Summary of Ph.D. thesis

STUDY OF AFRICAN SWINE FEVER VIRUS IN TISSUE CULTURE AND PRIMARY MACROPHAGES

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Introduction

African swine fever (ASF) is currently one of the most damaging viruses to pig populations, both economically and in terms of animal health. The disease causes haemorrhagic, febrile symptoms. The causative agent is the African swine fever virus (ASFV), which can cause up to 100% mortality in domestic pigs (*Sus scrofa domestica*) and wild boar (*Sus scrofa).*

Protection against the virus is hampered by the fact that we do not currently have an effective vaccine against ASFV, so the only protection is prevention.

For epidemiological studies, further research on the virus, verification of established vaccine candidates and vaccine development, it is essential to develop a simple and reproducible method to sequence the whole genome of ASFV. Due to the large size of the genome, the fastest way to sequence the virus is next-generation sequencing (NGS), but the sequencing of the whole genome of ASFV is complicated by a number of technical issues.

Although vaccine development trials began soon after the virus was discovered, conventional approaches have not had the desired effect. The best experimental results have been achieved with live attenuated viruses, but so far neither naturally attenuated strains nor genetically modified viruses with single gene deletions have led to complete success. Experiments with vaccine candidates

containing multigene deletions have shown encouraging results in a number of cases, but have not yet led to breakthroughs. A challenge in the production of vaccine strains containing multigene deletions is that the function of many ASFV proteins is still unknown. One of the most promising vaccine candidates against African swine fever is a naturally occurring attenuated strain, Lv17/WB/Rie1 (Lv17), but the strain cannot be used as a vaccine in its current form due to its side effects.

The development of attenuated vaccines and the study of proteins of unknown function are often carried out by targeted gene modifications based on homologous recombination. Double-strand breaks induced by the clustered regularly interspaced palindromic repeats /CRISPR-associated protein 9 (CRISPR-Cas9) system induce relatively high frequency recombination between ASFV and transfer plasmids, and there is also some indirect evidence that homologous recombination contributes to ASFV evolution, but experimental evidence for homologous recombination between ASFV genomes has not yet been published.

Vaccine development has been severely hampered by the lack of a host-derived cell line on which ASFV can be stably propagated in high titres. Although research on promising cell lines is increasing, most experiments requiring genetic stability of the virus are currently carried out on primary porcine macrophages. Working with

macrophages is complicated by the fact that cell isolation is expensive and time-consumingand experiments are difficult to reproduce. Adaptation of naturally occurring strains or vaccine candidates to immortalised cell culture almost always involves significant genetic changes, with major modification and rearrangement of the viral genome, which may also result in altered immunogenic properties of the parent strains.

Objectives

1) Our aim was to develop a protocol for the rapid and accurate determination of the complete genome of the African swine fever virus using next generation sequencing (NGS) methods.

2) Our other main objective was to increase the attenuation of Lv17 and to produce modified live virus vaccine candidates. We planned to achieve Lv17 attenuation in two ways; firstly by targeted gene modifications and secondly by exploiting genetic changes that occur during serial passaging of the strain on Cos7 cell line. We then planned to evaluate the generated viruses using the sequencing method we had developed.

3) For the targeted gene modifications, our first aim was to remove a gene of unknown function belonging to the multigene family (MGF), MGF 110-11L, using CRISPR-Cas9 method. Using the mutant strain we have generated (Lv17/d110-11L), our aim was to study the biological properties of the MGF 110-11L gene in porcine alveolar macrophages (PAM) and to test its effect on the virulence and replication of Lv17.

4) We also aimed to remove three well-studied genes encoding proteins involved in ASFV virulence (9GL, 8-CR, CD2v) from the Lv17 genome by viral crossing

demonstrating that homologous recombination of ASFV occurs in PAM.

5) Finally, we planned to study the genetic changes that occur during serial passage of the Lv17 strain in Cos7 tissue culture.

Materials and methods

Preparation and maintenance of porcine alveolar macrophages (PAM)

The PAMs were isolated according to the WOAH manual and stored at -72 °C. To maintain the cells, RPMI medium was used supplemented with 1% antibiotic-antimicrobial solution, 10% fetal bovine serum and 2mM L-glutamine. The cells were incubated at 37°C for 24 hours under 5% CO_2 until infection.

Developing a new generation sequencing method

The sequencing protocol was developed using the ASFV_HU_2018 isolate, which was obtained and propagated by the Functional Virology Group of the VMRI on PAM.

The susceptibility and infection dynamics of ASFV on PAM cells were investigated as the first step, and infection was standardized. To test susceptibility, cells were infected every 2 hours after plating for 24 hours and in a longer experiment for 144 hours. Infection was detected by immunofluorescence microscopy.

The sequencing protocol was developed initially enriching the ASFV genome and reducing the amount of contaminating host genome in the DNA samples. Firstly, a Virotype kit to was used to quantify the amount of host

and viral DNA in our initial samples. Next, we performed a DNase treatment to minimize the contaminating host DNA content. Viral DNA was purified using the High Pure Viral Nucleic Acid Kit. To increase the absolute amount of DNA for NGS sample preparation protocols, whole genome amplification (WGA) was performed using the REPLI-g Mini Kit. REPLI-g samples were purified using the NucleoSpin Gel and PCR clean-up Kit. DNA concentration was measured using a NanoDrop 2000. The ratio of host to viral DNA in our samples was also quantified after DNase treatment and WGA amplification.

After DNase and WGA treatment, the samples were sequenced using Iontorrent and Illumina methods. The resulting sequence reads were processed using Geneious Prime. The reads were mapped to the porcine genome to determine and screen for host DNA contamination. The resulting reads, now containing only viral reads, were mapped to ASFV Belgium strain 2018/1 and used to determine the consensus sequence.

Three regions that were difficult to determine were sequenced using Sanger sequencing, and PCR was designed to amplify these regions.

Production of modified viruses

Production of the Lv17/d110-11L construction

The Lv17/d110-11L construct was generated from the Lv17 strain by deletion of the MGF 110-11L gene with CRISPR-Cas9. The 110-11L gene was replaced with the green fluorescent protein (eGFP) under the regulation of the African swine fever virus p72 promoter. The pUC19 vector was used as a backbone to generate the recombinant transfer plasmid (p14L-eGFP) for the CRISPR-Cas9 system. The required primers to generate the transfer plasmid were designed using SnapGene.

PAM cells were plated on 6-well cell culture plates (5×10^6 /well) as described above. Each well was infected with 3 MOI (multiplicity of infection) Lv17 ASFV strain. For transfection, transfer plasmid and gRNA plasmid were mixed in medium and transfection was performed with 10 µl Fugene HD transfection reagent. After 10 min incubation at room temperature, the mixture was added to the infected PAMs. The transfected PAM cells were incubated at 37 °C with 5% CO_2 for 24 hours.

Production of the Lv17/dCD-dGL construct

Homologous recombination was tested by co-infection with Lv17/dGL (9GL coding region deleted) and Lv17/dCD (CD2v and 8-CR protein coding region deleted) mutant viruses carrying mCherry and eGFP marker gene. The

constructs were designed and isolated by the Functional Virology Group according to the method used for Lv17/d110-11L virus. PAMs were infected with 3-3 MOI with the two virus isolates. After 1 h of incubation, the supernatant was replaced with fresh PAM culture medium. The supernatant was collected after 3 days. The resulting cells containing recombinant Lv17/dCD-dGL viruses showing red and green fluorescence were manually selected under a fluorescence microscope.

Production of the Lv17/ d24 construction

The ASFV Lv7/d24 isolate was produced through serial passaging. The first eight passages were carried out in Cos7 cells with 80-100% confluence. In each passage, 3 MOI Lv17 strains were used to infect the cells. After 7 days, 200 µl of supernatant was transferred to new Cos7 cells. The next three passages were performed on PAMs, with 200 µl of supernatant transferred to fresh PAMs every 3 days. Finally, three additional passages were performed on PAMs to ensure a pure sample.

Isolation of viruses

The mutant viruses were isolated manually under a fluorescence microscope by using a pipette to separate fluorescently luminescent cells (infected with mutant virus containing the marker gene) from non-fluorescently luminescent cells. The isolated cells, i.e. infected with

mutant virus, were frozen and thawed three times for disruption. PAMs were infected with the isolated virus in 96-well plates and incubated at 37 °C for 24 hours. This procedure was repeated until the percentage of infected cells expressing the respective marker gene reached 100%.

Immunofluorescence staining (IF), titre and copy number determination

IF staining for testing was performed using a porcine anti-ASFV polyclonal antibody and an anti-pig secondary antibody at 1000-fold dilution.

To investigate how mutant viruses replicate *ex vivo*, PAMs were infected with Lv17/d110-11L, Lv17/dCD-dGL and Lv17/d24 viruses and Lv17 virus as control with 0.01 MOI and supernatant samples were taken at different time points (0, 12, 24, 36, 48, 60, 72, 84, 96 and 108 h post infection (hpi)). Then, the collected samples were titrated on PAMs and qPCR was used to determine the viral copy numbers in aliquots. The titer was determined using IF staining for the highest dilution level and the number of infected cells using a fluorescent focus unit (FFU)-based determination.

To determine the copy number of different gene-modified isolates of ASFV qPCR was used. The reaction

was designed for a 328 nucleotide long region of the conserved p72 gene of ASFV. A diluted supernatant of infected PAM cells was used directly as a template in the reaction after heating at 72 °C for 20 min. The specificity of qPCR was verified by melting curve analysis. Viral copy numbers were calculated using a standard curve of 10-fold dilution of purified amplicons. Differences between titres and copy numbers were also analysed statistically using the R program.

Results

Developing a new generation sequencing method

Testing the infectivity of primary alveolar macrophages (PAMs) was carried out in two experiments. The first experiment, which lasted 24 hours and included infections every 2 hours, showed that there is a slight peak in infectivity at 4-8 hours, but that the cells are most susceptible to APSV at 24 hours after plating the cells. The second trial, conducted over 108h, confirmed the findings. At 18 hours after plating, PAM infectivity was significantly higher than at any other time point in the experiment.

The primary challenge of sequencing is the low amount of viral DNA, therefore we focused our protocol development on enriching the ASFV genome and reducing the amount of contaminating host genome in DNA samples using the simplest methods available. We started with 3 samples and used them considering the results. After the initial determination of host and viral DNA in our samples, we focused on minimizing the DNA content of the contaminating host genome, which was achieved by increasing the efficiency of the DNase treatment. From the initial 3 samples, we performed 3-3 parallel treatments. In most cases, a minimal increase (Ct +1-2) in Ct values between untreated and DNase I treated samples was observed during virus DNA detection. In

contrast, the amount of host DNA decreased significantly, but the extent of the decrease varied from sample to sample. Whole genome amplification (WGA) was used to increase the absolute amount of DNA. qPCR showed a ~1000-fold increase in viral DNA content following the WGA reaction. In contrast, no host DNA could be detected.

To determine the most efficient method for ASFV NGS sequencing, two ASFV samples were sequenced on two platforms (Illumina and IonTorrent). Analysis of the sequencing data showed that the number of viral reads exceeded contaminating nucleic acid reads in all four samples, so it can be concluded that the method described above can be successfully used to minimize the number of contaminating genomes. In the homopolymeric sections of the samples sequenced on the Ion Torrent platform, a large number (~131) of nucleotides were found to be ambiguous compared to the reference sequence, mainly in the form of single- and double-nucleotide indels. In contrast, the Illumina platform results showed much fewer unidentifiable nucleotides. After analysing data from one sample, which contained approximately 7 million viral reads, unambigouty was limited to the three longest homopolymer regions that contained more than 10 C/G nucleotides. Sanger method was used to determine the exact sequence of the virus, in these three regions .

Production and characterisation of modified viruses

Production and characterisation of the Lv17/d110-11L construction

The function of the MGF-110 11L gene is currently unknown. This gene was selected for our studies due to its unique characteristics. The MGF-110 11L gene exhibits highly variable properties at the genetic level in different strains. It contains a homopolymeric G/C (hpG/C) region with a high degree of variability in length. Comparison of MGF-110 11L sequences from GenBank data reveals stretches from 4 to 19 bases in length in the homopolymeric region. Although the role of this region in ASFV is unclear, hpG/C regions play an important regulatory role in other viruses and cellular organisms.

Deletion of MGF-110 11L was achieved by CRISPR/Cas9 system-induced intercellular homologous recombination. The majority of the gene (760 of the 825 bp ORF were deleted) was replaced with an eGFP reporter gene regulated by ASFV-derived p72 promoter. After isolation, titration of the recombinant virus strain, revealed that its titer (3×10^6 FFU/ml) had approached that of the parental virus (7.2×10^6 FFU/ml). Using our sequencing method, we found that fourteen novel point mutations were found in different regions of Lv17/d110-11L virus compared to the parental genome. Two

mutations affected regulatory regions, one was a synonymous mutation, four caused amino acid substitutions, three caused early protein chain termination, and four sites could not be precisely identified, indicating the presence of quasispecies viruses in the isolate.

For *ex vivo* growth assays, PAM cells were infected with the Lv17/d110-11L mutant virus and Lv17/WB/Rie1 virus strain was used as the parental control. There was no significant difference in the growth kinetics of the mutant and parental virus up to 108 hpi, although the infectious viral titer of Lv17/d110-11L remained below that the parental strain. At 108 hpi, there was a sudden increase in the infectious titer of Lv17/d110-11L, the virus titer was 12-fold higher than Lv17. The specific infectivity (copy number/titer) of the virus, however, did not change during the experiment.

Production and characterisation of the Lv17/dCD-dGL construct

Two viruses containing marker genes previously generated by our laboratory using CRISPR-Cas9 were used to test homologous recombination. The first construct is an eGFP marker gene-containing virus (CD2v and 8-CR protein coding region were deleted). CD2v is homologous to the T-cell CD2 protein and is involved in red blood cell haemadsorption to infected macrophages.

The 8-CR protein contains a C-type lectin domain and is involved in stabilising the interaction between the viral CD2v protein and its cellular receptor and it is also required for haemadsorption. 9GL is highly conserved at protein and DNA levels in any virus isolate. The protein is involved in normal virion maturation and virus growth *in vitro* and may influence virulence *in vivo*. Deletion of the 9GL gene resulted in varying degrees of attenuation depending on the ASFV strain.

By repeating the isolation and the infection process five times after co-infection, a homogeneous isolate was obtained. All infected cells showed red and green fluorescence. NGS sequencing also confirmed the homogeneity of the viral population, no reads were found that indicated the presence of a parental sequence. Compared to the two parental strains, six novel mutations occurred in the left variable region. The disappearance of the Lv17/dGL unique sequence markers and the appearance of the Lv17/dCD unique markers in the genome of the recombinant Lv17/dCD-dGL suggest that at least two crossovers occurred, one between the endpoints of the two fluorescent markers and the other at the end of the genome.

The mutant virus showed a slower growth rate than Lv17, and its final titer ($6.9x10^3$ FFU/ml) was also significantly lower than that of Lv17 ($1.8x10^6$ FFU/ml) at

108 hpi. The genetic modifications of the mutant virus have weakened its ability to replicate in PAM, as indicated by its specific infectivity (SI) values. The SI of Lv17/dCD-dGL exceeded that of the parental Lv17 virus by an order of magnitude throughout the experiment, probably due to the reduction in infectivity.

Production and characterisation of the Lv17/d24 construct

To study the stability of Lv17, Cos7 cells were infected with the virus, and after 5 and 8 passages, samples were taken (Lv17_cos5, Lv17_cos8) and the viruses were characterized. Then, 3 passages were performed on PAM cells (Lv17_cos8_pam3). The resulting samples were sequenced by NGS. Sequence analysis of the three stocks revealed that the genome of Lv17 underwent significant rearrangements in the left variable region (LVR) in the form of large indels and, in addition, three point mutations were detected in the tested passages. The major viral component of the quasi-species in Lv17_cos5 and Lv17_cos8 was a viral genome that lost more than 40 kb (1-40568) of its left terminal region, a section containing 52 ORFs. The missing region was replaced by a viral fragment of almost the same size (38780 bp, encoding 51 genes) from the right terminal region of the genome. In Lv17_cos5 and Lv17_cos8, a relatively small number of viral reads (907 and 3 reads,

respectively) were still mapped between the proximal terminus of the reverse repeat region and the deletion breakpoint region of the genome (2352 and 40568), suggesting the presence of other genotypic variants. Another previously undetected deletion variant became the major component in Lv17_cos8_pam3. This contained a shorter deletion, now affecting the region between 187 and 26554 bp of the genome and involving the deletions of 42 genes. The region between 26554 and 40569 was not detectable in either Lv17_cos5 or Lv17_cos8, and was previously only detectable in Lv17. Since 63 reads could still be mapped to the deleted region, three more dilution and passaging cycles were performed, and the resulting Lv17/d24 virus was also sequenced and sent for animal experiments.

There were significant differences in the growth kinetics of the Lv17/d24 and the Lv17 viruses. Lv17/d24showed a slower growth rate than Lv17, and its final titer ($2.5x10^5$ FFU/ml) was lower than that of the parental virus ($1.8x10^6$) at 108 hpi. The SI values of Lv17/d24 were almost always higher than those of the parental Lv17, although their values remained slightly above the significance limit.

Discussion

Developing a new generation sequencing method

Our results on the susceptibility of PAM cells have clearly shown that work with these cells can be standardised to an acceptable level; infectivity can change rapidly and significantly, even within 2 hours. Additionally, the cells exhibit the highest susceptibility to ASFV approximately 24 hours after deposition.

The proper application of nuclease treatment, whole genome amplification and, most importantly, continuous monitoring and evaluation of the different steps of sample preparation allowed us to take full advantage of NGS for ASFV genome sequencing. The technique allows the rapid and accurate determination of the whole genome sequence of African swine fever virus on an Illumina platform potentially using only three Sanger sequencing.

Production and characterisation of modified viruses

Production and characterisation of the Lv17/d110-11L construction

To overcome problems related in live virus vaccine development, we deleted a gene from the MGF-110 gene family; this was expected to have a modest effect on the virulence of Lv17 and not to overattenuate the virus. We

hypothesised that deletion of the MGF-110 11L gene could cause significant attenuation through pleiotropic effects, both by removing the protein and by removing a potential regulatory function of the polyC/G tract. We indeed observed attenuation, but not to the desired level.

With a few exceptions, gene knockouts in ASFV are not associated with a large number of mutations in the genome. However, after isolation, sequencing of Lv17/d110-11L unexpectedly revealed a large number of mutations in the viral genome outside the deleted region. Many of the mutated genes are associated with processes that could potentially affect the immune system and virulence of the virus.

From the results of the *ex vivo* growth assays, we concluded that although we detected a significant change in the titer of the gene-deleted virus only by 108 h, the specific infectivity of the viruses did not change significantly in the last 24 h. This difference may be due to slower growth and potentially higher viral yield of Lv17/d110-11L.

Production of the Lv17/dCD-dGL construct

Although the sequence of some virus isolates and the success of techniques used to produce recombinant viruses clearly suggest that homologous recombination occurs between different ASFV strains, very little is known about this phenomenon in ASFV. To the best of our knowledge, our work is the first to provide direct experimental evidence that homologous recombination of ASFV occurs in macrophages. In our work, we were able to easily isolate a recombinant virus resulting from the crossing of two viruses carrying fluorescent markers, suggesting that, if coinfection occurs, homologous recombination may also occur with relatively high frequency between virus strains. The fact that at least two recombination events are clearly detectable in the genome of the recombinant virus based on unique parental genetic markers strongly supports the previous hypothesis.

Production and characterisation of the Lv17/d24 construct

Lv17 is genetically unstable in Cos7 cells, the stocks are not homogeneous, the majority of viruses losing about 40 kb of their genome in just five passages. However, even minor genetic variants that are undetectable or difficult to remove may persist in low dilution passages during adaptation and become the major variant in a few

passages under different culture conditions, in this case in PAM cells.

There are several examples in the literature showing that serial passaging of ASFV in cell lines can result in large deletions in the viral genome, most commonly in the left and right variable regions. In most cases, the virus loses the ability to replicate on the original target cells (macrophages) after serial passaging. The stability of the Lv17 virus has not been tested in Cos7 cells.

The sequencing technique used is obviously suitable for detecting single nucleotide changes, or even polymorphisms if they are dominant in the quasispecies. The additive distortions of whole genome amplification and the systematic distortions and inequalities inherent in the NGS procedure make it very difficult to estimate the proportion of minor genetic components in different samples. As a consequence of this disproportionality, it is generally not possible to determine with absolute certainty whether a virus population is genetically homogeneous (or not), as reads with a small number of components are unlikely to be detected in the sample. However, the detection of any small number of such reads clearly indicates the presence of genetic variants in the quasispecies.

In vivo experiments

A collaboration in Italy also allowed us to test these three strains *in vivo* in domestic pigs. The 110-11L gene-deleted strain showed a clear reduction in pathogenicity and lethality compared to the parental strain, but several clinical signs were observed, which make it unsuitable for use as a vaccine in its present form. Our in vivo results were consistent with the in vitro findings, demonstrating a decrease in the replication capacity of the Lv17/d24 and Lv17/dCD-dGL strains. Furthermore, the pigs exhibited mild clinical signs such as fever, diarrhoea, loss of appetite, and haemorrhages on the ears, despite the absence of ASFV in their blood, until they were infected with the Armenia/07 strain. The unacceptably high mortality rate following infection confirms that these viruses, in their current form, are not suitable for use as vaccines.

Overview of the new scientific results

1) We have developed a new generation of sequencing techniques that can easily and accurately determine the complete genome of ASFV.

2) Three different ASFV viruses were generated from the Lv17/WB/Rie1 strain (Lv17/WB/Rie1/d110-11L, Lv17/d24, Lv17/dCD-dGL) using three different recombination techniques. The MGF 110- 11L gene was deleted from the genome of the attenuated Lv17/WB/Rie1 strain using CRISPR-Cas9 and the virus was characterized.

3) We have demonstrated that homologous recombination events occur during coinfection of different ASFV strains on PAM cells and that the resulting viruses can be isolated.

4) We have found that the passage of Lv17 on Cos7 cells also results in significant changes in the viral genome.

5) The viruses produced are not suitable as vaccines in their current form, but we have demonstrated that the undesirable side effects of Lv17/WB/Rie1 can be mitigated by further mutations without a significant reduction in immunity.

Scientific publications

Peer-reviewed publications related to the topic of the thesis:

Mészáros I, Olasz F, Tamás V, Bálint Á, Zádori Z. **Az Afrikai Sertéspestis Vírusának Biológiája - Irodalmi összefoglaló.** Magyar Állatorvosok Lapja. 2019, 144./55-62.

Olasz F, Mészáros I, Tamás V, Bálint Á, Bruczynska M, Wozniakowski G, Zádori Z. **Az Afrikai Sertéspestis járványtana és a védekezés lehetőségei.** Magyar Állatorvosok Lapja. 2019, 141./101-115.

Olasz F, Mészáros I, Marton S, Kaján GL, Tamás V, Locsmándi G, Magyar T, Bálint Á, Bányai K, Zádori Z. **A Simple Method for Sample Preparation to Facilitate Efficient Whole-Genome Sequencing of African Swine Fever Virus. Viruses.** 2019 Dec 6;11(12):1129. doi: 10.3390/v11121129.

Tamás V, Righi C, Mészáros I, D'Errico F, Olasz F, Casciari C, Zádori Z, Magyar T, Petrini S, Feliziani F. **Involvement of the MGF 110-11L Gene in the African Swine Fever Replication and Virulence.** Vaccines, 2023 Apr 14;11(4):846. doi: 10.3390/vaccines11040846.

Franzoni G, Petrini S, Mészáros I, Dei Giudici S, Righi C, Olasz F, Zinellu S, Tamás V, Pela M, Gallardo C, Zádori Z, Oggiano A, Feliziani F. **Evaluation of Haematological and Immunological Parameters of the ASFV Lv17/WB/Rie1 Strain and its Derived Mutant Lv17/WB/Rie1/d110-11L against ASFV Challenge Infection in Domestic Pigs.** Vaccines. 2023; 11(7):1277. doi.org/10.3390/vaccines11071277.

Petrini S, Righi C, Mészáros I, d'Errico F, Tamás V, Pela M, Olasz F. Gallardo C, Fernandez-Pinero J, Göltl E, Magyar T, Feliziani F Zádori Z. **The production of recombinant ASFV Lv17/WB/Rie1 strains and their characterizations as vaccine candidates.** Vaccines. 2023, 11(12): 1860. doi:10.3390/vaccines11121860

Other peer-reviewed publications:

Olasz F, Tombácz D, Torma G, Csabai Z, Moldován N, Dörmő Á, Prazsák I, Mészáros I, Magyar T, Tamás V, Zádori Z, Boldogkői Z. **Short and Long-Read Sequencing Survey of the Dynamic Transcriptomes of African Swine Fever Virus and the Host Cells.** Front

Genet. 2020 Jul 28;11:758. doi: 10.3389/fgene.2020.00758.

Kiss I, Szigeti K, Homonnay ZG, Tamás V, Smits H, Krejci R. **Maternally Derived Antibody Levels Influence on Vaccine Protection against PCV2d Challenge.** Animals (Basel). 2021 Jul 29;11(8):2231. doi: 10.3390/ani11082231.

Tombácz D, Kakuk B, Torma G, Csabai Z, Gulyás G, Tamás V, Zádori Z, Jefferson VA, Meyer F, Boldogkői Z. **In-Depth Temporal Transcriptome Profiling of an Alphaherpesvirus Using Nanopore Sequencing.** Viruses. 2022 Jun 13;14(6):1289. doi: 10.3390/v14061289.

Tamás V, Mészáros I, Olasz F, Kiss I, Homonnay ZG, Mortensen P, Zádori Z. **Allele-Specific Dual PCRs to Identify Members of the 27a Cluster of PPV.** Viruses. 2022 Jul 8;14(7):1500. doi: 10.3390/v14071500.

Acknowledgements

I would like to thank my supervisor, Dr. Zoltán Zádori, for giving me the opportunity to do my research. I would like to thank him for his valuable suggestions and professional support, which contributed to the quality of my work and to the birth of my dissertation and scientific publications. I thank Dr. Ádám Bálint for the samples provided. I would like to thank to Pathogen Discovery Group of the HUN-REN Veterinary Research Institute for their help in sequencing and to the members of the Respiratory Bacteriology group for isolating PAMs. I would also like to thank the members of the HUN-REN Veterinary Research Institute's Functional Virology Group (Dr. István Mészáros, Dr. Ferenc Olasz) for their useful advice during my work and for their help and support.

I have to thank my friends who were patient with me when I was nervous and who always had a kind word or two for me. Special thanks to Ferenc Csizovszky for always cheering me up when I needed it and standing by me in difficult times.

Finally, I would like to thank my family for supporting me during my studies. A special thank you to my father, who sadly is no longer with us, for teaching me that with perseverance any goal can be achieved.

The work was funded by the H2020-VACDIVA, ÚNKP-20-3 and KDP-2020 grants.