KHV, CNGV, or CyHV-3, Which is the Koi/Carp Killer?

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ABSTRACT

Carp interstitial nephritis and gill necrosis virus (CNGV) is the cause of a lethal disease that afflicts koi and carps. Four genes of this virus are similar to those of Cyprinid herpesvirus 1 (CyHV-1) and Cyprinid herpesvirus 2 (CyHV-2) and so it was suggested that it be named CyHV-3. The virus induces gill necrosis and interstitial nephritis as early as 4-6 days post infection. The virus propagates well in the intestine and kidney and infected fish droppings contain high amounts of infectious virus. Thus, fish faeces is an appropriate source for non-invasive diagnosis by virus isolation in cell culture, PCR and ELISA tests. Strategies for controlling viral infection in fish are of limited use. Preventing introduction of CNGV into aquaculture facilities is desirable, but is not fail-safe. Live attenuated virus was developed in Israel and found to be an efficient and economic prophylactic vaccine. Morphologically CNGV resembles herpesvirus, but its genome size and its DNA sequences are different from herpesviridae members. Moreover, CNGV possesses genes resembling poxvirus rather than herpesvirus, indicating that additional information is required before classifying this virus.

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INTRODUCTION

Common carp (*Cyprinus carpio carpio*) is a fish species that is widely cultivated for human consumption, with 1.5 million metric tons harvested annually, principally by China, but also in other Asian countries, as well as several European countries (www.fao.org). Unlike the common carp, the subspecies koi (*Cyprinus carpio koi*) is a colorful ornamental fish, the focus of a hobby whose enthusiasts keep koi in backyard ponds and large display aquaria for personal pleasure and/or competitive showing.

A lethal viral carp disease was detected in the United Kingdom in 1996, but the first scientific reports did not appear until 1998, when Ariav *et al.* (1998) described the disease following major outbreaks in several carp farms along the Israeli coast. The disease was not restricted to the United Kingdom and Israel, and appeared in Germany with mass mortality (Bretzinger *et al*. 1999; Hoffman, 2000) and then in many countries all over the world (Crane, Sano and Komar, 2004; Haenen *et al.* 2004).

Although the morphology, the genome size and about 20% of the genomic sequences (see below) have been described, the International Committee on Virus Taxonomy has not yet determined the nomenclature of this virus. The virus has been designated by various names, first as koi herpesvirus (KHV), according to its morphological manifestation (Hedrick ett al. 2000), then as carp interstitial nephritis and gill necrosis virus (CNGV), according to the pathogenic effects it induces in fish (Pikarsky *et al*., 2004; Ronen *et al*., 2003). Recently it was shown that CNGV (KHV) possesses several genes whose sequences resemble those of CyHV-1 and CyHV-2 and that the CyHV-1 genome is approximately 295 kbp, similar in size to CNGV (KHV) (Hutonan *et al*., 2005; Ronen *et al*., 2003; Waltzek *et al*., 2005). These findings led to the suggestion that CNGV be designated cyprinid herpesvirus 3 (CyHV-3), recognising its unique characteristics (Waltzek *et al*., 2005).

THE DISEASE CAUSED BY CNGV

The disease caused by CNGV is seasonal, appearing in spring and autumn when the water temperature in open-air ponds is 17 to 28°C. It is highly contagious, spreads from infected to healthy fish sharing the same pond, and can induce 80 to 100% mortality rates. Mortality occurs within 6 to 22 days post infection (dpi), peaking at between 8 and 12 dpi (Perelberg *et al.*, 2003). Fish exposed to the virus at 20 to 24°C for 3 days and then transferred to a non-permissive temperature survived the disease (Perelberg *et al*., 2005; Perelberg *et al*., 2003; Ronen *et al*., 2003). On the other hand, many of the fish held at 13°C for 30 days developed the disease following a temperature shift up to 22 to 24°C. Persistence of the virus in the fish is limited, since fish transferred to the permissive temperature after 64 days at 13°C did not die (Gilad *et al*., 2003). Although the disease is highly contagious and extremely virulent, morbidity and mortality are restricted to koi and common carp (*Cyprinus carpio*) populations

(Bretzinger *et al*., 1999; Perelberg *et al*., 2003; Walster, 1999). The virus does not induce disease in other cyprinid or non-cyprinid species, and these virus-exposed fish do not

transfer disease to naïve carps, suggesting that species other than *Cyprinus carpio* are not carriers of CNGV (Perelberg *et al*., 2003; A. Perelberg, unpublished data).

Sick fish were apathetic and gathered close to the water surface, suffering from suffocation. Gill necrosis appeared as early as 3 dpi, coupled with an increase in the levels of external parasites and bacteria (Ariav *et al*., 1998). The skin showed a lack of lustre, with pale patches and increased mucus secretions (Haenen *et al*., 2004; Hedrick *et al*., 2000; Perelberg *et al*., 2005; Perelberg *et al*., 2003; Bergmann, unpublished data).

We examined the pathobiology of this disease in carp by using immunohistochemistry and PCR. We found large amounts of virus in the kidneys of sick fish and smaller amounts in liver and brain. Following the onset of disease a rapid increase in the viral load in the kidneys was detected by using both immunofluorescence and semiquantitative PCR. Histological analyses of fish at various times after infection revealed signs of interstitial nephritis as early as 2 dpi, which increased in severity up to 10 dpi. There was severe gill disease evidenced by loss of villi with accompanying inflammation in the gill rakers (Pikarsky *et al*., 2004).

Identification and characterization of CNGV

Hedrick and coworkers first isolated the virus after exposure of cultured KF-1 cells with cell extracts prepared from the organs of sick fish (Hedrick *et al*., 2000). Establishment of primary cultures of koi fin cells (KFC) and carp fin cells (CFC) allowed us to isolate the etiologic agent in Israel (Perelberg *et al.*, 2003; Ronen *et al.*, 2003). The isolated virus from sick carp was confirmed as the etiologic agent for this disease, based on the following data: (i) the virus was successfully isolated from infected fish but not from naïve specimens; (ii) inoculation of the virus propagated in KFC culture into naïve fish induced a similar disease; (iii) co-cultivation of kidney cells taken from specimens with the induced disease, but not from the mock-infected fish, yielded a similar virus, which propagated in KFC cultures; (iv) three cycles of transferring the virus between sick fish and cultured CFC ("ping-pong" technique) were successfully applied; (v) cloned virus isolated in tissue culture induced the same disease in fish; (vi) rabbit sera prepared against purified virus interacted specifically with tissues from experimentally infected fish as well as with sick fish from ponds; and (vii) viral DNA was identified in infected KFC and in sick fish but not in naive fish (Hutoran *et al*., 2005; Perelberg *et al*., 2003; Ronen *et al*., 2003). This initial identification of CNGV facilitated diagnosis of the disease by infection of KFC, PCR, and immunological methods.

Electron microscopy of negative-stained particles showed an icosahedral morphology with an average core diameter of 100 to 110 nm, resembling the core of herpesvirus (Hedrick *et al*., 2000; Hutoran *et al.*, 2005; Roizman, 1990). Thin sections of a purified virus pellet revealed enveloped particles with a thread-like structure (tegument) on the surface of the core. The cores bear atypically non-symmetrical electron-dense regions, which probably contain the viral genome (20) (Figure 1). The virus has a buoyant density of 1.16 g/ml and bands at 37 to 39% (wt/vol) sucrose following centrifugation in a sucrose gradient (Hutoran *et al.*, 2005). CNGV survived in pond water for 4 h at temperatures of around 22°C and probably survives for a longer period in droppings and pond mud (Crane, Sano and Komar, 2004; Dishon *et al.*, 2005; Hutoran *et al.*, 2005; Perelberg *et al.*, 2003).

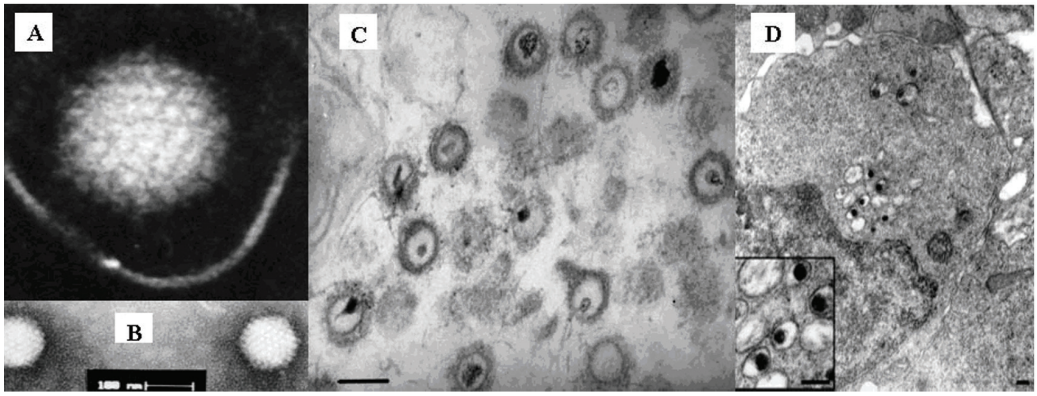

Figure 1. Electron micrographs of CNGV. CNGV harvested from infected KFC was purified by sucrose gradient and negatively stained with 2% phosphotungstate (panels A and B). Particle size was in the 96-105 nm range, with an average diameter of 103 nm (bar represents 0.1 μm). Thin sections (panel C) were made for ultrastructural analysis by transmission electron microscopy (TEM). Purified virus pellets were fixed with 3% glutaraldehyde in 0.1 M sodium cacodylate and stained with uranyl acetate and lead citrate. Panel D shows the ultrastructural appearance of CNGV particles in infected kidney 8 dpi. This cell harbors several cytoplasmic viral particles with round electron-dense cores (magnified in the insert). Bars represent 200 nm.

However, as a guideline for virus inactivation, infectivity is abolished after 2 days at 35°C (Perelberg *et al*., 2003) or after 30 min at 60°C (Crane, Sano and Komar, 2004). The virus is inactivated at pH values of below 3 and above 11, and it is also readily inactivated in chloroform, 25% ether, or 0.1% Triton X-100 (Crane, Sano and Komar, 2004; Hutoran *et al*., 2005).

Viral DNA

The CNGV genome is a large linear dsDNA of 270-295 kbp (Waltzek *et al*., 2005), as determined by pulsed-field gel electrophoresis (Hutoran *et al*., 2005; Waltzek *et al*., 2005) and is larger than those of vaccinia virus, herpes simplex virus type 1 (Figure 2), and all known members of the *Herpesviridae* except CyHV-1 (40). At present only ~20% of the viral sequences are present in GenBank, revealing that most of the viral genome (~80%) consists of DNA with no similarity to any sequences available in the databases. Moreover, the viral genome bears DNA sequences divergent from those of all other known avian and mammalian herpesviruses. Small genomic fragments of 16 to 45 bp show similarity mainly to members of the *Herpesviridae*, *Adenoviridae*, *Poxviridae*, and *Baculoviridae* families (Hutoran *et al*., 2005). One example is the DNA sequence of the CNGV thymidine kinase (TK) gene (accession #AJ535112) (Waltzek *et al*., 2005), of which only a 21-bp DNA fragment is similar to the Shope fibroma virus (a poxvirus) TK gene. However, recent studies found that the DNA sequences of the CNGV DNA polymerase, major capsid protein, helicase, and intercapsomeric triplex protein genes and of an additional open reading frame encoding an unidentified protein resemble those of CyHV-1 and CyHV-2 (Waltzek *et al*., 2005).

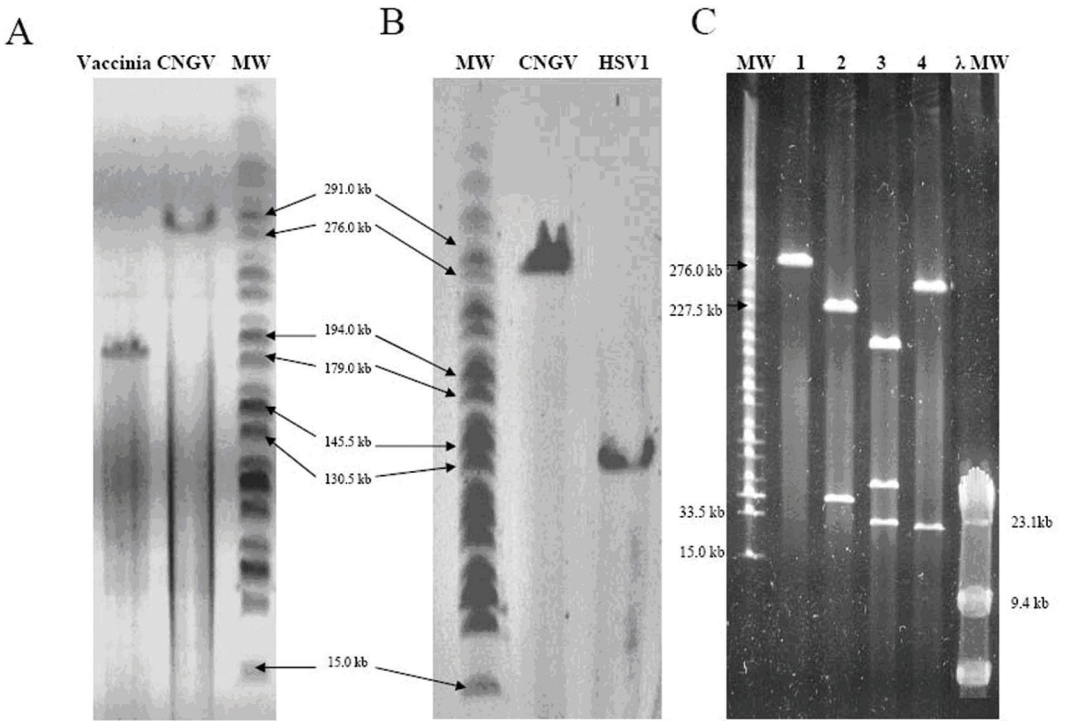

Figure 2. Analysis of CNGV DNA by pulsed-field gel electrophoresis (PFGE). Viral DNAs were obtained from purified viruses after incubation with proteinase K and SDS and phenol extraction. (A) Comparison of CNGV DNA and vaccinia virus DNA. (B) Comparison of CNGV DNA and HSV-1 DNA. (C) CNGV DNA cleaved with PmeI, PacI, and SwaI restriction enzymes. The molecular weight markers (MW) used were HindIII-cleaved λ phage DNA and a MidRange I PFGE marker (New England Biolabs).

Diagnosis of CNGV

The rapid dissemination of the virus in the fish body prompted us to develop efficient, rapid diagnostic means to identify the virus. Early diagnosis of the disease is extremely important to farmers, hobbyists, and veterinary authorities, allowing them to take appropriate precautions to prevent spread of the disease. It also allows hobbyists to save their fish by elevating the temperature and farmers to undertake early marketing of their fish. Several diagnostic techniques are currently in use, including isolation of the virus in cultured cells, histological and immunohistochemical methods, electron microscopy, enzyme-linked immunosorbent assay (ELISA), PCR, and *in situ* hybridization. These methods were described in detail by Haenen *et al*. (2004) and Pokorova *et al*. (2005).

The early appearance (3 to 5 dpi) of viral particles in carp droppings allows non-invasive detection of CNGV using PCR, ELISA, and infection of cultured cells (Dishon *et al*., 2005) (Figure 3). It is of great interest that virus present in sick fish droppings are infectious to fish, indicating that the virus is transmitted via faeces. It is not yet known whether infectious virus is preserved for long periods in the pool-bottom mud.

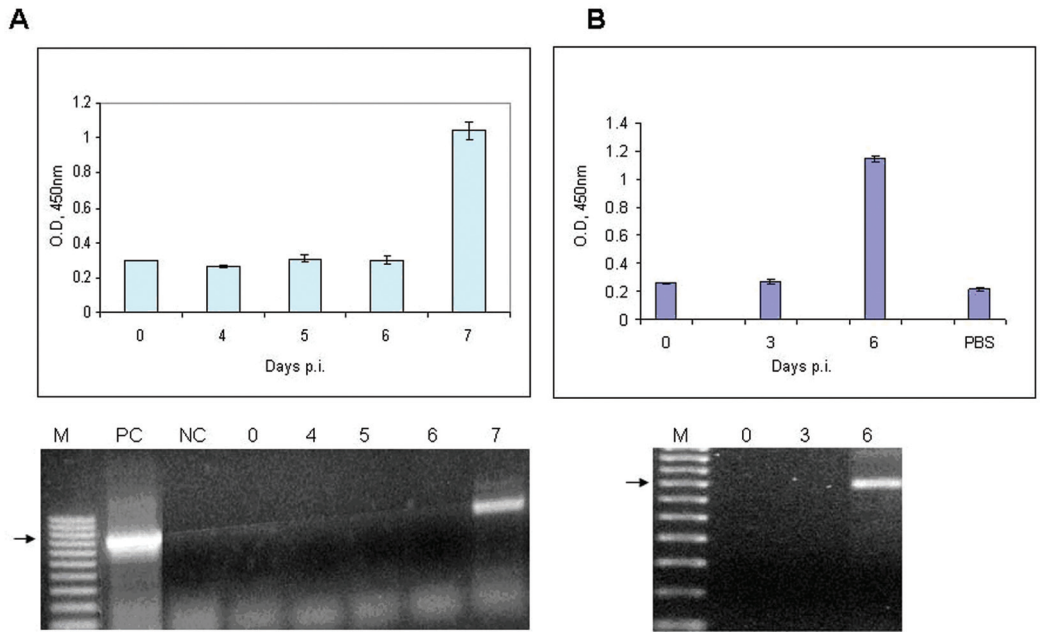

Figure 3. Detection of CNGV in fish-droppings by ELISA and PCR. Two fish (A and B) were kept separately, each in a tank, and their droppings were collected on the indicated days and frozen at -700C until processing. Each sample was assessed by ELISA and PCR. M=EZ load 100 bp molecular ruler (BIORAD). PC=positive control - purified CNGV DNA. NC=negative control – no template added. Arrow indicates the specific CNGV amplification product (see Materials and Methods). Data are shown as the mean OD values of wells ± the standard error of the mean.

Although most of the diagnostic methods are in use, they are time consuming and laborious and require specialized equipment. Recently, a "one-step ELISA kit" was developed (A.D. Thompson, pers. comm.), and a loop-mediated isothermal DNA amplification method was described by Gunimaladevi *et al.* (2004). and by Soliman and El-Matbouli (36). Both techniques are easy to use as rapid field diagnostic tools. It would be very helpful if these two new techniques were adapted to test for the presence of viral components in fish droppings. Detection of pre-vaccinated fish and/or fish surviving the disease following exposure to CNGV is crucial for veterinary authorities. Identification of viral DNA is not always successful, while detection of anti-CNGV antibodies in the fish serum by ELISA is relatively straightforward (Perelberg *et al.*, 2005; Ronen *et al.*, 2003). Unfortunately, a suitable kit for detecting anti-CNGV antibodies is not yet available.

Development of an efficient vaccine against CNGV

Currently, immunization of carp by injection of inactivated virus is ineffective. Live, attenuated vaccines potentially have many advantages in aquaculture (Benmansour and de Kinkelin, 1997). In general, live vaccine stimulates all phases of the immune system, resulting in balanced systemic and local responses involving both humoral and cellular

branches of the immune system. The advantages of using live attenuated virus vaccine are especially prominent in fish, where heat-inactivated virus is poorly immunogenic and large amounts of protein are required for achieving an efficient and durable immune response(Marsden *et al*., 1998; Marsden *et al*., 1996). With the use of live attenuated virus, the chance that reverted mutated virus will appear and threaten immunized populations is present but small. Experiments to achieve a nonpathogenic attenuated virus have been carried out in our laboratory since 2003 (Perelberg *et al.*, 2005; Ronen *et al*., 2003). The attenuated virus was isolated following serial transfer of the Israeli CNGV isolate in KFC. Viruses harvested after 20 passages in culture induced the disease in a small percentage of naïve fingerlings following injection or bathing (Perelberg *et al.*, 2005; Ronen *et al.*, 2003). It can be postulated, therefore, that the genetic alterations that accumulated in both the viral and host cell genomes facilitated the isolation of an attenuated virus. The attenuated virus was cloned in tissue culture in order to avoid undesired recombination, complementation, and reversion to a pathogenic virus. Several cloned viruses were UV irradiated and then re-cloned in order to insert additional mutations into the viral genome. This procedure was repeated twice. Currently, the selected attenuated virus clone does not induce lethal disease, and efficiently protects immunized fish against challenge infection (Perelberg *et al.*, 2005; Ronen *et al.*, 2003). Carp are very susceptible to pathogenic and attenuated viruses, and a short immersion of fish in water containing virus is sufficient for infection. The infection of fish with pathogenic and attenuated viruses is temperature restricted; fish held at the non-permissive temperature immediately following infection were not affected by the pathogenic virus and were not rendered resistant to the disease. The attenuated virus must propagate in the host fish in order to induce protection against the wild-type virus. Like the pathogenic virus, which induces the disease only at the permissive temperature, the attenuated virus requires the appropriate temperature to confer protection. Efficient protection is achieved by immersing the fish in water containing attenuated virus (10 to 100 PFU/ml) for 40 min, followed by incubation at the permissive temperature for an additional 48 to 72 hr (Perelberg *et al.*, 2005).

Protection against CNGV is associated with elevation of specific antibodies against the virus. The CNGV-specific antibody titer rises at 7 dpi and peaks at 21 dpi (Ronen *et al.*, 2003). Similar kinetics of antibody production was found in fingerlings immunized with the attenuated virus. The levels of anti-CNGV antibodies remained high in fish injected with either the pathogenic or the attenuated virus during the entire test period of 56 days (Fig. 4). These results point to a correlation between the survival rate and increased titers of anti-CNGV antibodies in the infected fish. The fish with naturally acquired immunity in ponds remain resistant for a long time (6 to 12 months). At present, we know that vaccination with the live attenuated virus confers resistance to a challenge infection for at least 8 months (unpublished data).

CNGV phylogenetics

Herpesviruses have complex and characteristic structures consisting of both symmetrical and nonsymmetrical components (Homa and Brown, 1997; Steven and Spear, 1997). The spherical virion comprises the core, capsid, tegument, and envelope (Nathanson and Murphy, 1997). The core consists of the viral genome, which is packaged as a single linear

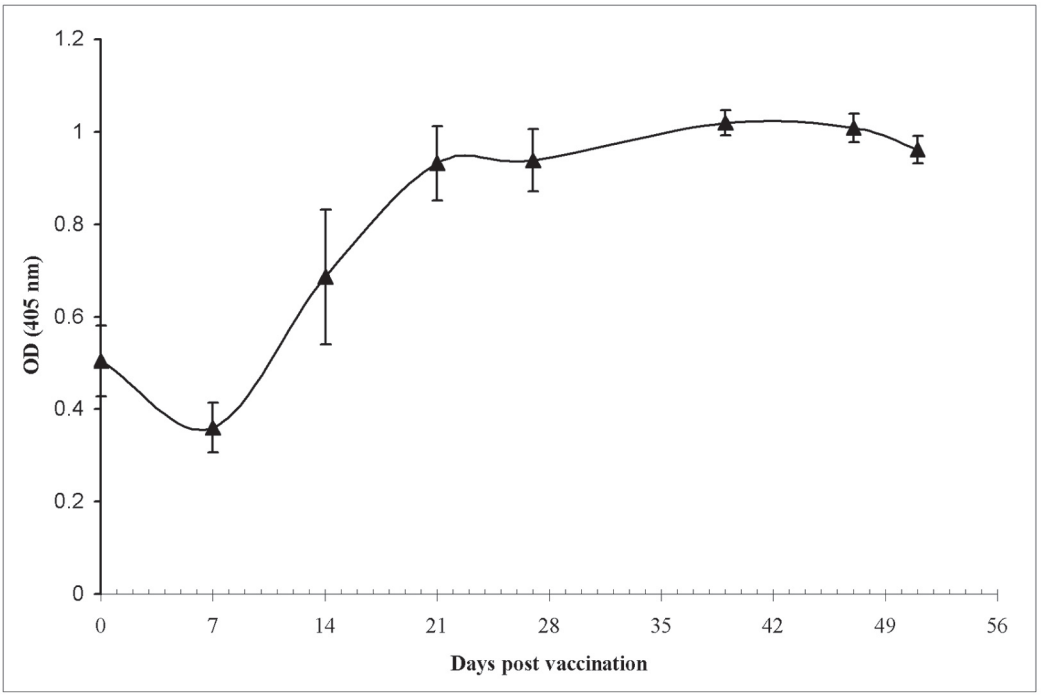

Figure 4. Induction of anti-CNGV antibodies in vaccinated fish. Fish (n=5 fish, average weight = 50 gr.) were immunized by intra-peritoneal injection of CNGV-P26 and thereafter, sera samples were collected, diluted 1:100 and analyzed by ELISA. Each point represents the average of 5 fish and the Error bars indicate ±S.E.

double-stranded DNA (dsDNA) molecule ranging from 125 to 240 kbp in size. Thus, the use of "herpes" in the CyHV may require either changing the definition of the herpesvirus family to include viruses with a genome larger than 240 kbp, or classifying these viruses with a large genome into a specific group. We and others estimate that about 80% of the CNGV genome is divergent from all the sequences available in the databases, making the classification of this virus very difficult.

Mammalian and avian herpesviruses are highly adapted to their hosts, and lethal infection occurs only in fetuses, in immuno-suppressed organisms, or following infection of an alternative host. Herpesviruses establish lifelong latent infection, a feature which is assumed to be the hallmark of all herpesviruses (Van Regenmortel *et al*., 2000). In contrast, infection with CNGV causes an acute disease with a higher than 90% mortality of both fry and adult carp. Assuming CNGV to be truly a member of the *Herpesviridae* family implies that it persists in the host as a latent virus. Solving the question of latency will contribute not only to the phylogenic classification of this virus, but also to understanding its epidemiology and improving prophylactic measures to prevent the spread of the disease.

The number of open reading frames in the viral genome and the number of proteins assembled in the virion are not yet known. Rough estimation made by sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of purified virions approximated the

number of proteins to be between 31 (Pokorova *et al*., 2005) and 80 (unpublished data). However, due to the large genomic size of this virus, the number of proteins expressed by CNGV may be even larger. Recent data showed that the CNGV genome allows 183 to 185 open reading frames (T. Aoki, pers. comm.).

Our early studies (Hutoran *et al.*, 2005) suggested that the DNA sequence of CNGV is divergent from all viral genomes available in the databases. However, following partial DNA sequencing of CyHV-1 and CyHV-2 genomes, it was demonstrated that CNGV and these viruses bear four similar genes (Waltzek *et al.*, 2005).

Comparison of the translation products of the viral DNA sequences to those available in the databases by using the BLAST X program showed that the major capsid protein, DNA polymerase, helicase and intercapsomeric triplex genes have some similarity to the proteins of anguillid herpesvirus 1 (Lee, Kobayashi and Miyazaki, 1999), ictalurid herpesvirus 1 (Hutoran *et al.*, 2005), ranid herpesvirus 1 (Davison *et al*., 1999), and salmonid herpesvirus 1 (Davison, 1998). Based on this similarity, Waltzek *et al.* (2005) suggested that these viruses can be included in a new group of aquatic herpes-like viruses. In agreement with this view, Rijsewijk *et al.* (2005) demonstrated by CyHV-3 (KHV) and AngHV-1 are closely related according to the sequences of their DNA polymerase proteins. Although the amino acid sequences of these two viruses are similar to those of ranid herpesvirus 1 and ictalurid herpesvirus 1, it has been suggested that they be categorized as a separate group (Rijsewijk *et al.*, 2005).

Although four genes of CNGV and CyHVs are similar to each other, there was no apparent homology with other DNA viruses. Providing additional CyHV-1, CyHV-2, and CNGV DNA sequences to GenBank will assist in the phylogenic classification of CNGV, which will be instrumental in developing strategies for controlling the lethal disease caused by CNGV. It is conceivable that fish viruses that evolve in distinct ecological environments are genetically highly divergent from each other. In this case it would be expected that CNGV, which thrives in captivity and is restricted to limited species of *Cyprinus*, bears only slight similarity to other viruses.

Importantly, the CNGV sequence encodes three enzymes involved in the processing of nucleotides for DNA synthesis, i.e., ribonucleotide reductase, thymidylate kinase (TmpK), and thymidine kinase (TK), which are similar to those encoded by the poxvirus genome (Table 1). It should be emphasized that B22R encoded by CNGV is a protein found exclusively in poxviruses. B22R and TmpK have not yet been found in herpesvirus (Meyer *et al*., 2005; Smith, Carlos and Chan, 1989). It would be of great interest to determine whether CyHV-1 and CyHV-2 code for these three proteins as well. Several other CNGV polypeptides show similarity to proteins derived from ictalurid herpesvirus 1, African swine fever virus, and shrimp white spot virus (Hutoran *et al.*, 2005). Iyer *et al*. (2001) showed that African swine fever virus, poxviruses, iridoviruses, and phycodnaviruses share a common evolutionary origin but have no direct evolutionary relationship to herpesviruses. Based on these analyses, we speculate that either CNGV evolved by intensive incorporation (probably by recombination) of genetic fragments derived from poxviruses and other DNA viruses or the CyHVs group descended from a unique common

Table 1. Comparison of CNGV to representatives of Pox viruses and other large DNA viruses. Four CNGV DNA sequences were analyzed using translating BLAST (http://www.ncbi.nlm.nih.gov/BLAST). The search was carried out against the Viruses subclass of the NR database. Representatives from different virus families are presented in the table. Identity (%) is the number of amino acids with identity/total number of amino acids of the protein; Similarity (%) is the number of conserved amino acids/total number of amino acids; Gaps (%) are numbers of gaps/total number of amino acids. a The assessed fragment does not represent the sequences of complete protein.

Gene and Acc#	Family	Genus	Acc#	Identity (%)	Similarity (%)	Gaps (%)
B22R like gene (AY661550)	Poxviridae	Fowlpox virus	CAE52648	78/262 (29%)[a]	133/262 (50%)	8/262 (3%)[a]
Thymidylate kinase (DQ118125)	Poxviridae	Canarypox virus	NP_955193	106/216 (49%)	143/216 (66%)	8/216 (3%)
	Nimaviridae	Shrimp white spot syndrome virus	AAK77840 TK-dTMP	82/191 (42%)	117/191 (61%)	1/191 (0%)
	Iridoviridae	Chilo iridescent virus	AAK82112	72/176 (40%)	117/176 (66%)	3/176 (1%)
	Asfarviridae	African swine fever virus	CAA79604	57/192 (29%)	89/192 (46%)	8/192 (4%)
Ribonucleotide reductase (AY786308)	Poxviridae	Monkeypox virus	AAU01269	447/803 (55%)	580/803 (72%)	35/803 (4%)
	Baculoviridae	Spodoptera litura nucleopolyhedrovirus	AAL01709	415/763 (54%)	555/763 (72%)	13/763 (1%)
	Phycodnaviridae	Feldmannia irregularis virus a	AAR26844	394/765 (51%)	529/765 (69%)	12/765 (1%)
	Mimivirus	Acanthamoeba polyphaga mimivirus	YP_142667	346/733 (47%)	489/733 (66%)	12/733 (1%)
	Nimaviridae	Shrimp white spot syndrome virus	AAL89096	379/845 (44%)	538/845 (63%)	49/845 (5%)
	Asfarviridae	African swine fever virus	P42491	285/737 (38%)	415/737 (56%)	64/737 (8%)
	Herpesviridae	Ostreid herpesvirus 1	YP_024594	263/741 (35%)	415/741 (56%)	58/741 (7%)
Thymidine kinase (AJ535112)	Poxviridae	Cowpox virus	AAF21104	71/171 (41%)	114/171 (66%)	3/171 (1%)
	Mimivirus	Acanthamoeba polyphaga mimivirus	YP_142612	60/181 (33%)	102/181 (56%)	14/181 (7%)
	Nimaviridae	Shrimp white spot syndrome virus	AAG40728	69/178 (38%)	100/178 (56%)	8/178 (4%)
	Asfarviridae	African swine fever virus	AAQ07945	52/177 (29%)	81/177 (45%)	10/177 (5%)

ancestor differing from the mammalian, avian, and reptile viruses, as well as from the clade of ostreid herpesvirus (Davison *et al.*, 2005). If the latter possibility proves to be true, it would be logical to include the CyHVs as a separate family under the umbrella of the order *Herpesvirales* (McGeoch, Rixon and Davison, 2006).

CONCLUSION

In conclusion, the morphology of CNGV is similar to that of the *Herpesviridae*. However, the genome of the isolated virus is composed of a 295 kbp linear dsDNA molecule, larger than that of the other *Herpesviridae* members and bearing highly divergent DNA sequences that encode polypeptides resembling those of other large dsDNA viruses. Analysis of the DNA and amino acid sequences of this high-molecular-weight viral genome revealed that CNGV could be a novel virus bearing a mosaic genome including genetic elements derived from phylogenetically distant DNA viruses.

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