Recent developments in the study and surveillance of koi herpesvirus (KHV) in Asia

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ABSTRACT

Koi herpesvirus infection causes significant mortalities in common carp (*Cyprinus carpio carpio*), koi carp (*Cyprinus carpio koi)* and ghost carp *(common x koi cross, Cyprinus carpio koi*). Outbreaks have been reported in many countries worldwide i.e. UK, Germany, Israel, USA, Belgium, South Africa, Switzerland, The Netherlands, France, Denmark, Austria, Italy, Luxemburg and Poland. The first outbreaks attributed to KHV in Asian countries were reported from Hong Kong in 2001; Indonesia in 2002; Taiwan in 2002; Japan in 2003; Thailand in 2005; and Singapore in 2005. Thereafter, research studies embarked on KHV focused on pathogenicity, cell line susceptibility, fish size susceptibility, predilection to fish organs, persistence in fish, vaccine development and application, surveillance and gene sequence analyses of KHV strains. To date, annual active surveillance of the virus in Cambodia, Lao PDR, Myanmar, the Philippines and Vietnam showed that these countries were free of KHV from 2004 to 2007. Several strains of KHV apparently affect koi and common carp in this region indicating that transboundary movement of the virus has occurred not only in Asia but also from Europe and the Americas. The extensive international trade in live ornamental koi fish has largely contributed to the global spread of KHV. Hence, KHV disease (KHVD) was recently added to the list of notifiable diseases of the World Organisation of Animal Health or the Office International des Epizooties (OIE), an indication of the global significance of this viral infection.

Key words: koi herpesvirus (KHV), *Cyprinid herpesvirus* 3 (CyHV-3); outbreaks, Asia, genotypes, pathogenicity, surveillance, vaccine

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INTRODUCTION

The common carp (*Cyprinus carpio carpio*) is the third most important farmed freshwater fish species in the world (FAO Yearbook, 2004). The main carp-producing countries are found in Europe, Asia, Russia, South America and parts of Africa. In many Asian countries, common carp are cultured widely as foodfish. In Indonesia, for instance, carp is the national fish species of the country. The ornamental variety, koi carp (*C. carpio koi*), is also popular worldwide, as pets. In fact, choice koi carp, which are very expensive, are bred in Japan, Israel and some Southeast Asian countries for ornamental fish hobbyists. As such, international trade in koi carp has provided routes for transboundary introduction of koi herpesvirus (KHV), also known as *Cyprinid herpesvirus* 3 (CyHV-3), into naïve populations in many countries and more recently in Southeast Asia (Haenen and Hedrick, 2006; Lio-Po, 2007).

Koi herpes virus infection causes significant mortalities in koi and common carp, *C. carpio*. The disease was earlier known as carp interstitial nephritis and gill necrosis (CNGV) in Israel (Ronen *et al.*, 2003; Pikarsky *et al.,* 2004). The first outbreaks of KHV were reported in Israel and Germany in 1998. Subsequent outbreaks were reported in USA, England and The Netherlands. To date, the disease has been reported in Belgium, South Africa, Switzerland, France, Denmark, Austria, Italy, Japan, Luxemburg and Poland (Haenen *et al*., 2004; Bergmann *et al*., 2006). Interestingly, polymerase chain reaction (PCR) testing of archived samples of an earlier unexplained mass mortality of koi and common carp in the UK confirmed that KHV was already present there as early as 1996 (Denham, 2003).

GEOGRAPHIC OCCURRENCES IN ASIA

In Asia, the first outbreak of KHV that caused mass mortalities of cultured common carp and koi carp occurred in Israel in the spring of 1998 when water temperatures were 18-28^{o}C. By the end of 2001, 90% of carp farms in Israel reported disease outbreaks attributed to KHV (Perelberg *et al.,* 2003). Other first reports of disease attributed to KHV occurred among koi in Hongkong in 2001; common carp in Indonesia, in 2002; koi in Taiwan, in 2002; common carp in Japan, in 2003; koi in Thailand and Singapore, in 2005 (Haenen and Hedrick, 2006; Lio-Po, 2007).

The initial case of KHV mortality in Indonesia occurred among koi carp cultured in Blitar, East Java, in March 2002. The site had a history of fish introduction with fish imported from China through Hong Kong in January 2002 (Sunarto *et al.*, 2005a). The following month, a second disease outbreak occurred among cultured common carp in Subang Regency, West Java. The infected fish manifested clinical signs similar to the infected koi in Blitar. By May and June of the same year, common carp cultured in floating net cages in Cirata Reservoir, West Java, were also hit by the virus. The following year, outbreaks were reported in several carp farms in other parts of the country and by 2006 the disease had spread to many more sites (Fig. 1). The outbreak was associated with 95% mortality and losses amounting to approximately US$0.5 million within a 3 month period (Sunarto *et al.*, 2005b).

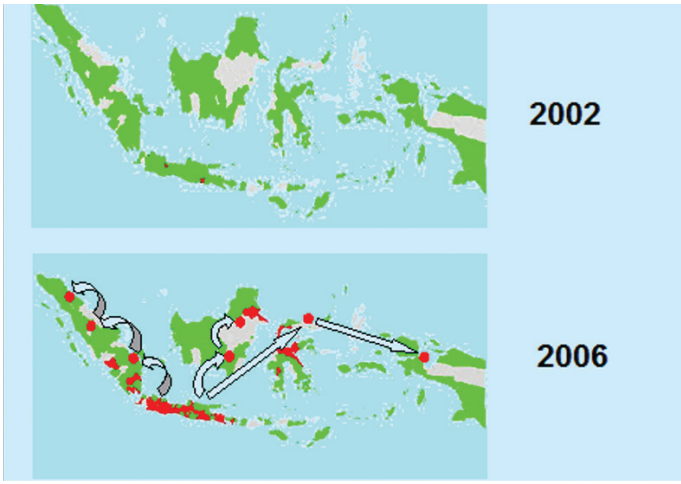

Figure 1. Sites of KHV occurrences in Indonesia in 2002 and 2006 (Photo courtesy of A. Sunarto)

At about the same time, the first occurrence of KHV infection emerged in two 2-year old koi carp in a private pond in Taipei County, Taiwan in December 2002 (Tu *et al.*, 2004a). During the following year, 3 more separate cases of 2-3 year old koi carp with populations of 20 (pond), 300 (artificial lake) and 700 (lake) occurred in Taipei, Taiwan and in 2004, another KHV outbreak occurred in a private koi hatchery in Taipei County, Taiwan (Tu *et al*., 2004b).

In Japan, KHV epizooties was first reported among cage-cultured common carp in Lake Kasumigaura, Ibaraki Prefecture in October 2003 when water temperature in the lake was 16-18°C (Sano *et al.*, 2004a). By mid-November, the virus caused mass mortality of approximately 1,200 metric tonnes of common carp cultured in the lake. Archived samples from an earlier case of >10,000 common carp mortalities in May to July 2003 in some rivers and a lake in Okayama Prefecture were subsequently confirmed as KHV outbreaks. By the end of that year, KHV was detected in 23 of 47 prefectures. From January to May 2004, KHV was detected in 24 of 47 prefectures (Sano *et al.*, 2004b). Moreover, mass mortality of carp attributed to KHV occurred in seven rivers in Kanagawa Prefecture in May/June 2004 (Hara *et al.*, 2006). By 2005, 42 of 47 prefectures had confirmed cases of the virus. By spring 2006, KHV was detected in 45 of 47 prefectures and involved koi carp for the first time (T. Iida, *pers. commun.*). Such dramatic spread of the disease in Indonesia and Japan created an acute awareness of its emergence as a new disease in the region with predictably alarming implications.

In Thailand, KHV was first diagnosed in koi carp of a fish hobbyist in Bangkok in 16 March 2005 (Tandavanitj *et al*., 2005). It was noted that only a few days earlier, the fish had participated in a koi competition held in Bangkok. After the contest, some of the koi

developed clinical signs and were confirmed as KHV-positive by PCR. Fortunately, the disease was limited to the koi companies that joined the competition and was reportedly contained. Mortalities ranged from 5 to 30%.

In Singapore, KHV was detected in two batches of Thai koi carp exported to Singapore, in February 2006. The fish tested KHV-positive by PCR and the virus was successfully isolated on KF-1 cells (S. Kueh, *pers. commun.*). During the period April to June 2006, koi carp imported to Singapore from Malaysia also tested positive for KHV. Earlier, the KHV was also detected among koi in Malaysia by nested PCR in 2004 but was not associated with epizootic outbreaks (Musa *et al.*, 2005). Even earlier, in another example of exported koi, the presence of KHV was detected among koi carp exported to the United Kingdom from Malaysia in 2000 and in 2001 (Gilad *et al.*, 2003).

Surveillance of the virus was carried out in both common and koi carp of Cambodia, Lao PDR, Myanmar, the Philippines and Vietnam through the auspices of the Government of Japan and the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD). The three-year active surveillance study covered 1481 common and koi carp sampled during the periods December 2004 to February 2005, September 2005 to February 2006 and October 2006 to February 2007. KHV was not detected using either cell culture, bioassay or PCR in any of the fish samples (Lio-Po *et al*., 2009). Moreover, no related epizooties among common and koi carp were observed by the fish farmers.

CAUSATIVE AGENT, GENOTYPES AND STRAINS

This disease is attributed to infection by Koi herpesvirus (KHV) or *Cyprinid herpesvirus* 3 (CyHV-3) of the family, *Herpesviridae* (Fig. 2) (Waltzek *et al.*, 2005). This double-stranded DNA virus has been isolated by cell culture from infected stocks of koi or common carp in many countries including the United States of America (USA), the UK, Israel, Japan, Indonesia, Malaysia, Thailand and Taiwan. The approximate diameter of KHV virion is 100-110 nm (Ronen *et al.,* 2005). The total genome length of KHV is approximately 295 kbp (Aoki *et al.*, 2007). Sequence analyses determined that KHV isolates from UK, USA and Israel were very similar. In other studies, the genotype of the USA strain was classified as E1, the Israel KHV as E6 strain while the Netherlands strains consisted of E1, E2, E3, E4 and E5 genotypes (Kurita *et al.*, 2008). Among the Asian KHV isolates, the Japanese KHV was classified as strain A1, the Indonesian strains as A1 and A2, the Taiwanese strains as A1 and A2 while the PR China KHV as an A1 strain (Lio-Po *et al.*, 2006; Kurita *et al.* 2008). Thus, it appears that the KHV strains found in The Netherlands, USA and Israel are closely related while those moving about Asian countries are, likewise, closely related.

Variation in the virulence of two strains of KHV was experimentally demonstrated by Yuasa *et al.* (2007). Strain 1 caused 50% mortality while strain 2 caused 60-75% mortality in common carp. In addition, strain 1 was detected by PCR in the gills, scales, kidney, spleen, liver, heart, intestine and brain 14 days post-infection (dpi) and in the gills, scales, kidney 28 dpi while strain 2 was initially detected in the 8 organs much later at 28 dpi and in the

gills, scales, kidney, intestine and brain by 60 dpi. Interestingly, strain 2 was detected in the brain over a long time period (360 dpi). At 70 dpi, >10,000 genome copies/mg wt. of the brain compared with a mean of <1 000 genome copies/mg wt. of the kidneys of surviving fish were detected.

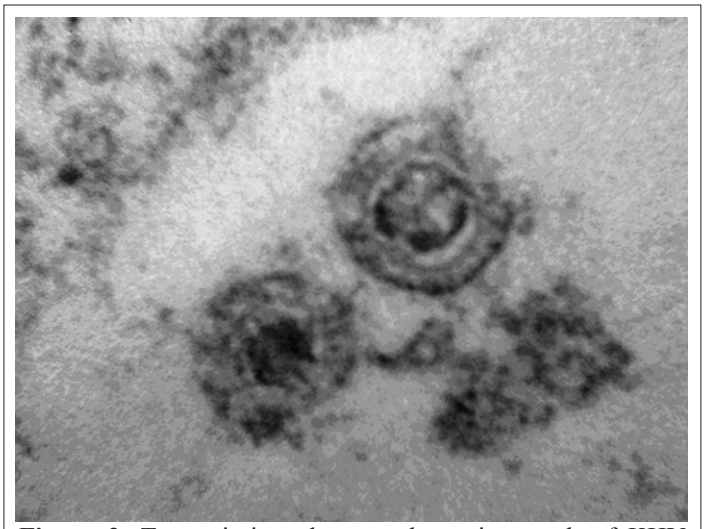

Figure 2. Transmission electron photomicrograph of KHV (Photo courtesy of K. Way).

FISH SPECIES AFFECTED

KHV infects common carp, koi carp and ghost carp (hybrid of koi and common carp) causing 30-100% mortality. Experimental transmission of KHV from common carp to goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*) or tilapia (*Oreochromis niloticus*) failed to induce KHV infection (Perelberg *et al.*, 2003). However, Hedrick *et al.* (2006) reported that goldfish x common carp hybrids were moderately sensitive to KHV infection. Likewise, tilapia (*O. niloticus*) and catfish (*Pangasius hypophthalmus*) upon cohabitation with KHV-infected common carp did not develop disease nor was KHV detected by PCR (Yuasa and Sano, 2009. In Indonesia, the common carp naturally-infected with KHV were cultured in cages adjacent to caged tilapia, but the latter fish did not succumb to infection.

GROSS CLINICAL SIGNS

Infected fish typically manifest white, necrotic patches on the gill filaments (Fig. 3). Thus, affected fish often swim on the surface and exhibit respiratory distress. Other clinical signs may consist of sunken eyes, pale patches on the body surface, excessive mucus production and rough pale patches of the skin. However, some infected fish may show no visible signs of disease. Microscopic examination of the gills often shows associated bacteria and parasites (Hedrick *et al.*, 2000).

Figure 3. Koi carp with KHV showing varying severity of whitish or necrotic gills (Photo courtesy of A. Sunarto).

PATHOLOGY

KHV is very virulent; natural infection often causes 80-90% mass mortality of koi and common carp within 1 week (Perelberg *et al.,* 2003; Sano *et al.,* 2004a; Tu *et al.,* 2004a; Sunarto *et al.,* 2005b). Experimental infection using cell-cultured KHV induced 75-95% mortalities in koi and common carp (Pikarsky *et al*., 2004). The disease is very contagious for koi and common carp but not for humans. The disease has an incubation period of 5-7 days and is characterized by sudden onset, rapid spread when water temperatures are in the range 15-25^{o}C (Gilad *et al*., 2003; Ronen *et al*., 2003).

In infected fish, large amounts of the virus are found in the kidneys and lesser amounts in the liver and brain as detected by immunofluorescence and by semi-quantitative PCR (Ronen *et al*., 2005). The same study reported that interstitial nephritis was detected as early as 2 days post-infection with increasing severity up to 10 days post-infection. In an earlier experimental study where real-time TaqMan PCR was used to quantify KHV DNA, the greatest amounts of viral DNA were found in the gill, kidney and spleen with virus genome equivalents of 10^{8} to 10^{9} per 10^{6} host cells (Gilad *et al*., 2004). High levels of KHV DNA were also found in mucus, liver, gut and brain. Yuasa *et al*. (2005b) experimentally immersed common carp fingerlings in 10^{3} $TCID_{50}$/ml at 23^{o}C and subsequently detected the virus in several organs including the gills from 3-40 days post-infection by PCR. However, the virus was not detected in any organ 60 days post-infection. The amount of the virus was higher, and peaked at 7-9 days, in the gills, fin, scales, kidney and intestine than in other organs. The virus could be isolated, in CCB cell cultures, from the gills and kidney 6-17 days post-infection but not after 20 days. Interestingly, KHV was detected in fish brain as long as 145 days after infection but was infectious only for up to 28 days (Yuasa *et al*., 2007).

Severe gill disease was associated with inflammation and loss of villi (Pikarsky *et al*., 2004). Other studies reported that the gills of infected fish develop necrotising branchitis, lamellar epithelial degeneration, focal areas of necrosis and exfoliation. Histologically, infected fish shows hyperplasia and fusion of the secondary gill lamellae (Tu *et al.*, 2004a; Cruz-Lacierda *et al.*, 2005). Intranuclear inclusions in the branchial epithelium may be observed. In addition, Ilouse *et al*. (2006) reported that the kidneys of infected fish exhibited heavy, interstitial, inflammatory infiltrates 6 days post-infection. This condition was associated with large cells having a foamy, distended cytoplasm and a few intranuclear inclusion bodies. By day 8, severe infiltration developed and was accompanied by a feathery degeneration of the tubular epithelium in many nephrons. Mild, focal inflammation was also noted in the livers and brains.

Temperature is a critical factor in the pathogenesis of KHV infections. KHV induces infection/mortalities at temperatures of 18-25^{o}C. Experimental infection showed that exposure of healthy fish to KHV at 22^{o}C can cause up to 82% mortality within 15 days (Ronen *et al*., 2003). Furthermore, fish exposed to KHV at 20-24^{o}C for 3 days then transferred to non-permissive temperatures survived the infection. However, fish held at 13^{o}C for 30 days and shifted to 22-24^{o}C developed disease and rapid onset of mortality. In another study, Yuasa *et al.* (2008) showed that common carp experimentally exposed to KHV at 16^{o}C, 23^{o}C and 28^{o}C died from infection at 21-54, 5-20 and 7-14 days post-virus exposure (dpe), respectively. Moreover, using cohabitation with naïve koi carp, infected carp were shown to continuously shed the virus for 34 days (7-40 dpe) at 16^{o}C, for 14 days (1-14 dpe) at 23^{o}C, and for 12 days (3-14 dpe) at 28^{o}C.

The virus has been isolated from KHV-infected common/koi carp in different carp cell lines: koi fin (KF-1), koi fin (KFC), common carp brain (CCB) and koi tail (KT-2) in USA, Israel, Germany, Indonesia, Japan, Singapore and Taiwan (Hedrick *et al*., 2000; Neukirch and Kunz 2001; Sano *et al.*, 2004a, Lio-Po and Orozco 2005; Ilouse *et al.*, 2006; NACA and FAO 2008). The KHV-infected cells cultured at 20-25^{o}C demonstrate typical cellular vacuolation. However, the vacuolated cells reverted to normal morphology and plaques disappeared following shift to the non-permissive temperature, and reappeared after transfer back to permissive temperature (Dishon *et al.*, 2007).

The virulence of the virus appears to be more potent in 14 g common carp (dying 6 to 9 days post exposure) than in 6 g fish (succumbed in 10 to 14 days) at 20-24^{o}C (Lio-Po *et al*., 2006). Similarly, recent comparative infection studies showed that three-day old common carp fry (mean TL: 7.5 and 8.7 mm) exposed to KHV were not susceptible to disease while 69-100% mortalities were observed in common carp juveniles (mean TL: 13.8 and 29.2 mm) reared at 24^{o}C (Ito *et al.,* 2007b).

Variation in susceptibility among different carp strains has been reported. In Europe, the ghost carp shows higher sensitivity to the virus than koi carp. In Japan, the indigenous strain of common carp shows higher sensitivity to the virus than the Eurasian strain of common carp or koi carp (Ito *et al.*, 2007a).

The virus can remain latent in the host for long periods of time, becoming active only at permissive temperatures. Thus, the virus persists in "carriers" which demonstrate no clinical signs of the disease. Active KHV infection recurred in recovered fish once they were stressed. Thus, even after 7 months post-infection the virus can be reactivated. Moreover, the virus can persist in the brain of its fish host for at least 360 days (Yuasa *et al*., 2007). Latent KHV can be reactivated by increasing the water temperature from non-permissive to permissive temperatures. Such reactivation was confirmed by virus transmission and detection to naïve carp cohabited with infected carp at 150 days following the initial infection. Moreover, the virus was transmitted horizontally from KHV-infected fish to naïve koi or common carp via intraperitoneal injection or by bath exposure at permissive temperatures (18-25^{o}C). Moreover, once infected, a pond will lose the majority of its fish within days, and no treatment is currently licensed.

In addition, non-specific secondary infections of bacterial, parasitic and fungal origin may be associated with KHV infections. For instance, secondary gill infections attributed to *Flavobacterium columnare* and *Aeromonas* spp. have often been associated with KHV infection (Sunarto *et al*., 2005b).

DIAGNOSIS

Several techniques have been used for detecting KHV in fish hosts. Among these are one-step and nested PCR, loop-mediated isothermal amplification (LAMP), *in situ* hybridization, cell culture, histopathology, transmission electron microscopy (TEM), enzyme-linked immunosorbent assay (ELISA), and bioassay in healthy, naïve, susceptible fish.

PCR is a more specific and sensitive method for KHV detection (Fig. 4) using the Sph and 9/5 primers (Gray *et al.,* 2002; Gilad *et al.,* 2002). More recently, Yuasa *et al.* (2005a) improved the PCR protocol using the *Sph* I-5 primer set that reduced non-specific reactions and reduced the reaction time by almost half. At about the same time, Bercovier *et al.* (2005) developed the TK PCR primer set to similarly improve the technique. In addition, a nested PCR has been developed with more sensitivity for KHV detection (Liu *et al.*, 2002; El-Matbouli *et al.*, 2007). Commercial PCR kits are already available in the market. A reflection of how widespread the use of PCR for KHV detection has become is illustrated by the broad participation in the international inter-laboratory proficiency test organised by Way *et al*. (2008) which involved 21 laboratories from 19 countries in 2006 and 32 laboratories from 27 countries in 2007. Results from these ring tests indicated that the Bercovier-TK (1) and modified Gray SpH (2) primer sets were the most robust for detection of KHV DNA. In addition, real-time PCR, useful for the quantitative estimation of the KHV virus, has been developed using the TaqMan PCR method (Gilad *et al.*, 2004).

More recently, the LAMP method was developed and reported to be as sensitive as PCR and to be more rapid than PCR for the detection of KHV. LAMP methods used to date include TK LAMP, 9/5 LAMP and Sph LAMP (Gunimaladevi *et al*., 2004; Soliman and

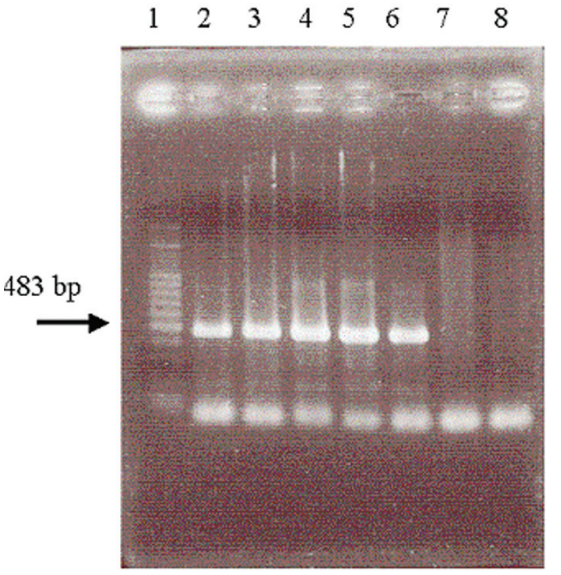

Figure 4. PCR test showing positive bands for KHV (483 bp) in lanes 3, 4, 5, 6 for koi carp samples collected in Taiwan. Lane 2 (positive control), lane 7 (koi carp sample negative for KHV) and 8 (negative control) (Photo courtesy of C. Tu).

El-Matbouli, 2005; Yoshino *et al.*, 2006). Other DNA based detection methods include *in situ* hybridization (Haenen *et al*., 2004) and the RT-PCR targeting the mRNA terminase (Yuasa *et al*., 2007).

The virus can be isolated in susceptible fish cell lines such as KF-1, KFC, KT-2 or CCB. Tissue filtrates are prepared from the gills, kidney, spleen or leukocytes by homogenization, centrifugation and filtration through 0.45 micron membrane filters. Inoculated cells are incubated at 20-25°C for 7-14 days. Infected cells develop cytopathic effect (CPE) consisting of typical cellular vacuolation (Fig. 5). The survival of KHV in infected CCB cells was maintained for 30 days at 30°C (Dishon *et al*., 2007).

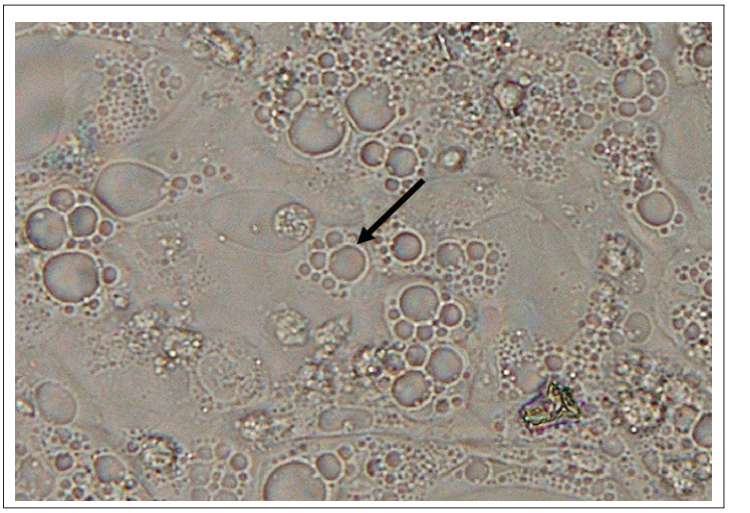

Figure 5. Cultured KF-1 cells inoculated with KHV showing typical vacuolations (arrow).

Histological examination of gills, liver, spleen and kidney of affected fish is also useful for diagnosis. The presence of severe gill hyperplasia with lamellar fusion and epithelial necrosis with prominent nuclear swelling and eosinophilic, intranuclear inclusions is diagnostic for KHV infection (Tu *et al*., 2004a; Cruz-Lacierda *et al*., 2005). Necrosis may also be observed in the liver, spleen and kidney parenchymal cells.

Electron microscopy used for visualizing KHV virions (Hedrick *et al.,* 2000; Miwa *et al.*, 2007) is another useful direct diagnostic method. Indirect methods such as the enzyme-linked immunosorbent assay (ELISA) allows detection of antibodies to KHV in the serum of koi and common carp previously exposed to the virus (Adkison *et al.*, 2005).

Where laboratory facilities for Level III Diagnosis are unavailable, the presence of KHV can also be confirmed by bioassay (Lio-Po *et al.*, 2009). Tissue filtrates prepared from KHV-infected fish are injected intraperitoneally to healthy koi or common carp which are maintained at the permissive temperature (23-28^{o}C) for at least 3 weeks. Development of typical clinical signs of KHV infection in the naïve fish is a presumptive indication of the presence of the virus. Confirmatory diagnosis is achieved by virus isolation in cell culture and identification by PCR. However, for expensive koi carp (where it is important to keep the fish alive), establishment of the KHV infection status can be determined by a modified "Sentinel" bioassay. This is a non-destructive technique wherein an expensive, koi carp, suspected of being infected with KHV, is cohabited with known KHV-free common carp at 23-28^{o}C for 2 to 4 weeks. The development of KHV clinical signs in the naïve common carp indicates KHV infection in the suspect koi carp. Conversely, if the common carp remain negative for KHV, then the expensive koi carp with which it was cohabited is also considered KHV-free.

PREVENTION AND CONTROL

The impact of KHV is very significant in the koi-keeping/breeding community because of its high pathogenicity. Unfortunately, there is no effective drug for KHV disease. The aquarium fish trade, especially of koi carp, most likely played a significant role in the transboundary movement of the virus. The annual fish trade shows featuring live ornamental fish sourced from many countries have contributed to the transmission of fish pathogens (Lio-Po, 2007). For example, there is a documented case of illegally imported live koi carp that was intercepted by the fish quarantine staff at Ninoy Aquino International Airport in Manila, Philippines in September 2004. Five days after stocking the fish in a contained facility, the koi developed clinical signs and tested positive for KHV by PCR. All koi died after two weeks in the holding tank (Somga *et al.*, 2010). Hence, health certification, quarantine and testing of imported koi or common carp for KHV should be instituted by the importing country before the imported fish gain entry into fish farms. In fish farms, biosecurity measures must be in place to mitigate against disease introduction, to ensure containment and to prevent dissemination of KHV among existing farm stocks.

In Israel, experimental cohabitation of naïve koi carp with KHV-infected fish induced the production of anti-KHV antibodies among exposed naïve carps (Ronen *et al.*, 2003). These findings led to studies on the development of a live, attenuated KHV vaccine that can induce high antibody titers in test fish after 7 dpi and peaks at 21 dpi (Ronen *et al.*, 2003 and 2005; Perelberg *et al.*, 2005; Ilouse *et al.*, 2006). Fish were immersed in 10 to 100 PFU/ml attenuated virus for 40 min followed by incubation at the permissive temperature for an additional 48 to 72 h (Perelberg *et al.*, 2005). Vaccinated fish survived subsequent challenge with the virus yielding a relative percent survival (RPS) of 80-95%. Field applications of the vaccine in carp in Israel from 2003 to 2007 resulted in increased carp production to 16,000 tonnes in 2007 compared to 8,000 tons in 2002 when the KHV vaccine was not in use. Moreover, protective immunity after vaccination extends for at least 8 months among vaccinated fish (Ilouse *et al.*, 2006). This vaccine is currently commercially available (M. Kotler, *pers. comm.*).

In Japan, Yasumoto *et al*. (2006) used a formalin-inactivated KHV vaccine entrapped within the liposomal membrane that was experimentally sprayed on dry pellets before feeding to common carp. The vaccinated fish yielded an RPS of 74.4% and 65% upon challenge with its homologous virus 22 days after vaccination. However, no further studies on field testing or commercialization of the vaccine were reported.

The viability of KHV under non-permissive conditions may also indicate a potential method to control KHV infections. For instance, cohabitation of fish with KHV infected fish for 3-5 days at permissive temperatures of 22-23^{o}C and then transferred to ponds with water temperature of approximately 30^{o}C for 30 days then re-challenged with the virus had significantly reduced mortalities of 39% compared to the control fish (not cohabited with sick fish) kept at 22^{o}C and then challenged with the virus yielding 82% mortalities within 15 days (Ronen *et al*., 2003).

The infectious titre of KHV kept in environmental water was significantly reduced within 3 days (Shimizu *et al.*, 2006). Experiments on survivability of KHV indicated that the virus cannot tolerate temperatures of 35^{o}C even for just 24 h (Lio-Po *et al*., 2006). Moreover, at 20 to 30^{o}C, the virus remains viable up to 3 days while at -5 to 0, 4 and 15^{o}C up to 5 days. However, KHV stored at -80^{o}C has maintained its viability even one year after storage. Moreover, KHV was shown to be completely inactivated when exposed to UV irradiation (4.0 x 10^{3} µWs/cm^{2}, 50^{o}C for 1 min, 200 mg/L iodophor, 60 mg/L benzalkonium chloride or 30% ethyl alcohol for 20 min (Kasai *et al*., 2005).

Successive outbreaks caused by KHV among several different countries are indications of the transboundary movement of this emerging viral pathogen (Lio-Po 2007). Moreover, a further indication of the global significance of this disease is its recent (since 2007) addition to the list of notifiable diseases of the World Organization of Animal Health or the Office International des Epizooties (OIE).

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