIE and a report was sent to OIE on 26 June 2002.

VIII. Recommendations for the Government of Indonesia

The following section outlines a list of recommendations for the Government of Indonesia, and for follow-up actions by interested supporting organizations such as ACIAR, FAO, NACA and OIE, which covers some, if not most of the subject areas listed in the Task Force terms of reference.

A. Implement a well-coordinated National Strategy for Aquatic Animal Health

- Develop and Implement Indonesia's National Strategy on Aquatic Animal Health Management
- Establish a National Response Plan (high priority)
- Develop and implement a comprehensive containment program and contingency plans to reduce further losses due to spread of the pathogen/disease concerned as well as outbreaks of other important exotic aquatic animal diseases (high priority)
- Enhance national regulatory frameworks and legislature, improve biosecurity measures, and strengthen national disease surveillance and reporting, to reduce the risk of movement of pathogens, both nationally and internationally, through movement of live aquatic animals.
- Develop and implement control measures
- Prevent uncontrolled spread in high risk systems by destocking
- Establish disease and disease free zones based on the results of the surveillance
- Lift the ban on export of fish from Java, by allowing controlled export from the identified free zones

B. Identification of Agent and Risk Factors (high priority)

- Conduct a detailed epidemiological and diagnostic assessment (pathology, virology, *in-situ* hybridization) of the disease outbreak to confirm the causative agent/s, identify root cause/s, analyze risk factors, and identify management measures for the identified risks.

C. Capacity Building and Improving Facilities for Disease Diagnosis and Control

- Establish and improve viral disease diagnostic facility at an appropriate state agency
- Develop risk analysis capacities for aquatic animal movement
- Improve disease diagnostic capacity at appropriate state laboratory/ies
- Develop human resources and provide training to fish health personnel in relevant state agencies on better management of health in freshwater aquaculture.
- Evaluate capacity for rural farmers to understand health management processes
- Improve capacity for vulnerable rural aqua-farmers to face future outbreaks in their culture systems.
- Develop and improve farmer awareness on better management of aquaculture practices (*e.g.* biosecurity and husbandry management measures at farm level) to reduce vulnerability to disease outbreaks.

D. Disease Information, Surveillance and Reporting

- Develop an improved information system to allow standardised reporting of disease information from the field to the central level, and practical reports designed to assist with control activities to flow back to the field.
- Develop surveillance and monitoring systems to evaluate the progress of control activities and provide early warning of spread to other islands, or the incursion of new exotic diseases.
- Establish disease and disease free zones based on the results of the surveillance for export and trading purposes.
- Develop surveillance programs to define the current distribution of the disease, economic impact and population at risk
- Establish national surveillance and disease reporting system for important aquatic animal diseases affecting freshwater aquaculture.
- Improve Indonesia's participation in the regional Asia-Pacific Quarterly Aquatic Animal Disease Reporting System

E. Extension

- Develop farmer guidelines and extension materials to prevent spread
- Undertake extension activities to inform farmers of the guidelines
- Develop and implement a system to promote producer cooperation in disease control activities (such as a farm accreditation scheme).
- Produce appropriate extension tools and materials to improve their ability to face disease outbreaks.

F. Research

- See also item B
- Research focus on defining appropriate control activities
    - Transmission of free-living virus through water, or by mechanical carriers / fomites / direct contact
    - Carrier, latent infection, period of infectivity after recovery
    - Persistence of agent in tissues after death, infectivity of tissues.
    - Spread - distance in clean water, water treatment factors (temperature, sunlight, UV light?), filtration
    - Drying of ponds and re-stocking – how to prevent re-infection, earthen ponds
    - On-farm control trials
    - Role of other fish and other aquatic animals as carriers
    - Assessment of potential risks to human health

G. Asian Regional Measures to Reduce Risks of trans-boundary spread of the disease, for consideration by the Asia Regional Aquatic Animal Health Advisory Group, and for OIE and FAO.

- Include the current disease 'Mass mortality of koi and common carp' in the regional list of diseases
- Prepare a disease template for use in the disease reporting

Dr. Angus Cameron's Epidemiology Report provides detailed and comprehensive phased recommendations to cover the following: Phase I – Emergency Response; Phase 2 –

Implementation of Control; Phase 3 – Developing Sustainable Management Systems. Most of them are included in the above recommendations.

In August 2002, the Government of Indonesia through the Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries (MMAF) officially requested an emergency technical assistance from FAO which approved on 17 September 2002, a project "Health Management in Freshwater Aquaculture" to be implemented from November 2002 to April 2004 under FAO's Technical Cooperation Programme. The assistance covers some of the recommendations outlined above. The objectives of the project, which are in line with the recommendations of the Task Force, are: (a) strengthen diagnostic and control capabilities within the responsible state agencies for major diseases of cultured freshwater fish, including koi and common carp; (b) improve capacity for vulnerable rural aqua-farmers to face future outbreaks in their culture systems; (c) enhance national regulatory frameworks and legislature, improve biosecurity measures, and strengthen national disease surveillance and reporting, to reduce the risk of movement of pathogens, both nationally and internationally, through movement of live aquatic animals; and (d) develop and implement Indonesia's National Strategy on Aquatic Animal Health Management. The mechanism of the FAO assistance to achieve the above objectives consists of fielding a number of consultants on epidemiology, virology, aquatic animal health management and aquatic animal health legislation with specific terms of reference, conduct of training programs and workshops on epidemiology, farm level diagnostics, surveillance and disease reporting for both government staff, extension workers and farmers, provision of local staff training at a regional resource center, preparation of extension materials and upgrading viral diagnostic capacities and facilities, to mention a few.

IX. References

Individual reports submitted by participating scientists and institutions as follows:

- Epidemiology Report of Dr. Angus Cameron (received on 24 July 2002)
- PCR Report of Dr. Agus Sunarto (received on August 5, 2002)
- Pathology Report of Prof. Hugh Ferguson (received on August 6, 2002)
- Virology, PCR and Histopathology/EM Report of InterVet Norbio Singapore/International (received on August 8 and 14, 2002, November 12, 2002))
- Virology and PCR Report of Dr. Somkiat Kanchanakhan (received August 29 and September 4, 2002)
- Pathology, Virology, PCR and EM Report of Prof. Ronald Hedrick (received on October 2, 2002)
- Pathology Report of Dr. Supranee Chinabut and Dr. Gary Nash (received on October 24, 2002)

Gray, M., S. LaPatra, Groff and A. Goodwin (2002). Detection of Koi Herpesvirus DNA in tissues of infected fish. *J. Fish Dis.* 25: 171-178.

Hedrick, RP, Gilad, O, Yun, S, Spangerberg, JV. (2000). A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. *J. Aquat. Anim. Health* 12:44-57.

Neukirch, M. and U Kunz. (2001). Isolation and preliminary characterisation of several viruses from koi (*Cyprius carpio*) suffering gill necrosis and mortality. *Bull. Eur. Ass. Fish Pathol.,* 21(4): 125 - 135

OATA. (2001). Koi Herpes Virus (KHV). Ornamental Aquatic Trade Association (OATA). United Kingdom. 33 pp. (http://www.ornamentalfish.org; http://www.aquaticsworldwide.org)