

Latent Infection of Koi Herpesvirus in Carp: Hidden Risk in Aquaculture

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ABSTRACT

Koi Herpesvirus (KHV) or Cyprinid herpesvirus-3 (CyHV-3) is considered among the most damaging viral diseases of common and koi carp aquaculture across the world. Koi herpes virus causes high mortalities, economic losses, and trade restrictions in aquaculture industry. One of the major problems related to KHV is its capability to cause latent infection in surviving fishes. In latent infection, the virus exists in hiding within the host organisms without showing any clinical signs; mainly within leukocytes and nervous tissues. Latently infected fishes serve as silent carrier of the virus that can be reactivated under certain stress conditions such as temperature change, transportation, crowding, and poor water conditions. Reactivation causes shedding of virus from latently infected fish and further spread of virus among clinically healthy fish populations. Research has shown that KHV DNA can be detected in blood leukocytes and many other organs even in healthy carp. Temperature changes have been reported as one of the most important factors that induce viral shedding from latently infected fishes. The detection of latent infection is very difficult due to presence of extremely low numbers of viral DNA during latency stage. PCR and Real-time PCR have been used successfully to detect latent KHV infection. It is necessary to have good surveillance, biosecurity measures, stress reduction, and periodic screening in order to avoid silent transmission of the virus. It is vital to understand the process of latent infection

and reactivation for sustainable carp farming.

INTRODUCTION

Koi herpesvirus (KHV) is an infectious pathogenic virus that affects the common carp (*Cyprinus carpio*) and ornamental koi carp species. The virus results in koi herpesvirus disease (KHVD) that features high mortality, gill necrosis, skin lesions, increased mucous secretion, respiratory disorders, and unusual swimming activity. Up to 80–100% mortality may be seen if optimal environmental conditions prevail (Amin *et al.*, 2018). KHV was initially identified in Israel and the United States towards the end of the 1990s and then became distributed globally due to the international koi and common carp trade (Cano *et al.*, 2020). The virus is classified into the family Alloherpesviridae and is equipped with a huge double-stranded DNA genome (Eide *et al.*, 2011). Latent infection is one of the critical traits of any type of herpesviruses. Latency is when the viral genome remains intact in infected host cells but does not replicate viruses. Infected fish seem normal but carry the virus genome for an extended period of time. Thus, infected individuals are a significant threat in aquaculture, as stress triggers the reactivation of the virus (Eide *et al.*, 2011).

KHV and Its Impact on Aquaculture

- ✓ Outbreaks of KHV have led to huge economic problems within the ornamental and edible carp industry. The disease is transmitted rapidly through ponds, hatcheries, and transportation. The clinical symptoms are characterized by pale gills, skin ulcers, lethargic behavior, eyes sinking into the skull, mucous secretion, and abnormal swimming (Amin *et al.*, 2018).
- ✓ The water temperature greatly influences the disease course. KHV usually breaks out

at temperatures from 16°C to 25°C, with peak mortality at 22–24°C (Cano *et al.*, 2020). If the temperature level is lower, the fish will become asymptomatic carriers of the virus. Asymptomatic infection may lead to uncontrolled spreading of the disease in the aquaculture system.

- ✓ The spread of ornamental koi throughout the world has had a great impact on the distribution of KHV. Latent infection of the disease in seemingly healthy fish is responsible for transferring the virus into the new environment.

Mechanism of Latent Infection

- ✓ Latency refers to a state where the virus remains within the body cells but does not replicate actively. Just like other viruses within the Herpesviridae family, KHV causes a lifelong infection once the fish becomes exposed to the virus (Eide *et al.*, 2011).
- ✓ There have been indications that white blood cells such as B lymphocytes provide the main sites for latency (Prescott *et al.*, 2016). Viral DNA was detected in the brain, spleen, kidney, liver, gills, intestine, and other organs of clinically healthy common carp (Eide *et al.*, 2011).
- ✓ When infection is in a latent form, there are no infectious viral particles, only few viral genes become active. As such, due to the inactive nature of the virus, infected fish do not exhibit any symptoms of the disease. However, the virus stays alive by residing within fish tissues for extended periods.
- ✓ Some researchers indicated that KHV DNA could always be found within white blood cells of koi from farms that had

experienced previous KHV infections (Eide *et al.*, 2011).

Reactivation of Latent KHV

Reactivation of latent KHV occurs when fish experience stressful environmental conditions. Stress suppresses the immunity of fish and stimulates viral replication. Temperature variation is one of the key drivers of viral reactivation (Cano *et al.*, 2020). Studies found that the fish kept at low temperatures could survive the viral infection but would develop symptoms once the water temperature was raised to 22°C. Virus reactivation leads to shedding and increased mortality rates (Cano *et al.*, 2020). (Eide *et al.*, 2011) have shown that temperature stress led to reactivation of the latent KHV in koi fish. The researchers noted that the infectious virus as well as viral DNA could be isolated from the gill mucus and feces following temperature stress exposure. It was concluded that reactivation of KHV due to stress provides carriers with an opportunity to infect other fish populations.

The list of stress factors that trigger reactivation includes:

- Overcrowding
- Transportation stress
- Poor water quality
- Handling stress
- Nutritional deficiency
- Sudden environmental changes

These stress conditions are common in aquaculture farms and ornamental fish trade systems, increasing the hidden risk of KHV outbreaks.

Immune Response During Latent Infection

- ✓ KHV infection triggers various immune responses in carp. Research has shown that

the immune response during KHV infection involves toll-like receptors, inflammatory cytokines, macrophages, and immune signaling pathways (Cano *et al.*, 2024).

- ✓ The KHV infection can lead to changes in the blood leukocytes and affect immune-related gene expressions. Cano *et al.* (2024) found an increase in the activation of pathogen recognition receptors, inflammatory pathways, and macrophage-associated genes among infected carp leukocytes.
- ✓ Moreover, KHV infection can suppress the immune system to remain persistent in the body. The suppression of the complement proteins and immunoglobulins during infection might assist the virus in evading the immune system (Cano *et al.*, 2020).
- ✓ Fish latently infected with KHV might eventually produce antibodies against KHV, especially when triggered by temperature changes. Nevertheless, immunity cannot clear the virus entirely from the host tissue.

Detection of Latent Infection

- ✓ Identifying carriers of KHV latent infection is not easy since very small numbers of viruses are present during latency. The conventional diagnostic tests may fail to determine such carriers.
- ✓ PCR is commonly used to diagnose KHV. The virus can be identified through the detection of its DNA in gill, kidney, mucous, and blood leucocytes (Amin *et al.*, 2018).
- ✓ Using advanced technologies like recombinase polymerase amplification (RPA) increases the ability to detect latent infections. This was shown by (Prescott *et al.*, 2016), who indicated the ability of RPA to detect very low copies of the KHV

genome from white blood cells of infected koi in 20 minutes.

- ✓ Real-time PCR, ELISA, and immunological tests are also used for monitoring purposes. However, identification of latent infection still remains difficult since there are very few copies of DNA in infected cells.
- ✓ Early diagnosis and continuous monitoring are necessary for preventing KHV infection.

Prevention and Control Measures

Management tactics should be put in place to mitigate the dangers of latent KHV infections in aquaculture systems. Some key preventive tactics include:

1. Screening broodstock and ornamental fish regularly
2. Conducting strict quarantine for fish that have been brought in from outside sources
3. Maintaining good water quality
4. Avoiding sudden temperature fluctuations
5. Reducing stocking density and handling stress
6. Proper disinfection and biosecurity practices
7. Monitoring carrier fish populations
8. Vaccination programs where available

Latently infected fish should not be introduced into disease-free culture systems. Proper farm management and health monitoring programs are essential to minimize disease outbreaks.

CONCLUSION

Infection of the koi herpesvirus in carp can lead to a latent infection that poses a great

danger to carp aquaculture. The fish that survives the first infection can retain the viral genetic material for many months without any manifestation of disease. Any stress, like temperature change, in the environment can reactivate the virus to result in viral shedding. White blood cells and other organs play a significant role in viral latency. Latent infections being hard to detect pose a risk for the spread of the disease from healthy carriers in aquaculture. Advances in molecular detection methods have made it possible to diagnose latent KHV infections. However, constant monitoring is still important in controlling and preventing the spread of the disease.

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