



UNIVERSITY OF VETERINARY SCIENCES BRNO



European Association of Fish Pathologists

**THE 1ST MEETING OF THE CENTRAL AND EASTERN EUROPEAN
EAFP BRANCHES**

4th to 6th December 2024 Křtiny, Czech Republic



ABSTRACT BOOK

Mendel University in Brno
University of Veterinary Sciences Brno

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Editor: Miroslava Palíková et al.



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Keynote: AN OVERVIEW OF BACTERIAL DISEASES OF FISH SPECIES, CULTURED IN EUROPE

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In this keynote, the most important bacterial diseases of fish cultured in Europe are shown, with some info on the pathogen, host range, environmental factors, geography, clinics, diagnostics, management, zoonotic potential and more. The information presented is based on over 38 years of fish disease laboratory experience of the author at Wageningen Bioveterinary Research of WUR, and on literature, including the upcoming FAO publication (lead by Dr. M. Reantaso of the Fisheries and Aquaculture Division (NFI) of the FAO) “*Responsible management of bacterial disease in aquaculture*”, to which a worldwide group of authors contributes, including the author of this abstract (see below). The bacterial fish diseases are shown one by one.

Regarding cyprinids, infections with motile *Aeromonas*, atypical *Aeromonas salmonicida*, (Carp Erythrodermatitis), and *Flavobacterium columnare* (columnaris disease) are shown. Regarding salmonids, infections with *Aeromonas salmonicida* (furunculosis), *Yersinia ruckeri* (Enteric Redmouth Disease), *Flavobacterium branchiophilum* (Bacterial Gill Disease), *Flavobacterium psychrophilum* (Rainbow Trout Fry Syndrome), *Aliivibrio salmonicida* (cold-water vibriosis, Hitra disease), *Moritella viscosa* (winter ulcer disease), *Piscirickettsia salmonis* (Salmonid Rickettsial Septicaemia), *Chlamydia* spp. (epitheliocystis), *Renibacterium salmoninarum* (Bacterial Kidney Disease), and *Lactococcus garvieae* are shown.

Regarding (other) freshwater cultured fish species in Europe, infections with motile *Aeromonas*, atypical *Aeromonas salmonicida*, *Edwardsiella tarda*, *Flavobacterium columnare* (columnaris disease), mycobacteria, and *Streptococcus agalactiae* are presented.

Regarding brackish/marine fish species, infections with *Tenacibaculum maritimum*, *Pseudomonas anguilliseptica*, *Vibrio anguillarum*, *Vibrio harveyi* clade, *Vibrio vulnificus*, *Photobacterium damsela*, *Francisella noatunensis* subsp. *noatunensis*, and mycobacteria are shown.

The impact of bacterial diseases in fish culture in Europe stays high. Reclassification of bacterial names happens, based in newest diagnostic methods. Some of the warmwater fish diseases have a zoonotic risk, and can be diagnosed fast and accurately with new methods like MALDI-TOF. To prevent for zoonoses, this risk and hygiene standards need to be communicated well from fish diagnosticians to fish farmers, processors and hobbyists, and to medics, who diagnose zoonoses.

Key words: bacterial diseases, FAO, freshwater, brackish, marine

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BEATING PRIMARY HEART CELL CULTURES AS A TOOL FOR MEASURING THE IMPACT OF ENVIRONMENTAL STRESSORS AND CARDIAC VIRUSES IN FISH

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The increasing prevalence of circulatory disorders in the heart represents a significant challenge to the sustainable development of salmonid aquaculture on a global scale. The main pathogens responsible for these problems are piscine orthoreoviruses (PRVs), which frequently result in Heart and Skeletal Muscle Inflammation (HSMI). In addition to pathogens, acute or chronic toxic exposures to micro- and nanometre-sized plastics (MNPs) and oil spills represent a significant risk to aquatic life. The objective of the present study was to develop novel cardiac cell cultures from salmonids with the aim of investigating the effects of viral and environmental stressors on cardiac cells. Furthermore, the gene expression profiles of these cardiac primary cultures of salmonids following infection with PRV-1, PRV-3, piscine myocarditis virus (PMCV) and salmonid alphavirus 3 (SAV-3) and exposure to MNPs were investigated to determine whether innate immune and oxidative stress responses could be used as markers to confirm virus replication and exposure to environmental stressors.

A novel method was implemented to culture contracting primary heart cell cultures, which are constantly beating for up to eight weeks. Three-week-old cultures from salmonids were infected with the viruses and then cultured at 8 °C and 15 °C for a further four weeks. The virus-infected cultures were sampled immediately after infection and at 3, 7, 14, 21 and 28 dpi. In addition, heart cell cultures and salmonid larvae were exposed to MNPs and crude oil. The number of contractions of the cultures was counted and samples of the cells were taken. Cell and media samples were used for virus detection, and cell samples were used to measure genes involved in antiviral and pro-inflammatory responses as well as oxidative stress response by RT-qPCR.

The results from the exposure experiments to environmental stressors indeed showed some species-specific differences in the response to particle challenges. Cell cultures from brown trout were found to be the most sensitive to the exposure. The results of the infections showed that the cardiac viruses did not replicate in the cells. Antiviral genes were upregulated when cardiac cells were actively replicating other viruses and that these genes are likely to play an important role in the cardiac immune response. Beating primary heart cell cultures may therefore be a valuable tool for monitoring host-pathogen or host-environment interactions involving cardiac cells and it could be concluded that experimentation with larvae and cell cultures might be used to reduce the number of fish in exposure studies.

Key words: cardiac cell cultures, salmonids, piscine orthoreoviruses

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IMMUNE RESPONSE OF DIFFERENT CARP STRAINS DURING THE KOI HERPES VIRUS INFECTION

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In recent years, there has been a tremendous increase in knowledge about koi herpesvirus (KHV) and the challenges faced in carp culture, but any new information can support overcoming the virus, which is important for the field, as it can cause a substantial rise in industry loss. Breeding more resistant breeds of carp is one way of minimizing losses due to this disease. The resistance of different strains of carp (koi; Amur wild carp AS; Hungarian mirror carp, M2; hybrid of M2 x Amur mirror carp, M2xAL), and grass carp, and their ability to transfer the infection to a naïve susceptible host have been evaluated in experimental conditions.

The infection was induced by cohabitation of tested fish with intraperitoneally infected koi. Samples of blood and tissues were taken every 7th day post-infection (dpi) till 35 dpi. The second phase was initiated by relocating the fish surviving the first phase (according to the strains/species) to aquaria with naïve koi. Samples of gill were taken at 7, 14, and 21 dpi non-lethally, at 28 and 35 dpi lethally together with other tissues. Clinical signs and mortality were observed during both phases.

In the first phase, no fish exhibited clinical signs, no mortality was noticed in naïve fish and there was low mortality even among the injected fish. In koi and grass carp, isolated positive PCR findings in gill were recorded as early as 7 dpi, and in other groups as early as 14 dpi. Overall, the highest viral load in the first phase was recorded in koi and the lowest in grass carp.

In the second phase, the naïve koi showed more clinical signs from the 7th dpi. The most turbulent course of infection, accompanied by high viral load and mortality, was observed in koi cohabited with M2. Relatively high viral loads were recorded at 28 and 35 dpi in koi cohabited with grass carp, but without clinical signs or mortality. Koi cohabited with AS, and surprisingly also with koi, showed overall the lowest virus copy numbers in gill.

Efforts to minimize the number of fish used to comply with the 3Rs and concerns about lack of samples for later sampling due to mortality have resulted in low sampling rates, which may reduce the predictive power of the results. To get more clarity on the immune response of fish depending on the groups, further analysis such as the detection of anti-KHV serum antibodies (ELISA) and analysis of specific immune gene expression level are being carried out and the results will be presented at the conference.

Key words: koi herpes virus, infection, resistant breeds of carp, immune response

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10+ YEARS WITH CARP EDEMAVIRUS IN CZECH CARP AQUACULTURE

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The first cases of spring disease in carp and koi, probably caused by Carp Edema Virus (CEV), were diagnosed in the Czech Republic in 2013. However, it is possible that similar cases, manifested by lethargy, discomfort, loss of reflexes and mortality, may have occurred previously but were attributed to other causes. Since these first documented cases of CEV in the Czech Republic, attention has been paid to the virus and suspected mortalities of carp and koi have also been tested for the presence of CEV DNA. Several positive cases occurred almost every year, and as it was known that a similar situation existed in other European countries, a proposal for a project on new viral diseases of carp was developed and accepted for funding by the National Agency for Agricultural Research in 2016.

As part of this project, which ran from 2017 to 2021, sampling was carried out on farms where increased mortality of carp or koi carp was reported. Through this screening, several cases of CEV were detected each year and material was collected for laboratory analysis. Even after the end of the project, we continue to test suspected spring and autumn carp mortalities for the presence of CEV. Although the number of cases investigated is lower because we rely on the willingness of farmers to share information about health problems on their farms and to provide us with fish samples, we still record some positive detections each year.

In total, 180 sites have been investigated since 2013. The majority of these have recorded mortality or clinical signs of disease, and some sites have been re-sampled in the years following a positive finding. Common carp (edible carp) were the main species tested (143 sites), sometimes including koi carp farms (37). A total of 52 CEV-positive cases (42 carp/10 koi) were detected by nested PCR or real-time PCR.

I have no illusion that we can detect all cases of CEV occurring in our territory. Certainly, this disease must continue to receive the attention it deserves, both in the scientific field and in raising awareness among farmers, whose behaviour can have a major impact on the survival and spread of pathogens in the environment.

In parallel with field studies, attempts have been made to induce infection under laboratory conditions. The only effective method so far has been to use field infectious material for gill or intraperitoneal application. Infection experiments with CEV are still limited by the inability to amplify it in vitro using standard cell lines. A light at the end of the tunnel could be the partial success in culturing CEV on primary carp cell lines.

Key words: carp edema virus (CEV), carp aquaculture, common carp, koi carp

Acknowledgements: The study has been funded by Czech Ministry of Education, Youth and Sports through the project PROFISH (CZ.02.1.01/0.0/0.0/16_019/0000869).

SEVERE METABOLIC DISTURBANCE IN FISH DISEASED WITH CARP EDEMA VIRUS INFECTION

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Significant mortalities associated with emerging viral diseases are challenging the economy of common carp aquaculture. As such, there is an increased need to disentangle how infected fish cope with progressive disease pathology and lose the ability for homeostatic maintenance of key physiological parameters. A natural carp edema virus (CEV) infection outbreak at a carp fish farm provided an opportunity to examine diseased and healthy carp in the same storage pond, thereby contributing to our better understanding of CEV disease pathophysiology. The disease status of fish was determined using PCR-based virus identification combined with analysis of gill pathology. Compared with healthy control carp, the blood chemistry profile of CEV-infected fish revealed major disruptions in electrolyte and acid-base balance (i.e., hyponatraemia, hypochloraemia, hyperphosphatemia, elevated pH, base excess, and anion gap and decreased partial dissolved carbon dioxide). In addition, we recorded hyperproteinaemia, hyperalbuminaemia, hypotonic dehydration, endogenous hyperammonaemia, and decreased lactate along with increased creatinine, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase. Red blood cell associated hematology variables were also elevated. The multivariate pattern of responses for blood chemistry variables (driven by sodium, pH, partial dissolved carbon dioxide, ammonia, and albumin in the principal component analysis) clearly discriminated between CEV-infected and control carp. To conclude, we show that CEV infection in carp exerts complex adverse effects and results in severe metabolic disturbance due to the impaired gill respiratory and excretory functioning.

Key words: carp edema virus (CEV), metabolic disturbance, blood chemistry profile

NEW INSIGHT INTO IMMUNOSUPPRESSION OBSERVED DURING CARP EDEMA VIRUS-INDUCED GILL DISEASE

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Gill disorders cause substantial losses in the aquaculture industry. Koi sleepy disease (KSD), caused by the gill infecting poxvirus carp edema virus (CEV), is a unique model to study an impact of branchial diseases on the immune response in fish. In the present study, we performed 2D-DIGE based proteomics of gills to evaluate biological pathways affected by the disease. Furthermore, using flow cytometry we measured the level of IgM⁺, CD4⁺, and CD8⁺ lymphocytes in the blood, gills, head kidney and spleen. Both results were supplemented by the analysis of the gene expression.

CEV-infected fish developed classical clinical signs of infection related to gill dysfunction, manifested as ion dysregulation: decrease of sodium level under 100 mmol L⁻¹ and an accumulation of ammonia to over 600 µmol L⁻¹, which can induce immunotoxic effect. The proteomic analysis indicated that the abundance of 86 proteins was significantly changed in gills during the infection. The IPA-analysis indicated an activation of regulatory networks responsible for the response to cellular compromise, infectious diseases, inflammatory response and connective tissues disorders. Furthermore, CEV-infection affected: regulatory networks for drug metabolism and glutathione depletion as well as activation of the xenobiotic metabolism CAR signaling pathway. The changes in these networks indicate a response to ammonia intoxication and point to the effort of gill cells to handle and eliminate this immunotoxic metabolic by-product. Increased concentrations of several heat shock proteins and activation of the upstream heat shock factor protein 2 (HSP2) suggest increased stress response in infected gills. Moreover, observed in infected animals, down-regulation of the antimicrobial peptide NK-lysin-like suggests a lower activity of NK cells. Together with an activation of anti-inflammatory transforming growth factor beta 1 (TGFB1), these results indicate infection-induced immunosuppression. This corroborates with flow cytometer analyzes in which we observed a drastic drop in IgM⁺, CD4⁺, and CD8⁺ lymphocyte counts in the blood and the spleen. All these changes are accompanied by the increased expression of viral genes responsible for immunosuppression like multiple paralogues of B22R and the viral HSP70.

Taken together, the results allowed to show connection between CEV-induced pathology and immunosuppression leading to a severe gill disease.

Key words: carp edema virus (CEV), immune response, branchial diseases

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STRESS RESPONSE DURING KOI SLEEPY DISEASE IN KOI

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Carp edema virus (CEV) is a fish poxvirus that primarily infects the gills of common carp (*Cyprinus carpio*) and its ornamental varieties called koi. CEV causes koi sleepy disease (KSD), which is highly contagious and can result in mortality of up to 100%. KSD is characterized by disturbances of the respiratory function, acid–base regulation, osmoregulatory function or nitrogenous waste excretion from the gills, resulting together in hypoxia, hyponatremia, and hyperammonemia. Due to its severe effect on the function of the gills, CEV infection might therefore cause physiological distress in infected fish, which can affect many biological functions, including the immune response.

In the present study, we analyzed the stress response during KSD in koi. We also analyzed the effect of supplementation of 0.6% NaCl into tank water, which prevents mortality of the CEV-infected fish (salt rescue model). We found that CEV infection resulted in the activation of stress response reflected by the upregulated expression of genes involved in stress response in the hypothalamo-pituitary interrenal (HPI) axis organs such as hypothalamic nucleus preopticus (NPO), pituitary gland (PIT) and head kidney (HK) and increased levels of cortisol and glucose in the blood plasma. These changes were clearly reduced by salt treatment. Moreover, the virus leads to a decrease in the percentage of neutrophils in head kidney suspension and an increase in the percentage of neutrophils in peripheral blood leukocytes. No significant differences in ROS production were observed between the control and infected CEV-infected fish. Histopathological analysis of the gills demonstrated nearly complete occlusion of the intralamellar space with the accumulation of cellular debris and an infiltration of eosinophilic cells in CEV-infected koi at 6 days post exposure. Despite showing no visible clinical signs of KSD, salt-treated CEV-infected koi showed almost as severe histopathological changes as CEV-infected koi.

In conclusions, CEV infection results in a high viral load in the gills, which causes a disruption in the functions performed by this organ. This leads to stress and cortisol production that can lead to suppression of the immune response, ultimately favoring virus replication.

Key words: koi sleepy disease, carp edema virus (CEV), stress response

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ENHANCING AQUATIC PARASITE MONITORING WITH eDNA METABARCODING TECHNIQUES

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The elusive nature of microscopic parasites poses significant challenges for monitoring their presence in hosts. Traditional microscopic examination is labor-intensive and only provides information on the parasite's presence during the sampling period. Consequently, parasites with complex life cycles may go undetected at other stages. Furthermore, direct observation of free-living stages in the environment is nearly impossible. Metabarcoding offers a solution to these challenges by enabling real-time monitoring of a broader range of parasitic organisms in the host or in environment.

The use of metabarcoding to assess myxozoan diversity is still in its early stages. The first study demonstrating the utility of environmental DNA (eDNA) analysis for myxozoan detection introduced myxozoan specific primers that successfully amplified myxozoan SSU rDNA. A species-specific eDNA approach was employed to detect e.g. *Tetracapsuloides bryosalmonae* or *Myxobolus cerebralis*. However, our investigation of myxozoan diversity in freshwater using eDNA metabarcoding revealed low detection rates of myxosporeans at sites where fish were heavily infected with these parasites. This low detection was suggested to be connected to the nature of the sampled eutrophic aquatic habitats. To address this limitation, we developed a novel method based on sediment analysis using group-specific myxozoan primers, which proved to be highly effective for exploring myxosporean diversity (Lisnerová et al. 2023).

We applied this method to monitor the presence of myxozoan parasites in nine South Bohemian hatcheries, particularly in the Šumava region, where salmonid fish such as brown trout and rainbow trout are bred. Our goal was to assess the impact of intensive fish farming on the myxozoan parasitofauna in the watercourses connected to these hatcheries.

We detected over ten *Myxobolus* species specific to salmonids, along with four additional species from the genera *Chloromyxum* and *Myxidium* associated with Salmoniformes. Additionally, we identified two new malacosporean species that may represent previously unknown salmonid pathogens, similar to the well-known *Tetracapsuloides bryosalmonae*. Myxozoans were detected both within the hatcheries and at four nearby sampling sites connected to the hatcheries. This represents a significantly higher number of detected myxozoan species compared to recent screening of Šumava watercourses distant from hatcheries within our Interreg Bayern – Česko project, suggesting a potentially high risk of parasite infection and possible high impact on fish fauna in areas closely connected to hatcheries.

Key words: environmental DNA (eDNA), metabarcoding, myxozoan parasites

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Reference: Lisnerová et al. 2023: Evaluation and optimization of an eDNA metabarcoding assay for detection of freshwater myxozoan communities. Environmental DNA 5: 312–325.

PREVALENCE OF PROLIFERATIVE KIDNEY DISEASE IN THE RIVERS OF THE CZECH REPUBLIC

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Proliferative kidney disease (PKD) affects both wild and farmed salmonid fish, causes losses in aquaculture and a substantial decline in brown trout populations throughout Europe. The causative agent, *Tetracapsuloides bryosalmonae*, is an endoparasite (Myxozoa: Malacosporea) and its life cycle involves freshwater bryozoans as invertebrate hosts. The kidney represents the main target organ in fish. In the lumen of the kidney tubules, *T. bryosalmonae* forms sporogonic developmental stages and can be detected in this organ with the use of molecular methods. With climate change, more severe PKD cases are to be anticipated, as the parasite is highly temperature-dependent.

The aim of this study was to evaluate the prevalence of *T. bryosalmonae* in wild brown trout (*Salmo trutta*) in the rivers of the Czech Republic. Rivers of three basins (Elbe, Morava and Oder) were investigated, and in total, fish from 62 locations from 42 unique streams were sampled. Where possible, at least ten fish per location were caught by electrofishing. The animals were euthanised, measurements were taken and the pathoanatomical and parasitological examinations (including collection and taxonomic determination of parasites from the skin, fins and gills by light microscopy) were conducted on-site. Tissue samples from each individual were collected and stored in 70% ethanol for parasitological examination (caudal kidney) and virological examination (haematopoietic organs: cranial kidney, spleen and heart) using molecular methods. Ethanol-stored samples of the caudal kidney were examined by real-time PCR for the presence of *T. bryosalmonae*. The fish were also examined for the presence of salmonid viruses (VHS, IHN, PRV-3, and IPN).

Swelling of the caudal kidney, indicative of the presence of *T. bryosalmonae*, was the most common pathoanatomical sign present in the fish, as confirmed by the PCR results. High prevalence of the parasite in wild brown trout in the Czech Republic was confirmed.

Key words: proliferative kidney disease (PKD), *Tetracapsuloides bryosalmonae*, brown trout

Acknowledgements: This research was funded by the Ministry of Agriculture of the Czech Republic (projects QK23020064 and RO0523).

EXPLORING THE NOTCH SIGNALLING PATHWAY IN EARLY-BRANCHING METAZOA: INSIGHTS FROM PARASITIC CNIDARIANS, MYXOZOA

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Cellular communication was crucial for the evolutionary diversification of cell types, enabling the development of complex body plans. In Metazoa, conserved signalling pathways, particularly the Notch pathway, play a central role. This pathway requires direct cell-cell contact for receptor and ligand activation and regulates fundamental processes such as cell fate determination, proliferation, differentiation, and apoptosis.

This study focuses on Myxozoa, an early-branching metazoan group of cnidarians that underwent a transition from free-living to microscopic endoparasites. We hypothesize that the dramatic changes in myxozoan body plans and those of some other cnidarians correlate with alterations in the Notch pathway, including changes in the structure and phylogeny of Notch receptors and ligands. Our analysis revealed a significant loss of 14 out of 28 Notch components in Myxozoa compared to the conserved pathway in free-living cnidarians. In particular, Myxozoa lack the coactivator MAML and the *Hes/Hey* target genes, while these components remain largely intact in free-living Cnidaria. Notably, hydrozoans, the only medusozoan group with a loss of MAML, share this trait with Myxozoa.

A phylogenetic analysis shows of Notch receptors exhibit significant sequence divergence in Myxozoa, resulting in long branches consistent with their rapid evolutionary rate. This divergence is accompanied by a reduction or absence of the RAM domain peptide structure in several myxozoan species. Furthermore, the Notch extracellular domain is notably reduced in Myxozoa and other early-branching Metazoa. Immunolocalization in the myxozoan *Sphaerospora molnari* showed the Notch receptor present in all primary, secondary, and tertiary cells, with the Notch intracellular domain detected in both the cytoplasm and nuclei of the cell-within-cell structure in *S. molnari* blood stages. Our research supports the hypothesis of a common cnidarian ancestry for delta-type Notch ligands, found in both invertebrates and vertebrates, as well as in placozoans and ctenophorans. Interestingly, delta ligands are present only in anthozoan cnidarians, suggesting their evolution predates the divergence of anthozoan and medusozoan lineages.

These findings illustrate extreme reduction in the Notch pathway in Myxozoa and provide insights into the early evolution of Notch signalling.

Key words: myxozoa, notch receptors, ligands

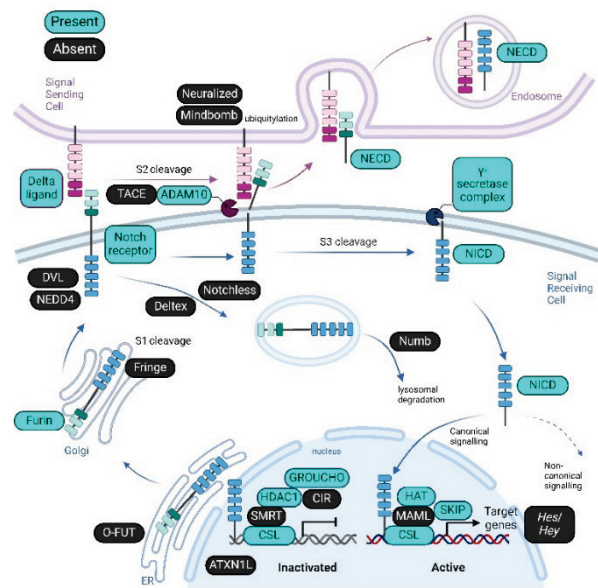


Fig. 1. Core components of Notch signalling in Myxozoa, components present (turquoise), absent (black).

THE COMMON CARP (*Cyprinus carpio*) MOUNTS AN IGT ANTIBODY RESPONSE AGAINST THE MYXOZOAN (CNIDARIA) *Sphaerospora molnari*

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Teleost fish express three immunoglobulin isotypes that serve as adaptive immune antigen receptors: IgM, IgT, and IgD. They are presumably non-redundant as fish can mount systemic IgM responses and mucosal IgT responses depending on the nature and mode of antigenic challenge. In the context of an infection, the antibody response to the myxozoan *Tetracapsuloides bryosalmonae* is predominantly IgT-mediated with little contribution from IgM or IgD. In contrast, we previously observed that the common carp (*Cyprinus carpio*) strongly upregulates *igm* expression and secretes IgM specific to another myxozoan, *Sphaerospora molnari*. Here, we compared the expression of three putative common carp *igt* subclasses to determine their relative contributions to the immune response against this parasite. Our results suggest that the different IgT subclasses have tissue-specific expression, and they are not redundant with each other nor with the IgM class in the response against *S. molnari*.

Key words: immune antigen receptors, myxozoan, common carp (*Cyprinus carpio*), antibody response

EVADING THE ANTIBODIES – INSIGHTS INTO ANTIGENIC VARIABILITY IN THE MYXOZOAN PARASITE *Sphaerospora molnari*

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With the emergence of adaptive immunity 400 mya ago, pathogens were faced with the ability of the host to recognize and remember any of the pathogens' surface antigens. To avoid antibody-mediated effector functions, pathogens across the tree of life developed various evasion strategies rendering the antibody responses ineffective. Using the model infection of common carp with myxozoan parasite *Sphaerospora molnari*, we aimed to shed more light on the protective capacity of *S. molnari*-specific antibodies and provide new information about the strategy of antigenic variability developed in one of the oldest clades of metazoan parasites.

To this end, we first investigated whether the *S. molnari*-specific antibodies conferred protection and elucidated the capacity of *S. molnari* to escape antibody-mediated elimination *in vitro* and *in vivo*. Furthermore, using the newly developed pan-*S. molnari* antibody, we studied the antigenic composition of independent parasite isolates and correlated it with the lytic potential of immune sera obtained during the experimental infection.

We demonstrated that, although the *S. molnari*-specific antibodies limit parasite proliferation through complement-mediated lysis, the immune sera do not prevent infection completely. Depending on the parasite's antigenic makeup, the parasite escapes the antibody-dependent elimination and establishes an infection even upon vaccination or secondary exposure, owing to the high variability of recognized surface antigens.

Collectively, the obtained results provide vital insights into the importance of antibody responses during myxozoan infections and point to the novel mechanism of antigenic variability in this ancient clade of cnidarian parasites.

Key words: myxozoan, *Sphaerospora molnari*, antibody responses

CAVIPLASMA TECHNOLOGY AS A TOOL FOR MANAGEMENT OF ICHTHYOPHTHIRIASIS

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Ichthyophthirius multifiliis is a parasitic ciliate causing one of the most serious fish diseases, white spot disease, which is associated with high morbidity, mortality and significant economic losses in the aquaculture. In the first part of the presented work, we tested the novel technology called CaviPlasma for treatment of water contaminated with free-swimming stages of *I. multifiliis*. Subsequently, we tested the treatment of water supplying recirculation tanks with fish suffering from ichthyophthiriasis. CaviPlasma technology is based on the synergetic effect of hydrodynamic cavitation in a fast-flowing liquid and electric discharges generated within the water vapour inside the cavitation bubbles. This leads to large-volume generation of UV radiation and reactive oxygen species with biocidal effect.

Experiment 1. Experiment was performed on healthy juvenile wells catfish (*Silurus glanis*), with a weight of $6,3 \pm 1,3$ g and total length of $102,7 \pm 8,9$ mm ($n = 8$). Fish were divided into four experimental groups and let to acclimatize for one week. After that, sample of tap water containing free stages of *I. multifiliis* was treated with CaviPlasma (Batch 1 for 80 s and Batch 2 for 420 s). Batch 3 was left untreated. Batch 4 was untreated tap water that did not contain *I. multifiliis*. Two hours later, four above mentioned batches of water were added into tanks with four experimental groups of fish. Sampling of fish was performed on the 6th day after the start of the experiment. Fish were euthanized, skin and gill smears were performed and numbers of *I. multifiliis* were detected microscopically. In fish from the group which obtained untreated water with *I. multifiliis*, massive ichthyophthiriasis was detected in both skin and gills. No parasites were found in any other group. **Experiment 2.** Treatment of inflow water with CaviPlasma was tested in recirculation tanks with adult common carp (*Cyprinus carpio*) with ongoing ichthyophthiriasis. Different modes of water treatment were tested; the length and frequency of the treatment was adjusted according to the concentration of reactive oxygen species detected in the treated water. Experiment lasted for three weeks. None of the treatment modes led to cure or suppression of ongoing infection in the diseased fish.

Conclusion: Experiment 1 shows that CaviPlasma was able to devitalize free-living stages of *I. multifiliis* completely and could be therefore a very effective tool for preventive treatment of inflow water. On the other hand, water treatment was ineffective in the case of ongoing ichthyophthiriasis (Experiment 2).

Key words: *Ichthyophthirius multifiliis*, CaviPlasma, water treatment

Acknowledgements: This work was supported by the Czech Ministry of Agriculture of the Czech Republic (project NAZV QK 21010030).

EFFICACY OF MEDICATED FEED TO CONTROL *Ichthyophthirius multifiliis* INFECTION IN RAINBOW TROUT

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Infection caused by *Ichthyophthirius multifiliis* (Ich) is one of the most commonly occurring eukaryotic diseases of freshwater fish. This parasitic disease can cause high economic losses in fish farming, especially in intensive aquaculture of rainbow trout and brook trout. Currently, there is no single treatment against Ich with sufficient efficiency. Historically, short-time treatment baths in malachite green have been used with good results, but the use of malachite green in food animals was banned due to carcinogenic and mutagenic properties.

The objective of this study was to examine the potential efficacy of drugs commonly used in livestock in the Czech Republic against the Ich infection. Four antiparasitics (amprolium, clopidol, praziquantel and toltrazuril) and one antibiotic (doxycycline) were tested and administered as medicated feed. The antiparasitic effect of individual drugs was verified in three separate trials considering the initial administration of the drugs 1) at the beginning of infection, 2) in the preclinical phase and 3) at the time of increased mortality. In all trials, mortality and the presence of the parasite were investigated by standard parasitological examination. In the trial with the application of drugs in the preclinical phase, the presence and intensity of the parasite in the water was also investigated by quantitative PCR.

Of all tested drugs, doxycycline and praziquantel appeared to be the most suitable candidates under experimental conditions, significantly decreasing mortality levels and the presence of the parasite when administered in the preclinical phase of the infection.

Key words: *Ichthyophthirius multifiliis*, antiparasitics, antibiotic

Acknowledgements: This work has been funded by the project Optimization of the management of parasitic diseases in fish farming with a focus on *Ichthyophthirius multifiliis* infection in rainbow trout (Grant: NAZV QK22010369).

THE EFFECT OF PROPHYLACTIC BATHS IN PERACETIC ACID AND HYDROGEN PEROXIDE ON PARASITIC AND BACTERIAL COLONIZATION OF FISH

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Fish diseases may be one of the factors slowing down the current development of aquaculture. Pathogenic bacteria and parasites are common disease agents. Treatment options for external parasitic and bacterial diseases are addressed in this paper.

The aim of the study was to test the influence of peracetic acid (PAA) and hydrogen peroxide (H_2O_2) on the bacterial and parasitic infection of fish. Three groups ($n=6$) of common carp (*Cyprinus carpio*) were exposed to PAA ($1.5\text{ mg}\cdot\text{l}^{-1}$) and H_2O_2 ($5\text{ mg}\cdot\text{l}^{-1}$) applied daily for a duration of 4 days. The test was triplicated. After exposition, bacterial and parasitological examinations of the gills and skin were conducted using bacteriological smear and microscopy of tissue scrapes. Parasitological and bacterial load of the skin and gills was classified into five categories and subsequently statistically evaluated using the Mann Whitney test.

Semiquantitative bacteriological examination revealed a statistically significant reduction ($p<0.05$) in the bacterial load of skin in both PAA and H_2O_2 exposed groups. Conversely, there was no statistically significant difference in the bacterial load of gills.

Parasitological examination revealed a statistically significant ($p<0.05$) effect of PAA on *Trichodina* sp. These parasites were eliminated on the skin and on the gills of the fish from PAA group. No other statistically significant differences were mentioned among group tested. The results show that the application of PAA and H_2O_2 in the tested concentrations for four days has antibacterial effect, while PAA showed also antiparasitic effects.

Key words: fish diseases, peracetic acid, hydrogen peroxide

Acknowledgements: The study has been funded by Czech Ministry of Education, Youth and Sports through the projects PROFISH (CZ.02.1.01/0.0/0.0/16_019/0000869) and CENAKVA (LM2018099).

VIABILITY OF *Metagonimus romanicus* (CIUREA, 1915) METACERCARIAE AFTER PHYSICO-CHEMICAL TREATMENTS

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Digenean (Heterophyidae) trematodes include several zoonotic species such as the genus *Metagonimus* (Katsurada, 1912), which are well known to cause infections in mammals, especially in humans in South Asia. *Metagonimus romanicus* (Ciurea, 1915) is a species widely distributed in Europe, whose metacercariae can be found on the scales of cyprinoid fishes, which are the second intermediate hosts. *Metagonimus romanicus*, which was formerly often identified as *Metagonimus yokogawai* (Katsurada, 1912), is abundant in the River Danube in Hungary and as well in the neighbouring countries. The study focused to assess the viability of *Metagonimus romanicus* metacercariae by physical (freezing, heating, desiccation) and chemical (acetic acid and NaCl solutions) treatments.

The methods were chosen to model procedures commonly used in traditional food preservation (such as freezing, salting, pickling and smoking) to estimate the survival rate of metacercariae under different physical and chemical conditions. The majority of physical treatments (freezing of metacercariae at -20 °C, keeping them at 40 °C and 60 °C and desiccation) and chemical treatments (2.5%, 5%, 10% acetic acid solution and 5% and 10% NaCl solution) killed the metacercariae in a relatively short time (from 30 min to 6 days depending on the treatment) so their effects eliminated the risk of human infection. Otherwise, a significant part of the metacercariae were able to survive at room temperature and at 4 °C up to one month.

In conclusion, storing unprepared fish in domestic refrigerators cannot prevent infection with metacercariae in humans. However, the chance of human infections is minimal, as the consumption of raw fish is unconventional in European culinary traditions and traditional preservation methods can destroy metacercariae with high efficiency. Humans can become infected during preparing fish for cooking, when the scales with embedded metacercariae get accidentally consumed. However, pets such as cats and dogs are more likely to be infected as they do not avoid raw fish.

Key words: *Metagonimus romanicus*, zoonotic infection, physical and chemical treatments

Acknowledgements: This study was funded by the Hungarian Scientific Research Fund (NKFI OTKA FK 140350).

INSIGHTS INTO FISH IMMUNOGLOBULIN RECEPTORS

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Teleost fishes, numbering over 32,000 extant species, constitute the largest portion of vertebrate species, showcasing significant taxonomic diversity and extensive genetic and phenotypic variation, including novel immunological strategies. Among the crucial components of both innate and adaptive immunity are Polymeric-Ig receptors (pIgRs) and fragment crystallizable (FcRL) receptors. Extensive genome searches conducted in cartilaginous (Chondrichthyes) and bony (Osteichthyes) fishes unveiled a substantial gene expansion of these receptors in these groups compared to higher vertebrates, including human. Utilizing the trout (*Oncorhynchus mykiss*) genome as a model, we delved into the evolutionary backdrop underlying the gene heterogeneity and diversification in bony fishes. Our analysis identified a total of 21 FcRL genes in trout, enabling us to characterize their domain architecture and chromosomal distribution. Further exploration into the impact of teleost-specific genome duplication (Ts3R) and salmonid-specific fourth vertebrate whole-genome duplication (Ss4R) involved comparative genomics of the trout genome with other salmonids and their ancestors. Intriguingly, we observed FcRL heterogeneity and gene expansion/contraction within the salmonid FcRL gene family. Comprehensive comparative genomics highlighted gene synteny of FcRLs and identified several ohnologs, particularly noting their retention after whole-genome duplication processes in salmonids. Notably, despite rediploidization following whole-genome duplication, FcRLs were not exclusive to subfamily-specific rediploidization regions but also classified within ancestral ohnolog resolution regions. Our genome collinearity analysis of trout unveiled the origin of the FcRL gene family, shaped not only by whole-genome duplication but also by transposition and tandem gene duplication processes. However, the functional impact of these genetic dynamics are yet to be discovered. Furthermore, genome synteny analysis revealed the retention of major salmonid FcRLs after whole-genome duplication, albeit with some gene losses during evolution. In summary, the fish Fc receptor repertoire seems intricately linked with radiation, exhibiting specificity for individual fish species and families with distinct evolutionary histories.

Key words: teleost fishes, polymeric-Ig receptors (pIgRs), fragment crystallizable (FcRL) receptors

UNVEILING FC RECEPTORS: UNLOCKING ANTIBODY EFFECTOR FUNCTIONS IN TELEOST FISH

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Fc receptors (FcRs) are pivotal in mediating immune responses by linking humoral and cellular immunity through their interaction with the Fc region of immunoglobulins (Igs). In mammals, these interactions are well-characterized and are known to drive processes such as phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), and inflammatory mediator release. However, the study of FcRs in teleost fish remains nascent, and the extent of their diversity, expression, and functional roles is not fully understood.

In this study, we conducted a phylogenetic analysis to identify potential homologs of known mammalian FcRs in teleost fish, focusing on rainbow trout (*Oncorhynchus mykiss*). Through a comprehensive genome search, we identified over 20 orthologs of polymeric-Ig receptors (pIgRs), Fc receptors, and Fc-like receptors, suggesting a more complex FcR system in fish than previously recognized. We performed molecular analyses on these newly identified receptors, examining their expression in various tissues from both naive and pathogen-challenged trout to gain insights into their functional relevance and potential binding partners.

Our findings reveal that FcRs in rainbow trout exhibit a broad and tissue-specific expression pattern, encompassing major leukocyte subpopulations such as B and T lymphocytes, myeloid cells, thrombocytes, and erythrocytes. This unexpected heterogeneity of Ig-binding receptors, despite the limited number of immunoglobulin isotypes in trout, points to a complex and specialized role of FcRs in teleost immunity. The observed diversity raises intriguing questions about the evolutionary pressures driving FcR specialization in fish and their potential adaptations to aquatic pathogens.

This study underscores the complexity of the FcR system in teleosts, challenging existing paradigms derived from mammalian models. Further research into the signaling pathways and immune functions associated with these receptors is crucial for advancing our understanding of vertebrate immunity and may have significant implications for disease management in aquaculture.

Key words: Fc receptors (FcRs), teleost fish immunity, polymeric-Ig receptors (pIgRs)

COMPARATIVE STUDY OF FISH AND HUMAN NUCLEAR RECEPTOR RESPONSES TO WATER POLLUTION

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To date, most of the *in vitro* bioassays available for monitoring biological activities in the aquatic environment are based on mammalian receptors. However, mammalian receptor-based assays may not accurately reflect the effects of waterborne contaminants on fish because of certain structural differences between fish and mammalian receptors.

The aim of this study was to investigate species-specific differences in the activation or inhibition of several human and zebrafish nuclear receptors by environmental water extracts. Two sampling campaigns were conducted. In the first one, four wastewater treatment plants (WWTP) influents, effluents and their respective receiving surface waters were analysed on the human and zebrafish progesterone receptor (PR). In the second sampling campaign, two wastewater treatment plants (WWTP) influents, effluents and their respective receiving surface waters, three ponds and two rivers were analysed on the human and zebrafish estrogen receptor α (ER α), androgen receptor (AR), PR, glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and pregnane X receptor (PXR).

In the first campaign, human PR agonistic activity was detected in all wastewaters and most receiving surface water samples, but zebrafish PR agonistic activity was found in only two influent wastewater samples. Human antagonistic PR activity was also detected in most wastewaters and some surface water samples, but no zebrafish anti-PR activity was found.

In the second campaign, both human and zebrafish ER α and AR agonistic activities were detected only in influents and the detected activities were similar. Zebrafish progestogenic activities were detected in two influent samples, but human PR activity was detected in only one sample, showing a difference between the activation of human and fish receptors. In the case of mineralocorticoid activities, no human mineralocorticoid activity was detected in any sample; however, zebrafish mineralocorticoid activities were detected in both WWTP influents. Zebrafish PXR agonistic activities were detected in both influents and surface waters, whereas human PXR agonistic activities were detected only in WWTP influents. None of the samples tested showed any activity on human or zebrafish GR.

The results of this study indicate that, for ER α and AR activities in the aquatic environment, even human-based nuclear receptor assays can provide relevant results. However, for PR, MR, and PXR activities in the aquatic environment, the zebrafish-based nuclear receptor assays should preferably be used.

Key words: nuclear receptors, zebrafish, *in vitro* bioassays

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FISH DISEASES AS AN IMPORTANT LIMITING FACTOR IN ORNAMENTAL FISH AQUACULTURE

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Ornamental fish production is a significant business worldwide. Some Asian countries, in particular, produce hundreds of species and colour variations of ornamental fish on a massive scale. The tradition of ornamental fish aquaculture in Central European countries serves as a historical foundation for global production, with the Czech Republic standing out as a leader in the field. According to statistics, more than 90 % of freshwater ornamental fish come from aquaculture production. In contrast, the marine ornamental fish sector has a smaller proportion of animals bred in aquaculture (about 10 %), though the number of species regularly produced in captivity is growing significantly.

Like all branches of aquaculture, ornamental fish production faces considerable economic losses due to disease. Although this sector generates substantial profits not only from the sale of fish but especially from the sale of accessories, there are limited studies to help producers effectively manage fish diseases. The vast array of species produced in the ornamental fish industry, often within family businesses where knowledge of fish diseases is minimal, has led to high mortality rates in some species that are nearly disappearing from the global market. For instance, the production of angelfish (*Pterophyllum scalare*) and guppies (*Poecilia reticulata*) faces severe challenges worldwide. Specific details about these cases will be shared at the conference. Notable exceptions include certain breeding facilities for KOI carp and Discus fish (*Symphysodon* sp.), which focus on identifying and treating specific diseases within their stocks at a high level.

Most of the theoretical and practical knowledge from food fish aquaculture is directly applicable to ornamental fish aquaculture. However, the spread of information on disease management in the ornamental fish sector is severely lacking. To ensure the future of ornamental fish production, establishing educational mechanisms on fish diseases for all workers in the industry and fostering close collaboration with fish pathologists is essential.

Keywords: aquaculture, ornamental fish, economic losses, diseases, education

CYPRINID BIOSECURITY - CURRENT KNOWLEDGE

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Common carp (*Cyprinus carpio*) is the most important farmed fish in Central and Eastern Europe, and one of the most farmed freshwater fish species worldwide. It is predominantly produced in the traditional shallow ponds, along with predatory fish and other cyprinids, such as grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*). Although some aspects of the cyprinid aquaculture date back to at least late medieval times and remain virtually unchanged, other risks and factors are a product of the last few decades.

Considering biosecurity, the pond system often presents an open and vulnerable system with poor control over the quality of water intake and interaction with wild fauna, especially mammal and avian predators. Additionally, complex systems including multiple ponds connected directly by water flow are often present in the carp rearing regions, increasing the disease-transfer potential.

Modern carp farming often requires stocking of ponds with biological material transported across large distances in the form of fish eggs or living fish. This, along with very little oversight over the biological safety of the transported material (eg. active surveillance, quarantine of imported animals, existence of SPF zones or farms) increases the ability of pathogens to travel across large distances and to overcome natural geographical barriers.

Antimicrobial resistance of bacterial pathogens has been observed regularly in recent years, especially against the few antimicrobials that are registered for use in farmed common carp (Oxytetracycline, Chloramphenicol).

Finally, factors negatively influencing the biosphere in general, mainly the lack of precipitation and increase in the average temperature due to climate change, contribute to the biosecurity risks as well. An example would be the spread of invasive species potentially carrying new pathogens, increased environmental stress and deterioration of fish immune capabilities and the introduction of pathogens previously limited by colder overall temperatures.

Key words: carp aquaculture, biosecurity in pond systems, fish pathogens

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PHYSICAL PARAMETERS OF FISHPONDS IN THE CONTEXT OF CLIMATE CHANGE

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This study evaluated changes in water temperature and their potential impact on productive fishponds in the Czech Republic. A model for monitoring and predicting water temperature in fishponds was developed and tested, using only the average daily air temperature as its sole variable for simplicity. The model assumes that the temperature of the surface water layer (depth 0.15 m) is linearly dependent on the average air temperature, with a slight delay in response.

The model predicts a sharp increase in unprecedented temperature regimes for Czech fisheries, which will present major challenges to traditional common carp farming practices in the coming decades. By 2050, we can expect very high water temperatures in all fishponds across the Czech Republic, at least in some years. In the South Moravian region, water temperatures above 30 degrees will likely become a regular occurrence.

Ongoing climate change may significantly impact the water balance. The combination of extremely warm water and minimal inflow could intensify the effects of both risk factors. The growing water deficit could affect all fish production areas and present a significant challenge to sustaining fishpond production. However, it remains uncertain to what extent this increased risk will lead to reduced oxygen levels, with potentially fatal consequences for the fish population. While temperature-related changes can be predicted with relatively high accuracy, the direct impact on fishpond management is complicated by the unique characteristics of each reservoir, its specific management, and the specific course of high-temperature episodes.

Key words: fishponds, climate change, water temperature

Acknowledgement: This study was supported by the project Národní Centrum Biotechnologií ve Veterinární Medicíně, TN02000017.

TESTING EPITHELIAL OR ENVIRONMENTAL SAMPLING FOR INTRA-VITAM DIAGNOSTICS OF GILL DISEASE IN COMMON CARP

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Recently, non-lethal or environmental sampling has been recognised as a tool for monitoring the presence of pathogens in aquaculture. Measurement of immune responses in the epithelium and the presence of pathogens in the epithelium and water appear to be particularly suitable for the detection of mucosal pathogens in fish. Gill and skin diseases are often multi-pathogenic, including co-infections with viruses, bacteria and parasites, and can induce a plethora of different immune responses. For example, carp edema virus (CEV) infection is indicative of immunosuppression of adaptive responses and often occurs with co-infections with ectoparasites such as *Ichthyobodo necator* and the bacterium *Flavobacterium branchiophilum*, which drive the proinflammatory responses and pathology, making diagnosis and treatment difficult. As carp cannot always be sacrificed for sampling during the production cycle, we tested the applicability and robustness of epithelial immune response monitoring methods and environmental DNA-based methods for the detection of pathogens associated with KDS.

To test the selected methods for rapid detection of KDS, water samples, gill swabs and gill biopsies were collected during disease outbreaks and experimental infections and stored frozen at -20°C . Several centrifugation speeds and different pore size filters were used to select the best method for concentrating pathogens from water. Detection of carp edema virus, *Ichthyobodo necator* and *Flavobacterium* sp. was performed by qPCR after DNA extraction using a Qiagen DNA mini kit. Immune responses were measured using a Fluidigm array and correlated with pathogen load and pathological changes.

Filtration (0.20 μm and 0.45 μm) appeared to be the most reliable method for concentrating the pathogens associated with KDS outbreaks. The detection of CEV, *I. necator* and *Flavobacterium* sp. was possible at very early stages of infection, and the concentration of CEV increased rapidly from day 4, when the first clinical signs appeared. Furthermore, the DNA of all pathogens could be detected in the water for at least 8 days after removal of the infected fish. Gill biopsies and swabs allowed the detection of immune responses previously measured during CEV infections: increased antiviral and proinflammatory responses and decreased levels of adaptive immunity markers.

Concentration of all pathogens involved in multi-pathogen gill disease associated with carp edema virus infection was possible with a single water filtration procedure using e.g. a 0.20 μm syringe filter. eDNA-based diagnosis could therefore be a very efficient method for detecting outbreaks of KSD, flavobacteriosis and ichthyobodiasis, at least in relatively small water bodies such as small ponds or tanks. Gill swabs appeared to be as reliable as gill biopsies or post-mortem samples for detecting immune responses characteristic of viral gill disease and may aid in differential diagnosis.

Key words: gill diseases, common carp, non-lethal sampling

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LAMP ASSAY DESIGNED FOR SCREENING eDNA AND FISH TISSUE SAMPLES FOR CEVD

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Carp edema virus disease (CEVD), also called Koi sleepy disease, is an emerging viral disease of common carp (*Cyprinus carpio*) occurring in Northern America, Asia and Europe. The symptoms include haemorrhagic skin lesions with underlying tissue edema, pale swollen gills and lethargic behaviour of infected animal. These symptoms are not specific to the disease, therefore diagnostics should be confirmed by molecular methods. We adapted existing assay with the goal to perform all necessary steps for pathogen confirmation in-situ at the pond.

Loop-mediated isothermal amplification (LAMP) is based on use of 4–6 primers targeting an area of DNA prone to single strand loop formation. Strand-displacing DNA polymerase attaches within the loop and as it amplifies new DNA it displaces strands of double strand DNA it encounters, thus providing new binding sites for primers and polymerase molecules. Compared to traditional end-point PCR, LAMP method is much faster (typically the result is observed in 20–30 minutes) and can be more specific and sensitive. We used six LAMP primers targeting p4A gene designed by Cano et al. (2021) with ISO-004 mastermix (OptiGene, UK) containing fluorescence dye, and portable Genie II machine (OptiGene, UK). This method was first tested in laboratory using cell culture of carp gills inoculated with the virus, that was confirmed to be positive by qPCR. Later (in field) we used samples of approx. 0.5 cm³ of carp gill that were collected directly from the fish on site.

One of the main challenges in field settings is how to perform DNA isolation. First we tested several approaches using PrepManTM Ultra (ThermoFisher Scientific, UK). However, the fish gill mucus seems to contain amplification inhibitors, and the sample needs to be 10× diluted to yield positive result, which is not very practical in field conditions. Adding bovine serum albumine (inhibition blockator) doesn't change the outcome. However simple boiling of the sample in distilled water (500 µl) led to uninhibited amplification.

We performed field test of isolation + LAMP detection *in situ* of confirmed CEVD outbreak in carp farm Pohořelice, Czech Republic. We were able to detect the disease in all samples collected from living ill animals as well as fresh carcasses. The detection was unsuccessful in carcasses in later stage of decomposition although CEVD was suspected as the cause of death. We also collected three water samples from the outbreak site for eDNA detection and forced it through microbiologic filters. The filters were cut into thirds and isolated in laboratory by three different methods – standard DNA kit isolation, boiling in 200 µl of PrepManTM Ultra and boiling in 500 µl of distilled water. The samples isolated by kit did not amplify in subsequent LAMP, but both the PrepMan and water boiled samples showed positive outcome. This could be expected as the amount of eDNA in sample is usually very small and some of it is lost during the kit isolation procedure. However, positive results from other methods of isolation show that eDNA can be considered viable tool for disease detection in ponds.

Key words: carp edema virus (CEV), loop-mediated isothermal amplification (LAMP), eDNA

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BARLEY AS A PRODUCTION PLATFORM FOR ORAL VACCINES IN SUSTAINABLE FISH AQUACULTURE

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Fish aquaculture, a sustainable source of global fish production, faces a significant challenge posed by disease spreading. The most effective measure to prevent disease outbreaks in aquaculture is fish vaccination, with oral vaccine administration emerging as the most practical approach. However, oral vaccines face a notable limitation due to insufficient stimulation of the complex gut-associated lymphoid tissue caused by factors such as vaccine degradation and poor absorption. A promising solution to these issues lies in the plant-based production of recombinant vaccines. Plant cells enable targeted storage of recombinant vaccines in specific cell organelles to ensure superior protection and contain natural compounds acting as adjuvants, which could lead to more efficient immune system activation.

Our study explores the potential of barley (*Hordeum vulgare*) for producing orally administered subunit vaccines based on the viral envelope proteins. Through *Agrobacterium*-mediated transformation of immature barley embryos, we have successfully generated transgenic barley in the T2 generation expressing recombinant antigens of spring viremia of carp virus and infectious salmon anaemia virus. Fluorescence detection with immunodetection confirmed the production of plant-based recombinant vaccines, specifically in the grain endosperm. The antigenicity of resulting plant material was evaluated using an intubation model of common carp (*Cyprinus carpio*), revealing a substantial upregulation of the immunoglobulin transcripts in both systemic and mucosal tissues over period of 28 days following a single dose of transgenic antigens. Collectively, these results underscore the potential of barley-based recombinant vaccines for disease prevention in aquaculture fish.

Key words: barley (*Hordeum vulgare*), common carp (*Cyprinus carpio*), vaccines

MAPPING KNOWLEDGE GAPS IN CURRENT BIOSECURITY PRACTICES

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The European Partnership on Animal Health and Welfare (represented by the abbreviation EUP AH&W or its acronym EUPAHW) is by far the most ambitious research and innovation initiative the European Commission (EC) has funded to control infectious diseases of animals, and to promote animal welfare.

The scope goes beyond the animal health and welfare actors to enhance cross sector collaboration and, through a One Health and One Welfare approach, to provide societal impact. The objectives of the EUP AH&W align with the European Green Deal and its associated Farm to Fork strategy for a fair, healthy, and environmentally friendly food system.

One of the task of the first two years of this initiative deals with Biosecurity of farmed animals. The goal of this task is to highlight and report current knowledge GAPS in the biosecurity of animal production.

For the aquatics, the focus has been on the five most important fin fish species farmed in Europe, namely Atlantic salmon, Rainbow trout, Carp (and cyprinids), European Sea bass and Gilthead sea bream.

For each species a specific subgroup has been established, and a network of stakeholders involved, as well a questionnaire (<https://freeonlinesurveys.com/>) addressing various aspects related to biosecurity has been produced and distributed to the stakeholders.

During this workshop, all participants will have opportunity to answer the questionnaire and results obtained will be discussed afterwards.

Key words: biosecurity, animal welfare, one health, aquaculture

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THE MOST SERIOUS HEALTH PROBLEMS IN THE BREEDING OF PIKEPERCH (*Sander lucioperca*) IN RAS (RECIRCULATING AQUATIC SYSTEM) AND THE POSSIBILITIES OF THEIR SOLUTION.

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The health status of pikeperch (*Sander lucioperca*) reared in RAS (recirculating aquatic system) is often negatively affected by **non-infectious causes**. These are impaired water quality (temperature, oxygen content, ammonia, nitrites, chlorine) and feeding with the composition of unsuitable feed (liver steatosis, avitaminosis, hypervitaminosis) or defective feed (ceroid degeneration of the liver, mycotoxicosis).

Bacterial infections are the biggest to solve health problem in fish farming in the RAS in general and in pikeperch farming in particular. In the RAS system, the requirement for bacterial growth in the biological filter (to maintain optimal water quality) is opposed to the need to minimize the pathological effect of bacteria on fish. This situation also complicates the possibility of antibacterial treatment in RAS. In practice, it is not possible to wait for the results of a bacteriological examination in the event of an outbreak of infection. Suspicious diagnosis is determined by a veterinarian based on clinical findings, who continuously checks the health status of the fish in the given farm. The priority measure is the improvement of animal health measures, water treatment with ozone, reduction of stocking density, vitamins and probiotics in feed, short-term bath in tosylchloramide (formerly Cloramin T, now Halamid) with draining the bath outside the RAS system.

The most common **parasitic infections** in pikeperch reared in RAS are infection with *Ichthyophthirius multifiliis* and *Gyrodactylus* sp. The parasites are diagnosed microscopically, and a long-term bath in formaldehyde has proven effective for their elimination, which is applied to the entire system - according to the literature and our experience, has no negative effect on biological filters.

If a **virological disease** of pikeperch is suspected, a virological examination can be performed for the presence of an unidentified virus (cytopathic effect on the tissue culture) or a rhabdovirus can be targeted directly using PCR analysis (according to our experience, this is currently only possible abroad). The solution to the problem of viral diseases in intensive pike breeding lies in the improvement of animal hygiene, feeding and, above all, the observance of preventive measures.

Key words: pikeperch (*Sander lucioperca*), recirculating aquatic system (RAS), diseases

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WILL WARM WINTERS MEAN ATYPICAL INCIDENCE OF DISEASES?

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Three case studies are described, two reporting not quite typical manifestation of diseases and one presenting an as-yet unexplained death of catfish.

In the first case, atypical behaviour of young breeding fish under the ice was observed in the first half of February 2024, followed by high mortality. The fish were congregating at the upper water layers along the edges of the pond. Initially, no fish kills were detected. The water temperature was 2 °C. The first comprehensive examination did not reveal the cause of this behaviour. After the thaw, clinical symptoms persisted, and the fish stock was in large part gathering at the shore showing signs of apathy. At a water temperature of 6 °C, additional gill samples were taken from 4 fish. The biochemical analysis of the water and the blood sampled excluded the possibility of ammonia auto-intoxication associated with toxic damage to the gills. However, all four gill samples were proved CEV-positive. This was the first detection of carp edema virus disease at the water temperature this low (2 °C), with clinical symptoms demonstrated already under ice cover. The total fish mortality exceeded 75 %.

In the second case study, there was a heavy infestation with *Ichthyophthirius multifiliis* in market carp detected in February after wintering. Considering the low water temperature (approximately 6 °C), the high intensity of parasite infestation was surprising, as its sharp increase along with increasing water temperature is typical for the agent with regard to its developmental cycle. This case was also accompanied by a very high mortality rate in the fish.

The subject of the third case study was death of catfish, both brood stock catfish reared in a pond and fish in a river, in May at a water temperature of about 20 °C. The fish in the river showed apathy together with loss of reflexes and moved close to the water surface, several individuals were found dead. In the first specimen delivered to our lab, an empty digestive tract and a full gall bladder were revealed, and one section of the intestine was obstructed with *Glanitaenia osculata* tapeworms (approx. 40 pcs). Monogeneans of the genus *Silurodiscoides* were present on the gills in medium intensity. Microbiological and virological examinations were negative. Similarly, an empty digestive tract and tapeworms present in the intestine, but in smaller quantities, were found in a surviving catfish from the pond. However, no tapeworms were detected in another catfish from the river and that is why the main cause of the catfish death remains unknown to us.

The 2023/2024 winter season had an average temperature of 2.4 °C, making it the second warmest after 2006/2007 (2.7 °C). February was the warmest month at 5.7 °C, rated exceptionally above normal, followed by December (2.1 °C, above normal) and January (−0.5 °C, normal). It is disputable whether the warm course of the winter could have played a fundamental role in the development of these seasonally atypical health problems, however, at the very least it could have acted as one of the negative factors. With regard to the temperature predictions for the future, it will probably be necessary to face such negative impacts.

Key words: case studies, carp edema virus, *Ichthyophthirius multifiliis*, *Glanitaenia osculata*

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FISH PARASITES IN SELECTED WATER SUPPLY RESERVOIRS

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Water supply reservoirs serve for ensuring water supplies for drinking water production, ensuring minimum flows under the dam, reducing culmination of flood flows and for production of energy by small hydropower plants. Water reservoirs are not part of fishing grounds. Their fisheries management uses so-called biomanipulation, where the fish stock is maintained and influenced by stocking suitable species of fish, removal of undesirable species and manipulating the water level in order to reduce the success rate of reproduction in some fish species. The abundance of plankton feeding cyprinid fish (e.g. common bream, common roach, common rudd) is decreased and predatory fish species (pike, zander, eel, etc.) are supported. This is called manipulated fish stock, whose composition affects the water quality in the reservoir.

University of Veterinary Sciences Brno (and their legal predecessor) has been dealing with the assessment of health status in fish from water supply reservoirs belonging to Povodí Moravy, s.p. since 1979. The reservoirs included are Bojkovice, Boskovice, Fryšták, Hubenov, Karolinka, Koryčany, Landštejn, Ludkovice, Mostiště, Nová Říše, Opatovice, Slušovice and Vír. The fish are caught by electrofishing or gillnets and transported live to a specialised lab at Department of Ecology and Diseases of Zoo Animals, Game, Fish and Bees at Faculty of Veterinary Hygiene and Ecology, where they are examined. The procedure is performed once a year, in late spring/summer.

The fish are euthanised, measured and weighed, and inspected macroscopically using a magnifying glass (skin, fins, gill cavity, gills, eyes, body openings). Their nutritional status is assessed. Scrapes from a half of the body and two gill arches are used for microscopical examination of the skin, fins and gills. This is followed by necropsy and examination of individual organs. Detected parasites are identified using specialized publications on the basis of their morphological traits and host specificity.

The results of parasitological examination of fish from water reservoirs Boskovice, Karolinka, Koryčany a Vír from 2007, 2011, 2015, 2019 and 2023 are further reported. With several exceptions the health status of the examined fish was considered as good or very good. The highest intensities of parasitological infections were determined especially in relation to gill ergasilosis.

Key words: *Ergasilus sieboldi*, fish stock, health, water reservoir

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HEALTH ISSUES IN RAS BRED AFRICAN CATFISH (*Clarias gariepinus*)

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The aim of the study was to examine the health status of farmed African catfish (*Clarias gariepinus*). The health assessment was conducted on three farms (two in the Czech Republic and one in the Slovak Republic). All farms use recirculating aquaculture systems. On each farm, at least 10 fish were sampled and subjected to the anatomical-pathological examination, haematological examination, parasitological examination of the gills and skin, bacteriological cultivation and histological examination of the gills, kidneys (head and trunk), liver and spleen.

On the first farm, skin lesions were observed in several fish, some of them (size approx. 3×4 cm) exposing the muscles and the head kidney. Additionally, there were small haemorrhages in the skin and fluid in the body cavity. Bacteriological cultivation proved high concentrations of *Aeromonas veronii* in one fish and *Aeromonas hydrophila* in another. Histological assessment showed a severe form of alteration in the kidneys (in 11 fish out of 28) and lamellar atrophy of gills, which were infected by Monogenea of *Quadricanthus* sp.

No macroscopic pathological changes were found by the necropsy of fish from the second farm. Bacteriological cultivation confirmed common bacteria on the gills (*Aeromonas caviae*, *Aeromonas veronii*, *Shewanella putrefaciens*, *Shewanella profunda*, *Citrobacter freundii*, *Hydrogenophaga pseudoflava* and *Proteus* sp.). Histological examination revealed granulomatous lesions in the head and trunk kidney of two fish.

On the third farm, organs of three fish showed macroscopic changes (atypical coloration of the organs, haematomas in the liver and nodular structures in the kidneys). Bacteriological cultivation proved only common bacteria on the gills (*Acinetobacter junii*, *Arthrobacter* sp., *Xanthomonas* sp., *Flavobacterium hibernum*, *Flavobacterium flevense*, *Aeromonas hydrophila*, *Pseudomonas* spp., *Sphingomonas* sp., *Chryseobacterium* sp., and *Sphingomonas* sp.). Relatively small foci of macrovesicular steatosis in the liver were detected by histological examination in five fish.

Serious health problems were revealed in the fish from the first farm, including changes in the organs, skin lesions and haemorrhages. No serious pathological changes were found in the fish from the second farm. Minor organ changes were revealed in several fish from the third farm.

Key words: African catfish (*Clarias gariepinus*), recirculating aquatic system (RAS), health issues

Acknowledgement: This study was funded by the project FVHE_Pikula_2024 ITA 24.

EFFECT OF BETA-GLUCAN ADDITIVE IN FEED ON HEALTH STATUS OF RAINBOW TROUT (*Oncorhynchus mykiss*) AFTER EXPERIMENTAL CHALLENGE WITH *Aeromonas salmonicida*

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Along with the rise in the intensity of fish farming, the risk of infectious disease outbreaks is also increasing. The most common infections are caused by bacteria. In salmonid farming, one of the most important diseases is furunculosis caused by *Aeromonas salmonicida*. Antibiotic therapy is usually the treatment of choice for controlling bacterial infections, but this carries a number of risks for product quality, human health and the environment. Therefore, preventive methods are used nowadays to limit the occurrence of infections, such as the addition of beta-glucans to fish feed. Beta-glucans are polysaccharide derivatives of plants, algae, fungi, bacteria and yeasts, which have a positive effect especially on the non-specific immune response in fish.

The aim of the experiment was to evaluate the effects of orally administered beta-glucans on juvenile rainbow trout (*Oncorhynchus mykiss*). The fish were divided into four groups with different concentrations of beta-glucan addition in the feed (0%, 0.2%, 0.5% and 1%). After eight weeks (a rearing part of the experiment), all groups of the fish were experimentally infected with *A. salmonicida* by immersion bath and monitored for another 4 weeks (an infectious part of the experiment), while they were still provided supplemented feed. Mortality, changes in fish behaviour and feed intake were assessed throughout the experiment. Both before the experimental infection and at the end of the experiment, blood was withdrawn for haematological, biochemical and immunological analyses, and body surface mucus was collected for lysozyme determination. After euthanasia of the fish, biometric parameters were determined and the intestine was removed for metagenomic analysis of the gut microbiome.

A non-significant increase in phagocytic activity was revealed in all fish groups at the end of the infectious part compared to the end of the rearing part of the experiment. Only 3 parameters (Leu, MCV, MCH) changed in the haematological and biochemical examinations at the end of the feeding part. At the end of the infectious part within the biochemical parameters, statistically significant differences in (phosphorus, total protein, triacylglycerids) were observed and results of haematological examination at the end of the infectious part did not show statistically significant differences in any of the measured parameters. The level of specific antibody titre and lysozyme activity were determined with no statistically significant changes. Metagenomic analysis of the composition of the gut microbiome is still ongoing.

Key words: beta-glucans, *Aeromonas salmonicida*, rainbow trout (*Oncorhynchus mykiss*)

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THE ANALYSIS OF KOI HERPESVIRUS DISEASE OUTBREAK IN KOJETIN IN CZECH REPUBLIC

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Koi herpesvirus disease (KHVD) is causing significant losses in common carp (*Cyprinus carpio*) and koi carp (*Cyprinus rubrofasciatus*) aquaculture worldwide. The disease caused by *Cyprinus carpio* herpesvirus (KHV) is highly infectious and spreads rapidly by water, direct contact with infected fish and also fishing equipment. Surviving fish are also considered a potential source of infection, due to occurrence of latent infections. According to the recent changes in the European legislation, KHVD is classified as a category E disease, so active surveillance is no longer carried out in Czech Republic. Additionally, only a location showing active mortality is now considered as an outbreak site and stamped out by the Veterinary Administration, regardless of the actual presence of the virus. This situation allowed us to study the long-term presence of KHV in the Czech pond aquaculture for the first time in almost 20 years.

The aim of our work was to monitor the persistence of KHV in a single common carp aquaculture pond (7,5ha) in the Olomouc region. The occurrence of KHVD was initially confirmed in the late summer of 2023, with mortalities sharply declining after two weeks, in correspondence with rapid decrease of air and water temperature. In order to verify whether the infection could be still present in the pond, we performed several additional samplings, namely in October 2023, November 2023 and in April 2024 using both molecular and serological methods.

From the group of ten common carps tested in October 2023, real time PCR confirmed the presence of KHV in three samples. A high antibody response was detected in each sample. In November 2023 three common carps and one koi were tested. We did not confirm any positive sample using real time PCR but a high antibody response was detected again in each sample. A group of 16 common carps was tested using real time PCR in April 2024 and only one sample was weakly positive. We tested 15 samples of common carps using ELISA method and we received a significant antibody response after 9 months from the beginning of infection.

This study confirmed the long-time perseverance of the virus in the common carp farmed in earth ponds typical for Central and Eastern Europe.

Key words: koi herpesvirus disease (KHVD), common carp (*Cyprinus carpio*), koi carp (*Cyprinus rubrofasciatus*)

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DIFFERENTIATION OF THE CLOSELY RELATED *Myxobolus tihanyensis* N. SP. AND *Myxobolus sandrae* REUSS, 1906

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Traditionally, the taxonomic descriptions of myxozoans are based on the morphology of the spores, together with biological features such as host specificity and tissue tropism. However, most features overlap, causing difficulties with species identification even if spores develop in the same organs or tissues of phylogenetically closely related fish hosts. The integrated application of molecular biological and phylogenetic analyzes can significantly facilitate the identification of taxonomic species, especially if it exceeds the limits of morphological distinction.

The present study conducted the morphological and molecular characterisations of a muscle-infecting *Myxobolus* species of *Perca fluviatilis* and compared it with *Myxobolus sandrae* REUSS, 1906 previously detected in phylogenetically closely related fish hosts (*Sander lucioperca* and *S. volgensis*). Fish specimens of perch, and pikeperch or Volga pikeperch were collected from Lake Balaton. Myxospores isolated from plasmodium situated in muscles in fresh and Lugol-stained smears were characterized morphologically and morphometrically, then preserved in ethanol for molecular analysis. A piece of the infected tissues was prepared for histological study.

The autopsy of the European perch revealed small plasmodia attached to the connective tissue in the muscle in various regions of the fish body, such as near the vertebral column or the base of the fins. However, the fish showed no abnormalities, lesions or deformities that could be related to the parasite. Morphological and morphometrical measurements indicated that myxospores isolated from the European perch could not be distinguished *M. sandrae* that was earlier recorded to cause infection of musculature in pikeperch (and in Volga pikeperch also demonstrated by our result). On the other hand, the molecular phylogenetic analysis of the 18S rDNA sequences confirmed that the myxosporean parasite infecting the European perch, and named *Myxobolus tihanyensis* n. sp. is different definitely from the *M. sandrae* species.

In summary, the *M. tihanyensis* n. sp., from the European perch and *M. sandrae*, inhabiting the pikeperch and Volga pikeperch possessing morphologically similar myxospores, could be merely differentiated by molecular data. The two species belong to phylogenetic sister groups and are the closest relatives of each other to date.

Our results support the significance of molecular markers combination with morphological analysis can play a crucial role in revealing the diversity of myxozoans.

Key words: Myxozoa, *perca fluviatilis* (*European perch*), morphological and molecular characterization

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CURRENT SITUATION OF AQUATIC FARMS IN ALBANIA BASED ON BIOSECURITY CONCEPTS

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The Albanian aquaculture sector is growing very fast in the last years. Mostly, this grownup is in marine aquaculture than in freshwater fishes. The total production is almost 16,000 tons/year, consisting of marine fish 79 % and rainbow trout 21 %. There has been a significant increase in trout production in the past few years, from 600 tons in 2017 to about 3,400 tons in 2023. There are currently 22 trout farms, which they produce for Albanians consumption on the domestic market and mostly for export to other third countries of the Balkans like Serbia, Bosnia Hercegovina, etc. Taken in consideration the freshwater fish's production, regarding our survey which was done during year 2023, we have included two farms which produce 2,850 tons/year or approx. 83% of the total rainbow trout produced in Albania.

Through this study we present a view of the current stage of aquatic farms in Albania regarding the concept of biosecurity and we mention that even in our days they have not reported outbreak cases. Considering the biosecurity, a very important pillar and also the lack of international biosecurity standard we have made and used a questionnaire with a question fund. We carefully examined the steps with a group of questions: the measures that the working staff do every day, the infrastructure and all veterinary intervention related with disinfections and medical treatment.

From all the visits we have made to the farms, it has resulted that disinfectants that are mostly used are as below: Inorganic chlorine compounds, Hth chlorine, Virkon, Chlorides and benzethonium chloride, Alcohols, Hydrogen peroxide. Good husbandry, good water quality/chemistry, nutrition, good preventive medical practices are some critical points to maintain high level of biosecurity on aquaculture farms

Referring to the fact that for years since these farms were established, there have been no outbreaks of infection or mass death, it undergoes that biosecurity measures work and yield result.

The amount of different veterinary prepareate used on these farms must be considered as in the long term we may have problems regarding the resistance concept or presence of the different types of pathogens, but for different reasons such as strain size or their virulence cannot create health problems.

There is an increased interest in farms to obtain detailed information from the veterinary service regarding the disease, measures and treatment of fish that are carried out on the farm.

Key words: aquaculture, farms, biosecurity, disinfectants, outbreaks

TEMPERATURE-DEPENDENT DICLOFENAC CYTOTOXICITY IN CULTURED CARP LEUKOCYTES

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Diclofenac is a drug commonly used in human and veterinary medicine for the treatment of diseases associated with inflammation and pain. Medicinal products enter waste and surface waters on an everyday basis and contaminate the aquatic environment. Fish are therefore permanently exposed to these chemicals dissolved in their aquatic environment. To simulate variable environmental conditions, the aim of our study was to examine adverse effects of diclofenac under different temperatures of cell incubation (18, 21, 24, 27 and 30 °C). Cytotoxic and cytostatic effects of diclofenac in concentrations of 0.001 µg/ml, 0.01 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml and 100 µg/ml for the carp (*Cyprinus carpio*) cultured leukocytes were quantified using detection of lactate dehydrogenase released from damaged cells. Overall diclofenac cytotoxicity was relatively low and its impact was pronounced at higher temperature and diclofenac concentration. Cells growth inhibition is changing more rapidly but it is high mainly at the highest concentration from low temperature. CYP450 increased diclofenac cytotoxicity only at the high concentration. Leukocyte viability is essential for immune functions and any change can lead to reduction of resistance against pathogens, mainly in cold year seasons, when the immune system is naturally suppressed.

Key words: diclofenac, cytotoxicity, cell culture

EFFECTS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS DICLOFENAC AND IBUPROFEN ON SELECTED INDICES IN COMMON CARP

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Non-steroidal anti-inflammatory drugs (NSAIDs), especially diclofenac (DCF) and ibuprofen (IBP) are the most commonly and regularly detected drugs in aquatic ecosystems. In addition to being abundant in the abiotic matrix of the aquatic environment, they can also be detected in the biota. There are numerous ecotoxicological reports on NSAIDs causing various deleterious effects in fish. It was summarized that exposure to these contaminants can affect physiological functions and processes in exposed fish, sometimes even at environmentally relevant concentrations.

Our study aimed to evaluate the effects of diclofenac and ibuprofen on selected parameters of freshwater fish – common carp (*Cyprinus carpio*). The test substances were administered in feed and the exposure period was six weeks. Either substance was tested at two concentrations, 20 or 2000 µg/kg, resulting in four different treatments (DCF 20, DCF 2000, IBP 20, IBP 2000). The potential toxicity of both substances was investigated comprehensively by using analyses of a wide range of biomarkers on cellular, molecular, and organ levels.

Carp exposure to high concentrations of NSAIDs (DCF 2000, IBP 2000) elicited numerous statistically significant changes ($p < 0.05$) in the endpoints investigated, with DCF being almost always more efficient than IBP. Compared to control fish, a decrease in total leukocyte count attributed to relative lymphopenia was observed. Plasma concentrations of total proteins, ammonia, and thyroxine, and enzyme activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were significantly elevated in either group, as were the activities of certain hepatic antioxidant enzymes (superoxide dismutase, glutathione-S-transferase) in the DCF 2000 group. The transcriptomic profile of selected genes in the tissues of exposed carp was affected as well. Significant changes in plasma total proteins, ammonia, ALT, and ALP, as well as in the transcription of genes related to thyroid function and the antioxidant defense of the organism, were found even in fish exposed to the lower DCF concentration (DCF 20).

Our research shows that long-term dietary exposure to both non-steroidal anti-inflammatory drugs IBP and DCF can significantly impair the physiological functions of common carp. To the best of our knowledge, this research is unique in its type of field, as other studies have focused primarily on exposure through the water.

Key words: diclofenac, ibuprofen, common carp (*Cyprinus carpio*), toxicity

ANTIMICROBIAL SUSCEPTIBILITY AND POPULATION STRUCTURE OF *Flavobacterium psychrophilum* ISOLATED IN CZECH FISH FARMS

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The salmonid pathogen *Flavobacterium psychrophilum* poses a significant economic threat to global aquaculture. Its control mainly relies on antibiotic treatments. This necessarily leads to the development of antibiotic resistance. Epidemiological analyses characterising the pathogen population may help to identify the most frequent routes of transmission of the pathogen and thus allow to introduce preventive measures. However, these data are still lacking in the Czech Republic, as well as in the whole region of Central Europe.

Therefore, in vitro activities of 8 selected antimicrobial agents against 70 *F. psychrophilum* isolates obtained from salmonid fish farms in the Czech Republic between 2012 and 2019 were determined using standard broth microdilution method. To better understand the population structure of this bacterium and the routes of its transmission, we have further examined the isolates using Multi-Locus Sequence Typing (MLST) and multiplex PCR (mPCR) serotyping. The results indicate that there is a high proportion of isolates with reduced susceptibility to enrofloxacin (100.0 %), flumequine (90.0 %), oxytetracycline (88.6 %) and oxolinic acid (75.7 %). Notably, all isolates classified as susceptible to oxytetracycline had minimal inhibitory concentration (MIC) at the calculated breakpoint for susceptibility. Sulfamethoxazole-trimethoprim testing showed reduced susceptibility in 32.9 % of isolates and erythromycin testing in 4.3 %. Florfenicol is currently considered the first choice of therapy in the management of *F. psychrophilum* infections. A total of 84.3 % of isolates were classified as susceptible. Surprisingly, 44.3 % of the isolates had MICs at the calculated breakpoint, suggesting that resistance to oxytetracycline and florfenicol may be developing in fish farms.

The results of mPCR serotyping revealed a diverse distribution among the serotypes, with most isolates classified as Type 1 (61.4 %), and Type 2 (32.9 %). Types 3 and 4 were detected only sporadically.

MLST revealed 12 sequence types (STs) among the isolates tested, 7 of which were newly described. Since these 7 STs have not been identified in other countries, they appear to be geographically specific to the Czech Republic. The most common sequence type was the recently described ST329 (34.3 %). The second most common was ST2, which is also the most common sequence type in other European countries. Our data from MLST and mPCR analysis show changes in serotype/sequence type prevalence over time, suggesting that the introduction of new strains plays a large role in recurrent outbreaks.

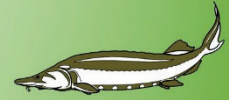
Our research provided important data about the structure and changes of the pathogen population over time in the Czech Republic. Our findings underscore the necessity of tracing *F. psychrophilum* clones, both within and between countries, using tools such as MLST and serotyping.

Key words: *Flavobacterium psychrophilum*, antibiotic resistance, Multi-Locus Sequence Typing (MLST), multiplex PCR (mPCR) serotyping

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DEPARTMENT OF FISHERIES AND HYDROBIOLOGY

The Department of Fisheries and Hydrobiology at the Faculty of Agriculture of Mendel University in Brno has been providing teaching and research since 1949.

The education of students focuses on freshwater fisheries (aquaculture and fish health, ichthyology, management of open waters) and the related hydrobiological issues, including ecotoxicology.

The studies in fisheries and hydrobiology are part of the three-year bachelor program and two-year master studies program. Graduated students are also eligible to apply for the PhD studies.

Research activities are focused on fish breeding (development of intensive fish culture technologies in controlled conditions), monitoring of the changes in aquatic environments due to anthropogenic activities (heavy metal contamination, contamination of freshwaters by cyanobacterial blooms, eutrophication of surface waters), and the study of natural fish populations. The students are closely involved in solving these issues during the writing of their final paper for the Bachelors studies, their Master's and PhD thesis.

The department is equipped with:

- a recirculation system for fish farming (accredited experimental facility)
- a hydrobiological laboratory which provides analyses of aquatic invertebrates
- a hydrochemical and toxicological laboratory that provides analyses of water and sediments
- equipment for sampling and basic processing of fish blood and tissues
- equipment for fish sampling in open waters (electrofishers, nets)





UNIVERSITY OF VETERINARY SCIENCES BRNO

FACULTY OF VETERINARY HYGIENE AND ECOLOGY

The Faculty of Veterinary Hygiene and Ecology (FVHE) is one of the two faculties of the University of Veterinary Sciences Brno, which as the only university in the Czech Republic educates future veterinarians and not only them. The FVHE specializes in veterinary hygiene, food safety and quality, veterinary public health, animal protection and welfare, environmental protection, and ecological aspects of all these areas.

Veterinary education at the university is bound by a more than 100-year tradition and the Faculty of Veterinary Hygiene and Ecology has been included in the prestigious list of international educational level assessments of the European Association of Establishments for Veterinary Education (EAEVE) for more than 20 years, which clearly demonstrates the quality of the education provided and ranks the faculty among the best that any European veterinary school offers to students.

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Moravian Fishing Union

The Union aims to:

- Gather people for the performance of fishing rights
- Get involved in nature and environment protection
- Get involved in out-of-school education of children and young people

Within its mission, the Union:

- Properly manages fishing grounds
- Protects fishery in fishing grounds and ponds
- Strives for water quality and environmental sustainability of aquatic communities
- Produces fish stocks for stocking of fishing grounds
- Enables its members to participate on the performance of fishing rights, mainly in sport fishery, and cultivates their interest in fishing
- Recruits and organizes young people for leisure activities
- Participates in the development and popularisation of sport fishing

Union membership:

- Currently, membership includes more than 76 thousand members including nearly ten thousand children and young people under the age of 15

Managed fishing grounds:

- 237 non-trout grounds with a total area of 6 803.6 ha
Largest fishing grounds:
 1. Dyje 5 (Dam Nové Mlýny) 1 600 ha
 2. Dyje 15 (Dam Vranov) 537 ha
 3. Dyje 7 (Dam Mušov) 530 ha
- Trout grounds – 77 with a total area of 491,2 ha
- Educates children and young people in leisure clubs organized by local branches of the Union
- Organizes summer fishing camps for children and youth
- Organizes training courses, basic examinations and regular re-examinations to obtain qualification for operating electro-fishing gear
- Organizes training courses and examinations for obtaining qualifications to perform the function of fishery manager and the representative of fishery manager of the fishing grounds
- Organizes training courses and examinations for obtaining qualifications for appointment to the position and performance of the function of a member of the fishing guard
- Organizes training courses and examinations for obtaining qualifications to perform the function of fishery manager and the representative of fishery manager of the fishing grounds
- Organizes training courses and examinations for obtaining a certificate of qualification for issuing a fishing license

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