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Invited Review Article

Current understanding of Cyprinid herpesvirus 2, and prospects in its management

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Abstract

Diseases are the major bottleneck for the successful development of the aquaculture industry. *Carassius auratus* (goldfish) is one of the major cultured and traded ornamental fish worldwide. Goldfish are frequently encountered with different disease conditions. Cyprinid herpes virus 2 (CyHV-2) is one of the most dreadful diseases of goldfish and its related species. CyHV-2 belongs to the genus *Cyprinivirus* under the family *Alloherpesviridae*. The virus consists of an icosahedral capsid of diameter 110-120 nm surrounded by an outer lipid envelope and gives a spherical appearance of 170-200 nm in diameter. The latency of the virus for a long period poses a greater threat of spreading. The immune reaction of the host against the virus is well studied and all are documented in the review. To date, no effective treatment or management strategy is available against the disease. Several trials have been made to develop effective vaccines against the virus. This review compiled and analyzed all the information available and how to move forward for effective management of the virus.

Keywords: *Carassius auratus*, Cyprinid herpesvirus 2, Host-pathogen interaction, Vaccines

Highlights

- *Carassius auratus* (goldfish) is one of the major cultured and traded ornamental fish worldwide.
- Cyprinid herpesvirus 2 (CyHV-2) is a major viral pathogen of goldfish and other cyprinids.
- Mortality with overt clinical signs can reach up to 100% during heavy infection.
- Several promising management tools *viz.* vaccines, antioxidants and other natural compounds showed encouraging results in laboratory conditions.

INTRODUCTION

The culture of ornamental fish not only provides financial advantages to farmers but also adds aesthetic value to the places they are kept in. The goldfish, *Carassius auratus*, is one of the most commonly cultured ornamental fish worldwide. Since goldfish can be co-cultured in ponds with Indian major carps (IMCs), it has gained immense popularity in India, especially in rural areas. In some parts of India, monoculture of goldfish is also a lucrative venture. Goldfish are reported to be affected by several pathogenic organisms. Herpes viral hematopoietic necrosis (HVHN) is a lethal disease of goldfish leading to 100% mortality. HVHN is caused by Cyprinid herpesvirus 2 (CyHV-2), which is a major viral pathogen of goldfish and other cyprinids (Thangaraj *et al.,* 2021). The first outbreak of CyHV-2 was reported in Japan during the spring of 1992 and 1993 (Jung and Miyazaki, 1995). Since then, it has been reported in the USA, Taiwan, Australia, UK, Hungary, Czech Republic, Italy, China, Netherlands, India, Switzerland, France, Germany, Turkey and Poland (Thangaraj *et al.*, 2021) and most recently in Korea (Jung *et al.*, 2022). Infection with CyHV-2 results in anorexia, lethargy, discolouration of skin and gills, gasping at the surface with erratic swimming, whitish bleached gills, exophthalmia, swollen vent, haemorrhages on the body, particularly at the posterior part, lepidorthosis, haemorrhages at the fin bases and mortality of all sizes of fish. Loss of pigmentation leading to a pale appearance is also prominent during infection. Further, necropsy examination reveals necrosis of gills, swollen kidney and spleen, whitish necrotic nodular foci in the spleen

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and haemorrhagic fluid in the abdominal cavity (Sahoo *et al.*, 2016). CyHV-2-infected crucian carp exhibits haemorrhages at different points of the body and fins, swollen anus and the presence of haemorrhages in gills and eyes since the virus attacks the haematopoetic tissues (Fichi *et al.*, 2013). Histopathological changes include necrosis and sloughing of epithelia in gill lamellae, necrosis of kidney tissue, lesions in the spleen involving necrosis of the lymphoid tissue and hypertrophied nuclei with marginated chromatin material (Sahoo *et al.*, 2016). Besides these, a significant reduction in total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin concentration and thrombocyte count has also been observed in CyHV-2-infected goldfish. Further, swollen erythrocytes and lysis of erythrocytes leading to pale red colouration of serum have also been observed (Lu *et al.*, 2016). Nuclei of necrotic monocytes and neutrophils displayed karyopyknosis and karyorrhexis, leading to the degeneration and disorientation in shape and structure of lymphocytes in Prussian carp (*Carassius gibelio*) after the CyHV-2 infection (Lu *et al.*, 2016).

Several cell lines *viz*. koi fin (KF-1), bluegill fibroblast 2 (BF-2), goldfish (GF-1), common carp brain (CCB), standard Ryukin Takafumi (SRTF), Ryukin fin (RKF), *Cyprinus carpio* koi fin (CCKF), goldfish fin (GFF) and gibel carp brain (GiCB) were used to study CyHV-2 pathogenesis *in vitro* (Xu *et al*., 2019; Thangaraj *et al.*, 2021). Lu *et al.* (2018) demonstrated that CyHV-2 induces apoptosis in the GiCF cell line (*C. auratus gibelio* caudal fin cell line). Chai *et al.* (2020) used a cell line of the gibel carp brain (GCBLat1) to study the latency of CyHV-2. Recently, three novel cell lines, Fantail goldfish gill (FtGG), Fantail goldfish liver (FtGL) and Fantail goldfish brain (FtGB), have been established and characterized from respective tissues of *C. auratus,* which are the target organs of CyHV-2 and have been proposed to be used for consistent propagation of CyHV-2 (Swaminathan *et al.*, 2021). The common cytopathic effects that are observed in cell lines include pyknosis, vacuolization, and rounding of cells leading to the destruction of monolayer (Sahoo *et al.*, 2016). Besides these, Thangaraj *et al.* (2021) described granulation, syncytium formation in focal areas, appearance of rounded bright cells and lysis of Fantail Goldfish Fin (FtGF), Fantail Goldfish Gill (FtGG), Fantail Goldfish liver (FtGL) and Fantail Goldfish brain (FtGB) cellines after infection with CyHV-2.

The occurrence of secondary bacterial infection during viral infection is very common. *Aeromonas hydrophila* is an opportunistic pathogen that has been frequently reported during CyHV-2 infection (Sahoo *et al*., 2016; Ren *et al*., 2021). Ren *et al*. (2021) also reported that the occurrence of mortality due to a single infection with *A. hydrophila* or CyHV-2 is below 40%, whereas co-infection of *A. hydrophila* with CyHV-2 infection can lead to mortality up to 100%. Since infection with CyHV-2 is a worldwide threat to the goldfish culture, several managemental methods such as health certification, quarantine of imported stocks and strict biosecurity measures is adopted widely. Till date there is no proven control measure available (Thangaraj *et al*., 2021). Vaccination seems to be the most effective way of controlling diseases including CyHV-2. In this review, we compiled the information regarding host-pathogen interaction and the vaccine trials conducted to date against this important virus.

Host range of CyHV-2

CyHV-2 can infect goldfish of all stages, among which juveniles are the most susceptible ones. Different cyprinids such as crucian carp *C. carassius*, Prussian carp *C. gibelio* and allo gynogenetic crucian carp are susceptible to CyHV-2 (Thangaraj *et al.*, 2021). Zhu *et al*. (2019) reported that CyHV-2 can also infect bighead carp *Aristichthys nobilis*, *Culter alburnus*, *Erythroculte rilishaeformis*, silver carp *Hypophthalmichthys molitrix* and black carp *Mylopharyngodon piceus* (Thangaraj *et al.*, 2021). One of the key areas of CyHV-2 study is understanding the host range. Earlier, CyHV-2 was thought to be limited to goldfish, but gradually, it has been found to infect many cyprinids, as mentioned already. So, we must focus on the ability of the virus to cross-infect other species (Thangaraj *et al*., 2021), how it adapts itself inside the host and its genome compatibility with other species. Additionally, there is no data available regarding the evolution of host preference of the virus which can be further investigated.

Virus structure and genome

Cyprinid herpesvirus 2 or the goldfish hematopoietic necrosis virus is a DNA virus that belongs to the genus *Cyprinivirus* under the family *Alloherpesviridae*. Electron microscopy of the CyHV-2-affected goldfish spleen and kidney tissues revealed that the virus consists of an icosahedral capsid of diameter 110-120 nm surrounded by an outer lipid envelope and gives a spherical appearance of 170-200 nm in diameter (Xu *et al*., 2014). With the advancement in bioinformatic tools, the double-stranded DNA genome of the virus has been analyzed and found to be comprised of 2,90,304 bp including 154 protein-coding Open Reading Frames (ORFs) (Davison *et al*., 2013). Depending upon the time of gene expression, the genes of the virus are divided into immediate-early (IE), early (E) and late (L) genes. Viral IE genes initiate transcription and simultaneously enable the efficient expression of E and L genes and

modulate host cell functions (Gruffat *et al*., 2016). Tang *et al*. (2020) identified 5 IE genes, 34 E genes and 39 L genes from the CyHV-2 genome. Out of these genes, the transcription of IE genes such as ORF54, ORF121, ORF141, ORF147 and ORF155 start soon after infection. Subsequently, the transcription of E and L genes occurs at 2 hours and 6 hours post-infection, respectively. Further studies showed that the virus possesses 74 viral proteins including 3 capsid proteins, 18 membrane proteins, 1 tegument protein, 6 types of proteinases and 46 other unclassified proteins (Gao *et al*., 2020). ORF104 of CyHV-2 belongs to the PKc superfamily and encodes a kinase-like protein that phosphorylates p38, leading to MAPK activation in the gill and muscles of gibel carp (Du *et al*., 2015). This enhances the infection in those particular tissues. As demonstrated by Du *et al*. (2015), the introduction of the p38 inhibitor SB203580 significantly reduces the mortality of fish. It indicates that the ORF104 might be playing a major role in CyHV-2 infection.

Fate of host immune genes during CyHV-2 infection

The innate immune response is a cell-intrinsic defense system that is promptly activated in most cell types when they are infected. It works by preventing pathogen multiplication while also alerting other cells about the infection. Modulation of various cellular pathways, including the generation of antiviral and inflammatory cytokines, activation of genes involved in pathogen restriction, and induction of cell death, are all part of this mechanism (Borden *et al*., 2007; Iwasaki, 2012a; 2012b). It has been demonstrated that the initial load of virus entering the host plays an important role in host–viral interactions, determining the fate of CyHV-2 infection (Xu *et al*., 2014). Viral infections start with interactions between the virus and its host. Fish's innate immunity is key in the initial defense against viral infections, and early recognition of the infecting virus is necessary to trigger an antiviral response. Understanding virus-host interactions can lead to a better understanding of disease development and the development of antiviral strategies (Table 1 and Fig. 1).

Myeloperoxidase (MPO) is involved in pro-

Table 1. Details of immune gene regulation during CyHV 2 infection

	Immune Gene	Tissue/Cell	Regulation	Host	References
CD8α	Cluster of differentiation 8	Fantail goldfish fin cell lines	↑	*C. auratus*	Das *et al*., 2021
GATA3		Fantail goldfish fin cell lines	↑		
IFNγ	Interferon gamma	Fantail goldfish fin cell lines	↑		
b2m	Beta 2 microglobulin	Fantail goldfish fin cell lines	↑		
MHC I	Major histocompatibility complex class I	Fantail goldfish fin cell lines	↑		
LMP 7	Low molecular mass polypeptide 7	Fantail goldfish fin cell lines	↑		
IL-10	Interleukin 10	Fantail goldfish fin cell lines	↑		
IL-12	Interleukin 12	Fantail goldfish fin cell lines	↑		
ULK2	Unc-51-like kinase 2	Spleen	↑	*C. auratus gibelio*	Zhang *et al*., 2020
RPS6KB	Ribosomal protein S6 kinase beta-1	Spleen	↑		
HIF1A	Hypoxia-inducible factor 1-alpha	Spleen	↑		
MYC	Myc proto-oncogene protein	Spleen	↑		
IL11	Interleukin 11	Spleen	↑		
CNTF	Ciliary neurotrophic factor	Spleen	↑		
TPOR	Thrombopoietin receptor	Spleen	↑		
IL6	Interleukin 6	Spleen	↑		
JUNB	Transcription factor jun-B	Spleen	↑		
JUN	Transcription factor AP-1	Spleen	↑		

Cont. Table 1.

Table 1., Cont. ...

	Immune Gene	Tissue/Cell	Regulation	Host	References
VCAM1	Vascular cell adhesion molecule 1	Spleen	↑	*C. auratus gibelio*	Zhang *et al.*, 2020
CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	Spleen	↑		
SOCS3	Suppressor of cytokine signaling 3	Spleen	↑		
TNFR2	Tumor necrosis factor receptor	Spleen	↑		
CFLAR	CASP8 and FADD-like apoptosis regulator	Spleen	↑		
EDN1	Endothelin 1	Spleen	↑		
GADD45	Growth arrest and DNA-damage inducible protein	Spleen	↑		
CDKN1A	Cyclin-dependent kinase inhibitor 1A	Spleen	↑		
PAI1	Plasminogen activator inhibitor 1	Spleen	↑		
MPO	Myeloid-specific peroxidase	Liver, spleen, gill and muscle	↑	*C. auratus gibelio*	Podok *et al.*, 2014
KRT-8	Keratin 8	Heart and kidney	↑		
DUSP-1	Dual specificity phosphatase 1	Liver, spleen, gill and muscle	↑		
ccBAX gene	BAX gene	Caudal fin cells	↑	*C. auratus gibelio*	Yu *et al.*, 2021
gabT	pyridoxal phosphate-dependent enzyme	Kidney	↑	*C. auratus gibelio*	Qiao *et al.*, 2020
sdhA	Succinate dehydrogenase (SDH)	Kidney	↑		
hsp70	Heat shock protein (Hsp)70	Kidney	↑		
RAF1		Kidney	↑		
JAK	Janus kinases	Kidney	↑		
PIK3R1	PIK3R1 phosphoinositide-3-kinase regulatory subunit 1	Kidney	↑		
MTOR	Mammalian target of rapamycin	Kidney	↑		
ACTB	actin beta	Gill, liver, kidney, spleen, muscle, heart and intestine	↑	*C. auratus*	Dharmara-tnam *et al.*, 2021
$EF1A_A$	Elongation factor 1 alpha	Gill, liver, kidney, spleen, muscle, heart and intestine	↑		
GADPH	glyceraldehyde-3P-dehydrogenase	Gill, liver, kidney, spleen, muscle, heart and intestine	↑		
18S	18S ribosomalRNA	Gill, liver, kidney, spleen, muscle, heart and intestine	↑		

Cont. Table 1.

Table 1., Cont. ...

	Immune Gene	Tissue/Cell	Regulation	Host	References
CagC3	*Carassius auratus gibelio* complement C3 gene	Brain cell line	↑/↓	*C. auratus gibelio*	Fan *et al*., 2020
SAA	Serum amyloid A protein	Kidney	↓		
β-actin		Kidney	↓		
LYZ C	Lysozyme C	Kidney	↑		
Mtap	Methylthioadenosine phosphorylase	Kidney	↑		
IL-10R	Interleukin-10 receptor	Kidney	↑		
nfkbia	NF-kappa light polypeptide gene enhancer in B cells inhibitor, alpha	Kidney	↑		
ccC7	Complement component C7	Kidney	↑		
PNP5a	Purine nucleoside phosphorylase 5a	Kidney	↑		
Intelectin	Intelectin	Kidney	↑		
gtanulin	Granulin	Kidney	↓		
Mta3	Metastasis associated family, member 3	Kidney	↓		
ANP32A	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	Kidney	↓	*C. auratus gibelio*	Xu *et al*., 2014
eno1a	Enolase 1a	Kidney	↑		
pyroxd2	Pyridine nucleotide-disulphide oxidoreductase domain 2	Kidney	↑		
tuba8l2	Tubulin, alpha 8 like 2	Kidney	↑		
SET B	SET (myeloid leukemia -associated) B	Kidney	↑		
CCT	Chaperonin containing T-complex polypeptide	Kidney	↑		
CXCR4	CXCR4	Kidney	↑		
NP	Nephrosin precursor	Kidney	↑		
IAP	Inhibitor of apoptosis protein	Kidney	↑		
mibp	Muscle-specific beta 1 integrin binding protein	Kidney	↑		
CALR	Calreticulin	Kidney	↑		
Ptpn6	Protein tyrosine phosphatase, non-receptor type 6	Kidney	↑		
pmt	Phosphoethanolamine methyltransferase	Kidney	↑		
lgmn	Legumain	Kidney	↑		
Cts L	Cathepsin L preproprotein	Kidney	↑		
capg	Capping protein (actin filament), gelsolin-like	Kidney	↑		

Cont. Table 1.

Table 1., Cont. ...

	Immune Gene	Tissue/Cell	Regulation	Host	References
TUBA2	Tubulin, alpha 2	Kidney	↑		
Cts C	Cathepsin C	Kidney	↑		
rac2	Ras-related C3 botulinum toxin substrate 2	Kidney	↑		
tubulin	Tubulin	Kidney	↑		
Cts B	Cathepsin B preproprotein	Kidney	↑		
eml2	Echinoderm microtubule associated protein like 2	Kidney	↑		
cxcl12	Chemokine ligand 12	Kidney	↑		
Rab-21	Ras-related protein Rab-21	Kidney	↑		
Tmsβ-11	Thymosin beta-11	Kidney	↑		
nfkbiaa	NF-kappaB inhibitor alpha-like protein A mRNA	Kidney	↑		

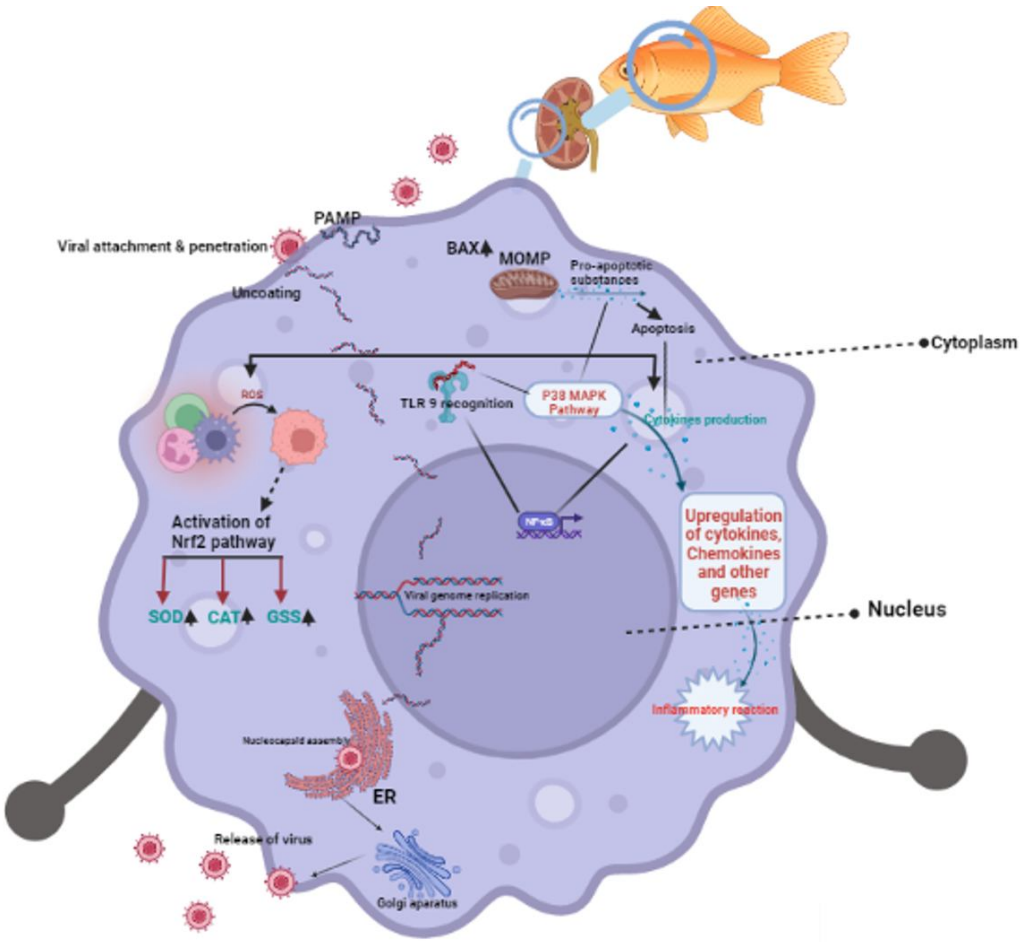

Fig. 1. Diagrammatic illustration of the hypothetical model of innate and adaptive immune reaction and network of interactions among different cellular and humoral molecules related to the response CyHV- 2 infection in goldfish. (h) **upregulation,** (i) **no significant difference. Image prepared using BioRender software.**

inflammatory reactions as well as several inflammatory disorders (Lau *et al*., 2005). MPO was shown to be highly elevated in crucian carp that survived CyHV-2 infection. Furthermore, KRT-8 and 18 are critical in protecting hepatocytes against mechanical and toxic stress; the expression level of KRT-8 was significantly downregulated in susceptible infection of CyHV-2. It was observed that the DUS-1 gene was upregulated during early CyHV-2 infection and further downregulated to normal (Podok *et al*., 2014). The DUS-1 gene has been reported as an activator of the production of cytokines and interleukins in humans. In contrast, it was found in the case of CyHV-2 infection, that although the DUS-1 gene gets downregulated, the production of IL-10 gets upregulated. Therefore, the cellular gene expressions could mitigate virus-induced cellular stress by inactivation of invading viral particles, maintaining cell shape and avoiding apoptosis, and signal pathway regulation by MPO, KRT-8 and DUSP-1 genes, respectively (Podok *et al*., 2014). Further, with enhanced expression of important genes such as CD8α, IFNγ, b2m, MHC I, LMP 7, IL-10, IL-12 and GATA3 except for tapasin, goldfish employs very complex T helper cell-mediated immunity against CyHV-2, with Th1 and Th2 responses equivalent to humans (Das *et al*., 2021).

A total of 1286 differentially expressed genes (DEGs) were obtained from the CyHV-2 infected gibel carp spleen tissue, with 1006 upregulated and 280 downregulated. After GO database annotation, the DEGs were primarily divided into molecular function and biological process to better understand their functions. Cellular process regulation, biological process regulation, G-protein coupled receptor signalling and biological regulation were the most common biological processes. Among the molecular functions, transcription factor activity, sequence-specific DNA binding, nucleic acid binding transcription factor activity, signal transducer activity, and sequence-specific DNA binding were the most abundant (Zhang *et al*., 2020). Forty-two different immune genes were studied from CyHV-2 infected goldfish kidney tissue samples, which showed that LYZ C, Mtap, IL-10R, nfkbiab, ccC7, PNP5a, intelectin, eno1a, pyroxd2, tuba8l2, SET B, CCT, CXCR4, NP, IAP, mibp, CALR, Ptpn6, pmt, lgmn, Cts L, capg, TUBA2, Cts C, rac2, tubulin, Cts B, eml2, cxcl12, Rab-21, Tms β-11 and nfkbiaa are the major immune genes having positive response against CyHV-2 infection while, SAA, β-actin, gtanulin, Mta3 and ANP32A have a negative response to the infection (Xu *et al*., 2014). The expression of the ccBAX protein was found to be upregulated in GiCF cells infected with CyHV-2 and has been suggested to play a regulatory role in GiCF cell apoptosis during viral infection, providing insight into CyHV-2 induced apoptosis (Yu *et al*., 2021). CyHV-2 has a major pathological influence on *C. auratus* in different organs like the gill, kidney, liver, spleen, brain, muscle, heart and intestine. The kidney and spleen among those seem to be hotspots for immune gene expression against the viral infection, as the majority of immune genes expressed against CyHV-2 infection are noticed either from the kidney or spleen tissue samples (Xu *et al*., 2014; Qiao *et al*., 2020; Zhang *et al*., 2020). This indicates that kidney and spleen tissues can be ideal sources for the detection of CyHV-2 infection. Many immune-related genes, such as intelectin, interleukin-11 (IL-11), and purine nucleoside phosphorylase, were overexpressed in crucian carp (*C. carassius*) in response to CyHV-2 infection. PNP5a, keratin8, dual specificity phosphatase 1 (dusp1), MHC-I, MPO, IRF3 and MAPK7 are all involved in the process (Goodwin *et al*., 2006; Xu *et al*., 2014; Xu *et al*., 2016). Interestingly, polyhydroxybutyrate (PHB) supplementation, a key component of Biofloc, dramatically increased transcriptional levels of eight immune-related genes, lowered cumulative mortality of gibel carp and promoted early CyHV-2 replication in the spleen *in vivo* (Qiao *et al*., 2020). Studies on host-pathogen interactions pave the way for disease treatment strategies. Hence, understanding the immune response to CyHV-2 will offer a foundation for the development of future viral vaccines and proper viral diagnostic procedures in goldfish.

Latency and reactivation

Latency is a remarkable feature of the Herpesviridae. The virus enters the host, mediates infection, and when the host begins to recover, the virus becomes dormant without losing its virulence. With the return of stressful conditions for the host, it reverts to its original virulent form, which is termed 'reactivation'. Different viruses under the family Herpesviridae such as Human herpes simplex virus (HSV), Epstein–Barr virus (EBV), Channel Catfish Virus (CCV or Ictalurid Herpesvirus 1), Cyprinid Herpesvirus 3 (CyHV 3) or Koi Herpesvirus (KHV) have been found to exhibit latency and subsequent reactivation (Wei *et al*., 2019). Also, the organs where the virus persists during latency vary from sensory neurons of the peripheral nervous system for HSV to memory B cells, monocytes, or lymphocytes for the rest (Chai *et al*., 2020). Wei *et al*. (2019) studied the latency of CyHV-2 in goldfish reared at a virus-permissive temperature (28°C) and found that it can remain inside the host fish spleen, trunk kidney and gills for up to 81 days. Stress, such as water temperature change, maturation,

transportation, injury and secondary infection, may induce virus reactivation in fish. Chai *et al*. (2020) reported the reactivation of CyHV-2 using heat stress and different chemicals such as trichostatin A (TSA) or phorbol 12-myristate 13-acetate (TPA). Besides, they demonstrated the latency and reactivation of CyHV-2 in the primary brain cell culture named the GCBLat1 cell line. Wei *et al*. (2020) also described the persistence of CyHV-2 in leucocytes of asymptomatic survivors and their reactivation using immunosuppressants like anti-inflammatory glucocorticoid, dexamethasone (Dex) and cyclosporine A (CsA), a lymphocyte immunosuppressant. All these studies indicate the unique adaptability of the virus to its host's internal environment.

Effect of temperature on the disease

Herpesviral hematopoietic necrosis (HVHN) disease usually occurs during spring and autumn. This indicates that CyHV-2 is virulent when water temperature ranges from 15 to 25°C as this temperature is suitable for viral replication. However, the virus does not lose its virulence at low temperatures. As described by Ito and Maeno (2014), the goldfish that survived CyHV-2 infection at 13-15°C water temperature functioned as a carrier of CyHV-2 and can transmit the disease with the return of favourable temperature. According to Liang *et al*. (2015), the mortality of Prussian carp due to CyHV-2 was recorded to be 6.67% at 10°C, and it gradually increased with the increase in water temperature. Mortality was the highest (100%) at 25°C, which gradually decreased to 16.7% at 30°C and finally nil at 32°C. Zhang *et al*. (2014) described that many immune genes and antioxidant enzymes of goldfish are temperature sensitive. This is crucial in establishing antiviral immunity against the virus. Ouyang *et al*. (2020) reported the occurrence of CyHV-2 outbreak at 10°C, which denotes that the virus has been able to overcome the temperature barrier. Thus, temperature affects both viral replication and the immune response of the fish towards the virus. Among other environmental parameters, Liang *et al*. (2015) found that pathogenicity is independent of salinity. They also found that the mortality is high at a pH of 5 to 10.

Vaccine development

Vaccines are the most effective tools for the prevention and control of viral diseases. In aquaculture, the ERM vaccine for salmonid fish (licensed in 1976 in the USA) was the first vaccine to be developed. With advances in the field of immunology, the number of commercial vaccines available for fish has expanded from 2 in the 1980s to 26 in 2019. At the advent of genetic engineering along with traditional live attenuated and inactivated vaccines, several new generation subunit vaccines were experimented against CyHV-2 (Table 2). The use of these vaccines and their effectiveness are described below.

Live attenuated vaccine: A live attenuated vaccine

Table 2. Details of vaccine developed against CyHV-2

Name of vaccine	Type of vaccine	Route of adm-inistration	species	Relative percentage survival	References
P7-P8	Live attenuated	Immersion	*C. auratus gibelio*	> 80	Saito *et al*., 2022
Heat inactivated	Inactivated vaccine	Intraperitoneal injection	*C. auratus gibelio*	66.7	Dharmaratnam *et al*., 2023
Formalin treated virus	Inactivated vaccine	Intraperitoneal injection	*C. auratus gibelio*	57	Ito and Ototake, 2013
β-propiolactone inactivated CyHV 2	Inactivated vaccine	intraperitoneal injection	*C. auratus gibelio*	71.4	Zhang *et al*., 2016
β-propiolactone inactivated CyHV 2 + β-glucan/β-propiolactone inactivated CyHV 2 + astragalus polysaccharide (APS)	Inactivated vaccine	Intraperitoneal injection	*C. auratus gibelio*	60 (β-glucan adjuvant)66 (APS adjuvant)	Huo *et al*., 2023
pcORF25/pcCCL 35.2	DNA vaccine	Intraperitoneal injection	*C. auratus gibelio*	50	Huo *et al*., 2020

Cont. Table 2.

Table 2., Cont. ...

Name of vaccine	Type of vaccine	Route of adm-inistration	species	Relative percentage survival	References
pEGFP-N1 -ORF25	DNA vaccine	Intramuscular injection	*C. auratus gibelio*	70	Yuan *et al*., 2020
BacCarassius-D4ORF	Recombinant vector vaccine	Oral and intraperitoneal injection	*C. auratus gibelio*	59.3 and 80.01	Li *et al*., 2019
EBY100/pYD1 -ORF132	Recombinant vector vaccine	Oral	*C. auratus gibelio*	64	Wang *et al*., 2023
pYD1-ORF25	Recombinant vector vaccine	Oral	*C. auratus gibelio*	66.7	Dong *et al*., 2022

contains a live pathogen with reduced virulence. It can induce both cell-mediated and humoral immunity. Besides, it can be administrated by immersion method that requires less labour and causes less stress to fish. To control CyHV-2, Saito *et al*. (2022) came up with a P7-P8 vaccine, which is a virulent CyHV-2 Saitama-1 isolate (CyHV-2 SaT-1) propagated 7 to 8 times in RyuF-2 cells followed by KF-1 cells. Its administration resulted in decreased DNA copy numbers in fish, 8 days after exposure along with no mortality and no signs of virulence reversion.

Inactivated vaccine: An inactive or killed vaccine contains the disease-causing pathogen, which has undergone physical or chemical changes without compromising its antigenicity. Heat inactivation is the cheapest method to produce a vaccine. Recently, Dharmaratnam *et al*. (2023) demonstrated a heat-inactivated CyHV-2 vaccine by simply keeping the viral inoculum in a water bath at 80°C for 1 hour before injection. The survival rate of the fish in the vaccine group (86.7%) was significantly higher compared to the control (20%). Ito and Ototake (2013) prepared a formalin-inactivated vaccine by propagating CyHV-2 Saitama-1 (SaT-1) isolate five times in GFF cells and then treating the cell culture supernatant with formalin (0.1% v/v) before application. β-propiolactone was also used as an inactivator by Zhang *et al*. (2016) and Huo *et al*. (2023) (Table 2). In contrast to live vaccines, inactivated vaccines are more stable and less expensive to produce. They do not persist within the environment or in the vaccinated fish, so they are usually considered to be safe. However, they induce weaker and shorter-lived humoral immunity (Ma *et al.*, 2019). The addition of booster immunizations or adjuvants may increase the vaccine potential of inactivated vaccines. Adjuvants such as β-glucan, anisodamine, astragalus polysaccharide (APS) and scopolamine can be used to enhance the effects of inactivated vaccines. Among these four adjuvants, β-glucan and APS combined with an inactivated vaccine give a better immune protection rate to gibel carp against CyHV-2 infection (Huo *et al*., 2023).

DNA vaccine: DNA vaccines contain an expression plasmid that carries a specific gene that codes for a selected antigenic protein, which when expressed in the host, elicits a strong immune response. The entire genome of CyHV-2 consists of 150 ORFs, each of which encodes for a unique protein. The DNA vaccine, pEGFP-N1-ORF25, was constructed based on ORF25 and was able to induce the production of specific antibodies along with upregulating the expression of MHC, IL-1β, C3, and TF-A (Yuan *et al*., 2020). Similarly, ORF25/CCL35.2 could also induce the expression of immune genes (IL-1β, IL-2, IFN-γ2, and viperin) and significantly suppress CyHV-2 replication in kidney and spleen tissues (Huo *et al*., 2020). Besides, the use of molecular adjuvant pcCCL35.2 led to minimal tissue lesions and higher protection (Huo *et al*., 2020; Yuan *et al*., 2020). Hence, the use of appropriate adjuvants is also essential for better efficacy of vaccines.

Recombinant vector vaccine: A recombinant baculovirus vector vaccine, BacCarassius-D4ORFs, containing D4ORFs genes of CyHV-2 along with the β-actin promoter of *Megalobrama amblycephala* was used as a vaccine candidate against CyHV-2. The relative survival of immunized *C. auratus gibelio* reached 59.3% and 80.01%, with oral and injection routes, respectively (Li *et al*., 2019). The yeast surface display technology is a relatively new method of recombinant vaccine preparation. In this method, foreign proteins are displayed on the yeast surface by the yeast-agglutinin receptor. Dong *et al*. (2022) synthesized pYD1-ORF25, and Wang *et al*. (2023) were able to prepare EBY100/pYD1-ORF132 by displaying ORF 25 and ORF132 of

CyHV-2 on the surface of *Saccharomyces cerevisiae*, respectively. Dong *et al*. (2022), however, found the relative percentage survival to be only 14.3 in the vaccine group. However, the booster vaccine effectively increased the RPS up to 66.7. Interestingly, vaccination with EBY100/pYD1-ORF132 showed long-lasting (up to 28 days) adaptive and innate immune responses in both mucosal and systemic tissues and improved survival rate against CyHV-2 infection.

Many vaccines ranging from traditional live attenuated vaccine to the newest recombinant vaccine has been employed against CyHV-2. But all these are limited to lab trials only. The traditional vaccines require multiple dosages, while the new-age vaccines are expensive to produce. Also, it is practically impossible to vaccinate a large number of fishes through injection. So, a proper channel to deliver the vaccine must be devised. Furthermore, the vaccines could be potentiated by using various adjuvant like ligands for toll receptors or different cytokines (Thangaraj *et al*., 2021) instead of traditional ones. Simultaneously, many natural antioxidants and antimicrobial products can be used as effective antiviral medicine against CyHV-2. Hence, further research in all these areas is required for an effective strategy against CyHV-2.

Treatment and management strategies

Treatment of any viral disease is always a matter of concern. Infection with CyHV-2 causes elevation of oxidative stress during the early stage of infection. Viral entry upregulates the Keap1-Nrf2 pathway, leading to the overproduction and accumulation of reactive oxygen species (ROS) in different tissues (Bender and Hildt, 2019). This breaks the oxidative homeostasis of the cell and exerts oxidative stress (Nathan and Cunningham-Bussel, 2013). Hence, it has been comprehended that oxidative stress might facilitate viral replication inside the cells (Lee, 2018). Hence, the application of suitable antioxidants would be effective against the virus (Lu *et al*., 2022). Berberine (BBR) is an isoquinoline alkaloid isolated from different plants belonging to the *Berberis* genus. It is a natural antioxidant with anti-cancer, anti-lipogenic, neuroprotective and antimicrobial properties. It was found to suppress the replication of CyHV-2 in RyuF-2 cells while increasing the survival rate of silver crucian carp (Zhang *et al*., 2022). Epigallocate-chin-3-gallate (EGCG) is derived from green tea, which is widely regarded for its antioxidant properties. The application of both antioxidants led to the inhibition of ROS accumulation. It also reduced viral gene expression and protein synthesis leading to decreased replication efficacy of CyHV-2 (Lu *et al*., 2022). Poly-β-hydroxybutyrate (PHB) is a polymer of short-chain fatty acid, which can be hydrolyzed to β-hydroxybutyrate through enzymes secreted by the intestinal microflora of many aquatic animals (Qiao *et al*., 2020). Thirty days of feeding of PHB to gibel carps was found to stimulate immune-related genes, suppress CyHV-2 replication, and decrease mortality compared to the control group. Hence, all these compounds i.e., BBR, EGCG and PHB have immense potential to be an effective antiviral medicine against CyHV-2 (Liu *et al*., 2022). Recently, it has been noticed that CyHV-2 infection modulated gut microbiome and metabolic functions of the gut leading to an increase in susceptibility to *Aeromonas* infections. Hence, it is suggested that modulation of the gut microbiome may be helpful in controlling or modulating this virus infection (Chen *et al*., 2023).

Conclusion

Recently, several viral diseases have been reported from ornamental fish in India. CyHV-2 is one of the major devastating viral infections mostly in goldfish and its varieties. The virus was first reported in 2016 in India, and since then, it has become widespread in the country. Quarantine and testing of new stock is one of the major precautionary measures that needs to be taken for better management of the disease. Further, information related to host-pathogen interaction is the key to the development of any control/management strategy. Although several research works are progressing, no commercial vaccine is available to date. Hence, the identification of strong antigenic proteins of the virus is very much needed. In addition, highly efficient new-generation adjuvants also need to be given priority. Strong surveillance and monitoring are needed for the effective management of these diseases.

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