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Research Article

Cyprinid herpesvirus 3 (CyHV-3), koi herpesvirus (KHV) disease and their current status in Iran: A review

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Abstract

Cyprinid herpesvirus 3 (CyHV-3) is a significant threat to the production of common carp and koi, resulting in high mortality rates and posing a risk to the long-term sustainability of carp aquaculture. The presence of this disease in Iran has been confirmed by the veterinary organization and reported to OIE, with evidence suggesting its presence since 2021 in Iranian Koi and carp farms. Given the economic importance and rapid spread of CyHV-3, this review aims to provide a comprehensive summary of the current knowledge and research on the virus and its disease in Iran and worldwide. The fish farmers and authorities in the country need to take necessary measures to prevent the spread of the disease and mitigate its impact on the aquaculture industry.

Introduction

Fish protein accounts for approximately 17% of the total animal protein consumed worldwide. Aquaculture, not hydroponics, accounted for 57.5% of the global food fish supply in 2018. Common carp (*Cyprinus carpio*) is one of the most economically valuable and widely cultivated fish species, with a global production of 4.2 million metric tons per year. As of 2018, common carp represented nearly 8% of worldwide aquaculture output (FAO, 2020).

Koi (*Cyprinus carpio* koi), known as the colorful subspecies of carp, is a highly prized fish species that is farmed for ornamental purposes in aquariums and competitive exhibitions. With its bright and vibrant colors, koi has become one of the most expensive individual freshwater fish on the market. Koi is widely regarded as a symbol of good luck, prosperity, and perseverance in many cultures around the world. The fish is also known for its longevity, with some specimens living up to 50 years or more. Due to its popularity and high demand, koi farming has become a lucrative industry worldwide, with breeders constantly striving to develop new and unique color patterns and markings.

In the late 1990s, an aquatic herpesvirus, designated cyprinid herpesvirus 3 (CyHV-3), as the causative agent of a new highly contagious viral disease, termed koi herpesvirus disease (KHVD), began to occur, rapidly spread and induce massive economic damage in both common carp and koi industries, as well as their hybrids. KHVD is distributed in most regions of the world, except Australia and South America, by unrestricted and unregulated fish transport and trade, mainly with latently infected but asymptomatic Koi. Although not transmissible to humans, the emergence of this disease negatively affects aquaculture and capture fish production, biodiversity, employment, and other wide ecological and socio-economic aspects. As a result, in 2007, OIE designated KHVD as a notifiable disease and outbreaks must be reported to this organization. Within the European Union, koi herpesvirus is now listed as a non-exotic disease. The rapid spread of KHVD to different countries, its broad host range, and insufficient current knowledge and research on this disease in Iran, highlight an emerging biosecurity risk and therefore, it is accepted that KHVD can be a potential hazard to Iranian aquaculture. In this article, scientific research on KHVD is reviewed and summarized in three major parts: Cyprinid Herpesvirus 3 (CyHV-3) and its main specific properties, description of koi herpesvirus disease and its current situation in Iran.

Characterization of CyHV-3

Classification and taxonomy

CyHV-3, also commonly known as koi herpesvirus (KHV) and formerly referred to as carp interstitial nephritis and gill necrosis virus (CNGV), is a member of the genus *Cyprinivirus*, family alloherpesviridae, order herpesvirales (King *et al.*, 2012). All herpesviruses are classified in the order herpesvirales and the family alloherpes viridae includes herpesviruses that infect fish and amphibians. The International Committee on Taxonomy of Viruses (ICTV) currently lists twelve species in the family alloherpesviridae, distributed among four genera, of which three contain fish viruses (*Cyprinivirus, Salmonivirus*,

and *Ictalurivirus*). The family Cyprini virus consolidates infections that contaminate common carp (cyprinid herpesvirus-1[1] and cyprinid herpesvirus-3[2], goldfish (Cyprinid herpesvirus 2[3]), and freshwater eel (Anguillid herpesvirus 1; Ang HV-1). According to phylogenetic analysis of specific genes, two major monophyletic clades are recognized in the family alloherpesviridae. CyHV-3, which possesses a large genome, is categorized in the first clade (Waltzek *et al*., 2009). The virus possibly originated from warm water areas in Asia and all other CyHV-3 variants can return to the Asian lineage, as concluded from CyHV-3 identification after 100 cell culture passages of virus isolates *in vitro* and *in vivo* (Klafack *et al*., 2019). The morphology of the CyHV-3 virion and its morphogenesis are entirely typical of the order herpesvirales. A capsid inner with icosahedral symmetry contains a single duplicate of a huge, straight, twofold abandoned DNA genome. The capsid is covered by a massive amorphous layer of proteinaceous matrix called the tegument, which is enveloped in a loosely applied, spherical to pleomorphiclipid bilayer derived from trans-golgi membrane of the host cell (Roizman and Pellet, 2007). The envelope has viral glycoproteins (Fig. 1).

The measurement of the whole CyHV-3 molecule is 167-230 nm, as per the infected cells (Miyazaki *et al.*, 2008). The morphology of the virus is very variable and is classified into three types: the first and most abundant type has a circular (spherical) structure inside the capsid, the second type has an electron-dense core with variable morphology, and the third type is empty-appearing capsids characterized by very low internal electron density (Miwa *et al*., 2007).

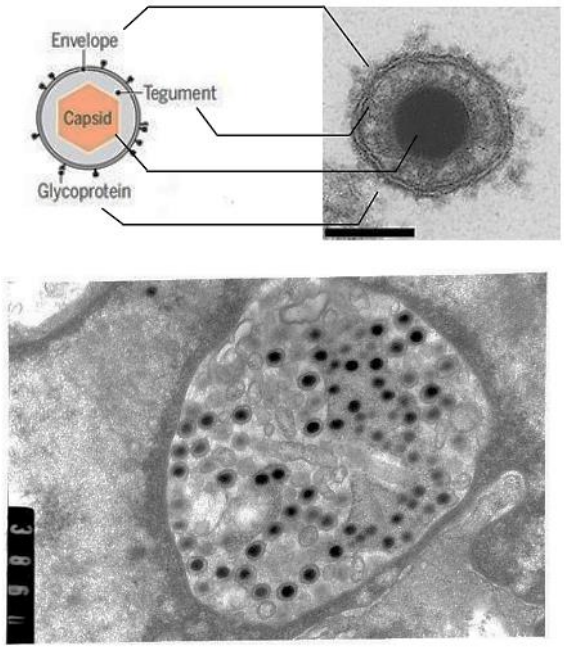

Figure 1: Schematic representation (left) and electron microscopy examination (right) of CyHV-3 virion. The bar represents 100 nm (Up; Mettenleiter *et al*., 2009). CyHV-3 viruses in the infected CCB cells, TEM (Bottom; Matras *et al*., 2012).

Viral DNA replication and capsid assembly occur in the nucleus with the acquisition of the lipid envelope and further processing to yield mature virions takes place in the cytosol of the host cell (Rakus *et al*., 2013). CyHV-3, like other herpesviruses, has the ability to establish both lytic and latent infections. The lytic cycle associated with the production of progeny virions results in the lysis of the infected cell and its membrane, while latency is characterized by the absence of regular viral transcription

[1] CyHV-1
[2] CyHV-3
[3] CyHV-2

and replication, the lack of production of infectious virus particles, the long-lasting support of the viral genome as a non-coordinated episome and the declaration of not many viral qualities and microRNAs (Donohoe, 2013). Upon reactivation, which is temperature and stress-dependent in CyHV-3, latency is replaced by lytic replication. The estimated level of latent genomes ranged from only 2-150 copies per microgram of total DNA (Eide *et al*., 2011). The main cells for persistence are the polymorphonuclear granulocytes (Bergmann *et al*., 2010) and IgM^+ B cells (Reed *et al*., 2014).

Molecular characteristics

Genomics and genotypes

Currently, there are 9–11 complete genomic sequences of CyHV-3 in the NCBI database and most of them are from the isolated virus in cell cultures (Bergmann *et al*., 2020). CyHV-3 has a 295 kbp genome, containing one large central unique region flanked by two 22 kbp left and right terminal direct rehashes (Aoki *et al*., 2007). Until now, this is the biggest known genome among all sequenced herpesviruses. This genome encodes 156 potential protein-coding open understanding casings (ORFs), including 148 ORFs (ORF9-ORF156) encoded by the focal locale and 8 ORFs (ORF1-ORF8) encoded by terminal rehashes. The latter are therefore duplicated in the genome of these 156 genes (Ilouze *et al*., 2012), have orthologues in CyHV-1 and CyHV-2 and 55 have orthologues in AngHV-1. In addition, twelve core genes are conserved across all members of the alloherpesviridae family, evidently inherited from a common ancestor (Davison *et al*., 2012). Twenty-one ORFs are unique to CyHV-3. CyHV-3 genome consists of five gene families, presumably emerged by gene duplication mechanism. These are the ORF2 family (ORF2, ORF3, ORF9, ORF129, ORF130, and ORF135), the TNFR family (ORF4 and ORF12, encoding proteins connected with growth rot factor receptor), the ORF22 family (ORF22, ORF24, and ORF137), the ORF25 family (ORF25, ORF26, ORF27, ORF65, ORF148, and ORF149, encoding potential film proteins containing an immunoglobulin space), and the RING family (ORF41, ORF128, ORF144, and ORF150) (Boutier *et al.*, 2015). All of these families are transcribed into mRNA.CyHV-3 genome also encodes several genes (*e.g.* TmpK and B22R-like genes) not found in any other herpesviruses, but instead, six ORFs are strongly associated with phylogenetically distant viruses, in particular, the members of Poxviridae and Iridoviridae families (Ouyang *et al*., 2019). Interestingly, some fragmented, and therefore probably non-functional, ORFs are present in CyHV-3 genome, with their precise set varies according to virus strain. It is conceivable that a deficiency of quality capabilities might have added to the rise of illness in carp species (Boutier *et al*., 2015). Three inconsequential types of CyHV-3, confined in Israel (CyHV-3 I), Japan CyHV- 3 J), and the US (CyHV-3 U), have been completely sequenced (Aoki *et al*., 2007). Despite their far-off geographic beginnings, these strains show close to 100% arrangement character (Radosavljević *et al.,* 2019). The low

diversity of successions among strains appears to be a characteristic of CyHV-3. Regardless of this low variety, atomic markers empowering separation among nine genotypes (seven from Europe, termed E1-E7, and two from Asia, called A1 and A2) have been identified (Kurita *et al*., 2009). It seems that small genomic changes, which occur frequently, if not always, are normal and are not essential for virus replication and virulence in carp (Bergmann *et al*., 2020). Recently, a fourth strain, CyHV-3 GZ11, was isolated and sequenced from a mass mortality outbreak in adult koi in China (Li *et al*., 2015). This isolate contained small genomic elements from the European lineage and the majority of its genome came from the Asian lineage.

Transcriptomics

During lytic replication, viral gene expression occurs as a classic regulatory cascade that is conserved across all herpesviruses (King *et al*., 2012). This is a coordinated temporal pattern that involves the initial expression of immediate early (IE) genes in the absence of de novo protein synthesis. These genes mainly encode gene trans-activators. This is followed by early (E) genes, whose expression is dependent on the expression of IE trans-activator genes and they encode enzymes and proteins involved in the modification of host cell metabolism and the viral DNA replication complex. In the final phase, late (L) genes, that code for structural proteins involved in the assembly of new virion components *i.e.* capsids, tegument, and envelope glycoproteins, dependent on viral DNA synthesis, will be expressed. The E and L genes, mainly located in the unique long region of the genome, were observed to be highly expressed during the acute and reactivation phases but to be expressed at very low levels during the persistent phase (Sunarto *et al*., 2012).

Proteomics

A sum of 46 underlying proteins of the viral beginning, containing 5 capsid, 11 covering, and 14 unclassified proteins, were portrayed (Fuchs *et al*., 2014). Also, 18–27 cellular proteins associated with extracellular CyHV-3 virions have been identified (Yi *et al*., 2014). These include proteins involved in stress response, signal transduction, vesicular trafficking, metabolism, cytoskeleton organization, translational control, immunosuppression, and cell-signaling regulation (Boutier *et al*., 2015). CyHV-3 ORF12 and ORF134 encoding separately a dissolvable TNFR homolog and an IL-10 homolog were among the most bountiful discharged viral proteins (Ouyang *et al*., 2013).

Koi herpesvirus disease

KHVD is seasonal, occurring mainly at water temperatures between 18 and 28°C (Gotesman *et al*., 2013). The 169 virus has a recurrent nature due to its ability to latent infection establishment.

History and global distribution

KHVD has a relatively short history. The disease was first recognized in 1997 in Koi in Germany (Bretzinger *et al*., 1999). In any case, examinations of tests from documents confirmed that the infection had been in wild normal carp beginning around 1996 in the UK (Haenen *et al*., 2004). The

first confirmed outbreak of KHVD occurred in 1998 on several carp farms in Israel, which was followed by a separate outbreak three months later in a Koi retail facility in the USA. In 2000, the causative agent was isolated and identified from samples of both locations (Hedrick *et al*., 2000). Before long, flare-ups of CyHV-3 happened in numerous nations in Europe, Asia, and Africa. Currently, KHVD has been reported from at least 38 different countries worldwide (Fig. 2). With a few exceptions, the virus appears to be present worldwide in areas where common carp and Koi are farmed or traded.

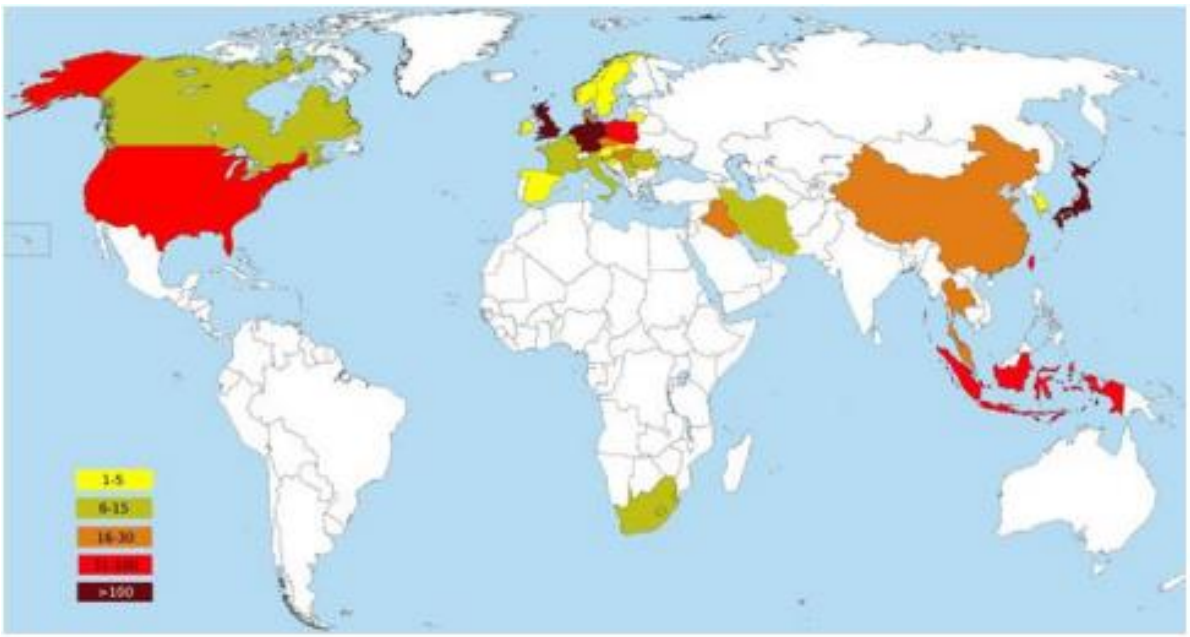

Figure 2: Heat map of KHVD outbreaks in farmed and wild fish populations based on WAHIS reports from 2007 to 2022. The number of outbreaks: Japan 162, Indonesia 31, Hong Kong 2, South Korea 5, Malaysia 21, Singapore 12, Thailand 16, China 28, Iraq 18,Iran 8, Taiwan 35, USA 47, Canada 10, South Africa 14, Denmark 20, France 6, Germany 2206, Croatia 4, Czech Republic 29, Belgium 15, Austria 4, England 232, Hungary 21, Ireland 3, Italy 8, Lithuania 1, Luxemburg 4, Netherlands 106, Norway 1, Poland 68, Romania 9, Serbia 7, Slovakia 4, Slovenia 13, Spain 1, Sweden 4.

Host range and Susceptibility

CyHV-3 principally causes intense mass mortality among common carp and Koi, which are the main species known to be impacted by this infection (Perelberg *et al*., 2003). Ghost carp (hybrids of common carp and koi) are also susceptible to the disease. Hybrids of common carp and koi with non-susceptible cyprinid species have shown mixed results in terms of resistance. Goldfish × koi hybrids show reduced mortality rates (35-42%) compared to crucian carp (*Carassius carassius*)×Koi hybrids (100%) (Bergmann *et al.,* 2010). Furthermore, Goldfish (*Carassius auratus*) common carp hybrids were shown some susceptibility to CyHV-3 infection, with limited mortality rates of ~5% under permissive conditions.

CyHV-3 typically affects carp; however, younger fish (1-3 months old) seems to be more susceptible to infection compared to mature fish (1 year old) (Michel *et al*., 2010). Carp have been shown to remain

unaffected while at the larval stage (Ito *et al.,* 2007). However, they do become susceptible once they mature past this stage to become fry. Ronsmas *et al*. (2014), utilizing CyHV-3 recombinant strain communicating luciferase (LUC) as a correspondent quality, shows that carp hatchlings are delicate and lenient to CyHV-3 contamination at all progressive phases. Nevertheless, the sensitivity of the early stages is reduced due to efficient inhibition of viral entry by the epidermal mucus.

Vector species and persistence in the natural environment

Cohabitation experiments suggest that some cyprinid and non-cyprinid fish species, previously co-habited with suspected latently infected carp, can carry CyHV-3 asymptomatically and spread it to naive carp. Furthermore, CyHV-3 DNA has been detected in some aquatic invertebrates and plankton, e.g. swan mussel (*Anodonta cygnea*), scud (*Gammarus pulex*) (Kielpinski *et al*., 2010) and *Rotifera* sp. (Minamoto *et al*., 2010). Recently, Panicz *et al.* (2020) confirmed the presence of CyHV-3 DNA in the tissues of five more aquatic invertebrates following episodes of KHVD mass mortality (Table 1a, b).

Table 1a: Aquatic organisms that act as asymptomatic reservoir for CyHV-3 (updated from Boutier *et al*., 2015).

Common name (species)	Detection of CyHV-3			Detection of CyHV-3 genome in carp after cohabitation
	DNA	Transcript	Antigen	
Goldfish (*Carassius auratus*)	Yes	Yes	Yes	Yes
Ide (*Leuciscus idus*)	Yes	nt	nt	nt
Grass carp (*Ctenopharyngodon idella*)	Yes	nt	nt	Yes
Silver carp (*Hypophthalmichthys molitrix)*	Yes	nt	nt	Yes
Prussian carp (*Carassius gibelio*)	Yes	nt	nt	Yes
Crucian carp (*Carassius carassius*)	Yes	nt	nt	Yes
Silver crucian carp (*Carassius auratus langsdorfii*)	Yes	No	nt	nt
Tench (*Tinca tinca*)	Yes	nt	nt	Yes
Vimba (*Vimba vimba*)	Yes	nt	nt	Yes
Common bream (*Abramis brama*)	Yes	nt	nt	Yes
Common roach (*Rutilus rutilus*)	Yes	nt	nt	Yes
Common dace (*Leuciscus leuciscus*)	Yes	nt	nt	No
Gudgeon (*Gobio gobio*)	Yes	nt	nt	Yes
Rudd (*Scardinius erythrophthalmus*)	Yes	nt	nt	Yes
European chub (*Squalius cephalus)*	Yes	nt	nt	nt
Common barbel (*Barbus barbus*)	Yes	nt	nt	nt
Belica (*Leucaspius delineatus*)	Yes	nt	nt	nt
Common nase (*Chondrostoma nasus*)	Yes	nt	nt	nt
Russian sturgeon (*Acipenser gueldenstaedtii*)	Yes	nt	nt	nt
Atlantic sturgeon (*Acipenser oxyrinchus*)	Yes	nt	nt	nt
Spined loach (*Cobitis taenia*)	Yes	nt	nt	nt
European bullhead (*Cottus gobio*)	Yes	nt	nt	nt
Pike (*Esox lucius*)	Yes	nt	nt	Yes
Brown bullhead (*Ameiurus nebulosus*)	Yes	nt	nt	No
Black bullhead (*Ameiurus melas*)	Yes	nt	nt	nt
Round goby (*Neogobius melanostomus*)	Yes	nt	nt	nt

Nt = Not tested

CyHV-3 remaining parts are irresistible in water for something like 4 h, yet not so much for 21 h, at water temperatures of 23-25°C (Perelberg *et al*., 2003). In addition,

CyHV-3 DNA can be present in approximately 100-fold higher concentration in sediment than in water, suggesting that the sediment can be a reservoir of the virus (Honjo *et al*., 2012). However, it is reported that in the absence of hosts, CyHV-3 can be rapidly inactivated in environmental water and sediment, possibly due to some anti-CyHV-3 bacterial activity (Yoshida *et al*., 2013).

Table 1b: Aquatic organisms that act as asymptomatic reservoir for CyHV-3.

Common name (species)	Detection of CyHV-3			Detection of CyHV-3 genome in carp after cohabitation
	DNA	Transcript	Antigen	
Three-spined stickleback (*Gasterosteus aculeatus*)	Yes	nt	nt	No
Ornamental catfish (*Ancistrus* sp.)	Yes	nt	nt	nt
European perch (*Perca fluviatilis*)	Yes	nt	nt	Yes
Ruffe (*Gymnocephalus cernua*)	Yes	nt	nt	Yes
Swan mussel (*Anodonta cygnea*)	Yes	nt	nt	nt
Scud (*Gammarus pulex*)	Yes	nt	nt	nt
Asiaticclam (*Corbicula fluminea*)	Yes	nt	nt	nt
Chinese pond mussel (*Sinanodonta woodiana*)	Yes	nt	nt	nt
Zebra mussel (*Dreissena polymorpha*)	Yes	nt	nt	nt
Mud bithynia (*Bithynia tentaculata*)	Yes	nt	nt	nt
Spiny-cheek crayfish (*Faxonius limosus*)	Yes	nt	nt	nt
Rotifer (*Rotifera* sp.)	Yes	nt	nt	nt

Nt = Not tested

Pathogenesis

Early explores have detailed that CyHV-3 may enter the host through contamination of the gills in view of the identification of viral particles and viral genome in this organ ahead of 215 schedules as 1-2 dpi (Pikarsky *et al*., 2004; Pokorova *et al*., 2005).

Later examinations involving *in vivo* bioluminescent imaging framework (IVIS) showed that the skin covering the fin and body is the major gateway of the passage of CyHV-3 and the site of early replication after immersion in virus-containing water (Costes *et al*., 2009). An extra method of passage for CyHV-3 is by ingestion of polluted materials and ensuing contamination of pharyngeal periodontal mucosa. Additionally, it is realized that CyHV-3 duplicates abundantly in the digestive system (Fournier *et al.*, 2012). After infection, CyHV-3 rapidly spreads within the body of infected fish through white blood cells. As soon as 1-2 dpi, CyHV-3 DNA was recuperated from practically all inside tissues, including liver, kidney, stomach, spleen, and cerebrum (Gilad *et al*., 2004), representing sources of viral shedding into the environment. Acute inflammation and severe dysfunction of osmoregulation in affected fish could be responsible for acute death (Negenborn *et al*., 2015).

Transmission

CyHV-3 is transmitted horizontally via direct physical contact with infected or carrier fish and also from the necrophagous behavior of carp. Indirect or vector-based modes of transmission include fish excrement, plankton, and aquatic invertebrates feeding by water filtration,

sediments, piscivorous birds, and finally the water as the major abiotic vector (Dishon *et al*., 2005; Honjo *et al.*, 2012; Torres-Meza *et al*., 2020). Mating may likewise build the predominance of CyHV-3 by conglomerating contaminated fish as well as causing a decrease in the safe reaction (Uchii *et al*., 2011). To date, there is no evidence of CyHV-3 vertical transmission.

Clinical signs

During a typical outbreak, morbidity among infected populations is usually ~100%. The first signs appear at 2-3 dpi (McDermott and Palmeiro, 2013). Infected fish exhibit general lethargy associated with anorexia and appetite loss. Other variable non-specific clinical signs can include gasping, aggregation near water inlet and other well-aerated areas, skin lesions and hyperemia, mucus hypersecretion, and bilateral enophthalmia (sunken eyes) (Rakus *et al*., 2013). Erratic swimming and loss of equilibrium may be seen at later stages (Hedrick *et al*., 2000). One of the principal external signs of clinical infection is swollen, pale, or patchy gills showing severe necrotic damage (Fig. 3).

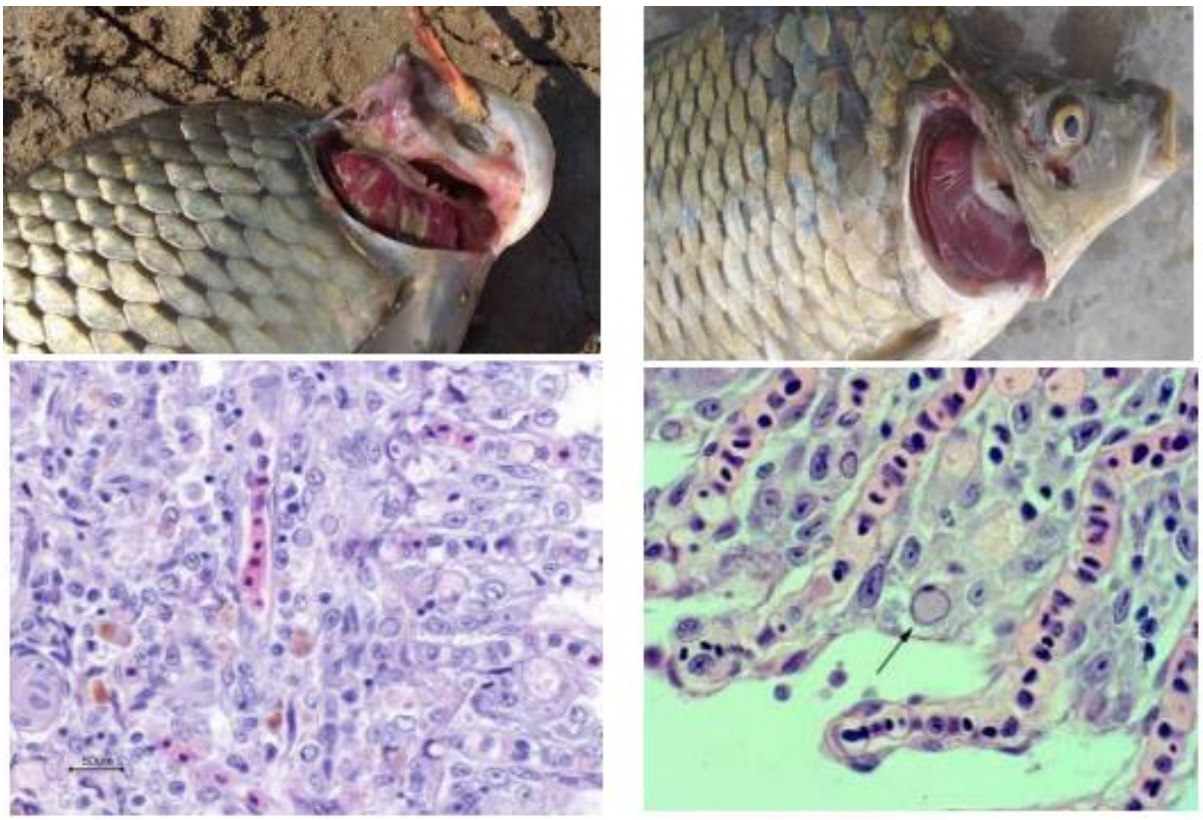

Figure 3: Severe gill necrosis in CyHV-3 infected common carp (up) Hyperplasia and fusion of secondary gill lamellae; intranuclear inclusion (arrow) in the branchial epithelium (gill section stained with hematoxylin and eosin) (bottom) (Hedrick *et al*., 2000).

Severe nephritis can be one of the most prominent internal pathological changes. Organs such as the liver, spleen, and gastrointestinal tract show necrosis, numerous microscopic lesions, and other forms of histopathological damage, thus

demonstrating the systemic nature of CyHV-3 245 infection (Miyazaki *et al.*, 2008). The course of disease is quite rapid and the first mortalities are frequently observed at 6-8 dpi, with a peak between 8 and 12 dpi. Mortality rates are usually up to 80- 247 100%.

CyHV-3-contaminated fish are more vulnerable to auxiliary diseases by bacterial, parasitic, or contagious microbes, which might add to the mortalities rate in the tainted populace (McDermott and Palmeiro, 2013). Recent studies reported CyHV-3 and carp edema virus (CEV) co-infection in association with mass mortalities of common carp and koi (Padhi *et al*., 2019; Kim *et al*., 2020).

Detection

Several direct and indirect methods of detection have been developed for CyHV-3. Introduced in 2000, virus isolation was the first diagnostic method and involves the culture of tissue extracts (especially gills, kidneys, and spleen) in susceptible cell lines such as KF-1, CCB, and KCF-1 and observation of cytopathic effects caused by virus replication (Michel *et al.*, 2010). This is a time-consuming method with low sensibility, and additionally, the virus is replicated in a limited number of established cell lines. Anti-CyHV-3 antibodies produced by hosts in response to the CyHV-3 challenge can be detected using an enzyme-linked immunosorbent assay (ELISA) (Adkison *et al*., 2005). This is a useful approach for the identification of fish that have survived CyHV-3 exposure and latent carriers, however, due to insufficient knowledge of the immune response in carp, it is difficult to definitively evaluate the potential usefulness of targeting anti-CyHV-3 antibodies as part of routine screening programs for CyHV-3 infection. ELISA methods to detect CyHV-3 antigens have also been developed (Dishon *et al*., 2005). These assays may be suitable for detecting CyHV-3 during clinical infections, but may not be sensitive enough to enable screening for carriers in healthy populations or latent infections (Way, 2019). A complete set of molecular techniques for detecting viral genes, with special emphasis on nucleic acid amplification-based methods has been developed. Including PCR-based methods, Taqman PCR based on the TK gene is reported to be the most sensitive. (Gilad *et al*., 2004) and isothermal amplification methods, i.e. loop-mediated isothermal amplification (LAMP) (Gunimaladevi *et al*., 2004) and recombinant polymerase amplification (RPA) (Prescott *et al*., 2016). The recommended oligonucleotide primer list is presented in Table 2.

Table 2: The list of recommended oligonucleotide primer for KHV.

Primer	Primer Sequence (5′-3′)	Primer Length (bp)	Source
KHV-TK-F	GGG TTA CCT GTA CGA G	16	Bercovier *et al.* (2005)
KHV-TK-R	CAC CCA GTA GAT TAT GC	17	
KHV9/5F	GAC GAC GCC GGA GAC CTT GTG	21	Gilad *et al.* (2002)
KHV9/5R	CAC AAG TTC AGT CTG TTC CTC AAC	24	
CEV-For-B	ATG GAG TAT CCA AAG TAC TTA G	22	Matras *et al.* (2017)
CEV-Rev-J	CTC TTC ACT ATT GTG ACT TTG	21	
KHV-F	GAC ACC ACA TCT GCA AGG AG	20	Way (2019)
KHV-R	GAC ACA TGT TAC AAT GGT CGC	21	

Prevention and Control

No treatment for KHVD is available. Quarantine of the new fish at a permissive temperature (23-28°C) for at least 4 weeks is reported to be an effective preventive way. If all fish appear healthy, blood samples of all fish should be tested for KHV antibodies using ELISA. Some researchers have shown that temperature manipulation up to 30°C was effective in reducing mortality in KHV-infected fish. Common disinfection protocols may be used to eliminate the virus from water systems and equipment. Recently, work has commenced on a DNA vaccine using a CyHV-3 glycoprotein to stimulate an immune response. Moreover, an RNA-mediated interference (RNAi)-based therapeutics was developed for the development of novel therapies against KHVD (Gotesman *et al.*, 2014). CRISPR/Cas9 gene editing method to repress CyHV-3 replication is confirmed to be effective (Zhao *et al*., 2015).

KHVD status in Iran and neighbouring countries

As mentioned earlier, CyHV-3 from Iranian carp farms has not been isolated and confirmed by the Iran Veterinary Organization until 2021, despite earlier evidence of its occurrence reported by some researchers. The first case was reported by Rahmati-Holasoo *et al.* (2016) who detected CyHV-3 from 2014 and 2015 Koi mass mortalities in ornamental fish supplying and propagation centers in Tehran, Iran using clinical signs, histopathological and molecular (Nested PCR) studies. Also based on complete nucleotide sequences of the TK gene, they concluded that CyHV-3 from Iran is closely linked to the A1 genotype of the Asian strain (CyHV-3 J). Koi importation from Asian countries without quarantine legislation was determined as the cause of native fish exposure to the virus and subsequent mortalities. In addition, it was presumed that CyHV-3 was introduced into Iranian koi farms earlier, but due to shortcomings in diagnostic methods, it has not been detected.

In 2015, a KHVD outbreak was reported from a common carp farm in Tehran province with a 60% mortality rate. Another incident was documented in 2016 from a carp farm in neighbouring Alborz Province with 80% cumulative mortality (Ahmadivand *et al*., 2020). Clinical examination, histopathology, and PCR

methods were used to confirm CyHV-3 as the aetiological agent. By gene sequencing examination of the TK quality, Marker I, and Marker II, it was uncovered that secludes had personality with CyHV-3 J strain. Since likenesses were seen between the distinguished secludes and those identified from koi in a similar district of Iran, it was close that CyHV-3 presumably communicated from Koi culture offices to cyprinid homesteads.

Iraq is a major export market of Iranian carp and Arvand River (Shat-al-Arab) acts as a Transboundary River basin between two countries. In Toffan *et al*. (2020) investigation, mass fish mortality occurred in Iraq, involving thousands of tons of cultured and wild common carp along the Euphrates and Tigris rivers. Virological investigation through real-time PCR and nested PCR were positive for koi herpesvirus (KHV) and carp edema virus. Results obtained were confirmed by the OIE reference laboratory of KHV disease (KHVD) at Cefas (UK) and by sequence analysis. The disease also was reported by Ababneh *et al*. (2020) and Al-Salih *et al.* (2020). In Ababneh *et al.* (2020) study, a semi-nested PCR assay coupled with sequencing confirmed the presence of koi herpesvirus disease in Iraq as a cause of mass mortality among the common carp of the Tigris River.

Seasonal mortalities have been reported annually from carp farms in other regions of Iran, notably Mazandaran and Gilan provinces with symptoms similar to KHVD, collectively termed carp summer mortality syndrome. Suspected carp fish deaths due to KHV in Khuzestan province occurred mostly in autumn. In 2018, a molecular (nested PCR) and pathological study on 140 samples from 14 carp farms (in Khuzestan, Mazandaran, and Gilan provinces) of these regions confirmed that some of these farms have been affected by KHVD (Taheri Mirghaed *et al.*, 2019).

In 2021, five KHV outbreaks were officially reported to OIE from carp farms of Mazandaran and Gilan provinces (OIE, 2022). Subsequently, KHV infection was detected by histopathological examination and phylogeny analysis from two koi and carp farms in Isfahan and Khouzestan provinces in the spring of 2022 (Shahvazi *et al.*, 2022). Results from sequence analysis of the TK gene obtained from moribund specimens showed a 97% identity to CyHV-3-J strain (Fig. 4).

Figure 4: KHVD outbreaks in Iran based on research papers and official reports to OIE (Taheri Mirghaed *et al.*, 2019).

Conclusion

KHVD is an emerging and fast-spreading disease, threatening the cyprinid culture industry. There is no current treatment strategy to save infected fish. Also, no commercial vaccine is yet available for worldwide use due to legislative restrictions concerning associated risks of reactivation or reversion to virulence. In Iran, the probability of CyHV-3 entering and establishing in koi and common carp would be high and the existence of this virus has already been reported in Iranian aquaculture. Moreover, the unregulated importation of ornamental fish and live aquatic animals, especially goldfish, increases the possibility of the virus spreading further to carp farms. Considering the above-mentioned risk factors, severe consequences of CyHV-3 establishment in Iranian aquaculture would warrant the study and implementation of specific risk management measures.

To prevent the spread of CyHV-3, it is crucial to implement strict biosecurity measures in fish farms and hatcheries. This includes disinfecting equipment, limiting the movement of fish, and screening for the virus before importing or exporting fish. Predictive models can also be developed to identify areas at high risk of infection and prioritize surveillance efforts. Eradicating the virus can be challenging as infected fish can shed the virus for an extended period, and there is no known cure. However, vaccination has shown promise in reducing mortality rates and limiting the spread of the disease. Import restrictions on live fish

from countries with known CyHV-3 outbreaks can also help prevent the introduction of the virus into new areas. Overall, a coordinated effort between government agencies, researchers, and industry stakeholders is necessary to effectively manage and control the spread of CyHV-3. Additionally, public awareness campaigns can educate fish farmers and the general public about the importance of biosecurity measures to prevent the spread of this virus and other diseases in aquatic animals.

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