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DETECTION OF KOI HERPES VIRUS (KHV) AND ITS PREVALENCE RATE IN COMMON CARP (*Cyprinus carpio*) COMMODITIES TRANSPORTED THROUGH THE ANIMAL, FISH AND PLANT QUARANTINE CENTER OF NORTH SULAWESI USING THE REAL-TIME POLYMERASE CHAIN REACTION (qPCR) METHOD

Deteksi Koi Herpes Virus (KHV) dan Tingkat Prevalensinya pada Komoditi Ikan Mas (*Cyprinus carpio*) yang Dilalu-Lintaskan di Balai Karantina Hewan, Ikan dan Tumbuhan Sulawesi Utara dengan Metode Real-Time *Polymerase Chain Reaction* (qPCR)

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ABSTRACT

Common carp (*Cyprinus carpio*) is one of the primary freshwater aquaculture commodities that plays a strategic role in supporting national fish production and contributing to the livelihoods and economic development of local communities. The high intensity of aquaculture practices, combined with the movement of live fish across regions, increases the risk of fish disease transmission, particularly highly contagious viral diseases. One of the most significant viral diseases of concern is Koi Herpes Virus (KHV), which can cause high mortality rates and substantial impacts on production, economic stability, and fish trade activities. This study aimed to detect the presence of Koi Herpes Virus (KHV) and to determine its prevalence in common carp (*Cyprinus carpio*) commodities transported through the Animal, Fish, and Plant Quarantine Center of North Sulawesi using the Real-Time Polymerase Chain Reaction (qPCR) method. The research employed a descriptive design with a surveillance approach. The study samples consisted of five common carp collected from quarantine traffic activities. Detection of KHV was conducted through DNA extraction followed by amplification of the viral target gene using the qPCR method. The prevalence rate was calculated based on the proportion of positive samples relative to the total number of examined samples. The results showed that out of five examined common carp samples, one sample tested positive for Koi Herpes Virus (KHV), while the remaining four samples were negative. Based on these findings, the prevalence rate of KHV in common carp transported through the Animal, Fish, and Plant Quarantine Center of North Sulawesi was 20%. These findings indicate that although the majority of transported fish were KHV-negative, the potential introduction and spread of the virus through fish movement remains present. In conclusion, the Real-Time PCR (qPCR)

method is effective as an early detection tool for Koi Herpes Virus (KHV) due to its high sensitivity and specificity. The detection of KHV in a portion of transported common carp emphasizes the importance of strengthening fish quarantine surveillance and consistently implementing laboratory examinations as preventive measures against the spread of fish diseases to other regions, in accordance with the national fish quarantine system.

Keywords: Common carp, *Cyprinus carpio*, Koi Herpes Virus, Fish quarantine, Real-Time PCR, Prevalence.

ABSTRAK

Ikan mas (*Cyprinus carpio*) merupakan salah satu komoditas perikanan budidaya air tawar yang memiliki peran strategis dalam mendukung produksi perikanan nasional dan perekonomian masyarakat. Tingginya intensitas budidaya serta aktivitas lalu lintas ikan hidup antarwilayah meningkatkan risiko penyebaran penyakit ikan, khususnya penyakit viral yang bersifat sangat menular. Salah satu penyakit viral yang menjadi perhatian serius adalah Koi Herpes Virus (KHV), karena dapat menyebabkan tingkat mortalitas yang tinggi dan berdampak signifikan terhadap produksi, ekonomi, serta kelancaran perdagangan ikan. Penelitian ini bertujuan untuk mendeteksi keberadaan Koi Herpes Virus (KHV) dan menentukan tingkat prevalensinya pada komoditas ikan mas (*Cyprinus carpio*) yang dilalu-lintaskan di Balai Karantina Hewan, Ikan dan Tumbuhan Sulawesi Utara menggunakan metode Real Time Polymerase Chain Reaction (qPCR). Penelitian ini menggunakan desain deskriptif dengan pendekatan surveilans. Sampel penelitian berupa lima ekor ikan mas yang berasal dari kegiatan lalu lintas karantina. Deteksi KHV dilakukan melalui tahapan ekstraksi DNA dan amplifikasi target gen virus menggunakan metode qPCR, sedangkan tingkat prevalensi dihitung berdasarkan perbandingan jumlah sampel positif terhadap jumlah total sampel yang diperiksa. Hasil penelitian menunjukkan bahwa dari lima sampel ikan mas yang diperiksa, satu sampel terdeteksi positif Koi Herpes Virus (KHV), sedangkan empat sampel lainnya menunjukkan hasil negatif. Berdasarkan hasil tersebut, tingkat prevalensi KHV pada ikan mas yang dilalu-lintaskan di Balai Karantina Hewan, Ikan dan Tumbuhan Sulawesi Utara sebesar 20%. Temuan ini menunjukkan bahwa meskipun sebagian besar ikan yang dilalu-lintaskan berada dalam kondisi negatif KHV, potensi masuk dan tersebarnya virus melalui lalu lintas ikan masih tetap ada. Dapat disimpulkan bahwa metode Real Time PCR (qPCR) efektif digunakan sebagai metode deteksi dini Koi Herpes Virus (KHV) karena memiliki sensitivitas dan spesifisitas yang tinggi. Keberadaan KHV pada sebagian sampel ikan mas yang dilalu-lintaskan menegaskan pentingnya penguatan pengawasan karantina ikan serta pelaksanaan pemeriksaan laboratorium secara konsisten sebagai upaya pencegahan penyebaran penyakit ikan ke wilayah lain, sebagaimana direkomendasikan dalam sistem karantina ikan nasional.

Kata kunci: Ikan mas, *Cyprinus carpio*, Koi Herpes Virus, Karantina ikan, Real Time PCR, Prevalensi

INTRODUCTION

Carp (Cyprinus carpio) is a key commodity in global freshwater aquaculture systems and has high economic value in various countries, including Indonesia. Carp production contributes significantly to food security, animal protein supply, and increases the income of small- to medium-scale fish farmers. The continued intensification of aquaculture, coupled with high mobility and movement of live fish between regions, has led to an increased risk of infectious disease transmission, particularly highly contagious viral diseases that have a significant impact on the sustainability of fisheries businesses.

One viral disease of serious concern in the fisheries industry is Koi Herpes Virus (KHV), taxonomically known as Cyprinid herpesvirus-3 (CyHV-3). This virus belongs to the Alloherpesviridae family and specifically infects carp and koi. Since its first report in the late 1990s, KHV has caused outbreaks in various countries, with mortality rates reaching 80–100% under favorable environmental conditions (Ronen et al., 2003; Haenen et al., 2004). KHV infection is highly contagious, transmitted through direct contact, contaminated water, and the movement of live fish between regions (Michel et al., 2010). In addition to causing significant economic losses, this disease also has the potential to disrupt the stability of aquatic ecosystems and the biodiversity of wild cyprinid fish (Daszak et al., 2000).

Biologically, optimal KHV replication occurs at temperatures of 18–25°C, while at higher temperatures the virus can experience decreased virulence, although it can remain latent in the host (St-Hilaire et al., 2009; Gotesman et al., 2013). This latent infection poses a serious challenge in the fish trade system because individuals without clinical symptoms remain potential carriers and sources of infection (Dishon et al., 2005). Therefore, early detection of the virus is a crucial component of fish disease control systems.

The World Organization for Animal Health (WOAH, formerly OIE) has designated KHV as a notifiable disease and recommends the use of molecular methods as the diagnostic standard (WOAH, 2022). The Real-Time Polymerase Chain Reaction (qPCR) method has been recognized as the gold standard for KHV detection due to its high sensitivity (up to 10^1–10^2 DNA copies per reaction) and specificity of >98% (Gilad et al., 2004; Bergmann et al., 2010). In addition to detecting subclinical infections, qPCR also allows for relative quantification of viral load through threshold cycle (Ct) analysis, providing more comprehensive epidemiological information than conventional PCR (Bustin et al., 2009).

In Indonesia, KHV cases were first reported in the early 2000s and have since become a serious threat to national carp production. Recent studies indicate that the prevalence of KHV in certain aquaculture centers remains in the range of 15–30%, depending on the aquaculture system and biosecurity level (Susilowati et al., 2018; Koesharyani et al., 2017). The high level of interprovincial fish distribution increases the risk of virus introduction and spread to previously disease-free areas. In this context, the role of quarantine centers is very strategic as the frontline in preventing the entry and exit of pathogens through fishery commodity traffic.

RESEARCH METHODS

Place and Time

This research was conducted at the Animal, Fish, and Plant Quarantine Center Laboratory in North Sulawesi. The study was conducted for one month, from November 1, 2025, to November 30, 2025.

Tools and Materials

The tools used in this study include dissecting set, pellet pastle, sterile micropipette and microtip, 1.5 ml microtube, refrigerated centrifuge, vortex mixer, thermoblock, spindown centrifuge, spectrophotometer, bio safety cabinet, freezer, real-time PCR machine. The materials used in this study include goldfish gill and kidney tissue, DNA extraction kit, KHV specific primer & probe, KHV standard control 10^4 copies, negative control, nuclease-free water, TE buffer, absolute ethanol and distilled water.

Research methods

This is a quantitative descriptive study using a laboratory survey approach. The aim was to detect the presence of Koi Herpes Virus (KHV) and determine its prevalence in transported carp (Cyprinus carpio). The descriptive approach was used because this study

did not provide any specific treatment to the research subjects, but rather described the health status of the carp against KHV infection based on laboratory test results (Sultan et al., 2018: 32).

The study design used was cross-sectional, where sampling was conducted over a specific time period, and each sample was tested only once to determine KHV infection status. This design is appropriate for disease prevalence studies because it can describe the proportion of infected individuals in a population at a specific observation time (Dong et al., 2014: 134).

Research Procedures

The research samples consisted of individual goldfish randomly selected from each shipment lot. The sample size was determined based on fish disease surveillance principles and adjusted to population availability and laboratory capacity. Random sampling was conducted to minimize bias and provide a representative picture of the KHV infection status of the goldfish population being transported (Koesharyani et al., 2017: 3). The goldfish samples were then taken to the Molecular Biology Laboratory of the North Sulawesi Animal, Fish, and Plant Quarantine Agency for further analysis.

Data Analysis

In this study, molecular-based testing was used with the real-time PCR (qPCR) method, which includes several stages, namely: DNA extraction using the DNeasy® Blood & Tissue Kit DNA Extraction Kit, DNA concentration measurement using the Nabi UV/Vis Nano Spectrophotometer, DNA amplification with the Rotorgene Real-Time PCR machine, interpretation of results and data analysis using Rotorgene Q software.

Prevalence Rate

The disease prevalence rate is calculated to determine the proportion of infected individuals in a population at a given time. The prevalence of KHV in this study was calculated using the following formula:

$$\text{Prevalensi } (\%) = \frac{\text{Jumlah sampel positif}}{\text{Jumlah total sampel}} \times 100\%$$

Description:

- Number of positive samples = number of individuals detected as infected (e.g. via qPCR)
- Total sample examined = all individuals tested in the study
- Multiply by 100 to get the value in percentage

RESULT

Based on the results of research on the detection of Koi Herpes Virus (KHV) and its prevalence rate in carp (Cyprinus carpio) transported at the North Sulawesi Animal, Fish, and Plant Quarantine Office using the real-time PCR (qPCR) method, the test results are presented in Table 1 below.

Table 1. Sample Test Results from Real-Time PCR Amplification

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (Copies)	Calc Conc (Copies)
1		K+ KHV 10^4	Positive Control	21.43		10,000.0	11,206.10
2		K - KHV	Negative Control				
3		067/KI/L-1/25	Unknown				
4		068/KI/L-1/25	Unknown				
5		069/KI/L-1/25	Unknown				
6		070/KI/L-1/25	Unknown				
7		071/KI/L-1/25	Unknown	30.35		1,000	1048.9

Legend:
NEG (NTC) - Sample cancelled due to NTC Threshold.
NEG (R. Eff) - Sample cancelled as efficiency less than reaction efficiency threshold.

This report was generated by Rotor-Gene Q Series Software 2.3.5 (Build 1)

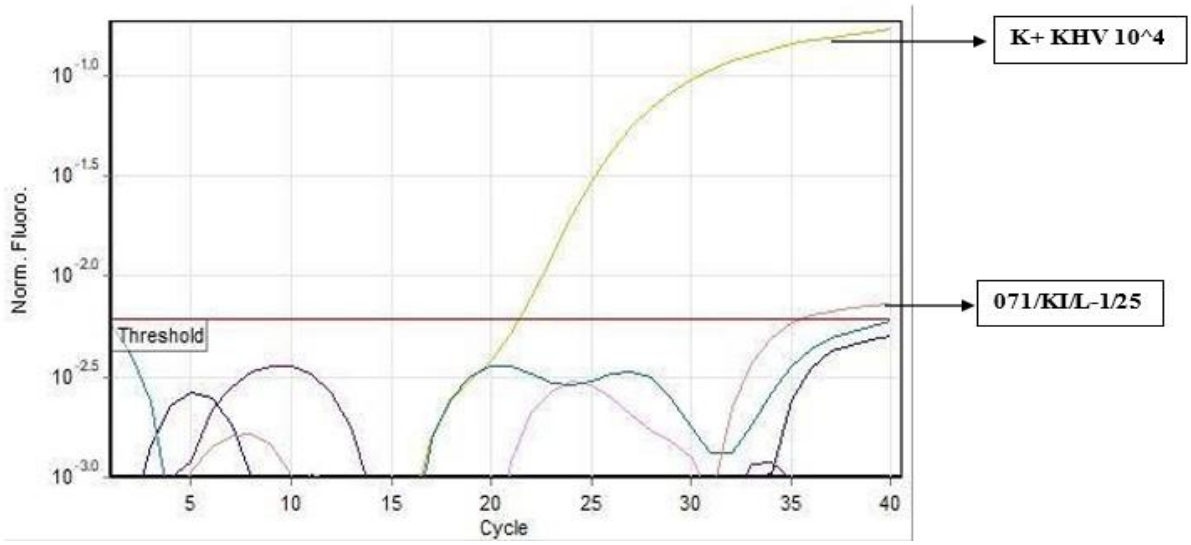

Figure 1. Real-time PCR (qPCR) test result curve

Based on Figure 1. above, it can be explained that the positive control curve of KHV 1.0 x 104 shows a clear sigmoid amplification pattern, the exponential phase begins at around cycle 18-20, the crossing threshold (Ct) is around ±20-22, this indicates a high virus concentration (10^4 copies), according to the control label. In the sample curve 071/KI/L-1/25, the crossing threshold (Ct) is shown at around cycle 34-36, the amplification pattern is slower than the positive control and the curve still shows an exponential shape, because the sample curve passes the threshold before cycle 38-40, the sample is declared positive for KHV.

Table 2. Results of KHV detection in carp samples using the real-time PCR (qPCR) method

No.	Kode Sampel	Daerah Asal	Organ Target	Nilai Ct	Status KHV
1	067/KI/L-1/25	Kota Manado	Insang dan Ginjal	Tidak terdeteksi	Negatif
2	068/KI/L-1/25	Kota Manado	Insang dan Ginjal	Tidak terdeteksi	Negatif
3	069/KI/L-1/25	Kota Manado	Insang dan Ginjal	Tidak terdeteksi	Negatif
4	070/KI/L-1/25	Kota Manado	Insang dan Ginjal	Tidak terdeteksi	Negatif
5	071/KI/L-1/25	Kota Bitung	Insang dan Ginjal	30,35	Positif

Based on Table 2 above, it can be explained that the number of samples examined was 5 (five) goldfish with target organs of gill and kidney tissue originating from the cities of Manado and Bitung. From the results of the Ct value analysis (not detected), there were 4 (four) sample codes 067-070/KI/L-1/25 originating from Manado with negative KHV status. Meanwhile, 1 (one) sample code 071/KI/L-1/25 originating from Bitung was detected with a Ct value of 30.35 and declared positive KHV status.

Based on the qPCR results, one sample tested positive for KHV out of five goldfish samples. Using the prevalence formula, the following calculation was obtained:

$$\text{Prevalensi KHV} = \frac{1}{5} \times 100\% = 20\%$$

The calculation results show that the prevalence rate of KHV in goldfish commodities transported through the North Sulawesi Animal, Fish and Plant Quarantine Center is 20%.

DISCUSSION

In this study, positive detection of KHV in one carp sample with a Ct value of 30.35 indicated KHV infection at a low to moderate viral load. This Ct value is below the standard detection threshold (Ct <35-38, depending on the OIE protocol, 2022), indicating significant amplification of KHV target genes (e.g., DNA polymerase or thymidine kinase genes). Biologically, a Ct of 30.35 reflects a viral concentration of approximately 103-104 copies/mL, which may be asymptomatic or latent, but still potentially transmissible through horizontal routes such as contaminated water or direct contact (Dishon et al., 2005; St-Hilaire et al., 2005).

The four negative samples (Ct >40 or undetectable) confirmed the absence of detectable KHV DNA, which can be interpreted as indicating the success of the initial quarantine procedures or low viral exposure in the host population. However, this interpretation should be cautious, as qPCR only detects viral genetic material, not its viability. False negatives can arise from factors such as sample degradation during transport or PCR inhibition by biological matrices (e.g., fish mucus), although internal controls (such as fish housekeeping genes) have been used to validate the assay (Bergmann et al., 2010). Overall, these results align with the principles of molecular diagnostics, where qPCR provides superior quantitative data compared to conventional methods such as end-PCR or viral culture, which are less sensitive for subclinical infections (Pokorova et al., 2005).

The prevalence rate of Koi Herpes Virus (KHV) in common carp (Cyprinus carpio) transported to the Animal, Fish, and Plant Quarantine Agency of North Sulawesi in this study was 20%, obtained from 1 positive sample out of a total of 5 samples examined. Comparatively, the 20% prevalence rate in this study is relatively lower than several international reports. Haenen et al. (2004) reported that the prevalence of KHV in European carp populations ranged from 40–60% during outbreak periods. In Southeast Asia, Sano et al. (2004) and Bondad-Reantaso et al. (2007) recorded prevalence rates between 25–50% in areas with high aquaculture intensity and fish traffic.

Differences in prevalence rates between regions can be influenced by various factors, including environmental conditions, aquaculture density, biosecurity systems, and the effectiveness of quarantine controls. Water temperature is an important determinant, as KHV is known to replicate optimally at 18–25°C, while the tropical waters of North Sulawesi, which range between 26–30°C, have the potential to suppress clinical disease expression, although not completely eliminate the virus (Gilad et al., 2003; St-Hilaire et al., 2009)

CONCLUSION

Koi Herpes Virus (KHV) was detected in carp (Cyprinus carpio) commodities transported through the Animal, Fish and Plant Quarantine Office of North Sulawesi based on the results of examination using the Real-Time Polymerase Chain Reaction (qPCR) method, namely in sample number 071/KI/L-1/25 with a Ct value of 30.35 indicating infection with a low viral load. The prevalence rate of KHV in carp transported was 20%, which indicates the continued risk of KHV transmission through live fish traffic.

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