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The outbreak of carp disease caused by CyHV-3 as a model for new emerging viral diseases in aquaculture: a review

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Abstract Aquacultured koi and common carp (*Cyprinus carpio*) are intensively bred for ornamental purposes and for human consumption worldwide. The carp and koi farming industries have suffered enormous economic losses over the past decade due to an epizootic disease caused by *Cyprinus herpesvirus-3* (CyHV-3) also known as koi herpesvirus and carp interstitial nephritis gill necrosis virus. CyHV-3 is a large dsDNA virus, morphologically similar to herpesviruses, yet contains genetic elements similar to those of pox, irido- and herpesviruses. Considering the phylogenetic distance between CyHV-3 and higher vertebrate herpesviruses, CyHV-3 represents the prototype of viruses assigned to the novel family *Alloherpesviridae*. Although emergence of a new virus rarely initiates a pandemic so severe that it reduces the life expectancy of a population, CyHV-3 is exceptional because of its enormous impact on the world carp population. High population density is the major contributing factor to the epizootic disease caused by CyHV-3.

Keywords CyHV-3 · KHV · Vaccine · Carp disease · *Alloherpesviridae*

Viruses of lower vertebrates

The growing demand for protein sources and the depletion of natural resources has pushed towards the rapid growth of the aquaculture industry. With an average annual growth rate of 8.8% since 1970, the

aquaculture industry has surpassed both the fish capture and the terrestrial farmed meat production systems (Nomura 2006). The rapid growth of this industry has come with a price. Viruses of fish have recently become a field of interest to the public due to increasing epizootic and economic losses (Essbauer and Ahne 2001). Under the usually crowded hatchery conditions, infectious agents easily spread within a susceptible species and cause high mortality rates. Outbreaks of mortal viral diseases afflicting fisheries have been reported worldwide. RNA and DNA viruses pose a threat to the growing aquaculture industry, causing severe financial losses worldwide. Moreover, viruses of lower vertebrates have proved to be of veterinary and public interest. Research on these viruses contributes to the phylogenetic definition of virus families, and the study of the epidemiology of viral diseases in fish may be used as a model for newly emerging diseases. Though evolutionarily distant, fish can serve as a convenient and cost-effective animal model to study host and virus interactions in higher vertebrates and humans.

General description of newly emerging diseases in carp

Common carp (*Cyprinus carpio carpio*) is a fish species widely cultivated for human consumption, principally by China and other Asian countries, Europe, and the USA (<http://www.fao.org>). In contrast to the common carp, the subspecies koi (*Cyprinus carpio koi*) is a beautiful and colorful fish, the focus of a hobby whose enthusiasts keep koi in backyard ponds and large display aquaria for personal pleasure or competitive showing. The hobby originated with the Romans in the first century A.D., matured into the present science and art practiced in Japan, and subsequently spread worldwide (Balon 1995).

A mortal viral carp disease was detected in the United Kingdom in 1996, but the first scientific reports did not appear until 1998, when Ariav and co-workers described the disease (Ariav et al. 1998) following major outbreaks

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in several carp farms along the Israeli coast. Now the disease afflicts carp in many countries all over the world, causing mass mortalities in Indonesia, Japan, Taiwan, and Thailand (Crane et al. 2004; Haenen et al. 2004; Pokorova et al. 2005; Sunarto et al. 2005; Takashima et al. 2005; Tu et al. 2004). The mortality rate in the affected Israeli ponds was 80–100% in fry as well as in older, market-size fish (Perelberg et al. 2003). Unlike other appearances of a new virus, which rarely causes a pandemic so severe that it reduces the life expectancy of a population, CyHV-3 is exceptional because of its enormous impact on the world carp population, resembling the global human influenza pandemic of 1918.

The characterization of CyHV-3 and the description of the disease it causes has already been described (Haenen et al. 2004; Ilouze et al. 2006; Pokorova et al. 2005). Here, we outline the characteristics of a new emerging virus, which first appeared 12 years ago and now afflicts carp in aquaculture farms and in environmental water such as lakes and rivers worldwide. The biological characteristics of the virus, such as persistency under non-permissive temperature, its ability to infect several closely related fish species, and its stability in water, provide the virus with the evolutionary advantages that are responsible for its rapid dissemination, and therefore present a novel model for new emerging diseases in aquaculture.

Virus morphology

Morphologically, CyHV-3 resembles a herpesvirus with a 100 to 110-nm-diameter icosahedral-shaped core (Hedrick et al. 2000; Hutoran et al. 2005; Mettenleiter et al. 2009; Miwa et al. 2007; Roizman and Whitley 2001). Thin sections of purified virus pellet revealed enveloped particles with a thread-like structure (tegument) on the surface of the core. The cores bear atypically nonsymmetrical electron-dense regions that contain the viral genome (Hutoran et al. 2005). Herpesvirus ultrastructural features (Fields et al. 2007) are identified in the infected cultured cells (Fig. 1). Cores containing the viral genome, surrounded by a slightly lighter capsid, form the viral nucleocapsid. Surrounding the capsid is an amorphous tegument, which is bounded by the outer envelope. The virus acquired a secondary envelope in the infected cytoplasm (Fig. 1), possibly due to association with membrane-bound cytoplasmic vesicles or membrane folds in the peripheral zone of the infected cell.

The CyHV-3 genome

The genome of the isolated CyHV-3 is a large dsDNA of 295 kbp, larger than the genomes of all the known members of *Herpesviridae* (Aoki et al. 2007; Waltzek et al. 2005). The viral genome consists of 156 open

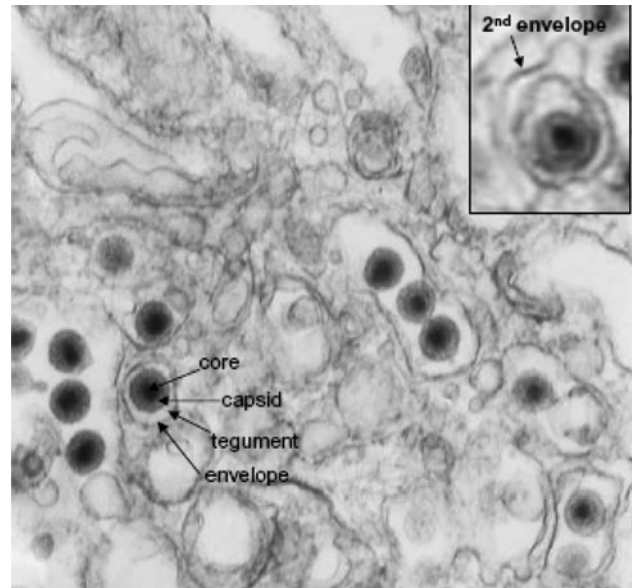


Fig. 1 Ultrastructure of mature virions in the cytoplasm of CyHV-3-infected carp cells. *Top right inset* shows a secondary envelopment of a mature virion at the periphery of the infected cytoplasm

reading frames and has two terminal repeats of ca. 22 kbp each (Aoki et al. 2007; Waltzek et al. 2005). The CyHV-3 156 ORFs are transcribed into mRNAs, which are classified into immediate early (IE or α), early (E or β), and late (L or γ), similar to all other herpesviruses (M. Ilouze, unpublished results).

The CyHV-3 genome is highly divergent from almost all other viruses, but small genomic fragments show similarity mainly with members of the *Herpesviridae*, *Adenoviridae*, *Poxviridae*, and *Baculoviridae* families (Hutoran et al. 2005). Interestingly, CyHV-3 codes for several proteins resembling those coded by some large DNA viruses, such as herpesviruses and the genetically distinct poxviruses. It also encodes for 15 genes, which have clear homolog counterparts in the distantly related Ictalurid herpesvirus-1 (IcHV-1) and Ranid herpesvirus-1 (RaHV-1) (Aoki et al. 2007), and it contains DNA and protein sequences resembling those of the closely related CyHV-1 and CyHV-2 (Waltzek et al. 2005). However, approximately 70% of CyHV-3 proteins do not show any similarity to known viruses. Forty viral and 18 cellular proteins are incorporated into the CyHV-3 virions (Michel et al. 2010). Analysis of the CyHV-3 genome could not resolve the evolutionary origin of the CyHVs, and therefore it was classified into a separate family in the order *Herpesvirales* (Davison et al. 2009; McGeoch et al. 2006).

The pathobiology of the disease caused by CyHV-3

Three isolates of CyHV-3, namely Japanese, American, and Israeli, distinguished by genetic variations, caused a similar disease (Aoki et al. 2007; Kurita et al. 2009).

The disease is seasonal, appearing in spring and autumn when the water temperature in open-air ponds is 18–28°C. It is highly contagious, spreads from infected to healthy fish sharing the same pond, and is lethal for 80–100% of the fish in infected open-air ponds. Mortality occurs within 6–22 days post-infection (d.p.i.), peaking at between 8 and 12 d.p.i. (Perelberg 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 the disease in fish other than *Cyprinus carpio*, yet recent findings suggest that cyprinids such as gold fish (*Carassius auratus*) may serve as carriers of CyHV-3 (Davidovich et al. 2007; El-Matbouli et al. 2007; Sadler et al. 2008). Sick fish are apathetic and gather close to the water's surface, suffering from suffocation. Gill necrosis appears as early as 3 d.p.i., coupled with an increase in the levels of external parasites and bacteria (Ariav et al. 1998). The skin shows a lack of luster, with pale patches and increased mucus secretions (Haenen et al. 2004; Hedrick et al. 2000; Perelberg et al. 2005, 2003).

In sick fish, the most prominent lesions were observed in the gill, skin, kidney, spleen, liver, and gastrointestinal systems (Hedrick et al. 2000; Pikarsky et al. 2004; Tu et al. 2004). Pathological changes were noted in the gills as early as 2 d.p.i., as evidenced by a loss of lamellae followed by complete effacement of the gill architecture accompanied by severe inflammation in nearly all of the filaments. The pathological signs in the gill rakers include increased subepithelial inflammation and congestion of blood vessels in the gill arch, accompanied by attenuation of the rakers' height. In addition to the gills, severe pathological changes were noted in the kidney. A mild peritubular inflammatory infiltrate was evident as early as 2 d.p.i. On day 6, a heavy interstitial inflammatory infiltrate was observed, along with congestion of blood vessels. Other organs are also affected: liver analysis showed mild inflammatory infiltrates located mainly in the parenchyma, while brain sections showed focal meningeal and parameningeal inflammation (Pikarsky et al. 2004).

Transmission of the virus

It is still unclear how viruses spread in an infected pond, from pond to pond, among remote farms, and where the virus is preserved between seasons. Virus harvested from tissue cultures remains infective in water for at least 4 h (Perelberg et al. 2003), explaining the highly contagious nature of the virus in ponds and open-air water. Shimizu and his coworkers (2006) suggested that CyHV-3 lost its infectivity within 3 days in environmental water, while its infectivity remained for more than 7 days in sterilized water.

It is not yet known how the virus enters the fish body, i.e., through the gills, the intestine (Hedrick et al. 2000; Perelberg et al. 2003), or as recently demonstrated, via the skin (Costes et al. 2009). Viral particles and/or viral DNA were detected in intestine, gill mucus, and lamellae as early as 1–2 d.p.i. (Gilad et al. 2004; Pikarsky et al. 2004), pointing at a possible route of infection.

Histological examination of sections derived from infected fish clearly demonstrated that the virus replicates in intestine, kidneys, and gills (Pikarsky et al. 2004). Cells expressing viral proteins were detected in the interstitial cells, most of which appeared foamy and large, corresponding to the cytomegali described in infected cultured cells, as early as 2–3 d.p.i. (Pikarsky et al. 2004). Large amounts of viral DNA were found in the gut early after infection (Gilad et al. 2004), and clusters of virus particles were detected by electron microscopy in the intestinal system (Perelberg et al. 2003).

It is assumed that the virus can be rapidly transferred from the gills to the kidneys, where it resides in white blood cells and induces severe interstitial nephritis. Localization of the virus within white blood cells raises the intriguing possibility that the virus is rapidly transported to the viscera via infected white blood cells and then multiplies in the epithelial cells of the kidney and intestine. Interestingly, recent findings suggest that CyHV-3 persists in the infected fish brain longer than in kidneys (Yuasa and Sano 2009).

The virus is released into the water either through shedding, or together with the sloughed epithelial and inflammatory cells resulting from severe local inflammation. The ability of the virus to invade the fish through the gills, multiply there, and then be released through the water is analogous to the case of respiratory viruses in mammals that infect the respiratory epithelium, replicate there, and are spread through air droplets and aerosols. This may turn out to be the most common means of the spread of aquatic viruses.

The possibility that the virus penetrates the fish body through the digestive system should also be considered. Based on these observations, we looked for viruses and/or viral components in the droppings of infected carp and we showed that: (i) fish droppings contain viral DNA, (ii) fish droppings contain viral antigens, which are useful for CyHV-3 diagnosis, and (iii) fish droppings contain active virus, which infects cultured common carp brain cells and induces the disease in naive fish following inoculation. Thus, we elaborate on the possibility that fish droppings, which contain epithelial cells, preserve viable CyHV-3 during the nonpermissive seasons (Dishon et al. 2005).

The next question is how the virus is transmitted from one fish farm to another. In Israel, the disease started in two farms on the Mediterranean shore, from which it spread sequentially to closely located ponds, infecting most of the country's farms within 3 years. Based on our observations that birds crowd around ponds with infected carp, easily catching sick fish gathered close to the

water's surface, we hypothesize that birds transfer the disease by moving sick fish from pond to pond.

On the other hand, the disease is probably transferred among distant countries by uncontrolled commercial trade of fishery equipment and fish lacking appropriate testing in accordance to quarantine rules. However, one of the crucial (and as of yet unsolved) questions is how and where the virus survives during non-permissive seasons. In this regard, we will discuss the possibility that the virus undergoes latent infection, and that other fish species serve as reservoirs of CyHV-3.

CyHV-3 host range

While lethal to common carp and koi, other closely related cyprinids and more distant fish species seem to be asymptomatic when exposed to the virus. Cohabitation of sick common carp (*Cyprinus carpio*) with silver carp (*Hypophthalmichthys molitrix*), goldfish (*Carassius auratus*), black carp (*Ctenopharyngodon idella*), tilapia (*Oreochromis niloticus*), and silver perch (*Bidyanus bidyanus*) showed no mortality in fish other than the common carp and koi (Perelberg et al. 2003). Furthermore, cohabitation of naive common carp with the exposed resistant species showed no mortality of the naive carp, suggesting that infection and transmission of CyHV-3 is restricted to koi and common carp (Perelberg et al. 2003). However, there is accumulated data demonstrating that goldfish are infected and may carry CyHV-3 (El-Matbouli et al. 2007; Sadler et al. 2008). While viral DNA was detected in non-symptomatic goldfish, as yet, we and others have not been able to demonstrate that goldfish disseminate the disease. Interestingly, carp larvae (Ito et al. 2007a, 2007b) and even juvenile carp are not (or are only to a lesser degree) susceptible to CyHV-3, but can easily be infected upon maturation.

Our studies showed that cultured cells derived from common carp brain (CCB), koi fins (KFC), silver carp (Tol/FL), and goldfish fins (Au) allow CyHV-3 propagation, while epithelioma papulosum cyprini (EPC) cells are infected but virus production is detected 3 weeks p.i. and in lower titers (our unpublished results). In contrast, cyprinid cells derived from fathead minnow (FHM) and non-cyprinid cells derived from the channel catfish ovary (CCO) are resistant to CyHV-3 infection (Davidovich et al. 2007). These results show that cyprinid species other than common carp and koi could be susceptible to the virus because they have no specific host restriction, suggesting that the virus indolently propagates in other cyprinids, sufficient to evoke efficient immunologic reactions, avoiding the manifestation of the disease.

Temperature sensitivity

In Israel, outbreaks of the disease occurred during the spring and fall. Exposure of captive naive carp to

infected specimens, or to a virus harvested from infected cultured cells, at temperatures ranging between 18 and 28°C resulted in a high mortality of 75–95% (Gilad et al. 2003, 2002; Hedrick et al. 2000; Hutoran et al. 2005; Perelberg et al. 2005, 2003). However, infected fish kept at a temperature of 13°C survived 60 days—the entire duration of the experiment—without manifesting any clinical signs of the disease (Gilad et al. 2003; Ronen et al. 2003). Moreover, infected carp maintained at 30°C did not develop the disease and became “naturally resistant” to a challenged infection (Ronen et al. 2003). These experiments indicate that the progression of the disease is temperature-dependent (Gilad et al. 2003; Ronen et al. 2003; Yuasa et al. 2008).

We observed that infectious virus is not propagated but preserved in cultured cells maintained for 30 days at 30°C. Cells infected with CyHV-3 at 22°C become morphologically deformed, but convert back to normal and plaques disappear upon increasing the temperature. The plaques reappear after transferring the cultures to the permissive temperature. Shifting cells from 22 to 30°C 2 h post-infection turned off viral propagation and halted the transcription of most viral genes (Dishon et al. 2007). Upon return of the cells to the permissive temperature, transcription of viral genes was reactivated in a sequence distinguished from that occurring in cells shifted to restricted temperature following infection.

We observed that although CyHV-3 penetrated cultured carp cells maintained at 30°C, viral DNA replication was completely halted. However, about 60% of CyHV-3 ORFs were transcribed shortly after infection. During incubation at 30°C, all these viral genes were down-regulated and then shut off, except three viral genes that were transcribed for 18–25 d.p.i. These genes could be involved in virus persistence (M. Ilouze, unpublished results). Although these experiments were carried out in cultured cells, they imply that CyHV-3 could persist for long periods in the fish body, enabling a new burst of infection upon shift to permissive temperature (Dishon et al. 2007).

Latency

Mammalian and avian herpesviruses are highly adapted to their hosts, and acute infection is usually observed only in fetuses, in immunosuppressed 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 CyHV-3 causes an acute disease with about 90% mortality of both fries and adult carp. If CyHV-3 is truly a member of the *Herpesviridae* family, it is plausible that it persists in the host as a latent virus. Recent publications suggest that the CyHV-3 genome is detected in survivor's brains 1 year post-infection (Yuasa and Sano 2009). Solving the question of latency will contribute not only to the phy-

logenic classification of this virus but also to understanding its epidemiology and thus improving prophylactic measures to prevent the spread of the disease.

It has been shown that some apparently related aquatic viruses exhibit latency in their hosts. For instance, latent CyHV-1 persists in carp that survive infection (Sano et al. 1993b), and AngHV-1 persists in surviving infected eels (Rijsewijk et al. 2005; Van Nieuwstadt et al. 2001). In these cases, the viral genome was identified in cranial nerve cells and spinal nerves by *in situ* hybridization (Morita and Sano 1990; Sano et al. 1993a, 1993b). It would be interesting to determine whether the pathogenic and attenuated CyHV-3 and CyHV-2 also persist in neurons of infected fish.

Mammalian herpesviruses bear specific genes that maintain the latent viruses in their host cells. The transcriptional profile of infected cells incubated at restrictive temperature is dramatically reduced compared to the permissive temperature, yet few genes are still expressed (Dishon et al. 2007). Do these genes serve similar functions to those of mammalian herpesviruses? Can we consider the virus preserved in cells at non-permissive temperatures as latent? These questions remain to be solved. Certainly this mechanism of persistence at non-permissive temperatures is advantageous to the virus, allowing it to survive between the seasons.

Protection of fish against CyHV-3

One way to reduce the threat caused by CyHV-3 is to select strains and crossbreds that are more resistant to viral infection. Screening of several edible carp strains and their crossbreds revealed that the Dor-70 × wild-type Sassan fish were quite resistant to CyHV-3 infection (60.7% survivors) (Shapira et al. 2005). However, the design and development of carp strains resistant to CyHV-3 will require the use of modern molecular genetic methodologies such as quantitative trait loci and microarrays (Gracey et al. 2004). It was recently suggested that resistance of common carp to CyHV-3 is linked to MHC II B gene polymorphism, and could be used as potential genetic markers in breeding carp for resistance to this virus (Rakus et al. 2009). Even so, the breeding system would not be appropriate for selecting resistant ornamental koi fish. Immunization of fish against the virus may be a useful tool for overcoming the CyHV-3 threat. Unfortunately, thus far, all efforts to immunize carp with inactivated virus or with viral proteins have proven to be unsuccessful. However, liposome vaccine, which includes inactivated CyHV-3 entrapped within liposome, seemed to be efficient for oral vaccination of carp against the disease (Yasumoto et al. 2006). To eradicate this disease from fish husbandries, two methods of fish immunization were developed in Israel: challenge of the fish with the pathogenic virus (see below) and fish immunization with an attenuated CyHV-3 (Perelberg et al. 2008, 2005; Ronen et al. 2003).

Fish with naturally acquired immunity

Based on the observation that the disease breaks out when the water temperature is between 18 and 28°C, Dr. I. Bejerano developed a protocol for selecting carp and koi fish with naturally acquired immunity. According to this procedure, healthy fingerlings were exposed to the virus by cohabitation with sick fish for 2–5 days at 22–24°C (permissive temperature). Thereafter the water temperature was elevated above 30°C for 25–30 days, and the fish were then transferred to open-air ponds. This procedure was found to be quite efficient, and 60% of the immunized fingerlings survived a challenge with sick fish (Ronen et al. 2003). We find that the fish with naturally acquired immunity remain resistant in ponds for a long time, as revealed by challenge infection even a year after exposure to the pathogenic virus (our unpublished results). Although this procedure was beneficial to Israeli fisheries, it has several disadvantages: (i) by using this method, farmers spread the pathogenic virus over many fisheries and risk spreading it into wild carp populations; (ii) the procedure involves a loss of 40% or more of the fingerlings; (iii) economically the procedure is costly, and (iv) most importantly, it involves a serious risk, because the pathogenic CyHV-3 used for immunization may persist in the fish body and could reproduce following stress, inducing the disease in the infected fish themselves and/or in the non-immunized fish. So far, using specific and efficient primers and conventional PCR, we and others have been unable to detect any latent viral DNA in organs of fish with naturally acquired immunity. However, Gilad et al. (2004) found small traces of viral DNA in surviving fish at 62–64 d.p.i. by using real-time TaqMan PCR.

Development of an efficient vaccine against CyHV-3

Immunization of carp by injection of inactivated virus has failed so far. Live, attenuated vaccines potentially have many advantages in aquaculture (Benmansour and de Kinkelin 1997). The availability of the CyHV-3 genome as an infectious BAC will allow the production of attenuated recombinant candidate vaccines (Costes et al. 2008). 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 proteins are required for achieving an efficient and durable immune response (Marsden et al. 1998, 1996). Moreover, the chance that reverted mutated virus will appear and threaten immunized populations is very small. Experiments to achieve a non-pathogenic attenuated virus have been carried out in Israel since 2003 (Perelberg et al. 2005; Ronen et al. 2005). The attenuated virus was

isolated following serial transfer of the Israeli CyHV-3 isolate in KFC. Viruses harvested after 20 passages in culture induced the disease in a small percentage of naive fingerlings following injection or bathing (Perelberg et al. 2005; Ronen et al. 2005). 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 (Drake 1969, 1976; Freese 1963). Currently, the selected attenuated virus clone does not induce the lethal disease and efficiently protects the immunized fish against challenge infection for quite long periods of time (Perelberg et al. 2005; Ronen et al. 2003). Carp are very sensitive 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 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 the attenuated virus (10–100 PFU/ml) for 40 min, followed by incubation at the permissive temperature for an additional 48–72 h (Perelberg et al. 2005). Protection against CyHV-3 is associated with elevation of specific antibodies against the virus. The CyHV-3-specific antibody titer rises 7 d.p.i. and peaks at 21 d.p.i. (Ronen et al. 2003). The levels of anti-CyHV-3 antibodies remained high in fish injected with either the pathogenic or the attenuated virus during the entire test period of 56 days. These results point to a correlation between the survival rate and increased titers of anti-CyHV-3 antibodies in the infected fish. The fish with naturally acquired immunity in ponds remain resistant for 6–12 months (Ronen et al. 2003). At present, we know that vaccination with the live attenuated virus confers resistance to a challenge infection for at least 8 months (Perelberg et al. 2008).

Prospects

The CyHV-3 disease has now spread to many countries worldwide, on four continents. The disease is not only restricted to the fish industry but was also found in lakes and rivers of Japan, Germany, England, the Netherlands, and the US (Bretzinger et al. 1999; Crane et al. 2004; Grimm et al. 2006; Haenen et al. 2004; Honjo et al. 2009). The disease is also not restricted to koi and

common carp but also infects goldfish (El-Matbouli et al. 2007; Sadler et al. 2008) and probably non-cyprinids and other aquatic organisms (Kielinski et al. 2010). It is plausible that CyHV-3 did not emerge within the aquaculture industry, but invaded the farms from natural water sources long before 1996. High population density is a major contributing factor to epizootics, with CyHV-3 being an example of the carp industry plagued by this virus. However, it is not yet clear whether CyHV-3 appears in a sole animal population, during a given period.

The transmission of persistent viral infections differs in many ways from that of acute infections. Herpesviruses (e.g., herpes simplex and *Varicella zoster* viruses) undergo persistent infection and are intermittently transmissible during periods of activation. One organism can continuously transmit the virus during its life span. This feature has important implications for perpetuation and eradication of infection.

It is most important to distinguish between persistence of a virus in a population and cell culture or individual host, and the term perpetuation emphasizes that distinction. All viruses, whether they cause short-duration acute or long-term persistent infections, are capable of perpetuating in populations because perpetuation is a prerequisite for survival. The parameter of perpetuation is determined by variables of both the population (size, density, susceptibility) and virus (transmissibility, multiplication, and duration of infection). Interestingly, viruses that spread rapidly can exhaust susceptible organisms rapidly and disappear more quickly from a population than a virus that moves indolently through the same population. The CyHV-3 seems to accommodate both characteristics; CyHV-3 is an acute lethal virus that propagates efficiently, is very contagious, is preserved in infected fish maintained at a non-permissive temperature, and may (but this has not yet been proved) remain as a latent virus in survivors (St-Hilaire et al. 2005).

These parameters should be taken into consideration when contemplating appropriate steps toward eradication of CyHV-3. However, before determining whether CyHV-3 latently infects carp, and whether cyprinid and non-cyprinid fish and other aquatic animals are reservoirs of CyHV-3, the difficult problem of determining the appropriate approach for virus eradication must be faced.

Eradication of smallpox is a classic example, demonstrating that abolition of a pathogenic virus is attainable. The features which make this eradication possible are: a relatively long incubation period (~14 days) and low infectiousness; its marked seasonality; a case/infection ratio approaching 1; and the lack of an alternative host reservoir. By using efficient vaccination in the case of each identified local outbreak the spread of the disease was averted.

On the other hand, much can be learned from the experience with the Newcastle disease virus (NDV) and the Marek's disease virus (MDV-a herpesvirus). These

viruses are transmitted and reintroduced into susceptible hosts in the fowl industry by migratory birds. It is obvious that these viruses will reinvade all un-immunized flocks. Therefore the solution to these threats is immunization. There is a possibility that the case of CyHV-3 is similar to that of fowl viruses.

Most acute viral infections confer lifelong immunity. On re-exposure after any interval from the initial infection, a re-infection often occurs with minimal virus replication and a specific immune response. Fish surviving the disease, or “naturally immunized” fish, although they have a very low level of antibodies, are resistant to a challenged infection.

So far, eradication and disinfection of infected ponds has failed to prevent reinvansion of CyHV-3. Searching for carp that are genetically resistant to CyHV-3 is impractical for ornamental fish and use of the natural immunization approach bears the danger of dissemination of pathogenic virus. Therefore, at present, the only way to reduce the threat of CyHV-3 is immunization. Improving the existing vaccine and/or developing new DNA vaccines are the only approaches to reduce dissemination of CyHV-3.

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