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REVIEW ARTICLE

Koi Herpes Virus: A Review and Risk Assessment of Indian Aquaculture

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Abstract Common carp (*Cyprinus carpio*) is a widely cultivated freshwater fish for human consumption, while koi carp, is a farmed colored sub species of common carp used for ornamental purposes. Since 1998, both common carp and koi carp are severely affected by a viral disease called as Koi herpes virus disease (KHVD). This disease is caused by Koi herpes virus (KHV), also known as cyprinid herpes virus-3. The virus causes interstitial nephritis and gill necrosis in carps, so it is also termed as carp interstitial nephritis and gill necrosis virus. KHV is a double stranded icosahedral DNA virus belonging to family *Alloherpesviridae*, with a genome size of 295 kbp, larger than any member of *Herpesviridae*. The viral genome encodes 156 potential protein coding open reading frames. Each virion consists of forty structural proteins, which are classified as capsid (3), envelope (13), tegument (2) and unclassified (22) structural proteins. Diagnosis of KHVD is mainly based on detection of viral DNA by polymerase chain reaction amplification using specific primers or loop mediated isothermal amplification. Temperature dependent latent infection is unique to KHV; and carrier fish are often not detected, thereby possibly resulting in spread of this pathogen to newer areas. The disease is now known to occur in, or has been recorded from at least 26 different countries of the world. Fortunately, KHVD has not been reported from India or from Indian major carps. To monitor the disease status of the country, a total of 254 fish samples collected from different parts of India were screened by PCR for the presence of KHV. None of the tested samples were found to be positive for KHV. These results demonstrate that tested samples from different parts of India were apparently free from KHV. Preliminary risk assessment of KHV suggest that in the event of unrestricted importation of koi carps into our country, there is a higher probability of risk to aquaculture as compared to natural waters. So there is strong need to develop diagnostic capabilities and launch surveillance programmes for KHV in India.

Keywords Koi carp · Common carp · Koi herpes virus

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Introduction

Common carp (*Cyprinus carpio*) is a widely cultivated freshwater fish, originally native to Eastern Europe and Central Asia. This fish species has been domesticated and introduced worldwide into freshwater environments for aquaculture. Koi, a subspecies of the common carp, is a colorful ornamental fish that is traded worldwide for display in aquaria [47]. In 1998, a viral disease outbreak was first noticed in cultured common carp in Israel and USA [34, 62] and in Germany in 1997–1998 [11]. This disease has been termed as Koi herpes virus disease (KHVD) and the disease causing agent was identified as Koi herpes virus (KHV). KHV was reported be present in England from

1996, as evident by analysis of preserved tissue samples [32]. Since then, outbreaks of KHVD are being regularly reported from Europe, South Africa, USA and Asia. In Asia, KHV is reported from Israel, Indonesia, Taiwan, China, Thailand, Japan and Malaysia. In view of its significance on fish trade, KHV is designated as OIE listed pathogen for finfish. Incidence of this disease is so far not reported from India, in spite of its occurrence in neighboring South East Asian countries. However, unregulated import of ornamental fish can lead to unintentional introduction of this pathogen. This can affect fish production from both culture and capture fisheries and also endanger biodiversity, thereby causing wide socio-economic impact.

Considering the international spread of KHV to different continents in a short span of time, it is well accepted that KHV can be a potential hazard to Indian fisheries that needs proper risk assessment. As per definition, risk assessment is evaluation of the likelihood and the biological and economic consequences of entry, establishment, or spread of a hazard within the territory of an importing country [61]. In brief, this process is the outcome of release assessment, exposure assessment and consequence assessment. Release assessment is dependent on the prevalence of infection in the imported commodity and the nature of etiological agent, while exposure assessment is the likelihood of exposure of the susceptible species to potential hazard/organism and its likely consequences upon establishment. Therefore, risk assessment requires a thorough knowledge of the virus and the disease in an analytical context. In this article, latest scientific developments on KHV are summarized with a view to carry out risk assessment of Indian aquaculture and possible implications on its presumed entry in our country. Accordingly, the article is divided into three major parts; first about the virus, second about the disease and finally about risk assessment.

Description of Koi Herpes Virus

Geographical Distribution

Internationally, KHV is considered as one of the most important pathogen of koi and common carp [41, 51]. KHV is now known to occur in, or has been recorded from at least 26 different countries of the world [40]. KHVD is reported from many European countries [10, 59, 81], and is now a listed disease in European Union legislation [2]. Information is available on outbreaks of KHVD from Canada [24], USA [30] and many south East Asian countries [28, 48] (Table 1). Recently isolation of KHV is reported from China using a new cell line developed from koi carp [18].

Virus Taxonomy

The nomenclature of KHV is based on its icosahedral structure which resembles herpesviruses and its host range appears to be limited to koi species of cyprinidae [34]. It is also named as cyprinid herpesvirus 3 (CyHV-3), following the nomenclature of other cyprinid herpesviruses: CyHV-1 (carp pox virus, fish papilloma virus) and CyHV-2 (goldfish haematopoietic necrosis virus) [85]. KHV is now formally assigned the genus cyprinivirus and species cyprinid herpesvirus 3, belonging to the newly defined family *Alloherpesviridae* in the order *Herpesvirales* [15]. On the basis of phylogenetic analysis, the family *Alloherpesviridae* comprises two distinct clades. The first clade comprises anguillid and cyprinid herpesviruses, which possess large genomes (245–295 kb). The second clade comprises ictalurid, salmonid, acipenserid, and ranid herpesviruses, which have smaller DNA genomes (134–235 kb) [86]. Interestingly, KHV resembles the poxviruses by the presence of thymidylate monophosphate, ribonucleotide reductase and a B22R-like genes [38].

Virion Structure

Like all members of the order *Herpesvirales*, KHV virions are composed of an icosahedral capsid containing the genome, a lipid envelope bearing viral glycoproteins, and an amorphous layer of proteins termed the tegument, which resides between the capsid and the envelope [56]. Nuclear capsids of KHV are classified into three different types: capsids containing an internal circular (spherical) structure, capsids containing an electron-dense core, and empty capsids. These morphologies represent the three different developmental stages of capsid morphogenesis of KHV [55].

Viral Genome

KHV is a large, linear, double-stranded DNA virus with an icosahedral core of 100–110 nm [34, 37]. DNA molecule consists of a central portion flanked by two 22-kb repeat regions, called the left and right repeats. The genome encodes 156 potential protein coding open reading frames (ORFs). Of these, 8 ORFs are encoded by the repeat regions and are consequently present as two copies in the genome [3]. Homologs of ORF2, tumor necrosis factor receptor, ORF22, ORF25, and RING families are present in the genome. Potential membrane glycoproteins of KHV are coded by ORF25 family, which consists of 6 ORFs (ORF25, ORF26, ORF27, ORF65, ORF148, and ORF149). Of these, proteins of ORF25, ORF65, ORF148, and ORF149 are detected in mature virions [51]. Several other genes involved in host immune evasion such as ORF16, which codes for a potential G-protein coupled receptor; ORF134, which codes for an IL-10 homolog; and ORF12, which

Table 1 Status of KHV in affected countries of the world

S. No.	Country	Year of reporting	Disease status	Reference
1	Belgium	2010	Reported present or known to be present	[40]
2	Canada	2010	First detection of KHV by PCR	[24]
3	China	2002	First detection of KHV by PCR	[49]
4	China (Hong-Kong)	2006	Reported present or known to be present	[57]
5	Chinese Taipei	2004	Reported in the country for the first time	[82]
6	Czech Republic	2010	First clinically apparent KHV infection	[59]
7	Denmark	2008	Reported present or known to be present	[40]
8	Germany	1999	Reported in country for the first time	[58]
9	Indonesia	2005	Reported present or known to be present	[79]
10	Ireland	2011	First detection of KHV in imported koi by PCR	[40]
11	Israel	1999	Reported in country for the first time	[34]
12	Japan	2004	Reported in the country for the first time	[72]
13	Korea	2001	First report of KHV	[60]
14	Luxembourg	2008	Reported present or known to be present	[40]
15	Malaysia	2008	Reported present or known to be present	[57]
16	Netherlands	2008	Reported present or known to be present	[40]
17	Philippines	2006	KHV associated mortalities in koi carp	[77]
18	Poland	2006	Reported in country for the first time	[7]
19	Romania	2010	First reported occurrence of KHV	[40]
20	Singapore	2006	Reported present or known to be present	[57]
21	Slovenia	2008	First reported occurrence of KHV	[40]
22	Spain	2011	First reported occurrence of KHV	[40]
23	Sweden	2011	Reported present or known to be present	[40]
24	Thailand	2009	Report of KHVD outbreak	[66]
25	United Kingdom	1999	First reported occurrence of KHV	[84]
26	United States of America	2000	First reported occurrence of KHV	[34]

codes for a tumor necrosis factor receptor homolog are also present in the genome. Two KHV-specific antigens corresponding to ORF62 (cysteine protease homolog) and ORF68 (myosin homolog) have been identified from a KHV genomic phage library using a KHV-specific rabbit polyclonal antibody. Of the two, protein encoded by ORF68 is antigenic and has been used to generate specific MAb for immuno-detection of KHV [4]. Deletions in thymidine kinase (ORF55), dUTPase (ORF123) and ribonucleotide reductase (ORF141) genes of KHV show that these genes are not essential for in vitro replication of virus [23].

Genotypes

Whole genomes of three strains of KHV from Japan, United States and Israel, have been completely sequenced, indicating >99 % identity among the isolates. Israel and USA strains (U/I) are more closely related to each other than to the Japan strain (J) [3]. There is a third genotype which is intermediate between the J and U/I lineages [9]. On the basis of difference between the variable numbers of tandem repeats (VNTR), two main genetic clusters of KHV are reported, each one divided in two subgroups including either U/I or J isolates. Indonesian isolate of KHV is distant from J isolate [5]. Variations in the thymidine kinase (TK) gene of the two genotypes are also reported. The C-terminal amino acids of TK gene of the Asian genotype varies greatly by frame-shift from the European genotype [46]. European genotypes of KHV have a 12 base-pair deletion in the ORF136 as compared to Asian genotype [18]. Hence, ORF136 can be also used as molecular marker to distinguish Asian isolates of KHV from European isolates. Recently, KHV from Indonesia is reconfirmed as new intermediate genotype between European and Asian genotypes [80].

Proteomic Analysis

Each virion of KHV consists of 40 structural proteins and 18 host cellular proteins. Structural proteins consists of capsid (3), envelope (13), tegument (2) and unclassified (22) structural proteins [51]. ORF092 and ORF136 are predicted to encode a major capsid protein (MCP) and a

viral envelope protein, respectively [3, 51]. ORF81 encodes a highly immunogenic type 3 membrane protein expressed on the envelope of CyHV-3 [70].

Physical Stability

KHV remains viable for at least 4 h in water with temperatures of 23–25 °C [62]. However, significant reduction in the infectious titre of KHV is observed within 3 days in environmental water or sediment samples at 15 °C [74]. Viral DNA is very stable and was detected in the water samples collected from the river even 4 months prior to observed disease outbreak; with water temperatures ranging from 9 to 11 °C [33]. The virus is inactivated by UV radiation and at temperatures above 50 °C for 1 min. The following disinfectants are also effective for viral inactivation: iodophor at 200 mg l^{-1} for 20 min, benzalkonium chloride at 60 mg l^{-1} for 20 min, ethyl alcohol at 30 % for 20 min and sodium hypochlorite at 200 mg l^{-1} for 30 s, all at 15 °C [42].

Latency in the Host

One of the unique feature of Herpesviridae is latency. Herpesvirus latency is characterized by restricted gene expression of the viral genome with no production of infectious virus [54]. KHV can become latent inside the leukocytes of healthy koi with probable exposure to the virus. In wild populations, KHV can remain as carrier in asymptomatic fish and act as reservoir of infection [83]. Several months after initial exposure to the virus, temperature-dependent reactivation of KHV infection can occur in exposed fish [19, 78]. This latency feature of KHV probably contributes in the spread of this pathogen to new geographic locations.

Viral Propagation

Cell lines derived from koi fin (KF-1), *C. carpio* carp brain (CCB), and *C. carpio* carp gills (CCG) are suitable for in vitro cultivation of KHV [34, 58, 69]. Other common fish cell lines such as FHM, RTG-2, CHSE-214 and EPC do not support the growth of KHV [14, 60, 65]. The optimal temperature for viral propagation in KF-1 cells is 15–25 °C; at 30 °C these cells do not produce virus [26]. The virus induces typical plaques in 3–4 days after inoculation in the cultured cells along with the formation of syncytia and increase in cytoplasmic vacuoles. Later, the cells become round and detach from the substrate [65]. KHV infected cells with deformed morphology can be converted to normal following shifting up of the temperature and can again deform after transfer to the permissive temperature. This is because viral propagation and viral gene transcription is turned on and off by shifting cells to the permissive and non-permissive temperatures. This suggests that virions persist for long periods in the fish body, enabling a new burst of infection upon a shift to a permissive temperature [18].

Koi Herpes Virus Disease (KHVD)

Susceptible Hosts

KHV causes KHVD in all varieties of common carp, including varieties such as mirror, leather, koi, and ghost koi [32, 34]. Other species, such as goldfish [20, 21, 71], crucian carp, grass carp, or tench [43, 50], are susceptible to infection and act as carriers in spreading infection to healthy carps. Hybrids of goldfish and carp or koi are also susceptible to experimental infection of KHV [8, 35]. KHV has been detected by PCR in Russian sturgeon and Atlantic sturgeon from fish farms in Northern Poland [44]. Susceptible host range of KHV and its nature of infection in different fish species are shown in Table 2. Studies also indicate that aquatic invertebrates such as swan mussels (*Anodonta cygnea*) and freshwater shrimp (*Gammarus pulex*) can also act as potential vector for KHV [45]. All age groups of carp, from juveniles upwards, are susceptible

Table 2 Host range of Koi Herpes Virus and its nature of infection in different fish species

S. No.	Host fish species	Nature of Infection	Reference
1	Common carp (*Cyprinus carpio carpio*)	Symptomatic	[32, 34, 62]
2	Koi carp (*Cyprinus carpio koi*)	Symptomatic	[32, 34, 62]
4	Goldfish (*Carassius auratus*)	Asymptomatic/carrier	[20, 21, 71]
5	Goldfish × koi carp hybrid	Symptomatic	[8]
6	Crucian carp × koi carp hybrid	Symptomatic	[8]
7	Goldfish × common carp	Symptomatic	[35]
8	Grass carp (*Ctenopharyngodon idella*)	Asymptomatic/carrier	[43, 50]
9	Ide (*Leuciscus idus*)	Asymptomatic/carrier	[43, 50]
10	Ornamental catfish (*Ancistrus* sp.)	Asymptomatic/carrier	[43, 50]
11	Russian sturgeon (*Acipenser gueldenstaedtii*)	Asymptomatic/carrier	[44]
12	Atlantic sturgeon (*Acipenser oxyrinchus*)	Asymptomatic/carrier	[44]

to KHVD [11, 72] but, under experimental conditions, 2.5–6 g fish are more susceptible than 230 g fish [62].

Clinical Signs and Cellular Changes

Grossly, the affected fish have pale patches or blisters on the skin along with sunken eyes and increased respiratory frequency. Later the fish becomes disoriented and swim erratically prior to death. Another characteristic sign seen in diseased fish is white patches on the gills or gill necrosis [34]. Internally, the most prominent cellular changes are seen in gill, skin, kidney, liver, spleen, gastrointestinal system and brain of diseased fish. Epithelial cells of the gill filaments exhibit hyperplasia, hypertrophy and severe inflammation, resulting in lamellar fusion. In kidney, congestion and degeneration of the tubular epithelium is seen in nephrons [65]. Congestion in the valvula cerebella and medulla oblongata is observed along with edematous dissociation of nerve fibers in the brain of diseased fish exhibiting neurological disorder [56]. Epithelial cells of gill and spleen also show eosinophilic intranuclear inclusion bodies and margination of chromatin [12].

Transmission

Mode of transmission of KHV is horizontal i.e. directly from infected fish or through contaminated water to susceptible fish. Bioluminescence imaging shows that KHV gains entry through the body surface of the host [13]. Removal of skin mucus and epidermal lesions facilitate the entry of virus in the host [67]. The incubation period of virus inside the host ranges between 7 and 10 days before the onset of clinical symptoms. The clinical signs are lethargy, loss of appetite, gill necrosis, haemorrhages on the body and uncoordinated swimming [29, 60]. The infected fish begin to die within 1 or 2 days after the onset of the symptoms [34]. During infection, virulent virus is shed continuously via feces, urine, gills and skin mucus for a longer period from infected common carp at 16 °C than those at 23–28 °C [90]. Massive mortalities occur within a week of onset of clinical signs, with the mortality rate reaching 80–100 % [84]. At temperatures above 30 °C or below 13 °C, KHV becomes dormant and clinical signs generally cease [12, 26]. Viral DNA can be detected in the blood and kidney, 1 day after virus exposure [17, 65]. Subsequently, DNA can be detected in the gill, intestinal tract, spleen and liver, but not in the brain [29]. Virus is most abundant in gill, kidney, and spleen during the course of infection [27]. KHV DNA has been detected in the environmental water before, during, and after an outbreak of the disease [33, 52]. After release from the host, the virus becomes associated with plankton and can be potentially involved in viral transmission [53].

Diagnostic Methods

Presently, several diagnostic techniques such as, isolation of virus in a susceptible cell line, histopathology, polymerase chain reaction (PCR) and ELISA are being used for detection of KHV. Virus isolation in cell culture is not as sensitive as the PCR based methods and therefore is not considered to be a reliable diagnostic method for KHVD [32]. Gill, kidney, and spleen tissues are the best organs for virus isolation. It is recommended to also include gut and encephalon in the test samples for PCR based detection methods. For clinical diagnosis, individual fish samples should be tested, while for surveillance testing, pooling of samples can be done to a maximum of five fish per pool.

Molecular Detection of Viral DNA

Polymerase chain reaction (PCR) is the most common molecular tool used for detection of KHV. Several diagnostic tests based on PCR [6, 25, 29, 89], nested PCR [7, 20], real-time PCR [27] and loop-mediated isothermal amplification (LAMP) [31, 75, 87, 88] have been developed for detection of KHV. A highly sensitive PCR method for detection of TK gene of KHV is able to detect 10 fg of KHV DNA [6]. The sensitivity of this method is ~10–1,000 times greater than other PCR methods [25, 29]. PCR primers targeting DNA polymerase gene and the major envelope protein gene of KHV have a sensitivity of 100 femtogram (fg) and 1,000 fg of KHV DNA, respectively in infected gills [41]. KHV can be detected in infected fish dropping by PCR assay with a detection limit of 40 fg of viral DNA [16]. MCP gene has been also used to develop PCR for detection of KHV [68].

Sensitivity of some of these diagnostic assays has been compared [8]. The real-time PCR recognizing KHV DNA fragment [27] with an internal [36] and external control system is considered as the "gold standard" for detection and absolute virus quantification. Nested PCR [7] and Bercovier's PCR [6] also have the same sensitivity as the "gold standard" which correspond to 10 fg DNA or 1–5 genomic KHV equivalents. A new on-tube semi-nested PCR (sn PCR) recognizing the KHV major glycoprotein gene is recently established [8], which can identify latent infection with a virus load between 5 and 10 KHV copies.

Besides PCR, LAMP primer sets are also available for detection of KHV [31, 75]. But these LAMP primers are either too high or too low, respectively, in terms of sensitivity [12]. Commercial LAMP assay named as Loopamp DNA Amplification Kit claims a comparable detection limit to TK gene PCR. A new LAMP assay in combination with nucleic acid lateral flow format is equally sensitive,

with a detection limit of 10 fg of KHV DNA or ~30 copies of the viral genome [76].

Detection of KHV Antigen/Antibodies

Viral antigen is detectable in infected tissues by immunoperoxidase staining or immunofluorescence staining [65, 73]. However, cross-reactivity with related cyprinid herpes virus or non-viral protein is a potential problem with immunostaining. ELISA is also useful in detection of KHV antigen from infected tissues or fish droppings [1, 16]. Detection of specific antibodies in serum by ELISA is an indirect method for diagnosis of KHV. A progressive increase in anti-KHV antibodies in naturally exposed koi is seen by ELISA [69]. These antibodies are detected in the serum at 3 weeks after experimental infection and in survivors after 1 year following a natural infection [1, 39, 78, 81]. KHV infection induces a high prevalence (54 %) of KHV specific antibodies in surviving wild fish population [83]; while studies in farmed fish of England show almost 85–93 % of fish are seropositive by ELISA, even 1 year after disease outbreak [81]. This indicates that ELISA is a valuable method of establishing previous exposure of KHV in apparently healthy fish. However, this method is not recommended as a primary diagnostic tool because it cannot determine whether fish is still infected with the virus. Cross reacting antibodies in fish serum can also lead to false positives.

Vaccines Against KHVD

In Israel, a live attenuated virus strain is known to induce high antibody titers in the vaccinated fish and offers protection against virulent virus. Vaccination is achieved by immersion of fish in viral suspension for 30 min, followed by a holding time of 2–3 days at permissive temperature to enable propagation of virus in vaccinated fish [39, 63, 69]. This vaccine confers resistance to a challenge infection for at least 8 months and is available in Israel for emergency use [64]; despite general safety concerns of reversion of attenuated virus to pathogenic virus.

Risk Assessment

Current Situation in India

Cyprinids largely contribute to freshwater aquaculture production in India. As a fish species, common carp is the single largest producer contributing to aquaculture production in India [22]. Entry of KHV in India can severely affect common carp production. Three species of Indian major carps (IMC) are the other cyprinids, which contribute ~58 % of total aquaculture production of India [22]. KHVD has never been reported in any of the IMC, in spite of their wide geographical distribution and culture in KHV affected countries of South-East Asia. This is a positive indication for Indian aquaculture as possible entry of KHV would probably not affect IMC production. However, studies are needed to prove that IMC are probably resistant to KHVD or they do not act as carriers of virus.

To monitor the disease status of KHV in India, a 3 year survey (2007–2009) was carried out to screen koi and common carps from different parts of the country. One hundred fifty fish samples were mainly collected from aquarium shops of four metros i.e. New Delhi ($n = 44$), Kolkata ($n = 32$), Chennai ($n = 38$) and Mumbai ($n = 36$), as most of the ornamental fish import is routed through these cities and supplied to whole sellers across the country. In addition, one hundred four fish samples were collected from nine other cities located in different regions of India. Each test sample was collected in 95 % ethanol and comprised of gills, kidney and gut tissues pooled from five fishes. The work was undertaken in a Department of Biotechnology funded joint project between National Bureau of Fish Genetic Resources, (NBFGR), Lucknow, India and Central Institute of Freshwater Aquaculture, Bhubaneswar, India. Diagnostic capability for detection of KHV by PCR was developed at NBFGR, using TK gene primers [6]. Positive DNA controls of KHV were kindly provided by Dr. Moshe Kotler, Israel and were used for standardization of TK gene PCR. Genomic DNA was isolated from pooled tissue samples through commercially available kits and subjected to PCR [6]. A total of 254 samples were screened by PCR during January 2007 to May 2009 for presence of KHV. None of the tested samples were found to be positive for KHV. These results demonstrate that tested samples from different parts of India to be apparently free from KHV. However, in the event of unrestricted importation of koi carp and gold fish from KHV affected areas, can pose a certain degree of risk for Indian aquaculture and needs risk assessment, which is as follows:

Release Assessment

Release assessment is the likelihood of KHV entering India via importation of koi and common carp, and will depend on following factors:

(a) The disease is reported from many Asian countries like Indonesia, Taiwan, China, Thailand, Japan, Korea, Singapore and Malaysia. Koi carp and gold fish are imported into India mostly from these South Eastern Asian countries.
(b) Detection of KHV is mainly done by PCR that requires a high level of technical expertise and

therefore the disease may go under-reported in the exporting countries. On the other hand, KHVD is generally associated with high morbidity and mortality. Therefore any disease outbreaks in the exporting country will be quickly recognized. However, disease manifests only at a water temperature of 18–25 °C and it subsides when water temperature rises above 30 °C.

(c) KHV can cause latent infections in susceptible species without showing any external signs of disease at non-permissive water temperatures. So there is likelihood that a fish in early stage of infection or latent infection may not be detected. Moreover, gold fish can also act as carrier to KHV.

Considering the above mentioned factors, the possibility of KHV entering India would be high, in absence of proper pre-border and border quarantine.

Exposure Assessment

If KHV manages to enter India through live fish import, it can reach aquaculture establishments or natural waters through various exposure pathways.

Aquaculture Establishments

The probability of KHV establishing in the aquaculture farms will depend on contact of live virus with susceptible host in the aquaculture establishment. The following factors have to be considered for exposure assessment:

(a) Generally, infected fish show external signs of disease at a permissible temperature. So there is every possibility that such fish gets detected during gross examination and destroyed at the port of entry. Only the import of a carrier fish would not be grossly detected and would pose a risk of initiating an infection in a susceptible fish in the aquaculture establishments.

(b) KHV latency is characterized by restricted gene expression of the viral genome with no production of infectious virus at non-permissive temperatures. Thus, carriers would pose a risk of initiating an infection in a susceptible fish, only at permissive temperatures.

(c) KHV remains active in water for at least 4 h, but not for 21 h, at water temperatures of 23–25 °C. A significant reduction in the infectious titre of KHV is seen within 3 days in environmental water or sediment samples at 15 °C. This implies that survival of virus in the susceptible host and in aquatic environment would be low in a warm tropical country like India as compared to cold temperate countries.

Taking these factors into account, it would be reasonable to assume that risk of a carrier fish initiating an infection in a susceptible fish being reared at a warmer temperature would be moderate.

Natural Waters

The probability of KHV establishing in the natural waters would depend on following:

(a) Escape of imported fish in natural waters: Koi carps are generally imported by the ornamental industry for either direct sale of fish in domestic market or for breeding of imported broodstock. The F1 progeny is either re-exported or sold to retail for display in aquarium. Of all the routes, there is a small probability of infected or asymptomatic carrier fish escaping from culture establishments to wild. Occasionally, aquarium fish including koi can be released by end users in natural waters due to a number of ethnic or religious beliefs.

(b) Presence of susceptible hosts and permissible water temperature of 18–25 °C in natural waters: KHV has a very narrow host range and only Koi and common carps are the natural hosts of KHV. Natural waters in India are comparatively warmer and have limited populations of these hosts, so the probability of susceptible fish being exposed to a dose sufficient to cause KHV infection would be low.

Analyzing these factors, there is a low probability of KHV reaching the natural waters and causing infection in susceptible hosts.

Consequence Assessment

KHVD is highly contagious, but morbidity and mortality is restricted to koi and common carp. Establishment of this virus will cause severe financial losses to mainly common carp farming as compared to koi carp. As such, koi carp is mainly farmed by ornamental fish industry, which is still a growing sector. On the other hand, in 2003, Common carp was the single largest species contributing to >20 % of total freshwater aquaculture production in India [22]. Therefore any disease affecting common carp can vastly influence the aquaculture production of our country. Moreover, presence of KHV in our waters can have negative effects on our finfish exports in the international market.

Conclusion

KHV is an emerging pathogen of finfish and is rapidly spreading across the world. In India, the probability of

KHV entering and establishing in the koi and common carp aquaculture industry would be higher as compared to natural waters. The consequences of such establishment of KHV in aquaculture would be high and would warrant the implementation of specific risk management measures. Considering this, it would be better to minimize the risk of KHV entering the country by holding the imported fish at a permissive temperature during aquatic animal quarantine and proper testing of imported ornamental fish for exotic pathogens.

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