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ORIGINAL ARTICLE

The susceptibility of silver crucian carp (*Carassius auratus langsdorfii*) to infection with koi herpesvirus (KHV)

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Abstract

Koi herpesvirus (KHV) infections cause high mortality in carp (*Cyprinus carpio*). This study compared the susceptibility of silver crucian carp (*Carassius auratus langsdorfii*), also called ginbuna, and koi carp to KHV infection. Silver crucian carp and koi carp were challenged with KHV by both intraperitoneal injection and immersion, respectively, and kept in tanks at 22°C. All KHV-exposed koi carp died within 14 days post-infection (dpi), whereas no clinics nor mortality was observed in the KHV-exposed silver crucian carp. KHV DNA was detected in both koi and silver crucian carp shortly after infection. At 7 dpi, the copy numbers of KHV genome were increased in koi carp but decreased in silver crucian carp. Using reverse transcriptase PCR, KHV mRNA was detected in koi carp but not in silver crucian carp. Cell cultivation on common carp brain (CCB) cell samples from koi carp caused KHV-associated cytopathic effects in CCB cells. Therefore, we concluded that KHV replicated in koi carp but not in silver crucian carp and that silver crucian carp is not susceptible to infection with KHV.

KEYWORDS

ginbuna, koi herpesvirus (KHV), mRNA-specific RT-PCR with KHV, real-time PCR, silver crucian carp, susceptibility

1 | INTRODUCTION

Koi herpesvirus (KHV; syn. cyprinid herpesvirus 3, CyHV-3) is a viral pathogen causing severe mortality in varieties of *Cyprinus carpio* such as the common carp and ornamental (koi) carp. *Cyprinus carpio* appears to be the only species susceptible to KHV disease (Hedrick et al., 2000). Moreover, the World Organisation for Animal Health (OIE, 2018) stated that the KHV host range includes carp hybrids and goldfish–carp hybrids, such as crucian carp × koi carp, koi × common carp (ghost carp), goldfish × common carp and goldfish × koi carp. However, KHV genome copies have been detected in goldfish (*Carassius auratus*) cohabiting with KHV-infected carp by conventional PCR and loop-mediated isothermal amplification (LAMP) (El-Matbouli, Saleh, & Soliman, 2007; Sadler, Marecaux, & Goodwin,

2008), and nested PCR has been used to confirm the presence of KHV genome copies in several goldfish varieties as well as in grass carp (*Ctenopharyngodon idella*), ide (*Leuciscus idus*) and ornamental catfish (*Ancistrus* sp.) (Bergmann et al., 2009; OIE, 2018). Moreover, KHV has been detected in tissue samples and separated leucocytes from goldfish by PCR, in situ hybridization (ISH) and immunofluorescence antibody technique (IFAT) (Bergmann et al., 2010). To demonstrate KHV replication in goldfish, El-Matbouli and Soliman (2011) attempted to detect viral mRNA by reverse transcriptase PCR (RT-PCR) using a KHV thymidine kinase gene primer set (Bercovier et al., 2005). Furthermore, an RT-PCR with improved specificity for mRNA has been developed using a primer set designed to cover an intron/exon junction in the KHV genome (Yuasa et al., 2012).

Interestingly, goldfish did not show any clinical signs after KHV exposure (Ronen et al., 2003). Hedrick, Waltzek, and McDowell (2006)

Hyoung Jun Kim and Se Ryun Kwon contributed equally to this work.

also reported detection of KHV genes in goldfish injected with KHV without mortality, and Yuasa, Sano, and Oseko (2013) reported that goldfish exposed to KHV did not transmit the virus to carp. Thus, there are contradictory results on the susceptibility of goldfish to KHV.

To propagate and isolate KHV, CCB (common carp brain) and KF-1 (koi fin) cell lines, which were derived from common carp and koi carp, respectively, are commonly used due to their susceptibility to the virus. The AU fin cell line from goldfish displayed cytopathic effects (CPEs) when inoculated with KHV but was not included in the study because the viral titre remained very low (Davidovich, Dishon, Ilouze, & Kotler, 2007).

However, a newly developed cell line derived from silver crucian carp, *Carassius auratus langsdorfii* (Cyprinidae), demonstrated to be as susceptible to KHV as CCB and KF-1 (K. Yuasa et al., unpublished). This could indicate that silver crucian carp is more susceptible to KHV than goldfish. Silver crucian carp is known to be highly or partly susceptible to infection with the goldfish herpesvirus CyHV-2 (Ito & Maeno, 2014; Nanjo et al., 2017). However, there are no reports on the susceptibility of silver crucian carp to KHV.

In this study, we investigated the replication of KHV in koi and silver crucian carp after experimental exposure to KHV in order to evaluate their susceptibility to this pathogenic virus.

2 | MATERIALS AND METHODS

2.1 | Virus

In this study, we used KHV-type strain NRIA0301 originally isolated from naturally KHV-infected common carp at a fish farm in Japan in 2003 (Sano et al., 2004). The virus was identified as genotype variant A1 using a method according to Kurita et al. (2009). The virus was propagated in CCB cells maintained at 20°C in MEM₁₀, a minimum essential medium (MEM, Gibco, Invitrogen Co.) supplemented with 10% foetal bovine serum (FBS) and 1 × concentrated antibiotic–antimycotic liquid (Gibco, Invitrogen Co.). Common carp brain cells inoculated with KHV were also incubated at 20°C. After 10 days, when CPE was observed in more than 80% of the cells, the cell culture supernatant was harvested and centrifuged at 2,000 g for 15 min at 4°C, divided into aliquots and stored at –80°C.

A 10-fold dilution series (up to 10^{–6}) of the supernatant in MEM₂ (MEM supplemented with 2% FBS and 1 × concentrated antibiotic–antimycotic liquid) was prepared, and 50 µl of each dilution was inoculated in eight replicates on 24-hr-old CCB cells seeded in 96-well plates. After incubation at 20°C for 14 days, the virus titres were measured as 50% of the tissue culture infective dose (TCID₅₀) per ml according to the method described by Karber (1931). The virus stock solution had a titre of 10^{5.8} × TCID₅₀ per ml.

2.2 | Fish

Koi herpesvirus-free koi carp (N = 25, body length range: 12–15 cm) were provided by the Niigata Prefectural Inland Water Fisheries

Experiment Station. Silver crucian carp (N = 80, body length, approximately 5 cm) were purchased from a commercial farm in Japan where silver crucian carp is cultured separately from other fish. Ten fish of each species were tested for KHV by PCR (Gilad et al., 2004) and cell culture using the CCB cell line to ensure that the fish were KHV-free.

2.3 | Infection experiment

Fish were intraperitoneally injected with 0.1 ml of KHV at a dose of 10^{3.8} × TCID₅₀ per fish, placed in 4-L aquaria with 10^{4.8} × TCID₅₀ per ml of KHV and transferred to a larger aquaria after 1 hr. Ten koi and silver crucian carp were kept separately in two 60-L aquaria to monitor mortality over 4 weeks. The remaining 70 silver crucian carp and 15 koi carp were kept separately in two 90-L aquaria for sample collection. Water temperature was kept at 22°C using a ceramic heater. The water was recirculated with an aeration filter. Adequate amounts of commercial dry pellet were given daily at maintenance level. Approximately 20 non-virus exposed koi carp and silver crucian carp were placed in separate 60-L aquaria as negative controls.

2.4 | Sampling

Ten silver crucian carp and three koi carp were randomly selected from the sampling aquaria at 0, 3, 5, 7 and 14 days post-viral inoculation (dpi) to determine KHV genome numbers and re-isolate the virus. The gills and fins (GF) as well as the spleens and kidneys (SK) were recovered and pooled for each fish as GF and SK tissue samples. The pooled organ samples were divided into two parts; approximately 15 mg was used for DNA/RNA extraction and the remainder for inoculation onto CCB cells.

2.5 | Extraction of nucleic acids and PCR

DNA/RNA AllPrep extraction kit (Qiagen) was used for both DNA and RNA extraction. KHV DNA was quantitatively analysed by the method of Gilad et al. (2004) on Mx3005P (Stratagene), and viral mRNA was detected according to the mRNA-specific RT-PCR method (Yuasa et al., 2012). Reverse transcriptase PCR was performed using SuperScript® One-Step RT-PCR System with Platinum® Taq (Life Technologies) and the forward primer (KHV RT F3: 5'-ATT GCC GCT GGA AGC CAG GT-'3) and reverse primer (KHV RT R1: 5'-TCA TGG GCA TCG ACA TCA TG-'3) at a final concentration of 0.5 µM each. The PCR programme was modified using 40 cycles and 65°C as annealing temperature. The amplified products (219 bp) were separated on 2% agarose gels (Agarose HS, Wako) and visualized using a Gel Doc/UV transilluminator (FAS-V; Nippon Genetics).

2.6 | Virus inoculation

The sampled organs were homogenized in 10 volumes of MEM containing 1% v/v of 10 × concentrated antibiotic–antimycotic liquid

using a disposal homogenizer (BioMasher II; Sarstedt). The homogenized samples were centrifuged at 2,000 g for 15 min at 4°C and the supernatants recovered. After incubation with 1% antibiotics at 4°C overnight, 10 and 50 µl of the supernatants were inoculated onto 24-hr-old CCB cells seeded in 1 ml MEM₂ per well using 24-well plates and incubated at 20°C for 14 days to monitor CPE.

3 | RESULTS

3.1 | Mortality

In the mortality monitoring aquariums, koi carp showed typical signs of KHV disease such as exfoliation of the epidermal cells and haemorrhage on the body surface and fins at 3 dpi. Moribund fish showed abnormal swimming, fin rot and enophthalmia followed by mortality at 5 dpi. Cumulative mortality in koi carp reached 100% at 13 dpi (Figure 1). In contrast, silver crucian carp showed neither signs of KHV disease nor mortality during the monitoring period (Figure 1). During this period, no clinic nor mortality was observed in non-exposed koi carp and silver crucian carp kept in separate tanks as negative controls (data not shown).

3.2 | Detection of viral DNA and copy numbers

The KHV genome copy numbers in the gill/fin (GF) pool and the spleen/kidney (SK) pool of each fish are shown in Table 1, and the average copy numbers of 10 silver crucian carp and three carp are shown in Figure 2. KHV DNA was detected in all individuals of both species at 0 dpi (Table 1 and Figure 2). The average copy numbers per mg tissue were 4.88×10^3 and 2.06×10^4 copies/mg in GF and SK silver crucian carp samples, respectively, and 1.15×10^2 and 3.38×10^2 copies/mg in GF and SK koi carp samples, respectively. In koi carp, the average KHV genome copy numbers in GF and SK samples increased to 1.36×10^5 and 1.23×10^6 copies/mg at 3 dpi and peaked at 7 dpi with 2.14×10^6 and 2.29×10^7 copies/mg, respectively. However, at 14 dpi, KHV genome copy numbers in GF and SK tissue samples from the only surviving koi carp significantly decreased to 5.60×10^4 and 6.58×10^4 copies/mg, respectively (Table 1). Contrastingly, in silver crucian carp, the average

KHV genome copy numbers detected in GF and SK samples decreased to 8.9×10^1 and 1.09×10^4 copies/mg at 3 dpi and to a minimum of 1.94 and 3.14×10^2 copies/mg at 7 dpi, respectively (Figure 2). However, at 14 dpi, KHV copies were still detectable at 6.59×10^2 and 8.58×10^2 copies/mg in GF and SK samples, respectively (Table 1).

3.3 | Re-isolation of infectious KHV using the CCB cell line

The supernatants of homogenized GF and SK from exposed koi carp and silver crucian carp were inoculated onto the CCB cell line. Infectious KHV were re-isolated from GF and SK pooled tissue samples of all exposed koi carp, while no KHV was isolated from any pooled tissue sample collected from silver crucian carp (Table 2).

3.4 | Detection of KHV mRNA in koi and silver crucian carp

Reverse transcriptase PCR confirmed the presence of KHV mRNA in GF and SK pooled tissue samples of koi carp at 3, 5 and 7 dpi, and the corresponding RT-PCR product bands became gradually stronger over time (Figure 3). In contrast, KHV mRNA was not detected in any of the pooled tissue samples collected from silver crucian carp within 7 dpi except for 1–2 faint RT-PCR product bands detected at 3 dpi in three fish (Figure 4).

4 | DISCUSSION

Koi herpesvirus is associated with significant morbidity and mortality in carp varieties. However, the susceptible host range among *Cyprinidae* species remains to be investigated. In this study, KHV-exposed koi carp by bath and intraperitoneal injection caused 100% mortality within 14 dpi (Figure 1). In contrast, silver crucian carp exposed to KHV in the same way dose showed neither mortality nor signs of KHV disease during the post-exposure monitoring period (Figure 1). Thus, we confirmed that silver crucian carp is not susceptible to KHV, although a cell line isolated from silver crucian carp displayed CPE after inoculation with KHV.

Similarly, it has been reported that goldfish did not show any clinical signs after KHV exposure by intraperitoneal injection or by cohabitation with KHV-infected koi carp (Hedrick et al., 2006; Yuasa et al., 2013), and KHV DNA was also not detectable in surviving goldfish at 25 dpi (Hedrick et al., 2006). In this study, we compared KHV genome copy numbers and the infectious KHV load of GF and SK pooled tissue samples from KHV-exposed koi and silver crucian carp up to 14 dpi (Table 1 and Figure 2). At 0 dpi, KHV DNA was detected by qPCR in all individuals of both fish species (Table 1). The calculated average KHV genome copy numbers in GF and SK samples of koi carp were 1.15×10^2 and 3.38×10^2 copies/mg, respectively (Figure 2). At 3 days, the average KHV genome copy numbers in GF and SK samples of koi carp increased 1.36×10^5 and 1.23×10^6

FIGURE 1 Mortality in KHV-exposed koi and silver crucian carp

TABLE 1 Calculation of KHV DNA genome copy numbers per tissue (mg) using qPCR

Fish species	Fish number	Sampling days											
		0 days			3 days			5 days			7 days		
		GF ^a	SK ^b		GF	SK		GF	SK		GF	SK	
Silver crucian carp (<i>Carassius auratus langsdorffii</i>)	1	3.27 × 10 ³	2.65 × 10 ⁴		1.17 × 10 ¹	6.98 × 10 ¹		5.12 × 10 ¹	3.01 × 10 ³		1.06 × 10 ¹	1.40 × 10 ²	UD
	2	3.00 × 10 ³	3.64 × 10 ⁴		7.86 × 10 ²	8.72 × 10 ⁴		1.26 × 10 ²	5.08 × 10 ²		8.86	1.54 × 10 ²	UD
	3	4.68 × 10 ³	4.06 × 10 ³		3.92	3.22 × 10 ²		UD	9.30 × 10 ²		UD	7.57 × 10 ¹	UD
	4	8.90 × 10 ³	9.77 × 10 ³		1.47 × 10 ¹	9.57 × 10 ²		6.96 × 10 ²	4.35 × 10 ³		UD	2.86 × 10 ²	2.55 × 10 ¹
	5	1.07 × 10 ⁴	1.11 × 10 ⁴		2.82	1.67 × 10 ⁴		6.87 × 10 ¹	2.86 × 10 ²		UD	7.46	UD
	6	1.66 × 10 ³	5.26 × 10 ³		UD	5.92 × 10 ²		UD	1.73 × 10 ³		UD	4.27 × 10 ²	4.04 × 10 ¹
	7	4.56 × 10 ³	1.09 × 10 ⁴		5.71	1.80 × 10 ³		1.84 × 10 ²	1.06 × 10 ³		UD	1.76 × 10 ³	UD
	8	1.23 × 10 ³	2.28 × 10 ⁴		1.12 × 10 ¹	7.29 × 10 ¹		UD	3.90 × 10 ²		UD	5.32 × 10 ¹	UD
	9	1.85 × 10 ³	1.43 × 10 ⁴		UD	1.18 × 10 ²		UD	8.00 × 10 ¹		UD	1.92 × 10 ²	UD
	10	9.04 × 10 ³	6.57 × 10 ⁴		6.39 × 10 ¹	1.58 × 10 ³		UD	1.65 × 10 ³		UD	5.46 × 10 ¹	UD
Koi carp (<i>Cyprinus carpio</i>)	1	1.31 × 10 ²	1.86 × 10 ²		3.08 × 10 ⁴	7.77 × 10 ⁵		9.49 × 10 ⁵	2.72 × 10 ⁶		4.73 × 10 ⁶	3.16 × 10 ⁷	5.60 × 10 ⁴
	2	1.21 × 10 ²	6.55 × 10 ²		3.39 × 10 ⁵	2.62 × 10 ⁶		4.64 × 10 ⁵	7.74 × 10 ⁵		6.25 × 10 ⁵	3.61 × 10 ⁷	-
	3	9.34 × 10 ¹	1.73 × 10 ²		4.04 × 10 ⁴	3.07 × 10 ⁵		3.78 × 10 ⁵	1.11 × 10 ⁶		1.07 × 10 ⁶	1.18 × 10 ⁶	-

Abbreviation: UD, under detection limit.

^aGill and fin.

^bSpleen and kidney.

FIGURE 2 Tissue-specific KHV load was determined by averaging KHV genome copy numbers per tissue (mg) using qPCR. (a) KHV copy numbers in gills and fin (GF) of silver crucian carp, (b) KHV copy numbers in gills and fin (GF) of koi carp, (c) KHV copy numbers in spleen and kidney (SK) of silver crucian carp, (d) KHV copy numbers in spleen and kidney (SK) of koi carp

per tissue, respectively (Figure 2). Thus, it was confirmed that the KHV genome copy number had significantly increased in koi carp 3 days after infection. At 7 dpi, the average KHV genome copy numbers in koi carp GF and SK were at a maximum level of 2.14×10^6 and 2.29×10^7 copies/mg, respectively (Figure 2). At 3, 5 and 7 dpi,

CPE caused by KHV was observed in CCB cells inoculated with supernatants of homogenized GF and SK samples of infected koi carp (Table 2). Thus, it was demonstrated that KHV genome copy number largely increased during the initial 7 dpi period and it showed a correlation between the mortality and the presence of infectious KHV

TABLE 2 CPE of inoculated CCB cells using GF and SK of KHV-exposed fish

Fish species	Fish number	Sampling days							
		3 days		5 days		7 days		14 days	
		GF ^a	SK ^b	GF	SK	GF	SK	GF	SK
Silver crucian carp (<i>Carassius auratus langsdorfii</i>)	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-
Koi carp (<i>Cyprinus carpio</i>)	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	N/A	N/A
	3	+	+	+	+	+	+	N/A	N/A

^aGill and fin.

^bSpleen and kidney.

FIGURE 3 KHV mRNA analysis in KHV-exposed koi carp. (a) Gills and fins (GF) and (b) spleens and kidneys (SK)

recovered from tissue samples. At 14 dpi, infectious virus was re-isolated using the CCB cell line from only one surviving koi carp, but the KHV genome copy numbers significantly decreased to 5.60×10^4 and 6.58×10^4 copies/mg of GF and SK tissue, respectively (Table 1). Thus, it was suggested that the surviving fish appeared to recover from the KHV infection.

In silver crucian carp, the KHV genome copy levels in GF and SK tissue samples were already reduced at 3 dpi and continuously decreased during the initial 7 dpi period (Figure 2). Moreover, the reduction in KHV genome copy numbers in the external organs of silver crucian carp (i.e. GF samples) was larger than in the internal organs (i.e. SK samples), which may be caused by water exchange and secretion of mucus. Furthermore, during the 14 dpi period, no

CPE was observed in CCB cells inoculated with homogenized supernatant from GF and SK samples of silver crucian carp (Table 2). Thus, the non-susceptibility of silver crucian carp to KHV infection was supported by the decrease and lack of detection of KHV DNA in GF and SK over time. Similarly, Hedrick et al. (2006) reported the absence of detectable KHV DNA in KHV-exposed goldfish at 25 dpi.

In this study, KHV-infected koi carps 1 and 3 had 3×10^4 and 4×10^4 copies/mg of the KHV genome, respectively, in GF samples at 3 dpi (Table 1) and caused CPE in the CCB cells. In contrast, samples from silver crucian carp 2 had a higher KHV copy number (8.72×10^4) at 3 dpi but did not cause any CPE in CCB cells (Tables 1 and 2). These results indicate that KHV lost infectivity in silver crucian carp and that the KHV genome copy numbers detected were

FIGURE 4 KHV mRNA analysis in KHV-exposed silver crucian carp. (a) Gills and fins (GF) and (b) spleens and kidneys (SK)

not related to infectivity in the cell cultures. These observations are equivalent to an earlier study by Yuasa et al. (2013) who showed that KHV-exposed goldfish did not transmit the virus after 48-hr cohabitation with naïve carp.

In this study, we investigated KHV mRNA levels as an indicator of virus replication in KHV-exposed koi and silver crucian carp (Figure 3). In koi carp, KHV mRNA was detected in GF and SK between 3 and 7 dpi (Figure 3). However, KHV mRNA was not detected in silver crucian carp except faint RT-PCR product bands obtained from some tissue samples at 3 dpi. Thus, it was demonstrated that KHV could not replicate in silver crucian carp. Bergmann et al. (2010) reported that KHV genome was detected in separated leucocytes from goldfish, suggesting that the infectious KHV was killed by phagocytosis of leucocytes in goldfish.

KHV DNA has been detected in infected carp, contaminated equipment (Fabian, Baumer, Adamek, & Steinhagen, 2016), plankton (Minamoto et al., 2011), molluscs (Kielinski et al., 2010), water plants and piscivorous birds such as herons or cormorants (Matsui et al., 2008). Our results indicate that the detection of viral DNA can be useful to check for evidence of non-replicating KHV in fish. However, the method cannot detect infectious KHV in fish. Therefore, the mRNA detection method is strongly suggested to confirm the existence of infectious virus and virus replication in fish or cell lines.

In conclusion, KHV DNA was detected in silver crucian carp exposed to KHV by combined injection and immersion. However, this species appeared not susceptible to KHV due to the lack of clinics, mortality and KHV replication in the fish. Koi herpesvirus is a DNA virus. However, except for *C. carpio*, it is difficult to determine the presence of infectious KHV in a fish species if only qPCR is used for detection. Furthermore, except for *C. carpio*, it is also difficult to determine the susceptibility of a fish species using only viral DNA detection methods. In this study, we suggest that positive KHV mRNA detection is evidence of KHV replication, but there is still a need to develop a method that distinguishes between infectious and non-infectious KHV at DNA level. In addition, the current OIE diagnostic manual only indicates molecular techniques for viral DNA detection except for the cell culture method. Therefore, we suggest the use of the mRNA detection and cell culture methods to verify infectious KHV for consideration to add as method into the OIE diagnostic manual.

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CONFLICT OF INTEREST

None.

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REFERENCES

- Bercovier, H., Fishman, Y., Nahary, R., Sinai, S., Zlotkin, A., Eynor, M., ... Hedrick, R. P. (2005). Cloning of the koi herpesvirus (KHV) gene encoding thymidine kinase and its use for a highly sensitive PCR based diagnosis. *BMC Microbiology*, 5, 13.
- Bergmann, S. M., Lutze, P., Schutze, H., Fisher, U., Dauber, M., Fichtner, D., & Kempter, J. (2010). Goldfish (*Carassius auratus auratus*) is a susceptible species for koi herpesvirus (KHV) but not for KHV disease (KHVD). *Bulletin of the European Association of Fish Pathologists*, 30, 74–84.
- Bergmann, S. M., Schutze, H., Fischer, U., Fichtner, D., Riechardt, M., Meyer, K., ... Kempter, J. (2009). Detection of koi herpes-virus (KHV) genome in apparently healthy fish. *Bulletin of the European Association of Fish Pathologists*, 29, 145–152.
- Davidovich, M., Dishon, A., Ilouze, M., & Kotler, M. (2007). Susceptibility of cyprinid cultured cells to cyprinid herpesvirus 3. *Archives of Virology*, 152, 1541–1546. <https://doi.org/10.1007/s00705-007-0975-4>
- El-Matbouli, M., Saleh, M., & Soliman, H. (2007). Detection of cyprinid herpesvirus type 3 in goldfish cohabiting with CyHV-3-infected koi carp (*Cyprinus carpio* koi). *Veterinary Record*, 161, 792–793.
- El-Matbouli, M., & Soliman, H. (2011). Transmission of Cyprinid herpesvirus-3 (CyHV-3) from goldfish to naïve common carp by cohabitation. *Research in Veterinary Science*, 90, 536–539. <https://doi.org/10.1016/j.rvsc.2010.07.008>
- Fabian, M., Baumer, A., Adamek, M., & Steinhagen, D. (2016). Transmission of Cyprinid herpes virus 3 by wild fish species—Results from infection experiments. *Journal of Fish Diseases*, 39, 625–628. <https://doi.org/10.1111/jfd.12399>
- Gilad, O., Yun, S., Zagmutt-Vergara, F. J., Leutenegger, C. M., Bercovier, H., & Hedrick, R. P. (2004). Concentrations of a Koi herpesvirus (KHV) in tissues of experimentally infected *Cyprinus carpio* koi as assessed by real-time TaqMan PCR. *Diseases of Aquatic Organisms*, 60, 179–187. <https://doi.org/10.3354/dao060179>
- Hedrick, R. P., Gilad, O., Yun, S., Spangenberg, J. V., Marty, G. D., Nordhausen, R. W., ... Eldar, A. (2000). A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. *Journal of Aquatic Animal Health*, 12, 44–57. [https://doi.org/10.1577/1548-8667\(2000\)012<0044:AHAWMM>2.0.CO;2](https://doi.org/10.1577/1548-8667(2000)012<0044:AHAWMM>2.0.CO;2)
- Hedrick, R. P., Waltzek, T. B., & McDowell, T. S. (2006). Susceptibility of koi carp, common carp, goldfish and goldfish × common carp hybrids to cyprinid herpesvirus-2 and herpesvirus-3. *Journal of Aquatic Animal Health*, 18, 26–34.
- Ito, T., & Maeno, Y. (2014). Susceptibility of Japanese Cyprininae fish species to cyprinid herpesvirus 2 (CyHV-2). *Veterinary Microbiology*, 169, 128–134. <https://doi.org/10.1016/j.vetmic.2014.01.002>
- Karber, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Naunyn-Schmiedeberg's Archiv Für Experimentelle Pathologie Und Pharmacologie*, 162, 480–483. <https://doi.org/10.1007/BF01863914>
- Kielinski, M., Kempter, J., Panic, R., Sadowski, J., Schutze, H., Ohlemeyer, S., & Bergmann, S. M. (2010). Detection of KHV in freshwater mussels and crustaceans from ponds with KHV history in common carp (*Cyprinus carpio*). *Israeli Journal of Aquaculture (Bamidgeh)*, 62, 28–37.
- Kurita, J., Yuasa, K., Ito, T., Sano, M., Hedrick, R. P., Engelsma, M. Y., ... Iida, T. (2009). Molecular epidemiology of koi herpesvirus. *Fish Pathology*, 44(2), 59–66. <https://doi.org/10.3147/jsfp.44.59>
- Matsui, K., Honjo, M., Kohmatsu, Y., Uchii, K., Yonekura, R., & Kawabata, Z. (2008). Detection and significance of koi herpesvirus (KHV) in

- freshwater environments. *Freshwater Biology*, 53, 1262–1272. <https://doi.org/10.1111/j.1365-2427.2007.01874.x>
- Minamoto, T., Honjo, M. N., Yamanaka, H., Tanaka, N., Itayama, T., & Kawabata, Z. (2011). Detection of cyprinid herpesvirus-3 DNA in lake plankton. *Research in Veterinary Science*, 90, 530–532. <https://doi.org/10.1016/j.rvsc.2010.07.006>
- Nanjo, A., Shibata, T., Saito, M., Yoshii, K., Tanaka, M., Nakanishi, T., ... Sano, M. (2017). Susceptibility of isogeneic ginebuna (*Carassius auratus langsdorfii* Temminck et Schlegel) to cyprinid herpesvirus-2 (CyHV-2) as a model species. *Journal of Fish Diseases*, 40(2), 157–168.
- OIE, World Organisation for Animal Health (2018). Koi herpesvirus disease. In Dr. Ingo Ernst (Ed.), *OIE, Manual of diagnostic tests for aquatic animals*. (pp. 1–18). OIE and Paris. Retrieved from http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/chapitre_koi_herpesvirus.pdf
- Ronen, A., Perelberg, A., Abramovitz, J., Hutoran, M., Tinman, S., Bejerano, I., ... Kotler, M. (2003). Efficient vaccine against the virus causing a lethal disease in cultured *Cyprinus carpio*. *Vaccine*, 21(32), 4677–4684. [https://doi.org/10.1016/S0264-410X\(03\)00523-1](https://doi.org/10.1016/S0264-410X(03)00523-1)
- Sadler, J., Marecaux, E., & Goodwin, A. E. (2008). Detection of koi herpesvirus (CyHV-3) in goldfish, *Carassius auratus* (L.), exposed to infected koi. *Journal of Fish Diseases*, 25, 171–178.
- Sano, M., Ito, T., Kurita, J., Yanai, T., Watanabe, N., Miwa, S., & Iida, T. (2004). First detection of koi herpesvirus in cultured common carp *Cyprinus carpio* in Japan. *Fish Pathology*, 39, 165–167. <https://doi.org/10.3147/jsfp.39.165>
- Yuasa, K., Kurita, J., Kawana, M., Kiryu, I., Oseko, N., & Sano, M. (2012). Development of mRNA-specific RT-PCR for the detection of koi herpesvirus (KHV) replication stage. *Diseases of Aquatic Organisms*, 100(1), 11–18. <https://doi.org/10.3354/dao02499>
- Yuasa, K., Sano, M., & Oseko, N. (2013). Goldfish is not a susceptible host of koi herpesvirus (KHV) disease. *Fish Pathology*, 48(2), 52–55. <https://doi.org/10.3147/jsfp.48.52>

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