Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

SPECIAL ISSUE ARTICLE

Koi herpesvirus (KHV) and KHV disease (KHVD) – a recently updated overview

S.M. Bergmann[1], Y. Jin[1], K. Franzke[1], B. Grunow[2], Q. Wang[3] and S. Klafack[1,4]

1 OIE and National Reference Laboratory for KHVD, Institute of Infectology, Friedrich-Loeffler-Institut (FLI), Greifswald-Insel Riems, Germany
2 Institute of Muscle Biology & Growth, Junior Research Group Fish Growth Physiology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany
3 Key Lab of Fishery Drug Development, Ministry of Agriculture, Key Lab of Aquatic Animal Immune Technology, Peal River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, PR China
4 Institute for Medical Biochemistry and Molecular Biology, University of Greifswald, Greifswald, Germany

Keywords
aquaculture, diseases, fish (live), genetic, KHV, KHVD, viruses.

Correspondence
Sven M. Bergmann, OIE and National Reference Laboratory for KHVD, Institute of Infectology, Friedrich-Loeffler-Institut (FLI), Südufer 10, 17493 Greifswald-Insel Riems, Germany.
Email: sven.bergmann@fli.de

2019/2229: received 11 December 2019, revised 6 February 2020 and accepted 7 February 2020

doi:10.1111/jam.14616

Abstract

Over the last years, there has been an enormous increase in the knowledge on koi herpesvirus (KHV), koi herpesvirus disease (KHVD), pathogenesis and virus variants. Different KHV lineages have clearly been identified, possible genomic changes during replication in different cell cultures at different temperatures but also in several hosts have been identified, a persistent stage of infection has been specified and it has been shown that infection with KHV is not host specific at all, but KHVD is. Additionally, it has been shown that it is possible to combat KHVD by immunization with inactivated and attenuated live vaccines using different delivery systems but also to benefit from alternative treatments with e.g. exopolysaccharids obtained from *Arthrospira platensis*.

Introduction

Over the last decades, a disease has occurred in cyprinid fish taxonomically grouped into the genus *Cyprinus carpio* L., the farmed but also the wild common carp and its ornamental relative, the koi. The causative agent of the disease was found to be an aquatic herpesvirus (Hedrick *et al.* 2000) belonging to the family Alloherpesviridae (Waltzek *et al.* 2009). The disease, named koi herpesvirus disease (KHVD), has occurred and is seen with more severe losses in *C. carpio*, common carp and koi as well as their hybrids (Hedrick *et al.* 2006; Bergmann *et al.* 2010a) only. Koi herpesvirus (KHV), scientifically cyprinid herpesvirus 3 (CyHV-3), has been distributed all over the world except Australia by trade mainly with latently infected but healthy appearing koi but also carp (Haenen *et al.* 2004). Due to the nature of this herpesviral disease to induce latency but most likely persistence, the diagnostic tools for virus detection were developed between ca 1999 and 2010. Even today, it is difficult under specific circumstances to identify the virus in fish samples due to its low concentration in the latent phase of the infection (Bergmann *et al.* 2010b). The complete genomic sequences of three KHV isolates (KHV-I, KHV-U and KHV-TUMST1) were first published in 2007 (Aoki *et al.* 2007). Meanwhile more than 11 complete genomic sequences have been published in the NCBI database. While some authors claim for different KHV genotypes based on only small sequenced areas (Bigarre *et al.* 2009; Sunarto *et al.* 2011; Kim and Kwong 2013), others have found by NGS analysis that 99·9% of the entire KHV genome are identical, but that it is possible to differentiate between a European and an Asian lineage (Bigarre *et al.* 2009; Avarre *et al.* 2011). This was confirmed by Klafack *et al.* (2017). Only in some cases the similarity of the entire genome deviated by up to 3% from the majority of the published complete genomes, which was designated as KHV-like or atypically reacting KHV (Engelsma *et al.* 2013). Furthermore, a KHV isolate or KHV detected from organ tissues directly is not a unique and

single virus but a bulk of diverse viruses including atypically reacting agents (Klafack *et al.* 2017; Klafack *et al.* 2019). KHV disease as well as infection with KHV are notifiable to the OIE and the EU.

Koi herpesvirus

Taxonomically, KHV or CyHV-3 belongs to the family of Alloherpesviridae showing a typical spherical to pleomorphic envelope and a particle size of up to 180–200 nm (Fig. 1). Morphologically and genetically, it is strongly related to the other cyprinid viruses such as CyHV-1 (carp or fish pox virus) and CyHV-2 (goldfish haematopoietic necrosis virus) (Waltzek *et al.* 2005, 2009) but more distant from the fourth member of the genus *Cyprinivirus*, *anguillid herpesvirus* 1 (AngHV-1) or *herpesvirus anguillae* (HVA). The genome size of 295 kbp encoding for at least 156 ORFs is the largest one known of all herpesviruses in the order Herpesvirales. Currently, there are 9–11 complete genomic sequences of KHV in the NCBI database, most of them from virus isolated in cell culture.

The virus probably originated from warm water areas in Asia as concluded from KHV identification after 100 cell culture passages of KHV-T (Taiwan), KHV-E (England), KHV-I (US isolate from koi imported from Israel) and KHV-G1 (Germany 1). While after 25–60 passages at 20°C incubation temperature onto common carp brain (CCB) cells (Neukirch *et al.* 1999) all viruses of the European lineage (KHV-E, KHV-I and KHV-G1) converted to the Asian lineage based on the VNTR sequences (Klafack *et al.* 2017) and NGS data covering the entire genome (data not shown), KHV-T always remained in the Asian lineage. Amazingly, KHV-E changed the lineage several times to end up as an Asian lineage variant, generally showing more variety than KHV-T during the passages (Klafack *et al.* 2017). The recently isolated KHV GZ11 (Dong *et al.* 2011) contained small genomic elements from the European lineage when first sequenced. The majority of the GZ11 genome came from the Asian lineage. After one passage onto CCB cells this European element was not detectable anymore, the virus was pure Asian, maybe again. On the one hand, this shows the unexpected ability of this large DNA virus to adapt to different conditions, e.g. temperatures, cells, hosts, very rapidly. On the other hand it reveals that there are always variants of the same virus with smaller or larger inclusions, deletions or substitutions present beside one most virulent variant, which is principally detected (Klafack *et al.* 2017, 2019). It seems that small genomic changes, which occur frequently, if not always, are normal and are not essential for virus replication and for virulence in *C. carpio* (common carp, koi and hybrids with them). The ancestor of KHV possibly originates from Asia and all other KHV variants can return to the Asian lineage after passages *in vitro* and in *vivo* (Klafack *et al.* 2019). The recently discovered so called intermediate lineages or variants of KHV in Europe (Bigarré *et al.* 2009) or Asia (Sunarto *et al.* 2011) may result from the changed, deleted or inserted or incomplete replication of KHV due to environmental conditions. This can be concluded in particular from detections in Europe where newly introduced Asian koi became ill. At first an Asian variant was discovered which later on, within the—for a big DNA virus—short time of one or two years changed into a European KHV lineage.

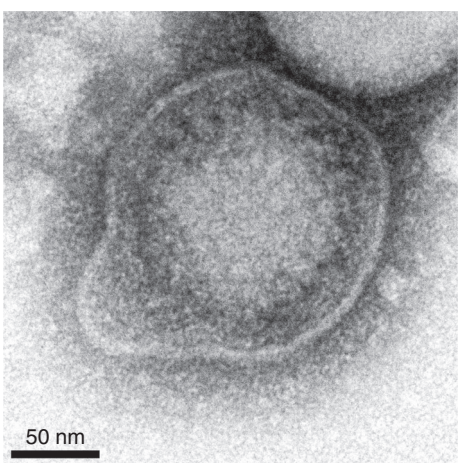

Figure 1 KHV isolate KHV-T by electron microscopy.

Koi herpesvirus disease

KHVD in common carp and koi is characterized by external clinical signs like lethargy and food refusal three to nine days post infection (dpi), followed by an enormous increase in mucus production on skin and gill tissues, frequently enophthalmus (Fig. 2b), later on focal and expanded necrosis of the gill tissues (Fig. 2a), round to massively expanded skin necrosis, petechial to expanded bleeding of the skin and fins up to sandpaper skin due to the release of mucus. The clinical symptoms can occur altogether or individually, but can also be absent in the case of a peracute disease event. The development of disease after infection can depend on e.g. the virulence of the virus, the virus concentration, the environmental conditions, the season, the stress level for the fish, the genetic lineage as well as the replicating tissue of the hosts. There are many more influencing factors on the virus side but also the host and environmental side.

As described in early publications (Haenen *et al.* 2004; Pikarsky *et al.* 2004), the main infection route for *C.*

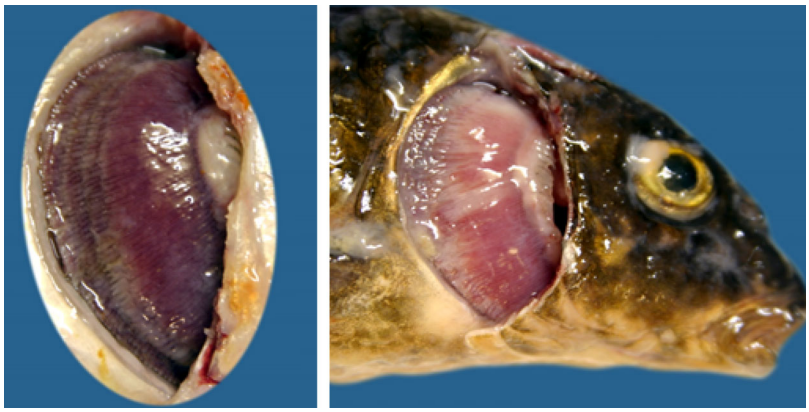

Figure 2 Symptoms of KHVD from infected koi. (a) Severe gill necrosis; (b) increased mucus production and enophthalmus. [Colour figure can be viewed at wileyonlinelibrary.com]

carpio is horizontal, from fish to fish including waterborne infections. The main routes of virus entrance are the gill tissues (Pikarsky *et al.* 2004; Gilad *et al.* 2004) followed by the gut tissues (Monaghan *et al.* 2015). In contrast, Costes *et al.* (2009) claimed that the entrance route is via the skin. Within two to five hours postinfection by immersion, virus particles were found in gill and gut tissues as well as after 4–6 h in mononuclear cells of the gills and in leucocytes connected to the gut (Monaghan *et al.* 2015). In these tissues, KHV mRNA was detected which implements virus replication. No KHV mRNA was detected in healthy mucus-covered skin without any injuries before the second or third dpi (Monaghan *et al.* 2015). After natural infection by immersion, KHV can be trapped by the mucus without any replication. Additionally, there might be a so far unknown response mechanism of carp, which can transport the virus into the skin within one hour after immersion without infecting a cell inside the host (C. Cobo, IGB Berlin, Germany, pers. comm.). KHV is spread to all organs via white blood cells from the gills and the gut. In the interstitium of the kidney, an explosive replication takes place between the second and fourth dpi. Again the virus is spread to all organs via the blood stream. Then the virus is replicated in epithelial cells of the skin, gut and gills after five to seven dpi. In the brain, KHV is found mainly in endothelial cells of the blood vessels. Only once the virus was identified in the granular layer of the brain by in-situ hybridization and PCR (Haenen *et al.* 2004) but never in the neuronal tissues of the white matter.

Afterwards, KHV is released via the gills, gut and skin. In skin and gill tissues, KHV occurs in club cells and epithelial cells, which can be found in increased numbers after infection, but not in mucus cells. This is often combined with necrosis and leucocyte infiltration. So far, cells which release KHV in the gut lumen are not well characterized. The main cells for persistence are the polymorphonuclear granulocytes (Bergmann *et al.* 2010b). Other peripheral blood leucocytes like B lymphocytes, non-B lymphocytes, thrombocytes, granulocytes or monocytes bear KHV proteins and nucleic acids only when there is an acute infection or in case of KHV re-activation from the persistence status (Bergmann *et al.* 2004; Bergmann *et al.* 2010b).

Distribution of KHV and KHVD

The virus and the disease are obviously present all over the world in areas where common carp and koi are cultured and/ or traded for human consumption or for pleasure (Haenen *et al.* 2004; Novotny *et al.* 2010; Ilouze *et al.* 2011). The only exception is perhaps Australia where it has been proven that KHV and KHVD are not present (Hampton and Hyndman 2019).

Hosts susceptible to KHV

Susceptibility to a virus infection is defined as replication in the host with a large variation among individuals regarding establishment of infection and development of disease (Sluijsm *et al.* 2017). In terms of KHV there is a large number of fish which can be infected by the virus, replicate it internally and release the agent infectious to common carp or koi but do not show any external clinical signs or mortality themselves.

For some fish it has been proven by cohabitation with common carp or koi that the virus is released infectious to other fish. Those fish are e.g. goldfish (*Carassius auratus*; Bergmann *et al.* 2009a), a wide range of *Cyprid*, *Ictalurid*, *Percid* and *Esocid* fish (Fabian *et al.* 2013), grass carp (*Ctenopharyngodon idella*), Crucian carp (*Carassius carassius*), tench (*Tinca tinca*) (Michel *et al.* 2010), Prussian carp (*Carassius gibelio*), brown bullhead (*Ameiurus nebulosus*) (Matras *et al.* 2019) but also salmonid fish-like

rainbow trout (*Oncorhynchus mykiss*) (Bergmann *et al.* 2016).

In some ornamental fish, e.g. goldfish variants, blue back ide (*Leuciscus idus*) *Ancistrus* sp. (Bergmann *et al.* 2009b) and *Acipenserids* (Russian sturgeon *Acipenser gueldenstaedtii* and Atlantic sturgeon *A. oxyrinchus*) (Kempter *et al.* 2009) KHV DNA was detected by different PCRs and confirmed by sequence analysis.

It seems that in the aquatic environment there is no species specificity for herpesviruses but for the occurrence of the disease in one species (Bergmann *et al.* 2016).

Combat against KHVD

While the emphasis is laid on hygienic and biosafety measures to prevent infection, there also are different vaccine formulations that can help to reduce the losses caused by KHV. Even with vaccination, there will be no possibility to prevent the infection with wild types of KHV in endemic KHVD areas. Once the fish is infected with KHV, the virus will remain present life-long.

Commercially there is only one live attenuated virus vaccine KV 3 from Israel (KoVax Ltd./Phibro Animal Health Corp.) (Ronen *et al.* 2003; Perelberg *et al.* 2005). In some countries, autogenous inactivated vaccines are produced and applied to protect the carp against KHVD (Germany, S.M. Bergmann unpublished data; Indonesia, Lusiastuti, 2020). Several recombinant vaccines, DNA vaccines and deletion mutant vaccines exist in experimental stages (e.g. Rosenkranz *et al.* 2008; Zhou *et al.*, 2014; Boutier *et al.* 2015; Klafack *et al.* 2019; Schröder *et al.* 2019). Alternatively it was shown that application of the exopolysaccharide (EPS) obtained from *Arthrospira platensis* (*Cyanobacteria*) in the water or in the food can stop a KHVD outbreak (metaphylaxis) and can also reduce the intensity of KHVD when delivered over four to six weeks (prophylaxis) prior to infection (Reichert *et al.* 2017).

Open question and ongoing work

The pathogenesis of KHVD is not fully understood. What is the reason that KHV as other aquatic herpesviruses as well is not species-specific by infection but by disease? It is still unclear how different virus variants or haplotypes can be present and can be developed in one fish. Is the water temperature the essential factor? What is the main entrance of the virus into the carp or koi? How the virus is transferred at different stages of the infection inside the fish? It is also not known whether the virus will be latent or persistent and for how long is KHV detectable present. How and why the re-activation works after stress induction? It needs also to be resolve how the antibodies against KHV are developed. Why are antibodies not always present? How safe can indirect detection methods be, e.g. antibody ELISA, serum neutralization assay or Western Blot? Are there any so far unknown mechanisms of defence or combat against aquatic herpesviruses? What are the best vaccines to reduce the losses in carp or koi? How the virus transfer can be prevented from never diseased carrier fish? Why carp or koi do not develop a sterile immunity that can prevent the virus uptake and eradicate it? What is the best application route for the fish? What season is most appropriate for vaccination? It will be still be a long way to solve all the gaps in the knowledge induced by KHV and KHVD.

Author contributions

All authors contributed to review material preparations, data collection, and analysis. The experiments were performed by Sandro Klafack, Yeonhwa Jin, Qing Wang and Sven M. Bergmann. Kati Franzke prepared and provided the picture from electron microscopy. Bianka Grunow and Qing Wang helped to select the publications used in the study. The first draft of the manuscript was written by Sven M. Bergmann and all authors commented on previous versions of this manuscript. All comments and correction were summarized by Sven M. Bergmann, Qing Wang and Bianka Grunow. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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