SECTION 2 - FINFISH DISEASES

F.1 GENERAL TECHNIQUES

General fish health advice and other valuable information are available from the OIE Reference Laboratories, Regional Resource Experts in the Asia-Pacific, FAO and NACA. A list is provided in Annexes F.AI and AII, and up-to-date contact information may be obtained from the NACA Secretariat in Bangkok (e-mail: naca@enaca.org). Other useful guides to diagnostic procedures which provide valuable references for regional parasites, pests and diseases are listed in Annex F.AIII.

F.1.1 **Gross Observations**

F.1.1.1 *Behaviour* (Level I)

At a time when there are no problems on the farm, "normal behaviour" of the animals should be observed to establish and describe the "normal" situation. Any change from normal behaviour should be a cause for concern and warrants investigation. Prior to the clinical expression of disease signs, individual finfish may exhibit increased feed consumption followed by cessation of feeding, or the fish may simply go off feed alone. Taking note of normal feed conversion ratios, length/weight ratios or other body-shape signs described below, is essential in order to detect impending disease.

Abnormal behaviour includes fish swimming near the surface, sinking to the bottom, loss of balance, flashing, cork-screwing or air gulping (non air-breathers) or any sign which deviates from normal behaviour. Bursts of abnormal activity are often associated with a generalised lethargy. Behavioural changes often occur when a fish is under stress. Oxygen deprivation leads to gulping, listlessness, belly-up or rolling motion. This can be due to blood or gill impairment. Flashing can indicate surface irritation, *e.g.*, superficial secondary infections of surface lesions. Corkscrew and other bizarre behaviour may also indicate neurological problems that may be disease related (see F.6 - Viral Encephalopathy and Retinopathy).

Patterns of mortalities should be closely monitored, as well as levels of mortality. If losses persist or increase, samples should be sent for laboratory analysis (Level II and/or III). Mortalities that seem to have a uniform or random distribution should be examined immediately and environmental factors during, pre- and post-mortality recorded. Mortalities that spread from one area to another may suggest the presence of an infectious disease agent and should be sampled immediately. Affected animals should be kept (isolated) as far away as possible from unaffected animals until the cause of the mortalities can be established.

F.1.1.2 *Surface Observations* (Level I)

Generally speaking, no surface observations can be linked to a single disease problem, however, quick detection of any of the following clinical signs, plus follow-up action (*e.g.*, removal or isolation from healthy fish, submission of samples for laboratory examination), can significantly reduce potential losses.

F.1.1.2.1 Skin and Fins (Level I)

Damage to the skin and fins can be the consequence of an infectious disease (*e.g.,* carp erythrodermatitis). However, pre-existing lesions due to mechanical damage from contact with rough surfaces, such as concrete raceways, or predator attack (*e.g.*, birds, seals, *etc.*, or chemical trauma) can also provide an opportunity for primary pathogens or secondary pathogens (*e.g.,* motile aeromonads) to invade and establish. This further compromises the health of the fish.

Common skin changes associated with disease, which should encourage further action include red spots (Fig. F.1.1.2.1a), which may be pin-point size (petechiae) or larger patches. These tend to occur around the fins, operculum, vent and caudal area of the tail, but may sometimes be distributed over the entire surface. Indications of deeper haemorrhaging or osmotic imbalance problem saredarkened colouration. Haemorrhagic lesions may precede skin erosion, which seriously affect osmoregulation and defense against secondary infections. Erosion is commonly found on the dorsal surfaces (head and back) and may be caused by disease, sunburn or mechanical damage. In some species, surface irritation may be indicated by a build up of mucous or scale loss.

Surface parasites, such as copepods, ciliates or flatworms, should also be noted. As with the gills, these may not be a problem under most circumstances, however, if they proliferate to noticeably higher than normal numbers (Fig. F.1.1.2.1b), this may lead to secondary infections or indicate an underlying disease (or other stress) problem. The parasites may be attached superficially or be larval stages encysted in the fins, or skin. Such encysted larvae (*e.g.*, flatworm digenean metacercariae) may be detected as white or black spots (Fig. F.1.1.2.1c)

in the skin (or deeper muscle tissue).

Abnormal growths are associated with tumourous diseases, which can be caused by disease, such as *Oncorhynchus masou* virus (see F.4 - *Oncorhynchus masou* Virus Disease) and Lymphocystis (see F.9 - Lymphocystis), or other environmental problems.

The eyes should also be observed closely for disease indications. Shape, colour, cloudiness, gas bubbles and small haemorrhagic lesions (red spots) can all indicate emerging or actual disease problems. For example, eye enlargement and distension, known as "Popeye", is associated with several diseases (Fig. F.1.1.2.1d).

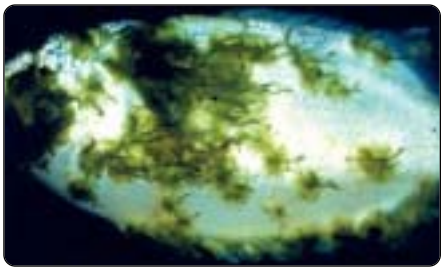
F.1.1.2.2 Gills (Level I)

The most readily observable change to soft tissues is paleness and erosion of the gills (Fig.F.1.1.2.2a). This is often associated with disease and should be of major concern. Red spots may also be indicative of haemorrhagic problems, which reduce the critical functioning ability of the gills. Fouling, mucous build-up or parasites (ciliate protistans, monogeneans, copepods, fungi, *etc.*) may also reduce functional surface area and may be indicative of other health problems (Fig.F.1.1.2.2b).These may affect the fish directly or render it more susceptible to secondary infections.

(MG Bondad-Reantaso)

Fig.F.1.1.2.1a. Red spot disease of grass carp.

(JR Arthur)



(K Ogawa)

Fig.F.1.1.2.1c. Ayu, *Plecoglossus altivelis*, infected with *Posthodiplostomum cuticola* (?) metacercariae appearing as black spots on skin.

(R Chong)

Fig. F.1.1.2.1d. Typical ulcerative, popeye, fin and tail rot caused by *Vibrio spp.*

(SE McGladdery)

Fig.F.1.1.2.2a. Example of gill erosion on Atlantic salmon, *Salmo salar*, due to intense infestation by the copepod parasite *Salmincola salmoneus.*

(MG Bondad-Reantaso)

Fig.F.1.1.2.2b. Fish gills infected with monogenean parasites.

Fig.F.1.1.2.1b. Surface parasites, *Lerneae cyprinacea* infection of giant gouramy.

F.1.1.2.3 Body (Level I)

Any deviation from normal body shape in a fish is a sign of a health problem. Common changes include "pinhead" which usually affects young fish indicating developmental problems; lateral or dorso-ventral bends in the spine (*i.e.*, lordosis and scoliosis) can reveal nutritional or environmental water quality problems. Another common, and easily detected, change in body shape is "dropsy". Dropsy is a distention of the abdomen, giving the fish a "pot belly" appearance. This is a strong indicator of disease problems which may include swelling of internal organs (liver, spleen or kidney), build up of body fluids (clear = oedema; bloody fluids = ascites), parasite problems, or other unknown cause. Dropsy is a common element in many of the serious diseases listed in the *Asia Diagnostic Guide* since it is commonly associated with systemic disruption of osmoregulation due to blood-cell or kidney damage.

F.1.1.3 *Internal Observations* (Level I)

As a follow up to behavioural changes, samples of sick fish should be examined and cut open along the ventral surface (throat to anus). This will allow gross observation of the internal organs and body cavity. A healthy-appearing fish should also be opened up the same way, if the person has little experience with the normal internal workings of the fish they are examining. Organ arrangement and appearance can vary between species.

Normal tissues should have no evidence of free fluid in the body cavity, firm musculature, cream-white fat deposits (where present) around the pyloric caecae, intestine and stomach, a deep red kidney lying flat along the top of the body cavity (between the spinal cord and swim-bladder), a red liver, a deep red spleen and pancreas. The stomach and intestine may contain food. Gonadal development will vary depending on season. The heart (behind the gill chamber and walled off from the body cavity) and associated bulbous arteriosus should be distinct and shiny.

F.1.1.3.1 Body Cavity and Muscle (Level I/II)

Clues to disease in a body cavity most commonly consist of haemorrhaging and a build up of bloody fluids. Blood spots in the muscle of the body cavity wall, may also be present. Body cavity walls which disintegrate during dissection may indicate a fish that has been dead for a while and which is, therefore, of little use for accurate diagnosis, due to rapid invasion of secondary saprobionts (*i.e.,* microbes that live on dead and decaying tissues).

Necrotic musculature may also indicate a muscle infection, *e.g.*, by myxosporean parasites. This can be rapidly investigated by squashing a piece of the affected muscle between two glass slides or between a Petri dish lid and base, and examining it under a compound or dissection microscope. If spore-like inclusions are present, a parasite problem can be reasonably suspected. Some microsporidian and myxosporean parasites can form cysts in the muscle (Fig.F.1.1.3.1a), peritoneal tissues (the membranous network which hold the organs in place in the body cavity), and organs that easily visible to the naked eye as clumps or masses of white spheres. These too, require parasitology identification. Worms may also be present, coiled up in and around the organs and peritoneal tissues. None of these parasites (though unsightly) are usually a disease-problem, except where present in massive numbers which compress or displace the organs (Fig.F.1.1.3.1b).

F.1.1.3.2 Organs (Levels I-III)

Any white-grey patches present in the liver, kidney, spleen or pancreas, suggest a disease problem, since these normally represent patches of necrosis or other tissue damage. In organs such as kidney and spleen, this can indicate disruption of blood cell production. Kidney lesions can also directly affect osmoregulation and liver lesions can affect toxin and microbial defense mechanisms. Swelling of any of these organs to above normal size is equally indicative of a disease problem which should be identified, as soon as possible.

Swollen intestines (Fig.F.1.1.3.2a and Fig.F.1.1.3.2b) should be checked to see if this is due to food or a build up of mucous. The latter is indicative of feed and waste disposal disruption, as well as intestinal irritation, and is commonly found in association with several serious diseases. This may also occur due to opportunistic invasion of bowels that have been irritated by rapid changes in feed, *e.g.*, by the flagellate protistan *Hexamita salmonis*. Mucous filled intestines can be spotted externally via the presence of trailing, flocculent or mucous faeces (casts).

F.1 General Techniques

(H Yokoyama)

Fig.F.1.1.3.1a. *Myxobolus artus* infection in the skeletal muscle of 0+ carp.

(K Ogawa)

Fig.F.1.3.1b. *Ligula* sp. (Cestoda) larvae infection in the body cavity of Japanese yellow goby, *Acanthogobius flavimanus.*

(H Yokoyama)

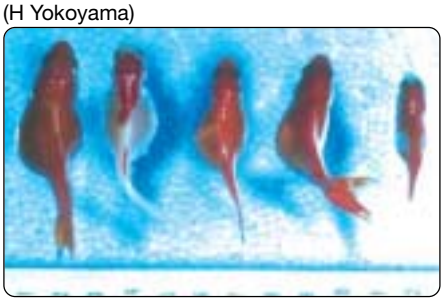


Fig.F.1.3.2a. Distended abdomen of goldfish.

F.1.2 **Environmental Parameters** (Level I)

Water quality and fluctuating environmental conditions, although not of contagious concern, can have a significant effect on finfish health, both directly (within the ranges of physiological tolerances) and indirectly (enhancing susceptibility to infections). This is especially important for species grown in conditions that bear little resemblance to the wild situation. Water temperature, salinity, turbidity, fouling and

(MG Bondad-Reantaso)

Fig.F.1.3.2b. Japanese Yamame salmon (*Onchorynchus masou*) fingerlings showing swollen belly due to yeast infection.

plankton blooms are all important factors. High stocking rates, common in intensive aquaculture, predispose individuals to stress as well as minor changes in environmental conditions that can precipitate disease. Accumulation of waste feed indicates either overfeeding or a decrease in feeding activity. In either situation, the breakdown products can have a direct toxic effect or act as a medium for microbial proliferation and secondary infections. Likewise, other pollutants can also have a significant effect on fish health.

F.1.3 **General Procedures**

F.1.3.1 *Pre-Collection Preparation* (Level I)

Wherever possible, the number of specimens required for laboratory examination should be confirmed *before* the samples are collected. Larger numbers are generally required for screening purposes than for diagnosis of mortalities, or other abnormalities. The diagnostic laboratory which will be receiving the sample should also be consulted to ascertain the best method of transportation (*e.g.*, on ice, preserved in fixative, whole or tissue samples). The laboratory will also indicate if both clinically affected, as well as apparently healthy individuals, are required for comparative purposes.

Inform the laboratory of exactly what is going to be sent (*i.e.*, numbers, size-classes or tissues and intended date of collection and delivery) so the laboratory can be prepared *prior* to sample arrival. Such preparation can speed up processing of a sample (fixative preparation, labeling of slides, jars, cassettes, test-tubes, Petri-plates, data-sheets, *etc*.) by as much as a day.

F.1.3.2 *Background Information* (Level I)

All samples submitted for diagnosis should include as much supporting information as possible including:
- reason(s) for submitting the sample (*i.e.* health screening, certification)
- gross observations,feed records,and envronmental parameters
- history and origin of the fish population date of transfer and source location(s) if the stock does not originate from on-site.

These information will help clarify whether handling stress, change of environment or infectious agents are causes for concern. It will also help speed up diagnosis, risk assessment, and husbandry management and treatment recommendations.

F.1.3.3 *Sample Collection for Health Surveillance*

The most important factors associated with collection of specimens for surveillance are:
- sample numbers that are high enough (see Table F.1.3.3 below)
- susceptible species are sampled
- sampling includes age-groups and seasons that are most likely to manifest detectable infections.
  Such information is given under the specific disease sections.

	Prevalence (%)						
Population Size	0.5	1.0	**2.0**	3.0	4.0	**5.0**	10.0
50	46	46	46	37	37	29	20
100	93	93	76	61	50	43	23
250	192	156	110	75	62	49	25
500	314	223	127	88	67	54	26
1000	448	256	136	92	69	55	27
2500	512	279	142	95	71	56	27
5000	562	288	145	96	71	57	27
10000	579	292	146	96	72	29	27
100000	594	296	147	97	72	57	27
1000000	596	297	147	97	72	57	27
>1000000	600	300	**150**	100	75	**60**	30

Table F.1.3.3[1]. Sample sizes needed to detect at least one infected host in a population of a given size, at a given prevalence of infection. Assumptions of 2% and 5% prevalences are most commonly used for surveillance of presumed exotic pathogens, with a 95% confidence limit.

F.1.3.4 *Sample Collection for Disease Diagnosis* (Level I)

All samples submitted for disease diagnosis should include as much supporting information as possible including:
- reason(s) for submitting the sample (mortalities, abnormal growth, *etc.*)
- handling activities (net/cage de-fouling, size sorting/grading, site changes, predators, new species/stock introduction, *etc.*)

[1] Ossiander, F.J. and G. Wedermeyer. 1973. Journal Fisheries Research Board of Canada 30:1383-1384.

- environmental changes (*rapid* water quality changes, such as turbidity fluxes, saltwater incursion into freshwater ponds, unusual weather events, *etc.*).

These information will help clarify whether handling stress, change of environment or infectious agents may be a factor in the observed abnormalities/mortalities. Such information is necessary for both rapid and accurate diagnosis, since it helps focus the investigative procedures required.

F.1.3.5 *Live Specimen Collection for Shipping* (Level I)

Collection should take place as close to shipping time as possible, to reduce mortalities during transportation. This is especially important for moribund or diseased fish.

The laboratory should be informed of the estimated time of arrival of the sample, in order to ensure that the laboratory has the materials required for processing prepared before the fish arrive. This shortens the time between removal of the fish from water and preparation of the specimens for examination (see F.1.3.1).

The fish should be packed in double plastic bags, filled with water to one third of their capacity with the remaining 2/3 volume inflated with air/oxygen. The bags should be tightly sealed (rubber bands or tape) and packed inside a styrofoam box or cardboard box lined with styrofoam. A plastic bag measuring 60 x 180 cm is suitable for a *maximum* of *four* 200-300 g fish. The volume of water to fish volume/biomass is particularly important for live fish being shipped for ectoparasite examination, so advance checking with the diagnostic laboratory is recommended. The box should be sealed securely to prevent spillage and may be double packed inside a cardboard carton. The laboratory should be consulted about the packaging required.

Containers should be clearly labeled as follows:

"LIVE SPECIMENS, STORE AT ___ to ___°C, DO NOT FREEZE"
(Insert temperature tolerance range of fish being shipped)

If being shipped by air also indicate

"HOLD AT AIRPORT AND CALL FOR PICK-UP"
(Clearly indicate the name and telephone number of the person responsible for picking up the package, or receiving it at the laboratory).

Where possible, ship ***early in the week*** to avoid delivery during the weekend which may lead to improper storage and loss of samples.

Inform the contact person(s) as soon as the shipment has been sent and provide the name of the carrier, flight number, waybill number and estimated time of arrival, as appropriate.

F.1.3.6 *Dead or Tissue Specimen Collection for Shipping* (Level I)

In some cases, samples may be unable to be delivered live to a diagnostic laboratory due to distance or slow transport connections. In such cases, diagnostic requirements should be discussed with laboratory personnel prior to sample collection. Shipping of non-preserved tissues or dead specimens may require precautions to prevent contamination or decay. In addition, precautions should be taken to protect ectoparasites, if these are of probable significance.

For bacteriology, mycology or virology:

- Small fish may be bagged, sealed and transported whole on ice/frozen gel-packs.
- For larger fish, the viscera can be aseptically removed, placed in sterile containers and shipped on ice/frozen gel-packs.
- For bacteriology or mycology examinations – ship fish individually bagged and sealed, on ice/frozen gel-packs.
- For virology examination - bag fish with five volumes of Hanks' basal salt solution containing either gentamycin (1,000 mg/ml) or penicillin (800 IU/ml) + dihydrostreptamycin (800 mg/ml). Anti-fungal agents such as Mycostatin or Fungizone may also be incorporated at a level of 400 IU/ml.

Note: Intact or live specimens are ideally best since dissected tissues rapidly start autolysis even under ice, making them useless for sterile technique and bacteriology, particularly for tropical climates. Fish destined for bacteriological examination can be kept on ice for a ***limited*** period. The icing should be done to ensure that the organs/tissues destined for examination using sterile technique are kept at temperatures below ambient water (down to 4°C is a standard low) but not freezing. Individual bagging is also recommended in order to prevent contamination by

[2] Further details are available in "Recommendations for euthanasia of experimental animals" Laboratory Animals 31:1-32 (1997).

one individual within a sample.

F.1.3.7 *Preservation (Fixation) of Tissue Samples* (Level I)

Fish should be killed prior to fixation. With small fish, this can be done by decapitation, however, this causes mechanical damage to the tissues and is unsuitable for larger fish. Alternatively, euthanasia with an overdose of anaesthetic is a better (unless examination is for ectoparasites, which may be lost) option. Benzocaine or Etomidate, administered at triple the recommended dose is usually effective for anaesthesizing fish. Injection of anaesthetic should be avoided, wherever possible, due to handling induced tissue trauma[2]. Putting fish in iced water is also recommended prior to killing of fish.

Very small fish, such as fry or alevins, should be immersed directly in a minimum of 10:1 (fixative:tissue) volume ratio.

For large fish (>6 cm), the full length of the body cavity should be slit open (normally along the mid-ventral line) and the viscera and swim bladder gently displaced to permit incision of each major organ, at least once, to allow maximum penetration of the fixative. Ideally, the organ, or any lesions under investigation, should be removed, cut into blocks (<1.0 cm^3) and placed in a volume of fixative at least 10 times the volume of the tissue. Length of time for fixation is critical.
For skin sample preparation, it will be best to cut out several large pieces with a scalpel avoiding pressing or distortion of the sample. Briefly soak the skin in fixative, then take each piece of skin and cut into smaller sections about 1.0 cm wide and return the pieces quickly to fixative for 24 hrs. For samples from lesions, it is advisable to cut out a sample which includes healthy tissue surrounding the lesion to allow for comparison between healthy and affected tissues, with a width of no more than 1.0 cm and immediately placed in the fixative for 24 hrs.

Most tissues require a minimum of 24-48 hr fixation time if optimal preparations are to be made. It should be noted that long-term storage in all fixatives, except 70% ethanol, renders tissues useless for *in situ* hybridization. Check with diagnostic laboratory if long term storage is required on-site, prior to delivery to the laboratory.

The most suitable fixative for preservation of finfish samples for histopathology is **Phosphate Buffered Formalin.**

Phosphate Buffered Formalin

37-40% formaldehyde	100.0	ml
Tap water	900.0	ml
$NaH_2PO4.H_20$	4.0	g
Na_2HPO_4	6.5	g

Note: Formaldehyde is a gas soluble in water and is supplied in a concentrated form of 40% by weight. In concentrated solution, formaldehyde often becomes turbid during storage due to the production of formaldehyde, thus warming the solution or adding a small amount of NaOH will aid depolymerization of the paraformaldehyde. Formaldehyde is not suitable for fixation in its concentrated form. All formaldehyde regardless of purity, will be acid when purchased (usually within the pH range of 3-5). Care should be taken to check the final pH of any formalin-based fixative.

F.1.3.8 *Shipping Preserved Samples* (Level I)

Samples should be transported in sealed, unbreakable, containers. It is usual to double pack samples (*i.e.* an unbreakable container within a second unbreakable or well-padded container). Many postal services and transport companies (especially air couriers) have strict regulations regarding shipping chemicals, including preserved samples. If the tissues have been adequately fixed (as described in F.1.3.7), most fixative or storage solution can be drained from the sample for shipping purposes. As long as sufficient solution is left to keep the tissues from drying out, this will minimise the quantity of chemical solution being shipped. The carrier should be consulted before samples are collected to ensure they are processed and packed according to shipping rules.

- Containers should be clearly labeled with the information described for live specimens (F.1.3.5).
- The name and telephone number of the person responsible for picking up the package, or receiving it at the laboratory, should be clearly indicated.
- Where possible, ship early in the week to avoid delivery at the weekend, which may lead to improper storage and loss of samples.
- Inform the contact person as soon as the shipment has been sent and provide the name of the carrier, flight number, waybill number and

estimated time of arrival, as appropriate.

F.1.4 **Record-Keeping** (Level I)

It is critical to establish, and record, normal behaviour and appearance to compare with observations made during disease events. Record-keeping is, therefore, an essential component of *effective* disease management. For fish, many of the factors that should be recorded on a regular basis are outlined in sections F.1.4.1, F.1.4.2 and F.1.4.3.

F.1.4.1 *Gross Observations* (Level I)

These can be included in routine records of fish growth that, ideally would be monitored on a regular basis, either by sub-sampling from tanks or ponds, or by estimates made from surface observations.

For hatcheries, critical information that should be recorded include:
- feeding activity
- growth
- mortalities

These observations should be recorded daily, for all stages, including date, time, tank #, broodstock (where there are more than one) and food source. Dates and times of tank and water changes, pipe flushing/back-flushing and/or disinfection, should also be recorded. Ideally, these records should be checked (signed off) regularly by the person responsible for maintaining the facility.

For pond or net/cage sites, observations which need to be recorded include:
- growth
- fouling
- mortalities

These should be recorded with date, site location and any relevant activities (*e.g.*, sample collection for laboratory examination). As elsewhere, these records should be checked regularly by the person responsible for the facility.

F.1.4.2 *Environmental Observations* (Level I)

Environmental observations are most applicable to open water, ponds, cage and net culture systems. Information that should be recorded include:
- weather
- water temperature
- oxygen
- salinity
- turbidity (qualitative evaluation or Secchi disc)
- algal blooms
- human activity (handling, neighbouring land use/water activities)
- pH

The frequency of these observations will vary with site and fish species. Where salinity or turbidity rarely vary, records may only be required during rainy seasons or exceptional weather conditions. Temperate climates may require more frequent water temperature monitoring than tropic climates. Human activity(ies) should also be recorded on an "as it happens" basis, since there may be time-lag effects. In all cases, date and time should be recorded, as parameters such as temperature and pH can vary markedly during the day, particularly in open ponds and inter-tidal sites.

It may not always be possible to monitor oxygen levels in the pond. However, the farmer should be aware that in open non-aerated ponds, oxygen levels are lowest in the early morning when plants (including algae) have used oxygen overnight. Photosynthesis and associated oxygen production will only commence after sunrise.

F.1.4.3 *Stocking Records* (Level I)

All movements of fish into and out of a hatchery or site should be recorded, including:
- the source of the broodstock/eggs/larvae/juveniles and their health certification
- the volume or number of fish
- condition on arrival
- date and time of delivery and name of person responsible for receiving the fish
- date, time and destination of stock shipped-out from a hatchery or site.

Such records are also applicable (but less critical) to movements between tanks, ponds, cages within a site. Where possible, animals from different sources should not be mixed. If mixing is unavoidable, keep strict records of which sources are mixed and dates of new introductions into the holding site or system.

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VIRAL DISEASES OF FINFISHES
F.2 EPIZOOTIC HAEMATOPOIETIC NECROSIS (EHN)

F.2.1 **Background Information**

F.2.1.1 *Causative Agent*

Epizootic Haematopoietic Necrosis (EHN) is caused by a double-stranded DNA, non-enveloped Iridovirus known as Epizootic Haematopoeitic Necrosis Virus (EHNV). This virus shares at least one antigen with iridoviruses infecting sheatfish (*Silurus glanis*) and the catfish (*Ictalurus melas*) in Europe and with amphibian iridoviruses from North America (frog virus 3) and Australia (Bohle iridovirus). Recently, the OIE included the two agents, European catfish virus and European sheatfish virus, as causative agents of EHN (OIE 2000a; http://www.oie.int). Current classification in the genus *Ranavirus* is under review (see http://www.ncbi.nlm.nih.gov/ICTV). More detailed information about the disease can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a).

F.2.1.2 *Host Range*

EHNV infects redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*). Other fish species found to be susceptible to EHNV after bath exposure are Macquarie perch (*Macquaria australasica*), mosquito fish (*Gambussia affinis*), silver perch (*Bidyanus bidyanus*) and mountain galaxias (*Galaxias olidus*).

F.2.1.3 *Geographic Distribution*

Historically, the geographic range of EHNV infections has been restricted to mainland Australia. However, a recent OIE decision to include sheatfish and catfish iridoviruses as causes of EHN, increased the geographic distribution to include Europe. A related virus recently isolated from pike-perch in Finland, was found to be immunologically cross-reactive but non-pathogenic to rainbow trout.

F.2.1.4 *Asia-Pacific Quarterly Aquatic Animal Disease Reporting System (1999-2000)*

Australia reported the occurrence of EHN in Victoria (last year 1996), New South Wales (last year 1996) and South Australia (1992). It was also known to have occurred in New South Wales during first quarter of 2000, with annual occurrence in the Australian Capital Territory (without laboratory confirmation) (OIE 1999, 2000b).

India reported EHN during last quarter of 1999 affecting murrels and catfishes (OIE 1999).

F.2.2 **Clinical Aspects**

There are no specific clinical signs associated with EHN. Mortalities are characterised by necrosis of liver (with or without white spots), spleen, haematopoietic tissue of the kidney and other tissues. Disruption of blood function leads to osmotic imbalance, haemorrhagic lesions, build up of body fluids in the body cavity. The body cavity fluids (ascites) plus enlarged spleen and kidney may cause abdominal distension (dropsy).

Clinical disease appears to be associated with poor water quality, as well as water temperature. In rainbow trout, disease occurs at temperatures from 11 to 17˚C (in nature) and 8 - 21˚C (experimental conditions). No disease is found in redfin perch at temperatures below 12˚C under natural conditions. Both juvenile and adult redfin perch can be affected, but juveniles appear more susceptible (Fig.F.2.2a). EHNV has been detected in rainbow trout ranging from fry to market size, although mortality occurs most frequently in 0+ - 125 mm fork-length fish.

F.2.3 **Screening Methods**

More detailed information on methods for screening EHN can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int, or selected references.

As with other disease agents, screening for the presence of an infectious agent in a sub-clinical population requires larger sample numbers than for a disease diagnosis. Numbers will vary according to the confidence level required (see F.1.3.3).

F.2.3.1 *Presumptive*

F.2.3.1.1 Gross Observations (Level 1) and Histopathology (Level II)

It is not possible to detect infections in sub-clinical fish, using gross observations (Level I) or histopathology (Level II).

F.2.3.1.2 Virology (Level III)

EHNV can be isolated on Bluegill Fin 2 (BF-2) or Fathead Minnow (FHM) cell lines. This requires surveillance of large numbers (see Table F.1.3.3)

F.2 Epizootic Haematopoietic Necrosis (EHN)

of sub-clinical fish to detect low percentage carriers.

F.2.3.2 *Confirmatory*

F.2.3.2.1 Immunoassays (Level III)

Suspect cytopathic effects (CPE) in BF-2 or FHM cell-lines require confirmation of EHNV as the cause through immunoassay (indirect fluorescent antibody test (IFAT) or enzyme linked immunosorbent assay (ELISA) or Polymerase Chain Reaction (PCR) (Level III).

F.2.4 **Diagnostic Methods**

More detailed information on methods for diagnosis of EHN can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int, or selected references.

EHNV is a highly resistant virus that can withstand freezing for prolonged periods, thus, fish may be stored and or/transported frozen without affecting the diagnosis.

F.2.4.1 *Presumptive*

F.2.4.1.1 Gross Observation (Level I)

As described under F.2.2, mass mortalities of small redfin perch under cool water conditions (< 11 °C), which include cessation of feeding, abdominal distension, focal gill and fin haemorrhage, as well as overall skin darkening, should be considered suspect for EHNV infection. Similar observations in rainbow trout fingerlings (11-17 °C) may also be considered suspect, but the conditions are not specific to EHN in either host.

Necropsy may reveal liver and spleen enlargement or focal pale spots on the liver, but these, again, are non-specific.

F.2.4.1.2 Histopathology (Level II)

Histopathology in haematopoietic kidney, liver, spleen and heart tissues are similar in both infected redfin perch and rainbow trout, although perch livers tend to have larger focal or locally extensive areas of necrosis. Gills of infected perch show focal blood clots, haemorrhage and fibrinous exudate. Focal necrosis occurs in the pancreas and intestinal wall. In the former tissue site necrosis can become extensive.

F.2.4.1.3 Virology (Level III)

Whole alevin or juvenile perch (<4 cm in length), viscera including kidney (4-6 cm body length) or kidney, spleen and liver from larger fish, are required for tissue culture. Presumptive diagnosis starts with viral isolation on BF-2 or FHM cell-lines. Cytopathic effect (CPE) is then cross-checked for EHNV using indirect fluorescence microscopy or ELISA (F.2.4.2).

F.2.4.1.4 Transmission Electron Microscopy (TEM) (Level III)

Icosahedral morphology, 145-162 *nm*, dsDNA non-enveloped viral particles are present in the cytoplasm of infected spleen, liver, kidney and blood cells.

F.2.4.2 *Confirmatory*

F.2.4.2.1 Immunoassay (Level III)

IFAT and ELISA are required to confirm EHNV in CPE from cell-line culture described under F.2.4.1.3. EHNV does not induce neutralising antibodies (Ab) in mammals or fish.

F.2.4.2.2 Polymerase Chain Reaction (PCR) (Level III)

PCR procedures and primers have been produced that can detect iridoviruses in isolates from redfin perch (*Perca fluviatilis*), rainbow trout (*Oncorhynchus mykiss*), sheatfish (*Silurus glanis*), catfish (*Ictalurus melas*), guppy (*Poecilia reticulata*), doctor fish (*Labroides dimidatus*), and a range of amphibian ranaviruses (unpublished data).

F.2.5 **Modes of Transmission**

Transmission of EHNV in rainbow trout is not fully understood. Infections may recur annually and this may be linked to redfin perch reservoirs of infection in the water catchment area. However, the disease is also known to occur at low prevalences in some infected trout populations, so mortality may not exceed "normal" background rates. This means infected fish may be overlooked among apparently healthy fish.

Another route of EHNV spread is by birds, either by regurgitation of infected fish, or mechanical transfer on feathers, feet or beaks. Anglers have also been implicated in EHNV transfer, either via dead fish or by contaminated fishing gear.

F.2 Epizootic Haematopoietic Necrosis (EHN)

F.2.6 Control Measures

Prevention of movement of infected fish between watersheds, and minimising contact between trout farms and surrounding perch populations is recommended. In addition, reducing bird activity at farm sites may be effective in reducing the chances of exposure and spread. Precautionary advice and information for recreational fishermen using infected and uninfected areas may also reduce inadvertent spread of EHN.

F.2.7 Selected References

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(AAHL)

Fig.F.2.2a. Mass mortality of single species of redfin perch. Note the small size of fish affected and swollen stomach of the individual to the centre of the photograph. Note the characteristic haemorrhagic gills in the fish on the left in the inset.

(EAFP)

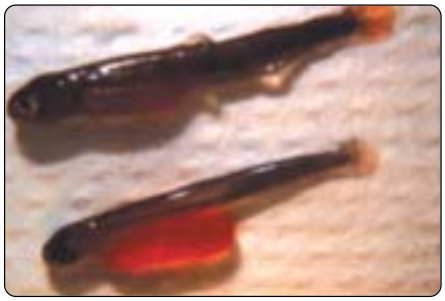

Fig.F.3.2a. IHN infected fry showing yolk sac haemorrhages.

(EAFP)

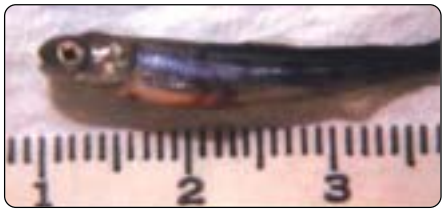

Fig.F.3.2b. Clinical signs of IHN infected fish include darkening of skin, haemorrhages on the abdomen and in the eye around the pupil.

F.3 INFECTIOUS HAEMATOPOIETIC NECROSIS (IHN)

F.3.1 **Background Information**

F.3.1.1 *Causative Agent*

Infectious Haematopoietic Necrosis (IHN) is caused by an enveloped single stranded RNA (ssRNA) Rhabdovirus, known as Infectious Haematopoietic Necrosis Virus (IHNV). It is currently unassigned to genus, but the International Committee on Taxonomy of Viruses (ICTV) is currently reviewing a new genus – Novirhabdovirus – which is proposed to include VHSV and IHNV (see http://www.ncbi.nlm.nih.gov/ICTV). More detailed information about the disease can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a).

F.3.1.2 *Host Range*

IHNV infects rainbow or steelhead trout (*Oncorhynchus mykiss*), sockeye salmon (*O. nerka*), chinook (*O. tshawytscha*), chum (*O.keta*), yamame (*O. masou*), amago (*O. rhodurus*), coho (*O. kisutch*), and Atlantic salmon (*Salmo salar*). Pike fry (*Esox lucius*), seabream and turbot can also be infected under experimental conditions.

F.3.1.3 *Geographic Distribution*

Historically, the geographic range of IHN was limited to the Pacific Rim of North America but, more recently, the disease has spread to continental Europe and Asia.

F.3.1.4 *Asia-Pacific Quarterly Aquatic Animal Disease Reporting System (1999- 2000)*

India reported occurrence of IHN during last quarter of 1999 affecting murrels and catfishes; Korea RO reported IHN among rainbow trout during 3rd and 4th (September) quarters of 2000 while Japan reported occurrence of IHN every month during 1999 and 2000 (OIE 1999, 2000b).

F.3.2 **Clinical Aspects**

Among individuals of each fish species, there is a high degree of variation in susceptibility to IHNV. Yolk-sac fry (Fig.F.3.2a) are particularly susceptible and can suffer 90-100% mortality. In rainbow trout, such mortalities are correlated with water temperatures <14°C. Survivors of IHNV demonstrate strong acquired immunity.

Susceptible fish show dark discolouration of the body (especially the dorsal surface and tail fin regions) (Fig.F.3.2b). The abdomen may be distended, with haemorrhaging at the base of the fins, on the operculum and around the eyes (which may show swelling – "pop-eye"). Weakened swimming capability may also be evident. Some fish may show a white discharge from the anus.

The IHNV multiplies in endothelial cells of blood capillaries, spleen and kidney cells, which results in osmotic imbalance, as well as systemic haemorrhagic lesions. These can be seen grossly as pale internal organs and/or pin-point bleeding in the musculature and fatty tissues. The kidney, spleen, brain and digestive tract are the sites where virus is most abundant during advanced infection.

F.3.3 **Screening Methods**

More detailed information on methods for screening IHN can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int or selected references.

F.3.3.1 *Presumptive*

F.3.3.1.1 Virology (Level III)

IHNV can be isolated from sub-clinical carriers on *Epithelioma papulosum cyprinae* (EPC) or BF-2 cell lines. The identity of the cause of any CPE on these cell lines, however, requires further confirmation (F.3.3.2).

F.3.3.2 *Confirmatory*

F.3.3.2.1 Immunoassay or Nucleic Acid Assay (Level III)

The cause of CPE produced on EPC or BF-2 cell lines by suspect IHNV carrier samples must be confirmed using immunological identification or PCR-based techniques (F.3.4.2.1).

F.3.4 **Diagnostic methods**

More detailed information on methods for diagnosis of IHN can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int or selected references.

F.3 Infectious Haematopoietic Necrosis (IHN)

F.3.4.1 *Presumptive*

F.3.4.1.1 Gross Observations (Level I)

Behavioural changes are not specific to IHN but may include lethargy, aggregation in still areas of the pond with periodic bursts of erratic swimming (see F.3.2) and loss of equilibrium.

Changes in appearance include dark discolouration of the body (especially the dorsal surface and tail fin regions), especially in yolk-sac fry stages (90-100% mortality). The abdomen can be distended due to accumulation of fluids in the body cavity (dropsy) and haemorrhaging may be visible at the base of the fins, on the operculum and around the eyes. The eyes may also show signs of water imbalance in the tissues by bulging ("pop-eye"). There may be vent protrusion and trailing white/mucoid casts.

F.3.4.1.2 Histopathology (Level II)

Tissue sections show varying degrees of necrosis of the kidney and spleen (haematopoietic) tissues, as well as in the brain and digestive tract.

F.3.4.1.3 Virology (Level III)

Whole alevins (body length ≤4 cm), viscera including kidney (fish 4 – 6 cm in length) or kidney, spleen and brain tissues from larger fish, are required for isolating the virus on EPC or BF-2 cell lines. Confirmation of IHNV being the cause of any resultant CPE requires immunoassay investigation, as described below.

F.3.4.2 *Confirmatory*

F.3.4.2.1 Immunoassays (IFAT or ELISA) (Level III)

Diagnosis of IHNV is achieved via immunoassay of isolates from cell culture using IFAT or ELISA, or immunological demonstration of IHNV antigen in infected fish tissues.

F.3.4.2.2 Transmission Electron Microscopy (TEM) (Level III)

TEM of cells infected in cell-culture reveals enveloped, slightly pleomorphic, bullet shaped virions, 45-100 *nm* in diameter and 100-430 *nm* long. Distinct spikes are evenly dispersed over most of the surface of the envelope (although these may be less evident under some cell-culture conditions). The nucleocapsids are coiled and show cross-banding (4.5 – 5.0 *nm* apart) in negative stain and TEM. Viral replication takes place in the cytoplasm with particle maturation at the cell membrane or the Golgi cisternae.

F.3.5 **Modes of Transmission**

IHNV is usually spread by survivors of infections, which carry sub-clinical infections. When such fish mature, they may shed the virus during spawning. Clinically infected fish can also spread the disease by shedding IHNV with faeces, urine, spawning fluids and mucus secretions. Other sources of infection include contaminated equipment, eggs from infected fish, and blood sucking parasites (*e.g.*, leeches, *Argulus* spp.). Fish-eating birds are believed to be another mechanism of spread from one site to another.

The most prominent environmental factor affecting IHN is water temperature. Clinical disease occurs between 8°C and 15°C under natural conditions. Outbreaks rarely occur above 15°C.

F.3.6 **Control Measures**

Control methods currently rely on avoidance through thorough disinfection of fertilised eggs. Eggs, alevins and fry should be reared on virus-free water supplies in premises completely separated from possible IHNV-positive carriers. Broodstock from sources with a history of IHN outbreaks should also be avoided wherever possible. At present, vaccination is only at an experimental stage.

As with viral haemorrhagic septicaemia virus (VHSV, see F.8), good over-all fish health condition seems to decrease the susceptibility to overt IHN, while handling and other types of stress frequently cause sub-clinical infection to become overt.

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F.4 *ONCORHYNCHUS MASOU* VIRUS (OMV)

F.4.1 **Background Information**

F.4.1.1 *Causative Agent*

Oncorhynchus masou virus disease (OMVD) is caused by *Oncorhynchus masou* virus (OMV) is believed to belong to the Family *Herpesviridae*, based on an icosahedral diameter of 120-200 *nm*, and enveloped, dsDNA properties. OMV is also known as Yamame tumour virus (YTV), Nerka virus Towada Lake, Akita and Amori prefecture (NeVTA), coho salmon tumour virus (CSTV), *Oncorhynchus kisutch* virus (OKV), coho salmon herpesvirus (CSHV), rainbow trout kidney virus (RKV), or rainbow trout herpesvirus (RHV). OMV differs from the herpesvirus of Salmonidae type 1, present in the western USA. Currently this salmonid herpesvirus has not been taxonomically assigned (see http://www.ncbi.nlm.nih.gov/ICTV). More detailed information about the disease can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a).

F.4.1.2 *Host Range*

Kokanee (non-anadromous sockeye) salmon (*Oncorhynchus nerka*) is most susceptible, followed, in decreasing order of susceptibility, by masou salmon (*O. masou*), chum salmon (*O. keta*), coho salmon (*O. kisutch*) and rainbow trout (*O. mykiss*).

F.4.1.3 *Geographic Distribution*

OMVD is found in Japan and, probably (as yet undocumented) the coastal rivers of eastern Asia that harbour Pacific salmon.

F.4.1.4 *Asia-Pacific Quarterly Aquatic Animal Disease Reporting System (1999-2000)*

Japan reported OMVD during all months of 1999 and 2000; and suspected by Korea RO for 1999, and during first two quarters of 2000 (OIE 1999, 2000b).

F.4.2 **Clinical Aspects**

OMV infects and multiplies in endothelial cells of blood capillaries, spleen and liver, causing systemic oedema and haemorrhaging. One-month-old alevins are the most susceptible development stage. Kidney, spleen, liver and tumours are the sites where OMV is most abundant during the course of overt infection.

Four months after the appearance of clinical signs, some surviving fish may develop epitheliomas (grossly visible tumours) around the mouth (upper and lower jaw) and, to a lesser extent, on the caudal fin operculum and body surface. These may persist for up to 1 year. In 1 yr-old coho salmon, chronic infections manifest themselves as skin ulcers, white spots on the liver and papillomas on the mouth and body surface. In rainbow trout, however, there are few (if any) external symptoms, but intestinal haemorrhage and white spots on the liver are observed.

Survivors of OMVD develop neutralising antibodies which prevent re-infection, however, they can remain carriers of viable virus.

F.4.3 **Screening Methods**

More detailed information on screening methods for OMV can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int or selected references.

F.4.3.1 *Presumptive*

F.4.3.1.1 Gross Observations (Level I)

Persistent superficial tumours are rare, but indicative of a potential carrier of viable OMV. Species, such as rainbow trout show no such lesions. Sub-clinical carriage cannot normally be detected using histology.

F.4.3.1.2 Virology (Level III)

OMV can be isolated from reproductive fluids, kidney, brain and spleen tissue samples on Chinook salmon embryo-214 (CHSE-214) or rainbow trout gonad-2 (RTG-2) cell lines. Any resultant CPE requires further immunological and PCR analyses to confirm the identity of the virus responsible (see F.4.3.2.1).

F.4.3.2 *Confirmatory*

F.4.3.2.1 Immunoassays and Nucleic Acid Assays (Level III)

Cytopathic effect (CPE) from cell cultures, as well as analyses of reproductive fluids, kidney, brain and spleen tissue samples from suspect fish can be screened using specific neutralisation antibody tests, indirect immunofluorescent antibody tests (IFAT) with immunoperoxidase staining, ELISA or Southern Blot DNA probe assays.

F.4 *Oncorhynchus Masou* Virus (OMV)

F.4.4 **Diagnostic Methods**

More detailed information on diagnostic methods for OMV can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000b), at http://www.oie.int or selected references.

F.4.4.1 *Presumptive*

F.4.4.1.1 Gross Observation (Level I)

Behavioural changes include lethargy and aggregation around the water inflow by young salmonids of susceptible species. Pin-point haemorrhaging or ulcers may be visible on the skin, along with darkened colouration. Popeye may also be present. Internally, white spots may be present on the liver (Fig.F.4.4.1.1a). After approximately 4 months, surviving fish may show signs of skin growths around the mouth (Fig.F.4.4.1.1b) or, less commonly, on the operculum, body surface or caudal fin area.

F.4.4.1.2 Histopathology (Level II)

Tissue sections from suspect fish may show lesions with enlarged nuclei in the epithelial tissues of the jaw, inner operculum and kidney.

F.4.4.1.3 Virology (Level III)

Whole alevin (body length A 4 cm), viscera including kidney (4 – 6 cm length) or, for larger fish, skin ulcerative lesions, neoplastic (tumourous tissues), kidney, spleen and brain are required for tissue culture using CHSE-214 or RTG-2 cell-lines. The cause of resultant CPE should be confirmed as viral using the procedures outlined in F.4.3.2.1.

F.4.4.1.4 Transmission Electron Microscopy (TEM) (Level III)

Detection of virions in the nuclei of affected tissues and tumours by TEM. The dsDNA virions are enveloped and icosahedral, measuring 120-200 *nm* in diameter (Fig.F.4.4.1.3).

F.4.4.2 *Confirmatory*

F.4.4.2.1 Gross Observations (Level I)

Gross behaviour and clinical signs at the onset of OMVD are not disease specific. Thus, confirmatory diagnosis requires additional diagnostic examination or occurrence with a docu-

(M Yoshimizu)

Fig.F.4.4.1.1a. OMV-infected chum salmon showing white spots on the liver.

(M Yoshimizu)

Fig.F.4.4.1.1b. OMV-induced tumour developing around the mouth of chum salmon fingerling.

(M Yoshimizu)

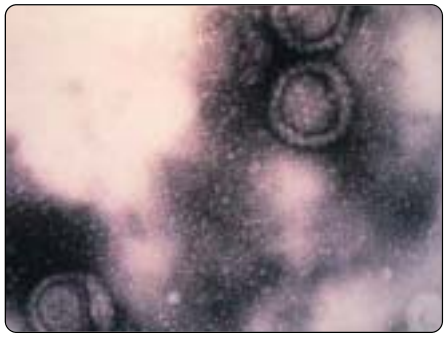

Fig.F.4.4.1.3. OMV particles isolated from masou salmon, size of nucleocapsid is 100 to 110 *nm*.

mented history of OMVD on-site or mortalities several months preceding the appearance of epithelial lesions and tumours.

F.4.4.2.2 Virology (Level III)

As described for F.4.3.1.2

F.4.4.2.3 Immunoassays and Nucleic Acid Assays (Level III)

As described for F.4.3.2.1.

F.4.5 Modes of Transmission

Virus is shed with faeces, urine, external and internal tumours, and, possibly, with skin mucus. Reservoirs of OMV are clinically infected fish as well as wild or cultured sub-clinical carriers. Maturation of survivors of early life-history infections may shed virus with their reproductive fluids ("egg associated", rather than true vertical transmission). Egg-associated transmission, although less frequent than other mechanisms of virus release, is the most likely source of infection in alevins.

F.4.6 Control Measures

Thorough disinfection of fertilised eggs, in addition to rearing of fry and alevins, in water free of contact with contaminated materials or fish, has proven effective in reducing outbreaks of OMVD.Water temperatures <14°C appear to favour proliferation of OMV.

F.4.7 Selected References

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F.5 INFECTIOUS PANCREATIC NECROSIS (IPN)

F.5.1 **Background Information**

F.5.1.1 *Causative Agent*

Infectious pancreatic necrosis (IPN) is caused by a highly contagious virus, Infectious pancreatic necrosis virus (IPNV) belonging to the *Birnaviridae*. It is a bi-segmented dsRNA virus which occurs primarily in freshwater, but appears to be saltwater tolerant. More detailed information about the disease can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a).

F.5.1.2 *Host Range*

IPN most commonly affects rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*) and several Pacific salmon species (*Oncorhynchus* spp.). Serologically related are reported from Japanese yellowtail flounder (*Seriola quinqueradiata*), turbot (*Scophthalmus maximus*), and halibut (*Hippoglossus hippoglossus*). Sub-clinical infections have also been detected in a wide range of estuarine and freshwater fish species in the families Anguillidae, Atherinidae, Bothidae, Carangidae, Cotostomidae, Cichlidae, Clupeidae, Cobitidae, Coregonidae, Cyprinidae, Esocidae, Moronidae, Paralichthydae, Percidae, Poecilidae, Sciaenidae, Soleidae and Thymallidae.

F.5.1.3 *Geographic Distribution*

The disease has a wide geographical distribution, occurring in most, if not all, major salmonid farming countries of North and South America, Europe and Asia.

F.5.1.4 *Asia-Pacific Quarterly Aquatic Animal Disease Reporting System (1999-2000)*

IPN was reported by Japan and suspected in Korea RO for 1999; in 2000, reported by Japan for the whole year except for the month of February, and by Korea RO in April (OIE 1999, 2000b).

F.5.2 **Clinical Aspects**

The first sign of IPN in salmonid fry is the sudden onset of mortality. This shows a progressive increase in severity, especially following introduction of feed to post-yolk-sac fry. IPN also affects American salmon smolt shortly after transfer to sea-cages. Clinical signs include darkening of the lower third of the body and small swellings on the head (Fig.F.5.2.a) and a pronounced distended abdomen (Fig.F.5.2b and Fig.F.5.2c) and a corkscrewing/spiral swimming motion. Some fish may also show 'pop-eye' deformities. Cumulative mortalities may vary from less than 10% to more than 90% depending on the combination of several factors such as virus strain, host and environment. Survivors of the disease, at early or late juvenile stages, are believed to be carriers of viable IPNV for life. Mortality is higher when water temperatures are warm, but there is no distinct seasonal cycle.

The pancreas, oesophagus and stomach become ulcerated and haemorrhagic. The intestines empty or become filled with clear mucous (this may lead to white fecal casts).

F.5.3 **Screening Methods**

More detailed information on screening methods for IPN can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000b), at http://www.oie.int or selected references.

As with other disease agents, screening for the presence of an infectious agent in a sub-clinical population requires larger sample numbers than for a disease diagnosis. Numbers will vary according to the confidence level required (see F.1.3.3).

F.5.3.1 *Presumptive*

F.5.3.1.1 Gross Observations (Level I) and Histopathology (Level II)

Carriers of sub-clinical infections show no external or internal evidence of infection at the light microscope level.

F.5.3.1.2 Virology (Level III)

Screening procedures use viral isolation on Chinook Salmon Embryo (CHSE-214) or Bluegill Fin (BF-2) cell lines. The cause of any CPE, however, has to be verified using confirmatory techniques (F.5.3.2.2). Fish material suitable for virological examination include whole alevin (body length A 4 cm), viscera including kidney (fish 4 – 6 cm in length) or, liver, kidney and spleen from larger fish.

F.5 Infectious Pancreatic Necrosis (IPN)

F.5.3.2 *Confirmatory*

F.5.3.2.1 Immunoassays and Molecular Probe Assays (Level III)

The viral cause of any CPE on CHSE-214 or BF-2 cell lines has to be confirmed by either an immunoassay (Neutralisation test or ELISA) or

(EAFP)

Fig.F.5.2a. IPN infected fish showing dark colouration of the lower third of the body and small swellings on the head.

(J Yulin)

Fig.F.5.2b. Rainbow trout fry showing distended abdomen characteristic of IPN infection. Eyed-eggs of this species were imported from Japan into China in 1987.

(EAFP)

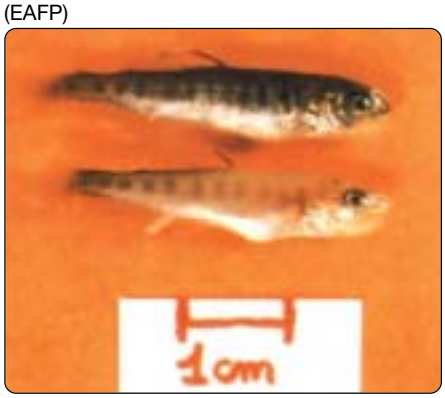

Fig.F.5.2c. Top: normal rainbow trout fry, below: diseased fry.

(J Yulin)

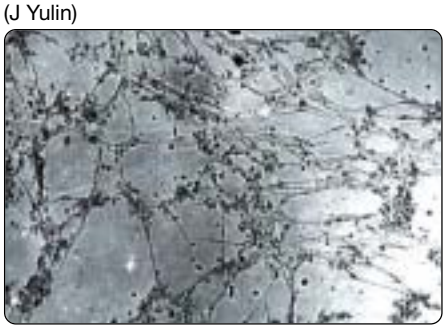

Fig.F.5.4.1.3. CPE of IHNV.

(J Yulin)

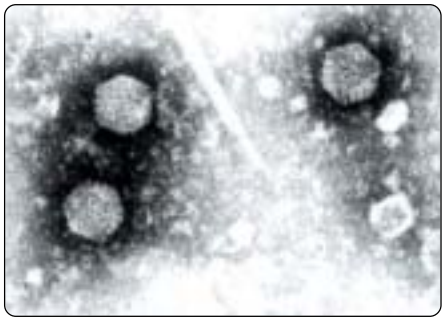

Fig.F.5.4.1.4. IPN Virus isolated from rainbow trout fimported from Japan in1987. Virus particles are 55 *nm* in diameter.

PCR techniques, including reverse-transcriptase PCR (RT-PCR) and *in situ* hybridization (ISH).

F.5.4 **Diagnostic Methods**

More detailed information on diagnostic methods for IPN can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000b), at http://www.oie.int or selected references.

F.5.4.1 *Presumptive*

F.5.4.1.1 Gross Observations (Level I)

Clinical signs in salmonid fry and parr include lying on the bottom of tanks/ponds, or showing cork-screw swimming behaviour. High mortalities may occur when fry are first fed or in smolt shortly after transfer to seawater. Chronic low mortalities may persist at other times. Dark discolouration (especially of the dorsal and tail surfaces) may be accompanied by swollen abdomens, pop-eye and/or pale faecal casts.

F.5 Infectious Pancreatic Necrosis (IPN)

F.5.4.1.2 Histopathology (Level II)

Tissue pathology is characterised by necrotic lesions and ulcers in the pancreas, oesophagus and stomach. The intestines may be empty or filled with clear mucus (NB difference from parasite infection by *Hexamita inflata* (Hexamitiasis), where there is a yellowish mucus plug).

F.5.4.1.3 Virology (Level III)

As described for screening (F.5.3.1.2), fish material suitable for virological examination include whole alevin (body length A 4 cm), viscera including kidney (fish 4 – 6 cm in length) or, liver, kidney and spleen for larger fish. The virus (Fig.F.5.4.1.3) can be isolated on CHSE-214 or BF-2 cell lines, but the cause of resultant CPE has to be verified using confirmatory techniques (F.5.3.2).

F.5.4.1.4 Transmission Electron Microscopy (TEM) (Level III)

The ultrastructural characteristics of IPNV are shared by most aquatic birnavididae, thus, immunoassay or nucleic acid assays are required for confirmation of identity. Birnaviruses are non-enveloped, icosahedral viruses, measuring approximately 60 *nm* in diameter (Fig.F.5.4.1.4). The nucleic acid component is bi-segmented, dsRNA, which can be distinguished using standard histochemistry.

F.5.4.2 *Confirmatory*

F.5.4.2.1 Virology and Immunoassay (Level III)

As described for screening (F.5.3.2.1), the viral cause of any CPE on CHSE-214 or BF-2 cell lines has to be confirmed by either an immunoassay (Neutralisation test or ELISA) or PCR techniques, including RT-PCR and ISH.

F.5.5 Modes of Transmission

The disease is transmitted both horizontally through the water route and vertically via the egg. Horizontal transmission is achieved by viral uptake across the gills and by ingestion. The virus shows strong survival in open water conditions and can survive a wide range of environmental parameters. This, in addition to its lack of host specificity, provides gives IPNV the ability to persist and spread very easily in the open-water environment.

F.5.6 Control Measures

Prevention methods include avoidance of fertilised eggs from IPNV carrier broodstock and use of a spring or borehole water supply (free of potential reservoir fish). Surface disinfection of eggs has not been entirely effective in preventing vertical transmission.

Control of losses during outbreaks involves reducing stocking densities and dropping water temperatures (in situations where temperature can be controlled).

Vaccines are now available for IPN and these should be considered for fish being grown in IPNV endemic areas.

F.5.7 Selected References

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F.5 Infectious Pancreatic Necrosis (IPN)

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F.6 VIRAL ENCEPHALOPATHY AND RETINOPATHY (VER)

F.6.1 Background Information

F.6.1.1 *Causative Agents*

Viral Encephalopathy and Retinopathy (VER) is caused by icosahedral, non-enveloped nodaviruses, 25-30 *nm* in diameter. These agents are also known as Striped Jack Nervous Necrosis Virus (SJNNV), Viral Nervous Necrosis (VNN) and Fish Encephalitis Virus (FEV). All share serological similarities with the exception of those affecting turbot (F.6.1.2). More detailed information about the disease can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a).

F.6.1.2 *Host Range*

The pathology of VER occurs in larval and, sometimes, juvenile barramundi (sea bass, *Lates calcarifer*), European sea bass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*), halibut (*Hippoglossus hippoglossus*), Japanese parrotfish (*Oplegnathus fasciatus*), red-spotted grouper (*Epinepheles akaara*), and striped jack (*Pseudocaranx dentex*). Disease outbreaks with similar/identical clinical signs have been reported in tiger puffer (*Takifugu rubripes*), Japanese flounder (*Paralichthys olivaceus*), kelp grouper (*Epinephelus moara*), brown spotted grouper (*Epinephelus malabaricus*), rock porgy (*Oplegnathus punctatus*), as well as other cultured marine fish species.

F.6.1.3 *Geographic Distribution*

VER occurs in Asia, the Mediterranean and the Pacific.

F.6.1.4 *Asia-Pacific Quarterly Aquatic Animal Disease Reporting System (1999-2000)*

Australia reported VER occurrence during 8 of 12 months in 1999, and 7 of 12 months in 2000. Japan reported VER in 6 of 12 months in 2000, and 3 of 12 months in 1999. Last major outbreak reported by Singapore was in 1997 and recently in April 1999 and November 2000 among seabass. Korea RO suspected VER occurrence for whole year of 1999 and half year of 2000 (OIE 1999, OIE 2000b).

F.6.2 Clinical Aspects

VER affects the nervous system. All affected species show abnormal swimming behaviour (cork-screwing, whirling, darting and belly-up motion) accompanied by variable swim bladder hyperinflation, cessation of feeding, changes in colouration, and mortality (Fig.F.6.2). Differences between species are most apparent with relation to age of onset and clinical severity. Earlier clinical onset is associated with greater mortality, thus onset at one day post-hatch in striped jack results in more severe losses than suffered by turbot, where onset is up to three weeks post-hatch. Mortalities range from 10-100%.

Two forms of VER have been induced with experimental challenges (Peducasse *et al*.1999):

i) acute – induced by intramuscular inoculation, and
ii) sub-acute – by intraperitoneal inoculation, bath, cohabitation and oral routes.

F.6.3 Screening Methods

More detailed information on screening methods for VER can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000b), at http://www.oie.int or selected references.

F.6.3.1 *Presumptive*

There are no obvious diagnostic lesions that can be detected in sub-clinical carriers.

F.6.3.2 *Confirmatory*

F.6.3.2.1 Virology (Level III)

The nodavirus from barramundi has been cultured on a striped snakehead (*Channa striatus*) cell line (SSN-1) (Frerichs *et al*. 1996). The applicability of this cell line to other nodaviruses in this group is unknown.

F.6.3.2.2 Nucleic Acid Assays (Level III)

A newly developed polymerase chain reaction (PCR) method has shown potential for screening potential carrier striped jack and other fish species (*O. fasciatus, E. akaara, T. rubripes, P. olivaceus, E. moara, O. punctatus* and *D. labrax*).

F.6.4 Diagnostic Methods

More detailed information on diagnostic methods for VER can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000b), at http://www.oie.int or selected references.

F.6 Viral Encephalopathy and Retinopathy (VER)

(J Yulin)

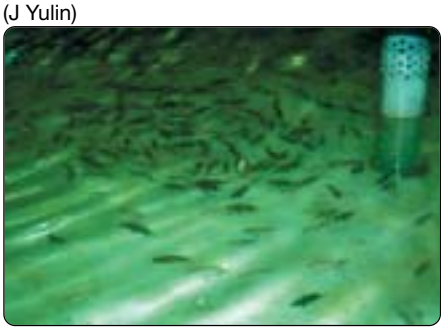


Fig.F.6.2. Fish mortalities caused by VER.

F.6.4.1 *Presumptive*

F.6.4.1.1 Gross Observations

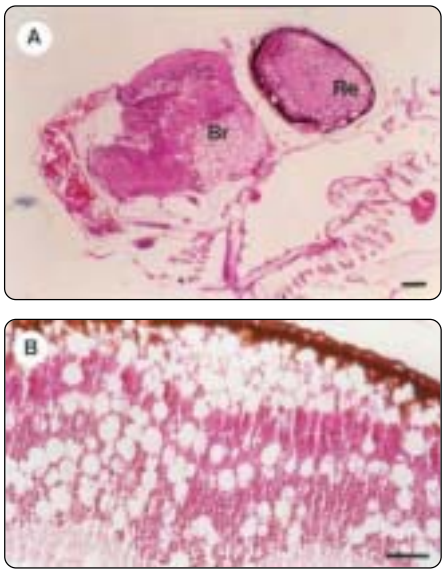
Abnormal swimming behaviour and swim-bladder inflation in post-hatch larvae and juvenile stages of the host. Species described above, along with associated mortalities are indicative of VER. Different species show different gross clinical signs (Table F.6.4.1.1). Non-feeding, wasting and colour changes in association with behavioural abnormalities, should also be considered suspect.

F.6.4.1.2 Histopathology (Level II)

Normal histological methods may reveal varying degrees of vacuolisation in the brain or retinal tissues (Fig.F.6.4.1.2a and Fig.F.6.4.1.2b). Small larvae can be embedded whole in paraffin blocks and serially sectioned to provide sections of brain and eyeballs. Larger fish (juvenile) usually require removal and fixation of eyes and brain.

All the diseases described/named under F.6.1.1 demonstrate vacuolisation of the brain, although some species (*e.g.*, shi drum, *Umbrina cirrosa*) may show fewer, obvious, vacuolar lesions. In addition, vacuolisation of the nuclear layers of the retina may not be present in Japanese parrotfish or turbot. Intracytoplasmic inclusions (A 5 μm diameter) have been described in sections of European sea bass and Australian barramundi, Japanese parrotfish and brown-spotted grouper nerve tissue. Neuronal necrosis has been described in most species. Vacuolisation of the gut is not caused by VER nodaviruses, but is typical.

(S Chi Chi)



Figs.F.6.4.1.2a, b. Vacuolation in brain (Br) and retina (Re) of GNNV-infected grouper in Chinese Taipei (bar = 100 mm).

F.6.4.2 *Confirmatory*

F.6.4.2.1 Virology (Level III)

As described under F.6.3.2.1.

F.6.4.2.2 Immunoassays (Level III)

Immunohistochemistry protocols for tissue sections fixed in Bouin's or 10% buffered formalin and direct fluorescent antibody test (DFAT) techniques use antibodies sufficiently broad in specificity to be able to detect at least four other viruses in this group. An ELISA test is only applicable to SJNNV from diseased larvae of striped jack.

F.6.4.2.3 Transmission Electron Microscopy (TEM) (Level III)

Virus particles are found in affected brain and retina by both TEM and negative staining. Positive stain TEM reveals non-enveloped, icosahedral, virus particles associated with vacuolated cells and inclusion bodies. The particles vary from 22-25 *nm* (European sea bass) to 34 *nm* (Japanese parrotfish) and form intracytoplasmic crystalline arrays, aggregates or single particles (both intra- and extracellular). In negative stain preparations, non-enveloped,

F.6 Viral Encephalopathy and Retinopathy (VER)

Species	Behaviour Changes	Appearance Changes	Onset of Clinical Signs
Barramundi	Uncoordinated darting and corkscrew swimming; off feed	Pale colouration, anorexia and wasting	Earliest onset at 9 days post-hatch. Usual onset at15-18 days post-hatch
European Sea Bass	Whirling swimming; off feed	Swim-bladder hyperinflation	Earliest onset at 10 days post-hatch. Usual onset 25-40 days post-hatch
Japanese Parrotfish	Spiral swimming	Darkened colour	First onset anywhere between 6-25 mm total length
Red-spotted Grouper	Whirling swimming	-	First onset at 14 days post-hatch (7-8 mm total length). Usual onset at 9-10 mm total length
Brown-spotted Grouper	-	-	20-50 mm total length
Striped Jack	Abnormal swimming	Swim-bladder hyperinflation	1-4 days post-hatch
Turbot	Spiral and/or looping swim pattern, belly-up at rest	Darkened colour	< 21 days post-hatch

Table F.6.4.1.1 – adapted from OIE (1997)

round to icosahedral particles, 25-30 *nm*, are detectable. These are consistent with VER nodaviruses.

F.6.4.2.4 Nucleic Acid Assay (Level III)

Reverse transcriptase PCR assays have been developed for VER nodavirus detection and identification.

F.6.5 Modes of Transmission

Vertical transmission of VER virus occurs in striped jack, and ovarian infection has been reported in European sea bass. Other modes of transmission have not been clearly demonstrated, but horizontal passage from juvenile fish held at the same site, and contamination of equipment cannot yet be ruled out. Experimental infections have been achieved in larval stripe jack and red-spotted grouper using immersion in water containing VER virus. Juvenile European sea bass have also been infected by inoculation with brain homogenates from infected individuals.

F.6.6 Control Measures

Control of VNN in striped jack and other affected species is complicated by the vertical transmission of the virus(es). Strict hygiene in hatcheries may assist in controlling VNN infection. Culling of detected carrier broodstock is one control option used for striped bass, however, there is some evidence that reduced handling at spawning can reduce ovarian infections and vertical transmission in some carrier fish. Control of clinical disease in striped bass using the following techniques has also shown some success:

- no recycling of culture water
- chemical disinfection of influent water and larval tanks between batches, and
- reduction of larval density from 15-30 larvae/litre to <15 larvae/litre (preferably fewer than 10 larvae/litre).

Anderson *et al.* (1993) reported that non-recycling of water, chemical sterilization of influent seawater and disinfection of half of the tanks during each hatching cycle was successful in a barramundi hatchery.

Extensive culture in 'green ponds' has also been related to low prevalences of clinical disease and/or histological lesions.

Arimoto *et al.* (1996) recommended the following measures: (a) disinfection of eggs (iodine or ozone) and materials (chlorine); (b) rearing of each batch of larvae/juveniles in separate tanks supplied with sterilized (UV or ozone) seawater; and (c) rigorous separation of larval and juvenile striped jack from brood fish.

F.6 Viral Encephalopathy and Retinopathy (VER)

F.6.7 Selected References

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F.7 SPRING VIRAEMIA OF CARP (SVC)

F.7.1 **Background Information**

F.7.1.1 *Causative Agents*

Spring viraemia of carp (SVC) is caused by ssRNA Vesiculovirus (Rhabdoviridae), known as Spring viraemia of carp Virus (SVCV) or *Rhabdovirus carpio* (RVC) (Fijan 1999). More detailed information about the disease can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a).

F.7.1.2 *Host Range*

SVCV infects several carp and cyprinid species, including common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), crucian carp (*Carassius carassius*), goldfish (*C. auratus*), tench (*Tinca tinca*) and sheatfish (*Silurus glanis*).

F.7.1.3 *Geographic Distribution*

SVC is currently limited to the parts of continental Europe that experience low water temperatures over winter.

F.7.1.4 *Asia-Pacific Quarterly Aquatic Animal Disease Reporting System (1999 – 2000)*

No reported case in any country during reporting period for 1999 and 2000 (OIE 1999, 2000b).

F.7.2 **Clinical Aspects**

Young carp and other susceptible cyprinids (F.7.1.2), up to 1 year old, are most severely affected. Overt infections are manifest in spring when water temperatures reach 11-17 °C. Poor physical condition of overwintering fish appears to be a significant contributing factor. Mortalities range from 30-70%.

Viral multiplication in the endothelial cells of blood capillaries, haematopoietic tissue and nephron cells, results in oedema and haemorrhage and impairs tissue osmoregulation. Kidney, spleen, gill and brain are the organs in which SVCV is most abundant during overt infection. Survivors demonstrate a strong protective immunity, associated with circulating antibodies, however, this results in a covert carrier state.

F.7.3 **Screening Methods**

More detailed information on methods for screening SVC can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int or selected references.

F.7.3.1 *Presumptive*

There are no methods for detection of sub-clinical infections using gross observations or routine histology.

F.7.3.1.1 Virology (Level III)

Screening for sub-clinical carriers uses tissue homogenates from the brain of any size fish or the ovarian fluids from suspect broodstock fish. Cell lines susceptible to SVCV are EPC and FHM. Any resultant CPE requires molecular-based assays as described under F.7.3.2.

F.7.3.2 *Confirmatory*

F.7.3.2.1 Immunoassays (Level III)

CPE products can be checked for SVCV using a virus neutralisation (VN) test, indirect fluorescent antibody tests (IFAT), and ELISA. IFAT can also be used on direct tissue preparations.

F.7.4 **Diagnostic Methods**

More detailed information on methods for diagnosis of SVC can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int
or selected references.

F.7.4.1 Presumptive

F.7.4.1.1 Gross Observations (Level I)

Sudden mortalities may occur with no other clinical signs. Behavioural clues are non-specific to SVC and include lethargy, separation from the shoal, gathering at water inlets or the sides of ponds and apparent loss of equilibrium.

External signs of infection are also non-specific, with fish showing varying degrees of abdominal distension (dropsy), protruding vents and trailing mucoid faecal casts. Haemorrhaging at the bases of the fins and vent, bulging eye(s) (pop-eye or exophthalmia), overall darkening and pale gills may also be present (Figs.F.7.4.1.1a, b, c and d).

Internal macroscopic signs of infection include an accumulation of body cavity fluids (ascites)

which may lead to the dropsy visible as abdominal distension, bloody and mucous-filled intestines, swim-bladder haemorrhage and gill degeneration.

F.7.4.1.2 Transmission Electron Microscopy (TEM) (Level III)

Detection of enveloped, bullet-shaped, viral particles measuring 90-180 nm in length and with a regular array of spicules on the surface in spleen, kidney and brain tissues, or in isolates from CPE in the cell-lines described under F.7.4.1.3, should be considered indicative of SVC in susceptible carp species showing other clinical signs of the disease. Viral replication takes place in the cytoplasm with maturation in association with the plasma membrane and Golgi vesicles.

F.7.4.1.3 Virology (Level III)

Whole fish (body length A4 cm), or viscera including kidney (fish 4 - 6 cm in length) or kidney, spleen and brain of larger fish, can be prepared for tissue culture using *Epithelioma papulosum cyprinae* (EPC) or FTM cell lines. Resultant CPE should be examined using the diagnostic techniques outlined below and under F.7.3.2.1 to confirm SVCV as the cause.

F.7.4.2 *Confirmatory*

F.7.4.2.1 Immunoassays (Level III)

As described under F.7.4.1.3, SVCV can be confirmed in CPE products using a virus neutralisation (VN) test, indirect fluorescent antibody tests (IFAT), and ELISA. IFAT can also be used on direct tissue preparations.

F.7.4.2.2 Nucleic Acid Assay (Level III)

RT-PCR techniques are under development.

F.7.5 Modes of Transmission

Horizontal transmission can be direct (contact with virus shed into the water by faeces, urine, reproductive fluids and, probably, skin mucous) or indirectly via vectors (fish-eating birds, the carp louse *Argulus foliaceus* or the leech *Piscicola geometra*). Vertical transmission is also possible via SVCV in the ovarian fluids (however, the rarity of SVC in fry and fingerling carp indicates that this may be a minor transmission pathway).

SVCV is hardy and can retain infectivity after exposure to mud at 4°C for 42 days, stream water at 10°C for 14 days, and after drying at 4-21°C for 21 days. This means that avenues for establishing and maintaining reservoirs of infection are relatively unrestricted. This, plus the broad direct and indirect mechanisms for transmission, makes this disease highly contagious and difficult to control.

F.7.6 Control Measures

No treatments are currently available although some vaccines have been developed. Most effort is applied to optimising the overwintering condition of the fish by reducing stocking density, reduced handling and strict maintenance of hygiene. New stocks are quarantined for at least two weeks before release into ponds for grow-out.

Control of spread means rapid removal and destruction of infected and contaminated fish immediately on detection of SVC. Repeat outbreaks may allow action based on presumptive diagnosis. First time outbreaks should undertake complete isolation of affected fish until SVC can be confirmed.

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(EAFP)

Fig.F.8.4.1.1. Non-specific internal sign (petechial haemorrhage on muscle) of VHS infected fish.

(EAFP)

Figs.F.7.4.1.1a, b, c, d. Non-specific clinical signs of SVC infected fish, which may include swollen abdomen, haemorrhages on the skin, abdominal fat tissue, swim bladder and other.

F.8 VIRAL HAEMORRHAGIC SEPTICAEMIA (VHS)

F.8.1 **Background Information**

F.8.1.1 *Causative Agent*

Viral haemorrhagic septicaemia (VHS) is caused by ssRNA enveloped rhabdovirus, known as viral haemorrhagic septicaemia virus (VHSV). VHSV is synonymous with Egtved virus. Although previously considered to fall within the Lyssavirus genus (Rabies virus), the ICTV have removed it to "unassigned" status, pending evaluation of a proposed new genus – Novirhabdovirus – to include VHSV and IHNV (see http://www.ncbi.nlm.nih.gov/ICTV). Several strains of VHSV are recognised. More detailed information about the disease can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a).

F.8.1.2 *Host Range*

VHS has been reported from rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), white fish (*Coregonus* spp.), pike (*Esox lucius*) and turbot (*Scophthalmus maximus*). Genetically distinct strains of VHSV have also been associated with disease in Pacific salmon (*Oncorhynchus* spp.), Pacific cod (*Gadus macrocephalus*) and Pacific herring (*Clupea pallasi*). These strains show little virulence in rainbow trout challenges (OIE 2000a). VHSV has also been isolated from Atlantic cod (*Gadus morhua*), European sea bass (*Dicentrarchus labrax*), haddock (*Melanogrammus aeglefinus*), rockling (*Rhinonemus cimbrius*), sprat (*Sprattus sprattus*), herring (*Clupea harengus*), Norway pout (*Trisopterus esmarkii*), blue whiting (*Micromesistius poutassou*), whiting (*Merlangius merlangius*) and lesser argentine (*Argentina sphyraena*) (Mortensen 1999), as well as turbot (*Scophthalmus maximus*) (Stone *et al.* 1997). Among each species, there is a high degree of variability in susceptibility with younger fish showing more overt pathology.

F.8.1.3 *Geographic Distribution*

VHSV is found in continental Europe, the Atlantic Ocean and Baltic Sea. Although VHSV-like infections are emerging in wild marine fish in North America, VHS continues to be considered a European-based disease, until the phylogenetic identities of the VHSV-like viruses which do not cause pathology in rainbow trout can be clearly established.

F.8.1.4 *Asia-Pacific Quarterly Aquatic Animal Disease Reporting System (1999-2000)*

Japan reported the disease during second quarter of 2000, no other reports from other countries (OIE 1999, 2000b).

F.8.2 **Clinical Aspects**

The virus infects blood cells (leucocytes), the endothelial cells of the blood capillaries, haematopoietic cells of the spleen, heart, nephron cells of the kidney, parenchyma of the brain and the pillar cells of the gills. Spread of the virus causes haemorrhage and impairment of osmoregulation. This is particularly severe in juvenile fish, especially during periods when water temperatures ranging between 4 – 14°C.

F.8.3 **Screening Methods**

More detailed information on methods for screening VHS can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int or selected references.

F.8.3.1 *Presumptive*

F.8.3.1.1 Gross Observations (Level I) and Histopathology (Level II)

There are no gross visible clues (Level I) or histopathology clues (Level II) to allow presumptive diagnosis of sub-clinical VHS infections. Subclinical carriers should be suspected, however, in populations or stocks which originate from survivors of clinical infections or from confirmed carrier broodstock.

F.8.3.1.2 Virology (Level III)

VHSV can be isolated from sub-clinical fish on Bluegill Fry (BF-2), *Epithelioma papulosum cyprinae* (EPC) or rainbow trout gonad (RTG-2). Any resultant CPE requires further immunoassay or nucleic acid assay to confirm VHSV as the cause (F.8.3.2).

F.8.3.1.3 Immunoassay (Level III)

Immunohistochemistry can be used to highlight VHSV in histological tissue samples (which on their own cannot be used to screen sub-clinical infections). Due to the wide range of hosts and serotypes, however, any cross-reactions need to be confirmed via tissue culture and subsequent viral isolation as described under F.8.3.1.2.

F.8 Viral Haemorrhagic Septicaemia (VHS)

F.8.3.2 *Confirmatory*

F.8.3.2.1 Immunoassay (Level III)

Identification of VHSV from cell-line culture can be achieved using a virus neutralisation test, indirect fluorescent antibody test (IFAT) or ELISA.

F.8.3.2.2 Nucleic Acid Assay (Level III)

RT-PCR techniques have been developed.

F.8.4 Diagnostic Procedures

More detailed information on methods for diagnosis of VHS can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int or selected references.

F.8.4.1 *Presumptive*

F.8.4.1.1 Gross Observation (Level I)

There are no VHS-specific gross clinical signs. General signs are shared with bacterial septicaemias, IHN, osmotic stress, handling trauma, *etc.*, and include increased mortality, lethargy, separation from the shoal, gathering around the sides of ponds, nets or water inlets.

The skin may become darkened and haemorrhagic patches may be visible at the case of the fins, the vent and over the body surface. Gill may also be pale. Internal organ changes may or may not be present depending on the speed of onset of mortalities (stressed fish die quicker). Where present these include an accumulation of bloody body cavity fluids (ascites), mucous-filled intestines and pale rectal tissues. Pin-point haemorrhages may also be present throughout the muscle (Fig.F.8.4.1.1), fat (adipose) tissue and swim-bladder.

F.8.4.1.2 Virology (Level III)

VHSV can be isolated from whole alevin (body length A 4 cm), viscera including kidney (fish 4 – 6 cm in length) or kidney, spleen and brain tissue samples from larger fish, using BF-2, EPC or RTG-2 (as described under F.8.3.1.2). Any resultant CPE requires further immunoassay or nucleic acid assay to confirm VHSV as the cause (F.8.3.2.1/2).

F.8.4.1.3 Immunoassay (Level III)

Immunohistochemistry can be used to highlight VHSV in histopathological lesions (however, histology is not a normal method of diagnosing VHS). Due to the wide range of hosts and serotypes, however, any cross-reactions need to be confirmed via tissue culture and subsequent viral isolation as described under F.8.3.1.2.

F.8.4.2 *Confirmatory*

As described under F.8.3.2.

F.8.5 Modes of Transmission

VHSV is shed in the faeces, urine and sexual fluids of clinically infected and sub-clinical carrier fish (wild and cultured). Once established at a site or in a water catchment system, the disease becomes enzootic because of the virus carrier fish. Water-borne VHSV can be carried 10-26 km downstream and remain infective. Mechanical transfer by fish-eating birds, transport equipment and non-disinfected eggs from infected broodstock, have all been demonstrated as viable routes of transmission (Olesen 1998).

F.8.6 Control Measures

No treatments are currently available, although DNA-based vaccines have shown some success under experimental conditions. Most control methods aim towards breaking the transmission cycle and exposure to carriers, as well as reducing stress. Pathogenic proliferation occurs at temperatures <15°C and periods of handling stress in sub-clinical populations.

Isolation, destruction and sterile/land-fill disposal of infected fish, as well as susceptible fish exposed downstream, along with disinfection of sites and equipment, has proven effective in controlling losses from this disease. Disinfection requires a minimum of 5 minutes contact with 3% formalin or 100 ppm iodine, 10 minutes with 2% sodium hydroxide and 20 minutes with 540 mg/L chlorine. Fallowing for at least 4 weeks when water temperatures exceed 15°C has also proven effective for re-stocking with VHSV-negative fish. These approaches have led to elimination of VHS from several areas in Europe.

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F.8 Viral Haemorrhagic Septicaemia (VHS)

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F.9 LYMPHOCYSTIS

F.9.1 **Background Information**

F.9.1.1 *Causative Agent*

Lymphocystis is caused by dsDNA, non-enveloped, iridoviruses, with particles measuring 200±50 *nm*, making them among the largest of the Iridoviridae. The iridovirus associated with gilthead sea bream (*Sparus aurata*) is known as Lymphocystis Disease Virus (LDV).

F.9.1.2 *Host Range*

Lymphocystis occurs in many marine and some freshwater fish families, including, herring (Clupeidae), smelt (Osmeridae), sea bass (Serranidae), flounder (Paralichthidae), snappers (Lutjanidae), perch (Percidae), drum (Sciaenidae), butterfly fishes (Chaetodontidae), cichlids (Cichlidae), gobies (Gobiidae) and sole (Soleidae).

F.9.1.3 *Geographic Distribution*

The geographic range of lymphocystis is probably global. The disease has been reported from Europe, North and Central America, Australia, Africa, Hawaii, the South Pacific and Asia.

F.9.2 **Clinical Aspects**

Lymphocystis is a common chronic and benign infection by an iridovirus that results in uniquely hypertrophied cells, typically in the skin and fins of fishes. The main clinical signs are white (occasionally pale red), paraffin-like nodules covering the skin and fins of sick fish (Fig. F.9.2a). Some particulate inclusions may be observed in the lymphoma lesion.

At maturity the lesions are irregularly elevated masses of pebbled texture. The colour is light cream to grayish, but covering epithelial tissue may be normally pigmented. Vascularity sometimes gives large clusters of cells a reddish hue. Considerable variation occurs in size, location, and distribution of the masses. Infected cells may also occur singly.

Although the infection is rarely associated with overt disease, mortalities can occur under culture conditions, possibly due to impaired gill, swimming or feeding capability with mechanically intrusive lesions. The primary effect, however, is economical, as fish with such grossly visible lesions are difficult to market.

F.9.3 **Screening Methods**

Currently there are no detection techniques that are sensitive enough to detect or isolate this group of iridoviruses from sub-clinically infected fish. To date, cell-culture techniques have been limited to isolation of virus from evident lymphoma lesions.

F.9.4 **Diagnostic Methods**

F.9.4.1 *Presumptive*

F.9.4.1.1 Gross Observations (Level I)

The main external signs associated with lymphocystis are white (or occasionally pale pink), paraffin wax-like nodules or growths over the skin and fins. Such growths may contain small granular-like particles, and some may show signs of vascularisation (extension of blood capillaries into the tissue growth) (Fig. F.9.4.1.1a and Fig. F.9.4.1.1b). The presence of the granular inclusions is an important for distinguishing lymphocystis from Carp Pox disease (caused by a *Herpesvirus*) (Fig.F.9.4.1.1c). The wax-like appearance is also an important feature which distinguishes Lymphocystis from fungal (mycotic) skin growths (Fig.F.9.4.1.1d).

F.9.4.2 *Confirmatory*

F.9.4.2.1 Histopathology (Level II)

Light microscopy of tissue sections of lymphoma reveal that the particulate inclusions are virus-induced giant cells of connective tissue origin, enveloped with a thick capsule. The diameter of each giant cell is about 500 Îm, a magnification of normal cell volume of 50,000 to 100,000-fold (Fig.F.9.4.2.1a). The distinctive capsule (Fig.F.9.4.2.1b), enlarged and centrally located nucleus and nucleolus, and cytoplasmic inclusions, are unique. No such cell alterations occur with Carp Pox *Herpesvirus* infections. In addition, there may be some eosinophilic, reticulate (branching) inclusion bodies in cytoplasm of the giant cell. These correspond to the viral replicating bodies which have a light refractive density which renders them “cytoplasmic-like” and demonstrate one of the few instances when a viral aetiology can be diagnosed at the light microscope level with a high degree of confidence. Identification of the exact virus(es) involved requires further investigation, however, this level of diagnosis is sufficient to allow control advice to be made (F.9.6).

F.9 Lymphocystis

(MG Bondad-Reantaso)

Fig.F.9.2a. Wild snakehead infected with lymphocystis showing irregularly elevated masses of pebbled structure.

(J Yulin)

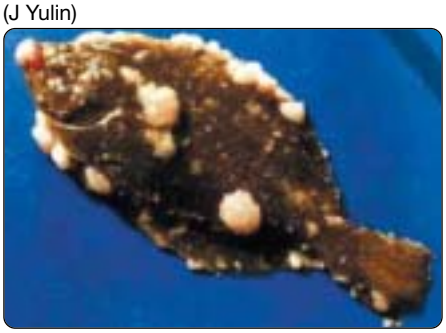

Fig.F.9.4.1.1a. Flounder with severe lymphocystis.

(J Yulin)

Fig.F.9.4.1.1b. Lymphocystis lesions showing granular particle inclusions.

(J Yulin)

Fig.F.9.4.1.1c. Carp Pox Disease caused by Herpesvirus.

(J Yulin)

Fig.F.9.4.1.1d. Goldfish with fungal (mycotic) skin lesions

(J Yulin)

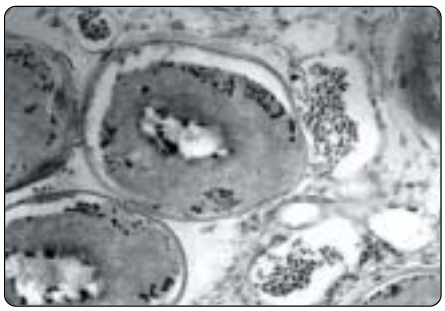

Fig.F.9.4.2.1a. Giant (hypertrophied lymphoma cells with reticulate or branching inclusion bodies around the nuclei.

(J Yulin)

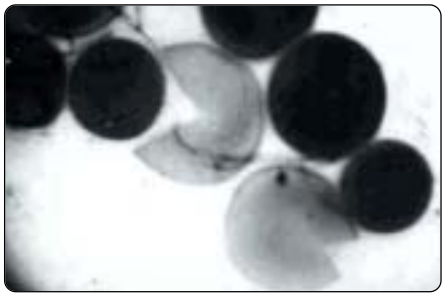

Fig.F.9.4.2.1b. Impression smear of lymphocystis showing some giant cells, and hgaline capsules (membrane).

F.9 Lymphocystis

F.9.4.2.2 Transmission Electron Microscopy (TEM) (Level III)

TEM of ultra-thin section of lymphocystis tissue is the principal method of further confirming gross and light microscope observations. The viral particles show up as large icosahedral (roughly hexagonal-spherical) particles measuring 150-300 *nm* within the encapsulated cell cytoplasm. Ultrastructural features of lymphocystis iridovirus particles include a dense core within two unit membranes making up the capsid (Fig.F.9.4.2.2a and Fig.F.9.4.2.2b). Note the difference from *Herpesvirus* in Carp Pox, which are enveloped and smaller virions (Fig.F.9.4.2.2c).

F.9.4.2.3 Virology (Level III)

Due to the relative ease of finding and identifying the viruses associated with lymphocystis lesions (compared with the viral agents of other fish diseases), there has been little emphasis on cell culture as a means of confirming diagnosis of the disease. However, the growing impact of this disease in aquaculture situations around the world has increased interest in differentiating between the iridoviral agents involved and enhancing apparent acquired immunity to infection. A new cell-line from gilt-head sea bream is currently under investigation and has shown promise for isolating lymphocystis iridoviruses.

F.9.5 Modes of Transmission

Horizontal contact and water-borne transmission appear to be the principal mechanism for lymphocystis virus spread. This is reinforced by proliferation of the problem under intensive culture conditions. High population density and external trauma enhance transmission. External surfaces including the gills appear to be the chief portal of epidermal entry. The oral route seems not to be involved, and there is no evidence of vertical transmission.

F.9.6 Control Methods

At present, there is no known method of therapy or of immunization. There is some evidence of antibodies in at least one flatfish species, however, this remains to be investigated further. Avoidance of stocking with clinically infected fish, early detection through monitoring and sterile (land-fill or chemical) disposal, along with minimising stocking densities and handling skin-trauma, have proven to be effective controls.

(J Yulin)

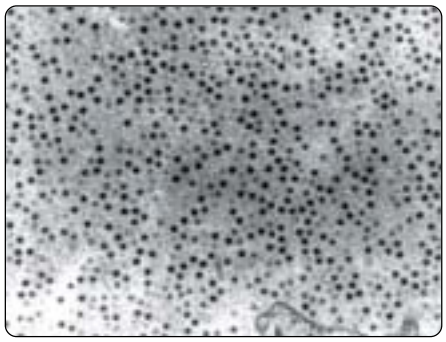

Fig.F.9.4.2.2a. Electron micrograph showing numerous viral particles in cytoplasm.

(J Yulin)

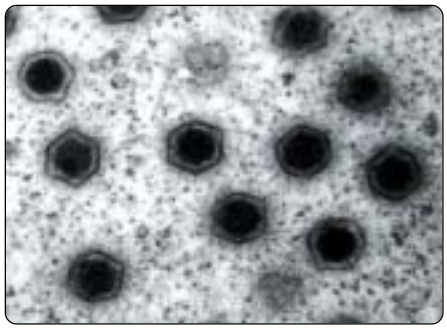

Fig.F.9.4.2.2b. Enlarged viral particles showing typical morphology of iridovirus (100 mm = bar).

(J Yulin)

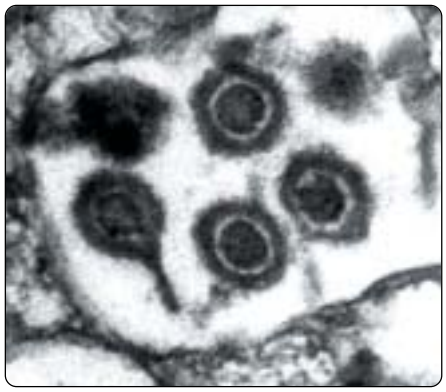

Fig.F.9.4.2.2c. *Herpervirus* in Carp Pox showing enveloped and smaller virions compared to lymphocystis virus.

F.9 Lymphocystis

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BACTERIAL DISEASE OF FINFISH
F.10 BACTERIAL KIDNEY DISEASE (BKD)

F.10.1 **Background Information**

F.10.1.1 *Causative Agent*

Bacterial kidney disease (BKD) is caused by *Renibacterium salmoninarum*, a coryneform, rod-shaped, Gram-positive bacterium that is the sole species belonging to the genus *Renibacterium*. More detailed information about the diseases can be found in the OIE Manual for Aquatic Animal Diseases (OIE 2000a).

F.10.1.2 *Host Range*

Fish of the Salmonidae family are clinically susceptible, in particular the *Oncorhynchus* species (Pacific salmon and rainbow trout).

F.10.1.3 *Geographic Distribution*

BKD occurs in North America, Japan, Western Europe and Chile.

F.10.1.4 *Asia-Pacific Quarterly Aquatic Animal Disease Reporting System (1999-2000)*

Japan reported BKD occurrence for whole year except for the month of December for both 1999 and 2000 reporting period. Pakistan suspected the disease from July to December of 1999 (OIE 1999, 2000b).

F.10.2 **Clinical Aspects**

Renibacterium salmoninarum infections can build up over a long period of time, with clinical disease only appearing in advanced infections, usually when the fish have completed their first year of life. Virulence of *R. salmoninarum* varies with:

- the strain of bacterium
- the salmon species infected
- environmental and holding conditions.

The bacteria can evade lysosomal breakdown by the blood cells that engulf them, thus avoid destruction by the fishes' primary defence mechanism. Nutrition and seawater transfers can also affect the pathogenicity of *R. salmoninarum* infections and broodstock infection levels are believed to have a direct correlation to susceptibility in their offspring. Progeny of parent stock with low levels or no infection with *R. salmoninarum* show better survival than offspring from BKD compromised fish. This may reflect greater transmission titres by the latter (F.10.5).

F.10.3 **Screening Methods**

Detailed information on methods for screening BKD can be found at the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int, or selected references.

F.10.3.1 *Presumptive*

F.10.3.1.1 Gross Observations (Level I) and Histopathology (Level II)

There are no gross signs or histological lesions that can be detected in sub-clinical carriers of *Renibacterium salmoninarum*.

F.10.3.1.2 Bacteriology (Level II)

When no lesions are present, the kidney should be selected for culture. In mature females, coelomic fluids may also be used. Specialised growth media, such as, kidney disease medium enriched with serum (KDM2) or charcoal (KDMC), or selective kidney disease medium (SKDM) are required due to the fastidious nature of *Renibacterium salmoninarum*.

Growth requires 2-3 weeks, but may take up to 12 weeks. Colonies are pinpoint to 2 mm in diameter, white-creamy, shiny, smooth, raised and entire (Fig.F.10.3.1.2a). The rods (Fig.F.10.3.1.2b) are 0.3-1.5 x 0.1-1.0 mm, Gram-positive, PAS-positive, non-motile, non acid-fast, frequently arranged in pairs or chains or in pleiomorphic forms ("Chinese letters"). Old cultures may achieve a granular or crystalline appearance. Transverse sections through such colonies will reveal the presence of Gram-positive rods in a crystalline matrix. Although few other bacteria have these growth characteristics, identification of the bacteria should be confirmed by immunoassay (F.10.3.2.1) or nucleic acid assay (F.10.3.2.2).

F.10.3.2 *Confirmatory*

F.10.3.2.1 Immunoassays (Level II/III)

Agglutination tests, direct and indirect fluorescent antibody tests (DFAT, IFAT) and ELISA kits are now available that can be used to detect *R. salmoninarum* antigen in fish tissues, as well as from bacterial cultures. The ELISA tests are believed to be the most sensitive to low-titre infections, hence they are recommended for screening for sub-clinical carriers (such as ovarian fluids from broodstock salmonids). Commercially produced kits are

F.10 Bacterial Kidney Disease (BKD)

(M Yoshimizu)

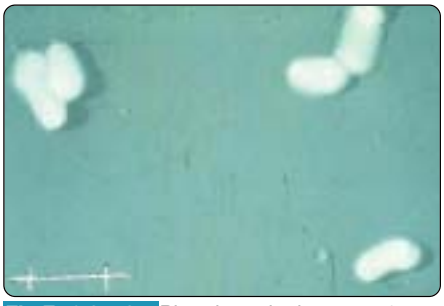

Fig.F.10.3.1.2a. Pinpoint colonies up to 2 mm in diameter of *Renibacteriium salmonimarum,* white-creamy, shiny, smooth, raised and entire; three weeks after incubation at 15°C on KDM-2 medium.

(M Yoshimizu)

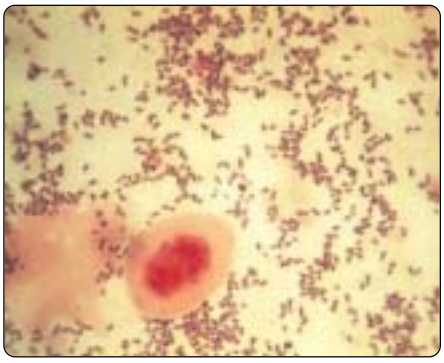

Fig.F.10.3.1.2b. Renibacterium salmoninarum rods, isolated from masou salmon.

(M Yoshimizu)

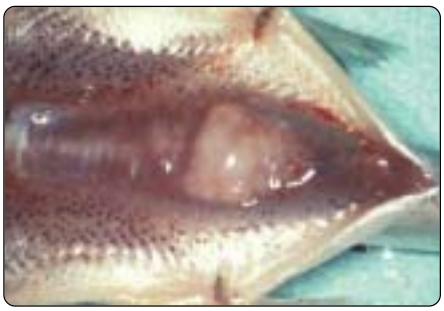

Fig.F.10.4.1.1a. Kidney of masou salmon showing swelling with irregular grayish.

also available, which contain specific instructions. Positive ELISA results, using either polyclonal or monoclonal antibodies, should be corroborated with other diagnostic tests,

(EAFP)

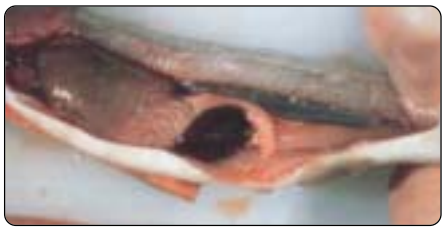

Fig.F.10.4.1.1b. Enlargement of spleen is also observed from BKD infected fish.

especially for sub-clinical cases, or first time isolations (Griffiths *et al*. 1996).

F.10.3.2.2 Nucleic Acid Assays (Level III)

Renibacterium salmoninarum primers have been developed for PCR-probes. These can detect *R. salmoninarum* DNA in tissue homogenates. The primers have been published and some kits are now commercially available.

F.10.4 **Diagnostic Methods**

Detailed information on methods for diagnosis of BKD can be found at the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int, or selected references.

F.10.4.1 *Presumptive*

F.10.4.1.1 Gross Observations (Level I)

Gross clinical signs are not usually evident until infections have become well-advanced (usually after at least 1 year). These include exophthalmia (pop-eye), varying degrees of abdominal distension (dropsy) due to disruption of the kidney excretory function, skin lesions and haemorrhaging.

Internally, there is evidence of grey/white lesions (granulomas) in all the organs, but especially the kidney (Fig.F.10.4.1.1a); enlargement of spleen (Fig.F.10.4.1.1b) is also observed. The greyish spots may show signs of multiplication and coalescence until the whole kidney appears swollen and bloated with irregular greyish patches. BKD can be distinguished from **proliferative kidney disease (PKD)** in salmonids, where the kidney becomes enlarged but there is no associated grey discolouration. Another salmonid kidney disease – **nephrocalcinosis** – only affects the urinary

ducts, which develop a white porcelain texture and colour.

F.10.4.1.2 Smears (Level I)

Smears from tissue lesions of susceptible hosts stained with Gram's stain or other metachromatic stain may reveal large numbers of small Gram-positive, rod-shaped, bacteria. Care should be taken not to confuse these with the melanin granules commonly present in kidney tissues. Other Gram-positive bacteria, such as *Lactic* species, may also be present, so further bacteriological identification methods are required.

F.10.4.1.3 Bacteriology (Level II)

Whenever possible, culturing should be used for confirmation despite the difficulties imposed by the slow, fastidious growth of *Renibacterium salmoninarum*. Presumptive diagnosis is also possible from bacterial culture due its slow growth (2-3 weeks) at 15°C. Kidney and other organs with suspicious lesions should be sampled. Protocols for culture are as described under F.10.3.1.2. Although few other bacteria have these growth characteristics, identification of the bacteria should be confirmed by immunoassay (F.10.3.2.1) or nucleic acid assay (F.10.3.2.2)

F.10.4.2 *Confirmatory*

F.10.4.2.1 Immunoassay (Level II/III)

Slide agglutination tests can be used for rapid identification of culture colonies. Bacterial agglutination is determined by comparison with duplicate suspension containing rabbit serum, as a control. Co-agglutination with *Staphylococcus aureus* (Cowan I strain) sensitised with specific immunoglobulins is also effective at enhancing the agglutination process (Kimura and Yoshimizu 1981).

For immunofluorescence (direct and indirect) and ELISA tests, MAbs against specific determinants are recommended to avoid cross-reactions with other bacteria. As noted under F.10.3.2.1, positive results, using either polyclonal or monoclonal antibodies, should be corroborated with other diagnostic tests, especially for first time isolations (Griffiths *et al*. 1996).

F.10.4.2.2 Nucleic Acid Assays (Level III)

As described under F.10.3.2.2, *Renibacterium salmoninarum* PCR-probes are now available. Cross-checking positive samples with other diagnostic methods (bacteriology, immunoassay), however, is highly recommended, especially for first time isolations (Hiney and Smith 1999).

F.10.5 **Modes of Transmission**

Renibacterium salmoninarum is widely distributed in both freshwater and marine environments. It can be transmitted horizontally by water-borne release and faecal contamination, as well as via reservoir hosts which span all salinity ranges. Indirect vertical transmission via reproductive fluids and spawning products is also possible for sub-clinical carriers of the bacteria.

F.10.6 **Control Measures**

Due to its intracellular location in the host fish, BKD is difficult to treat with antibiotics. Injection of female broodstock with erythromycin at regular intervals prior to spawning appears to have some success in preventing vertical transmission to eggs. Vaccination and medicated feeds have also shown some success in reducing the occurrence of BKD, however, results have varied with strain of *R. salmoninarum* and host species.

Most emphasis is placed on breaking vertical and horizontal transmission routes (F.10.5). Culling of high BKD-titre broodstock, reducing stocking density, avoiding contact with sub-clinical carriers/reservoirs, reducing handling stress and avoiding unacclimatised transfer from fresh to saltwater, have all proven effective in reducing BKD pathogenicity.

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FUNGUS ASSOCIATED DISEASE
F.11 EPIZOOTIC ULCERATIVE SYNDROME (EUS)

F.11.1 Background Information

F.11.1.1 *Causative Factors*

The mycotic granulomas in EUS-affected tissues are caused by the Oomycete fungus *Aphanomyces invadans* (also known as *A. invaderis, A. piscicida*, Mycotic Granuloma-fungus (MG) and ERA [EUS-related *Aphanomyces*]). It is also known as Red spot disease (RSD). More detailed information about the disease can be found in the OIE Diagnostic Manual for Aquatic Animal Diseases (OIE 2000a).

F.11.1.2 *Host Range*

EUS affects freshwater and estuarine warm water fish and was first reported in farmed ayu (*Plecoglossus altivelis*) in Japan (Fig.F.11.1.2a). Severe outbreaks occurred in Eastern Australia affecting estuarine fish, particularly grey mullet (*Mugil cephalus*). Region-wide, over 50 species (Fig.F.11.1.2b) have been confirmed affected by histopathological diagnosis (Lilley *et al.*, 1998), but some important culture species including tilapia, milkfish and Chinese carps have been shown to be resistant.

F.11.1.3 *Geographic Distribution*

EUS was first reported in Japan and subsequently in Australia. Outbreaks have shown a westward pattern of spread through Southeast and South Asia. EUS has also spread westward with major outbreaks reported in Papua New Guinea, Malaysia, Indonesia, Thailand, Philippines, Sri Lanka, Bangladesh and India. EUS has most recently been confirmed in Pakistan. The pathology demonstrated by ulcerative mycosis (UM)-affected estuarine fish along the Atlantic coast of USA is indistinguishable from EUS, but further work is required to compare the causal agents involved in each case.

F.11.1.4 *Asia-Pacific Quarterly Aquatic Animal Disease Reporting System (1999-2000)*

Australia, Bangladesh, India, Japan, Lao PDR, Nepal, Philippines, Sri Lanka, and Thailand reported the disease on various months for the reporting year 1999; for the year 2000, Australia, Bangladesh, India, Japan, Lao PDR, Nepal, Pakistan, Philippines and Thailand reported positive occurrence of EUS (OIE 1999, OIE 2000b).

F.11.2 Clinical Aspects

Affected fish typically show necrotic dermal ulcers, characterised histologically by the presence of distinctive mycotic granulomas in underlying tissues. The mycotic granulomas in EUS-affected tissues are caused by the Oomycete fungus *Aphanomyces invadans*. Initial lesions may appear as red spots (Fig.F.11.2a), which become deeper as the infection progresses and penetrate underlying musculature (Fig.F.11.2b). Some advanced lesions may have a raised whitish border. High mortalities are usually associated with EUS outbreaks but, in certain cases, where fish do not succumb to secondary invasion of these gaping wounds, ulcers may be resolved.

F.11.3 Screening Methods

There are no screening methods for sub-clinical animals available.

F.11.4 Diagnostic Methods

More detailed information on methods for diagnosis of EUS can be found in the OIE Manual for Aquatic Animal Diseases (OIE 2000a), at http://www.oie.int, or selected references..

F.11.4.1 *Presumptive*

F.11.4.1.1 Gross Observations (Level I)

The gross appearance of lesions varies between species, habitat and stage of lesion development (Fig.F.11.4.1.1a). The most distinctive EUS lesion is the open dermal ulcer. However, other diseases may also result in similar clinical lesions (Fig.11.4.1.1b) and it is, therefore, important to confirm the presence of *A. invadans* to ensure accurate diagnosis.

F.11.4.1.2 Rapid Squash Muscle Preparation (Level I)

Presumptive diagnosis of EUS in susceptible fish showing dermal lesions can be made by demonstrating aseptate hyphae (12-30 ?m in diameter) in squash preparations of the muscle underlying the visible lesion (Fig.11.4.1.2). This can be achieved using a thin piece of muscle squashed between two glass plates or microscope slides, examined using a light or dissecting microscope under field conditions.

F.11 Epizootic Ulcerative Syndrome (EUS)

(K Hatai)

Fig.F.11.1.2a. Ayu, *Plecoglatus altivelis,* infected with mycotic granulomatosis.

(RB Callinan)

Fig.F.11.1.2b. EUS affected farmed silver perch *Bidyanus bidyanus* from Eastern Australia.

(MG Bondad-Reantaso)

Fig.F.11.2a. Catfish showing initial EUS red spots.

(MG Bondad-Reantaso)

Fig.F.11.4.1.1a. Wild mullet in Philippines (1989) with EUS.

(MG Bondad-Reantaso)

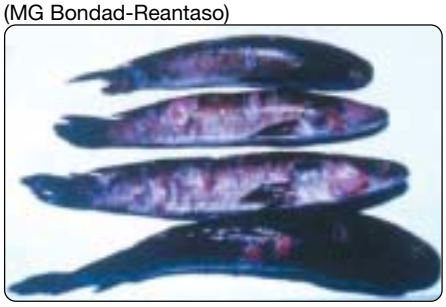

Fig.F.11.2b. Snakehead in Philippines (1985) showing typical EUS lesions.

(MG Bondad-Reantaso)

Fig.F.11.4.1.1b. Red spot disease of grass carp in Vietnam showing ulcerative lesions.

F.11.4.2 *Confirmatory*

F.11.4.2.1 Histopathology (Level II)

Confirmatory diagnosis requires histological demonstration of typical granulomas and invasive hyphae using haematoxylin and eosin (Fig.F.11.4.2.1a) or a general fungus stain (*e.g.* Grocott's) (Fig.F.11.4.2.1b). Early EUS lesions show shallow haemorrhagic dermatitis with no obvious fungal involvement. Later lesions demonstrate *A. invadans* hyphae penetrating the skeletal muscle tissues and increasing inflammation. The fungus elicits a strong inflammatory response and granulomas are formed around the penetrating hyphae, a typical characteristic of EUS. The lesion progresses from a mild chronic dermatitis, to a severe, locally pervasive, necrotising dermatitis, with severe degeneration of the muscle. The most typical lesions are large, open, haemorrhagic ulcers about 1-4 cm in diameter. These commonly show secondary infections with bacteria, and pathogenic strains of *Aeromonas hydrophila* have been isolated from lesions.

F.11.4.2.2 Mycology (Level II)

Moderate, pale, raised, dermal lesions are most suitable for fungal isolation attempts. Remove

F.11 Epizootic Ulcerative Syndrome (EUS)

the scales around the periphery of the lesion and sear the underlying skin with a red-hot spatula to sterilise the surface. Using a sterile scalpel blade and sterile, fine pointed, forceps, cut through skin underlying the seared area and cut horizontally to lift the superficial tissues and expose the underlying muscle. Ensure the instruments do not contact the external surface and contaminate the underlying muscle. Aseptically excise 2 mm^3 pieces of muscle, approximately 2 mm^3, and place on a Petri dish containing Czapek Dox agar with penicillin G (100 units/ml) and oxolinic acid (100 mg/ml). Seal plates and incubate at room temperature examining daily. Transfer emerging hyphal tips onto fresh plates of Czapek Dox agar until cultures are free of contamination.

The fungus can be identified to genus by inducing sporogenesis (Fig.F.11.4.2.2a) and demonstrating the asexual characteristics of *Aphanomyces* as described in Lilley *et al.* (1998). *A. invadans* is characteristically slow growing in culture (Fig.F.11.4.2.2b) and fails to grow at 37°C on GPY agar (GP broth with 0.5 g/l yeast extract and 12 g/l technical agar). Detailed temperature-growth profiles are given in Lilley and Roberts (1997). Confirmation that the isolate is *A. invadans* can be made by injecting a 0.1 ml suspension of 100+ motile zoospores intramuscularly in EUS-susceptible fish (preferably *Channa striata*) at 20°C, and demonstrating histologically growth of aseptate hyphae 12-30 μm in diameter in muscle of fish sampled after 7 days, and typical mycotic granulomas in muscle of fish sampled after 14 days.

F.11.5 Modes of Transmission

The spread of EUS is thought to be due to flooding and movement of affected and/or carrier fish. *Aphanomyces invadans* is considered to be the **"necessary cause"** of EUS, and is present in all cases, however, an initial skin lesion is required for the fungus to attach and invade underlying tissues. This lesion may be induced by biotic or abiotic factors. In Australia and Philippines, outbreaks have been associated with acidified water (due to acid sulfate soil runoff), along with low temperatures, presence of susceptible fish and *A. invadans* propagules. In other areas, where acid water does not occur, it is possible that other biological (*e.g.*, rhabdovirus infection) or environmental factors (*e.g.*, temperature) may initiate lesions.

F.11.6 Control Measures

Control in wild populations is impossible in most cases. Selection of resistant species for culture purposes currently appears to be the most effective means of farm-level control. Where changing culture species is not an option, measures should be taken to eradicate or exclude the fungus through:

- drying and liming of ponds prior to stocking
- exclusion of wild fish
- use of prophylactically-treated, hatchery-reared fry
- use of well-water
- salt bath treatments
- disinfection of contaminated nets and equipment.

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F.11 Epizootic Ulcerative Syndrome (EUS)

(MG Bondad-Reantaso)

Fig.F.11.4.1.2. Granuloma from squash preparation of muscle of EUS fish.

(MG Bondad-Reantaso)

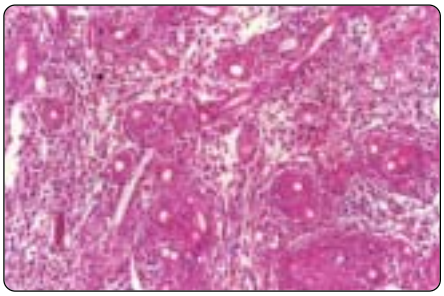

Fig.F.11.4.2.1a. Typical severe mycotic granulomas from muscle section of EUS fish (H & E).

(MG Bondad-Reantaso)

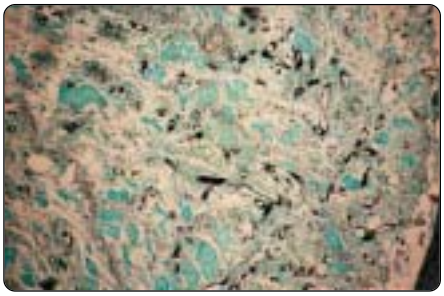

Fig.F.11.4.2.1b. Mycotic granulomas showing fungal hyphae (stained black) using Grocotts stain.

(K Hatai)

Fig.F.11.4.2.2a. Typical characteristic of *Aphanomyces* sporangium.

(MG Bondad-Reantaso)

Fig.F.11.4.2.2b. Growth of *Aphanomyces invadans* on GP agar.

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ANNEX F.AI. OIE REFERENCE LABORATORIES FOR FINFISH DISEASES

Disease	Expert/Laboratory
Epizootic haematopoietic necrosis virus (EHNV)	**Dr. A. Hyatt** Australian Animal Health Laboratory Geelong, Victoria 3213, AUSTRALIA Tel: 61-3-52275000 Fax: 61-3-52275555 E-mail: alex.hyatt@dah.csiro.au
	Dr. R. Whittington Elizabeth MacArthur Agricultural Institute PMB 8, Camden NSW 2570, AUSTRALIA Tel: 61-2-46293333 Fax: 61-2-46293343 E-mail: Richard.Whittington@smtpgwy.agric.nsw.gov.au
Infectious haematopoietic necrosis virus (Rhabdoviruses)	**Dr. J. A. Leong** Oregon State University Department of Microbiology Nash Hall 220, Corvallis, Oregon 93331-3804 UNITED STATES of AMERICA Tel: 1-541-7371834 Fax: 1-541-7370496 E-mail: leongj@orst.edu
	Dr. J. Winton Western Fisheries Research Center 6505 N.E. 65th Street Seattle, Washington 98115 UNITED STATES of AMERICA E-mail: jim_winton@nbs.gov
Onchorhynchus masou virus	**Dr. M. Yoshimizu** Laboratory of Microbiology Faculty of Fisheries Hokkaido University 3-1-1, Minato-cho, Hakodate Hokkaido 041-0821 JAPAN Tel./Fax: 81-138-408810 E-mail: yosimizu@pop.fish.hokudai.ac.jp
Spring viremia of carp virus	**Dr. B.J. Hill** The Centre for Environment, Fisheries and Aquaculture Sciences (CEFAS) Barack Road, the Nothe, Weymouth, Dorset DT4 8UB UNITED KINGDOM Tel: 44-1305-206626 Fax:44-1305-206627 E-mail: b.j.hill@cefas.co.uk
Viral haemorrhagic septicaemia virus	**Dr. N.J. Ollesen** Danish Veterinary Laboratory Hangovej 2, DK-8200 Aarhus N DENMARK Tel: 45-89372431 Fax:45-89372470 E-mail: njo@svs.dk

Annex F.AI. OIE Reference Laboratories for Finfish Diseases

Channel catfish virus	**Dr. L.A. Hanson** Fish Diagnostic Laboratory College of Veterinary Medicine Mississippi State University Box 9825, Spring Street Mississippi 39762 UNITED STATES of AMERICA Tel: 1-662-3251202 Fax: 1-662-3251031 E-mail: hanson@cvm.msstate.edu
Viral encephalopathy and retinopathy	**Dr. G. Bovo** Instituto Zooprofilaticco Sperimentale delle Venezie Dipartimento di Ittiopatologia, Via Romea 14/A 35020 Legnaro PD ITALY Tel: 39-049-8830380 Fax: 39-049-8830046 E-mail: bovo.izs@interbusiness.it
	Dr. T. Nakai Fish Pathology Laboratory Faculty of Applied Biological Sciences Hiroshima University Higashihiroshima 739-8528 JAPAN Tel: 81-824-247947 Fax: 81-824-227059 E-mail: nakaitt@ipc.hiroshima-u.ac.jp
Infectious pancreatic necrosis	**Dr. B.J. Hill** The Centre for Environment, Fisheries and Aquaculture Sciences (CEFAS) Barack Road, the Nothe, Weymouth, Dorset DT4 8UB UNITED KINGDOM Tel: 44-1305-206626 Fax:44-1305-206627 E-mail: b.j.hill@cefas.co.uk
Infectious salmon anaemia	**Dr. B. Dannevig** National Veterinary Institute Ullevalsveien 68 P.O. Box 8156 Dep., 0033 Oslo NORWAY Tel: 47-22-964663 Fax: 47-22-600981 E-mail: birgit.dannevig@vetinst.no
Epizootic ulcerative syndrome	**Dr. Kamonporn Tonguthai** Aquatic Animal Health Research Institute Department of Fisheries Kasetsart University Campus Jatujak, Ladyao, Bangkok 10900 THAILAND Tel: 662-5794122 Fax: 662-5613993 E-mail: kamonpot@fisheries.go.th
Bacterial kidney disease	**Dr. R.J. Pascho** Western Fisheries Research Center U.S. Geological Survey Biological Resources Division 6505 N.E. 65th Street Seattle, Washington 98115

Annex F.AI. OIE Reference Laboratories for Finfish Diseases

	UNITED STATES of AMERICA Tel: 1-206-5266282 Fax: 1-206-5266654 E-mail: ron_pascho@usgs.gov
Enteric septicaemia of catfish	**Dr. L.A. Hanson** Fish Diagnostic Laboratory College of Veterinary Medicine Mississippi State University Box 9825, Spring Street Mississippi 39762 UNITED STATES of AMERICA Tel: 1-662-3251202 Fax: 1-662-3251031 E-mail: hanson@cvm.msstate.edu
Piscirikettsiosis	**Dr. J.L. Fryer** Distinguished Professor Emeritus Department of Biology 220 Nash Hall Oregon State University Corvallis, Oregon 97331-3804 Tel: 1-541-7374753 Fax:1-541-7372166 E-mail: fryerj@bcc.orst.edu
Gyrodactylosis (*Gyrodactylus salaris*)	**Dr. T. Atle Mo** National Veterinary Institute Ullevalsvein 68 P.O. Box 8156 Dep., 0033 Oslo NORWAY Tel: 47-22-964722 Fax: 47-22-463877 E-mail: tor-atle.mo@vetinst.no
Red sea bream iridoviral disease	**Dr. K. Nakajima** Virology Section, Fish Pathology Division National Research Institute of Aquaculture Fisheries Agency 422-1 Nakatsuhama, Nansei-cho Watarai-gun Mie 516-0913 JAPAN Tel: 81-599661830 Fax: 81-599661962 E-mail: kazuhiro@nria.affrc.go.jp

ANNEX F.AII. LIST OF REGIONAL RESOURCE EXPERTS FOR FINFISH DISEASES IN ASIA PACIFIC[1]

Disease	Expert
Epizootic ulcerative syndrome (EUS)	**Dr. Richard Callinan** NSW Fisheries, Regional Veterinary Laboratory Wollongbar NSW 2477 **AUSTRALIA** Tel (61) 2 6626 1294 Mob 0427492027 Fax (61) 2 6626 1276 E-mail: richard.callinan@agric.nsw.gov.au
	Dr. C.V. Mohan Department of Aquaculture College of Fisheries, UAS Mangalore-575002 **INDIA** Tel: 91 824 439256 (College); 434356 (Dept), 439412 (Res) Fax: 91 824 438366 E-mail: cv_mohan@yahoo.com
	Prof. Kishio Hatai Divison of Fish Diseases Nippon Veterinary and Animal Science University 1-7-1 Kyonan-cho, Musashino, Tokyo 180 **JAPAN** Tel: 81-0422-31-4151 Fax: 81-0422-33-2094 E-mail: hatai@scan-net.ne.jp
	Ms. Susan Lumanlan-Mayo Fish Health Section Bureau of Fisheries and Aquatic Resources Arcadia Building, 860 Quezon Avenue Quezon City, Metro Manila **PHILIPPINES** Tel/Fax: 632-372-5055 E-mail: slmayo99@yahoo.com
	Mr. Jose O. Paclibare Fish Health Section Bureau of Fisheries and Aquatic Resources Arcadia Building, 860 Quezon Avenue Quezon City, Metro Manila **PHILIPPINES** Tel/Fax: 632-372-5055 E-mail: jopac@edsamail.com.ph
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	Dr. Somkiat Kanchanakhan Aquatic Animal Health Research Institute Department of Fisheries Kasetsart University Campus

[1] The experts included in this list have previously been consulted and agreed to provide valuable information and health advise concerning their particular expertise.

Annex F.AII.List of Regional Resource Experts for Finfish Diseases in Asia Pacific

	Jatujak, Ladyao, Bangkok 10900 **THAILAND** Tel: 662-5794122 Fax: 662-5613993 E-mail: somkiatkc@fisheries.go.th
	Dr. Supranee Chinabut Aquatic Animal Health Research Institute Department of Fisheries Kasetsart University Campus Jatujak, Ladyao, Bangkok 10900 **THAILAND** Tel: 662-5794122 Fax: 662-5613993 E-mail: supranee@fisheries.go.th
	Dr. Melba B. Reantaso Network of Aquaculture Centres in Asia Pacific Department of Fisheries Compound Kasetsart University Campus Jatujak, Ladyao, Bangkok 10900 **THAILAND** Tel: 662- 561-1728 to 9 ext. 113 Fax: 662-561-1727 E-mail: Melba.Reantaso@enaca.org
Viral nervous necrosis (VNN) **Viral encephalopathy and retinopathy (VER)**	**Dr. Kei Yuasa** Fisheries and Aquaculture International Co., Ltd. No. 7 Khoji-machi Bldg., Room B105 4-5 Khoji-machi, Chiyoda-ku Tokyo 102-0083 **JAPAN** Tel: 81-3-3234-8847 Fax:81-3-3239-8695 E-mail: fai@faiaqua.com; yuasakei@hotmail.com
	Dr Myoung-Ae Park Pathology Division National Fisheries Research and Development Institute South Sea Regional Fisheries Research Institute 347 Anpo-ri, Hwayang-myun, Yeosu-City Chullanam-do, 556-820 **KOREA RO** Tel: 82-662-690-8989 Fax: 82-662-685-9073 E-mail : mapark@haema.nfrda.re.kr
	Dr. Lin Li Guangdong Daya Wan Fisheries Development Center Aotou Town Huizhou City, Guangdong Province **PEOPLE's REPUBLIC OF CHINA** Tel: 86-752-5574225 Fax: 86-752-5578672 E-mail: hzgdfdc@pub.huizhou.gd.cn; linli39@hotmail.com
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Annex F.AII.List of Regional Resource Experts for Finfish Diseases in Asia Pacific

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	Dr Nguyen Huu Dung Center for Bio-Tech and Environment Research University of Fisheries 02 Nguyen Dinh Chieu St. Nha Trang City **VIETNAM** Tel: 84 58 83 2065 Fax: 84 58 83 1147 E-mail : huudung@dng.vnn.vn
Lymphocystis	**Prof. Jiang Yulin** Shenzhen Exit and Entry Inspection and Quarantine Bureau 40 Heping Road, Shenzhen 518010 **PEOPLE's REPUBLIC OF CHINA** Tel: 86-755-5592980 Fax:86-755-5588630 E-mail: szapqbxi@public.szptt.net.cn
Diseases of Grass Carp	**Prof. Jiang Yulin** Shenzhen Exit and Entry Inspection and Quarantine Bureau 40 Heping Road, Shenzhen 518010 **PEOPLE's REPUBLIC OF CHINA** Tel: 86-755-5592980 Fax:86-755-5588630 E-mail: szapqbxi@public.szptt.net.cn
Parasitic Diseases	**Dr. Robert D. Adlard** Queensland Museum PO Box 3300, South Brisbane, Queensland 4101 **AUSTRALIA** Tel: +61 7 38407723 Fax: +61 7 38461226 E-mail: RobertAd@qm.qld.gov.au; http://www.qmuseum.qld.gov.au
	Professor R.J.G. Lester Department of Microbiology and Parasitology The University of Queensland, Brisbane 4072 **AUSTRALIA** Tel: +61-7-3365-3305 Fax:+61-7-3365-4620 E-mail:R.Lester@mailbox.uq.edu.au; http://www.biosci.uq.edu.au/micro/academic/lester/lester.htm
	Dr. Ian D. Whittington Department of Microbiology and Parasitology The University of Queensland Brisbane, Queensland 4072 **AUSTRALIA** Tel: 61-7-3365-3302 Fax:61-7-3365-4620 E-mail: i.Whittington@mailbox.uq.edu.au
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Annex F.AII.List of Regional Resource Experts for Finfish Diseases in Asia Pacific

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	Dr. Bo Young Jee Pathology Division National Fisheries Research and Development Institute 408-1,Shirang-ri, Kitang-up Kitang-gun, Pusan **KOREA RO** Tel: 82-51-720-2498 Fax:82-51-720-2498 E-mail: protjee@nfrdi.re.kr
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	Dr. Susan Lim Lee-Hong Institute of Biological Sciences Institute of Postgraduate Studies and Research Universiti of Malaya 50603 Kuala Lumpur **MALAYSIA** Tel: 603-7594502 Fax: 603-7568940 E-mail: susan@umcsd.um.edu.my
	Prof. Mohammed Shariff Faculty of Veterinary Medicine Universiti Putra Malaysia 43400 Serdang, Selangor **MALAYSIA** Tel: 603-9431064; 9488246 Fax: 603-9488246; 9430626 E-mail: shariff@vet.upm.edu.my

Annex F.AII.List of Regional Resource Experts for Finfish Diseases in Asia Pacific

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Bacterial Diseases	**Dr. Indrani Karunasagar** Department of Fishery Microbiology University of Agricultural Sciences Mangalore – 575 002 **INDIA** Tel: 91-824 436384 Fax: 91-824 436384 E-mail: mircen@giasbg01.vsnl.net.in
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Viral Diseases	**Mr. Jeong Wan Do** Pathology Division National Fisheries Research and Development Institute 408-1, Shirang-ri, Kitang-up Kitang-gun, Pusan **KOREA RO** Tel: 82-51-720-2481 Fax:82-51-720-2498 E-mail: jwdo@nfrdi.re.kr
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Annex F.AII.List of Regional Resource Experts for Finfish Diseases in Asia Pacific

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	Dr. Kei Yuasa Fisheries and Aquaculture International Co., Ltd. No. 7 Khoji-machi Bldg., Room B105 4-5 Khoji-machi, Chiyoda-ku Tokyo 102-0083 JAPAN Tel: 81-3-3234-8847 Fax:81-3-3239-8695 E-mail: fai@faiaqua.com; yuasakei@hotmail.com
Finfish Diseases	**Dr. Mark Crane** AAHL Fish Diseases Laboratory Australian Animal Health Laboratory CSIRO Livestock Industries Private Bag 24, Geelong Vic 3220 **AUSTRALIA** Tel : +61 3 52 275118 Fax: +61 3 52 275555 E-mail: mark.crane@li.csiro.au
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F.AIII. LIST OF USEFUL GUIDES/MANUAL OF FINISH DISEASES IN ASIA-PACIFIC

- **Atlas of Fish Diseases (1989) by Kishio Hatai, Kazuo Ogawa and Hitomi Hirose (eds.) Midori Shobo, Tokyo, 267 p. (in Japanese)**
  Information: Prof. Kazuo Ogawa
  Department of Aquatic Bioscience
  Graduate School of Agricultural and Life Sciences
  The University of Tokyo
  Yayoi, Bunkyo, Tokyo 113-8657
  Tel: +81-3-5841-5282/5284
  Fax: +81-3-5841-5283
  E-mail: aogawak@mail.ecc.u-tokyo.ac.jp

- **Parasites and Diseases of Culture Marine Finfishes in Southeast Asia (1994) by Leong Tak Seng**
  Information: Dr. Leong Tak Seng
  No. 3 Cangkat Minden, Lorong 13
  11700 Glugor, Pulau Pinang
  Malaysia
  E-mail: mhpg@pc.jaring.my

- **Asian Fish Health Bibliography III Japan by Wakabayashi H (editor).** Fish Health Special Publication No. 3. Japanese Society of Fish Pathology, Japan and Fish Health Section of Asian Fisheries Society, Manila, Philippines
  Information: Japanese Society of Fish Pathology

- **Checklist of the Parasites of Fishes of the Philippines by J. Richard Arthur and S. Lumanlan-Mayo.** 1997. FAO Fisheries Technical Paper 369. 102p.
  Information: Dr. Rohana P. Subasinghe
  FAO of the United Nations
  Viale delle Terme di Caracalla
  Rome 00100 Italy
  E-mail: Rohana.Subasinghe@fao.org

- **Manual for Fish Diseases Diagnosis: Marine Fish and Crustacean Diseases in Indonesia (1998) by Zafran, Des Roza, Isti Koesharyani, Fris Johnny and Kei Yuasa**
  Information: Gondol Research Station for Coastal Fisheries
  P.O. Box 140 Singaraja, Bali, Indonesia
  Tel: (62) 362 92278
  Fax: (62) 362 92272

- **Diagnostic Procedures for Finfish Diseases (1999) by Kamonporn Tonguthai, Supranee Chinabut, Temdoung Somsiri, Pornlerd Chanratchakool and Somkiat Kanchanakhan**
  Information: Aquatic Animal Health Research Institute
  Department of Fisheries
  Kasetsart University Campus
  Jatujak, Ladyao, Bangkok 10900
  THAILAND
  Tel: (66.2) 579.41.22
  Fax: (66.2) 561.39.93
  E-mail: aahri@fisheries.go.th

- **Pathology and Histopathology of Epizootic Ulcerative Syndrome (EUS) by Supranee Chinabut and RJ Roberts**
  Information: Aquatic Animal Health Research Institute
  Department of Fisheries
  Kasetsart University Campus
  Jatujak, Ladyao, Bangkok 10900
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F.AIII. List of Useful Guides/Manual of Finfish Diseases in Asia-Pacific

E-mail: aahri@fisheries.go.th

- **Fish Health for Fisfarmers (1999) by Tina Thorne**
  Information: Fisheries Western Australia
  3rd Floor, SGIO Atrium
  186 St. Georges Terrace, Perth WA 6000
  Tel: (08) 9482 7333
  Fax: (08) 9482 7389
  Web: http://www.gov.au.westfish

- **Australian Aquatic Animal Disease – Identification Field Guide (1999) by Alistair Herfort and Grant Rawlin**
  Information: AFFA Shopfront – Agriculture, Fisheries and Forestry – Australia
  GPO Box 858, Canberra, ACT 2601
  Tel: (02) 6272 5550 or free call: 1800 020 157
  Fax: (02) 6272 5771
  E-mail: shopfront@affa.gov.au

- **Manual for Fish Disease Diagnosis - II: Marine Fish and Crustacean Diseases in Indone sia (2001) by Isti Koesharyani, Des Roza, Ketut Mahardika, Fris Johnny, Zafran and Kei Yuasa, edited by K. Sugama, K. Hatai, and T Nakai**
  Information: Gondol Research Station for Coastal Fisheries
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