Final report

Please note that papers published within the frameshift of the present grant acknowledge K140348 or K127916 grant, since its number was changed when our Institute separated from the Centre for Agricultural Research.

Detailed results of the virus discovery/characterization part of the project are the following:

In the early spring of 2018, in Lake Balaton, a roach (*Rutilus rutilus*) and an asp (*Leuciscus aspius*) were found in an fish trap at the estuary of River Sió showing typical signs of the so called carp pox disease, such as foci of epidermal hyperplasia on the head and the whole body surface including the fins. The molecular investigations revealed the presence of the DNA of a yet unknown fish herpesvirus. Three genes encoding the DNA-dependent DNA polymerase, major capsid protein and ATPase subunit of terminase were amplified and sequenced from the alloherpesviral genome. The gene sequences of the viruses obtained from the two different fish species shared 94.4% nucleotide identity (98.1% amino acid identity) suggesting that they belong to the same virus species. Phylogenetic tree reconstruction based on the DNA polymerase (and the concatenated sequences of the amplified genes as well) implied that the detected virus obviously belongs to the genus *Cyprinivirus* within the family *Alloherpesviridae*. The sequences of the novel alloherpesvirus diverge from those of the five cyprinivirus species described previously, so it is putatively representing the sixth virus species in the genus. (Published: Boglárka Sellyei, Ferenc Baska, Ádám Varga, Réka Borzák, Andor Doszpoly (2020) Molecular detection of a novel cyprinid herpesvirus in roach (*Rutilus rutilus*) and asp (*Leuciscus aspius*) showing typical signs of carp pox disease. Arch Virol 165(7): 1569-1576)

The complete genome characterization of the CyHV-5 was also carried out. Using next generation sequencing method, the genome of CyHV-5 was completely sequenced and analysed. The complete genome sequence proved to be 242,925 bp in size containing 151 predicted protein-coding regions, including 12 ORFs that seems to have a homologue in every alloherpesvirus genome sequenced to date. The genome organization of the CyHV-5 shows similarity to that of the other CyHVs. The whole genome analysis confirms the formerly established taxonomic position of CyHV-5. (The manuscript will be submitted soon to the Archives of Virology journal.)

As for other alloherpesviruses, two adult barbels (*Barbus barbus*) with visible skin tumours were subjected to histopathological and molecular examinations. The fish were caught in the River Danube near Budapest. Papillomas were found around their oral cavity, at the operculum and at the pectoral fins, while epidermal hyperplasias were seen on the body surface. *Cyprinid herpesvirus 1* (CyHV-1) was detected in the kidney of the specimens by PCR, and *Barbel circovirus 1* (BaCV1) was found in all internal organs and in the tissues of the tumours. The whole genome of BaCV1 and three conserved genes from the genome of CyHV-1 were sequenced. Previously, BaCV1 had been reported only once from a mass mortality event among barbel fry. The whole genome sequence of our circovirus shared 99.9% nucleotide identity with that of the formerly reported BaCV1. CyHV-1 is known to infect common carp and coloured carp, and has been assumed to infect other cyprinid fish species as well. We found the nucleotide sequences of the genes of CyHV-1 to be identical in 98.7% to those of the previous isolates from carp. To the best of our knowledge, this is the first molecular confirmation of the presence of CyHV-1 DNA in cyprinid fish species other than carp (Published: Borzák R, Sellyei B, Baska F, Székely C, Doszpoly A (2020) Detection of cyprinid herpesvirus 1 (CyHV-1) in barbel (*Barbus barbus*): First molecular evidence for the presence of CyHV-1 in other fish than carp (*Cyprinus carpio*). Acta Vet Hung 68(1): 112-116).

We also revealed the presence of a novel herpesvirus (HV) in the specimen of a wels catfish (*Silurus glanis*) presenting disseminated pox-like dermal lesions all over its body. A broad-range PCR, designed for the detection of large DNA viruses, gave positive result. The sequence analysis of the 463-bp PCR product from the viral DNA polymerase gene indicated the presence of a hitherto unknown virus, a putative member of the family *Alloherpesviridae* in the sample. With specific primers, designed according to the genomic maps of the cyprinid and anguillid HVs, a genomic fragment of 15 kb was also amplified and sequenced by primer walking. In phylogeny inferences, based on several genes, the putative wels catfish HV clustered closest to various cyprinid HVs or to AngHV-1. The novel virus, named as silurid herpesvirus 2, represents a distinct species in the genus *Cyprinivirus*. However, its association with the skin disease remains unclear. (Published: Tarján Z, Doszpoly A, Eszterbauer E, Benkő M (2022) Partial genetic characterisation of a novel alloherpesvirus detected by PCR in a farmed wels catfish (*Silurus glanis*) presenting skin lesions. Acta Vet Hung 70: 321-327)

Complete genome sequencing of the silurid herpesvirus 2 has been carried out as well by next generation sequencing (Illumina). Majority of the genome (approximately 95%) has been assembled, filling of the gaps by PCRs is in the pipeline.

Besides the formerly mentioned alloherpesvirus, a novel papillomavirus (PV) was detected in farmed wels catfish in Hungary, showing clinical signs that resemble to those of the wels catfish herpesvirus disease. The whole genome of the Silurus glanis papillomavirus 1 (SgPV1) was identified using next-generation sequencing. The 5,612-bp complete genome contains four predicted protein-coding regions (E1, E2, L1 and L2), which seem to have homologues in every PV genome sequenced to date. Five complete fish PV genomes are available in the GenBank. Their genomes range between 5,748 and 6,086 bp comprising the minimal PV backbone genes E1-E2-L2-L1, unlike PVs of higher vertebrates having larger genomes (6.8-8.6 kbp) and additional (onco)genes. Considering the actual species demarcation criteria for the family *Papillomaviridae,* the establishment of a novel species named *Nunpapillomavirus siluri* is proposed for the SgPV1 in a novel genus *Nunpapillomavirus,* under the subfamily *Secondpapillomavirinae*. (Published: Surján A, Fónagy E, Eszterbauer E, Harrach B, Doszpoly A (2021) Complete genome sequence of a novel fish papillomavirus detected in farmed wels catfish *(Silurus glanis*). Arch Virol 166:2603-2606)

Regarding the pathology of SgPV1, further studies are needed to ascertain whether SgPV1 can cause any clinical signs or disease alone, or it could be found always in co-infection with wels catfish herpesvirus. Concurrence of fish PVs with other DNA viruses in gilt-head seabream was previously reported. We have carried out a retrospective monitoring on samples originating from Hungarian fish farms from the last 7 years. The preliminary results showed high prevalence, 42% for SgPV1 and 20% for silurid herpesvirus 2. It seems that the 77% of the herpesvirus infected individuals also carry the SgPV1. (Manuscript preparation is in the pipeline.)

Poxviruses of fish can cause severe economical losses. One of them is the salmon gill poxvirus endemic in Northern Europe, and the carp edema virus is worldwide distributed causing substantial losses in aquaculture. No other poxvirus originating from fish was known until we have discovered a novel poxvirus in black bullhead (*Ameiurus melas*) in Lake Balaton. Approximately 200 juvenile black bullhead were caught and transferred to our animal facility showing no signs of any diseases. After the arrival, FMC (formalin, malachite green, methylene blue) treatment at 0.6-0.75 ml/50-L concentration was conducted for three consecutive days to prevent the proliferation of ectoparasites. One week later an outbreak of mass mortality happened, within a few days all animal died. Specimens were free of all known catfish viruses (ictalurid herpesviruses, European catfish virus, etc), however a novel poxvirus was detected by consensus PCR. Phylogenetic tree reconstruction based on the DNA

polymerase implied that the detected virus obviously belongs to the family *Poxviridae* and probably represents a novel genus. (Manuscript preparation is in the pipeline.)

Very recently we were able to show the presence of the Siberian sturgeon herpesvirus firstly in Hungary, causing heavy losses in a fish farm among Siberian sturgeon (*Acipenser baeri*) and Russian sturgeon (*Acipenser güldenstaedti*). Further investigations are in the pipeline.

Primary cells were successfully developed and stored in liquid nitrogen from all the fish species listed in the work plan of the grant proposal. Eight of the primary cells (NPL-3; northern pike larvae cells, NPF-1 northern pike fin, PF-1; perch fin, AF-1 asp fin, TF-2 tench fin, CCG-7 common carp gill, RL-10 roach larvae and RF-2 rudd fin) have been immortalized, and became permanent cell lines. Their characterization (growth curves, chromosome analysis) have been carried out. The NPL-3 cell line is susceptible for at least three virus species: pike fry rhabdovirus (PFRV), European catfish virus (ECV), ictalurid herpesvirus 2 (IcHV-2). The ECV and IcHV-2 were able to replicate in the PF-1 cell line as well. While the AF-1 line were permissive for all of them and the spring viremia of carp virus as well. The investigations for the susceptibility of the rest of the cell lines are in the pipeline. These newly established cell lines could be used as a diagnostic tool for viral diseases in these fish species and also for the isolation and study of novel emerging freshwater fish viruses in the future. For example, the attempts for the isolation of the CyHV-5 originating from roach (see above) in the RL-10 cell line are in the pipeline. (After finishing the rest of the investigations, manuscript will be submitted to the Acta Veterinaria Hungarica.)

In the frameshift of an informal collaboration with German colleagues, we are examining the role of the JUN-related oncogenes, encoded by cyprinid herpesvirus 1, in the development of skin tumours. Usually CyHV-1 is able to cause only epidermal hyperplasias, however it was already reported in skin tumours. We were testing koi skin tumours for presence of the CyHV-1 and 35 of 79 tumours were highly positive for CyHV-1, but when we started to measure the mRNA expression of oncogenes JunB, JunC and JunD (these gene products are considered as the putative agents in the development tumours) we were able to find their expression only in four skin tumours. We commenced the complete genome investigation of these isolates without JUN genes, and we found some other genes, instead of the JUN genes, which might have role in the oncogenesis. A homolog of the human CD276 was found, which is preferentially expressed only in tumour tissues. This protein may participate in the regulation of T-cell-mediated immune response. It may play a protective role in tumour cells by inhibiting natural-killer mediated cell lysis. Another gene was found,

which contains an N-terminal immunoglobulin-like domain of T-cell surface antigen. It was reported as an carcinoembryonic antigen-related cell adhesion molecule 1-like isoform in *Carassius auratus.* mRNA expression study of these genes is in the pipeline. (Joint paper with the German colleagues is expected to be ready for the next year.)

Also, the potential possibility of the vertical transmission of the CyHV-1 was investigated. During the insemination procedure eggs of common carp were infected with CyHV-1. Three groups of eggs were included in the experiment. One control (uninfected), one infected, and one which was treated with divosan forte (DF) for disinfection of the surface of the eggs after the infection. Overall, CyHV-1 was detectable from eggs in both DF treated and untreated infected groups, but not from the fry. The sequencing results confirmed that our experimental virus strain is the one that infected the eggs. Compared to the negative control, there was no difference in the number of non-hatched eggs. There was some delay in the hatching between the groups (a few hours difference), but all groups hatched within a day. The control group started to hatch in the morning, then the DF treated infected group did it, and finally the untreated infected group started to hatch in the afternoon. No differences were seen between the groups, with only 1-2 mortalities, which was negligible considering the large number of individuals (about 2400-3000 per group in 3-3 parallel clutches). One week after hatching, the infected group seemed to be more lethargic, with some mortality, but this was not observed in the following days. (Manuscript preparation is in the pipeline.)

We also carried out the characterization of some fish viruses from abroad. In 2015 an episode of lymphocystis disease (LCD) was detected in wild and cultured populations of whitemouth croaker (*Micropogonias furnieri*) at the coast of Uruguay. Fish of both origins were collected to our laboratory for histopathological and molecular investigations. Macroscopically, multinodular tumorlike masses were observed in the skin. Histological examination of these masses revealed enlarged cells with a hyaline capsule and basophilic inclusion bodies in the cytoplasm. The inclusion bodies were further examined by electron microscope showing icosahedral virions, with a median diameter of 182 nm. The routine molecular investigations targeting the DNA polymerase and major capsid protein genes showed the presence of the DNA of an unknown lymphocystis disease virus (LCDV) in all specimens showing the external signs of LCD. Subsequently, four other core genes were amplified and sequenced from the viral genome. Phylogenetic tree reconstruction, based on the concatenated sequence of six core genes, implied that the virus undoubtedly belongs to the genus *Lymphocystivirus*. However, the core gene sequences of the whitemouth croaker LCDV differ markedly from those of the three known LCDVs; putatively representing the fourth

LCDV species. (Published: Perretta A, Doszpoly A, Puentes R, Bessonart M (2020) Diagnosis of lymphocystis disease in a novel host, the whitemouth croaker (*Micropogonias furnieri*), associated with a putatively novel Lymphocystivirus species (LCDV-WC). Dis Aquat Org 137 (3): 185-193).

We have continued the work on this novel lymphocystivirus. In this part of the research, using next generation sequencing method, the whole genome of the above-mentioned virus has been sequenced and analysed. The complete genome sequence proved to be 211,086 bp in size containing 148 predicted protein-coding regions, including the 26 core genes which seem to have a homologue in every iridovirus genome sequenced to date. Considering the actual species demarcation criteria for the family *Iridoviridae* (genome organization, G+C content, amino acid identity and phylogenetic relatedness of the core genes), the establishment of a novel species (*Lymphocystis disease virus 4*) in the genus *Lymphocystivirus* was suggested, and the International Committee on Taxonomy of Viruses have accepted our proposal. (Published: Doszpoly A, Kaján GL, Puentes R, Perretta A (2020) Complete genome sequence and analysis of a novel lymphocystivirus detected in whitemouth croaker (*Micropogonias furnieri*): *Lymphocystis disease virus 4*. Arch Virol 165(5): 1215-1218).

Adenoviruses are commonly found in members of almost every vertebrate lineage except fish and amphibians, from each of which only a single isolate is available as yet. In this work, the complete genomic sequence of a fish adenovirus, originating from the white sturgeon (*Acipenser transmontanus*), was determined and analyzed. Several exceptional features were observed including the longest hitherto known genome size (of 48,395 bp) and a strange location of the putative fiber genes resulting in an unconventional organization pattern. The left genome end contained four fiber-like genes, three of them in a tandem position on the r (rightward transcribed) strand, followed by a fourth one on the l strand. Rightward from these, the conserved adenoviral gene cassette, encompassing 16 family-common genes, was identified. In the right-hand part, amounting for more than 42% of the entire genome, the presence of 28 ORFs, with a coding capacity of larger than 50 amino acids, was revealed. Interestingly, most of these showed no similarity to any adenoviral genes except two ORFs, resembling slightly the parvoviral NS gene, homologues of which occur in certain avian adenoviruses. These specific traits, together with the results of phylogeny reconstructions, fully justified the separation of the white sturgeon adenovirus into the recently established new genus *Ichtadenovirus*. Targeted attempts to find additional adenoviruses in any other fish species were to no avail as yet. Thus the founding member, WSAdV-1 still remains the only representative of ichtadenoviruses. (Published: Doszpoly A,

Harrach B, LaPatra SE, Benkő M (2019) Unconventional Gene Arrangement and Content Revealed by Full Genome Analysis of the White Sturgeon Adenovirus, the Single Member of the Genus *Ichtadenovirus*. Infect Genet Evol 75:103976)

Detailed results of the prototype DNA vaccine development part of the project are the following:

We had some unexpected hindrance with the large-scale animal experiments, which has decelerated our progress. Two breeding seasons (hence two years) have fallen out. Due to the Covid pandemic the spring of 2020, and in another case the budget of the present grant was not available from March till September of 2022 due to re-organization of our Institute.

At the beginning, for the development of prototype DNA vaccines we chose the European catfish virus (ECV) for the following reasons: According to the literature good immune response were gained by DNA vaccines against other ranaviruses. Secondly, it is easy to work with this virus. Thirdly, ECV is responsible for heavy losses in wels catfish (*Silurus glanis*) farming in European countries. And according to the plans of the Hungarian Department of Agriculture, a significant development and strengthening of the wels catfish production and fishery is expectable in the near future. Our ECV strain was isolated from black bullhead (*Ameiurus melas*), we decided to use this species in the animal experiments (smaller size than wels catfish, no cannibalism).

We have developed three DNA vaccine constructions. The backbone of the construct was the pcDNA3.1 plasmid, containing the major capsid protein (MCP) or the ORF2 or ORF39 of the ECV (all of these genes proved to be efficient against giant salamander ranavirus). Subsequently, *in vitro* transfection experiments were carried out in order to check the expression level of the above-mentioned plasmids. The intensity of the expression of the MCP and ORF2 was similar, however the ORF39 was expressed two order magnitude less. Hence this latter construct was excluded from the following experiments. Complete genome sequence of the black bullhead is not available in the GenBank, hence we had to amplify (by consensus primers designed to closely related fish species) and sequence its immune genes (Mx1, MHC-I, MHC-II, Il-8, INFa, IgM, TNFa, ZAP70), which we wanted to monitor after delivering the DNA vaccine. Then, in small scale animal experiment (three fish/group; 4 groups: MCP, ORF2, empty pcDNA3.1, PBS), the gene expression induced by the DNA vaccine candidates was investigated by real-time RT-PCRs. Seven and 16 days post intramuscular injection (25 μg DNA/fish), blood samples were collected and investigated.

The results were encouraging, in case of both candidates we could observe significant overexpression of some immune related genes involved in both the innate and adaptive immune response. In small scale animal experiments the optimal challenge method was also determined (water bath for 48 hours, $TCID_{50}$/ml=1,78x10^4). Then large scale (n=25/group) vaccination and virus challenge experiments were planned. The juvenile bullheads were obtained from Lake Balaton (fish farms do not breed bullhead species in Hungary). Fish were treated by FMC (formalin, malachite green, methylene blue) at 0.6-0.75 ml/50-L concentration for three consecutive days to prevent the proliferation of ectoparasites. However, bacterial and other viral diseases (poxvirus, herpesvirus) emerged in the fish stock causing almost 100% mortality in two consecutive years. Then we decided to artificially inseminate the black bullhead, for this we asked the help of colleagues of the MATE Institute of Aquaculture and Environmental Safety. Induced maturation and ovulation of black bullhead from an intensive system was achieved with repeated carp pituitary treatment (5 mg carp pituitary/kg body weight/24 h, n = 3-6 times). With the same treatment, it was not possible to induce a larger amount of sperm production from the males, sperm collection could be solved by surgery on the testicle. On one occasion, it was possible to achieve fertility and raise small number of offspring from spawning. Further studies are necessary to explore the reproductive biological characteristics of mother fish prepared under intensive rearing conditions and to develop their induced reproduction.

After this we decided to change the host species to wels catfish. Some Western-European strains of ECV isolated from black or brown bullhead were reported to induce high mortality rates in wels catfish as well. There have not been previous reports about the infectivity of any Hungarian ECV isolate and exact information about the presumed/probable age dependent mortality. So, we designed and carried out some experiments to investigate the effect of Hungarian ECV on wels catfish at different age. The results of our study demonstrate that an ECV strain isolated from bullhead in Hungary could cause devastating losses among juvenile wels catfish. Furthermore, the age-related mortality rate following ECV infection was investigated in three virus challenge experiments at two different virus dosages. Eight-week-old (ca. 3 g), ten-week-old (ca. 8 g), and sixteen-week-old (ca. 55 g) catfish were infected with ECV at 21°C. In the youngest age group, 96% (at 10^6 $TCID_{50}$/ml dosage) and 100% (at 10^5 $TCID_{50}$/ml) mortality rates were observed, while these rates were reduced to 56% and 68% in the ten-week-old groups, respectively. The mortality was significantly higher in virus-exposed groups than in the control ones. In the sixteen-week-old group, 23% mortality was detected at 10^5 $TCID_{50}$/ml concentration of ECV. Here, significant difference was not found

between the exposed and control groups. The performed experiments show that different age-groups of wels catfish may have various susceptibility to ECV. These findings draw attention to the importance of the prevention/protection against virus infections of juvenile (up to 3-month-old) wels catfish in aquaculture. (Published: Abonyi F, Varga, Á, Sellyei B, Eszterbauer E, Doszpoly A (2022) Juvenile wels catfish (*Silurus glanis*) display age-related mortality to European catfish virus (ECV) under experimental conditions. Viruses 14(8), 1832)

In the wels catfish vaccine experiments, the same vectors were used as in the black bullhead trials (the pcDNA 3.1 vector with cytomegalovirus (CMV) promoter was used with the surface or structural protein of the ECV (MCP, ORF2). These vaccine constructions were tested in a small-scale trial in juvenile wels catfish: two vaccinated groups (n=6 per group) with two control groups (injected with PBS and empty pcDNA3.1 plasmid) were used for gene expression analysis. The fish were sampled three- and six-days post-vaccination from muscle and spleen tissues, and the innate and adaptive immune gene expression was followed by real-time reverse transcription PCR. We found upregulation of the IgM and interferon γ with both vaccines. To determine the exact effectivity of the vaccines, juvenile wels catfish (n=25 per group) were vaccinated with ORF2, MCP, pcDNA3.1 empty vector (25 μg DNA/fish) and PBS. The virus challenge was planned three weeks after the vaccination. Unfortunately, we had to terminate the experiment after two weeks because of severe cannibalism. The experiment will be repeated in the next season, when we can obtain juvenile catfish again. This time we are going to separate each individual by small cages in the tank.

The development of a DNA vaccine against the spring viraemia of carp virus (SVCV) using its glycoprotein (G) gene has been carried out. The SVCV belonging to the family *Rhabdoviridae* has a considerable economic impact in Europe, as it infects cyprinids such as common carp. In previous studies, DNA vaccines containing the G gene of the virus induced moderate to full protection against SVCV even in a single low dose in juvenile carp. The backbone of these vaccines was the pcDNA3.1 plasmid with CMV promoter. This promoter works less effectively under 25ºC. Another promising nucleic acid vaccine construction against viral diseases is the DNA-layered Salmonid alphavirus-based replicon (SAV), which was tested successfully against infectious salmon anemia virus in Atlantic salmon (*Salmo salar*). The DNA-layered SAV replicon has not been tested in non-salmonid fish species yet. In our study, a DNA-layered SAV replicon expressing the G gene of SVCV was designed, and its efficacy was compared with the previously described pcDNA3-SVCV-G construct in common carp. The two different vaccine prototypes were injected i.m. in a 0.1 μg/g of fish

dosage (n=25 per group) at water temperature 20ºC. Fish were kept at this temperature for two weeks, then the temperature was gradually decreased to 13ºC at a rate of 1ºC/day. The three vaccinated groups and the control group (injected with empty pcDNA3.1 plasmid) were challenged by immersion with SVCV, three weeks after vaccination. 30 days after virus challenge, the cumulative mortalities were: 44% in control, 52% in pcDNA3-SVCV-G group, while in pSAV-DNA-SVCV-G group, the mortality was 8% only. According to these results, the pcDNA3.1-based vaccine did not induce any protection against SVCV under these conditions, however, the DNA-layered SAV raised significant protection after a single low dose of i.m. injection. Our results show that the SAV-based replicon may serve as a potential vaccine candidate for non-salmonid fish aquaculture as well in the future if further clinical and field trials confirm its efficiency. (Manuscript preparation is in the pipeline.) In the next season we are going to test DNA-layered SAV vaccine candidates against koi herpesvirus and carp edema virus. The construction and large-scale production of these plasmids (2 constructs against both viruses) have been carried out.

Although the following topics were not included originally in the research plan, these experiments also related to the prevention/cure of viral diseases in the aquaculture.

The efficacy of silver nanoparticles (AgNPs) was tested in vitro against three different fish viruses causing significant economic damage in aquaculture. These viruses were the SVCV, ECV and the Ictalurid herpesvirus 2 (IcHV-2). The safe concentration of the AgNPs, which did not cause cytotoxic effect in EPC cells, proved to be 25 ng/ml. This dose of AgNPs decreased significantly (5-330x) the viral load of all three viruses in three different types of treatments (virus pre-treatment, cell pre-treatment, and cell post-treatment with the AgNPs). In higher concentration, the AgNPs proved to be efficient against ECV and IcHV-2 even in a delayed post cell-treatment experiment (AgNPs treatment was applied 24 hours after the virus inoculation). These first in vitro results against three devastating fish viruses are encouraging to continue the study of the applicability of AgNPs in aquaculture in the future. (Published: Doszpoly, A. Shaalan, M. El-Matbouli, M. (2023) Silver Nanoparticles Proved to Be Efficient Antivirals In Vitro against Three Highly Pathogenic Fish Viruses. Viruses 15, 1689. https://doi.org/ 10.3390/v15081689)

The goal of another study was to investigate the impact of the acyclovir (ACV) on ranaviruses in cell cultures. Acyclovir is known to inhibit the replication of different herpesviruses. However, it was never tested against ranaviruses. The virus encoded thymidine

kinase monophosphorylates the ACV with much greater rate than the cell-encoded enzymes do, the concentration of the ACV-triphosphates (ACT-TP) in virus-infected cells reaches much higher values than in non-infected cells. The ACV-TP is a substrate, inhibitor and inactivator for the herpesviral DNA polymerase, which is much more sensitive to the ACV-TP than the cell-encoded DNA polymerases. We carried out *in silico* comparison of the thymidine kinase genes of some ranaviruses and herpesviruses, and we concluded that ACV might work with ranaviral thymidine kinase as well. Subsequently, we carried out *in vitro* experiments with two ranaviruses ECV and FV3 (frog virus 3) and Ictalurid herpesvirus 2 as control. Virus titration on EPC cells were performed in the presence or absence of ACV. The ACV decreased the virus titer of both ranaviruses with three order of magnitudes, while in case of the herpesvirus it was four order of magnitude. Another *in vitro* experiments are in the pipeline (viral load and RNA transcription measurement by qPCR and RT-qPCR in the presence or absence of ACV). Presented results provide a good reason to continue the research on anti-ranavirus activity of nucleoside analogues.