KOI HERPESVIRUS DISEASE - Aquatic Manual Chapter

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Appendix XXII

Aquatic Manual Chapter

CHAPTER 2.1.17.

KOI HERPESVIRUS DISEASE

1. Case definition

Koi herpesvirus disease (KHVD) is a herpesvirus infection (17) capable of inducing a contagious and acute viraemia in common carp (Cyprinus carpio) and varieties such as koi carp and ghost carp (15).

2. Information for the design of surveillance programmes

a) Agent factors

The aetiological agent is koi herpesvirus (KHV) in the family Herpesviridae (17, 40) although it has also been given the name carp interstitial nephritis and gill necrosis virus (CNGV) (19, 28). Waltzek *et al.* (39) provided evidence to support the classification of the virus as a herpesvirus, and named it cyprinid herpesvirus 3 (CyHV-3) following the nomenclature of other cyprinid herpesviruses: CyHV-1 (carp pox virus, fish papilloma) and CyHV-2 (goldfish haematopoietic necrosis virus). Estimates of the genome size of KHV vary from at least 150 kbp (11) to 277 kbp (19) to 295 kbp (39). Four genes coding for a helicase, an intercapsomeric triplex protein, DNA polymerase, and major capsid protein have been identified, and sequence analysis of these genes

has shown that KHV is closely related to CyHV-1 and CyHV-2, and distantly related to channel catfish virus virus (Ictalurid herpesvirus: IcHV-1) (39). Estimates of virion size also vary. Nucleocapsids of negative stained virus have been measured at 103–112 nm diameter surrounded by an envelope (17, 19, 37). The nucleocapsids of thin sectioned virus have been measured at 80– 110 and 110–120 nm in diameter (4, 17, 26).

Serum from koi carp containing antibodies to KHV have been shown to cross-react with CyHV1, a further indication that these viruses are closely related. Evidence of cross reacting antibodies was demonstrated in reciprocal enzyme-linked immunosorbent assay (ELISA) and western blot analyses of serum from koi infected with CyHV-1 or KHV (1).

Comparisons of the genomes of KHV isolates from different geographical areas by restriction enzyme analysis (9, 15) or nucleotide sequence analysis (13, 20, 29) have shown them to be practically identical. Likewise, the polypeptides of KHV isolates from different geographic areas were similar, although one isolate from Israel had two additional polypeptides (7, 9).

The virus is inactivated by UV radiation and temperatures above 50°C for 1 minute. The following disinfectants are also effective for inactivation: iodophore at 200 mg/litre for 20 minutes, benzalkonium chloride at 60 mg/litre for 20 minutes, ethyl alcohol at 30% for 20 minutes and sodium hypochlorite at 200 mg/litre for 30 seconds, all at 15°C (21).

b) Host factors

Naturally occurring KHV infections have only been recorded from common carp (*Cyprinus carpio carpio*), koi carp (*Cyprinus carpio koi*) and ghost carp (*Cyprinus carpio goi*) and hybrids of these varieties. All age groups of fish appear to be susceptible to KHVD (4, 29, 36), but under experimental conditions, 2.5–6 g fish were more susceptible than 230 g fish (26). Differential resistance to KHVD has been shown among different common

carp strains (32) and other studies have suggested an age-related resistance (26). Morbidity of affected populations can be 100%, and mortality 70-80% (4, 38), but the latter can be as high as 90 or 100% (4, 37).

Carp are often raised in polyculture with other fish species, but no signs of disease or mortalities have been observed in those other fish, during KHVD outbreaks, under normal polyculture conditions. Refractory species include goldfish (*Carrassius auratus*), grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitrix*), tench (*Tinca tinca*), sturgeon (*Acipenser* sp.) Nile tilapia (*Oreochromis niloticus*), silver perch (*Bidyanus bidyanus*) and channel catfish (*Ictalurus punctatus*) (4, 17, 26, 35).

The disease is temperature dependent, occurring between 16–25°C (6, 17, 26, 29, 36, 37). Under experimental conditions the disease has caused high mortality at 28°C (10) but not at 29 or 30°C (19, 25), nor at 13°C (10). However, viral DNA was detected in the fish by PCR at 13°C, and it is possible that infected fish surviving at low temperatures may be reservoirs of the virus (10). The disease course can be rapid. The disease manifested itself in 3 days following the addition of naïve fish to a pond containing diseased fish (38), but usually under those circumstances it takes 8– 21 days for the disease to be observed in the naïve fish (4, 17). It is not known whether under natural conditions survivors of KHVD are persistently infected with virus, and if so, whether they shed the virus or for how long the fish retain the virus. Some of these aspects have been investigated in experimentally infected fish where it was shown that virus could persist in common carp infected at a permissive temperature and subsequently maintained at a lower than permissive temperature (33).

Common carp (*Cyprinus carpio*) strains are currently the only reported host of KHVD and therefore considered to be most susceptible to KHV infection. Goldfish x common carp hybrids, produced by hybridising male goldfish with female carp, have been reported to show some susceptibility to KHV infection. Approximately 50% of these hybrids examined at 25 days after intraperitoneal

injection with a high dose of KHV possessed viral genomic DNA, as detected by PCR (18). In contrast to findings elsewhere, recent experimental data from Germany suggests a susceptibility of goldfish and grass carp to KHV but further confirmation of these findings are needed (14, 18). When sampling during surveillance programmes for KHV, common carp or strains such as koi or ghost (koi × common) carp should be preferentially selected followed by any common carp hybrids present on the site such as goldfish × common carp. Cyprinid species are commonly mixed together in polyculture systems and the risk of transmission of virus between species, during disease outbreaks, is high. If the findings from Germany were confirmed then, for disease surveillance purposes, all cyprinid species would need to be considered as potential covert carriers of KHV.
The reservoirs of KHVD are clinically infected fish and covert virus carriers among cultured, feral or wild fish. Virulent virus is shed via faeces, urine, gill and skin mucus. However, gill, kidney, and spleen are the organs in which KHV is most abundant during the course of overt infection (10).

The mode of transmission of KHV is horizontal but ‘egg-associated’ transmission (usually called ‘vertical’ transmission) cannot currently be ruled out. Horizontal transmission may be direct (fish to fish) or vectorial, water being the major abiotic vector. However, animate vectors (e.g. parasitic invertebrates and piscivorous birds and mammals) and fomites may also be involved in transmission.

c) Disease pattern

Disease patterns are influenced by water temperature, virulence of the virus, age and condition of the fish, population density and stress factors. The immune status of the fish will also be an important factor with both non-specific (interferon) and specific immunity (serum antibodies, cellular immunity) having important roles in herpesvirus infections. Clinical disease dominates at water temperatures above 18°C when the host immune response is at its optimum. Infected carp produce antibodies against the virus,

which have been detected by ELISA methods at high serum dilution. Antibody has been detected in the serum at 3 weeks after experimental infection and in survivors after 1 year following a natural infection (1, 28, 33). Secondary and concomitant bacterial and/or parasitic infections are commonly seen in diseased carp and may affect the mortality rate and display of signs (15).

Following the first reports of KHVD in Israel and Germany (4, 16, 26) the geographical range of the disease has become extensive. The disease has been spread to many countries world -wide, predominantly through the trade in Koi carp before the current knowledge of the disease and means to detect it were available. It is now known to occur in, or has been recorded in fish imported into at least 21 different countries. In Europe this includes Austria, Belgium, Denmark, France, Italy, Luxembourg, The Netherlands, Poland, Switzerland and the United Kingdom (3, 6, 15, 30). In Asia, China (Hong Kong) (15), Indonesia (35), Japan (29), Malaysia (15, 22, 23), Singapore (in fish imported from Malaysia), Taipei China (37) and Thailand (in fish imported into Germany, 15). Elsewhere, South Africa (15) and the United States of America (11, 16, 36) have reported occurrence of KHVD. It is likely that the virus is present in many more countries, but has not yet been identified there or reported.

d) Control and prevention

Methods to control KHVD should mainly rely on avoiding exposure to the virus coupled with good hygiene and biosecurity practices. This is feasible on small farms supplied by spring or borehole water and a secure system to prevent fish entering the farm via the discharge water. Biosecurity measures should also include ensuring that new introductions of fish are from disease free sources and a quarantine system where new fish are held with sentinel fish at permissive temperatures for KHVD. The fish are then quarantined for a minimum of 4 weeks to 2 months before transfer to the main site and mixing with naïve fish. Hygiene measures on site should be similar to those recommended for SVC and include disinfection of eggs by iodophore

treatment (21), regular disinfection of ponds, chemical disinfection of farm equipment, careful handling of fish to avoid stress and safe disposal of dead fish.

In rearing facilities with a controlled environment, elevation of water temperature above 26–28°C can reduce mortalities during KHVD outbreaks (7, 28). Lowering the stocking density, and treating secondary infections may also help reduce the severity of the disease (35) A safe and effective vaccine is not currently widely available. However, attenuated virus has been used to vaccinate carp and protect the fish from virus challenge (25, 28). The vaccine preparation induced antibody against the virus, but the duration of the protection is unknown. The vaccine is currently licensed for use in Israel and has been widely used in carp farms across the country.

3. Diagnostic methods

Diagnosis of KHVD in clinically affected fish can be achieved by virus isolation. However, the virus is isolated in only a limited number of cell lines and these cells can be difficult to handle. Also, cell culture isolation is not as sensitive as the published PCR-based methods to detect KHV DNA and is not considered to be a reliable diagnostic method for KHVD (15). Immunodiagnostic methods, similar to those used for diagnosis of SVC (e.g. immunofluorescence [IF] tests or ELISAs), may be suitable for rapid identification and diagnosis of KHVD but have not been extensively reported, compared or validated. Until such time as validated tests are available, diagnosis of KHVD should not rely on just one test but a combination of two or three tests (15).

KHV infection produces a detectable antibody response in carp and enzyme immunoassays that reliably detect these antibodies have been published (1, 28). These methods can be used as rapid presumptive tests during the acute disease, however various parameters, such as antibody sensitivity and

specificity and sample preparation, can influence the results and therefore a negative result should be viewed with caution.

Detection of antibodies may prove to be a valuable method of establishing previous exposure to KHV in apparently healthy fish, and until PCR-based methods have been developed that are able to reliably detect persistent virus in exposed fish, antibody assays may be the only surveillance tools available. However, due to insufficient knowledge of the serological responses of fish to virus infections, the detection of fish antibodies to viruses has not thus far been accepted as a routine screening method for assessing the viral status of fish populations. Validation of some serological techniques for certain fish virus infections could arise in the near future, rendering the use of fish serology more widely acceptable for health screening purposes.

Fish material suitable for virological examination is:

- • **Asymptomatic fish** (apparently healthy fish): Gill, kidney, spleen, and encephalon (any size fish).
- • **Clinically affected fish:** Gill, kidney, spleen, gut and encephalon (any size fish).

a) Field diagnostic methods

During a KHVD outbreak there will be a noticeable increase in mortality in the population. All age groups of fish appear to be susceptible to KHVD, although, generally, younger fish up to 1 year are more susceptible to clinical disease. Fish become lethargic, separate from the shoal and gather at the water inlet or sides of a pond and gasp at the surface of the water. Some fish may experience loss of equilibrium and disorientation but they may also show signs of hyperactivity. On closer examination of individual fish, typical clinical signs include pale discolouration or reddening of the skin, which may also have a rough texture, focal or total loss of epidermis, over-or under-production of mucus on the skin and gills. Other gross signs include enophthalmia (sunken eyes) and haemorrhages on the skin and base of the fins and fin erosion.

b) Clinical methods

There are no pathognomic gross lesions. Final diagnosis must await direct detection of viral DNA or antigen in tissues or virus isolation and identification. However, the most consistent gross pathology is seen in the gills and this can vary in extent from pale necrotic patches to extensive discolouration, severe necrosis and inflammation. Further examination can reveal erosion of primary lamellae, fusion of secondary lamellae, and swelling at the tips of the primary and secondary lamella. Other internal lesions are variable in occurrence and often absent in cases of sudden mortality. Other gross pathologies that have been reported include adhesions in the abdominal cavity with or without abnormal colouration of internal organs (lighter or darker). The kidney or liver may be enlarged, and they may also exhibit petechial or focal haemorrhages.

Presence of gross pathologies may also be complicated because diseased fish, particularly common carp, are also infested with ectoparasites such as *Argulus* sp., *Chilodonella* sp., *Cryptobia* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., *Ichthyobodo* sp., *Ichtyophthirius* sp., *Trichodina* sp. and gill monogeneans, as well as numerous species of bacteria.

The histopathology of the disease can be non-specific and variable, but inflammation and necrosis of gill tissues is a consistent feature. Gills also exhibit hyperplasia and hypertrophy of branchial epithelium, and fusion of secondary lamellae and adhesion of gill filaments can be seen. Necrosis, ranging from small areas of necrotic epithelial cells of secondary lamellae to complete loss of the lamellae is observed. Branchial epithelial cells and leucocytes may have prominent nuclear swelling, margination of chromatin to give a “signet ring” appearance and pale diffuse eosinophilic intranuclear inclusions have been observed. Inflammation, necrosis and nuclear inclusions have been observed (individually or together) in other organs, particularly the kidney, but also in the spleen, pancreas, liver, brain, gut and oral epithelium.

c) Agent detection and identification methods

Detailed methods are not presented here because there have not been extensive comparison and validation of detection and identification methods for KHV. However, a short description of available published methods is provided. Method reco mmendations will rely on further testing and validation and further data being obtained from laboratories that have developed the methods to decide if they are ‘fit-for-purpose’.

Direct detection methods i) Isolation of KHV in cell culture

The virus can be isolated in a limited number of cell cultures, but cell culture isolation is not as sensitive as PCR and is not considered to be a reliable diagnostic method for KHVD (15).

The virus replicates in koi fin cells (KF-1) (17), carp fin (CaF-2) and carp brain (CCB) cells (24), and in primary cells from fins of common or koi carp (19, 26, 28). Other cell lines used routinely for isolation of fish pathogenic viruses such as EPC, FHM, BF-2, CHSE214 and RTG-2 cells are refractory to the virus (4, 19, 24, 37). The virus is most abundant in gill, kidney, and spleen tissues during the course of overt infection (10) and it is recommended to sample these tissues for virus isolation. The optimum incubation temperature for virus isolation in KF-1 or CCB cells is 20°C but 8–12 days’ incubation may be required before a cytopathic effect (CPE) is observed (7).

ii) Identification of virus isolated in cell culture

Viruses isolated in cell culture must be definitively identified, as a number of different viruses have been isolated from carp exhibiting clinical signs resembling those of KHVD (5, 15).

Rapid presumptive methods

Immunodiagnostic methods, similar to those used for presumptive identification of SVC
(e.g. IF tests or ELISAs), may well be suitable for rapid identification and diagnosis of KHVD (27, 32).

Confirmatory identification methods

The most reliable method for confirmatory identification is by PCR, or one of its variants, which have also been used to identify KHV DNA directly in fish tissues (2, 8–11, 13, 19, 20, 27, 40).

A PCR based on the thymidine kinase (TK) gene of KHV was reported to be more sensitive than PCR methods described by Gilad *et al.* (9) and Gray *et al.* (11), and could detect 10 fg of KHV DNA (2); the PCR of Ishioka *et al.* (20), based on the DNA polymerase gene, detected 100 fg of KHV DNA. The loop-mediated isothermal amplification (LAMP) method (13) was also based on the KHV TK gene, and was as sensitive as a PCR method developed by the same authors, but was more rapid than the PCR. The PCR described by Gray *et al.* (11) was improved by Yuasa *et al.* (40), and has been incorporated in the official Japanese guidelines for the detection of KHV. New improved diagnostic PCR tests will continue to be developed and it is hoped that they will be validated as recommended in Chapter 1.1.3 of this *Aquatic Manual*. The DNA extraction and PCR protocols detailed below for direct detection of KHV in fish tissues are also suitable for confirmatory identification of infected cell culture supernatants.

iii) Diagnostic methods for clinically diseased fish

Direct detection in fish tissues

KHV has been identified in touch imprints of liver, kidney and brain of infected fish by IF. Highest levels of positive immunofluorescence was seen in the

kidney and the virus could be detected by IF on a kidney imprint 1 day post-infection (27, 32). Virus antigen has also been detected in infected tissues by an immunoperoxidase staining method. The virus antigen was detected by 2 days post infection in the kidney, and was also observed in the gills and liver (27). However, the detection of KHV by immunostaining must be interpreted with care, as positive staining cells could result from cross-reaction with serologically related virus (e.g. CyHV-1) or a non-viral protein (27).

ELISA-based methods for direct detection of KHV antigen in infected tissues are under development in a number of laboratories worldwide but no methods have been published.

The most commonly used method for detection of KHV directly in fish tissues is using PCR assays specific for KHV (see above, under confirmatory identification).

In studies carried out at the Cefas Weymouth laboratory, published primer sets were compared using a standard PCR protocol for detection of KHV DNA in carp tissues (K. Way, unpublished data). The primer set targeting the TK gene (2) was the most sensitive with a detection limit three log greater than Gilad primers. CNGV primers (27) and modified Gray SpH primers that target short regions of the genome (109 bp and 151 bp, respectively) also performed well, particularly on decomposed tissues. The TK primer set later performed well in a method ring-trial carried out in 21 laboratories in 19 countries around the world (K. Way, unpublished data).

The same study at Cefas and the method ring-trial also compared commercial DNA extraction kits for their ability to provide KHV DNA of sufficient quality for the PCR. Of the commercial kits tested at Cefas, EasyDNA (Invitrogen), DNeasy (Qiagen) and DNAzol reagent (Invitrogen) all extracted DNA of suitable quality. In the ring-trial, the High Pure PCR template preparation kit (Roche), QIAamp DNA blood minikit (Qiagen) and the Puregene DNA

purification kit, all performed well. However, some laboratories found the DNAzol reagent not to be as reliable.

The sample preparation protocol detailed below uses the DNAzol reagent for extraction of KHV DNA. This is an easy to use, short duration protocol that is also relatively inexpensive compared to some kits. Laboratories that are not familiar with DNAzol extraction may find the method less reliable in their hands. However, a number of DNA extraction kits are available commercially (including those listed above) that will produce high quality DNA suitable for use with the PCR protocol detailed.

The PCR protocol detailed below uses the TK primer set developed by Bercovier and colleagues at the Hebrew University-Hadassah Medical School in Israel (2). Of the published single-round-PCR methods, this is currently considered to be the most sensitive for detection of KHV DNA in fresh tissue samples from clinically diseased carp. This protocol may also allow detection of subclinical levels of virus. If the tissue shows evidence of decomposition then primer sets (see above) targeting shorter regions of the genome may need to be used in place of the TK primer set.

General notes

PCR is prone to false-positive and false-negative results. Therefore each assay and tissue extraction should include a negative control to rule out contamination. To further minimise the risk of contamination, aerosol-preventing pipette tips should be used for all sample and PCR reaction preparation steps.

Sample preparation

i) Virus extraction from organ tissues should be carried out using the procedure described in Chapter I.1 (Section B.3.2).

ii) Add 100 µl of tissue homogenate (1/10 [w/v]) or virus culture supernatant to a 1.5 ml microcentrifuge tube containing 1 ml DNAZOL® reagent.

iii) Mix gently by inverting the tube five times and stand at room temperature for 5 minutes then centrifuge at 10,000 rpm for 10 minutes using a microcentrifuge.

iv) Remove 1 ml of the supernatant to a new 1.5 ml microcentrifuge tube containing 0.5 ml of ethanol.

v) Mix gently by inverting the tube five times and stand at room temperature for 5 minutes, then centrifuge at 13,000 rpm for 30 minutes using a microcentrifuge. vi) Remove the supernatant and rinse the pellet with 250 µl of 70% ethanol in molecular biology grade water. vii) Spin samples for 5 minutes at 13,000 rpm. viii) Remove the ethanol using a pipette and air-dry the pellet by leaving the tubes open on the bench for 5 minutes.

ix) Resuspend the pellet in 50 µl molecular biology grade water, prewarmed at 60°C, and incubate at 60°C for 5 minutes. Samples can be stored at –20°C until required.

PCR

All PCR reactions are prepared in a clean area that is separate from the area where the amplifications are performed. This will minimise the risk of contamination.

i) For each sample prepare a master mix containing: For Go Taq Polymerase: 10 µl Reaction buffer (×10 conc.) 5 µl $MgCl_2$ (25 mM stock)
0.5 µl dNTPs (25 mM mix) [Promega Cat.no.U1240]
0.5 µl Forward primer (100 pmol/µl stock)
0.5 µl Reverse primer (100 pmol/µl stock)

- 0.25 µl Go Taq polymerase 500 µ (5 µ/µl) [Promega Cat.no.M8305]
- 30.75 µl Molecular biology grade water

Bercovier TK primers:

Forward = 5'-GGG-TTA-CCT-GTA-CGA-G-3'

Reverse = 5'-CAC -CCA-GTA-GAT-TAT-GC-3'

Product size = 409 bp

For each sample dispense 47.5 µl into a 0.5 ml thin walled microcentrifuge tube.

Overlay with two drops of mineral oil.

ii) Add 2.5 µl of the DNA extracted DNAzol®. Store the remainder of the DNA at –20ºC. iii) Place tubes in a thermal cycler and perform programme: 1 cycle of: 5 minutes at 94ºC 40 cycles of: 1 minute at 95ºC 1 minute at 55ºC 1 minute at 72ºC Followed by a final extension step of 10 minutes at 72ºC.

iv) Electrophorese 20 µl volumes of PCR product on a 2% ethidium bromide stained agarose gel (4% when separating smaller amplification products of <300 bp) at 120 V for 20 minutes and visualise under UV light. An appropriate molecular weight ladder should be included on the gel to determine the size of the product.

v) Products of the correct size should be confirmed as KHV in origin by sequence analysis.

4. Rating of tests against purpose of use

The methods currently available for surveillance, detection and diagnosis of KHVD are listed in Table 1. The designations used in the table indicate: A = the method is currently the recommended method for reasons of availability, utility and diagnostic sensitivity and specificity; B = the method is a standard method with good diagnostic sensitivity and specificity; C = the method has application in some situations, but cost, accuracy or other factors severely

limits its application; D = the method is currently not recommended for this purpose. Although not all of the tests listed as category A or B have undergone formal standardisation and validation (at least stages 1 and 2 of figure 1 of Chapter 1.1.2), their routine nature and the fact that they have been used widely without dubious results makes them acceptable.

Table 1. KHVD surveillance, detection and diagnostic methods

Method	Surveillance to declare freedom from infection	Presumptive diagnosis of infection or disease	Confirmatory diagnosis of infection or disease
Gross signs	D	B	D
Histopathology of tissues and organs	D	B	C
Isolation of in cell culture	D	C	D
Antibody-based assays to detect KHV antigen (IFAT, ELISA)	D	B	C
Transmission EM of tissues	D	B	C
PCR of tissue extracts*	C	A	A
PCR – sequence analysis	NA	C	A
Detection of KHV antibodies in exposed fish (ELISA)**	C	C	D

IFAT = Indirect fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay; EM = electron microscopy; PCR = polymerase chain reaction.

*Diagnostic virologists should be aware that fish recently vaccinated against KHV may test positive by PCR. No information is currently available to indicate any genome sequence differences between the attenuated vaccine strain and wild-type (w.t.) KHV. Until this sequence information is provided, diagnostic laboratories will not be able to distinguish between w.t. and vaccine strain of KHV and this could lead to a false diagnosis.
**Diagnostic virologists should be aware that fish recently vaccinated against KHV may test positive by ELISA. There may also be a low-level cross reaction with antibodies to CyHV-1.

NOTE: Many diagnostic laboratories may encounter difficulties in obtaining antibodies against KHV that are suitable for use in immunodiagnostic tests. However, a limited number of monoclonal and polyclonal antibodies may be very soon available from commercial sources. It is quite likely that diagnostic kits will also soon be available from the same sources.

5. Corroborative diagnostic criteria

a) Definition of suspect case

A suspect case of KHVD is defined as the presence of typical clinical signs of the disease in a population of susceptible fish OR presentation of typical histopathology in tissue sections OR typical CPE in cell cultures without identification of the causative agent OR a single positive result from one of the diagnostic assays described above.

b) Definition of confirmed case

A confirmed case is defined as a suspect case with subsequent identification of the causative agent by one of the serological or molecular assays described above OR a second positive result from a separate and different diagnostic assay described above.

6. Diagnostic/detection methods to declare freedom

There are no currently recommended methods for surveillance of susceptible fish populations for declaration of freedom from KHV. However, many laboratories are investigating further development of molecular-based methods to increase sensitivity (e.g. real-time and nested PCR) or to reliably detect low levels of persistent virus DNA. These assays may well prove suitable for surveillance programmes.

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NB: There are OIE Reference Laboratories for Koi herpesvirus disease (see Table at the end of this *Aquatic Manual* or consult the OIE Web site for the most up-to-date list: www.oie.int).