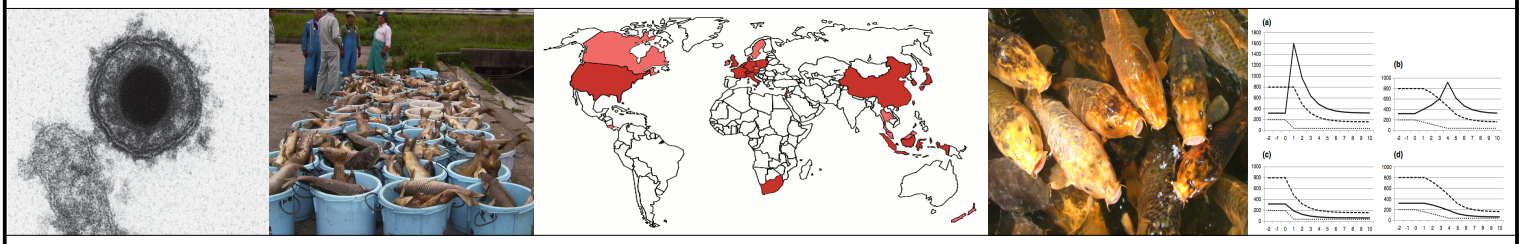
Westfälische Wilhelms-Universität Münster

Emergence of Viral Diseases: Ecology and Evolution of Koi Herpes Virus



International Symposium

July 04 (Mon.) - 05 (Tue.), 2011

Venue: Institute for Evolution and Biodiversity
Hüfferstr. 1,
48149 Münster, Germany

No attendance fee - registration is appreciated

Schedule:			
	Session 1: KHV distribution in wildlife populations	July 04	15:00 – 18:00
	Session 2: Stress, immunocompetence & disease	July 05	09:00 – 12:00
	Session 3: Linkage of environment, pathogen and human	July 05	13:30 – 18:30

Speakers: Zen'ichiro Kawabata (Kyoto), Toshifumi Minamoto (Kyoto), Gisep Rauch (Münster), Mikolaj Adamek (Hannover), Dieter Steinhagen (Hannover), Arndt Telschow (Münster), Kimiko Uchii (Tokyo)

Organizer: Arndt Telschow, Institute for Evolution and Biodiversity, Münster
Email: KHV.symposium@wwu.de
URL: http://ieb.uni-muenster.de/genevol/events.html

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Foreword

The emergence of infectious diseases has increased over the past decades and is now viewed as one of the greatest threats to biodiversity. The Institute for Evolution and Biodiversity is therefore delighted to host the International Symposium *Emergence of Viral Diseases: Ecology and Evolution of Koi Herpes Virus*.

The focus of the symposium is on one of the most devastating examples for emerging diseases, Koi Herpes Virus (KHV) also known as Cyprinid Herpes Virus 3 (CyHV-3). KHV is an infectious agent in common carp (*Cyprinus carpio L.*) and its ornamental strain koi (*Cyprinus carpio koi*). Despite thousands years of carp aquaculture by humans, the first known KHV outbreak occurred at a koi farm in England in 1996. Since then, KHV had spread to fish farms around the world by the early 2000s, causing mass mortality of cultivated carp. After KHV was introduced to Japan in 2003, it spilled over to wild populations of common carp with the largest outbreak in the wild happening in spring 2004 in Lake Biwa with 70% of the wild carp population (> 100.000) dying within a few weeks. Although no additional major outbreak has occurred in Lake Biwa, cases of death due to KHV are reported every year.

The symposium brings together researchers from Europe and Japan working on KHV. The variety of talks ranges from studies on disease pathology and vaccination over KHV infection dynamics to viral transmission in the wild. Among the speakers are fish veterinarians, microbial ecologists and evolutionary biologist. We hope that these different expertises result in many fruitful discussion that will lead to a better understanding not only of KHV and the causes for its outbreaks but also of other emergent diseases. We welcome the speakers and participants in Münster and wish an interesting symposium.

Münster, June 2011
Arndt Telschow

Time schedule

Monday, July 4th

$15^{\underline{00}} - 16^{\underline{00}}$	Welcome and registration	
$16^{\underline{00}} - 17^{\underline{00}}$	**Toshifumi Minamoto**	KHV dynamics in natural freshwater environments
$17^{\underline{00}} - 17^{\underline{45}}$	**Gisep Rauch**	An RNAi-therapy against KHV in carp
$17^{\underline{45}} - 18^{\underline{30}}$	**Arndt Telschow**	KHV as model for emerging disease: a mathematical modeling perspective
$19^{\underline{00}}$	*Dinner*	

Tuesday morning, July 5th

$09^{\underline{00}} - 10^{\underline{00}}$	*Coffee*	
$10^{\underline{00}} - 11^{\underline{00}}$	**Kimiko Uchii**	Transmission dynamics of KHV and its impact on the genetic structure of the host population
$11^{\underline{00}} - 12^{\underline{00}}$	**Mikolaj Adamek**	Susceptibility of carp to CyHV-3: Is it influenced by host genetics and/or pond environment?
$12^{\underline{00}} - 13^{\underline{30}}$	*Lunch*	

Tuesday afternoon, July 5th

$13^{\underline{30}} - 14^{\underline{30}}$	**Dieter Steinhagen**	Why do carp get sick from an infection with KHV? A pathophysiological study
$14^{\underline{30}} - 15^{\underline{30}}$	**Sven Bergmann**	Investigation on early pathogenesis of KHVD in carp (Cyprinus carpio L.)
$15^{\underline{30}} - 16^{\underline{00}}$	*Coffee*	
$16^{\underline{00}} - 17^{\underline{00}}$	**Zen'ichiro Kawabata**	Linkage of environmental alteration, pathogen and human.
$17^{\underline{00}} - 18^{\underline{30}}$	Discussion	
$19^{\underline{00}}$	*Dinner*	

Monday, $16^{\underline{00}}$

KHV dynamics in natural freshwater environments

Toshifumi Minamoto
Research Institute for Humanity and Nature, Kyoto, Japan
Based on joint collaborations with: M. Honjo, H. Yamanaka K. Uchii, and Z. Kawabata.

A newly recognized fish disease caused by cyprinid herpesvirus-3 (KHV; also known as cyprinid herpesvirus-3 or CyHV-3) has spread worldwide and is a large threat for carp and koi cultivators and for freshwater ecosystems. However, little is known about KHV dynamics in natural environments. To investigate where / how much the KHV exists in natural environments, we established the quantification method of KHV in environmental waters. To elucidate the situation of KHV contamination in Japan, a nationwide survey of all national class-A rivers was conducted, and 4-years monitoring in a natural lake (Lake Biwa, Japan) and river (Yura River, Japan) was performed. The virus in environmental water was concentrated by the cation-coated filter method and virus quantification was carried out by realtime PCR. Resultantly more than 90% of rivers in Japan are contaminated with KHV, although only 5 years have passed since its initial detection. Our results indicate that virus invasion does not consistently cause an outbreak and that several environmental factors may be involved in its occurrence. The patterns of seasonal dynamics of KHV differed between lake and river, possibly affected by the different patterns of temperature fluctuation. Our study demonstrates that virus detection using molecular biology techniques is a powerful tool for monitoring the pathogenic virus in natural environments.

Monday, $17^{\underline{00}}$

An RNAi-therapy against KHV in carp

Gisep Rauch
Institute for Evolution and Biodiversity WWU, Münster, Germany
Based on joint collaborations with: M. Adamek, J. Scharsack, A. Telschow and D. Steinhagen.

Common carp is a key source for human food production. Koi-herpes virus (KHV known also as Cyprinid herpesvirus 3 or CyHV-3) is thereby one of the most devastating diseases in common carp and koi aquaculture. Contemporary, there is no treatment against KHV to cure diseased fish in a fast and effective way. However, several groups work on the development of a vaccine against KHV. Vaccines have the important disadvantage that they rely on the up-regulation of the immune system, which may take several weeks in fish and the effect of the vaccination will come too late in an acute outbreak. In this study we used for the first time the RNA interference technology (RNAi) to successfully treat a KHV-infection in cell cultures in a fast and effective way (patent application submitted). At the moment we are testing our RNAi- therapy in KHV-infected carp. Our RNAi-therapy represents a promising approach to deliver for the first time a tool able to eradicate KHV during an acute outbreak.

Monday, $17^{\underline{45}}$

Koi herpes virus as model for emerging disease: a mathematical modeling perspective

Arndt Telschow
Institute for Evolution and Biodiversity WWU, Münster, Germany
Based on joint collaborations with: N. Yamamura, K. Uchii, T. Minamoto, M. Honjo, S. Metelmann, B. Wieczorek and Z. Kawabata.

The emergence of infectious diseases has increased over the past few decades and is now viewed as one of the greatest threats to biodiversity. However, little is known about the transmission routes and infection dynamics in the wild, making it difficult to predict their emergence and develop effective countermeasures against outbreaks. To gain a better understanding of the basic properties of emerging diseases in the wild, a combination field studies and mathematical modeling are of major importance. We present results from theoretical studies with the focus on the highly virulent cyprinid herpesvirus 3 (CyHV-3) which emerged less than two decades ago and now causes mass mortality in common carp populations worldwide. In the first part, we present a basic equation for the population dynamics of animals which migrate to local habitats for reproduction. Examples are ubiquitous among crabs, freshwater fishes, amphibians, migratory birds, and sea animals. Using this equation, we will discuss the vulnerability of populations to epidemic diseases due to temporal local high densities with decreasing breeding habitats by human activities, exemplifying an outbreak of cyprinid herpesvirus 3 for wild carps in Lake Biwa (Japan). In the second part, we present a simple mathematical model which describes the CyHV-3 dynamics in freshwater ecosystems during and after an outbreak.

Tuesday, $10^{\underline{00}}$

Transmission dynamics of koi herpesvirus disease and its impact on the genetic structure of the host population

Kimiko Uchii
The University of Tokyo, Tokyo, Japan

Based on joint collaborations with: A. Telschow, N. Okuda, T. Minamoto, H. Yamanaka, M.N. Honjo, K. Matsui and Z. Kawabata.

Emerging infectious diseases are major threats to wildlife populations. To enhance our understanding of the dynamics of these diseases, we investigated how host reproductive behavior and seasonal temperature variation drive transmission of infections among wild hosts, using the model system of koi herpesvirus (KHV) disease in a wild common carp population of Lake Biwa, Japan. Our main findings were: (1) a seroprevalence survey showed that KHV infection occurred mostly in adult hosts, (2) a quantitative assay for KHV in the host population demonstrated that KHV was most abundant in the spring when host reproduction occurred and water temperature increased simultaneously, and (3) an analysis of the dynamics of KHV in water revealed that the KHV concentration increased markedly in breeding habitats during host group mating. These results indicate that breeding habitats can become hot spots for transmission of infectious diseases if hosts aggregate for mating and the activation of pathogens occurs during the host breeding season. We also investigated how the genetic structure of the common carp population in Lake Biwa was affected by the outbreak of KHV. We found that: (1) the KHV outbreak would have reduced the occurrence of mitochondrial DNA haplotypes that are derived from Japanese lineages, and (2) genetic introgression of introduced Eurasian strains into Japanese native strains increased after the KHV outbreak. These results suggest that Japanese native common carp are facing the greatest threat of extinction by the combined effect of biological invasions and emerging infectious diseases.

Tuesday, $11^{\underline{00}}$

Susceptibility of carp to CyHV-3: Is it influenced by host genetics and/or pond environment?

Mikolaj Adamek
Insitut for Aquaculture, Polsih Academy of Sciences, Golyz, Polen
Based on joint collaborations with: K.L. Rakus, D. Steinhagen and I. Irnazarow.

Within the last 15 years mass mortality of common carp, which have a viral aetiology, has been observed in numerous countries worldwide. The disease-causing virus has been identified as Cyprinid herpesvirus 3 (CyHV-3). Since CyHV-3 was described it became one of the fastest spreading viruses in aquaculture and currently is present in many common carp populations. Resistance to CyHV-3 is genetically determined but the interaction of the pathogen and the environment has not been taken in to account. The disease itself is an interaction between the host, pathogen and the environment. Infection experiments are designed in order to limit the environmental factor of the disease as much as possible but what happens when the "resistant" individuals will be challenged in the natural environment?
We created an artificial population of common carp containing 12 diallelic F1 crossbred groups originating from 4 breeding lines and introduced them into two different carp farms (in one CyHV-3 outbreaks occur annually, the second is CyHV-3 free). The third group was kept in Specific Pathogen Free conditions. After one production season surviving fish were screened for polymorphisms in blood serum proteins by means of polyacrylamide gel electrophoresis. A PCR based diagnostic CyHV-3 test was also performed followed by sequencing of PCR products. Furthermore we conducted series of experimental trials in order to estimate how high the genetic factor of resistance is and what the survival rates are of the fish after challenge in controlled conditions.

The comparison of genotype frequencies was performed between predicted F1 generation populations introduced into the three different environments; SPF, CyHV-3 negative and CyHv-3 positive. The virus positive environment caused a shift in frequencies of TF and ES genotypes. The results from laboratory experimental trials correlated with the environmental samples with the most resistant cross carrying the genotype with the highest change in frequency between CyHV-3 positive and negative environment.
The results in these studies indicate that the selection of the most resistant line shown in the experimental trials can be useful in the reduction of mortalities in culture ponds. Furthermore these studies also suggest that the genetically divergent common carp population can be changed by the CyHV-3 outbreak. The genetic approach can be also useful in environmental studies. The introduction of a highly virulent pathogen to the environment leads to a shift in genetic diversity of the affected population. This mechanism is based on a genetically associated tolerance or resistance to the pathogen. Looking at the changes in the genetic diversity of the population can also lead to determination of resistant individuals which can be used in aquaculture.

Tuesday, $13^{\underline{30}}$

Why do carp get sick from an infection with Koi Herpesvirus? A pathophysiological study

Dieter Steinhagen
Univ. of Veterinary Medicine, Centre for Infection Medicine, Hannover, Germany
Based on joint collaborations with: J. Negenborn, and M. Ganter.

In carp, an infection with Koi Herpesvirus often is associated with severe alterations in skin, gills and kidney and may lead to morbidity and mortality. In the gill tissue, changes such as hyperplasia, hypertrophy and necrosis of epithelial cells, fusion of secondary lamellae up to a complete destruction of the structure of secondary lamellae occurs in infected carp. In the kidney of these carp a progressive interstitial nephritis associated with a degeneration of the renal tubule epithelium was seen.
For a study on pathophysiologial effects of these alterations to gills and kidney, juvenile carp were infected with cell culture derived KHV and serum and urine samples were collected at different time intervals after infection. In these body fluids, the osmolarity, electrolyte concentrations, and the level of the enzymes alkaline phosphatase and gamma glutamyl transferase were determined.
First signs of infection in the form of depressed swimming behaviour were already seen 2 days after infection. At the same time changes haematology and blood chemistry could be observed as well. In particular osmolarity and the sodium concentration was altered in serum and urine of infected carp throughout the observation period. Later during the course of infection, reduced protein concentrations were also measured in the plasma of carp. The results of these experiments suggest that in carp under KHV infection, a renal efflux of sodium occurs, which is associated with progressing deterioration of the renal tissue. Our data suggest that carp under KHV infection suffer from a challenge of their electrolyte balance.

Investigation on early pathogenesis of KHVD in carp (Cyprinus carpio L.)

Sven M. Bergmann
Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Insel Riems, Germany
Based on joint collaborations with: S. Monaghan, K.D. Thompson, A. Adams and D. Fichtner.

SPF Carp (Cyprinus carpio L.), free from koi herpesvirus (KHV, CyHV-3) and carp pox virus (CyHV-1), were infected with KHV, isolate KHV-E (D 182, provided by Dr. Keith Way, CEFAS, UK), by immersion with 103 TCID50 / ml for 1 hour at 20[U+FFFD]C. After incubation, carp were divided into two groups. Carp were checked twice a day for KHVD. Samples were collected lethally (skin, gill, spleen, kidney, gut, liver and brain) and non-lethally (gill, fins and skin swabs, blood for leukocytes separation) from both groups 1 to 8 hours post infection. From the 1st dpi, samples were collected from both groups until the 4th dpi, where 100% mortality occurred in group 1. Then only group 2 was sampled further on. Mortality started in group 2 from 7th dpi.
KHV copy number quantification was carried out by real-time PCR, modified according to Gilad et al., 2004 and Bergmann et al., 2010. In both cases of KHVD development, the virus was found to be at very high levels of infection to the skin and gill mucus in the first two hours. Up to 4 hours p.i. a massive decrease of viral DNA took part on those superficial organs. There was obviously no replication in these tissues as KHV mRNA was not detected by RT-PCR. Internally KHV replication took place first in gut tissue, then in leukocytes and was brought via blood stream into all other organs. An enrichment of viral DNA was detected in spleen but with an explosive replication in kidney tissue at this stage of infection. During acute KHVD development, a 10,000 times higher viral DNA load was observed from 4th dpi followed by an enormous increase of KHV DNA in all organ tissues, swabs and leukocytes preparations.

Tuesday, $16^{\underline{00}}$

Linkage of environmental alteration, pathogen and human.

Zen'ichiro Kawabata
Research Institute for Humanity and Nature, Kyoto, Japan
Based on joint collaborations with: T. Minamoto, M. Honjo, K. Uchii, H. Yamanaka, A. Suzuki, Y. Kohmatsu, K. Asano, Y. Shirae, T. Itayama, T. Ichijo, K. Omori, N. Okuda, M. Kakehashi, M. Nasu, K. Matsui, M. Matsuoka, H. Kong, T. Takahara, D. Wu, R. Yonekura.

The rapid spread of infectious diseases is threatening humans, wildlife, and livestock worldwide. There is an important environmental component to infectious diseases. While pathological studies inform effective disease treatment, the study of disease ecology - the interactions between environment, pathogen, host and humans that may create or alleviate fertile disease environments - is necessary for prediction and prevention of disease outbreaks.
Koi herpes virus (KHV, denoting CyHV-3) is a pathogen responsible for episodic mass mortality of common carp (Cyprinus carpio carpio) since the late 1990s. Our RIHN project is describing the links between lakeshore, modified by humans, KHV, common carp, and humans to make a conceptual linkage model which is applicable to other infectious diseases. I will introduce main findings which have been done by the members of Environmental Disease Project of the RIHN and integrate them to construct a linkage. Based on this linkage, I would like to discuss how we could reduce unfavorable affects of diseases on life.
Further information on the project of Research Institute for Humanity and Nature (Kyoto, Japan) can be found at the following website: http://www.chikyu.ac.jp/rihn_e/project/C06.html.

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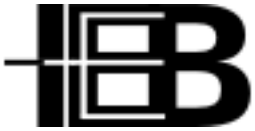

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