

Thesis

Martin Grünbeck

2019

**Department of Exotic Animal and Wildlife Medicine
University of Veterinary Medicine**

**Koi-Herpesvirus Infection of
ornamental and cultivated carp and
the current epidemiological
situation in Germany**

**By
Martin Grünbeck**

**Supervisor
Dr. Ferenc Baska**

Budapest, 2019

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1. Abbreviations:

AciHV2	Acipenserid herpesvirus 2
AngHV1	Anguillid herpesvirus 1
AP-activity	Alkaline Phosphatase activity
ATPase	Adenosintriphosphatase
CCB	Common Carp Brain Cell Line
CEV	Carp Edema Virus
CNGV	Carp Interstitial Nephritis and Gill necrosis virus
CyHV1	Cyprinid herpesvirus 1
CyHV2	Cyprinid herpesvirus 2
CyHV3	Cyprinid herpesvirus 3
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FischSeuchV	Fischseuchenverordnung, Eng. = Animal-Epidemics-Regulations
IBB	Intranuclear Inclusion body
IcHV1	Ictalurid herpesvirus 1
IcHV2	Ictalurid herpesvirus 2
ICTV	International Committee on Taxonomy of Viruses
kbp	kilobase pairs
KF-1	Koi Fin cell line
KHV	Koi Herpes Virus
KHVD	Koi Herpes Virus Disease
KHV-I	Koi Herpesvirus Infektion
OIE	World Organization for Animal Health
qPCR	real-time quantitative PCR
PCR	polymerase chain reaction
RaHV1	Ranid herpesvirus 1
RaHV2	Ranid herpesvirus 2
SalHV1	Salmonid herpesvirus 1
SalHV2	Salmonid herpesvirus 2
SalHV3	Salmonid herpesvirus 3
SVC	Spring Viremia of Carp
TEM	transmission electron microscopy
TierGesG	Tiergesundheitsgesetz Eng. = Animal-Health Law

2. Introduction:

Over hundreds of years fish is an important source for human food. To cover the demand of fresh fish, more and more fish farms are found over the world. Next to salt water fish also the amount of freshwater fish farms are increasing. Additional to that also the keeping of petfish in aquariums or private ponds got more and more spread in the last decades. The connected international trade of alive fish made global spread of fish diseases possible.

In my work I am going to focus on the disease caused by the Koi Herpes Virus (KHV) which is an excellent example for the rapid international spread of fatal diseases.

Due to dramatic losses and the rapid spread combined with missing knowledge regarding this disease, Koi Herpes Virus Disease (KHVD) came into the focus of researchers. Till today the facts about the virus and the disease change continuously. That's why I want to give an overview over the current knowledge regarding this disease and what knowledge gaps still have to be closed.

As my family is owning a company retailing fishing tackle I grew up with fishing and fishkeeping. Next to that due to my studies also the health and diseases of fish came into my focus. Especially of carps. That's why I decided to write my thesis about fishes.

The targets of my thesis are:

1. The disease caused by KHV including taxonomy of the virus, symptoms, spread, vaccination and prevention
2. Prevention of the spread and occurrence of the disease and measures implemented in case of an outbreak
3. Analysis of statistical data regarding the occurrence and frequency of the disease in Germany
4. Evaluation of the results

3. Literature review:

3.1 Herpesviruses:

Herpesviruses are viruses with large DNA genomes (Kibenge and Godoy 2016).

The virion consists of four morphological units with a center containing double stranded DNA wrapped around a protein spool. The enveloped virion has a particle size of 120-200 nm. 162 capsomeres, each consisting of several proteins, build a nucleocapsid with isocahedral symmetry. On top of it a so-called Tegument is formed. This amorphous layer with a fibrous appearance is unique for herpesviruses.

As Herpesviruses are enveloped viruses, the Tegument is covered by a lipid bilayer containing minimum six glycoproteins, spikes are formed in the envelope for protection.

To cause an infection the virions attach to specific cell receptors. Followed by the fusion of the envelope and the cytoplasmic membrane, the nucleocapsid enters the cell (Roberts 2012).

Different members of the Herpesvirus family can cause diseases in many species with various clinical symptoms. Genetically there are 3 families known causing diseases in different species.

- Herpesviridae: infect birds, reptiles and mammals
- Malacoherpesviridae: infect mollusks
- Alloherpesviridae: infect fish and amphibians

Interesting is, that there is nearly no similarity between the amino acid sequence of the mentioned families. The only gene showing homology is the ATPase subunit of the Terminase (Kibenge and Godoy 2016).

3.1.1 Alloherpesviridae:

The international Committee on Taxonomy of Viruses (ICTV) recognized after many genetic investigations 4 genera each containing several species:

- Batrachovirus with RaHV1 and RaHV2
- Cyprinivirus with CyHV1, CyHV2, CyHV3 and AngHV1
- Ictalurivirus with IcHV1, IcHV2 and AciHV2
- Salmonivirus with SalHV1, SalHV2, SalHV3

(Kibenge and Godoy 2016)

3.2 Koi Herpesvirus Disease:

3.2.1 History:

The first outbreak of the disease in cultivated carps was seen 1998 in Israel. Till the end of the millennium the disease was present in up to 90 % of the country's fish farms and caused yearly costs of 300 million US-Dollars. In the USA the disease was first seen 1998 in a Koi-carp imported from Israel.

In Germany mass mortalities in connection with the causative agent in cultivated carp were observed in 1997 and 1998 (Friedrich-Loeffler-Institut 2016).

The disease also spread to Asia and Africa. 2002 in Indonesia and 2003 in Japan, where cage-cultured carp was affected. The spread of the disease extended to Thailand, Malaysia, Singapore, South Korea, China and Taiwan. Also cases in South Africa were known (Woo and Cipriano 2017).

The reason why Koi Herpes Virus was detected in many countries all over the world in the following years (Friedrich-Loeffler-Institut 2016) was the unregulated trade and movement of infected fish and due to other unknown means (Gilad et al. 2004). Additional to the trade of cultivated cyprinid species the high global trade volume of ornamental cyprinids created a big concern for the official authorities to establish measures to limit the

global spread (Roberts 2012). Important in the spread of the disease are also symptomless carrier fish. These are latent infected fishes without clinical symptoms, which can shed and transmit the virus under special conditions (Steinbrück 2017).

The OIE published in 2012, that the disease has been reported or occurred in at least 30 countries (Woo and Cipriano 2017). In the following table you can see in which European country and when the disease was officially confirmed.

Bull. Eur. Ass. Fish Pathol., 24(6) 2004, 297

Country	Current situation	Source of information
<i>European countries with KHV confirmed</i>		
Austria	First outbreak reported summer 2003 - confirmed by PCR.	Reported at Munich KHV Meeting, December 2003.
Belgium	First outbreak 1999 - not confirmed. Outbreaks in 2002 & 2003 - confirmed by PCR.	CER, Marloie, Belgium; CEFAS, Weymouth.
Denmark	First outbreak in 2002 and two further in 2003 - confirmed by PCR.	DFVF, Aarhus.
France	Two suspicions in 2001; first confirmed outbreak in 2003.	Munich meeting, 2003.
Germany	First outbreak in 1997 - over 80 cases in 2002 and 100+ in 2003 - confirmed by PCR, RT-PCR and <i>in situ</i> hybridization.	FLI, Insel-Riems; University of Munich.
Italy	Strong suspicion by EM and Histology.	Munich meeting, 2003.
Luxembourg	Outbreak in 2003.	Munich meeting, 2003.
The Netherlands	First detected in 2001 by PCR; Disease cases confirmed in 2002; Further outbreaks in 2003 and 2004.	CIDC-Lelystad.
Switzerland	First report in 2003 - suspicion before in 2001.	Centre for Fish and Wildlife Health, Univ. of Berne; Munich meeting, 2003.
UK - England & Wales	First isolated in 2000 - suspicion in 1998 and 1999 & earlier in 1996 - confirmed by PCR.	CEFAS Weymouth.
Poland	First detected in 2003 in carp, 2 outbreaks in 2004 in carp farms. Confirmed by PCR's, RT-PCR and <i>in situ</i> hybridization.	Insel-Riems/ Vet.Res.Inst., Pulawy.
<i>Countries where no KHV has been reported (to date)</i>		
Czech Republic, Finland, Greece, Hungary, Republic of Ireland, Russia, Slovenia, Spain, Sweden, parts of UK (Scotland & N.Ireland).		NVFRI (EELA), Helsinki / A.Prapas (pers.comm.) / CVI, Budapest / Marine Inst., Abbotstown/VNIIPRH/ Univerza V.Ljubljani/ J.Barja (pers.comm.) /A.Hellström (pers.comm.)/ FRS, Aberdeen & DARDNI, Belfast.

Table 1 Confirmed cases of Koi Herpes Virus in Europe till 2004 (Haenen et al. 2004)

3.2.2 Koi Herpes Virus:

As mentioned above, the koi herpesvirus (KHV) belongs to the genus Cyprinivirus of the family Alloherpesviridae (OIE 2019). Within the family we differentiate between Cyprinid Herpesvirus 1 (CyHV1) also called “carp pox virus”, Cyprinid Herpesvirus 2 also called “herpesviral haemopoetic necrosis virus” or “goldfish herpesvirus” and the Cyprinid Herpesvirus 3, which is the causative agent of the Koi Herpesvirus disease (Friedrich-Loeffler-Institut 2016). It is also known as “carp interstitial nephritis and gill necrosis virus” (CNGV) (OIE 2019). The disease internationally is called KHV-Disease (KHVD), in Germany it’s called “Koi Herpesvirus Infektion” (KHV-I) (Friedrich-Loeffler-Institut 2016).

Sequencing the genome showed, that CyHV-3 is closely related to CyHV-1 and CyHV-2. Also distant relation to the channel catfish virus (Ictalurid Herpesvirus: IchV-1) and the frog herpesvirus (Ranid herpesvirus: RaHV-1) could be observed (OIE 2019).

A complete genome sequencing was possible for 3 Isolates. The U-Isolate (from the USA), the J-Isolate (from Japan) and the I-Isolate (from Israel). Only slight differences of the genomes were observed. Especially the U-strain and the I-strain show strong similarities. These facts are the fundament of the theory, that originally 2 strains were present, an Asiatic and an European one. New studies showed, that with the evolution the Asiatic strain split into two and parallelly seven different variations of the European strain have developed. A new strain was isolated in 2011 in Indonesia. As it has characteristics of both the Asiatic and the European strain it was assigned to a new intermediary line. With 295 kbp the KHV is considered to be the biggest Herpesvirus. Nowadays also 40 structure proteins are identified (Steinbrück 2017).

The first isolation and amplification of the virus was performed with a cell line of the fin of a koi carp (koi fin cell line- KF-1). Also a cell line of the brain of a carp can be used (common carp brain cell line- CCB). Both cell lines can be ordered for research at the Friedrich-Loeffler-Institute with the registration number 843 and 816. As the efficacy of cultivating the virus with this cell lines is not very high, this method is not recommended for the diagnosis of the disease anymore (Friedrich-Loeffler-Institut 2016) .

3.2.3 Host spectrum:

According to the Aquatic Animal Health Code all varieties, subspecies and hybrids of the common carp (*Cyprinus carpio*) are considered to be susceptible to the disease (Table 2) (OIE 2019). This means that high mortalities and morbidities can be observed (Steinbrück 2017).

S. No.	Host fish species	Nature of Infection
1	Common carp (<i>Cyprinus carpio carpio</i>)	Symptomatic
2	Koi carp (<i>Cyprinus carpio koi</i>)	Symptomatic
4	Goldfish (<i>Carassius auratus</i>)	Asymptomatic/carrier
5	Goldfish × koi carp hybrid	Symptomatic
6	Crucian carp × koi carp hybrid	Symptomatic
7	Goldfish × common carp	Symptomatic
8	Grass carp (<i>Ctenopharyngodon idella</i>)	Asymptomatic/carrier
9	Ide (<i>Leuciscus idus</i>)	Asymptomatic/carrier
10	Ornamental catfish (<i>Ancistrus</i> sp.)	Asymptomatic/carrier
11	Russian sturgeon (<i>Acipenser gueldenstaedtii</i>)	Asymptomatic/carrier
12	Atlantic sturgeon (<i>Acipenser oxyrinchus</i>)	Asymptomatic/carrier

Table 2 Host range and nature of infection of Koi Herpes Virus (Rathore et al. 2012)

Also the Aquatic Animal Health Code mentions species with incomplete evidence for susceptibility. This includes the goldfish (*Carassius auratus auratus*), the Siberian crucian carp (*Carassius auratus gibelio*) and the Grass carp (*Ctenopharyngodon idella*) (OIE 2019).

Current studies showed, that goldfish (*Carassius auratus auratus*) is a susceptible species to KHV and does not only function as a potential transmitter. This means, that the virus can replicate in goldfish (Steinbrück 2017). Antigens and viral DNA could be isolated from goldfish leucocytes by PCR and by fluorescent antibody test (OIE 2019).

3.2.4 Carrier fish:

Animals which survived the disease, but also other cyprinid and non-cyprinid species not showing clinical signs and mortalities can be carrier of the virus. A carrier fish is defined as latent infected individual with the virus without showing any clinical symptoms. Under special conditions (stress factors) these animals can shed and transmit the virus. The persistence of the virus potentially takes place in the intestinal epithelium and the Leukocytes (Steinbrück 2017).

In an experiment goldfishes were exposed to KHV. 60 days after infection they were kept together with koi carps at 19°C. At the end of the experiment the virus was detected in the kois leukocytes via PCR. This experiment was the prove, that extra-susceptible species represent a risk of transmitting the disease (Roberts 2012).

Experiments with Koi-hybrids were performed to investigate, whether these hybrids have a higher resistance against the disease.

Sadly, experiments showed, that no lowered mortality could be observed in hybrids of kois with goldfish or crucian carp. All hybrids developed KHV-Disease after infection.

This means that the breeding of different hybrids of *Cyprinus carpio* are no options in decreasing the losses of KHVD (Bergmann et al. 2010).

3.2.5 Susceptibility according to the age of the host:

KHV can affect susceptible species of all ages. Young fishes are more susceptible than older ones. This might be due to their immature immune system. In experiments 2,5-6g fish were more prone to KHVD than fishes with 230g.

Only carp larvae are resistant to a KHV infection. With maturation the susceptibility increases (OIE 2019).

3.2.6 Resistance:

As Herpesviruses are enveloped viruses, their resistance is generally low. This means, that KHV is sensitive to drying agents and disinfectants. UV radiation and heating are also possibilities for viral inactivation.

For example : UV radiation at a dose of $4 \times 10^3 \mu \text{ W/cm}^2$, temperatures of 50°C or more for 1 min, 200ppm iodophors and 60ppm benzalkonium (Roberts 2012).

3.2.7 Life cycle

As the resistance of the virus is quite low, KHV remains active in water for at least 4 hours (Steinbrück 2017). Other sources describe activity of the virus for not more than 21 hours at 23-25°C water temperature (OIE 2019). The exact way of transmission of KHV is not completely clear yet. The theory, that the gills are the major portal for infection have been proven wrong. An experiment showed via bioluminescent imaging, that infection takes place percutaneously but is restricted to the posterior part of the fish.

Different than in mammals, the skin of teleost fish consists of squamous stratified epithelium and is capable of mitotic division. Even the outermost layer. This makes the initial viral replication in the epidermis possible and is followed by rapid spread in the infected fish (Michel et al. 2010). The spread inside the animal may happen via the bloodstream as viral DNA could be isolated from Leukocytes (Steinbrück 2017). Due to this systemic spread KHV DNA can be isolated from kidney, spleen, liver and gut tissue. Hypersecretion of mucus in an early stage of infection in experimentally infected carp gave evidence, that the skin is highly involved in the pathogenesis of the disease. From this mucus KHV DNA could be isolated. Excretion of the virus via urine and faeces may play an important role in virus shedding as well (OIE 2019).

3.2.8 Transmission:

Transmission of the disease on a horizontal way has been demonstrated. Either with faeces or viral particles in the water. The skin of the carp is the site of entry and early replication. The early replication at the point of entry may be a factor in spreading the disease within the fish population. 2-3 days after infection, the fishes start rubbing themselves against objects or other fish. This could contribute to a skin-to-skin infection. Also the picking of uninfected fish at the skin lesions caused by the KHV may play a role in transmission. The possibility of vertical transmission is not known (Michel et al. 2010).

Additional to that also piscivorous birds and mammals but also parasitic invertebrates may play a role in transmission of the disease. According to studies from Japan KHV DNA in plankton samples was found. Especially the amount of present Rotifera species positively correlated with the KHV in the plankton. In Poland KHV was also found in freshwater shrimp (*Gammarus pulex*) and swan mussels (*Anodonta cygnea*) (OIE 2019).

3.2.9 Water temperature:

The clinical manifestation of koi herpes virus disease (KHVD) does not only depend on viral dose, virus type, and age and condition of the fish but also on the water temperature (Steinbrück 2017). During spring and autumn the disease usually occurs, as a water temperature between 18-26 °C is needed (Gilad et al. 2004). The permissive water temperature is the temperature range, at which clinical manifestation of the disease occurs (Steinbrück 2017). Laboratory trials showed, that infections at lower water temperatures resulted in absence of clinical signs of the disease (Gilad et al. 2004). Also at temperatures above 30°C clinical signs cease, as the virus becomes dormant. Also the shedding of the virus via faeces, urine, gills and skin mucus takes place for a longer period at 16°C than at 23-28°C (Rathore et al. 2012). In cell culture optimal viral growth can be achieved by keeping a temperature of 15-25 °C (Gilad et al. 2004).

Experiments with changing the water temperature from 13°C to 23°C provoked clinical manifestation of the disease in infected fish. This proved the latency of the virus in infected fish at too low or too high temperature. If susceptible fish is exposed to the virus at

permissive water temperature with a following rise of the water temperature over 30°C a decreased mortality rate can be observed. Some breeders and keepers use this method for preventing KHVD. In Israel this method was even a requirement for export of koi carps. All in all the feasibility and effectivity of this “immunization therapy” is highly questionable (Steinbrück 2017).

3.2.10 Pathogenesis:

After the above mentioned routes of infection the virus starts to spread within the animal towards the kidney via the blood stream. With time a generalization occurs as viral DNA concentrations can be observed in gills, kidneys, spleen, skin mucus, liver, intestines and skin. Main viral replication probably takes place in gills and kidneys. But also high concentrations of viral DNA in faeces of infected fish makes an intestinal virus replication most likely (Steinbrück 2017).

3.2.11 Incubation period and mortality patterns:

Fishes are poikilotherm animals. Consequently, the water temperature highly influences the incubation period and the course of the disease. Usually with an acute course of the disease first clinical signs and mortalities can be observed 7-10 days after infection (Steinbrück 2017). Other sources describe that the incubation period can vary between 4 days and several months. Infection of carrier fish may have happened even years before (Friedrich-Loeffler-Institut 2016). With a peracute or acute course of the disease, morbidities of 80-100% and mortalities of 70-80% can be observed. The fast and often fatal progress of the disease can obliterate whole animal populations within a few days.

Other factors next to the water temperature influencing the course of the disease are water quality, age and condition of the fishes, population density, status of the immune system and the amount of the exposed virus. Secondary bacterial or parasitic infections have influence on the disease as well and mortality rates can be increased (Steinbrück 2017).

3.2.12 Clinical signs and pathological alterations:

Usually gross lesions of KHVD are never pathognomonic and can be caused by other diseases or conditions. Consequently, in case of observation of the following mentioned lesions in a fish population, a laboratory investigation has to be carried out for final diagnosis. Either by detection of viral DNA or viral isolation and identification (OIE 2019).

The first signs can be seen 2-3 days post infection (Michel et al. 2010). Loss of appetite, fatigue, disorientation and lethargy combined with frequent ventilation (gasping) and erratic swimming are the first symptoms (Haenen et al. 2004). Often the animals lie at the bottom of the tank with their dorsal fin folded (Michel et al. 2010). Fishes may die within a few hours after the first signs appeared, but the course of the disease can be protracted in case of lower temperatures as well.

The most characteristic gross pathological lesions can be seen in the gills. With increasing severity it can vary from pale necrotic patches to extensive discoloration, severe necrosis and inflammation (OIE 2019). This necrosis is caused by the massive swelling of the gills, leading to a dyspnoe in the affected fish (Figure 1) (Steinbrück 2017). Important to know is as well, that the primary gill infection can be obscured by secondary parasitic infections.

Infections with *Ichthyobodo sp.*, *Trichodina sp.*, *Ichthyophthirius sp.*, *Dactylogyrus sp.*, *Chilodonella cyprini* and monogenean parasites have to be mentioned (Roberts 2012). Also several bacterial species can complicate the external gross lesions. Especially infections with *Flavobacterium columnare* at warmer water temperatures (OIE 2019), but also *Aeromonas sp.*, *Pseudomonas sp.* and *Shewanella putrefaciens* have been observed.

Due to the property of the possible secondary and opportunistic bacterial and parasitic pathogens, to obscure virus specific pathologic lesions, these may cause problems in the diagnostic work of fish veterinarians and pathologists (Haenen et al. 2004).



Figure 1: Gill necrosis in a late-stage KHV infected koi (Roberts 2012)

Additionally pale, irregular patches on the skin can be observed (Woo and Cipriano 2017). At the beginning the fishes usually show a hypersecretion of mucus of the skin and gills accompanied by hemorrhages on the skin.



Figure 2 Haemorrhages on the hyperaemic skin of a common carp (Woo and Cipriano 2017)

With progressing course of the disease, the mucus production decreases leading to a development of irregular patches on the skin with a “sandpaper”-like texture (Steinbrück 2017).



Figure 3 Irregular patches on the skin with sandpaper-like texture (Woo and Cipriano 2017)

In addition to the mentioned signs, sunken eyes (enophthalmia) often occur as well (OIE 2019). In the final stage of the disease the fishes often show neurological signs, characterized by disorientation and loss of the equilibrium (Michel et al. 2010).



Figure 4 Enophthalmia in koi carp (left) (Woo and Cipriano 2017)

Simultaneously with the external lesions, also internal gross pathological lesions can be observed. Enlarged organs, abdominal adhesions and abdominal fluid accumulation can be found but are as well not pathognomonic for KHVD (Woo and Cipriano 2017). Other sources describe a prominent cachexia, pallor and anemia (Roberts 2012).



Figure 5 Chronically infected koi carp with anemia and cachexia (Roberts 2012)

The severity and prominence of the pathological lesions depend highly on the course and stage of the disease. The gill necrosis can be manifested from single small necrotic spots up to severe erosions of the primary lamella in the complete branchial arch. Often the inner organs show no gross lesions but petechial hemorrhages in the liver and kidney may be found. Most prominent next to the lesions of the gills, are the often enlarged kidneys due to an interstitial nephritis (Steinbrück 2017). Other sources describe, that a swollen spleen and a flaccid heart with mottled appearance can be found as well (Haenen et al. 2004).

3.2.13 Histopathological alterations:

In Japan a big study was carried out, where Koi Carps were experimentally infected with KHV. In this study the fishes were infected by direct application of the virus on the gills. Although its mentioned above, that the gills are probably not the port of entry, the fishes sickened. After the induced outbreak the diseased fishes were killed, samples were taken and a histological investigation was carried out of the different organs.

Gills:

The main histological changes were seen in the gills, where the majority of the respiratory epithelial cells of the lamellae were swollen or vacuolated. Also a nuclear degeneration in form of pale coloration, karyorrhexis and intranuclear inclusion body formation (IIB) could be seen. This IIB-formation was described as a basophilic material with marginal hyperchromatosis due to heterochromatin deposition on the inner nuclear membrane (Figure 6A). This was confirmed by electron microscopy as well.

Lamellar fusion and clubbing of the gill filaments could be seen in the tissue as well as the affected respiratory epithelial cells fused with cells of neighboring lamellae (Figure 6B) (Miyazaki et al. 2008). Reason for this lamellar fusion is the hyperplasia, hypertrophy and severe inflammation of the epithelial cells of the gill filament (Rathore et al. 2012).

Heart:

In the investigated heart tissues bundles of myofibrils with disappearance of cross-striations were dilated or coagulated. Several myocardial cells showed nuclear degeneration in form of intranuclear inclusion bodies or pyknosis (Figure 6C).

In moribund fish the myocardial infiltration by macrophages and lymphocytes could be seen (Miyazaki et al. 2008).

Kidney:

In the kidneys the tubular epithelium in the nephrons is congested and undergoes degeneration (Rathore et al. 2012). Already 2 days after infection peritubular infiltration with inflammatory cells can be observed (Michel et al. 2010). These tubular cells had a cloudy appearance and some cells showed even IIB. Nevertheless, in the kidneys the main affected

cells were the hematopoietic cells. In severe cases they showed necrosis and intranuclear inclusion bodies accompanied by a pyknotic nucleus (Figure 6D) (Miyazaki et al. 2008).

Spleen:

The splenocytes are the most obvious infected cells (Michel et al. 2010). Intranuclear inclusion bodies could be found, while ellipsoidal cells showed less affection. In severe cases an extensive amount of splenocytes showed necrosis. In addition hemorrhages in the splenic pulp could be seen (Figure 6E) (Miyazaki et al. 2008). The epithelial cells of the spleen are characterized by eosinophilic inclusion bodies and margination of chromatin (Rathore et al. 2012).

Liver:

In the liver the hepatocytes are the most obvious infected cells (Michel et al. 2010). Nevertheless, only a small number of hepatocytes showed intranuclear inclusion bodies. A cloudy appearance of most hepatocytes was quite evident (Miyazaki et al. 2008).

Brain:

Fishes which stand out by spiral swimming showed capillary and small vein congestion in the valvular cerebelli and medulla oblongata (Michel et al. 2010). Additional to that edematous dissociation of the nerve fibers contribute to the development of neurological signs (Rathore et al. 2012). In the affected vessels no obvious endothelial lesions were found. Only small round cells and a perivascularitis could be detected around some vessels. In the medulla oblongata only some small nerve cells showed intranuclear inclusion bodies (fasciculus cells or putative internal cells) but also megalocells (Miyazaki et al. 2008).

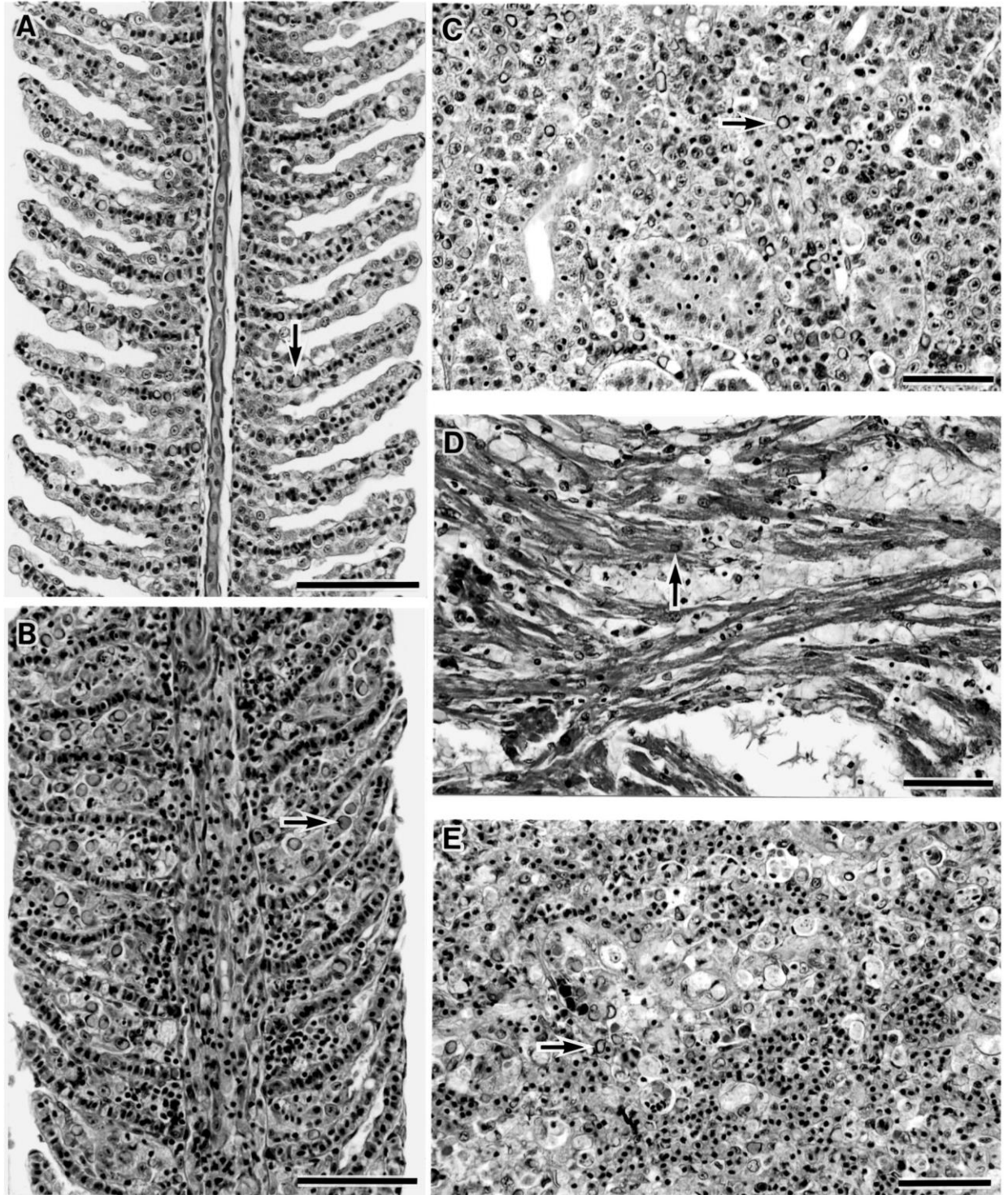


Figure 6: Histopathological features of moribund *Cyprinus carpio* infected with KHV (Miyazaki et al. 2008)

3.2.14 Electron microscopic alterations:

The usage of transmission electron microscopy (TEM) is not a reliable diagnostic method when tissue from infected carp is examined (OIE 2019).

The respiratory epithelial cells of the gills and the interlamellar epithelial cells showed a nuclear assembly of immature capsids and nucleocapsids of KHV (Figure 7 A,C).

The affected nuclei show heterochromatin deposition on the inner nuclear membrane and filamentous nucleoproteins could be observed. Additional to that intranuclear inclusion bodies consisting of electron-dense granules were found (Figure 7 A,C).

Next to infected respiratory epithelial cells, also macrophages showed assembly of nucleocapsids and virions in the nucleus and cytoplasm. These ones also migrated into the lamellar capillaries. Also virions are released into the bloodstream indicating a viremia and systemic infection (Figure 8E) (Miyazaki et al. 2008).

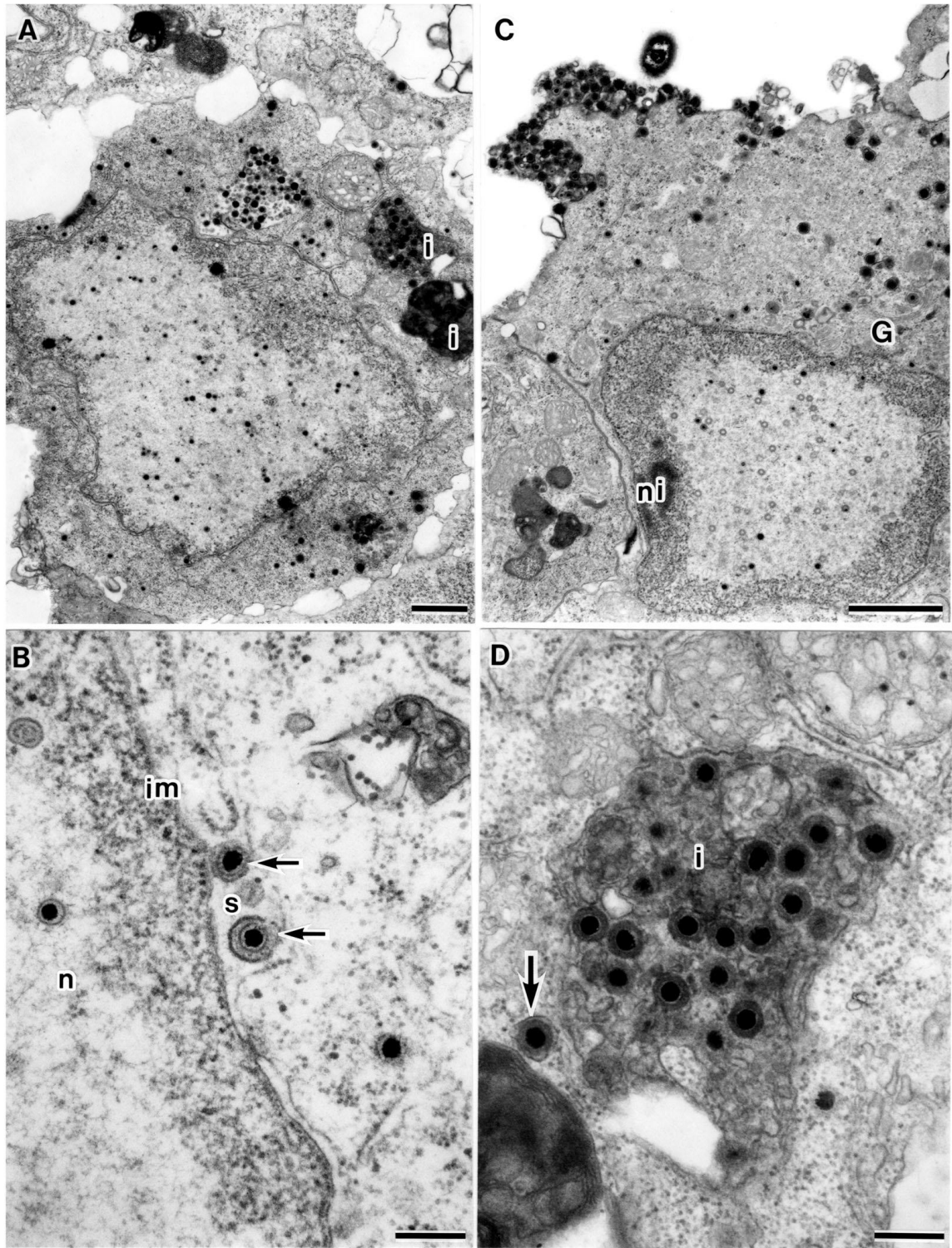


Figure 7: *Cyprinus carpio*: Gill lamellae of KHV-infected respiratory epithelial cells (Electron micrography) (Miyazaki et al. 2008)

i: inclusion bodies, *ni*: intranuclear inclusion bodies, *s*: perinuclear space, *im*: inner nuclear membrane, *n*: nucleus, *G*: Golgi apparatus

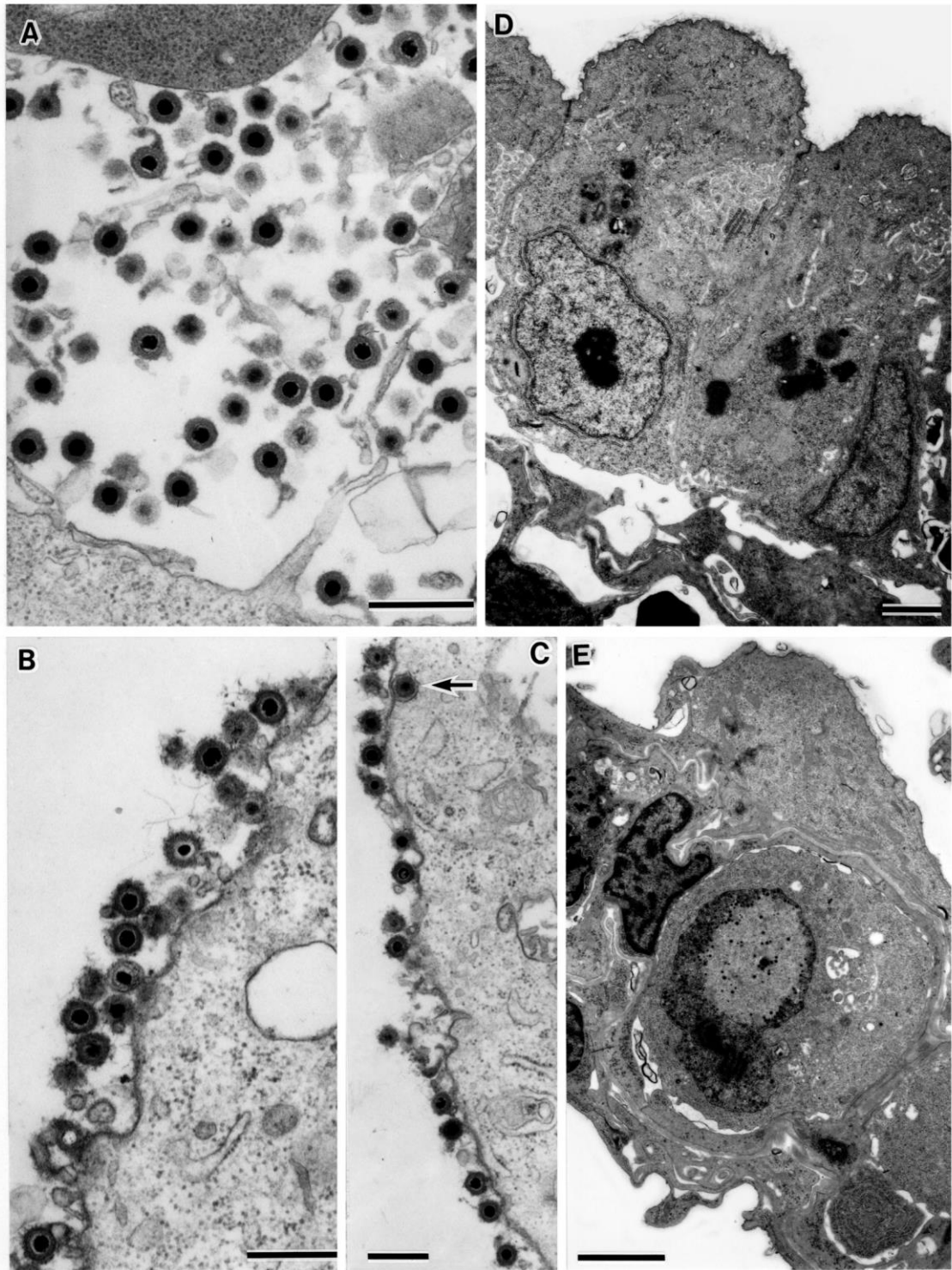


Figure 8 electron micrographs of respiratory cells of *Cyprinus carpio* (Miyazaki et al. 2008)

3.2.15 Laboratory alterations:

In a study experimentally infected fish with KHV was investigated histologically but also blood and urine samples were taken and examined.

Blood:

During the experiment blood was drawn and investigated from fishes 2, 5, 8 and 13 days post infection. The evaluated parameters were compared to blood parameters from blood of the same fishes taken 10 days prior infection.

The blood parameters of fishes under CyHV-3 infection changed significantly. The affected fishes developed an anemia. A severe reduction of the hematocrit and red blood cell counts could be observed from day 2 and 5 post infection.

In comparison the amount of leukocytes increased significantly and remained on a high level throughout the observation period. Also percentages of monocytes and neutrophilic granulocytes were altered. To the disadvantage of the leukocytes their percentages increased dramatically.

Investigating the blood plasma, a decreased osmolality and sodium concentration was measured, while potassium and creatinine concentrations and the AP-activity was significantly increased. However the total protein concentrations were stable and the Ca^{2+} and Mg^{2+} divalent ions and phosphate concentrations remained unaltered.

Urine:

Urine was sampled in the control group and in the infected fish population 2, 5, 8, 13 days post administration of the virus or for the control group a phosphate buffer solution. A significance between the groups was defined to be significant in case of $p < 0.05$.

Comparing the two groups, a progressive decrease in urine flow from day 5 post infection on was seen in the infected fish.

Investigating the chemical parameters of the urine an increase of the osmolality and sodium concentrations could be seen parallel with the decrease of these parameters in the blood. Significant alterations in the concentration of divalent Ca^{2+} and Mg^{2+} ions as well as phosphate and creatinine were not observed. However slight variations of these

concentrations were detected. 5 days post infection the activity of alkaline phosphatase, which is physiologically found in the renal epithelium of carp, started to elevate.

Next to that also the elevated activity of gamma glutamyl transferase in the urine was measured. Nevertheless due to strong variations of the values a meaningfulness of this parameter shall be doubt.

To summarize the mentioned parameters the lack of regulation of the osmotic balance probably is caused by the severe tissue damage of the gills and kidneys.

Its believed, that the osmotic imbalance in the organism is the cause of death especially in case of an acute progression of the disease (Negenborn et al. 2015).

3.2.16 Differential diagnosis:

In case of differential diagnosis we have to differentiate between following diseases: Spring Viremia of Carp (SVC) , Carp Edema Virus (CEV) infection causing the “Koi Sleepy Disease”, different bacterial infections (e.g *Aeromonas sp.*, *Vibrio sp.*) and severe parasitosis (gill worms (*Dactylogyrus*), skin worms (*Gyrodactylus*), white spot disease (*Ichthyophthirius multifiliis*)). Also a negative change of the water quality (drop in oxygen, increased ammonia concentration) has to be taken into consideration.

Especially an infection with CEV can cause the same symptoms and gross pathological lesions. This means that Koi Herpes Virus Disease and the Koi Sleepy Disease can not be differentiated on a clinical point of view (Friedrich-Loeffler-Institut 2016) .

3.3 Diagnosis of KHV:

In the following lines I want to mention when and with which method sampling shall take place and what diagnostic methods are used nowadays and how reliable they are.

Perquisition for a suspicion for a KHV-infection are increased mortalities with characteristic gross pathological lesions and observation of typical clinical symptoms in alive fish. Whether there is a connection or not to other officially confirmed KHV cases plays an important role as well (Tauros-Diagnostik 2010).

3.3.1 Time of sampling:

Sampling shall be performed in common carp, koi carp and other potential susceptible species (e.g. Hybrids of crucian carp and goldfish with carps or kois) (Friedrich-Loeffler-Institut 2016).

In case of new purchased fishes the incubation period has to be considered. According to that a quarantine for 3-4 weeks at a water temperature of minimum 20°C is required.

A stress-provocation of the fishes is emphasized, but in a animal welfare point of few highly questionable (Tauros-Diagnostik 2010).

In common it has to be mentioned that if possible, fishes shall be sampled, which were kept for a longer period at the permissive water temperature (2-3 weeks at 15-26°C). In carp farms usually its not possible to collect useful samples during the production period in summer (Friedrich-Loeffler-Institut 2016). When putting fish from winter to summer ponds some fishes can be taken and kept isolated till the permissive water temperature is reached. Easiest is to collect fish together within the normal fish farming operations. All production units of the fish farm (ponds, channels, tanks, net-cages) have to be checked for moribund, disturbed or recently died fish (without signs of autolysis) (Tauros-Diagnostik 2010). Samples have to be taken proportionally of fishes of all production units and ages. Also the water supplies have to be considered (Friedrich-Loeffler-Institut 2016).

Samples shall be taken in following ways:

1. Alive fish shall be transported to the laboratory as fast as possible (max. 10°C)
2. Dead fish shall be transported chilled but not frozen (max.10°C)
3. In case of valuable fish or fish not intended to be killed, gill samples or blood samples can be sent
4. Organs or parts of organs shall be taken individually and analyzed separately (no collection samples)
5. Blood samples have to be taken for each animal individually (no group samples)
(Tauros-Diagnostik 2010)

Samples taken of organs or blood samples (with anticoagulant) have to be taken under sterile conditions and shall be chilled immediately below 10°C.

Unchilled sending of samples is also possible if they are stored in Isopropanol (100%) or in a PCR-Lysis-buffer (Friedrich-Loeffler-Institut 2016).

3.3.2 Sampling:

Lethal sampling:

From clinically symptomless fishes, samples shall be taken 24-72 hours after fishing out, caging or transport.

In fishes with clinical signs of KHVD or fishes with suspicious post mortem lesions a sample pool taken from minimum 10 individuals is required (Organ samples have to be pooled from maximum 5 fishes). In case of investigation of clinically symptomless fishes, organ samples have to be taken from 30 individuals. Only samples of 2 fishes are allowed to be pooled in this case. The volumes of the taken samples in case of official confirmation or rule out of the disease are regulated by the commission implementing decision (EU) 2015/1554 laying down rules for the application of Directive 2006/88/EC regarding the requirements for the surveillance and diagnostic methods.

From fishes gill and kidney samples shall be taken. Additionally samples of the spleen, brain and intestines can be sent for investigation (Friedrich-Loeffler-Institut 2016).

Non-lethal sampling:

In special cases (for example very expensive fishes) sample taking can be performed, which do not require the killing of the fish. This can be blood samples, gill smears, gill biopsies or mucus smears. Nevertheless, a non-lethal sampling shall always be performed according to the instructions of the responsible authorities.

Gill smears from alive fish shall be sampled with sterile swabs a have to be stored in a PCR-Lysis buffer (e.g. ATL buffer with Proteinase K, Qiagen) (Friedrich-Loeffler-Institut 2018) .

On the swabs blood has to be visible. If the PCR-Lysis buffer is used the DNA extraction has to be performed latest 48 h post sampling (Friedrich-Loeffler-Institut 2016).

Blood can be sampled for serum examinations or with an anticoagulant for Leukocyte separation. Sent material immediately shall be chilled after sampling (Friedrich-Loeffler-Institut 2018). Blood is usually sampled from the tail vein or directly from the heart.

When biopsies of the gills are used for investigation, the fishes are anesthetized and the tissue sample is removed with a sterile scissor. That no severe damage is caused it has to be considered that in larger individuals only smaller parts of the gills can be removed. In smaller fish the biopsy consists of one whole gill arch. Transportation takes place unchilled or chilled in alcohol, PCR-Lysis buffer or other appropriate transport media (Friedrich-Loeffler-Institut 2016).

3.3.3 Direct detection of the causative agent:

Molecular biological methods:

For a routine laboratory diagnostic test a standardized, highly sensitive and reliable method has to be used. For the detection of the genome different methods of the Polymerase-chain-reaction (PCR) are used (Steinbrück 2017).

Following methods shall be used:

- Real-time PCR according to Gilad et al., 2004
- As an alternative the PCR developed by Engelsma et al. 2013 with a subsequent sequence analysis of the amplified fragment and if required a following nested PCR with sequence analysis of the fragment of the viral polymerase gene can be performed
- Also a PCR according to Bercovier et al. 2005 can be used

If there is a positive result with real-time quantitative PCR (qPCR) or PCR according to Bercovier KHV is officially confirmed. The PCR developed by Engelsma the sequencing and following comparison with data bases is needed. KHV is officially confirmed if the PCR products are to 98% identically with the sequences in the data bases.

Other qPCR and PCR methods can be used as well, if it they can be used with similar reliability (Friedrich-Loeffler-Institut 2016).

Nowadays as gold standard a quantitative TaqMan real-time PCR developed by Gilad et al, 2004 is used. The Primer KHV-86f and KHV-163r amplifies a 78 bp long fragment.

As an example the protocol for the q PCR according to Gillard et al. 2004 shall be mentioned (Friedrich-Loeffler-Institut 2016):

Primer:	Sequence:	Program:
KHV-86f	5'- GACGCCGGAGACCTTGTG - 3'	One cycle: 50°C, 2min 95°C, 10min 40 Cycles: 95°C, 15s 60°C, 1min
KHV-163r	5'- CGGGTTCTTATTTTGTCTTGTGTT - 3'	
KHV-109p	5'-FAM-CTTCCTCTGCTCGGCGAGCACG-TAMRA 3'	

The current PCR methods are only limited usable for the surveillance and confirmation of disease-free areas and countries. In the latent phase of the infection a detection of the virus is extremely difficult, as the virus hides from the immune system of the fish and no immune reaction takes place. The amount of viruses in samples taken during the latent phase is probably too low that current PCR methods can detect them. Nevertheless, a frequent sampling of potential carrier fishes is required. Without a reduction or eradication of the disease is not possible at all. Additionally, the usage of serological methods seems to be useful.

Cell culture:

As mentioned above KHV can be cultured in different cell lines. “Common Carp Brain” (CCP) and “Koi-Fin” (KF-1) cell lines can be used. As the isolation of the virus from the different organs rarely is successful the usage of cell cultures as a diagnostic tool is unsuited (Steinbrück 2017).

Electron microscopy:

With electron microscopy of KHV infected tissue the most pathognomonic characteristic lesions were intranuclear inclusion bodies (Steinbrück 2017). Also different stadiums of virus production characteristic for herpesviruses can be seen. Nevertheless a high number of accumulating nucleocapsids in the nucleus and cytoplasm are characteristic findings (Friedrich-Loeffler-Institut 2016). Limiting factors for using electron microscopy as a diagnostic tool are a high virus load, a good condition of the to be examined tissue and the high technical effort.

Further direct methods:

The in-situ hybridization is a molecular biological method to detect nucleic acids in histological preparates. Sadly it is not usable for routine diagnosis but is an important tool for KHV detection in the different tissues (Steinbrück 2017).

3.3.4 Indirect detection of KHV:

Enzyme-Linked Immunosorbent Assay (ELISA):

The ELISA is a diagnostic method, with which KHV-Antibodies can be detected. Commercial ELISA tests are available nowadays as commercial available test kits (Steinbrück 2017). These kits can detect anti-CyHV-3 antibodies in the blood of carps and CyHV-3 antigens in other samples (Michel et al. 2010). Even though KHV specific antibodies can be detected in the individual up to one year post infection, this test has to be used carefully as the antibody kinetics in case of KHV are not investigated enough yet. The generation of antibodies for example is highly temperature dependent. Additionally to that cross reactions with CyHV-1 are possible as well if serum dilutions of 1:2500 or lower are used. In case of serum dilutions above 1:2500 a specific reaction to KHV can be seen (Roberts 2012). Important to know as well is, that a detected antibody titer is not a protection for a reoccurrence of the disease.

Nevertheless, the ELISA test is established nowadays as the most important diagnostic tool for the monitoring of populations (Steinbrück 2017).

3.4 Therapy and vaccination:

In case of an KHV outbreak there is no possibility of therapy. The only way to lower the mortality is to manipulate the keeping conditions. So is a strict hygiene, continuous water change and a removal of dead fish highly emphasized. By adding salt to the water secondary infections can be decreased as well.

The possibility to increase the water temperature to reduce mortality rates is not emphasized, as next to the decreased mortality, latent infected fishes are produced. These picture a big risk for further spread of the disease (Steinbrück 2017).

Regarding vaccination against KHV there is no current authorized vaccine against KHV in Germany (Steinbrück 2017). However, in Israel live attenuated virus strains are used for vaccination. High antibody titers of vaccinated fish providing protection against virulent virus is reported. Keeping fish for 30 minutes in a viral suspension followed by a keeping for 2-3 days at permissive water temperature enables viral propagation in the vaccinated fish.

This vaccine can induce protection for at least 8 months, but is available in Israel for emergency use only, as the possible reversion of attenuated virus to a pathogenic one pictures a severe general safety concern (Rathore et al. 2012).

3.5 Legal framework:

The KHVD was first admitted 2006 into the Aquatic Animal Health Code. Provided by the OIE (Office International des Epizooties) it sets international standards for disease control objectives for KHV. The member states of the OIE are notifying confirmed cases of KHVD. The collected data is available for all national veterinary authorities and provide a disease monitoring within the member states (Steinbrück 2017).

In Germany the KHVD was declared as a notifiable disease in December 2005 already. In the EU directive 2006/88/EG the disease is listed in appendix IV as a non-exotic disease of fish. Also, minimum eradication measures which have to be implemented in case of an official confirmation of the disease are mentioned.

This directive was transposed into national law as the “Fischseuchenverordnung” (FischSeuchV) which translated means “Fish-Epidemics-Regulation”. It came into force on

24th November 2008. Additionally to that the commission implementing decision (EU) 2015/1554 is regulating the requirements of methods regarding monitoring and diagnosis of the disease (Friedrich-Loeffler-Institut 2016). Basis of this law is the duty of acquiring a registration and approval of each fish farm before business. After approval of the farm, it is placed into a category regarding the risk of an occurring diseases. The categories reach from I-V and are defined in the following lines.

- Category I: officially disease free
- Category II: no cases of KHV and a monitoring program is applied
- Category III: no cases of KHVD are reported without a monitoring program
- Category IV: cases of KHV are known and an approved eradication program is implemented
- Category V: cases of KHV are known but only the minimum measures are applied

According to the annual report of 2017 published by the Friedrich-Loeffler institute giving an overview of the epidemiological situation of notifiable diseases in Germany only 9 KHV-free fish farms belong to Category I and none to Category 2. The majority was placed into Category III. No farms were Category IV and minimum 35 carp producing facilities fell under Category V.

This categorization is mainly used to determine the frequency of controls which are needed and to organize the movement of alive fish.

This means that fishes kept in a farm with certain Category are only allowed to be introduced to enterprises with the same or higher category number. Additionally, enterprises belonging to Category IV and II are only allowed to purchase fish from Category I fish farms.

In comparison to the prohibition of the use of vaccines against KHV in enterprises of Category I and II, vaccination may be used in Category III, IV and V fish farms (Friedrich-Loeffler-Institut 2018).

3.6 Measures in case of suspicion and confirmation of KHV in Germany:

There is a suspicion of an outbreak of KHV if increased mortalities with unclear reason can be observed. Any suspicion of KHVD has to be notified immediately to the responsible authority according to the upper mentioned national law (§ 7 fish-epidemic-regulation).

Laboratory tests for confirmation or rule out of the presence of KHV have to be ordered.

According to §23 the official authorities have to carry out following epidemiological investigations in case of an outbreak or suspicion of an outbreak on fish farms. This includes:

- The possible time frame, in which the causative agent could be present in the enterprise in question prior the notification of suspicion
- The possible source of introduction of the disease
- Determination of other enterprises which received or delivered fishes from or to the enterprise suspicious of KHVD

Additionally to that the enterprise in question and the enterprises with possible epidemiological connection have to be put under official surveillance. Regarding these fish farms, strict movement restrictions for fishes are applied. Only if the official authority approves it, healthy fishes without suspicion of having KHVD, may be slaughtered or transported to other enterprises affected by KHVD. The produced offal of the above slaughtered fish has to be disposed properly.

Diseased fishes or fishes suspicious to KHVD have to be killed immediately.

Dead or killed fish is only allowed to be removed for diagnostic purpose or for proper disposal.

If required, the authorities can order additionally following control measures:

- Movement restrictions of the personal on the fish farm
- Disinfection of shoes of persons, vehicles, tanks and instruments leaving or entering the enterprise in question

Is KHVD officially confirmed, additionally a restriction zone and a surveillance zone are established. Sizes of these zones are chosen according to the transmissibility of KHVD and the geographical conditions (especially to the possible water sources).

The within the restriction zone located fish farms have to be investigated for KHV. All of them are also put under official surveillance. Fishes from a in the restricted zone located enterprise are only allowed to be moved if officially authorized.

In the surveillance zone, an area outside the restriction zone established for prevention of the spread of KHV, additionally investigations can be ordered as well. The upper mentioned measures can be canceled if the presence of the disease can be ruled out.

This can be either due to official rule out of the presence of the disease in case of suspicion, or if the measures indicate the successful elimination of the agent. The agent is absent, if all fishes from the enterprise are dead or killed and removed and thorough cleaning and disinfection has been carried out.

Restriction and surveillance zone can be lifted if the in the zones carried out investigations are negative. Anyone who is acting intentionally or due to negligent against the implemented measures is guilty a misdemeanor (Bundesministerium der Justiz und für Verbraucherschutz 2008).

3.7 State compensations:

In case of an outbreak/ confirmation of a notifiable disease on a fish farm, financial support is possible and is regulated by the TierGesG (Tiergesundheitsgesetz =“Animal-Health-Law”). Section1, §1 defines, that financial support is only applied for fishes, which are kept for agricultural production, with the aim to prevent and eradicate diseases.

Section 6, 15§ regulates, when exactly the in §16 mentioned 20 € compensation per kg lost live fish takes place. This state compensations are paid only, if the farmer acted according to the rules. When state compensations do not or only partially cover the losses due to killing of the animals or cover losses due to unrealized profits, is mentioned in §17 (exclusion from state compensation) and §18 (omission from state compensations) (Bundesministerium der Justiz und für Verbraucherschutz 2013).

4. Material and methods:

The severe losses caused by an outbreak of KHVD and the global distribution are the reasons, why the disease till today is a big concern for the fish industry and the official authorities. In Germany, beside strict control of movement and laboratory investigation of ornamental and cultivated carps, several outbreaks can be observed yearly.

The epidemiological data regarding KHV is provided by an annual report published by the Friedrich-Loeffler-Institute. By reading through each annual report between 2007 and 2017, collecting the important data, and creating different graphs for visualization, I was able to write following lines regarding the epidemiological situation of KHV in Germany and Bavaria.

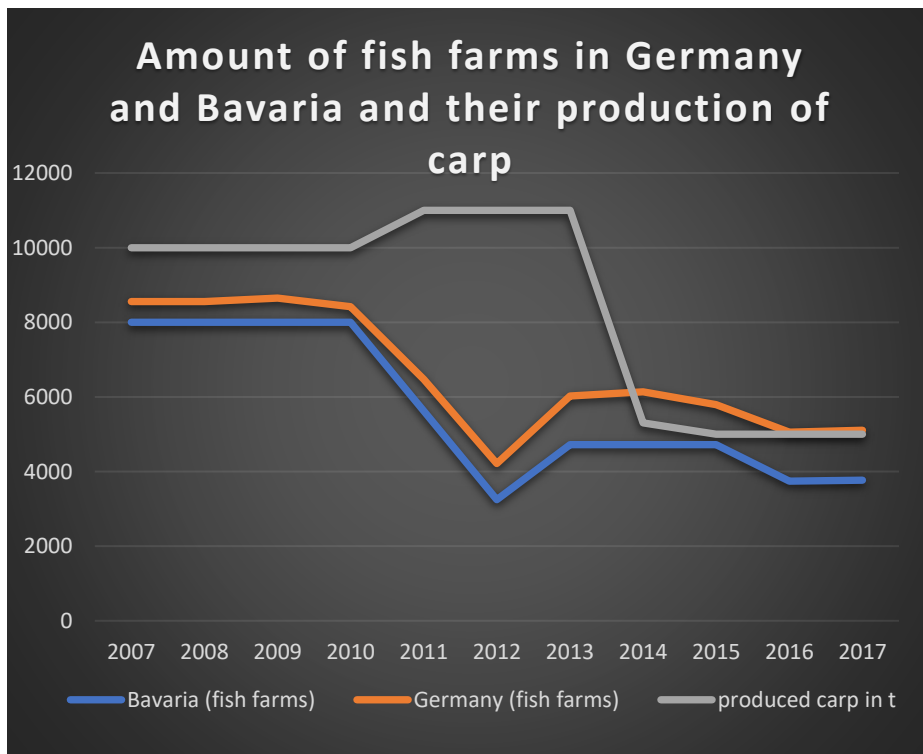
5. Results

5.1 Statistics from 2007 to 2017 in Germany:

The following statistics are collected by the Friedrich-Loeffler Institute in collaboration with the diagnostic center of each state of the Federal Republic Germany. One outbreak equals an official confirmation of KHVD on one fish farm.

Bavaria among the 16 states of the federal republic of Germany has the greatest amount of fish farms. Even the strong decrease of fish farms from 2010 to 2012 in Bavaria and in whole Germany didn't change the fact, that Bavaria is the most important state regarding cultivation of carp (Graph 1). This is the reason why I want to focus on the epidemiological situation of KHVD in Bavaria in comparison to the state Saxony and whole Germany.

Also in Graph 1 we can see the annual carp production of Germany in tons. With delay together with the decrease of the fish farms the annual production of cultivated carps decreased to approximately the half within a few years.

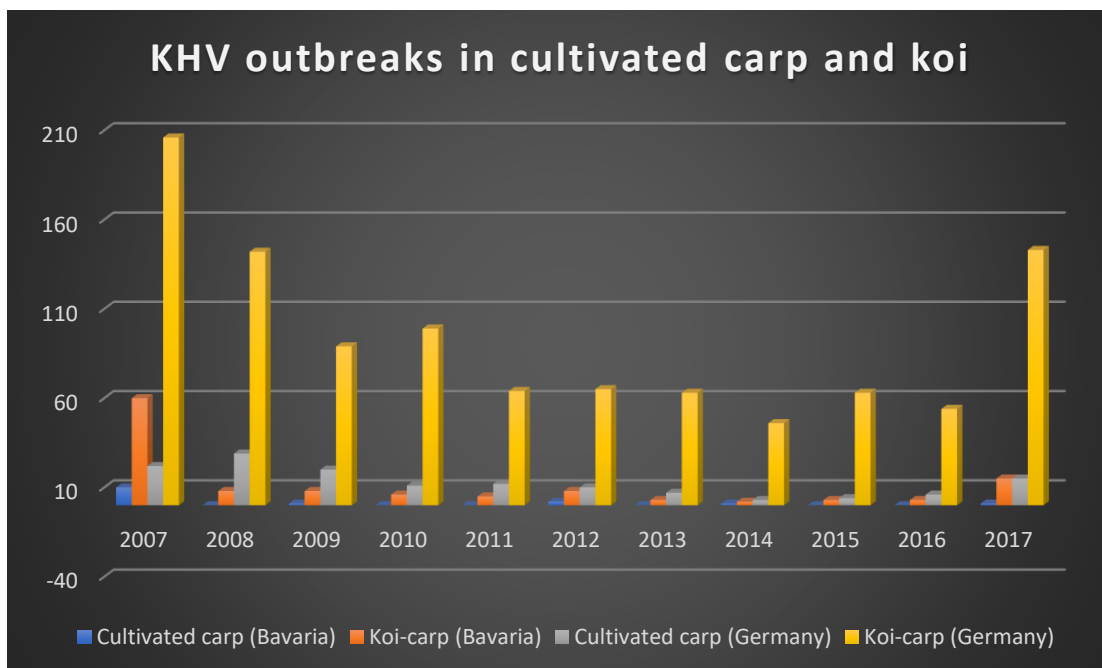


Graph 1 Amount of enterprises cultivating carp in Bavaria and Germany and the national annual production in tons

Year	Bavaria	Germany	produced carp in t
2007	8000	8555	10000
2008	8000	8555	10000
2009	8000	8648	10000
2010	8000	8414	10000
2011	5625	6473	11000
2012	3245	4214	11000
2013	4723	6021	11000
2014	4723	6136	5300
2015	4720	5791	5000
2016	3743	5053	5000
2017	3768	5106	5000

Table 3 Numbers written, as shown in Graph 1

When focusing on the statistics regarding KHV outbreaks in Germany and Bavaria, we have to keep in mind, that outbreaks are reported from cultivated carp and koi carps separately.



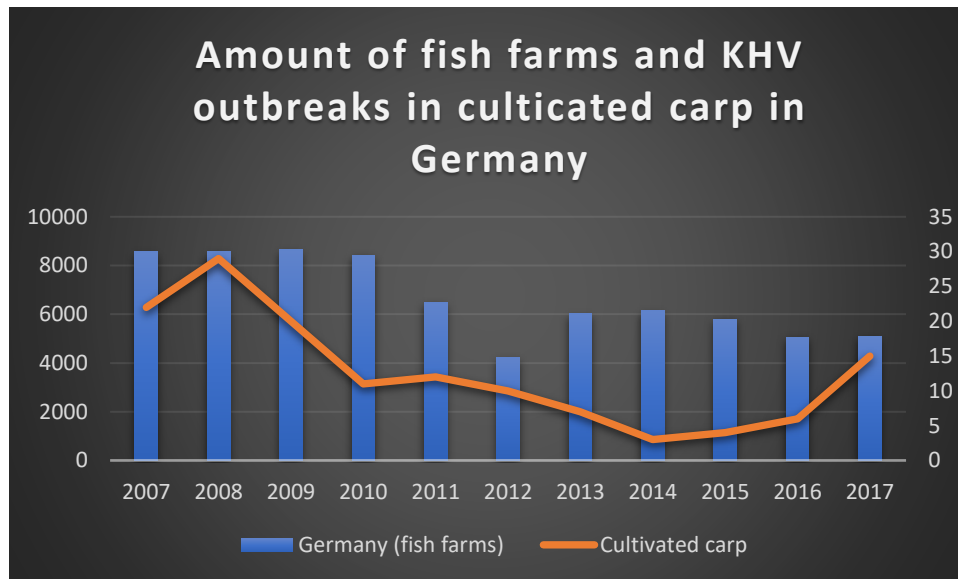
Graph 2 KHV outbreaks in Germany and Bavaria regarding cultivated carp and koi

In an epidemiological point of view, it is important to separate these 2 groups as the disease usually occurs in koi carps due to the trade and transport of latent infected fish (pet shops etc.). This means that these outbreaks have no influence on the epidemiological situation of KHV in the territory in question. More with this statistic it is visible, how well the ornamental fishes are tested and whether latent infected fish is consequently detected. Except 2017 we can observe a steady decrease of reported KHV cases in koi carps, both in Germany and Bavaria. This could be the consequence of the increasing sensitivity of the test methods over the years and the frequent testing of the fish, leading to a stamping out of latent infected fishes picturing a potential source of infection. A critical point of this interpretation is, that also a decrease of the trade and transport of koi could be an explanation for this trend.

year	Cultivated carp (Bavaria)	Koi-carp (Bavaria)	Cultivated carp (Germany)	Koi-carp (Germany)
2007	10	60	22	206
2008	0	8	29	142
2009	1	8	20	89
2010	0	6	11	99
2011	0	5	12	64
2012	2	8	10	65
2013	0	3	7	63
2014	1	2	3	46
2015	0	3	4	63
2016	0	3	6	54
2017	1	15	15	143

Table 4 Numbers written as presented in Graph 2

If we focus on the statistics regarding KHV in cultivated carp, we can see in Graph 3 that with the decrease of the enterprises producing carp also a decrease of the KHV outbreaks is visible.



Graph 3 KHV outbreaks in Germany's fish farms cultivating carp

Year	Cultivated carp	Fish farms in Germany
2007	22	8555
2008	29	8555
2009	20	8648
2010	11	8414
2011	12	6473
2012	10	4214
2013	7	6021
2014	3	6136
2015	4	5791
2016	6	5053
2017	15	5106

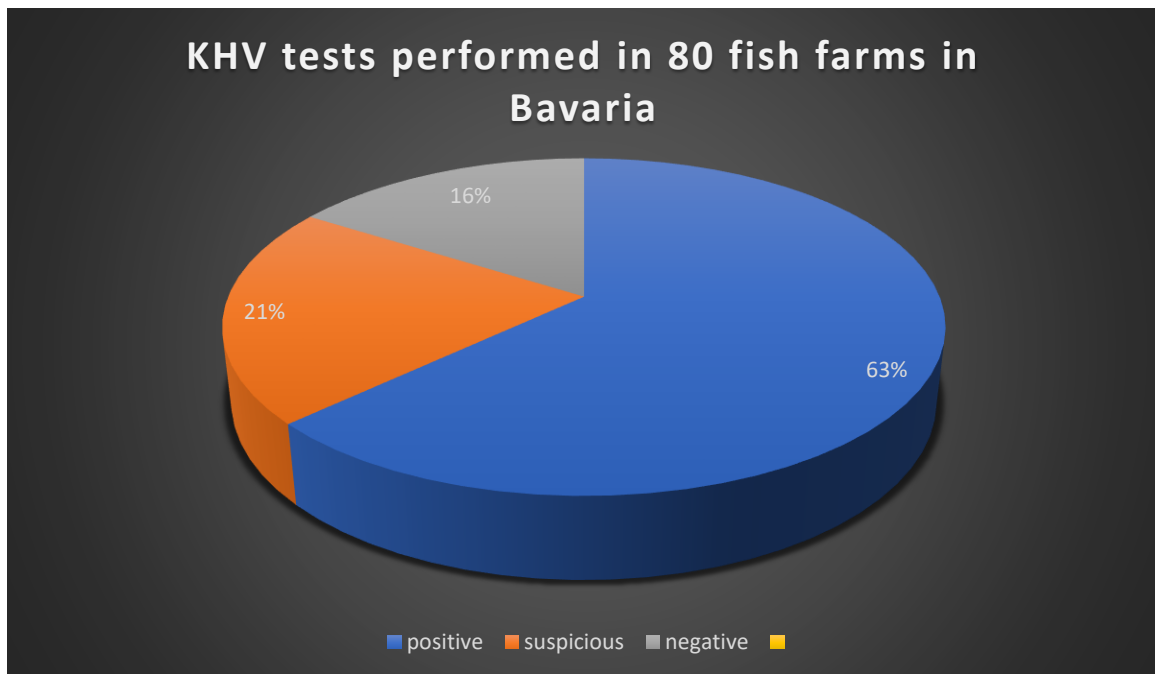
Table 5 Numbers for Graph 3

This Graph indicates, that the decrease of KHV outbreaks in the last decade is mostly influenced by the decreasing amount of fish farms and consequently the decreased production of carp accompanied by a decreased movement, trade and breeding. It does not

necessary show, whether the frequent testing and epidemiological measures for the prevention and eradication of KHV in Germany are successful or not.

Interesting is also, that all in all we can see, that KHV outbreaks in cultivated carp as well as in koi originate only in a low percentage from the Bavarian state.

As Bavaria is the state producing the highest amount of carps in Germany, the low numbers of KHV outbreaks are an interesting finding. This is especially interesting, as field studies in Bavaria showed a high infection rate of the mainly symptomless fish populations (Graph 4).

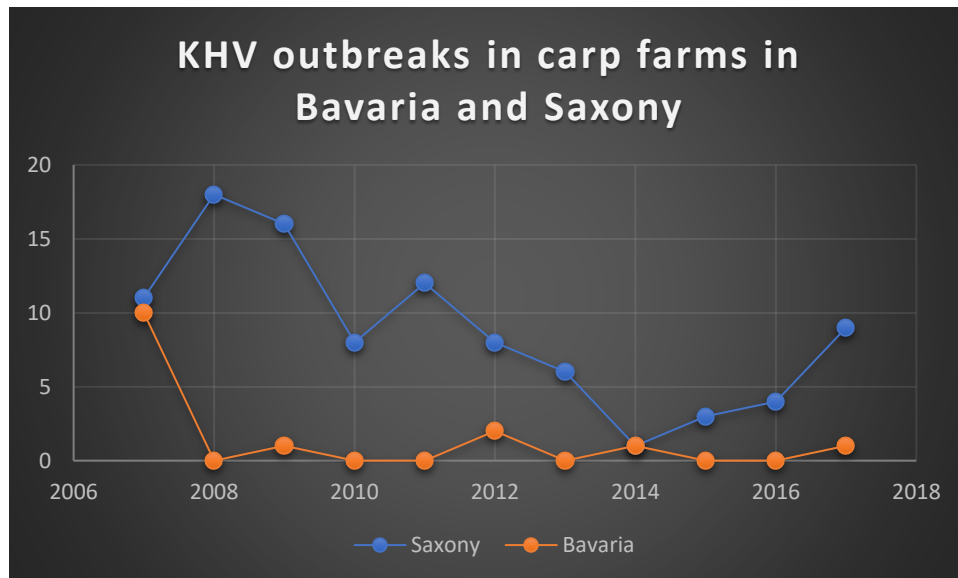


Graph 4: KHV monitoring of 80 carp farms in Bavaria (Wißmath 2019)

In comparison the state Saxony, which is producing a high amount of carp as well, is recording a much higher number of KHV cases in its fish farms cultivating carp (Graph 5). As the legal framework and measures for disease prevention and eradication is the same for all sates in Germany other fundamental factors have to be the reason for this severe difference.

Probably several factors like structural differences, different farming methods and farm managing can explain the strong variation of the epidemiological situation of KHV in

the 16 states of Germany. For example, the water supply, purchase or breeding of the new population or the disinfection frequency of fish farms can be mentioned. Any of these factors probably can explain, why especially in Bavaria such low numbers of outbreaks are confirmed yearly.



Graph 5 KHV outbreaks in Saxony and Bavaria

Year	Saxony	Bavaria
2007	11	10
2008	18	0
2009	16	1
2010	8	0
2011	12	0
2012	8	2
2013	6	0
2014	1	1
2015	3	0
2016	4	0
2017	9	1

Table 6 Number of KHV outbreaks in carp farms in Bavaria and Saxony

6. Discussion:

As already mentioned, statistics regarding KHVD outbreaks in ornamental carp in Germany cannot be interpreted regarding the geographic epidemiological situation. It shows only how well the measures regarding the trade of alive fish are working. As the number of notified KHVD cases in ornamental carp decreased over the years and stayed on a low level (except 2017), the measures seem to be effective.

Regarding the fish industry in Germany we can observe a decrease of the amount of fish farms cultivating carp followed by a decreased production. As running such an enterprise requires a lot of energy, time and working power many quit, as a proper work-life balance cannot be met. Especially these ones, who produced carp as a hobby or for a second income are affected. Also the increasing strictness of measures and guidelines which have to be met regarding epidemics, natural protection & preservation and consumer safety etc. maybe made several people decide to stop their business. The strict regulations and the fact that the virus is so widespread, drives many enterprises into ruin as sooner or later there will be a positive test result during the screening programs. The banning of movement and possible stamping out policies in case of positive tested but often not diseased fishes cause tremendous financial losses.

All in all, as a consequence the annual carp production decreased to approximately the half. Whether this is affecting the epidemiological situation regarding KHV is questionable. With decreasing amount of fish farms the trade of alive fish between the enterprises decreases as well combined with larger distances for transportation. In an epidemiological point of view its important to note, that with the decreasing amount of breeding facilities also more and more fish fattening farms will purchase young fish from the same origin. Due to this created network, the consequences and measures in case of one outbreak of KHVD have to be implemented for a higher number of enterprises.

Additionally it has to be mentioned, that the decreased production together with the fish demand of Germanys population consequently leads to an import of already slaughtered and alive fish.

As the statistics show a decrease of KHV outbreaks over the years, a success of the regulation regarding control and eradication of KHVD seem reasonable. This might be a

false interpretation as parallelly the amount of carp producing enterprises decreased over the years. Except 2016 and 2017 where an increased number of outbreaks were notified.

Interesting is when this whole process is compared to the fact, that a high percentage of the fish populations are tested positive regarding KHV, but no outbreaks can be observed. This highly questions the effectivity of the legal regulations over the last years but also the need of it. It seems, that diseases of aquatic animals cannot be controlled as easy as diseases of land-living animals especially if the use of vaccination is not allowed or even available. The fact that ponds are not like stables, which can be isolated quite easily, makes the use of stamping-out methods highly questionable. Also the water supply and the following possibility of contact with infected feral fish has to be taken into consideration. In case of ponds supplied by water from rivers it could be interesting to estimate the infection rate of feral living fish and consequently the risk of introduction of KHV via water.

Most important is also that by the regular monitoring program latent infected carrier fishes may be not detected. This means that even in official free populations, carrier fish can be present and picture a permanent unknown potential for transmission.

If we consider the instability of the virus, also an increased awareness of the importance of good hygienic practice could have led to the decreased number of outbreaks. Still the idea of producing islands of sterile pathogen free holdings is illusory as introduction from surrounding waters with high or unknown infection rate is most likely. Preventing this would require a non-justifiable killing of all infected fish but also the destruction of other water organisms in the surrounding lakes and rivers.

The with the help of the FLI created statistics show, that all in all the amount of outbreaks are on a relatively low level in Germany. This means, that with the epidemiological measures and good hygienic practice only low losses are caused by KHVD nowadays.

Nevertheless, the increasement of KHVD outbreaks in 2017 in both ornamental and cultivated carp can be worrying. If there is an epidemiological connection behind it only future statistics will show.

7. Summary:

Till today KHV outbreaks with tremendous losses are recorded all over the world every year. Due to the trade of carrier fish the disease could spread to a global extend and is till today one of the most important infectious diseases of carps.

As the diagnostic identification of latent infected fish till today is not reliable enough and the fact that often fish populations are already infected with the virus but don't show clinical signs, the main aim should not be the eradication of the disease by stamping out.

Instead a prevention of clinical manifestation and the reduction of the losses shall be in the foreground in case of infected populations, and the prevention of infection in case of KHV free populations. Good keeping conditions and hygienic management shall be mentioned as key points.

Regarding ornamental carp, with global trade and transport of alive and often highly valuable fish, the main focus should be put on keeping and trading of KHV free fishes. To realize that, frequent and conscientious testing together with strict regulations and surveillance regarding the global shipment of KHV susceptible fishes are needed.

Maybe soon safe, easily applicable and effective vaccinations are invented, and the tremendous problems regarding the prevention and eradication of KHV are solved. Hopefully further research, with new knowledge about the virus and the disease will contribute to the mentioned problematic as well.

As due to failure to prevent the spread of the disease It could be possible that like in case of Eel-Herpes-Virus after severe losses an immunized and healthy rest population remains.

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9. Acknowledgements

My high appreciation shall be expressed to all authors, researchers and institutes for their scientific work and publications. Especially Dr. Nelly Scuda from the LGL (“Bayrisches Landesamt für Gesundheit und Lebensmittelsicherheit”) who helped me with my research and understanding regarding Koi Herpes Virus.

Especially I have to express my gratitude to Dr. Baska Ferenc, who gave me as my supervisor patient support and advices.

As an end I want to thank my family for their love and support. Without them my studies at the University of Veterinary Medicine in Budapest wouldn't have been possible.