Hervesvirus

by Muhamad Ali

Submission date: 05-Oct-2019 12:07PM (UTC+0700)
Submission ID: 1186492134
File name: Hervesvirus.pdf (624.07K)
Word count: 5445
Character count: 27399

VirusDis.
https://doi.org/10.1007/s13337-018-0488-7

ORIGINAL ARTICLE

Detection of koi herpesvirus in healthy common carps, *Cyprinus carpio* L.

Muhamad Amin[1] · Dian N. Adrianti[2] · Ni Luh A. Lasmika[2] · Muhamad Ali[3]

Received: 7 April 2018 / Accepted: 14 September 2018

Abstract Koi herpesvirus (KHV), a member of *Hervesviridae,* has been frequently reported to cause mass mortality (80–100%) in common carps (*Cyprinus carpio* L.). A unique feature of *Herverviridae* members is latent infection, maintaining their genetic information for an extended period in the absence of productive infection, and reactivate when environmental conditions are favorable for their growth. To prevent this occurs, a monitoring program should be done for early detection. This study aimed at detecting the presence of KHV in healthy common carps reared in West-Nusa Tenggara Province, Indonesia. A total of 80 healthy fish was collected randomly from eight fish farms (Lingsar, Batu Kumbung, Narmada, Tanjung, Lenek, Aik Mel, Brang Rea and Rhee) across West-Nusa Tenggara Province, Indonesia. The presence of KHV genome was detected using a PCR with a commercial kit, IQ 2000TM. The result showed that common carps collected from four farms (Aik Mel, Lenek, Rhee and Brang Rea) were positive KHV. The size of an amplified gene was ~ 550 bp which was the same as positive KHV control. The obtained result suggests that KHV as other member of *Hervesviridae* shows a latent infection in common carps, and should be anticipated for their reactivation. Based on this result it is highly recommended that common carps cultured in this region should be vaccinated. In addition, transporting common carp out from Lombok and Sumbawa Islands should be carefully regulated to prevent the spread of the disease to other areas.

Keywords Common carp · Detection · KHV · Latent infection

Introduction

Koi herpesvirus (KHV) is a lethal disease which has caused mass mortalities in common carp (*Cyprinus carpio*), and fancy carp or koi (*Cyprinus carpio* koi) in many countries worldwide [38]. Taxonomically, the virus is included in family *Herpesviridae* [4, 44]. Fish infected by KHV generally shows several gross pathological signs including discoloration of skin and gills [7, 17], the bases of fins were congested, and secreting massive mucus on their skin and gills [17, 26]. The massive production of mucus on gill cause disturbance of oxygen transfer, which could lead to the low oxygen content in their blood which leads to gasping behavior of fish on the surface of ponds. Other clinical signs are that the infected fish showed poor appetite, and lethargy abnormal swimming behaviors [15]. Mortality of KHV infected fish can be up to 100% within 2 or 3 weeks [17], therefore being regarded as one of the most dangerous fish pathogen in the world.

Since firstly reported in the USA and Israel in 1998 [14, 16, 17], KHV disease has been spread across wide geographical areas. In 2000, mass mortality of common carp due to the virus was reported from several European countries including Germany, Holland, Belgium, and United Kingdom [42]. In the same year, it was reported to

✉ Muhamad Amin
muhamad.amin81@gmail.com

[1] Fisheries Faculty, University of 45 Mataram, West-Nusa Tenggara, Indonesia

[2] Fish Quarantine Inspection Agency Selaparang, Ministry of Marine Affairs and Fisheries Republic of Indonesia, Mataram, West Nusa Tenggara, Indonesia

[3] Laboratory of Microbiology and Biotechnology, Faculty of Animal Science, University of Mataram, West-Nusa Tenggara, Indonesia

Published online: 25 September 2018

infect different life stages of Koi in Taiwan, which were juvenile and adult stages [43]. In 2002, the virus has reach several Asian countries including Thailand, Vietnam, Singapore, Cambodia, Philippine and Indonesia [19, 21–23, 31, 35, 39]. The virus has also been reported to infect various stages of carp, from fry (less than 2 months) to juvenile stages in 2003 in Japan [20, 37, 41].

In Indonesia, the first case of KHV infection was reported in 2000 in Blitar, East Java [25, 40]. The disease has caused vast economic losses in koi and common carp industries. Due to the disease outbreak, it affected 5000 farmers with economic losses of more than IDR 5 billion within the first 3 months. Since then, the disease outbreak continued to spread to new areas in Indonesia. KHV infection has spread to several areas in Indonesia, including Surabaya (East Java), Subang Regency (West Java) during April 2003, Lubuk Lingau Regency (South Sumatera) in February 2003, Bali, East Kalimantan and Central Sulawesi in February 2003 [40]. Around 2009, there was mass mortality of common carp and koi in West-Nusa Tenggara. Based on its clinical sign, the mass mortality was due to the KHV infection. However, no scientific study has been done about the KHV outbreak in West-Nusa Tenggara Province.

A unique feature of the virus is a latent infection, which is maintaining genetic information in certain hosts for an extended period in the absence of productive infection [11]. When environmental conditions are favorable for their growth especially temperature and stress condition, the virus can be reactivated and cause mass mortality. To prevent this occurs, a monitoring program should be done regularly to those farms which are presumed to have previously been exposed to KHV for early detection of the virus. Thus, this study aimed at detection of KHV in West-Nusa Tenggara province, Indonesia. The study result would provide important information about the current state of KHV in this region to prevent the mass mortality in fish farmers or the spread of this contagious disease, to other areas.

Materials and methods

Fish samples

Eighty common carps collected by fish health professionals were obtained directly from 8 fish farms (@10 fish) across West-Nusa Tenggara Province which were Narmada (S8°34′21; E116°11′27.1), Lingsar (S8°34′26.4″; E116°11′19.5), Batu Kumbung (S8°34′18.7; E116°11′44.8″), Tanjung (S8°21′46.6; E116°09′28.6), Lenek (S8°34′42.1″; E116°31′02.2), Aik Mel (S8°35′10.0″ S; E116°30′26.8), Brang Rea (S8°28′36″; E117°16′54.4764″) and Rhee (S8°56′4908″ E116°09′7678″), Fig. 1. The fish with an average weight of 95.32 ± 2.54 g (Fig. 2), and a mean length of 13.45 ± 1.23 cm were caught from ponds in which water temperatures ranged from 27 °C to 29 °C. Live fish placed in aerated water bags were transported to a Fish Health Laboratory of Fish Quarantine Inspection Agency, Mataram, West-Nusa Tenggara, Indonesia. As soon as arriving, the fish were placed in a 3000L-concrete tank and reared until sampling processes.

Physical examinations

Physical condition of fish sample was examined according to a protocol developed by Hedrick et al. [17] with a slight modification. In brief, fish samples were firstly anesthetized with clove oil at a concentration of 50 µl l^{-1} rearing water. After opercula movement was ceased, the fish were killed and immediately subjected to physical, examinations. The physical appearance of the fish was observed individually for detection of any gross clinical signs. The main organ targets to be observed were gills and skin as these organs are the most common pathological signs in KHV infections [17].

DNA extraction

Afterwards, gill was removed aseptically from each fish samples, and the gills from the same location (10 fish) were placed on a sterile Petridisk, pooled together for further use. Thereafter, DNA was extracted from the organ target (gill) which was pooled based on sample locations using an IQ 2000^{TM} kit according to the manufacturer's instructions. In brief, the pooled gills were cut into pieces and placed in a 1.5 sterile tube containing 600 µl extract solution of 8% dedocyltrimethilammonium bromide (DTAB), 1.5 M NaCl, 100 mM TRis–Cl (pH8.8), 50 mM EDTA and incubated at 68 °C water bath overnight. The tube was agitated several times to mixed the sample and the buffer solutions. Afterwards, 700 µl chloroform was added to the tube and followed by centrifugated 10.000×*g* for 5 min. Thereafter, 200 µl aqueous layer (supernatant) was transferred to a new sterile 1.5 ml tube containing 900 µl dH_2O and 100 µl cetyltrimethyl-ammonium bromide (CTAB) solution (5% CTAV, 0.4 M NaCl). The tube was then inverted gently several times and allowed to sit at room temperature. After 5 min, the tube was centrifuged at 10,000 x *g* for 10 min. The supernatant was discarded and the pellet was resuspended in 300 µl M NaCl to exchange the CTAB. The DNA was precipitated by adding 300 µl ethanol (95%) and centrifuged again, dried in a speed-vac for 10–15 min and resuspended in 50 µl of 0.5 × Tris EDTA buffer pH 8.0. This DNA solution was then used as

Fig. 1 Eight locations of fish sampling for detecting KHV in West-Nusa Tenggara, Indonesia. **a** Lingsar, **b** Batu Kumbung, **c** Narmada, **d** Tanjung, **e** Lenek, **f** AikMel, **g** Brang Rea, and **h** Rhee

Fig. 2 Fish samples, collected from fish farms at West-Nusa Tenggara Province, Indonesia. All fish samples showed no symptoms of KHV infected fish; no abnormal changes on their body surfaces and gills

a template for polymerase chain reaction (PCR) amplification.

Molecular detection of KHV

Detection of KHV DNA from fish tissues was conducted using a commercial kit (IQ 2000^{TM}) with a pair of specific primers (F: 5′-GGGTTACCTTACGAG-3′; R: 5′-CACCCAGTAGATTATGC-3′). A master mix was made containing the following: A total of 7.5 µl of first PCR pre-mixed was combined with 0.5 µl IQzyme DNA polymerase ^{TM}2U/µl 2 µl of extracted DNA sample. The DNA Engine Peltier Thermal Cycler Model (Tetrad2, MJ Research) was used for amplification using 1 cycle of 94 °C for 2 min followed by 15 cycles of 20 s at 94 °C, 20 s at 62 °C, and 30 s at 72 °C, followed by 1 cycle of 30 s at 72 °C. Eight to ten µl were loaded onto a 2% agarose gel for electrophoresis. The duplicate samples were run with positive and negative controls and a DNA marker.

According to the manufacturer protocol, the amplified KHV gene of interest was about 550 bp. Representative amplicon (PCR amplicon from AikMel) was then purified according to a protocol of Ali et al. [1], and send for sequencing.

Sequence identity and phylogenetic analysis

The identity of PCR amplicon was searched according to Amin et al. [3] with some modifications. In brief, the sequence of PCR amplicon was run for sequence homology at https://blast.ncbi.nlm.nih.gov/Blast.cgi with the archived 16S rDNA sequences from GenBank. Thereafter, multiple alignments of some KHV sDNA sequences from different countries were performed with the ClustalW2 program. A phylogenetic tree was constructed using the neighbor-joining DNA distance using Geneious software version 5.3.6. The resultant tree topology was evaluated by bootstrap analysis of neighbor-joining data sets based on 100 resamplings.

Water quality

Physical–chemical parameters of rearing water which were pH, dissolved oxygen (DO), temperature (T), ammonia (NH_3^-) and nitrite (NO_2^-) were measured in situ at each sampling location. Water temperature, and DO were measured with Dissolved oxygen meter (DO Meter AZ-8403), pH with pH Meter Hanna Instruments HI99162/HI99162. While ammonia (NH_3) and (NO_2) were monitored with ammonia and nitrite test kits (Hanna products).

Results

Physical examination

The 80 fish samples collected from eight fish farms across West-Nusa Tenggara Province, Indonesia showed no signs of KHV infection such as depression, lethargy, abundant mucus production on the body, behavioral abnormalities and disorientation. In addition, gross physical examination of the 80 common carps showed no abnormal changes on their body surfaces and gills. Literally, all fish were healthy and free from any pathological signs of KHV infection.

Molecular detection

Fish samples collected from four fish farms (Lenek, Aikmel, Brang Rea and Rhee) were positive KHV, indicated by the amplification of KHV DNA as presented in Fig. 3. The size of the amplicon was ~ 550 bp which was the same size as the positive control. Meanwhile, there was no amplified product from fish samples collected from the other four fish farms which were Lingsar, Batu Kumbung, Narmada and Tanjung, Fig. 3. This result may indicate that the four fish farms were free from KHV.

Sequence identity and phylogenetic analysis

Through partial 16S rRNA sequencing and phylogenetic analysis revealed that strain the amplified gene sequence showed 95% similarity to KHV 3 strain T (Acc. Nb. MG925491.1). The 16S rDNA sequence of the isolate was deposited in GeneBank under accession number MG976611. The phylogenetic position of the bacterial strain was compared with other *Bacillus* spp. in a dendogram. The dendogram analysis revealed a close relatedness between the amplified gene and KHV 3 strain T (Fig. 4).

Water quality

Water quality parameters (pH, ammonia, nitrite, dissolved oxygen, and temperature) in the rearing water were all within normal condition, as presented in Table 1. Temperature ranged from 27 to 29 °C, which was normal temperature of rearing water during dry season in West-Nusa Tenggara area of Indonesia. Dissolved oxygen was recorded at 6.5–7.6 ppm, which were in a range of a normal concentration for common carp. Furthermore, pH, ammonia and nitrite were all below toxic concentration for common carp.

Discussion

The first outbreak of KHV in Indonesia was reported in 2002 in Blitar, East Java [40], which caused 90–100% mortality in fancy carp (koi) and common carp. The disease outbreak has caused enormous economic loses for fish farmers. Since then, the disease had been reported to spread in several islands across Indonesia: Java Island, Bali, Southern Part of Sumatra, East Kalimantan and Central Sulawesi [8]. However, no specific study has reported KHV infection from West-Nusa Tenggara Province which consisted of 2 main islands, Lombok and Sumbawa Islands, as one of the main culture center of common carps. Nonetheless, according to fish farmers in the area, there was a mass mortality in common carp occurring in Lombok Island (West and Central Lombok) in 2009. Based on gross pathological signs on the dead fish, it was presumed that the mass mortality was due to KHV infection. Nevertheless, there was no specific study to validate this question yet.

One of the most important features of KHV which is under family of *Alloherpesiviridae* within the order of *Herpesvirales* is latent infection. The latency means that the virus could maintain their DNA in fish hosts but remaining dormant and showing no clinical signs. The latency of KHV has been reported especially in those fish

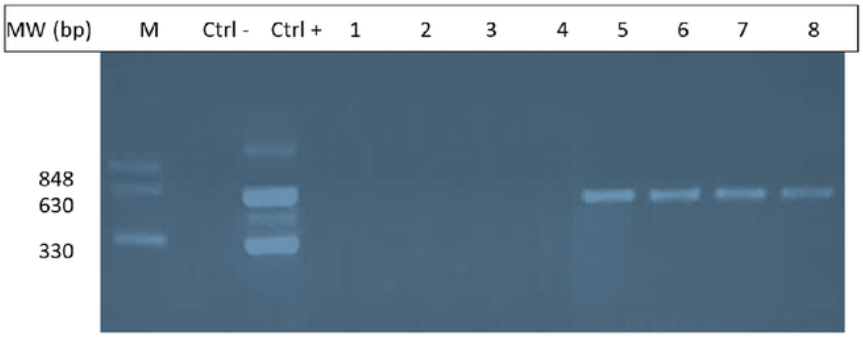

Fig. 3 Amplified gene of KHV from common carp. M: marker, Ctrl −: Negative Control, Ctrl +: Positive control. Amplified gene of sample collected from Lingsar (1), Batu Kumbung (2), Narmada (3), Tanjung (4), Lenek (5), Aik Mel (6), Brang Rea (7) and sample from Rhree (8). Size of the amplified gene in all sample was 550 bp. MW; molecular weight of marker

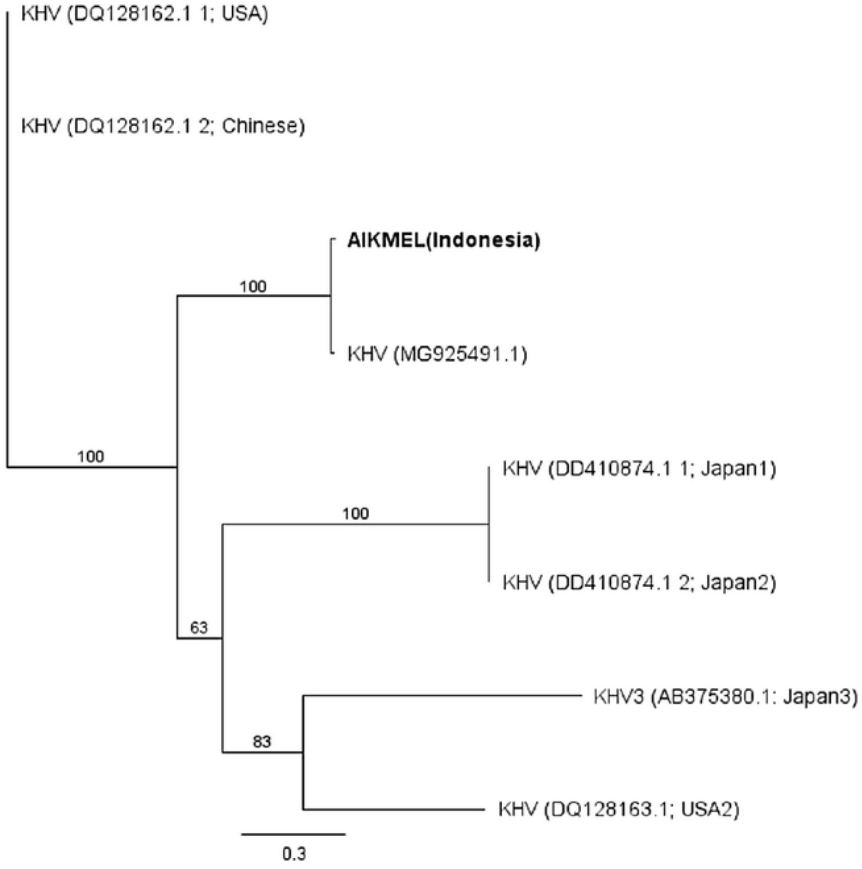

Fig. 4 A phylogentic tree of KHV based on 16S rRNA sequence isolated from carp reared in Aikmel, Lombok Island, West Nusa Tengara, Indonesia. Sequences were aligned using ClustalW Alignment

Table 1 Physical-chemical parameters of water quality measured during sampling in 8 fish farms, in West-Nusa Tenggara Province, Indonesia

No	Locations	Water parameters				
		T (°C)	pH	DO (ppm)	NH_3 (ppm)	NO_2^- (ppm)
1	Lingsar	28.5 ± 0.71	7.0 ± 0.00	6.5 ± 0.71	0.02 ± 0.01	< 0.01
2	Batu Kumbung	27.5 ± 0.71	7.0 ± 0.00	6.9 ± 0.14	0.01 ± 0.01	< 0.01
3	Narmada	28.5 ± 0.71	7.3 ± 0.35	6.9 ± 0.07	0.02 ± 0.01	< 0.01
4	Tanjung	29.5 ± 0.71	7.2 ± 0.28	7.5 ± 0.71	0.01 ± 0.01	< 0.01
5	Aik Mel	29.5 ± 0.71	7.1 ± 0.14	7.5 ± 0.71	0.01 ± 0.01	< 0.01
6	Lenek	28.5 ± 0.71	7.1 ± 0.57	7.6 ± 0.57	0.02 ± 0.01	< 0.01
7	Rhee	27.5 ± 0.71	7.2 ± 0.21	7.2 ± 0.49	0.01 ± 0.00	< 0.01
8	Brang Rea	28.5 ± 0.71	7.4 ± 0.57	6.9 ± 0.14	0.03 ± 0.01	< 0.01

which were presumed to have been previously exposed to KHV [11, 45]. The virus can be reactivated when environmental conditions such as temperature stress were favorable for their growth [12, 27, 30, 38]. One way to prevent the outbreak occur is by performing early detection. Therefore, this study was conducted to detect the presence of KHV especially in common carp reared in West-Nusa Tenggara Province using a robust and powerful PCR technique. The technique is considered to be a powerful method to detect the latency of KHV in common carp due to its ability to detect a low number of viral copies [24].

According to Sunarto et al. [40], KHV infected fishes showed several gross pathological signs such as sloughing off the epithelium with loss of mucus, hemorrhages of operculum, fins, tail and abdomen, and severe gill damage. Thus, this study randomly selected 80 healthy common carps (showing not any clinical infection signs of KHV)

Table 2 Detection of KHV from eight fish farms in West-Nusa Tenggara Province, Indonesia

No	Sampling locations	Physical examination	Molecular detection
1	Lingsar	−	−
2	Batu Kumbung	−	−
3	Narmada	−	−
4	Tanjung	−	−
5	Aik Mel	−	+
6	Lenek	−	+
7	Rhee	−	+
8	Brang Rea	−	+

"−": negative KHV, "+": positive KHV detected using PCR

from 8 fish farms across two islands of West-Nusa Tenggara Province as fish samples. Physical examination on these fish samples also indicated that all fish were free from KHV disease. However, based on PCR result, fish collected from 4 farms were positive KHV, indicated by the amplification of ~ 550 bp bands, which was the same size as the positive control. This result in agreement with a previous study reporting that health carps (no pathological signs of KHV infection), might contain or carry the virus [11]. The present study used a commercial KHV kit (IQ 2000^{TM}) which targeted 2 band sizes, 550 bp, and 330 bp gene. However, the PCR amplicon sizes for KHV in the present study were also longer than previously published, including 484 bp [14], 409 bp by Bercovier et al. [5], and 414 bp by Bergmann et al. [6]. These differences might be due to the different primers which targeted different genes [2]. Based on on Primer-BlAST program, the primers used in the current study targeted 410 bp, located at 96,160 and 96,553. According to Gray et al. [16], DNA fragment of KHV was at least 150.000 bp, and different protocols and primers may give different results. For instance, 2 pairs of primer developed by Gray et al. [16] amplified 2 different sizes of PCR products, 365 bp with BamHI-6 primer and 290 bp with SphI-5 primer. Yuasa et al. [46] reported almost similar size which was 292 bp with a corrected SphI primer. Thus, the amplified gene obtained in the present study may amplify correct gene.

Meanwhile, there was no amplified product from fish samples collected from the other four fish farms (Lingsar, Batu Kumbung, Narmada and Tanjung), Table 2 and Fig. 3. This result may indicate that the fish were free from KHV. However, according to Meyer et al. [24] negative result of PCR might also mean that the number of DNA target was too low in the samples. Since the organ target used in this study only gill, it is highly recommended to take more organs, such as kidney, brain, heart, eye, liver, and pancreas. Taken more sample organ sources might give more accurate and comprehensive results.

This study is the first study to report KHV infection in these two islands, Lombok and Sumbawa which are part of West-Nusa Tenggara Province. Thus, it may be assumed that mass mortality of common carp reported in 2009 in Lombok Islands could be the first outbreak of KHV in Wes-Nusa Tenggara area. Some fish might able to survive and carry the virus afterward. This assumption was built based on several previous studies which demonstrated the ability of the virus to cause a latent infection in carp and koi [11, 32–34]. The virus has not productive since all water quality parameters (pH, ammonia, nitrite, dissolved oxygen, temperature) were all within normal condition for common carps, as [9, 10, 13]. The optimum environmental conditions might prevent the occurrence of KHV, especially temperature which was recorded between 27.5 and 29.5 °C, in all sample locations. According to Perelberg et al. [28], when temperature reaches 26 °C or below during the rainy season, reactivation of KHV may occur [40].

Based on this study result, Lombok and Sumbawa Islands should be listed as KHV-infected area together with Blitar, Surabaya (East Java), Subang Regency (West Java), Lubuk Lingau Regency (South Sumatera), Bali, East Kalimantan and Central Sulawesi [40]. Therefore, transporting live fish between farms and islands should be carefully done, acknowledging the virus characteristics. In addition, it is highly recommended that every fish farm in West-Nusa Tenggara Province should be vaccinated to prevents outbreak of the disease in these areas. Several studies have successfully prevented the disease infection through vaccination in carps [29, 36] and koi [18]. Thus, the approach could also be applied to prevent outbreak of KHV in West-Nusa Tenggara Province.

In conclusion, the result indicates that KHV shows a latent infection to common carps collected from four fish farms, Lenek, Aikmel, Brang Rea and Rhree. Although showing no clinical signs of infections, the present of KHV gene was detected using PCR. This result suggests that the two islands, Lombok and Sumbawa, have been infected by koi herpesvirus (KHV). Based on this result, it is highly recommended that every farm culturing common carps of koi to be vaccinated. In addition, these two islands should be included in the list of KHV infected area, and transportation of koi or common carp out of this regions should be strictly monitored.

Acknowledgements This work was supported by Laboratory of Aquatic Microbiology, University of 45 Mataram and Fish Quarantine Inspection Agency, Mataram, West-Nusa Tenggara, Indonesia. We also wish to thanks Farhan Ramli for providing fish samples and technical support during our laboratory work.

References

1. Ali M, Hidayatullah TA, Alimuddin Z, Sabrina Y. Sequence diversity of pfmdr1 and sequence conserve of pldh in Plasmodium falciparum from Indonesia: its implications on designing a novel antimalarial drug with less prone to resistance. Iran J Parasitol. 2013;8(4):522.
2. Amin M, Adams M, Bolch CJS, Burke CM. *In vitro* screening of lactic acid bacteria isolated from gastrointestinal tract of Atlantic Salmon (*Salmo salar*) as probiont candidates. Aquacult Int. 2016;23:1–14. https://doi.org/10.1007/s10499-016-0045-6.
3. Amin M, Bolch CJS, Adams MB, Burke CM. Isolation of alginate lyase-producing bacteria and screening for their potential characteristics as abalone probionts. Aquac Res. 2017;48(11):5614–23. https://doi.org/10.1111/are.13383.
4. Aoki T, Hirono I, Kurokawa K, Fukuda H, Nahary R, Eldar A, et al. Genome sequences of three koi herpesvirus isolates representing the expanding distribution of an emerging disease threatening koi and common carp worldwide. J Virol. 2007;81(10):5058–65. https://doi.org/10.1128/JVI.00146-07.
5. Bercovier H, Fishman Y, Nahary R, Sinai S, Zlotkin A, Eyngor M, et al. Cloning of the koi herpesvirus (KHV) gene encoding thymidine kinase and its use for a highly sensitive PCR based diagnosis. BMC Microbiol. 2005;5(1):13.
6. Bergmann S, Kempter J, Sadowski J, Fichtner D. First detection, confirmation and isolation of koi herpesvirus (KHV) in cultured common carp (*Cyprinus carpio* L.) in Poland. Bull Eur Assoc Fish Pathol. 2006;26(2):97.
7. Bergmann SM, Stumpf P, Schutze H, Fichtner D, Sadowski J, Kempter J. Similarities and heterogenicity of koi herpes virus (KHV) genome detected in ornamental fish without clinical signs. Aquaculture. 2007;272:1. https://doi.org/10.1016/j.aquaculture.2007.07.034.
8. Bondad-Reantaso M, Sunarto A, Subasinghe R. Managing the koi herpesvirus disease outbreak in Indonesia and the lessons learned. Dev Biol (Basel). 2007;129:21–8.
9. Dabrowska H, Własow T. Sublethal effect of ammonia on certain biochemical and haematological indicators in common carp (*Cyprinus carpio* L.). Comp Biochem Physiol Part C: Comp Pharmacol. 1986;83(1):179–84.
10. De Boeck G, De Smet H, Blust R. The effect of sublethal levels of copper on oxygen consumption and ammonia excretion in the common carp, *Cyprinus carpio*. Aquat Toxicol. 1995;32(2):127–41.
11. Eide KE, Miller-Morgan T, Heidel JR, Kent ML, Bildfell RJ, LaPatra S, et al. Investigation of koi herpesvirus latency in koi. J Virol. 2011;85(10):4954–62. https://doi.org/10.1128/JVI.01384-10.
12. Eide K, Miller-Morgan T, Heidel J, Bildfell R, Jin L. Results of total DNA measurement in koi tissue by Koi Herpes Virus real-time PCR. J Virol Methods. 2011;172(1–2):81–4. https://doi.org/10.1016/j.jviromet.2010.12.012.
13. Gan L, Liu YJ, Tian LX, Yue YR, Yang HJ, Liu FJ, et al. Effects of dissolved oxygen and dietary lysine levels on growth performance, feed conversion ratio and body composition of grass carp, *Ctenopharyngodon idella*. Aquac Nutr. 2013;19(6):860–9.
14. Gilad O, Yun S, Andree KB, Adkison MA, Zlotkin A, Bercovier H, et al. Initial characteristics of koi herpesvirus and development of a polymerase chain reaction assay to detect the virus in koi, *Cyprinus carpio* koi. Dis Aquat Organ. 2002;48(2):101–8.
15. Gomez DK, Joh SJ, Jang H, Shin SP, Choresca CH Jr, Han JE, et al. Detection of koi herpesvirus (KHV) from koi (*Cyprinus carpio* koi) broodstock in South Korea. Aquaculture. 2011;311(1–4):42.
16. Gray WL, Mullis L, Lapatra SE, Groff JM, Goodwin A. Detection of koi herpesvirus DNA in tissues of infected fish. J Fish Dis. 2002;25(3):171–8. https://doi.org/10.1046/j.1365-2761.2002.00355.x.
17. Hedrick RP, Gilad O, Yun S, Spangenberg JV, Marty GD, Nordhausen RW et al. A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. Journal of Aquatic Animal Health. 2000;12(1):44–57. https://doi.org/10.1577/1548-8667(2000)012<0044:ahawmm>2.0.co;2.
18. Hedrick R, Gilad O, Yun S, Spangenberg J, Marty G, Nordhausen R, et al. A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. J Aquat Anim Health. 2000;12(1):44–57.
19. Huat LK, Kueh S, Kwang PY, Nagasawa K. Current status of transboundary fish diseases in Singapore: occurrence, surveillance, research and training. Aquaculture Dept., SEAFDEC, Tigbauan, Iloilo (Philippines), [mailto:aqdchief@seafdec.org.ph]; 2004.
20. Ishioka T, Yoshizumi M, Izumi S, Suzuki K, Suzuki H, Kozawa K, et al. Detection and sequence analysis of DNA polymerase and major envelope protein genes in koi herpesviruses derived from *Cyprinus carpio* in Gunma prefecture. Jpn Vet Microbiol. 2005;110(1–2):27–33. https://doi.org/10.1016/j.vetmic.2005.07.002.
21. Kanchanakhan S. Current status of transboundary fish diseases in Thailand: occurrence, surveillance, research and training. Aquaculture Dept., SEAFDEC, Tigbauan, Iloilo (Philippines), [mailto:aqdchief@seafdec.org.ph]; 2004.
22. Laila H, Hamid H. Current status of transboundary fish diseases in Brunei Darussalam: occurence, surveillance, research and training. Aquaculture Dept., SEAFDEC, Tigbauan, Iloilo (Philippines), [mailto:aqdchief@seafdec.org.ph]; 2004.
23. Latiff FA. Current status of transboundary fish diseases in Malaysia: occurrence, surveillance, research and training. Aquaculture Dept., SEAFDEC, Tigbauan, Iloilo (Philippines), [mailto:aqdchief@seafdec.org.ph]; 2004.
24. Meyer K, Bergmann SM, Van der Marel M, Steinhagen D. Detection of Koi herpesvirus: impact of extraction method, primer set and DNA polymerase on the sensitivity of polymerase chain reaction examinations. Aquac Res. 2012;43(6):835–42. https://doi.org/10.1111/j.1365-2109.2011.02897.x.
25. Nagasawa K. Koi herpesvirus. A new threat to fish culture: mobilizing Southeast Asian capacity. Fish for the people. 2004;2(3):28–32.
26. Neukirch M, Kunz U. Isolation and preliminary characterization of several viruses from koi (*Cyprinus carpio*) suffering gill necrosis and mortality. Bull Eur Assoc Fish Pathol. 2001;21(4):125–35.
27. Omori R, Adams B. Disrupting seasonality to control disease outbreaks: the case of koi herpes virus. J Theor Biol. 2011;271(1):159–65. https://doi.org/10.1016/j.jtbi.2010.12.004.
28. Perelberg A, Smirnov M, Hutoran M, Diamant A, Bejerano Y, Kotler M. Epidemilogical Description of A New Viral Disease Afflicting Cultured *Cyprinus Carpio* In Israel. 2003.
29. Perelberg A, Ronen A, Hutoran M, Smith Y, Kotler M. Protection of cultured *Cyprinus carpio* against a lethal viral disease by an attenuated virus vaccine. Vaccine. 2005;23(26):3396–403.
30. Pokorova D, Vesely T, Piackova V, Reschova S, Hulova J. Current knowledge on koi herpesvirus (KHV): a review. Vet Med. 2005;50(139):V147.
31. Racy B. Current status of transboundary fish diseases in Cambodia: occurrence, surveillance, research and training. Aquaculture Dept., SEAFDEC, Tigbauan, Iloilo (Philippines), [mailto:aqdchief@seafdec.org.ph]; 2004.
32. Reed AN, Izume S, Dolan BP, LaPatra S, Kent M, Dong J, et al. Identification of B cells as a major site for cyprinid herpesvirus 3

latency. J Virol. 2014;88(16):9297–309. https://doi.org/10.1128/JVI.00990-14.
33. Reed AN, Putman T, Sullivan C, Jin L. Application of a nanoflare probe specific to a latency associated transcript for isolation of KHV latently infected cells. Virus Res. 2015;208:129–35. https://doi.org/10.1016/j.virusres.2015.06.003.
34. Reed A, Lin L, Ostertag-Hill C, Wang Q, Wu Z, Miller-Morgan T, et al. Detection of ORF6 protein associated with latent KHV infection. Virology. 2017;500:82–90. https://doi.org/10.1016/j.virol.2016.09.030.
35. Regidor SE, Albaladejo JD, Somga JR. Current status of transboundary fish diseases in the Philippines: occurrence, surveillance, research and training. Aquaculture Dept., SEAFDEC, Tigbauan, Iloilo (Philippines), [mailto:aqdchief@seafdec.org.ph]; 2004.
36. Ronen A, Perelberg A, Abramowitz J, Hutoran M, Tinman S, Bejerano I, et al. Efficient vaccine against the virus causing a lethal disease in cultured *Cyprinus carpio*. Vaccine. 2003;21(32):4677–84.
37. Sano M, Ito T, Kurita J, Yanai T, Watanabe N, Miwa S, et al. First detection of koi herpesvirus in cultured common carp *Cyprinus carpio* in Japan. Fish Pathol. 2004;39(3):165–8.
38. St-Hilaire S, Beevers N, Way K, Deuff R, Martin P, Joiner C. Reactivation of koi herpesvirus infections in common carp *Cyprinus carpio*. Dis Aquat Organ. 2005;67(1):15–23.
39. Sunarto A, Widodo, Taukhid, Koesharyani I, Supriyadi H, Gardenia L et al. Current status of transboundary fish diseases in Indonesia: occurrence, surveillance, research and training. Aquaculture Dept., SEAFDEC, Tigbauan, Iloilo (Philippines), [mailto:aqdchief@seafdec.org.ph]; 2004.
40. Sunarto A, Rukyani A, Itami T. Indonesian experience on the outbreak of koi herpesvirus in koi and carp (*Cyprinus carpio*). Bull Fish Res Agen. 2005;2(395):15–21.
41. Takashima Y, Watanabe N, Yanai T, Nakamura T. The status of koi herpesvirus disease outbreaks in Lake Kasumigaura and Kitaura. Bull Ibaraki Prefect Freshw Fish Exp Stn. 2004;39:1–8.
42. Thiery R, Pozet F, Kinkelin P. Two viral diseases emerging in the fishes: the nodavirosis of the marines species and the koi herpesvirosis of the carp, *Cyprinus carpio*. Epidemiol Sante Anim. 2003;44:83–94.
43. Tu C, Lin S-Y, Sung H-T. Current status of koi herpesvirus disease in Taiwan. Aquaculture Dept., SEAFDEC, Tigbauan, Iloilo (Philippines), [mailto:aqdchief@seafdec.org.ph]; 2004.
44. Waltzek TB, Kelley GO, Stone DM, Way K, Hanson L, Fukuda H, et al. Koi herpesvirus represents a third cyprinid herpesvirus (CyHV-3) in the family *Herpesviridae*. J Gen Virol. 2005;86(6):1659–67. https://doi.org/10.1099/vir.0.80982-0.
45. Xu J-R, Bently J, Beck L, Reed A, Miller-Morgan T, Heidel JR, et al. Analysis of koi herpesvirus latency in wild common carp and ornamental koi in Oregon, USA. J Virol Methods. 2013;187(2):372–9. https://doi.org/10.1016/j.jviromet.2012.11.015.
46. Yuasa K, Sano M, Kurita J, Ito T, Iida T. Improvement of a PCR method with the Sph I-5 primer set for the detection of koi herpesvirus (KHV). Fish Pathol. 2005;40(1):37–9. https://doi.org/10.3147/jsfp.40.37.

Hervesvirus

ORIGINALITY REPORT

17%	6%	15%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Muhamad Amin. "Marine protease-producing bacterium and its potential use as an abalone probiont", Aquaculture Reports, 2018 Publication	3%
2	Phillips, A. J., and C. Simon. "Simple, Efficient, and Nondestructive DNA Extraction Protocol for Arthropods", Annals of the Entomological Society of America, 1995. Publication	3%
3	St-Hilaire, S, N Beevers, K Way, RM Le Deuff, P Martin, and C Joiner. "Reactivation of koi herpesvirus infections in common carp Cyprinus carpio", Diseases of Aquatic Organisms, 2005. Publication	3%
4	ss.nria.affrc.go.jp Internet Source	1%
5	K. E. Eide. "Investigation of Koi Herpesvirus Latency in Koi", Journal of Virology, 05/15/2011 Publication	1%
6	Gomez, D.K.. "Detection of koi herpesvirus	

	(KHV) from koi (Cyprinus carpio koi) broodstock in South Korea", Aquaculture, 20110203 Publication	1%
7	Muhamad Amin. "Screening of Cellulose - Degrading Bacteria Associated with Gastrointestinal Tract of Hybrid Abalone as Probiotic Candidates", International Journal of Aquaculture, 2016 Publication	1%
8	Submitted to Universiti Kebangsaan Malaysia Student Paper	<1%
9	Gilad, O, S Yun, KB Andree, MA Adkison, A Zlotkin, H Bercovier, A Eldar, and RP Hedrick. "Initial characteristics of koi herpesvirus and development of a polymerase chain reaction assay to detect the virus in koi, Cyprinus carpio koi", Diseases of Aquatic Organisms, 2002. Publication	<1%
10	www.nature.com Internet Source	<1%
11	Tanmoy Das, Amrita Banerjee. "Distribution, molecular characterization and diversity of banana bunchy top virus in Tripura, India", VirusDisease, 2018 Publication	<1%
12	Gotesman, M, H Soliman, and M El-Matbouli. "Antibody screening identifies 78 putative host	<1%

proteins involved in Cyprinid herpesvirus 3 infection or propagation in common carp, *Cyprinus carpio L*", Journal of Fish Diseases, 2013.
Publication

---

13 Avarre, J.C.. "Investigation of Cyprinid herpesvirus-3 genetic diversity by a multi-locus variable number of tandem repeats analysis", Journal of Virological Methods, 201105 <1%
Publication

---

14 eprints.keele.ac.uk <1%
Internet Source

---

15 bictel.ulg.ac.be <1%
Internet Source

---

16 Submitted to Coventry University <1%
Student Paper

---

17 Kalamathy Murugananthan, Pethirupillai A. D. Coonghe, Natkunam Ketheesan, Faseeha Noordeen. "Comparison of a rapid immuno-chromatography assay with a standard ELISA for the detection of IgM and IgG antibodies against dengue viruses", VirusDisease, 2018 <1%
Publication

---

18 veterinaryresearch.biomedcentral.com <1%
Internet Source

---

nepis.epa.gov

19	Internet Source	<1%
20	Submitted to Sparsholt College, Hampshire Student Paper	<1%
21	www.ncbi.nlm.nih.gov Internet Source	<1%
22	www.scribd.com Internet Source	<1%
23	Submitted to National University of Singapore Student Paper	<1%
24	repository.seafdec.org.ph Internet Source	<1%
25	www.ornamentalfish.org Internet Source	<1%
26	T Haetrakul, S G Dunbar, N Chansue. " Antiviral activities of (Burm.f.) Lindau extract against Cyprinid herpesvirus 3 in koi (koi) ", Journal of Fish Diseases, 2018 Publication	<1%
27	Submitted to Murdoch University Student Paper	<1%
28	Muhamad Amin, Mark Adams, Christopher J. S. Bolch, Christopher M. Burke. "In vitro screening of lactic acid bacteria isolated from	<1%

gastrointestinal tract of Atlantic Salmon (Salmo salar) as probiont candidates", Aquaculture International, 2016

Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		