Introduction to Koi Herpes Virus (KHV) Disease

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Abstract

Koi herpesvirus (KHV), scientifically recognized as Cyprinid herpesvirus 3, is an extremely contagious pathogen accountable for KHVD in carp and its ornamental variant, koi. The virus, belonging to the family *Alloherpesviridae*, was first recognized in the USA and Israel in 1990s. KHV infects a broad spectrum of fish species, including goldfish, grass carp, and sturgeon. However, common carp and koi are the most affected, suffering severe mortalities during outbreaks. KHV is a dsDNA virus with a linear genome encoding one 156 possible protein-coding ORFs, several of which are involved in host immune evasion. The virus has a complex structure, including a capsid, envelope, and tegument. Infection typically results in clinical signs such as decrease in appetite, erratic swimming, and discoloration, with gill necrosis, hemorrhages, and skin lesions. Transmission is horizontal, primarily via direct contact with infected fish, water, and contaminated surfaces. Diagnosis of KHVD relies on various methods, including virus isolation, PCR, ELISA, and immunofluorescence. Despite advances in diagnostic techniques, there is currently no widespread commercial vaccine for KHV, although live attenuated vaccines have shown effectiveness in certain regions. KHV is a problem to the carp and koi industry globally, and among the economic consequences are large deaths during outbreaks.

Keywords: Cyprinid herpesvirus 3, Contagious, Erratic swimming, Gill necrosis, Immunofluorescence, Live attenuated

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Introduction

Koi herpesvirus (KHV) is one of the most contagious pathogens which causes morbidity and deaths in common carp (*Cyprinus carpio*) varieties (Hedrick et al., 2000, Haenen et al., 2004). Typical of the majority of countries, the common carp is trained for food consumption as well as purposes of ornamental fish farming; such species here are known as koi (Haenen et al., 2004). Among the diseases credited to a herpesvirus, aquatic herpesvirus (Hedrick et al., 2000), a member of the *allo-herpesviridae* family, is considered as the cause of the disease (Waltzek et al., 2009). The illness, known as KHVD, has only affected *C. carpio*, common carp, koi, and their hybrids, causing more severe mortality (Hedrick et al., 2006; Bergmann et al., 2010). Classification of KHV describe in Table 1.

Table 1: Taxonomic Position of KHV (Bergmann et al., 2020)

Order	*Herpesvirales*
Family	*Alloherpesviridae*
Genera	Cyprinivirus
Spp	Cyprinid herpesvirus-3 (CyHV-3)

After illness outbreaks in Israel and the USA in 1998, KHV was initially isolated in the United States. Initial reports of illness outbreaks with symptoms resembling KHV infection came from the UK in 1998, and the virus was discovered in 2000. According to Ariav et al., (1999) KHV was initially identified in Israel in 1998 and subsequently published in 1999 describe in Table 2.

Except for Australia, Koi herpesvirus (KHV)), has transmitted globally through trade, primarily with carp and latently ill but healthy-looking koi (Haenen et al., 2004).

KHV Characterization

KHV is a pathogen that resembles double stranded DNA herpes-virus. It is composed of at least eight glycosylated proteins and thirty-one virion polypeptides, twelve of which share molecular weights with CHV-Herpesvirus cyprini and ten of which share molecular weights with channel catfish herpesvirus (CCV) polypeptides. According to Miyazaki et al. (2008) KHV virions are made up of an icosahedral capsid

that contains the genetic material, a lipid envelope that contains virus associated glycoproteins, and an amorphous structure of amino acids called the tegument that connects the capsid and the envelope. KHV nuclear capsids are divided into 3 categories: empty capsids, capsids with an electron-dense core, and capsids with an internal circular (spherical) structure.

Table 2: Name of countries and years of first case of KHV

Europe	America	Asia	Africa
United Kingdom (1998)	USA (1998)	Israel (1998)	S. Africa (2003)
Belgium (1999)		Indonesia (2002)	
Denmark (2002)		China (2002)	
Germany (2002)		Taipei China (2002)	
The Netherlands (2002)		Japan (2003)	
Switzerland (2003)			
Luxemburg (2003)			
Italy (2003)			
Austria (2003)			
France (2003)			

According to Miwa et al. (2007) these morphologies correspond to the 3 distinct developmental phases of KHV capsid morphogenesis. According to Hutoran et al. (2005), KHV is a big, linear, dsDNA virus with an icosahedral shape measuring 100–110nanometer. The left and right repeats are two 22-kb repeat sections that flank the core portion of a DNA molecule. 156 putative protein-coding open reading frames (ORFs) are encoded by the genome. Eight of these ORFs are held in two copies in the genome for being encoded by the repeat regions (Aoki et al., 2007). In the manner of most countries, common carp is reared for the dual purposes of being a food organism and an ornamental fish, wherein such species are dubbed as koi (Haenen et al., 2004). The diseases attributed to herpesvirus, aquatic herpesvirus (Hedrick et al., 2000) is considered the causative agent of the disease (Waltzek et al., 2009).

Each KHV virion contains forty structural proteins and eighteen host cell proteins. According to Michal et al. (2010) structural proteins are categorized as capsids (three), envelopes (thirteen), teguments (two), and unclassified (twenty-two). KHV can be propagated in vitro with the cell lines of koi fin (KF-1), C. carpio carp brain (CCB), and C. carpio carp gills (CCG) (Hedrick et al., 2000; Neukirch and Kunz, 2001; Ronen et al., 2003). According to Gilad et al. (2003), KF-1 cells do not produce virus at temperatures below 15 to 25°C, which is the ideal temperature span for viral development. In the three to four days following inoculation, the virus causes the cultured cells to develop characteristic plaques, syncytia, and an elevation in cytoplasmic vacuoles. According to Pikarsky et al. (2004) the cells eventually round out and separate from the substrate. When the temperature is raised, KHV-illed cells with distorted structure can return to normal, but once they reach the permissive temperature, they can deform once more. This is due to the fact that moving cells between permissive and non-permissive temperatures activates and deactivates viral gene transcription and propagation. This implies that virions remain in the fish body for extended periods of time, allowing for a fresh infection burst when the temperature changes to a permissive level (Dong et al., 2011).

Koi Herpes Virus Disease (KHVD)

Hosts Species

All usual carp species, are susceptible to KHVD (Hedrick et al., 2000; Haenen et al., 2004). Additionally vulnerable to experimental KHV infection are hybrids of goldfish, carp or koi (Hedrick et al., 2006; Bergmann et al., 2010). Russian and Atlantic sturgeon from aquatic farms in Poland have been found to carry KHV by PCR (Bergmann et al., 2009). Table 2 lists the susceptible host variety of KHV additionally to the type of infection that occurs in various fish species. According to recent studies, fresh-water shrimp (*Gammarus pulex*) and swan mussels *(Anodonta cygnea)* are examples of aquatic invertebrates that could function as KHV vectors (Kielpinski et al., 2010). Carp of all ages, from juveniles to adults, are vulnerable to KHVD (Bretzinger et al., 1999; Sano et al., 2004). In contrast, in clinical trials, fish weighing two to six grams are more susceptible than fish over 230 g (Perelberg et al., 2003).

Host of Koi Herpes Virus and its type of Infection in Fish Species

The common carp (*Cyprinus carpio carpio*) and koi carp (*Cyprinus carpio koi*) both show signs of infection, as noted in studies by Haenen et al. (2004), Hedrick et al. (2000), and Perelberg et al. (2003). Similarly, goldfish (*Carassius auratus*) act as carriers without showing any signs of infection, according to El-Matbouli et al. (2007), Sadler et al. (2008), El-Matbouli and Soliman (2011). Hybrid species, such as goldfish × koi carp and crucian carp × koi carp, show signs of infection, as reported by Bergmann et al. (2010). Additionally, the goldfish × common carp hybrid shows signs of infection, as indicated by Hedrick et al. (2006). In contrast, species like grass carp (*Ctenopharyngodon idella*), ide (*Leuciscus idus*), and ornamental catfish (*Ancistrus sp.*) do not show any signs of infection and act as carriers, as documented by Kempter and Bergmann (2007) and Mayer (2008). Both the Russian sturgeon (*Acipenser gueldenstaedtii*) and the Atlantic sturgeon (*Acipenser oxyrinchus*) are also carriers without showing symptoms, as per the findings of Bergmann et al. (2009).

Clinical Signs and Histopathology

The afflicted fish experienced a drop in appetite and would swim erratically before dying. The disease's most dependable symptoms include skin lesions, enlarged, pale, patchy gills, discoloration, and increased respiratory frequency (Oh et al., 2001; Gray et al., 2002).

Histological results show mass gill epithelial cells proliferations with necrotic and degenerative alterations. Internally, diseased fish have the most noticeable cellular alterations in the gills, skin, renal, hepatic, spleen, digestive system and nervous system. Lamellar fusion is the

outcome of increased inflammation, elevated cell numbers, and enlarged cell size of the gill filament epithelial cells. Nephrons in the kidney exhibit congestion and tubular epithelial degradation (Pikarsky et al., 2004). The brains of ill species with neurological disorders show edematous disconnection of nerve fibers and congestion in the medulla oblongata and valvula cerebella (Miyazaki et al., 2008). Additionally, chromatin margination and eosinophilic intranuclear inclusion bodies are seen in gill and splenic epithelial cells (Cheng et al., 2011).

Routes of KHV Spread

KHV is spread horizontally, that is, directly from affected fish, their fluids, water, mud, or other inanimate objects that have come into exposure to contaminated systems. According to Costas et al. (2009) bioluminescence imaging demonstrates that KHV enters the body through the host's surface. Viral entry into the host is facilitated by the removal of skin mucous and epidermal lesions (Raj et al., 2011). Before any clinical signs appear, the virus must incubate inside the host for seven to ten days. Lethargy, appetite loss, gill necrosis, body hemorrhages, and uncoordinated swimming are the clinical symptoms (Oh et al., 2001; Gray et al., 2002). Within a day or two of the signs appearing, the infected fish start to die (Hedrick et al., 2000). The virulent virus is continually secreted by infected common carp at 16°C for a longer duration than those at 23–28°C through their excretions, gills, and skin secretions (Yuasa et al., 2008). Within a week after the start of clinical symptoms, there are massive deaths, with a death rate of eighty to hundred percent (Walster, 1999). Outbreaks of KHV disease (KHVD) usually happen in the spring and autumns when the water temperature is between 60° and 77°F (16° and 25°C). KHV becomes dormant and clinical symptoms usually stop at temperatures more than 30°C or less than 13°C (Gilad et al., 2003; Cheng et al., 2011). One day following viral exposure, viral DNA can be found in the kidney and blood (Pikarsky et al., 2004; Dishon et al., 2007). Deoxyribonucleic acid can then be found in the liver, spleen, digestive system, and gills, whereas it is not in the cerebral cortex itself (Gray et al., 2002). During an infection, the virus is most prevalent in the renal, spleen, and gills (Gilad et al., 2004). Prior to, during, and following a disease outbreak, KHV deoxyribonucleic acid has been found in environmental water (Minamoto et al., 2009). Following its discharge from the host, the pathogenic virus attaches itself to plankton and may serve in the transmission of viruses (Minamoto et al., 2011).

Diagnostic Methods

KHV is currently detected using a number of diagnostic methods, including histopathology, PCR, ELISA, and virus isolation in a susceptible cell line (Haenen et al., 2004). The finest organs for viral isolation are the tissues of the gills, kidney, and spleen. For PCR-based detection techniques, it is advised that test samples also include the intestines and encephalon. Individual samples of fish should be evaluated for clinical diagnosis, but samples from up to 5 fish per pool may be pooled for surveillance testing.

a) Cell Culture for Virus Isolation

KHV has been isolated utilizing the KF-1 and CCB cell lines (Hedrick et al., 2000). Nevertheless, other labs have experienced difficulties in sustaining the KF cell line, and few have documented a spontaneous pseudo-cytopatic impact in the negative control cells. Also, KHV isolation usually takes 10–12 days when the cells and pathogens are in excellent condition. On the other hand, it takes five to eight days at 26°C for the virus to create a well-recognizable CPE in CCB cells. A pathogen negative outcome based solely on virus isolation is not dependable, nevertheless, as the sensitivity of virus isolations is significantly lesser than that of the PCR test. Initial cultures of koi fin cells have also been found to harbor the virus in Israel (Ronen et al., 2003).

b) Recognition of KHV DNA

The PCR assay is a quick and sensitive method used by most diagnostic labs to detect and amplify certain KHV DNA sequences. For the detection of KHV, a lots of detection tests have been proposed that are focused on PCR (Gilad et al., 2002; Bercovier et al., 2005; Yuasa et al., 2005), loop-mediated isothermal amplification (LAMP) (Gunimaladevi et al., 2004; Soliman and El-Matbouli, 2005; Yoshino et al., 2006; Yoshino et al., 2009), nested PCR (Bergmann et al., 2006; El-Matbouli et al., 2007) and real-time PCR (Gilad et al., 2004).

According to Bercovier et al. (2005) a very sensitive PCR technique can identify the TK gene of KHV at the level of identification of KHV DNA at a concentration of 10fg. This is about 10-1000 times further sensitive than former PCR techniques (Gilad et al., 2002). KHV can be noticed in sick organism's excreta by PCR techniques with an exposure limit of forty fragment viral DNA (Ishioka et al., 2005).

Sensitivity evaluation has been performed for some of these diagnostic tests (Bergmann et al., 2010). Real-time PCR detecting the KHV segment of DNA (Gilad et al., 2004) is measured the gold standard for recognition and absolute pathogen quantity with an external and an internal control system. The "gold standard," which is comparable to 10 fragments DNA or one to five genomic KHV equivalents, is likewise as sensitive as Bercovier's PCR (Bercovier et al., 2005) and nested PCR (Bergmann et al., 2006). With a pathogen quantity ranging from 5 to 10 KHV copies, a novel on-tube in PCR that recognizes the KHV main glycoprotein gene has just been developed (Bergmann et al., 2010).

Recently, novel methods known as loop-mediated isothermal amplification (LAMP) was created that can quickly, efficiently, and with high specificity amplify and detect KHV DNA in isothermal conditions. Focused on the sequencing of the KHV thymidine kinase (tk) gene, a set of 4 primer, 2 inner and 2 outer was created. Routine diagnoses and surveillance/quarantine operations may benefit greatly from this diagnostic approach (Gunimaladevi et al., 2004).

c) Detection of KHV Antigen/Antibodies

Immunoperoxidase staining or immunofluorescence staining can identify viral antigen in infected tissues (Pikarsky et al., 2004; Shapira et al., 2005). ELISA can be used to detect KHV antigen from fish droppings or infected tissues (Adkison et al., 2005; Dishon et al., 2005). KHV can be indirectly diagnosed by ELISA, which detects certain antibodies in serum. Since the KHV ELISA requires a blood specimen, it is a harmless detection method. Indication that an organism is mounting or has straddling an immune response (i.e., the

generation of Abs) toward KHV can be seen in ELISA data. ELISA shows that naturally exposed koi have a steadily rising level of anti-KHV antibodies (Ronen et al., 2003). Following a natural infection, these antibodies are found in the blood one year later in survivors and three weeks after an experimental illness (Adkison et al., 2005; Taylor et al., 2010; Ilouze et al., 2011). Nevertheless, since this approach cannot identify if fish are still infected with the pathogen, it is not advised as an initial detection method. Another source of false positives is cross-reacting antibodies in fish serum.

d) Other Detection Methods

i. In-situ Hybridization

Research applications have employed in-situ hybridization (ISH) on isolated fish leucocytes for KHV identification, confirmation, or detection (Le Deuff et al., 2001).

ii. Indirect Fluorescent Antibody Test (IFAT)

By using immunofluorescence (IF), KHV can be found in the touch imprints of the hepatic, renal, and skull of fish with the infection. One day after infection, the virus could be identified by IF on a kidney impression, and the kidney showed the highest amounts of positive IF. Care must be taken when interpreting immunostaining results for KHV detection because positive-staining cells may be the consequence of cross-reaction with a non-viral protein or a serologically related virus (e.g., CyHV-1) (Pikarsky et al., 2004).

Biosecurity and Disease Control Strategies

A. Vaccination

There is not a commercial vaccine that is both safe and effective yet. However, carp have been vaccinated using a live attenuated virus. At least eight months of protection were provided by the vaccine formulation, which produced an immune response against the virus (Ilouze et al., 2011). The vaccination has been routinely utilized in carp farms throughout Israel since it was granted a license for emergency use. According to research conducted in Japan, carp were also protected against clinical sickness by consumption of a liposome-based vaccination containing inactivated KHV (Miyazaki et al., 2008; Ilouze et al., 2011). BAC cloning technology was used to create an immune individual that utilized the dual deletion of ORF56 and ORF57, and experimental challenge trials have shown that attenuated recombinant vaccines are efficacious (Boutier et al., 2015).

Chemotherapy Including Blocking Agents

Although there is currently no approved chemotherapy, exopolysaccharides have been shown to have antiviral effect beside KHV in vitro (Reichert et al., 2017).

B. Breeding Resistant Strains

Different carp strains have been found to exhibit varying resistance to KHV infection, but not to virus entrance (Shapira et al., 2005; Dixon et al., 2009; Ito et al., 2014). Artificial or natural infection was used to test the offspring of crosses between two farmed carp strains and one wild carp strain. According to Shapira et al. (2005), a highly resistant strain had a survival rate of 60.7% after laboratory contact and 63.5% after regular introduction in ponds, whereas the shortest survival rate was roughly 8%. In a more contemporary resistance investigation, KHV was experimentally challenged in 96 families that were produced by di-allele crossing four common carp strains from Europe and Asia. In the conclusion of the virus challenge study, the five of the most resistant crosses had survival rates ranging from 42.9 percent to 53.4 percent (Dixon et al., 2009).

C. General Husbandry

The majority of outbreaks happened after new fish from polluted areas were brought in or after people contracted the disease from tainted water and equipment. Thus, the idea of biosecurity may be used to stop or lessen the risk of contracting KHV. Just KHV-free germ and broodstock should be used on non-infected farms, and new fish, especially those from infected areas, should not be introduced. It is advised that all new fish, together with sentinel fish, be quarantined for a minimum of two weeks at a permissive water temperature of 18 to 28°C if the introduction of new fish cannot be avoided.

Prior to stocking with KHV-free livestock, farms or areas where KHV is currently endemic should eradicate KHV from the farm. It should involve draining out and the liming the ponds, disinfecting contaminated equipment, and removing all fish especially those that are susceptible from water bodies, reservoirs and water canals before replenishing.

Avoiding the virus's reappearance is crucial after it has been eliminated from a location. Affected fish are probably the main source of infection, along with tainted water and equipment. Therefore, it is important to think about the following measures: obtaining seed and broodstock from KHV-free areas; strictly excluding all wild carps from the farms in endemic areas; obtaining water only from KHV-free sources; and disinfecting any equipment that may have been applied at infected sites. These methods might work well for isolated farms like koi farms, but not for raceway culture in rivers or cage culture in lakes.

D. Good Management Practices

In the culture of carp and koi, good management practices (GMPs) should be used. GMPs appear to have the potential to reduce KHV infection-related mortality. Reducing stocking density and using only KHV-free seed are advised. According to sporadic reports, moving sick fish from a raceway to an earthen pond will reduce mortality and stop more losses. By lowering the quantity of stockings and maintaining a constant water temperature, these methods improved the conditions for the sick fish. Vitamin C and immunostimulants may improve fish's

non-specific immunity to KHV.

Since lifeless and moribund fish are an excellent reservoir for the virus, it is advised to remove them from the pond. Curative methods were also used to treat secondary bacterial, fungal, and parasitic infections in an effort to lower the mortality rate. Secondary bacterial infections were treated with enrofloxacin (5–10 ppm). Benzalkonium chloride (BKC) at one drop per 100 L and dyvon (Dichlorphos) at 1–5 ppm was administered to combat Argulus along with additional parasite infections. GMPs and the biosecurity concept appear to offer an alternate management technique at the farm level, notwithstanding the challenges in managing outbreaks in common water bodies, such as raceways and net cages in reservoirs or lakes. However, more investigation is required to determine additional crucial elements of biosecurity and GMPs that effectively contain the KHV outbreak.

Conclusion

Koi herpesvirus disease caused by CyHV-3, is a foremost threat to the global carp and koi fish industry, leading to significant economic losses. The virus primarily affects koi and common carp, but it can also infect other cyprinid species. CyHV-3 is a DNA double strand virus that inflicts the death of various organs in fish with tissue damage, the infected fish appear lethargic, unusual swimming, and discoloration, histopathological examination also reveals characteristic intra-nuclear inclusion bodies. Improved biosecurity, reliable diagnostic tools, and preventative measures such as vaccines and antiviral treatments are required for effective management of KHVD. At present, PCR assays are the most reliable diagnostic method, but the lack of a commercial vaccine is a significant challenge. One of the possible strategies to deal with KHV effectively has to do with selective breeding of carp strains resistant to KHV. Studies have reported such strains that are resistant to the virus. Thus, using these genetic traits in breeding may be beneficial in reducing the impact of KHVD. International collaboration and strict regulations regarding live fish movement will also help prevent KHVD cross-border spread. In conclusion, whilst KHVD management and understanding have shown some progress in terms of advances, now with research into vaccines, antivirals, and resistant carp strains combined with vigorous biosecurity and international cooperation, there is need for management and protection of the carp and koi industries against the disease.

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