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ABSTRACT BOOK

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ORAL PRESENTATIONS

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 the 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

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).

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 ± 1,3 g and total length of 102,7 ± 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).

CYPRINID BIOSECURITY - CURRENT KNOWLEDGE

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Common carp (*Cyprinus carpio*) is the most important farmed fish in Central and Easter 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 aspect 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 and 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 the 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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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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POSTER PRESENTATIONS

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

Acknowledgement: Supported by project FVHE_Pikula_2024 ITA 24.

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

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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 rubrofuscus*) aquaculture worldwide. The disease caused by *Cyvirus cyprinidallo* (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 rubrofuscus*)

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