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Investigation of the infectivity of the porcine parvovirus SAT⁻ mutant strain and waterfowl viruses using real-time PCR and immunofluorescence methods

PhD thesis

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2017

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Introduction and aims of the thesis

The generally accepted view about parvoviruses is that proteins are translated only from the two major Open reading frames (ORF) which code a small set of non-structural (NS) and two or three viral capsid (VP) proteins. During the years apart from the two major ORFs a few shorter ORFs were identified in different parvovirus genomes which overlap with the NS and VP ORFs. Some of these alternative overlapping ORFs were shown to code proteins.

A small, alternative ORF was revealed with computer analysis, overlapping the amino terminal region of the VP2 gene in all members of the *Protoparvovirus* genus. A small alternatively translated protein (SAT) is translated from this genus-specific ORF and it is localized in the endoplasmic reticulum of the host cell. The loss of the SAT function results "slow-spreading" mutants in the porcine parvovirus (PPV) attenuated NADL-2 strain. This can be explained by the large decrease of the cell lysis.

The molecular mechanism of the function of SAT did not know at the start of our work. Because it accumulates in the ER of the cells, we assumed that it

plays a role in the development or modification of the ER stress response.

The waterfowl husbandries are getting more industrialized in each continent. Large-scale waterfowl production in industrialized farms demands constant animal health monitoring and quick reaction to biological hazards in order to avoid massive economic losses, thus the development of simple and rapid methods for pathogen detection becomes more important.

A common feature of the duck circovirus (DuCV), the duck hepatitis A virus type 1 (DHAV-1), the goose haemorrhagic polyomavirus (GHPV) and the goose parvovirus (GPV) is that they do not or poorly replicate in continuous cell lines, and their propagation requires the use of primary cells or embryonated eggs. However, the preparation of primary cells and the use of embryonated eggs are laborious and cumbersome processes with several technical complications. The experiments with injected eggs take relatively long time, the primary cells have limited dividing potential and life span, furthermore eggs and animals used for inoculation or primary cell preparation should come from specific pathogen free (SPF) flocks, which make the investigation of these viruses expensive.

Recently, the AGE1.CR, AGE1.CR.pIX and AGE1.CS cell lines have been created from primary Muscovy duck cells. The cell lines were immortalized by stable expression of the E1A and E1B genes of the human adenovirus serotype 5. The CR and CRpIX cell lines are permissive modified vaccinia virus Ankara, for avian influenza A virus and for Newcastle disease virus. However, we assume that these Muscovy duck-origin cell lines may be suitable for the detection and investigation of the mentioned waterfowl viruses.

The aims of our work was the investigation of the effect of the SAT protein on the spreading of PPV Kresse strain using immunofluorescence (IF) and real-time PCR methods in order to we explore that the “slow spreading” phenotypes commonly feature in the SAT^- PPV strain.

We wanted to study the influence of SAT for the cytopathogenic effect in the infected cells using mutant PPV Kresse strain. Our aim was that we confirmed the slow spreading of the SAT^- virus associated with the weaker lysis of the infected cells.

We wanted to prove that the SAT can influence the ER stress response in the infected cells. We wanted to

verify that the emergence of the stress effect associated with the faster spread of the virus using by ER stress inducer chemical agents.

We wanted to determine the effect of SAT on the cells without presence of other virus proteins using by transfection experiments.

We wanted to study that the DuCV, the DHAV-1, the GPV and the GHPV can replicate in the AGE1.CR, AGE1.CR.pIX or AGE1.CS cell lines using by immunofluorescence staining and real-time PCR methods.

We wanted to teste the combined studies of the high and low multiplicity infection for the time of infectious cycle determination of the viruses.

We wanted to determine the growth curve of the viruses and the necessary number of the viral copy for the one successful infection in the different cell lines using by immunofluorescence staining and real-time PCR methods.

Materials and methods

The investigation of the SAT protein

The infectious clone of SAT$^-$ virus (pSAT$^-$) was created from the pUC19 cloned wild type infectious clone (pKresse). A STOP codon was inserted to the open reading frame and the following (potential initiation codon) methionine was changed (T-2842→A and T-2845→C). The modifications did not change the amino acid sequences of the VP. In order to create the stock culture of the wild type and the mutant PPV Kresse strains, the pKresse and the pSAT$^-$ plasmids were transfected to the 50% confluence PT cells using by TurboFect Transfection Reagent.

50% confluence PT cells were infected in 24-well plate with high and low multiplicity (MOI: 3 and 0.01). After 2 hours, the medium were changed and the cells were fixed (using 3% formaldehyde) at every 2 hours. The first infected cells and the first fluorescence foci (as sing of secondary infection) were detected using by IF staining, the replication of the viruses were followed with real-time PCR. The High Pure Viral Nucleic Acid Kit was used for the purification of the viral nucleic acid. Cytotoxicity Detection Kit was used for the measuring of

the lactate dehydrogenase enzyme (LDH) activity, the lysed and apoptotic cells were detected with propidium iodide (PI) and Hoechst staining.

The infected cells were treated with ER stress inducer chemical agents (MG132, ditiotreitol and thapsigargin). Real-time PCR was used for the following of the replication. The ER stress marker Xbp1 and CHOP and the morphological change of the ER were detected with IF staining in infected cells. The results were compared with the effect of the 5, 10 and 20 minute long UV stress.

The genes of SAT and the CHOP were cloned to Ds Red-Monomer-N1 using by TurboFect Transfection Reagent. The cells were fixed with 3% formaldehyde at every 4 hours between 16 and 48 hours after the transfection. The activation of the Xbp1 the CHOP and the morphological changes of the ER were detected with IF staining.

The CHOP-DsRed plasmid was co-transfected with the pKresse or the pSAT– and the cells were fixed 12, 16, 24, 32, 40, 48, and 96 hours after the transfection. The spread of the viruses and the localisation of the CHOP were investigated with IF staining

Investigation of the waterfowl's viruses

The virus samples (DuCV, DHAV-1, GHPV, and GPV) were created from tissue samples what originated from the Veterinary Diagnostic Directorate National Food Chain Safety Office. The original virus samples were homogenized by pestle, clarified by centrifugation (10000× g, 5 minutes), filtered through 0.22 µm syringe filters and diluted in culture media used for the cells 3×, 10×, 30×, and 100× times.

The AGE1.CR, the AGE1.CRpIX and the AGE1.CS cells were grown in 75 cm^3 tissue culture flask in 11 ml medium (DMEM high Glucose 4,5 g/l, 10% Fetal Bovine Serum Gold, 5% penicillin-streptomycin and 5% sodium pyruvate), at 37 °C in presence of 5% CO_2. Freshly plated, approximately 50% confluent CR, CRpIX and CS cells (1×10^5 cells/well) were infected with 100 µl of the viral dilutions. At 72 hours post infection (p.i.) 100 µl of the infected supernatant was transferred to a new plate loaded with freshly seeded cells. This step was repeated ten times, and the viral nucleic acid were purified with High Pure Viral Nucleic Acid Kit and the viral copy number were determined with real-time PCR. The original virus sample was used as positive control.

The AGE1 cells were infected with the known copy number samples with 0.01 multiplicity of infection. The supernatants were replaced 2 hours later. The plates were incubated for 4, 24, 48, 72 hours. The changes of copy number of viral nucleic acid were established from the supernatant. The infections were followed IF staining as well using viral specific sera (no sera for DuCV was available).

The cells were infected high and low multiplicity (MOI: 2 and 0.01) and time of the infection cycle were determined. The cells were fixed (with 3% formaldehyde) at every two hours and the infected cells were detected with IF staining. The cycle was started at the first detection of an infected cell in the high multiplicity infected wells and it was ended at the detection of the fluorescence foci in the low multiplicity infected wells (as sing of the secondary infection).

The specific viral infectivity and the limit of detection by IF were also determined by low multiplicity infection. The original virus samples were serially diluted (ten times dilution), and titrated on the three cell lines. Cells were fixed at 22 hours p.i., IF staining was performed and the positive cells were counted in the wells infected with the most diluted virus samples. The

specific infectivity of the viruses was calculated by dividing the number of genome copy with the number of the IF positive cells in a well.

Results

The spreading of the wild type and the SAT⁻ mutant PPV Kresse strain

We could detect the first infected cells at the 12th hour of the low multiplicity infection. The secondary infected cells were appeared at the 20th hour at the wild type virus infected cells and at the 24th hour at the mutant virus infected wells. The different between the two strains increased gradually. The result was equivalent with the change of the copy number ant the infectious titre. The values did not change after 48 hours at the wild type virus while it slower, but continuously increased to 88th hour at the SAT⁻ virus.

The increase of the viral copy number started between 22-24 hours at the SAT– virus, however it began grow between 18-20 hours at the wild type virus. The copy number of the mutant virus was lower during the monitoring period, but the different did not rise above one magnitude (it was the higher at the 22 and between 46-48 hours). The different was slower after the DNase treatment. It was the higher at the 48th hour and it decreased gradually and we measured practically equal

values at the 84 hours p.i. (2.1×10^{10}/ml and 1.99×10^{10}/ml).

The cytopathogenic effect of the wild type and the SAT⁻ mutant strains

The LDH activity grew rapidly between 24-64 hours p.i. in supernatant of the wild type virus infected cells, after the growth was slight at the end of the monitoring period. The enzyme activity shows slight grow to the 64 hours. The growth was rapid between 64-88 hours, therefore the enzyme activities were near equal at the 88th hour.

The most cell lysed or did not attached to bottom of the well at 48th hour of the wild type virus infection (just the 6% of the confluence cell number were detectable). However, the number of the SAT^- virus infected cells decreased between 48-72 hours. Because of this, the cell numbers were near equal at the different virus infection (4% and 10%).

The PI staining revealed the SAT^- infected cells preserve the integrity of the membrane for a long time like the wild type virus infected cells. The rate of the apoptotic nuclei was lower than the rate of the PI positive cell during the monitoring period and higher values were

measured in the wild type virus infected cells to the 48 hours p.i. The different was significant between 28-48 hours ($p<0,046$).

The change of the ER morphology and the activation of the ER stress response at the PPV infection

The PPV infection caused condensation of the ER membrane in the cells based on the IF staining of the high multiplicity infected cells (MOI: 3). Knot and clod developed in the perinuclear region and relevant different were not showed between the wild type and the SAT– mutant virus infected cells.

The Xbp1 was detectable from 14th hour of both virus infections in the nuclei and in the cytoplasm sometime. The number of the infected cells which it activated rapidly increased between 16-18 hours. This number peaked at 18th hour of the infection (95%) and after the rate decreased and the protein was detectable in 5% of the infected cells at the 20th hour.

The CHOP was detected first at the 22th hour in the 20% of the wild type virus infected cells in the nuclei. This value peaked at 75% at the 24th hour and did not change to 36th hour. The protein was detectable in the SAT^- infected cells from the 22th hour as well (in 20% of the infected cells) but the rate of the CHOP expressed

cells peaked at lower level (41%) and the protein localised perinuclear. The number of the CHOP expressed cells gradually decreased between 36-48 hours and the protein was undetectable after 48 hours.

ER stress induction by chemical agents and its effects to the PPV infection

The secondary infection formed fluorescent foci not just at the wild type virus but at the SAT^{-} virus infected cells as well after the 10 mM DTT, 20 μM MG132 or 10 μM thapsigargin treatments. The strongest positive effect was seeing at the MG132 treatment (3-8 hours p.i.).

The chemical agents could increase the copy number and the infectious titer of the viruses in the supernatant (MOI: 3). The 7th hour of the infection started DTT treatment was the most positive effect to the copy number of the wild type virus (4.41× growth), while the 3-8 hours MG132 treatment resulted the higher copy number (71.67× higher than at the untreated cells) and titer at the SAT^{-} virus infection. The copy numbers of the mutant virus were higher than of the wild type virus in almost every case after the chemical induced ER stress. UV-C light did not cause neither the Xbp1 nor the CHOP

activation. Its effect for the copy numbers was softly negative but for the titre was strong negative.

The transfections of the SAT and CHOP to the infected cells

The fusion protein what produced from CHOP-DsRed plazmid localised in the nuclei of the transfected cells and it did not change when the plasmid was co-transfected to the cells with the pKresse or with the pSAT$^-$ infectious clone. The appearance of the protein in the nuclei caused development of the apoptotic specific, fragmented nuclei at relatively early (from 18th hour of the transfection) and practically the all transfected cells dead to the 48th hour. Furthermore, we could not experience speeder virus spreading at the co-transfection of the CHOP-DsRed plasmid and pSAT$^-$ infectious clone.

Copy number of the waterfowl viruses in the Muscovy duck origin cell lines

The DuCV did not caused cytopathogenic effect in the infected cells and the final copy number was relatively low except two outliers (10^6 and 10^7/ml) and did not reach the measured value of the starter homogenates. Cytopathogenic effects were observed at

the DHAV-1 infected cells during the passage and the copy number was 10^7-10^8/ml in the last supernatant, which was higher almost two magnitude than was measured in the tissue homogenise. The GHPV could not develop cytopathogenic effect in every case and the final copy numbers scattered in a relatively great interval (between 10^4 and 10^7/ml). The GPV caused strong cytopathogenic effect at every three cell lines. The final copy numbers were near equal to value of the tissue homogenise (10^6-10^7/ml), expected in the CS cell line where we observed higher copy numbers.

The growth curve of the DuCV, DHAV-1, GHPV and GPV

The growth curve of the DuCV was almost similar at all three cell lines. The copy number increase started after 24 hours and between 48-72 hours it did not change substantially. The copy number of the DAHV-1 was very similar in the three cell lines as well. However the increase started already after 4th hour, it was especially fast between 24-48 hours and it slowed down after the 48th hour. The form of the growth curves of the GHPV was different depending on the cell line. The increase of the copy numbers started after 4th hour of

the infection, but it was moderate in the CR and CS cell lines, therefore one order of magnitude more virus could detected at this time. The copy number did not change essentially between 48-72 hours at the infection of CR cells, it increased slower at the CRpIX cells and the copy number similarly grew like between 24-48 hours. The number of the newly produced viruses was substantially less (MOI: 0.01) than at the end of serial passage (10^7-10^9/ml).

Define of the time of the infectious cycle and the susceptibility of the cell lines

The stained cytoplasm and the shiny, perinuclear clods what accumulated in one side of the nuclei made the infected cells easy distinguish. The first infected cells were detectable at the 12th hour of the high multiplicity (MOI: 2) infection (two hours earlier than at the low multiplicity), while the secondary infection formed fluorescent foci were visible from the 24th hour of the low multiplicity (MOI: 0.01) infection. Therefore the length of the infectious cycle is 10-14 hours at the DHAV-1. Strong stained nuclei were proved the replication of the viruses at the GHPV and GPV infection. The first infected cells (MOI: 2) were observed at the 16th hour

and at the 14th hours in case of the GHPV and GPV respectively. The GHPV re-infected cells appeared from the 30th hours while the secondary GPV infected cells were detectable from the 28th hour. Therefore, times of the infectious cycles were 12-16 hours both at the GHPV and GPV. The susceptibility of the cell lines and the infectivity of the viruses were calculated based on the viral copy number which were needed for the IF detection of the one infected cells. The cell lines were the most susceptible for the DHAV-1 infection and the values were almost equal. One magnitude more GHPV was needed for one infected CS cell compared to the CR and CRpIX. The CRpIX cells were more susceptible with one magnitude for the GPV infection. Generally, the CRpIX cell line was the most susceptible however we measured very similar values at the CR cells (excepted at the GPV infection).

Discussion

The effect of the SAT protein to spread of the PPV Kresse strain and to the lysis of the infected cells

The “slow spreading” phenotype seems general property of the SAT$^-$ PPV strains. The first infected cells were detectable using capsid-specific antibody from the 12th hour of the infection based on the *in vitro* infection experiments. This suggests that the speed of the viral entry, the decapsidation, the replication of the nucleic acid, the synthesis of the proteins and the assembly is same regardless of the presence of SAT.

There is a great different between the copy numbers of the wild type and the mutant viruses at the 40-64 hours of the low multiplicity infection (MOI: 0.01). This different could explained by the three hours later viral release of the SAT$^-$ viruses, which accumulate during the re-infection and by the decrease of number of the dividing cells which are essential for the parvovirus. There is a less different between the copy numbers at the high multiplicity infection. Our studies showed that the final copy number of the SAT$^-$ and the wild type virus is near equal. However, substantial different was observed between the LDH activities in favour of the wild

type virus. The number of the lysed cells is higher presumably therefore more viruses released from the cells. The higher different (6.5×) was detectable at the 48th hour between the LDH activities. The 95% of the wild type virus infected cells were de-attached from the plate at this time, while 15% of the SAT^- virus infected cells. The rate of these is 6.33 what near equal to the different of the copy numbers (6.5). This suggests that the SAT protein do not influence the number of the produced viruses.

The rate of the apoptotic nuclei stays low at the PPV Kresse infection regardless of the presence of SAT protein. We did not observed fragmented, apoptotic like nuclei contrast by the CHOP transfection. The PI staining and the LDH release indicated cell membrane damaged suggest that the main form of the cell death is the necrosis. The cell keep there integrity more time in the absence of SAT, decrease the number of both the apoptotic necrotic cells and the so the virions later released from the infected cells.

The effect of ER stress response for spreading of PPV

The Xbp1 expressed and the CHOP activated at least 95% at least 41% of the infected cells respectively. These verify that the PPV infection can induce the ER stress. However, the wild type virus expressed SAT protein can activate the CHOP in significantly more cell than in the mutant virus infected cells (76% and 41%). Furthermore, the SAT can influence the localisation of the CHOP in the cell. Based on the literature, the nuclear localisation of the SAT is the sign of the strong ER stress therefore, possibility that the virus can induce only weaker ER stress in the absence of SAT. The CHOP is one of the most important regulator protein of the ER stress induced cell death, which suggested that there is a connection between the wild type virus infection observed stronger ER stress and the stronger cytopathogenic effects.

The used chemicals (DDT, MG132 and thapsigargin) can induce the expression of Xbp1 and CHOP and lead to the death of the treated cells. The short term (five and two hours) treatment of the low multiplicity infected cells had positive effect for the spreading of the wild type and the SAT^- viruses.

Furthermore, the DTT and MG132 treatment increased the copy number and the titre of the viruses which released from the high multiplicity infected cells. This suggested that the infection inhibitory effect of the chemicals can fully compensate the positive influence of the UPR. Based on our results, the ER stress or the response of the cell helps the virus release.

The artificially, different way induced ER stress could compensate the loss of SAT. This confirm that the development of the ER stress in the infected cells associates with the faster spreading of the PPV Kresse virus and this suggested that the function of SAT protein is not limited to the influence of the CHOP, it more generally related to the ER stress response.

The effect of the SAT-DsRed and CHOP-DsRed fusion proteins

The expression of SAT caused morphological changes in the ER of the transfected cells and induced the apoptosis but, it could not activate the expression of Xbp1 or CHOP, and did not influence the localisation of CHOP.

The expression and nuclear localisation of CHOP could not neutralize the “slow spreading” phenotype of

the pSAT^{-} infectious clone. This suggested that the protein alone cannot activate the transcription changes which cause strong lysis and accelerated spreading. This result confirms that the SAT protein is not direct function the stimulation of the expression of CHOP.

Since severe ER stress emulates the effect of SAT protein, one of the most likely mechanisms of action of SAT is that in the ER it influences protein interactions, which make the UPR response more severe. This process may lead to the activation of the PERK-eIF2α-ATF4-CHOP pathway where one or more of the proteins upstream of CHOP also induce alternative pathways. These may supplement the effect of CHOP leading to early cell death. In an alternative scenario, SAT may induce other ER stress response pathway/s beside UPR, and this effect alone or synergistically with the CHOP pathway cause early cell death.

Permissiveness of Muscovy duck origin cell lines

High copy number of viral nucleic acid was observed in the supernatant of DHAV-1 infected cells (10^8/ml). Copy number changes of the DHAV-1 during the monitored period were very similar in the three cell types at the low multiplicity infection. The genome copy

numbers at 72 hours p.i. in this low MOI infection were as high as the measured values at the end of the original serial passages. The ascent of the viral growth curve of the DHAV-1 is steeper than that of the investigated DNA viruses, which can be attributed to the shorter cycle time of the virus and to the independence of RNA genome replication from the cellular replicative machinery that allows DHAV-1 replication also in quiescent cells.

DuCV did not induce cytopathic effects on the examined cell monolayers, and it was associated with the lowest copy number in the supernatants of the ten-fold passaged viruses in each of the cell types (around 10^5 copies/ml). Therefore, we assumed that the AGE1 cell lines are semi-permissive for the DuCV.

GHPV also produced with variable copy numbers (10^4-10^7copies/ml) and the virus showed markedly different growth rates in the different cell types. These suggested the GHPV is more sensitive to the small, biological different of the cells. The presence or absence of the cytopathogenic effect did not specific for the cell line, which suggested for the importance of the other circumstances of the infection.

The GPV caused cytopathogenic effect on the all cell lines. The DNA copy number of GPV in the tissue

supernatants was the highest at the CS cell line (MOI: 3). However, the virus exhibited the fastest growth also in the CRpIX cells (MOI: 0.01).

The susceptibility of the AGE1 cell lines and the time of the infectious cycle in the CRpIX cells

Was generally observed that the first infected cells were detectable two hours later at the low multiplicity than at the high multiplicity infection. It is another proof of that the course of infection depend on the quantity of the viruses. The possibility is raised (especially at the DNA viruses which cannot replicate independent) that the lower rate of the cell in appropriate phase of the cell cycle may be responsible for the later appearance of the infected cells. This was confirmed by the fact that the DHAV-1 (what can replicate its genome independent of the replication machinery of the cell) had the shorter time of infectious cycle and the higher infectivity. 55 copies were sufficient for one infection however we could not observed high different between the cell lines. The independent replication for the cell cycle, the speeder time of infectious cycle and the higher infectivity of the DHAV-1 can explain the accelerated growing of the copy number.

The times of the infectious cycle were similar at the GHPV and GPV (12-16 hours), however the first infection and the first secondary infection were detected at two hours later. The infectivity were very similar as well in the CS and CRpIX cell lines, however the CR cell line was the most susceptibility for the GHPV infection. Nonetheless, the CRpIX seems to be the most applicable for the studies of the investigated viruses based on the growth curve.

The results of the infectivity could interpreted as the detection limit of the IF staining. The susceptibility of the used SYBR Green based real-time PCR was half-one magnitude higher (24-154 copy) than we observed at the IF staining.

The AGE1 cell lines stable expressed the E1A and E1B proteins of the human adenovirus-5. The expression pattern influencing and the cell cycle progression ability of these proteins are known. The DuCV, GHPV and GPV are small DNA viruses, which (absence of own polymerase enzyme) depend on the replication machinery of the cells. However the active replication machinery cannot explain alone the permissive of the AGE1 cell lines, because primer cell or immortalised cell lines did not supported the replication

of these viruses at some times. Earlier studies were shown that the E1A regulator protein can modify the anti-viral response of the cells. The CRpIX cell line was the most applicable and it expressed the IX protein. This is a multifunction protein, which modify the anti-viral process, regulated the apoptosis, influence transcription of some genes and induce the heat sock chaperons. So can be assumed, that the influence of the adenovirus genes contribute to the susceptibility of the cells.

New scientific results

1. We confirmed that the “slow spreading” phenotype develops in the PPV Kresse strain in the absence of the SAT as well, so it seems general property of the SAT^- PPV strains. The reason of the “slow spreading” is that the SAT^- infected cells maintain their integrity for a longer period of time and the amount of the lysis decrease in case of the absence of SAT protein.

2. We detected that the ER stress response related Xbp1 activates in PPV Kresse infected cells regardless of presence of the SAT and the cell death related CHOP activates in less infected cells and changes its localisation in absence of SAT.

3. We demonstrated by using ER stress inducing chemical agents (DDT, MG132, thapsigargin) that the development of the ER stress in the infected cells associates with the faster spreading of the PPV Kresse virus.

4. We concluded that the SAT protein alone cannot activate the transcription of the Xbp1 or the CHOP, furthermore the expression and the nuclear

localisation of the CHOP do not explain the faster spreading of the wild type virus.

5. We confirmed that the AGE1.CR, AGE1.CRpIX and AGE1.CS cell lines are permissive for the DHAV-1, GHPV and the GPV and semi-permissive for the DuCV.

6. We defined the growth curve of the DuCV, DHAV-1, GHPV and GPV and revealed the CRpIX and the CR cell line is the more susceptible for the DHAV-1 and GPV and for the GHPV.

Acknowledgement

I would like to thank my supervisor, Dr. Zoltán Zádori for the elaboration of the research plan and for management of my work. I am grateful for his support and patience.

I thank Dr. Ádám Bálint for his suggestions and help during the research and for the tissue samples and sera. I am grateful to Dr. Ádám Dán for made the primers available to us, to Dr. Tamás Tuboly for his encouraging words and suggestions.

I would like to thank all my colleagues in the Functional Virology Research Team, Enikő Kádár-Hürkecz, Ferenc Olasz, Renáta Tóth, Andrea Viszovszki for I could always count on their help. I thank Dr. Andor Doszpoly, Barbara Ujvári and all my colleagues in the IVMR CAR HAS who helped my work.

I would like to gratefully thank my parents and my family for their support.

Publications related to the present dissertation

Publications in peer-reviewed journal

Mészáros, I., Tóth, R., Olasz, F., Tijssen, P., Zádori, Z.: **The SAT protein of porcine parvovirus accelerates viral spreading through irreversible ER stress induction**, Journal of Virology, doi: 10.1128/JVI.00627-17, 2017.

Mészáros, I., Tóth, R., Bálint, Á., Dán, Á., Jordan, I., Zádori, Z.: **Propagation of viruses infecting waterfowl on continuous cell lines of Muscovy duck origin**, Avian Pathology, 43(4). 379–386, 2014.

Mészáros, I., Tóth, R., Bálint, Á., Thuma, Á., Zádori, Z.: **Víziszárnyas-parvovírusok**, Magyar Állatorvosok Lapja, 136. 599–609, 2014.

International conference abstracts

Mészáros, I., Tóth, R., Tijssen, P., Zádori, Z.: **The SAT protein of ungulate protoparvovirus-1 accelerate viral spreading through irreversible ER stress induction.** XVI. International Parvovirus Workshop. France, Ajjacio, 2016.

Zádori, Z., Tóth, R., Bálint, Á., Dán, Á., Jordan, I., Mészáros, I.: **Propagation of viruses infecting waterfowl on continuous cell lines immortalized by adenoviral genes.**

International Union of Microbiological Societies Congresses. Canada, Montreal, 2014.

Publications do not related to the present dissertation

Cságola, A., Zádori, Z., Mészáros, I., Tuboly, T.: **Detection of porcine parvovirus 2 (Ungulate tetraparvovirus 3) specific antibodies and examination of the serological profile of an infected swine herd**, PLoS One, 11(3). e0151036, doi: 10.1371/journal.pone.0151036, 2016.

Tóth, R., Mészáros, I., Farsang, A., Zádori, Z.: **Az állatorvosi vakcinák adjuvánsainak hatásmechanizmusai**, Magyar Állatorvosok Lapja. 138. 31–46, 2016.

Tóth, R., Mészáros, I., Stefancsik, R., Bartha, D., Bálint, Á., Zádori, Z.: **CpG distribution and methylation pattern in porcine parvovirus**, PLoS ONE, 8(12). e85986, doi:10.1371/journal.pone.0085986, 2013.