Susceptibilities of three kinds of hybrids between crucian carp and common carp, Carassius cuvieri × Cyprinus carpio, Carassius buergeri grandoculis × Cyprinus carpio and Carassius buergeri subsp.1 × Cyprinus carpio to cyprinid herpesvirus 3 (CyHV-3)

Susceptibilities of three kinds of hybrids between crucian carp and common carp, *Carassius cuvieri* × *Cyprinus carpio*, *Carassius buergeri grandoculis* × *Cyprinus carpio* and *Carassius buergeri* subsp.1 × *Cyprinus carpio* to cyprinid herpesvirus 3 (CyHV-3)

Kei Yuasa[1*] and Takafumi Ito[2]

[1]*Production Engineering Division, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Iwate 027-0097, Japan*

[2]*Pathology Division, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Mie 519-0193, Japan*

ランニングタイトル：Susceptibilities of　crucian carp x common carp to KHV

キーワード：Susceptibilities, hybrids, crucian carp, common carp, KHV

連絡先：湯浅　啓、〒027-0097、岩手県宮古市崎山 4-9-1、TEL0193-63-8121、FAX0193-64-0134、Email: yuasa_kei32@fra.go.jp

Susceptibilities of three hybrids between crucian carp including *Carassius cuvieri* (*Ccu*), *Carassius buergeri grandoculis* (*Cbg*) and *Carassius buergeri* subsp.1 (*Cbs*) and common carp *Cyprinus carpio* (*Cc*) to KHV were evaluated in an infection experiment. Infection rates of KHV in each hybrid were 30% and 20% in *Ccu* × *Cc*, 40% and 30% in *Cbg* × *Cc*, and 10% and 0% in *Cbs* × *Cc*, at 14 and 44 dpe, respectively. This indicates that *Cbs* × *Cc* shows lower sensitivity to KHV than the other two hybrids. More importantly, *Ccu* × *Cc* and *Cbg* × *Cc* transmitted the virus to co-habited naïve koi.

ゲンゴロウブナ、ニゴロブナおよびナガブナとコイとの交雑種のコイヘルペスウイルス（KHV）に対する感受性

湯浅　啓・伊東尚史

ゲンゴロウブナ、ニゴロブナおよびナガブナとコイとの交雑種のコイヘルペスウイルス（KHV）に対する感受性について、ウイルス暴露後の感染状況および暴露 2 日後に各水槽に投入した健康ニシキゴイへのウイルス伝播の可否により評価した。試験期間中、ゲンゴロウブナ交雑種のみに死亡が認められた。ゲンゴロウブナおよびニゴロブナとの交雑種の KHV 検出率はナガブナ交雑種より高く、同居したニシキゴイへのウイルス伝播も認められた。以上より、ゲンゴロウブナおよびニゴロブナとの交雑種に KHV への感受性が認められ、かつウイルスの感染源になり得ると考えられた。

Cyprinid herpesvirus 3 (CyHV-3), known as koi herpesvirus (KHV), is a pathogen of koi and common carp *Cyprinus carpio* that causes significant disease and mortality in juvenile to adult of the species (Hedrick *et al*., 2000; Perelberg *et al*., 2003). Regarding host range of the virus, there has been a disagreement on infection status of the species in genus *Carassius* such as goldfish *Carassius auratus* and crucian carp *Carassius carassius*: they are susceptible to the virus or can be carriers of the virus. The previous reports mentioned that KHV DNA was detected from not only species in genus *Carassius* but also fish in other families (Sadler *et al.*, 2008; Bergmann *et al*., 2010). However, Yuasa *et al*. (2013) demonstrated that the goldfish exposed by the virus cannot transmit the virus to koi after 2 days viral exposure. Subsequent reports indicated that the virus did not replicate in crucian carp experimentally infected by the virus (Kim *et al*., 2019) as well as in goldfish inhabiting with common carp infected by KHV (Isaiah *et al*., 2021). Based on such information on viral detection or replication, the World Organisation for Animal Health (WOAH, 2022a) mentions that goldfish and crucian carp as well as grass carp *Ctenopharyngodon idella* are categorized in species for which there is insufficient evidence to fulfil the criteria for listing as susceptible to infection with KHV according to Chapter 1.5 of the Aquatic Animal Health Code (WOAH, 2022b). Thus, detecting viral replication in target fish or viral transmit from the fish to naïve fish is a key to determine whether the fish is a susceptible species to the pathogen.

There are several species or subspecies in genus *Carassius* in Japan endemic such as gengorobuna *Carassius cuvieri,* nigorobuna *Carassius buergeri grandoculis* and nagabuna *Carassius buergeri* subsp.1. Nigorobuna and nagabuna are endemic to Lake Biwa in Shiga Prefecture and Lake Suwa in Nagano Prefecture, respectively. On the other hand, a habitat area of gengorobuna has been spread to many freshwater sheds in Japan by releasing for game fishing. Although the WOAH categorizes common carp hybrids (e.g. *Cyprinus carpio* × *Carassius auratus*, *Cyprinus carpio* × *Carassius carassius*) as susceptible species to KHV, information of the susceptibility on hybrids of *Cyprinus*

carpio × species in genus *Carassius* is limited.

In this study, we evaluated susceptibilities of three kinds of hybrids between common carp and three species/subspecies of crucian carp to KHV by not only detecting viral DNA in the hybrids that were experimentally infected with KHV, but also finding viral transmission from the infected hybrids to naïve koi, and we further discussed on possibilities whether these hybrids can be a source of KHV infection.

Materials and methods

Fish

Adult hybrids of three species or subspecies of genus *Carassius* in female × common carp in mail; *Carassius cuvieri* (*Ccu*) × *Cyprinus carpio* (*Cc*), *Carassius buergeri grandoculis* (*Cbg*) × *Cc* and *Carassius buergeri* subsp.1 (*Cbs*) × *Cc*, which were produced by Masaoka *et al.* (2014), were used in this study. These hybrids were bred and reared in 10 t raceway tanks using running system of groundwater at Tamaki Field Station of Fisheries Technology Institute. The ranges of body weights of hybrids, *Ccu* × *Cc*, *Cbg* × *Cc* and *Cbs* × *Cc* were 26 – 43 g, 83 – 121 g and 47 – 78 g, respectively. Each hybrid including *Ccu* × *Cc* (n=60), *Cbg* × *Cc* (n=60) and *Cbs* × *Cc* (n=50) were divided in three 60 L aquaria by 20, 20 and 20 or 15, respectively and acclimatized to experimental environment with free-flowing groundwater adjusted at 20℃ by ceramic heater and aeration for 2 days prior to experimental infection.

For cohabitation with hybrids post viral exposure in each aquarium, KHV-free koi fingerlings (n=45, 13 – 19 g in a range of body weights) reared at Tamaki Field Station were transferred and acclimatized in 60 L aquarium under the same condition as each aquarium for the hybrids for 4 days prior to cohabitation.

Virus

KHV NRIA0301 that was isolated from cultured common carp was used in the experimental infection. The isolate (6 passages) stocked at -80℃ was thawed quickly, inoculated into newly sub-cultured CCB cells with 15 mL minimal essential medium (Gibco) supplemented with 2% foetal bovine serum in 75 cm^2 culture flask and incubated at 20℃ for 7 days. The viral supernatant was collected and used for experimental infection.

Experimental infection

Total 115 fish (40 of *Ccu* × *Cc*, 40 of *Cbg* × *Cc* and 35 of *Cb*s × *Cc*) in six aquaria (*Ccu*-Ex1 or 2, *Cbg*-Ex1 or 2 and *Cbs*-Ex 1 or 2, respectively) with 10 L grand water each were exposed to a dilution of the isolate ($1 \times 10^{5.8}$ $TICD_{50}$/10 L) for an hour with aeration. After viral exposure, each aquarium was supplied by the free-flowing groundwater adjusted at 20℃ with ceramic heater. Fish for non-infection group (20 of *Ccu* × *Cc*, 20 of *Cbg* × *Cc* and 15 of *Cb*s × *Cc*) were also prepared in three 60 L-aquaria (*Ccu*-C, *Cbg*-C and *Cbs*-C, respectively). Fish were fed with commercial dried pellet every day and died fish were monitored for 44 days.

Sampling and viral detection

At 14 and 44 days post viral exposure (dpe), a part of the pectoral fin (approximately 20 mg in wet tissue weight) of 10 fish in each hybrid (5 fish in each aquarium) was excised using anatomical scissors to perform biopsy. The fin tissue was homogenized in a homogenizer, and DNA was extracted using Qiagen DNA extraction MiniKit (Qiagen). The DNA extraction was used for quantitative analysis of KHV by a real-time PCR (Gilad *et al*., 2004). In addition, the amount of KHV DNA in the 20 mg gills of all sacrificed fish in aquarium Cc-Ex1 and Cc-C was analysed by the real-time PCR.

Cohabitation

To observe viral transmission from the infected hybrids to naive koi by detecting KHV DNA from koi with the real-time PCR, each 5 koi fingerling was cohabitated with the hybrids in 9 aquaria between 2 dpe and 28 dpe.

Results

Cumulative mortalities and KHV detection in dead fish

Cumulative mortalities in two infected aquaria, *Ccu*-Ex1 and *Ccu*-Ex2 were 40% and 20%, respectively and unexpectedly mortality in non-infected aquarium, *Ccu*-C was also observed (10% in cumulative mortality). All moribund or dead fish in aquaria *Ccu*-Ex1 and *Ccu*-Ex2 showed clinical signs including sluggish and hemorrhage in fins, but individuals in aquarium *Cc*-C did not show any clinical signs. In contrast, no mortality was observed in neither infected nor non-infected (control) aquaria for *Cbg* × *Cc* and *Cb*s × *Cc*. Individuals in these aquaria did not show any clinical signs. (Table 1).

KHV DNA was detected from the fins of all dead fish in aquarium *Ccu*-Ex1. The numbers of KHV DNA in the fins were $5.45 \times 10^3 - 3.81 \times 10^5$ copies/20 mg tissues, which were much lower than those in the gills of dead koi cohabitated in *Ccu*-Ex1 and *Ccu*-Ex2. No KHV DNA was detected from the fins of 2 deceased dead hybrids in *Ccu*-C aquarium (Table 2) .

KHV DNA detection from survival fish post viral exposure

Table 3 shows KHV DNA copies number in 20 mg fin tissue of 5 individuals in each hybrid sampled at 14 and 44 dpe. Numbers of fish positive for KHV in hybrids *Ccu* × *Cc*, *Cbg* × *Cc* and

*Cb*s × *Cc* were 3, 4 and 1 at 14 dpe and 2, 3 and 0 at 44 dpe, respectively. Individuals detected with KHV DNA over 1 $\times 10^5$ copies/20 mg tissue were observed in *Ccu* × *Cc* and *Cbg* × *Cc*. In an individual of *Cb*s × *Cc*, 1.92 x10^4 copies/20 mg tissue of KHV DNA were observed at 14 dpe, but not at 44 dpe.

Cohabitation

Table 4 shows numbers of sacrificed koi post cohabitation with each hybrid in 9 aquaria and KHV DNA copies in the gills of the koi. All koi cohabited with *Ccu* × *Cc* were died with a high number of KHV DNA copies in the gills. KHV DNA was detected from the gills of koi cohabited with hybrid *Cbg* × *Cc*, although no mortality was observed in the koi. In contrast, neither mortality nor positive for KHV was found in koi cohabited with hybrid *Cb*s × *Cc* (Table 4).

Discussion

In this study, three kinds of hybrids between crucian carp and common carp, *Carassius cuvieri* (*Ccu*) × *Cyprinus carpio* (*Cc*), *Carassius buergeri grandoculis* (*Cbg*) × *Cc* and *Carassius buergeri* subsp.1 (*Cb*s) × *Cc* were exposed to KHV and mortalities, the virus load in the hybrids and infectious virus shed from the hybrids were monitored to evaluate their susceptibilities to the virus and possibilities that the hybrids be a source of the viral infection.

Although the present study did not include an experimental infection to common carp or koi, at least two hybrids, *Cbg* × *Cc* and *Cbs* × *Cc*, each of which showed no morality in the present study, were thought to be lower susceptible to the virus than common carp. Also, the amount of KHV DNA in the fins of dead *Ccu* × *Cc* was much lower than that in the gills of dead koi cohabited with *Ccu* × *Cc* in the same aquarium (Table 2, 4). Furthermore, KHV was detected from the fins of not more

than 40 % of each hybrid at 14 dpe (Table 3) in contrast with that all of koi exposed to the virus were infected by the virus at 2-21 dpe in our previous study (Yuasa *et al*., 2021). These show differences in susceptibility to KHV between the three hybrids used in the present study and common carp or koi. An experimental infection with KHV to the hybrid of goldfish × common carp in other laboratory also indicated that the hybrid showed much lower sensitivity to virus than common carp or koi (Hedrick *et al*., 2012). On the other hand, a report mentioned that hybrids of crucian carp × koi and goldfish × koi exposed to KHV showed high and intermediate mortalities, respectively (Bargmann *et al*., 2010). It seems that susceptibilities of common carp/koi-hybrids to KHV may depend on species or subspecies in crucian carp used for crossbreed.

In the present study, mortality was observed in a hybrid *Ccu* × *Cc* even though without viral exposure, *Ccu*-C group. Therefore, it is difficult to evaluate the difference in susceptibility to the virus among three kinds of hybrids refer to cumulative mortalities in the test. However, detection rates of KHV DNA in *Cbs* × *Cc* hybrid were lower than those in the other two hybrids at both 14 and 44 dpe (Table 3), indicating that susceptibility of *Cbs* × *Cc* to the virus was lower than the other two hybrids. Further, the result that KHV DNA was not detected from koi cohabited with KHV-exposed *Cbs* × *Cc* also shows lower possibility of the hybrid to become a virus carrier. On the other hand, detection rates of KHV DNA in survivors of *Ccu* × *Cc* and those in *Cbg* × *Cc* were almost same, suggesting that their susceptibility to KHV is equivalent. Although only *Ccu* × *Cc* shed infectious virus in number enough to kill the koi, the hybrids were reared under the condition with a presence of mortality even without KHV infection. This suggests that *Cbg* × *Cc* hybrid may shed infectious virus in number enough to kill koi under some kinds of stress condition.

KHV DNA was detectable in subclinical common carp sampled in rivers where KHV occurred (Fujioka *et al*., 2015), but the origin of the infection has not been found out hitherto. Although KHV DNA was detected in the brain of common carp for >1 yr post viral exposure in an experimental

infection (Miwa *et al*., 2015), whether these fish can transmit the virus to other fish as a carrier of the virus is unknown. On the other hand, it is known that hybrids of common carp × crucian carp or goldfish have been appeared in natural habitats from way back (Suzuki, 1966). Take the fact that *Carassius cuvieri* widely habitats in natural water system in Japan into consideration, it is possible that hybrid of *Carassius cuvieri* × *Cyprinius carpio* also nationally habitat. In addition, a novel hybrid of common carp *Cyprinus carpio* × a species of genus *Carassius* has been found in a creek in Japan, recently (Hibino and Kukita, 2019). Such information in addition to our results in the present study suggests that hybrids of *Cyprinus carpio* × specie/subspecies of genus *Carrasius* can be carriers of KHV and transmit the virus to *Cyprinus carpio* in natural water system of Japan.

In this study, we revealed that at least two hybrids of species/subspecies in crucian carp, *Carassius curvieri* and *C. buergeri grandoculis*, × common carp *Cyprinus carpio* can be in a source of infection with KHV. On the other hand, it is suggested that crucian carp as well as goldfish does not be a carrier of KHV in the previous studies (Yuasa *et al*., 2013; Kim *et al*., 2019). A hybrid of crucian carp or goldfish × common carp shows intermediate in morphology between them (Talor and Mahon, 1977). Therefore, it is necessary to carefully identify species/subspecies of fish used in experimental infection when a range of KHV-susceptible species in Cyprinidae is investigated. Furthermore, when a hybrid of crucian carp/ goldfish × common carp that may be a carrier of the virus is introduced in KHV-free area, inspection to prove their free from KHV is essential to avoid contamination of the virus.

Acknowledgement

We thank Dr. Motohiko Sano, Professor of University of Marine Science and Technology for reviewing the manuscript. We also thank Dr. Hiroyuki Nagoya, National Research Institute of Aquaculture, Japan Fisheries Research and Education Agency in former for providing hybrids

between crucian carp and common carp.

References

Bergmann S.M., H. Schutze, U. Fischer, D. Fichtner, M. Riechardt, K. Meyer, D. Schrudde and J. Kempter (2009): Detection of koi herpesvirus (KHV) genome in apparently healthy fish. *Bull. Eur. Assoc. Fish Pathol.*, **29**: 145–152.

Bergmann, S. M., J. Sadowski, M. Kiełpiński, M. Bartłomiejczyk, D. Fichtner, R. Riebe, M. Lenk and J. Kempter (2010): Susceptibility of koi × crucian carp and koi × goldfish hybrids to koi herpesvirus (KHV) and the development of KHV disease (KHVD). *J. Fish Dis.*, **33**: 267–272.

Fujioka H., K. Yamasaki, K. Furusawa, K. Tamura and others (2015): Prevalence and characteristics of Cyprinid herpesvirus 3 (CyHV-3) infection in common carp (*Cyprinus carpio* L.) inhabiting three rivers in Kochi Prefecture, Japan. *Vet. Microbiol.*, **175**: 362–368.

Gilad O., S. Yun, F.J. Zagmutt-Vergara, C.M. Leutenegger, H. Bercovier and R.P. Hedrick (2004): Concentration of a koi herpesvirus (KHV) in tissues of experimentally infected Cyprinus carpio koi as assessed by real-time TaqMan PCR. *Dis. Aquat. Org.*, **60**: 179-187.

Hedrick R.P., O. Gilad, S. Yun and V. Spangenberg (2000): A herpesvirus associated with mass mortality of juvenile and adult koi, a strain of common carp. *J. Aquat. Anim. Health*, **12**: 44-57.

Hedrick, R.P., T.B. Waltzek and T.S. McDowell (2012): Susceptibility of koi carp, common carp, Goldfish, and Goldfish × common carp hybrids to Cyprinid herpesvirus-2 and herpesvirus-3. *J. Aquat. Anim Health*, **18**: 26–34.

Hibino, Y. and N. Kukita (2019): A record of a hybrid specimen of Carassius sp. And Cyprinus carpio (Cypriniformes:Cyprinidae), collected from Kagoshima Prefecture, Japan. *Nature of Kagoshima*, **46**: 235–238 (In Japanese).

Isaiah E.T., K.P. Soumesh, W. Keifer, S. Vikash, H. Sophie, K.M. Sunil and B.D.P. Nicholas (2021): Susceptibility of Pimephales promelas and Carassius auratus to a strain of koi herpesvirus isolated from wild Cyprinus carpio in North America. Scientific Reports, 11: 1985, https://doi.org/10.1038/s41598-021-81477-0.

Kim H. J., S.R. Kwon, N.J. Olesen and K. Yuasa (2019): The susceptibility of silver crucian carp (*Carassius auratus langsdorfii*) to infection with koi herpesvirus (KHV). *J. Fish Dis*., **42**: 1333-1340.

Masaoka T., H. Nagoya, H. Okamoto, K. Araki, A. Fujiwara, T. Kobayashi (2014): Identification of the hybrid between Cyprinus carpio and Japanese Carassius species using nuclear genes and mitochondrial gene region. *DNA polymorphism*, **22**: 80-83.

Miwa S, I. Kiryu, K. Yuasa, T. Ito, T. Kaneko (2015): Pathogenesis of acute and chronic diseases caused by cyprinid herpesvirus-3. *J. Fish Dis.*, **38**: 695–712.

Perelberg A., M. Smirnov, M. Hutoran, A. Diamant, Y. Bejerano and M. Kotler (2003): Epidemiological description of a new viral disease afflicting cultured Cyprinus carpio in Israel. *Isr. J. Aquac. - Bamidgeh*, **55**: 5-12.

Sadler J., E. Marecaux and A.E. Goodwin (2008): Detection of koi herpes virus (CyHV-3) in goldfish, Carassius auratus (L.), exposed to infected koi. *J. Fish Dis.*, **31**: 71–72.

Suzuki R. (1966): Thremmatological aspects on the hybridization in fish. *Bull. Jap. Soc. Sci. Fish.*, **32**: 677-688 (in Japanese).

Taylor J. and R. Mahon (1977): Hybridization of Cyprinus carpio and Carassius auratus, the first two exotic species in the lower Laurentian Great Lakes. *Env. Biol. Fish.*, **1**: 205-208.

World Organisation for Animal Health (WOAH) (2022a): Chapter 2.3.6. Koi herpesvirus disease. In: Manual of diagnostic tests for aquatic animals.

World Organisation for Animal Health (WOAH) (2022b): Chapter 1.5 Criteria for listing species as susceptible to infection with a specific pathogen. In: Aquatic Animal Health Code.

Yuasa K., S. Motohiko and N. Oseko (2013): Goldfish is not a susceptible host of koi herpesvirus (KHV) disease. *Fish Pathol.*, **48**: 52-55.

Yuasa K., M. Kawana, T. Ito, I. Kiryu, N. Oseko and M. Sano (2021): *Intra vitam* assays for detecting fish infected with cyprinid herpesvirus 3 (CyHV-3). *Dis. Aquat. Org.*, **149**: 77–82.

Table 1. Cumulative mortalities of 3 hybrids with/without experimental infection by KHV in each aquarium

Group*	*Ccu* x *Cc*			*Cbg* x *Cc*			*Cb*s x *Cc*		
	viral exposure		Cont	viral exposure		Cont	viral exposure		Cont
Aquaria	*Ccu*-Ex1	*Ccu*-Ex2	*Ccu*-C	*Cbg*-Ex1	*Cbg*-Ex2	*Cbg*-C	*Cbs*-Ex1	*Cbs*-Ex2	*Cbs*-C
Numbers of fish	20	20	20	20	20	20	20	15	15
Cumulative mortality	40%	20%	10%	0%	0%	0%	0%	0%	0%

* *Ccu*, *Carassius cuvieri*; *Cbg*, *Carassius buergeri grandoculis*; *Cb*s, *Carassius buergeri* subsp.1; *Cc*, *Cyprinus carpio*

Table 2. Numbers of KHV DNA copies in the fins of dead hybrids in two aquaria, *Ccu*-C and *Ccu*-Ex1

Aquaria	Date of mortality (dpe)	KHV DNA number in 20 mg of the fins
Ccu-C	7	nd
Ccu-C	9	nd
Ccu-Ex1	16	2.92E+05
Ccu-Ex1	19	7.84E+03
Ccu-Ex1	19	5.45E+03
Ccu-Ex1	20	4.89E+04
Ccu-Ex1	22	1.29E+05
Ccu-Ex1	33	6.59E+04
Ccu-Ex1	36	1.29E+05
Ccu-Ex1	40	3.81E+05

nd: not detected

Table 3. Numbers of KHV DNA copies in 20 mg of the fins of 3 hybrids at 14 and 44 days post viral exposure (dpe). Five fish in each aquarium of 3 hybrids were randomly sampled for the detection

dpe	Aquaria					
	Ccu-Ex1	*Ccu*-Ex2	*Cbg*-Ex1	*Cbg*-Ex2	*Cb*s-Ex1	*Cb*s-Ex2
14 dpe	5.80E+05	3.09E+03	1.49E+02	1.88E+05	1.92E+04	nd
	1.80E+05	nd	nd	8.82E+01	nd	nd
	nd	nd	nd	8.08E+01	nd	nd
	nd	nd	nd	nd	nd	nd
	nd	nd	nd	nd	nd	nd
44 dpe	3.19E+02	1.97E+03	3.31E+02	1.71E+04	nd	nd
	nd	nd	2.94E+02	nd	nd	nd
	nd	nd	nd	nd	nd	nd
	nd	nd	nd	nd	nd	nd
	nd	nd	nd	nd	nd	nd

nd: not detected

Table 4. Numbers of sacrificed koi post cohabitation with each hybrid in 9 aquaria and KHV DNA copies in the gills of sacrificed koi or survival at the end of experiment

Hybrids*	Aquaria	Number of dead fish	KHV DNA number in 20 mg of the gills**
Ccu x *Cc*	*Ccu*-Ex1	5	9.15E+07
	Ccu-Ex2	5	1.66E+08
	Ccu-C	0	nd
Cbg x *Cc*	*Cbg*-Ex1	0	1.35E+03
	Cbg-Ex2	0	2.44E+03
	Cbg-C	0	nd
*Cb*s x *Cc*	*Cb*s-Ex1	0	nd
	*Cb*s-Ex2	0	nd
	*Cb*s-C	0	nd

* *Ccu*, *Carassius cuvieri*; *Cbg*, *Carassius buergeri grandoculis*; *Cb*s, *Carassius buergeri* subsp.1; *Cc*, *Cyprinus carpio*

**KHV DNA in the gill tissues pooled by 5 fish was analyzed for quantification.