THE CURRENT PROBLEM : KOI HERPESVIRUS (KHV)

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Abstract

Koi herpesvirus (KHV) is a pathogen agent that predominantly affects common and koi carp (Cyprinus carpio and Cyprinus carpio koi, respectively) causing disease and mass mortality in farmed and wild populations of these breeds. Following the first reports of KHV in Israel and Germany in 1998, the geographical range of this disease has become extensive. The disease has been spread to many countries worldwide, predominantly through the trade in koi carp and it is now known to occur at least 28 countries. Most recently KHV outbreaks have been reported to the World Organization for Animal Health from Romania, Slovenia, Spain and Sweden. Morbidity of affected populations can be 100% and mortality 70–80%, but the latter can be as high as 90 or 100% . Disease patterns are influenced by virulence of the virus, age and condition of the fish, population density and stress factors. The disease is temperature dependent, too, occurring between 16 and 25°C. Symptoms include mottled gills, bleeding gills, blisters or pale patches on the skin, sunken eyes, constant, lethargic and uncoordinated swimming.

Key words: common carp, koi carp, koi herpesvirus (KHV)

This information paper is intended to alert fish producers and hobbyists about a very important problem at the moment internationally, namely koi herpesvirus (KHV). At present, this highly contagious pathogen is considered to be one of the most risky factors affecting populations of common carp and koi carp [13]. The author believes that accurate information on the specificity of the disease and prevention methods will guard dealers by severe financial losses and, at the same time, will give courage persons who wish to practice the trade of ornamental carp but are reserved because this disease.

Common carp (*Cyprinus carpio*) is a widely cultivated freshwater fish for human consumption, while koi carp, is a farmed colored subspecies 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 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 [14]. With a better capacity to detect KHV and a need to limit transboundary movement of virus-infected fish, the World Organisation for Animal Health (OIE) in 2006 added KHV disease to the list of notifiable diseases, thereby requiring detection of KHV to be reported to the OIE [1]. Since August 2008, the disease is notifiable also for the European Community (EC) [2].

Following the first reports of KHVD in Israel and Germany in 1998 and detection of KHV DNA in tissue samples taken during a mass mortality of carp in the UK in 1996, the geographical range of the disease has become extensive. Rapid spread of KHV is predominantly connected with a worldwide fish trade and koi carp shows that are held without previous health examinations or requirements for health certificates [13]. It is now known to occur in, or has been recorded in fish imported into, at least 28 different countries. In Europe KHV has been detected in many countries across the continent [11]. Most recently KHVD outbreaks have been reported to the OIE from Romania, Slovenia, Spain and Sweden. In Romania, the disease was semnalated in August 2010, in Bucharest and Constanta [5]. In the table 1 is presented the currrent global situation, according to Rathore et all, 2012.

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It is likely that the virus is present in many more countries, but has not yet been identified or reported. Consequently, in many areas of the world, signifiacant resources are currently being directed toward developing control strategies for the disease [9].

Table 1 Status of KHV in affected countries of the world (after Gaurav Rathore et all, 2012)

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

(1) Koi herpesvirus
(2) Polymerase chain reaction
(3) Koi herpesvirus disease

Temperature is a dominant environmental factor that affects the onset and severity of KHV outbreaks. Most outbreaks occur during the spring and autumn (in the Northern Hemisphere) at water temperatures from 18 – 26^{o}C. At lower water temperatures the virus can infect fish without inducing clinical signs of disease but when permissive water

temperatures are again experienced the fish undergo typical disease and mortality[3].

KHVD affects carp of all ages, but younger fish (1–3 months, 2.5–6 g) seem to be more susceptible to infection than mature fish (1 year, ≈230 g) [12]. Recently, the susceptibility of young carp to KHV infection was analyzed by experimental infection. Most infected juveniles (>13 days posthatching) died of the disease, but the larvae (3 days posthatching) were not susceptible [16].

Suspicion of KHV infection is based on clinical signs and histopathologic findings. Clinical signs of KHV are often non-specific. Mortality may begin very rapidly in infected populations, with deaths starting within 24 to 48 hours after the initial onset of clinical signs. In experimental studies, 82% of fish exposed to the virus at a water temperature of 22°C died within the first 15 days [15].

KHV infection may produce severe gill lesions which exhibit as gill mottling with red and white patches (may be similar to columnaris disease signs). The white patches are due to necrosis of the gill tissue. Gill lesions caused by KHV disease are the most common clinical signs in affected koi. Other external signs of KHV may include bleeding gills, sunken eyes, pale patches or blisters on the skin. In some cases, secondary bacterial and parasitic infections may be the most obvious problem, masking the damage caused by the primary viral infection. Microscopic examination of gill biopsies often reveals high numbers of bacteria and various parasites [7]. Gilad et all sugested that death is due to loss of the osmoregulatory functions of the gills, kidneys and gut [10].

Histopathologic changes appear in the gills as early as two days post infection and involve the epithelial cells of the gill filaments. These cells exhibit hyperplasia, hypertrophy, and/or nuclear degeneration. Severe inflammation leads to the fusion of respiratory epithelial cells with cells of the neighboring lamellae, resulting in lamellar fusion. In the kidney, a weak peritubular inflammatory infiltrate is evident as early as two days post infection and, along with blood vessel congestion and degeneration of the tubular epithelium in many nephrons, increases with time. In the spleen and liver, splenocytes and hepatocytes, respectively, are the most obviously infected cells. In brain of fish that showed neurologic signs, congestion of capillaries and small veins are apparent in the valvula cerebelli and medulla oblongata, associated with edematous dissociation of nerve fibers [10]. Morbidity of affected populations can be 100%, and mortality 70–80%, but the latter can be as high as 90 or 100% [8].

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 for 20 min, benzalkonium chloride at 60 mg/ l for 20 min, ethyl alcohol at 30 % for 20 min and sodium hypochlorite at 200 mg/l for 30 s, all at 15°C [14].

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. 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. As with other herpes viral infections, KHV is believed to remain in the infected fish for life; therefore, exposed or recovered fish should be considered as carriers of the virus [7]. This latency feature of KHV probably contributes in the spread of this pathogen to new geographic locations [14].

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 is the major abiotic vector. Between animate vectors it were confirmed after the ADN viral identification by nested PCR following species: goldfih, with varieties red, lion-head and shubunkin, grass carp (*Ctenopharyngodon idella*), ide (*Leuciscus idus*), ornamental catfish (*Ancistrus sp.*), Russian sturgeon (*Acipenser gueldenstaedtii*) and Atlantic sturgeon (*Acipenser oxyrinchus*) [8].

Diagnostic identification of KHV may be accomplished by several direct and indirect methods. Direct methods are procedures that

detect actual virus or "pieces" of virus. Indirect methods are procedures that quantitate the immune response by measuring antibody levels [4]. Direct methods used to identify KHV include: virus isolation and identification (i.e., growing the virus) using a susceptible cell line such as Koi Fin (KF) cell lines and PCR techniques (i.e., testing for the presence of KHV DNA material). Indirect tests for KHV include enzyme-linked immunosorbent assay (ELISA) and virus neutralization (VN) testing. A positive ELISA or VN test for KHV indicates that the fish has produced antibodies against KHV and is either experiencing an outbreak or is a carrier [7].

There is no treatment for KHV. Dealers and hobbyists can help protect themselves by [6]:

1. Buying from reputable sources that adequately quarantine all fish arriving at their facilities and operate under best health management practices.
2. Adequately quarantining new fish prior to introducing them into the general fish populations. This is the single most effective action within the fish owners' control.
3. Disinfecting, or otherwise verify the safety of everything that comes in contact with water of existing pond/ system or new fish.
4. Depopulating all koi and carp exposed to KHV.
5. Supporting efforts to educate the koi community and to find new ways to control and ultimately eradicate this disease.
6. Buying vaccinated koi if and when proven safe and having existing fish vaccinated.

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