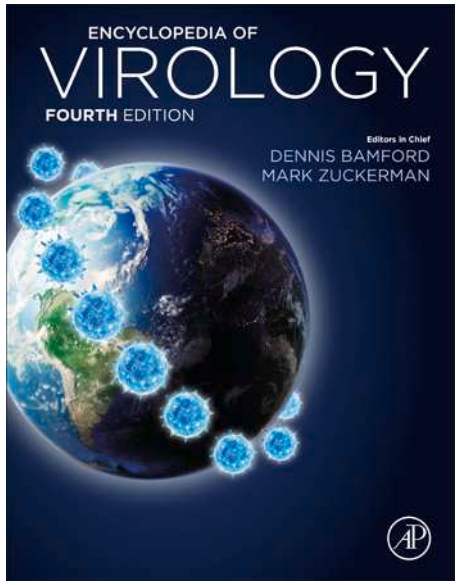

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Fish and Amphibian Alloherpesviruses (*Herpesviridae*)

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Nomenclature

AciHV Acipenserid herpesvirus
AngHV Anguillid herpesvirus
BAC bacterial artificial chromosome
BfHV Bufonid herpesvirus
CyHV Cyprinid herpesvirus
dsDNA double-stranded DNA
EsHV Esocid herpesvirus
GaHV Gadid herpesvirus
IcHV Ictalurid herpesvirus
ICTV International Committee on Taxonomy of Viruses
kbp kilobase pair
ORF open reading frame
PeHV Percid herpesvirus
RaHV Ranid herpesvirus
SalHV Salmonid herpesvirus
SiHV Silurid herpesvirus
TK thymidine kinase
TR terminal repeat

Glossary

Alloherpesvirus Herpesvirus classified in the family *Alloherpesviridae*, order *Herpesvirales*, grouping all herpesviruses infecting fish and amphibians described until now.
Bacterial artificial chromosome Large DNA vector that can allow the stable maintenance and efficient mutagenesis of herpesvirus genome in *Escherichia coli*, followed by the reconstitution of progeny virions after its transfection into permissive eukaryotic cells.
Behavioral fever Phenomenon by which ectotherms increase their body temperature by moving to warmer places in response to an infection.
Ectotherm Organism unable to internally self-regulate its body temperature, therefore relying on external sources of heat.
Hyperplastic lesion Increase of the size of a tissue or organ as a result of cell proliferation.
Latency Maintenance of viral genome as a non-integrated episome associated with minimal viral protein expression and absence of infectious particle production.
Viral reactivation Phenomenon by which the state of viral infection switches back from latency to productive infection.

Introduction and Classification

Herpesviruses form a diverse group of viruses initially described by a conserved virion structure containing a large, linear double-stranded DNA (dsDNA) genome. Indeed, the unifying property of herpesviruses is virion morphology rather than genetic content. Herpesviruses are now classified in the order *Herpesvirales* and share only one convincing conserved gene, i.e., the ATPase subunit of the terminase. Phylogenetic analysis of this gene has led to the current classification of herpesviruses by the International Committee on Taxonomy of Viruses (ICTV). The order *Herpesvirales* is now composed of three viral families, namely the families *Herpesviridae*, *Alloherpesviridae* and *Malacoherpesviridae*. Until now, herpesviruses of amniotes (reptiles, birds and mammals) have been grouped in the family *Herpesviridae*. Herpesviruses of molluscs are encompassed within the family *Malacoherpesviridae*. Finally, herpesviruses of fish and amphibians are classified in the family *Alloherpesviridae*.

The family *Alloherpesviridae* contains four genera with 13 virus species currently accepted by the ICTV (**Table 1**). The alloherpesviruses of amphibians (*Ranid herpesvirus 1*; *2* and *3* [RaHV]) belong to the genus *Batrachovirus*, while fish alloherpesviruses have been clustered into three genera. The genus *Cyprinivirus* comprises the alloherpesviruses of cyprinids (*Cyprinid herpesvirus 1*; *2* and *3* [CyHV]) and that of eel (*Anguillid herpesvirus 1* [AngHV]). The genus *Ictalurivirus* includes the alloherpesviruses isolated from catfish (*Ictalurid herpesvirus 1* and *2* [IcHV]) and sturgeon species (*Acipenserid herpesvirus 2* [AciHV]), while the genus *Salmonivirus* consists of herpesviruses of salmonids (*Salmonid herpesvirus 1*; *2* and *3* [SalHV]) (**Fig. 1(A)**). Interestingly, viruses of the genera *Cyprinivirus* and *Ictalurivirus* are able to infect fish from two different superorders (Elopomorpha, Ostariophysi) or subclasses (Chondrostei, Neopterygii), respectively. This observation supports a more recent evolution of the family *Alloherpesviridae*, associated with a lesser degree of coevolution of some alloherpesviruses with their hosts. In addition to the accepted viral species, more than 10 putative alloherpesviruses were detected by PCR from a wide range of fish species (**Table 1**). Unfortunately, only partial genome sequences are available. The official taxonomical classification of these viruses is still pending. However, according to phylogenetic analyses, these viruses undoubtedly belong to the family *Alloherpesviridae*. Some of them clearly cluster into already existing genera (**Fig. 1(B)**), e.g., SalHV-4 and SalHV-5 are likely going to expand the genus *Salmonivirus*. Other viruses (e.g., AciHV-1; EsHV-1) cannot be grouped with viruses of any existing genera (**Fig. 1(B)**). Further genomic data are needed from these virus species in order to establish their evolution and taxonomic classification. The

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Table 1 Classification of fish and amphibian alloherpesviruses according to the ICTV (Order *Herpesvirales*, Family *Alloherpesviridae*)

Genus	*Viral species (ICTV list)*	*Viral name*	*Viral acronym*	*Alternative viral name (s)*	*Susceptible host (s)*
Batrachovirus	*Ranid herpesvirus 1*[a]	Ranid herpesvirus 1	RaHV-1	Lucké tumor herpesvirus	Northern leopard frog (*Lithobates pipiens*), green frog *(L. clamitans),* pickerel frog *(L. palustris)*
	Ranid herpesvirus 2	Ranid herpesvirus 2	RaHV-2	Frog virus 4	Northern leopard frog (*Lithobates pipiens*)
	Ranid herpesvirus 3	Ranid herpesvirus 3	RaHV-3		Common frog (*Rana temporaria*)
Cyprinivirus	*Anguillid herpesvirus 1*	Anguillid herpesvirus 1	AngHV-1	European eel herpesvirus	Japanese and European eel (*Anguilla japonica* and *A. anguilla*)
	Cyprinid herpesvirus 1	Cyprinid herpesvirus 1	CyHV-1	Carp pox herpesvirus	Common carp (*Cyprinus carpio*)
	Cyprinid herpesvirus 2	Cyprinid herpesvirus 2	CyHV-2	Goldfish haematopoietic necrosis virus	Goldfish (*Carassius auratus*), gibel carp (*Carassius gibelio*)
	Cyprinid herpesvirus 3[a]	Cyprinid herpesvirus 3	CyHV-3	Koi herpesvirus	Common carp (*Cyprinus carpio*)
Ictalurivirus	*Acipenserid herpesvirus 2*	Acipenserid herpesvirus 2	AciHV-2	White sturgeon herpesvirus 2	Sturgeon (*Acipenser spp.*)
	Ictalurid herpesvirus 1[a]	Ictalurid herpesvirus 1	IcHV-1	Channel catfish virus	Channel catfish (*Ictalurus punctatus*), striped catfish (*Pangasius hypophtalmus*)
	Ictalurid herpesvirus 2	Ictalurid herpesvirus 2	IcHV-2	Ictalurus melas herpesvirus	Black bullhead catfish (*Ameiurus melas*)
Salmonivirus	*Salmonid herpesvirus 1*[a]	Salmonid herpesvirus 1	SalHV-1	Herpesvirus salmonis	Rainbow trout (*Oncorhynchus mykiss*)
	Salmonid herpesvirus 2	Salmonid herpesvirus 2	SalHV-2	*Oncorhynchus masou* herpesvirus	Salmon and trout (*Oncorhynchus spp.*)
	Salmonid herpesvirus 3	Salmonid herpesvirus 3	SalHV-3	Epizootic epitheliotropic disease virus	Lake trout (*Salvelinus namaycush*)
Unclassified	N/A	Acipenserid herpesvirus 1	AciHV-1	White sturgeon herpesvirus 1	White sturgeon (*Acipenser transmontanus*)
	N/A	Bufonid herpesvirus 1	BfHV-1		Common toad (*Bufo bufo*)
	N/A	Cyprinid herpesvirus 4	CyHV-4	Sichel herpesvirus	Sichel (*Pelecus cultratus*)
	N/A	Esocid herpesvirus 1	EsHV-1	Blue spot disease virus	Northern pike (*Esox lucius*)
	N/A	Gadid herpesvirus 1	GaHV-1	Atlantic cod herpesvirus	Atlantic cod (*Gadus morhua*)
	N/A	Percid herpesvirus 2	PeHV-2	European perch herpesvirus	European perch (*Perca fluviatilis*)
	N/A	Salmonid herpesvirus 4	SalHV-4	Atlantic salmon papillomatosis virus	Atlantic salmon (*Salmo salar*)
	N/A	Salmonid herpesvirus 5	SalHV-5	Namaycush herpesvirus	Lake trout (*Salvelinus namaycush*)
	N/A	Silurid herpesvirus 1	SiHV-1		Glass catfish (*Kryptopterus bicirrhis*)

[a]Designates the type species of each genus (ICTV, 2018).

complexity and precision of the current classification are likely to rapidly evolve in the future based on the description of new alloherpesvirus members. Finally, many fish species have experienced integration of alloherpesvirus sequences fused with transposons into their genomes.

Virion Structure

In terms of virion structure, alloherpesviruses are no exception to the remarkably conserved and morphologically distinct architecture found throughout the *Herpesvirales* order. It was demonstrated, for example, that the nucleocapsid of IcHV-1 was strikingly similar to that of human herpesvirus 1 (HHV-1 also known as herpesvirus simplex 1). The structure of herpesviruses is, from the center outwards, as follows: (i) a densely packed genome consisting of a single copy of linear dsDNA encapsulated in an icosahedral nucleocapsid (T = 16) with a diameter of 100–130 nm; (ii) an amorphous proteinaceous tegument layer; and (iii) a lipid bilayer envelope acquired from the host and bearing various viral glycoproteins.

Genome

Until now, 11 complete alloherpesvirus genomes have been sequenced and partial genome sequences are available from 11 more viruses. The full-length genomes of SiHV-1 (potential ictalurivirus) and BfHV-1 (potential batrachovirus) were released recently.

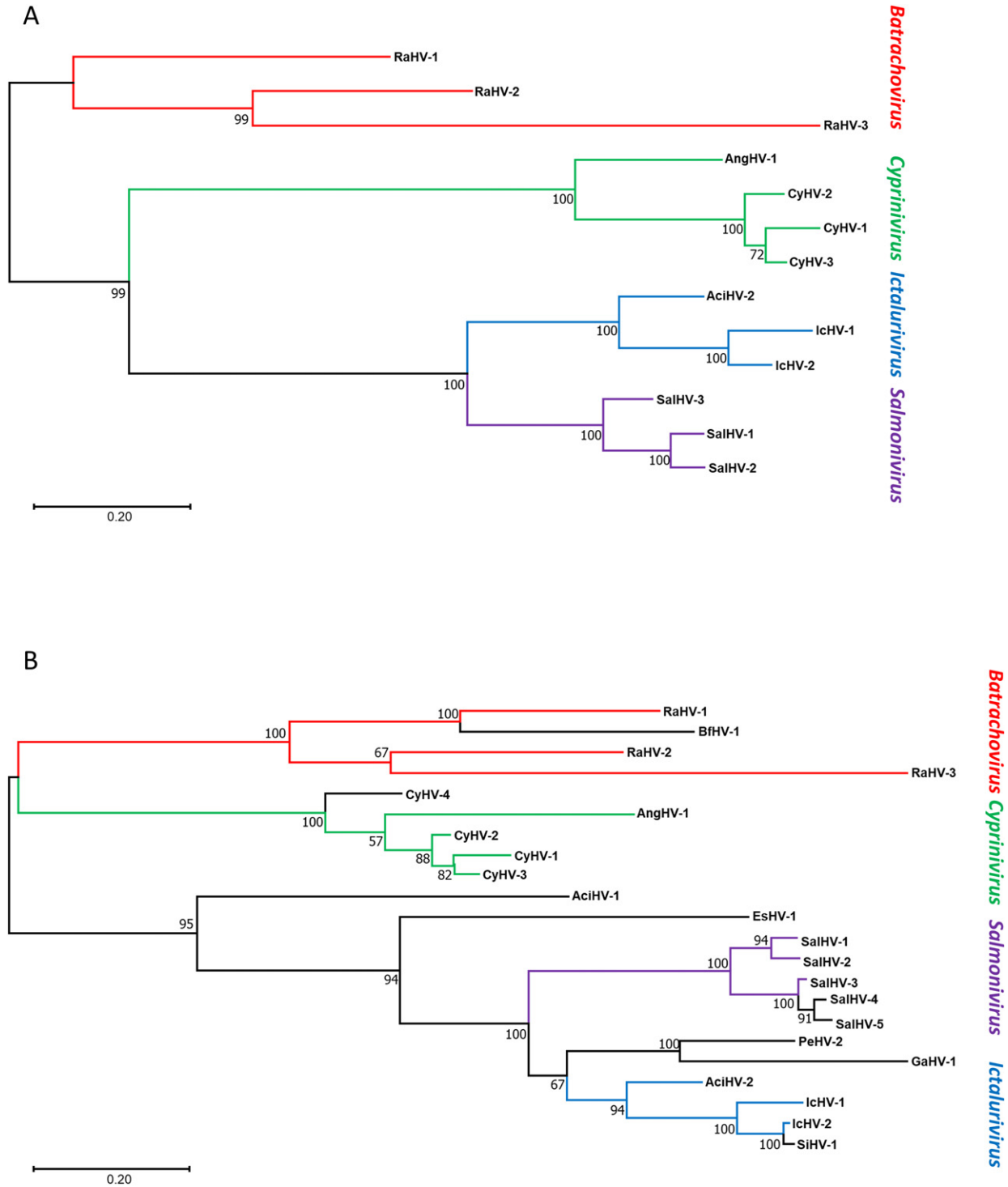


Fig. 1 Phylogenetic tree of the family *Alloherpesviridae*. The analyses were based on the Bayesian analysis (WAG amino acid model) of the deduced amino acid sequences of DNA polymerase genes (134 and 113 amino acid residues, respectively for panel A and B). High statistical values confirm the topology of the trees. The four main genera within the family *Alloherpesviridae* are designated by different colored lines on the trees. Panel A: phylogenetic tree of the classified viral species in the *Alloherpesviridae* family. Panel B: phylogenetic tree of the classified and unclassified potential members of the *Alloherpesviridae* family. AciHV: acipenserid herpesvirus; AngHV: anguillid herpesvirus; BfHV: bufonid herpesvirus; CyHV: cyprinid herpesvirus; EsHV: esocid herpesvirus; GaHV: gadid herpesvirus; IcHV: ictalurid herpesvirus; PeHV: percid herpesvirus; RaHV: ranid herpesvirus; SalHV: salmonid herpesvirus; SiHV: silurid herpesvirus. GenBank and RefSeq accession numbers: AciHV-1: EF685903; AciHV-2: FJ815289; AngHV-1: NC_013668; BfHV-1: MF143550; CyHV-1: NC_019491; CyHV-2: NC_019495; CyHV-3: NC_009127; CyHV-4: KM357278; EsHV-1: KX198667; GaHV-1: HQ857783; IcHV-1: NC_001493; IcHV-2: NC_036579; PeHV-2: MG570129; RaHV-1: NC_008211; RaHV-2: NC_008210; RaHV-3: NC_034618; SalHV-1: EU349273; SalHV-2: FJ641908; SalHV-3: EU349277; SalHV-4: JX886029; SalHV-5: KP686090; SiHV-1: MH048901.

Table 2 Data on complete genome sequences of fish and amphibian alloherpesviruses

Viral name	*Genome size (kbp)*	*Genome structure*	*Structure sizes (kbp)*	*GC%*	*ORFs (No.)*[a]	*Genbank accession number*
Anguillid herpesvirus 1	248.53	TR-U-TR	U: 226 TR: 11	53.0	134	NC_013668.3[b]
Cyprinid herpesvirus 1	291.14	TR-U-TR	U: 224 TR: 33	51.3	143	NC_019491.1[b]
Cyprinid herpesvirus 2	290.3	TR-U-TR	U: 260 TR: 15	51.7	154	NC_019495.1[b]
Cyprinid herpesvirus 3	295.15	TR-U-TR	U: 250 TR: 22	59.2	163	NC_009127.1[b]
Ictalurid herpesvirus 1	134.23	TR-U-TR	U: 97 TR: 18	56.2	90	NC_001493.2[b]
Ictalurid herpesvirus 2	142.92	TR-U-TR	U: 101 TR: 20	53.8	91	NC_036579.1[b]
Silurid herpesvirus 1	149.34	TR-U-TR	U: 100 TR: 25	53,7	94	MH048901.1
Ranid herpesvirus 1	220.86	TR-U-TR	U: 219 TR: 0.6	54.6	132	NC_008211.1[b]
Ranid herpesvirus 2	231.8	TR-U-TR	U: 230 TR: 1	52.8	147	NC_008210.1[b]
Ranid herpesvirus 3	207.91	U	U: 208	41.8	186	NC_034618.1[b]
Bufonid herpesvirus 1	158.25	U	U: 158	40.6	152	MF143550.1

[a]Predicted to encode functional proteins. Includes ORFs duplicated in TR.
[b]Designates Reference Sequences (RefSeq) in the GenBank.
Note: U: Unique region; TR: Terminal repeat; kbp: kilobase pair.

The genome size of alloherpesviruses range between 134 and 295 kilobase pairs (kbp) containing 90–186 Open Reading Frames (ORFs). There are significant differences in the genome size of viruses belonging to different genera. The (potential) ictaluriviruses have the smallest genomes ranging from 134 to 149 kbp, while the estimated genome size of salmoniviruses is around 170 kbp. The genomes of the (potential) batrachoviruses are comprised within the range 158–232 kbp, and the largest genomes are presented by cypriniviruses ranging from 245 to 295 kbp (**Table 2**). The genome of CyHV-3 is the largest of all herpesviruses sequenced so far. The structure of alloherpesvirus genomes is simpler than that of most of the *Herpesviridae*. Most of their genomes contain only one unique region which is flanked by direct terminal repeats (TR). On the contrary, several of the members of the *Herpesviridae* family are more complex and composed of one long region associated with one or sometimes two short unique regions flanked by internal and terminal repeats. Fish alloherpesviruses have long TR, while frog herpesviruses have very short or even no detectable TR (**Table 2**). Alloherpesviruses differ from the members of the *Herpesviridae* family in the number of conserved genes. The latter have a subset of about 40 convincingly conserved genes in members of the family *Herpesviridae*. While alloherpesvirus genomes share 40–60 homologous genes within a genus, there are only 12 genes which have homologs in all completely sequenced alloherpesvirus genomes. These encode major structural proteins and enzymes involved in replication and packaging of viral DNA into virions.

Life Cycle

Infection begins with viral entry, where surface glycoproteins attach to cellular receptors, leading to cell penetration. Two non-exclusive mechanisms of viral penetration have been described in the *Herpesviridae* family, i.e., direct fusion with the plasma membrane, or endocytosis followed by fusion of the viral envelope with the endosome membrane, both resulting in the release of the nucleocapsid, coated by tegument proteins, into the cytoplasm (**Fig. 2**). Nevertheless, the entry mechanisms of alloherpesviruses are still poorly known. Retrograde transport ensues along microtubules to the nuclear pores, where the viral linear genome is delivered into the nucleus. After circularization of the genome, cellular transcription machinery is used for expression of viral genes as a cascade, starting with immediate early genes, followed by early and late categories. As a result of early gene expression, replication of virus DNA is mediated by viral DNA polymerase, leading to the synthesis of branching concatemers. Finally, late gene expression results in the generation of structural proteins that assemble in the nucleus as capsids. Concatemers are then cleaved before the viral genomes are loaded into capsids. Nuclear egress begins with primary envelopment whereby budding at the inner nuclear membrane occurs, with release into the perinuclear space. From there, nucleocapsids exit the nucleus by fusion of the primary envelope with the outer nuclear membrane (de-envelopment). Naked capsids then get to sites of virion assembly and bud into vesicles belonging to the *trans*-Golgi network, where tegument addition and secondary envelopment with acquisition of the glycoprotein-studded envelope arise. Lastly, enveloped virions undergo egress via exocytosis.

Increasing evidence points to alloherpesviruses displaying the capacity, as found in the *Herpesviridae* family, to establish latent infections. The balance between productive and latent infection is intricately linked to host biology, as latency may be interrupted by reactivation that reverts to a productive infection. In alloherpesviruses, water temperature could be a key factor in switching between productive and latent infections.

Epidemiology

Fish Alloherpesviruses

Alloherpesviruses share the general tendency of herpesviruses to possess a narrow host range, although limited infection can sometimes occur in non-natural host species. For example, CyHV-1 is usually restricted to common carp but was detected in

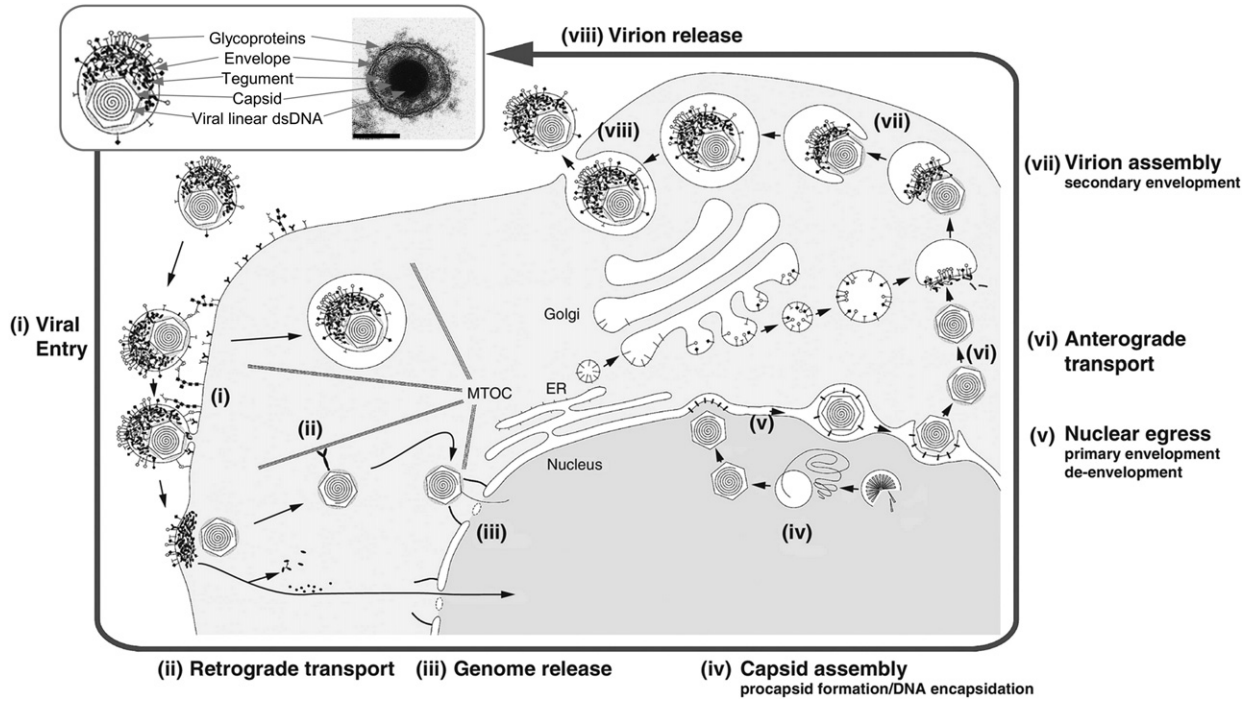


Fig. 2 Schematic representation of herpesvirus productive life cycle. Schematic representation and electron microscopy examination of CyHV-3 virion (upper left panel, bar represents 100 nm). Typical replication cycle of herpesviruses. Diagrammatic representation of the herpesvirus replication cycle, including virus entry and dissociation of the tegument, transport of incoming capsids to the nuclear pore, and release of viral DNA into the nucleus. Capsid assembly, DNA packaging, primary, secondary envelopment and egress are also illustrated. MTOC: Microtubule Organizing Center; ER: Endoplasmic Reticulum. Modified from Zeev-Ben-Mordehai, T., *et al.*, 2014. Current Opinion in Virology 5 (100), 42–49. Electron microscopy picture adapted with permission from Mettenleiter, T.C., *et al.*, 2009. Virus Research 143 (2), 222–234 Copyright Elesvier.

golden ide (*Leuciscus idus*) imported from Germany into North America. For CyHV-3, multiple vertebrate and invertebrate species have been identified as bearing viral DNA and being a potential epidemiological risk factor of viral spreading.

The geographical distribution of alloherpesviruses is variable: for instance, whereas members of the *Cyprinivirus* genus are found worldwide, others have only been associated with restricted geographical areas and contexts. Such is the case for IcHV-2 that caused mass mortalities in black bullhead catfish farms in Italy in the late 1990s. In the case of CyHV-3, the first outbreaks in 1997 in Germany and its rapid worldwide distribution were attributed to international trade of common and koi carp and notably the methods used in koi carp competitions before awareness of this pathogen was commonplace. It can therefore be stated that the epidemiology of some alloherpesviruses is tightly interwoven with the precise use and trade of the domesticated host species.

Deadly outbreaks of CyHV-3 have occurred in many natural habitats such as in some North American lakes, but have not caused any long-term decrease of common carp populations. Recently, the high virulence of CyHV-3 associated with its believed absence in Australia has led to the hypothesis of using this virus to control common carp populations which are considered as an invasive species in this country. However, the predicted safety and efficacy of this method have recently been under important scientific debate.

Amphibian Alloherpesviruses

The real causes of alloherpesvirus emergence and spread in free-ranging amphibians are mostly unknown. However, the impact of human activities on their habitat and the climate may play a relevant role. Of the known batrachoviruses, RaHV-1 has been detected in free-ranging leopard frogs (*Lithobates pipiens*) in specific areas of the North American continent where, to the best of our knowledge, it has remained confined. The prevalence of RaHV-1 associated tumors ranged between 1% and 9% according to the collection site. A drastic reduction of the infected populations of leopard frogs was recorded in the 70's. Currently, no information is available concerning possible reemergence of the virus. Differently, RaHV-3 and BfHV-1 have been recorded in Europe only, in the common frog (*Rana temporaria*) and common toad (*Bufo bufo*), respectively. Their actual and current significance in the context of amphibian disease ecology is currently unknown.

Pathogenesis

Fish Alloherpesviruses

Fish alloherpesviruses are characterized by their temperature dependency, a consequence of the ectothermic nature of their hosts. The optimal temperature for viral replication and disease is variable among alloherpesviruses. Whereas AngHV-1 infection is

promoted by high temperatures (25°C), SalHV-3 outbreaks are reported when temperatures are low (8–10°C). In natural conditions, if the environmental water temperature is suboptimal for virus replication, the induced disease will be less severe or even asymptomatic, explaining the seasonal occurrence of alloherpesvirus diseases. Interestingly, expression of behavioral fever was described in common carp infected by CyHV-3.

Studies of CyHV-3 pathogenesis mimicking natural conditions demonstrated that the skin is the major portal of entry of the virus after inoculation of carp by immersion, whereas the virus would penetrate through the pharyngeal periodontal mucosa following oral contamination. Viral spreading within the infected host is probably promoted by infected white blood cells allowing rapid spread in almost all tissues soon after inoculation.

Horizontal transmission of fish alloherpesviruses can occur either directly between fish or indirectly, through several potential vectors (e.g., water, fish droppings). Salmoniviruses were detected in ovarian fluids, suggesting a possible vertical transmission. Vertical transmission was demonstrated for IcHV-1 after detecting the virus in offspring from IcHV-1 positive broodstocks.

The hallmark of herpesviruses is their ability to establish latency in their host after initial productive infection. Some evidence of latency has been described for CyHV-1, CyHV-3, AngHV-1, SalHV-2 and IcHV-1 fish alloherpesviruses. The latency of alloherpesviruses in fish surviving the primary infection allows viral transmission to naïve fish upon disease reactivation. This aspect of the virus biology results in a certain spatial and temporal flexibility for viral maintenance in the host population.

Amphibian Alloherpesviruses

Pathogenesis of the *Batrachovirus* associated disease is virtually unknown. RaHV-1 has been shown to be the etiologic agent of Lucké's adenocarcinoma in leopard frogs. However, it is not known how the virus induces tumor development although environmental temperature may play a role. The disease could be reproduced by only infecting frogs during their larval stage with tumor extract. Viral replication appears to be dependent on low environmental temperatures, whereas tumor growth and metastasis would be promoted at higher temperatures. Regarding temperature and seasonality, the proliferative dermatitis associated with RaHV-3 and BfHV-1 are observed right after hibernation, during the mating season, between the end of winter and beginning of spring, when the environmental temperature is low. No virus-associated lesion has been reported during the later spring and summer. The molecular bases of the associated diseases are not known and Koch's postulates have not been fulfilled for these two viruses.

Clinical Features

Fish Alloherpesviruses

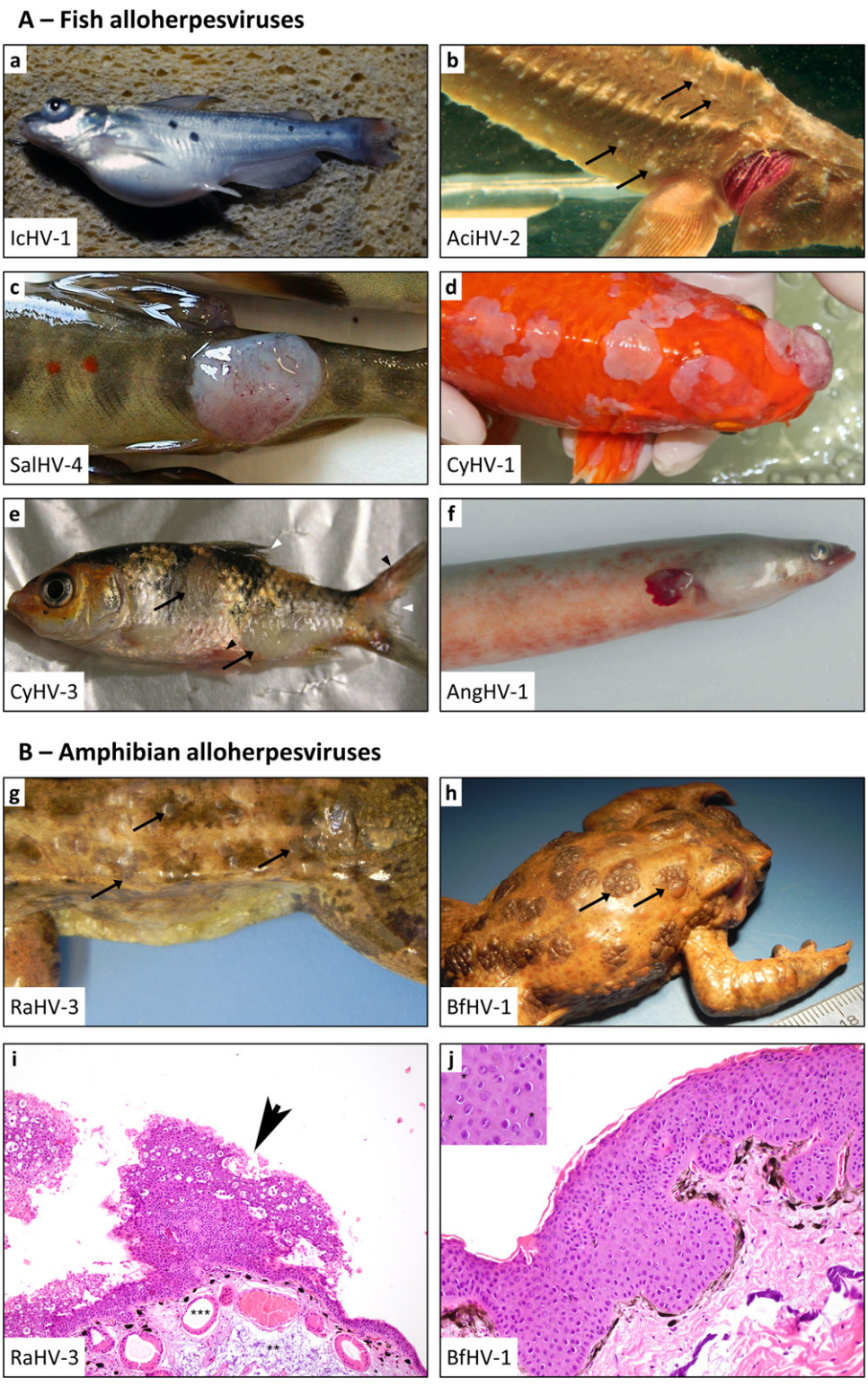
Clinical aspects induced by fish alloherpesviruses can vary substantially among outbreaks or individuals. Indeed, variations are observed in the severity of clinical signs according to diverse factors including, but not restricted to, those related to the environment (temperature, density of the population) and host (age of the individuals, genetics, immune status). Primary infection involves productive viral replication that may result in viremia and acute disease associated with high morbidity and mortality. In general, fish exhibit non-specific behavioral changes including lethargy, hyperexcitability, erratic swimming or gasping at the water surface. Diseases associated with infection of naïve individuals by alloherpesviruses can include epithelial lesions, diffuse multisystemic hemorrhages, ascites, kidney and liver necrosis (**Fig. 3(A)**, **Table 3**).

Contrarily to their *Herpesviridae* family counterparts, alloherpesviruses usually induce severe diseases in their hosts. This may result from a milder degree of coevolution with their respective hosts. Alloherpesvirus infections might nevertheless be artificially enhanced by aquaculture methods. Among the most important fish alloherpesviruses, CyHV-2, CyHV-3 and IcHV-1 cause a systemic and lethal disease (**Fig. 3(A)**; **Table 3**) characterized by mass outbreaks associated with high mortalities. During the summer, when temperature is optimal for the occurrence of IcHV-1 disease, 90% of fry and fingerlings may die in less than two weeks. During CyHV-3 infection, infected individuals may begin to die at 6–8 days post infection. Mortality rates are in general up to 70%–80% of infected individuals but they can reach 100% in some cases. Mass mortalities within 24 h were also recorded in CyHV-2 infected gibel carp. CyHV-1, IcHV-2, AciHV-2, SalHV-2 and SalHV-3 primary infections can also induce lethal diseases.

Development of a benign epithelial hyperplasia, also called herpetic lesion or papilloma is another clinical pattern largely observed in alloherpesvirus infections. This clinical feature can be observed during primary productive infection with AciHV-2, SalHV-3 and SalHV-4 (**Fig. 3(B)**). In addition, this lesion can arise during recrudescence of a previous infection. Indeed, while CyHV-1 and SalHV-2 primary infection is typically characterized by an acute systemic disease, the recrudescence of the disease in survivors is associated with epithelial tumors. Papillomas associated with CyHV-1 infection appear 5–6 months after primary exposure in most survivors and are more commonly observed on the skin (body, fins, mouth) and at the site of intraperitoneal inoculation. Similarly, epitheliomas in SalHV-2 disease are observed around the mouth, at the caudal fin, operculum, and the body surface. These lesions may persist for one year. Experimental challenges show that tumor occurrence varies between species (12%, 40%–60%, and 100% in rainbow trout, chum salmon and masu salmon, respectively).

Amphibian Alloherpesviruses

RaHV-1 causes adenocarcinomas in leopard frogs (Lucké's adenocarcinoma). The tumor can grow extensively and metastasize, involving and invading other organs. Papillary projections are often observed in the neoplastic mass. Differently, the only reported



lesion associated with RaHV-2 is the occurrence of severe edema in frogs infected at larval stages. RaHV-3-associated disease is characterized by multifocal to coalescent gray skin patches scattered throughout the integument of affected frogs, which correspond to areas of prominent epidermal hyperplasia during microscopic examination (Fig. 3(B)). Large numbers of intranuclear eosinophilic inclusions are seen in the thickened epidermis. Toads infected with BfHV-1 show similar but distinct skin lesions. Relatively flat to slightly elevated, dark brown, cauliflower-like skin proliferations are observed grossly. Histologically, the lesions correspond to areas of epidermal hyperplasia, frequently associated with hyperkeratosis. Intranuclear inclusions are seen in infected toads, but less numerous and prominent than in frogs infected with RaHV-3. Interestingly, minimal to no associated inflammation is observed in both infections.

Diagnosis

Diagnosis of alloherpesvirus infection can rely on various methods. First, diagnosis can be oriented by analysis of the clinical signs, anatomopathological and histopathological lesions. However, the latter are only indicative of the alloherpesvirus infection due to the absence of known pathognomonic signs and the high frequency of multipathogen co-infection. Still, hyperplastic epidermal lesions are typical and very indicative of some alloherpesvirus infections (SalHV-4, CyHV-1, RaHV-3, BfHV-1).

Specific diagnosis involves the use of viral isolation using specific cell lines, viral detection using molecular methods or serological methods. Viral isolation of most alloherpesviruses has been achieved using cell lines. Nevertheless, viral isolation is still restricted by the absence of permissive cell lines: for example, among batrachoviruses, only RaHV-2 has been successfully isolated in cell culture. Specific molecular methods are now available for detection of most alloherpesviruses (PCR, qPCR, monoclonal and polyclonal antibodies). These methods can be tentatively used to diagnose productive, but also potentially latent infection (PCR, qPCR). Regarding the latter, the specific sites of alloherpesvirus latency are frequently unknown. Viral DNA can be detected in the brain and B lymphocytes of fish previously exposed to CyHV-3. Note that the sensitivity of the molecular tools developed could be decreased by the limited knowledge about genetic coalescence within each alloherpesvirus species. Detection of the antibody response against alloherpesviruses (ELISA, seroneutralization) can help to diagnose a previous (latent) infection (e.g., screening of broodstocks). Nevertheless, interpretation should be cautious, as specific antibody half-life is rather short in fish. Specific antibodies against CyHV-3 can barely be detected from sera of fish 280 days post infection. Finally, on-site diagnostic methods including lateral flow devices and loop-mediated isothermal amplification have been developed for some alloherpesviruses.

Management and Treatment

Alloherpesviruses are not equally threatening the aquaculture sector. Some alloherpesvirus infections, like those induced by SalHV-1, were found to have limited impact on aquaculture. Other alloherpesviruses (e.g., IcHV-1), can be controlled by management procedures (screening of broodstocks, disinfection of facility and incoming water, microbiological independence of produced fish batches), improving rearing conditions (fish density, water quality, stress reduction) or by regulating the water temperature.

As explained above, alloherpesvirus replication and disease expression correlates strongly with environmental temperature. This peculiarity has been exploited for the treatment of alloherpesvirus infection by changing environmental temperature to levels suboptimal or non-permissive for viral replication. For example, fish infected by CyHV-3 can be transferred to non-permissive

Fig. 3 Illustration of typical clinical features observed during some alloherpesvirus infection. Panel A. Illustration of clinical features induced by fish alloherpesviruses. (a) channel catfish (*Ictalurus punctatus*) infected by IcHV-1, typical exophthalmia and distended abdomen caused by ascite. (b) Multiple plaques of epidermal proliferation (arrows) in a fingerling of Siberian sturgeon (*Acipenser baeri*) infected AciHV-2. (c) Severe papillomas caused by SalHV-4 in wild young Atlantic salmon (*Salmo salar*) captured at 10°C. (d) Proliferative cutaneous lesions in adult koi carp (*Cyprinus carpio*) infected by CyHV-1, multifocal to coalescing, slightly raised to nodular, white cutaneous proliferations along the dorsum, mouth, and pectoral fins. (e) Koi carp (*Cyprinus carpio*) infected by CyHV-3, extensive circular necrosis of the skin covering the body (black arrows), fin erosion (white arrowheads), and hemorrhages on the skin and fins (black arrowheads). (f) Diseased cultured European eel (*Anguilla anguilla*) with AngHV-1 infection. The eel shows a patchy pattern of hemorrhages in the skin, and severe hemorrhagic fins. Panel B. Illustration of clinical features induced by amphibian alloherpesviruses. (g) Multifocal to coalescent light tan to gray patches of skin are present, more frequently on the dorsum and along the flanks of a common frog (*Rana temporaria*) (black arrows). (h) Bufonid herpesvirus 1 infected common toad (*Bufo bufo*) associated gross skin lesions. Multifocal raised, dark brown, patchy areas of skin are scattered over the dorsum of the toad (black arrows). (i) Skin, Common frog (*Rana temporaria*) naturally infected with Ranid herpesvirus 3. The epidermis is focally, severely elevated (hyperplastic), forming a papillary projection (arrowhead) characterized by several clear spaces (epidermal vacuoles) partially filled with degenerating and necrotic keratinocytes. The underlying dermis shows loosely arranged collagen fibers (edema-two asterisks) and variably ectatic mucous glands (three asterisks). (j) Skin, Common toad (Bufo bufo) naturally infected with Bufonid herpesvirus 1. The epidermis is diffusely thickened (hyperplasia) up to 5 times as normal. Among the several nuclei are a number of intranuclear eosinophilic inclusions (inset, asterisks). The underlying dermis is characterized by loose collagen fibers (edema). (a) Courtesy of J. A. Plumb. (b) Adapted with permission from Shchelkunov, *et al.*, 2009. Diseases of Aquatic Organisms 86 (3), 193–203. Copyright Inter-Research. (c) Adapted with permission from Doszpoly, *et al.*, 2013. Diseases of Aquatic Organisms 107 (2), 121–127. Copyright Inter-Research. (d) Adapted with permission from Crossland, *et al.*, 2018. Journal of Aquatic Animal Health 30 (3), 185–190. Copyright John Wiley and Sons. (e) Adapted from Michel *et al.*, 2010. Emerging Infectious Diseases 16 (12), 1835–1843. (f) Adapted from Haenen, O.L.M., *et al.*, 2002. Bulletin of the European Association of Fish Pathologists 22 (4), 247–257. (g, i, j) Courtesy of F.C. Origgi. (h) Adapted from Origgi, *et al.*, 2018. Scientific Reports 8 (1), 14737.

Table 3 Clinical features caused by some alloherpesviruses

Viral name	*Susceptible host*	*Clinical signs (non-exhaustive)*	*Mortality*
Batrachovirus			
Ranid herpesvirus 1	Northern leopard frog (*Lithobates pipiens*)	Renal adenocarcinoma	Unknown
Ranid herpesvirus 2	Northern leopard frog (*Lithobates pipiens*)	Unclear, severe edema when infected at larval stage	Unknown
Ranid herpesvirus 3	Common frog (*Rana temporaria*)	Proliferative dermatitis	Unknown
Cyprinivirus			
Cyprinid herpesvirus 1	Common carp (*Cyprinus carpio*)	Systemic disease in fry, hyperplastic cutaneous proliferation (papillomas, pox-like lesions) in surviving/older fish	Up to 87.5% (fry)
Cyprinid herpesvirus 2	Goldfish (*Carassius auratus*), gibel carp (*Carassius gibelio*)	Lethargy, anorexia, pale patches and hypersecretion of mucus in the gills, hemorrhages on the body and gills	Up to 100% (all ages)
Cyprinid herpesvirus 3	Common carp (*Cyprinus carpio*)	Lethargy, anorexia, hypersecretion of mucus on the skin, hyperemia of the fins, skin fin & gill necrosis, fin erosion, hemorrhages on the skin, neurological symptoms	Up to 100% (all ages)
Anguillid herpesvirus 1	Japanese and European eel (*Anguilla japonica* and *A. anguilla*)	Decreased growth rates, hemorrhages and ulcerative lesions on the skin (head, beak and caudal abdominal surface) fin and gills	Up to 10%
Ictalurivirus			
Ictalurid herpesvirus 1	Channel catfish (*Ictalurus punctatus*), striped catfish (*Pangasius hypophtalmus*)	Reduced growth rates, erratic swimming, distended abdomen, hemorrhages, exophthalmia	>90% (fry and fingerling channel catfish), 30%–40% (striped catfish)
Ictalurid herpesvirus 2	Black bullhead catfish (*Ameiurus melas*)	Erratic swimming, hemorrhages	High mortality (fry and juveniles)
Acipenserid herpesvirus 2	Sturgeon species (*Acipenser spp.*)	Erratic swimming, lethargy, hyperplastic skin lesions, hyperemia of the ventral scutes, mouth and anus	Up to 80% (juvenile white sturgeon), up to 100% (juvenile Siberian sturgeon)
Salmonivirus			
Salmonid herpesvirus 1	Rainbow trout (*Oncorhynchus mykiss*)	Darkened pigmentation, exophthalmia, abdominal distention, haemorrhages in the fins, pale gills	Low
Salmonid herpesvirus 2	Salmon and trout (*Oncorhynchus spp.*)	Lethargy, anorexia, darkened appearance, skin ulcers, focal epithelial proliferation on the mouth and body, exophthalmia Epithelial tumors (papilloma), preferably on the mouth, in surviving fish	Up to 100%. High losses in juveniles
Salmonid herpesvirus 3	Lake trout (*Salvelinus namaycush*)	Lethargy, swim near the surface, behavioral modification, mucoid patches on the skin, hemorrhages on eyes and jaw	Up to 100%. High losses in juveniles
Unclassified			
Acipenserid herpesvirus 1	White sturgeon (*Acipenser transmontanus*)	Few external clinical signs associated with a diffuse hyperplastic dermatitis	Up to 35% (juvenile white sturgeons)
Bufonid herpesvirus 1	Common toad (*Bufo bufo*)	Proliferative dermatitis	Unknown
Esocid herpesvirus 1	Northern pike (*Esox lucius*)	Whitish circular plaques on the fins and dorsal parts	NA
Salmonid herpesvirus 4	Atlantic salmon (*Salmo salar*)	White plaques of epithelial tumors (papilloma) on skin, or caudal peduncle	>50% when secondary infections involved
Salmonid herpesvirus 5	Lake trout (*Salvelinus namaycush*)	Abnormal swimming, discolored musculature	

water temperatures (>30°C), allowing fish to clear viral infection and develop an adaptive immune response. This method was also used as a natural immunization method following iatrogenic CyHV-3 contamination. Unfortunately, fish infected with these methods may become latently infected by CyHV-3. Interestingly, fish infected by CyHV-3 have been shown to naturally seek for warmer environments (behavioral fever), thereby surviving viral infection; this feature could be exploited in aquaculture. The specific temperature range allowing alloherpesvirus replication and disease is variable among viral species. For instance, eel farms experiencing AngHV-1 infection usually decrease environmental temperature to decrease clinical signs and mortality.

Antiviral treatments were tested against alloherpesviruses, but to the best of our knowledge, no commercial treatment is currently available. Extracts of the *Clinacanthus nutans* (Burm. f.) Lindau medicinal plant used as a prophylactic or therapeutic treatment against CyHV-3 infection showed positive effects on common carp survival. Nucleoside analogues such as acyclovir are able to limit IcHV-1, CyHV-3 or SalHV-2 replication in cell culture, and to be effective against SalHV-2 *in vivo*.

Prevention

Facing the lack of efficient therapeutic methods, safe and efficacious vaccines represent the most promising control approach against alloherpesviruses. Vaccination of fish should involve delivery methods adapted to mass vaccination of the target species, minimizing the cost for vaccination and maximizing the duration of immunity with an early in life and limited number of vaccine administrations.

Multiple vaccine candidates against alloherpesviruses have been studied but very few are commercially available nowadays. Inactivated vaccines consist of the entire killed viral particle, thus containing all structural viral antigens. Inactivated vaccine candidates have been described for SalHV-2, CyHV-2 and CyHV-3. Injection of inactivated CyHV-2 vaccines to gibel carp induced partial protection. A formalin-inactivated CyHV-3 vaccine, trapped within a liposomal compartment and used for oral administration, induced partial protection. The high amount of viral antigen required, the vaccine delivery method, and a generally low efficacy are the main concerns and restrictions for inactivated vaccine development.

DNA vaccines consist of genetically engineered DNA plasmids encoding specific antigens from pathogens. DNA vaccines were tested against IcHV-1 and CyHV-3. Injectable DNA vaccines consisting of plasmids encoding CyHV-3 ORF25 envelope glycoprotein were shown to be partially efficacious against immersion challenge, but not against a cohabitation challenge. Attempts to deliver the DNA plasmids encoding ORF25 orally failed to induce an efficient protection. DNA vaccines against IcHV-1 showed equivocal results, even when cocktails of plasmid DNA encoding multiple ORFs were used. Studies on DNA vaccines against alloherpesviruses highlighted two main challenges for future research, namely selecting the best antigens for immunization and improving vaccine delivery methods.

Attenuated vaccines consist of live viral strains with a virulence that has been reduced or suppressed by serial passages in cell culture (conventional attenuated vaccine) or by targeted viral genome editing (recombinant attenuated vaccine). Attenuated vaccines have the advantages of being compatible with mass vaccination and simulating a natural viral infection of the host. However, they raise safety concerns including residual virulence, reversion to virulence, and spread from vaccinated to naïve subjects. Conventional and recombinant attenuated vaccines were developed against IcHV-1 and CyHV-3. A conventional anti-CyHV-3 attenuated vaccine has been developed by serial passages in cell culture and UV irradiation. This vaccine is commercialized in Israel for the vaccination of koi and common carp. The determinism of the attenuation is unknown, and consequently, reversions to a pathogenic phenotype cannot be ruled out. The attenuated strain has residual virulence for fish weighing less than 50 g.

Due to scientific advances in molecular biology and molecular virology, the development of attenuated vaccines is evolving from empirical to rational design. A viral genome can be edited to delete genes encoding virulence factors in such a way that reversion to virulence cannot occur. This approach has been tested for CyHV-3 by targeting different genes such as the thymidine kinase (TK) and deoxyuridine triphosphatase. The previous cloning of IcHV-1 and CyHV-3 as infectious bacterial artificial chromosomes (BAC) allows viral mutagenesis. Recently, a vaccine candidate based on the double deletion of ORF56 and ORF57 was produced using BAC mutagenesis. This strain is safe for vaccinated fish, is limitedly transmitted to naive fish and induces full protection against a lethal cohabitation challenge. ORF57 was found to be the crucial virulence factor and is conserved in the genus *Cyprinivirus*. A recombinant attenuated vaccine candidate was also developed for IcHV-1 by deletion of the TK gene and was shown to be effective. Attenuated recombinant vaccines, associated with the tools provided by BAC mutagenesis, allow efficient development of vector vaccine strategies. Fish immunized with an IcHV-1 TK deleted recombinant attenuated strain expressing a reporter gene developed an antibody response against the transgene product.

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