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# Analysis of koi herpesvirus latency in wild common carp and ornamental koi in Oregon, USA

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Koi herpesvirus (KHV) infection is associated with high mortalities in both common carp (*Cyprinus carpio carpio*) and koi carp (*Cyprinus carpio koi*) worldwide. Although acute infection has been reported in both domestic and wild common carp, the status of KHV latent infection is largely unknown in wild common carp. To investigate whether KHV latency is present in wild common carp, the distribution of KHV latent infection was investigated in two geographically distinct populations of wild common carp in Oregon, as well as in koi from an Oregon-based commercial supplier. Latent KHV infection was demonstrated in white blood cells from each of these populations. Although KHV isolated from acute infections has two distinct genetic groups, Asian and European, KHV detected in wild carp has not been genetically characterized. DNA sequences from ORF 25 to 26 that are unique between Asian and European were investigated in this study. KHV from captive koi and some wild common carp were found to have ORF-25–26 sequences similar to KHV-J (Asian), while the majority of KHV DNA detected in wild common carp has similarity to KHV-U/-I (European). In addition, DNA sequences from IL-10, and TNFR were sequenced and compared with no differences found, which suggests immune suppressor genes of KHV are conserved between KHV in wild common carp and koi, and is consistent with KHV-U, -I, -J.



## 1. Introduction

Koi herpesvirus (KHV) is highly contagious and pathogenic to both koi and common carp. The first formal descriptions of the disease were from Israel and the USA in 1998 (Calle et al., 1999; Hedrick et al., 1999) and from Germany in 1997–1998 (Bretzinger et al., 1999). The latter group designated the virus as carp nephritis and gill necrosis virus (CNGV) and recently confirmed that CNGV is the same as KHV (Hutoran et al., 2005). The virus has been reported in many countries of Europe, Asia, and North America since 2000. The clinical signs of an active KHV infection include red and white mottling of the gills, gill hemorrhage, sunken eyes, pale patches or blisters on the skin, and external hemorrhages (Gilad et al., 2002). The virus can be found in the kidney, gill, spleen, fin, intestine, and brain (Gilad et al., 2004). More recently, latent KHV has been detected in circulating white blood cells from koi exposure to KHV 10–15 years prior to testing (Eide et al., 2011a,b). In other experimental studies, 82% of fish died within 15 days when they were exposed to the virus at a water temperature of 22 °C (Perelberg et al., 2005).

KHV is formally known as Cyprinid herpesvirus 3 (CyHV-3) and has been proposed to be a member of *Alloherpesviridae* (Waltzek et al., 2005). Members of *Alloherpesviridae* also include CyHV-1 (carp pox herpesvirus) and CyHV-2 (goldfish hematopoietic necrosis virus). Herpes viruses are known to be well adapted viruses in their natural host and can be found in many different species. KHV may have existed in wild common carp before emerging as a highly pathogenic virus. KHV latency sites have been investigated in koi recovered from KHV infection, and no consistent detection of KHV DNA has been found in tissues, such as liver, kidney, or spleen (Eide et al., 2011b; Gilad et al., 2004). However, KHV DNA can be detected consistently in white blood cells of koi that have recovered from a clinical KHV infection (Eide et al., 2010, 2011b), which suggests that white blood cells are the preferred latency site for KHV. To investigate KHV latency in wild carp populations, fish from a flood pond in the Willamette Valley of Oregon and from the Barnyard Springs in the USFWS-Malheur National Wildlife Refuge in Oregon were examined for the presence of KHV genome. In addition, KHV latent infection was investigated in ornamental koi from a fish retailer in Oregon.

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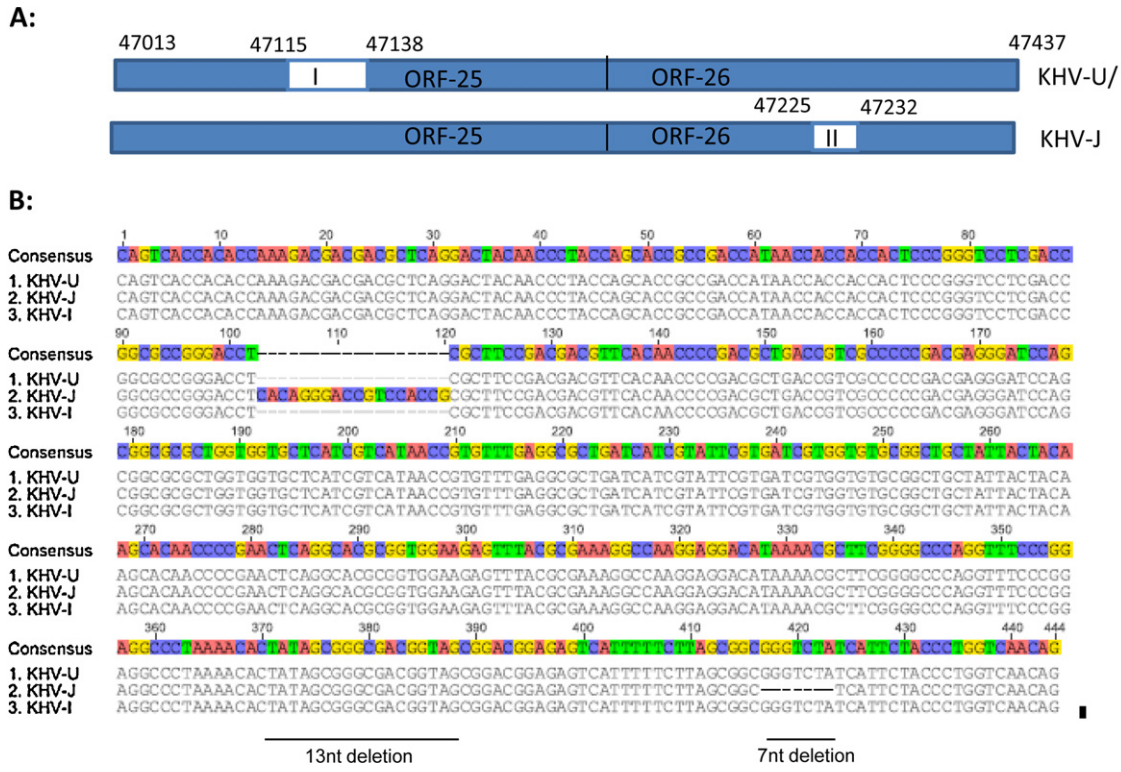


**Fig. 1.** DNA sequence variations within ORF-25–ORF-26 of KHV-I, KHV-J and KHV-U. (A) Schematic of ORF-25–26 with 13 nt deletion in ORF-25 of KHV-U, -I and 7 nt deletion in ORF-26 of KHV-J. Open box I: 13 nt deletion. Open box II: 7 nt deletion. (B) DNA sequence alignment flanking 13 nt deletion in KHV-U and KHV-I, 7 nt deletion in KHV-J.

There are two genetically distinct KHV groups, European and Asian (Avarre et al., 2011). KHV-I (Israel) and KHV-U (United States) are members of the European group. KHV-U is considered to have originated from KHV-I because of genetic similarity between the two isolates. KHV-J (Japan) belongs to the Asian group, and is genetically different from KHV-U and KHV-I (Aoki et al., 2007; Avarre et al., 2011). The size of the KHV genome is 295, 271; 295, 146, and 295, 138 bp for J, U, and I, respectively (Aoki et al., 2007). Although most genes of the three isolates are similar, ORF-25 and ORF-26 of KHV-J are different from those of KHV-U and KHV-I. As shown in Fig. 1, there is a 13 nt deletion in ORF-25 of both KHV-I and KHV-U that is not observed in ORF-25 of KHV-J. Additionally, KHV-J has a 7 nt deletion in ORF-26 that is not observed in either KHV-I or KHV-U. To investigate whether KHV DNA of wild common carp or ornamental koi is similar to Asian or European KHV, DNA sequences of KHV ORF-25 and ORF-26 from wild carp and koi were investigated and compared in this report.

## 2. Materials and methods

### 2.1. Source of wild common carp and sampling

Five wild common carp were obtained from a pond formed from back-water after a flood in Linn County, Oregon, in late spring of 2010. The carp were designated as C1–C5, with ages estimated at 2–5 years old based on morphometric measurements (length and weight). Approximately 2–3 ml of whole blood were collected from the caudal tail vein of each fish and stored in heparin-coated tubes in accordance with National Institutes of Health guidelines and the Oregon State University Animal Care and Use Committee (IACUC). An additional 24 adult wild common carp were collected from Barnyard Springs located in USFWS-Malheur National Wildlife Refuge in Princeton, Oregon in two groups: the first group of 10, designated as A1–A10, was collected in late winter of 2010, the second group of 14, designated as B1–B14, was collected in early spring of 2011. Approximately 1–2 ml of whole blood were collected from each fish and stored at 4 °C in EDTA coated tubes prior to shipping.

### 2.2. Source of ornamental koi and DNA sampling

Eight 1–2-year old koi ranging from 7 to 10 cm in length, designated as K1–K8, were obtained from a fish supplier in Corvallis, Oregon in the summer of 2010. Because the fish were still very small, DNA was extracted from the entire fish. All koi were euthanized by MS222 overdose at 500 ppm in accordance with National Institutes of Health guidelines and the Oregon State University Animal Care and Use Committee (IACUC). The koi were homogenized in 2 ml 1X lysis buffer, and 0.5 ml of tissue homogenates were digested with 100 μg proteinase K at 55 °C overnight. Genomic DNA was extracted from the tissue lysates with a High Pure PCR Template Preparation Kit according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN, USA).

### 2.3. Separation of peripheral white blood cells (WBC) and total DNA extraction from WBCs and plasma

Whole blood from wild common carp was centrifuged at 650 × *g* at 4 °C for 10 min, the buffy coat was collected and exposed to 3–4 volumes of red blood cell lysis buffer (Tris–$NH_4Cl$). The white blood cells (WBCs) were washed twice in sterile DMEM by centrifugation at 650 × *g* at 4 °C for 10 min. WBCs were subjected to total DNA extraction using a High Pure PCR Template Preparation Kit (Roche Diagnostics, Indianapolis, IN, USA).

### 2.4. Primers and probes

Selection of primers for KHV sequence amplification was based on KHV genome sequence data available through Genbank