

**University of Veterinary Medicine Budapest
Doctoral School**

**Multifactorial viral enteric diseases of swine,
with emphasis on coronaviruses**

Brief Summary of PhD Thesis

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Introduction

Infectious enteric diseases of swine are highly prevalent and have great economic importance worldwide. The disorder have been associated with many viruses, although their role as an enteropathogen is not clear in several cases. Numerous prevalence surveys are available for certain viruses with a growing number of complex studies, as the development of diarrhea is assumed to have a complex aetiology. This infectious disease affects mostly young animals, therefore most studies concentrate on the examination of suckling piglets. However, older animals can have a role in the sustainment of the pathogen on a herd level, so it is expedient to involve more age groups in these kinds of studies. There are prevalence surveys about certain diarrhea associated viruses in suckling piglets and fatteners in Hungary, but a complex study is not implemented, yet.

There are known enteropathogens among coronaviruses with great importance nowadays, deserving particular attention. Transmissible gastroenteritis virus (TGEV) is not accentuated from this viewpoint, as it was only present sporadically in the last few decades in Europe. However, it reappeared in a clinical case in Hungary a few years ago, which questioned the immunity of swine herds against this virus in the country. In contrast to TGEV, porcine epidemic diarrhea virus (PEDV) is one of the most important enteropathogens at present causing great economic losses in North-America and Asia, while Europe is less affected for now. There is no clear explanation for the difference between the continents, which makes the prevalence studies of PEDV relevant along with the genetic analysis of detected viruses in order to compare differently located viruses and explore mutations and recombination characteristic for coronaviruses.

Aims of the thesis

The aim of our research was to get a comprehensive view on the actual status of Hungarian swine herds in relation to diarrhea associated viruses. We designed a prevalence survey to detect such viruses with the intention of achieving an improved knowledge about the complexity of the disease and a recommendation of protection strategies for different examined age groups.

In relation to TGEV our aim was along with the prevalence survey to determine the level of protection of swine herds by elevating serological examinations.

The prevalence of PEDV according to its highlighted importance was intended to assess with special care, extending the range of samples with routine diagnostic specimen. The aim of our study was to analyze PEDV from other countries genetically in comparison with viruses detected by us. We also intended to isolate the identified viruses in order to take the first steps toward antigen structure studies and vaccine development.

Materials and Methods

Sample collection

A total of 384 fecal samples from 17 farms were collected for the prevalence survey from May 2016 to February 2018 throughout Hungary. Out of the compilation 239 samples were collected from diarrheic pigs and 145 from asymptomatic animals, as controls. Samples were collected from one, two and three to four week-old piglets, their related sows and fatteners shortly after weaning.

The collection of 908 serum samples for TGEV serology was selected from samples sent to the Veterinary Diagnostic Directorate (VDD) of the National Food Chain Safety Office (NFCSO) in the framework of the eradication plan of porcine reproductive and respiratory syndrome (PRRS). The samples were sent from 93 different farms and 10 samples per farm were chosen, if possible. These samples were collected mainly from sows, therefore we included our own archive samples in the survey and examined 174 samples collected from farm 'F' in North-East-Hungary in 2013.

Unrelated to the prevalence survey different samples were collected from four cases of PEDV occurred in 2016 and 2018.

In January 2016 watery diarrhea and occasional vomiting was observed in a 60-sow farrow-to-finish pig farm located in western Hungary. Morbidity reached 100% and the mortality of suckling piglets rose to a peak of 30%. The carcass of a piglet and 12 rectal swabs from different age groups (boars, sows and piglets) were submitted from this case.

In January 2018 diarrhea and inappetence was observed in all age groups in a 1800-sow farrow-to-finish pig farm (farm 'A') located in North-East Hungary. Mortality of less than a week-old piglets reached 40%. Four weeks after the preliminary test of two carcasses a selection of samples were collected from boars, sows, gilts, four and one week-old piglets, respectively. Eight rectal swabs, eight blood and five to eight fecal samples were collected from the same animals along with three environmental swabs from each age group.

In January 2018 diarrhea and considerable weight variability was observed in a fattening unit (unit 'B2') of a pig farm (farm 'B') located in mid-Hungary. Six oral fluid samples were collected from six groups of approximately 30 animals in the 1000-sow farrow-to-finish unit (unit 'B1') without any symptoms. Five oral fluid, five rectal swab, four environmental swab and five blood samples were collected from five different pigsties holding 25-30 pigs each in unit 'B2'. Samples were collected mainly from symptomless animals four weeks after the first clinical signs appeared.

In March 2018 six carcasses were submitted for routine diagnostic examinations from a fattener only pig farm (farm 'C') located in eastern Hungary.

Oral fluid samples of farm 'B' and samples from farm 'C' were tested with WITNESS PED-TGE-Rota quick test at the site.

Molecular biology methods

Nucleic acid extraction was carried out with MagAttract Virus Mini M48 Kit (Qiagen) on a King Fisher 96 Flex (Thermo Fisher Scientific Inc.) instrument according to the manufacturers' instructions.

Coronaviruses were detected with real time PCR using Vioreal Kit PEDV&SDCV and Vioreal Kit TGEV (Ingenetix GmbH) on a Rotor-Gene Q 5plex Platform (Qiagen) following the manufacturers' instructions. PEDV-positive samples were also tested with PEDV N gene PCR and the full genome sequence of two PEDV was determined based on previously reported methods.

Adeno-, astro-, boca-, calici-, kobu-, rota- and Torque teno viruses were detected with conventional PCR on a TGradient Thermocycler (Biometra) or a 2720 Thermocycler (Applied Biosystems) based on primers reported previously. Reverse transcription was carried out to detect RNA viruses with RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc.) according to the manufacturers' instructions.

Beyond the fecal samples collected for the prevalence study 30 fecal samples from farm 'A', 25 rectal swabs from farm 'B' and five fecal samples from farm 'C' were tested for viruses listed above.

Sequencing, sequence analysis

Approximately one third of the PCR positive products of the prevalence survey, adequate products of the PEDV N gene PCR and fragments necessary to determine the full genome sequence of two PEDV were submitted to BaseClear B.V. or Biomi Ltd. for sequencing. Different versions of BioEdit (Ibis Biosciences), DNASTAR (DNASTAR Inc.) and MEGA were used to edit and analyze sequences, then RDP4 and SimPlot were utilized to detect recombination. The complete genome sequences of two PEDV were submitted to GenBank under the accession numbers (Acc. No.) KX289955 and MH593900, respectively.

Serology methods

Serum samples collected for TGEV serology and our archive samples were examined with immunofluorescence test (IFT). Swine testis cells infected with the cell culture adapted Purdue-115 TGEV strain were incubated with the diluted samples, followed by an incubation with a fluorochrome-labeled anti-pig IgG (Sigma-Aldrich) and dyed for contrast with Evans blue at last. Cell staining was examined with a fluorescence microscope (Nikon). Positive samples

of farm 'F' representing more age groups (piglets, sows and fatteners) were also tested quantitatively by preparing a two-fold dilution.

In order to differentiate antibodies produced against TGEV and porcine respiratory coronavirus (PRCV) the IFT-positive serum samples were also tested with INgezim Corona Diferencial 2.0 (Ingenasa) according to the manufacturers' instructions.

Blood samples of farm 'A' and 'B' involved in the PEDV cases of 2018 were tested with Ingezim PEDV (Ingenasa) following the manufacturers' instructions.

Virus isolation

Virus isolation of PEDV was attempted with intestinal samples from the farm involved in the PEDV case of 2016 and the following samples of 2018: two intestinal, two fecal and two rectal swab samples from farm 'A', three oral fluid and two rectal swab samples from farm 'B' and five fecal samples from farm 'C'. Isolation was performed on the basis of a previously described method with slight modifications. Vero cells were inoculated with centrifuged and filtered samples. Flasks containing inoculated cells were frozen and thawed three times on the seventh or earlier day based on the cytopathogen effect (CPE) observed daily. The obtained suspension was tested with the above mentioned PEDV N gene PCR to determine the success of the isolation.

Statistical analysis

The statistical analysis of the results of the prevalence survey was conducted by using GraphPad Prism 7.0 (GraphPad Software). Fisher's exact test was used for the comparison of diarrheic and control sample rates. The difference in the number of detected viruses in different age groups was examined with chi-square test. The relations between age groups and viruses were analyzed by using binomial distribution with a null hypothesis that there is no difference between examined groups.

Results

Prevalence survey

Out of 384 fecal samples collected for the prevalence survey 196 contained at least one examined virus and positive samples were detected on all 17 farms. There were 126 single and 70 mixed infections involving two (63%), three (27%) or four (10%) viruses at the same time. Kobuviruses were detected most frequently in 55.1% of the positive samples followed by bocaviruses with 33.2% and rotaviruses with 20.9% detection rates. The prevalence of other examined viruses did not reach 15% in proportion to all positive samples (adenovirus – 14.3%, astrovirus – 13.8%, porcine circovirus type 2 – 7.7%, calicivirus – 5.6%, Torque teno sus virus 1 and 2 – 1.0-1.0%). Coronaviruses and porcine rotavirus (PRV) B were not found in any samples collected for the prevalence study.

Positive samples were found in 63.8% of cases from diarrheic animals and all detected viruses were more frequent in diarrheic samples by count except for Torque teno sus virus (TTSuV) 1, which was found only in control samples. However, after a correction in relation to the total number of diarrheic (239) and control (145) samples the frequency of porcine kobuviruses (PKV) was higher in the control group (24.7% diarrheic and 33.8% control). The positivity rate of diarrheic samples was higher than control samples with all other examined viruses (bocaviruses – 17.6% vs. 15.9%, rotaviruses – 13.4% vs. 6.2%, adenovirus – 8.4% vs. 5.5%, astrovirus – 7.5% vs. 6.2%, calicivirus – 5.0% vs. 2.1%, porcine circovirus type 2 – 3.3% vs. 2.1%, Torque teno sus virus 2 – 0.8% vs. 0.0%), although the only significant difference ($p=0,0275$) was found in case of rotaviruses.

The proportion of positive samples in correlation to age groups in a decreasing order was as follows: three to four week-old piglets 69.9% (48/69), two week-old piglets 64.5% (49/76), weaned pigs 63.9% (46/72), one week-old piglets 40.4% (36/89) and sows 21.8% (17/78). The order in relation to the number of detected viruses changed to weaned pigs 108, three to four week-old piglets 65, two week-old piglets 58, one week-old piglets 48 and sows 20 detected viruses. Summarizing these data it was determined that mixed infection was the most frequent in weaned pigs with 76.1%, while single infection was dominant in all other groups. The rate of mixed infection was 30.6% in one week-old piglets, 16.3% in two week-old piglets, 29.2% in three to four week-old piglets and only 11.8% in sows. There was no significant difference in the proportion of positive samples of different age groups, but an overall significant difference ($p<0,0001$) was found in the number of detected viruses and also in the comparison of each age group with the exception of two week-old and three to four week-old piglets.

The most frequent PKV was detected usually in the samples of suckling piglets and in a significantly ($p<0,0026$) lower number in weaned pigs, while it was not found in any samples

of sows. In contrast, porcine bocaviruses (PBoV) second in frequency were detected in a significantly ($p < 0,0001$) higher rate in weaned pigs compared to other age groups. The third in frequency, PRV only showed a significant difference ($p = 0,0029$) compared to other age groups in one week-old piglets, where the only PRVC positive samples were found. Other examined viruses were detected mostly from weaned pigs with the exception of TTSuV1, which was identified in two samples collected from a two week-old and a three to four week-old piglet, respectively.

TGEV serology

Out of the examined 93 pig farms 41 had positive results distributed evenly throughout the country. Only one positive sample was detected on most (in total 12) farms, while only one farm had TGEV specific antibodies in all its examined samples. Out of 908 serum samples 140 contained anti-TGEV antibodies. The differentiating ELISA showed that almost all of these antibodies found in the IFT were produced against PRCV, only one sample contained antibodies produced against TGEV.

Out of 174 samples from farm 'F' representing more age groups 31 was positive by IFT. Positive samples were distributed to 20 suckling piglets and 11 fatteners. The titer of the samples as a result of the quantitative IFT reached an average of 28.4 between the values of four and 128. The differentiating ELISA showed antibodies produced against PRCV in all IFT positive samples.

PEDV cases

In the PEDV case of 2016 the presence of the virus was confirmed in an intestinal sample of the carcass of a piglet and five rectal swabs.

In relation to the PEDV cases of 2018 both PCR and ELISA positive samples were obtained. Out of 85 samples from farm 'A' 26 were positive for PEDV with PCR and 34 of 40 blood samples contained PEDV specific antibodies. ELISA-positive samples were found in all age groups. Positive fecal samples were identified with the exception of sows and positive environmental samples with the exception of one week-old piglets in all age groups, while positive rectal swabs were only detected in one week-old piglets. Out of 30 fecal samples 12 were positive for PEDV, including five cases of mixed infections, while ten samples were positive only for other viruses. Adeno-, astro-, boca-, calici-, kobu- and Torque teno viruses were found apart from PEDV.

All oral fluid samples from farm 'B' were negative with WITNESS PED-TGE-Rota quick test and all six samples from unit 'B1' were negative for PEDV with PCR as well, but it was detected with this method in five of 25 oral fluid samples collected in unit 'B2'. Moreover, all 25 blood samples of unit 'B2' were positive for PEDV specific antibodies and there were seven of

25 rectal swabs and 14 of 25 environmental swabs positive for PEDV with PCR involving all pigsties. Rectal swabs tested for other viruses as well resulted in four single PEDV cases, three mixed infections with PEDV and nine samples were only positive for other examined viruses. Adeno-, astro-, boca-, calici- and kobuviruses were found apart from PEDV.

Pathological examination of the six carcasses from farm 'C' revealed high amounts of watery intestinal content in two animals, but all cases were diagnosed with other disorders. Samples from five animals were positive for PEDV with WITNESS PED-TGE-Rota quick test, which was verified by PCR. All five samples contained other examined (circo-, kobu- and Torque teno) viruses as well.

Similarity of PEDV N gene sequences from the four farms involved in the PEDV cases ranged between 99.4% and 99.7%.

Virus isolation was successful from an intestinal sample collected from the PEDV case of 2016 and three of 16 samples chosen from cases of 2018. CPE characterized by rounded, fused and detached cells was observed from 48 hours after the inoculation of cells and the presence of the virus was confirmed by PEDV N gene PCR.

Complete genome sequence of PEDV from the case of 2016 was assembled by using an intestinal sample as a base material. The obtained PEDV HUN/5031/2016 showed higher than 99% nucleotide (nt) similarity with European PEDV sequences of 2014-2015 available at the time with the most differences in the S gene. These differences concentrated on an approximately 400 nt long section, which shared the highest identity of 95-96% with swine enteric coronaviruses (SeCoV). A significant ($p < 0.05$) recombination was detected by RDP4 in this section between positions 248 and 640 nt involving PEDV 15V010/BEL/2015 (major parent, Acc. No.: KR003452) and SeCoV Italy/213306/2009 (minor parent, Acc. No.: KR061459), which was verified by SimPlot.

One virus isolate from the cases of 2018 showing the strongest band after PEDV N gene PCR was chosen for full-length genome analysis. The isolated PEDV HUN/S236/2018 shared the highest identity of 99.6% with the Hungarian PEDV HUN/5031/2016 and a Slovenian recombinant (SLOreBAS-1/2015, Acc. No.: KY019623) virus. Analysis of the section, where a recombination event was detected in PEDV HUN/5031/2016 showed high similarity with the above mentioned PEDV and SeCoV, but recombination was not clearly identified by RDP4 in this case with unknown origin of parental viruses. However, SimPlot detected a possible recombination similarly to the PEDV case of 2016.

Discussion

Prevalence survey

A total of 384 fecal samples were collected from 17 Hungarian swine farms in a two-year period to determine the prevalence of diarrhea associated viruses. As the end result, at least one examined virus was detected in 52.3% of diarrheic samples and 49% of control samples, which is slightly lower from the data of similar studies, although the range of examined viruses differ in each report. Single infection was found in 64% of the total of 196 positive samples, which again contradicts certain reports and also our hypothesis of higher prevalence of co-infection with different viruses at the same time. After ruling out the technical problems we assume that the lower and less complex virus prevalence should be considered as a local characteristic, which may be clarified by the examination of larger sample numbers and more viruses or using other, for example metagenomic methods.

Interestingly, coronaviruses (PEDV, TGEV and porcine deltacoronavirus, PDCoV) were not found in the samples collected for the prevalence survey, although PEDV appeared in the country during the examined period. However, more than one study aiming to assess the prevalence of coronaviruses in neighboring countries had similar negative results, which suggests that these viruses are not widespread in this area.

Porcine kobuviruses (PKV) were detected most frequently with a rate of 55.1% in all positive samples, which is similar to the data found in the relevant literature, but there are differences in the results in relation to health status. We detected PKV in 33.8% of 145 samples collected from symptomless animals and 24.7% of 239 samples of diarrheic pigs. In contrast, a previous Hungarian study showed 54.5% PKV positivity in 13 samples of healthy animals and a rate of 92.3% in 37 pigs with diarrhea. The higher PKV prevalence in symptomless animals in our study is still not ostentatious, since the virus is often found in samples of healthy pigs, which was the origin of its first Hungary-related description as well. Out of 108 detected PKV 99 were identified in suckling piglets and may have caused disease in this susceptible age group, while PKV positive piglets without symptoms may have received a higher level of protection by maternal immunity.

PBoV was second in line in detection frequency with 33.2% of all positive samples and was found mostly in weaned pigs, similarly to another study examining pigs with respiratory symptoms.

The third in frequency detection, PRV was detected in 13.4% of diarrheic samples and 6.2% of control samples, which rates showed the only significant difference ($p=0,0275$) in health status with a higher rate in diarrheic samples compared to other examined viruses in accordance with another Central-European study.

The prevalence of other viruses (adenovirus – 14.3%, astrovirus – 13.8%, porcine circovirus type 2 – 7.7%, calicivirus – 5.6%, Torque teno sus virus 1 and 2 – 1.0-1.0%) was lower in general compared to other studies showing a low rate of viral load in the examined Hungarian swine herds during the time of this study. However, the collection of samples positive for certain viruses could be useful in future studies planned in our institute aiming for example to determine the causative role of viruses with unclarified pathogenesis.

The result of the prevalence study in relation to age showed no significant difference between the number of positive samples among certain age groups, but there was a significant difference ($p < 0,0001$) regarding the complexity of infections by examining all age groups and also during the comparison of each age group except for two week-old and three to four week-old piglets. Most viruses, a total of 108 virus, i.e. 36% of all detected viruses were found in 46 samples of weaned pigs. In contrast, single infections were dominant in suckling piglet groups. This means that mixed infections were more frequent in weaned pigs, which led to the assumption that the hypothesis of higher prevalence of co-infection with different viruses at the same time can depend on age. The lowest number of positive samples and detected viruses were found in sows, which shows that even if their piglets were infected with a virus, the infection did not reach such a level in sows to shed the virus in a detectable extent.

The pathogenicity of examined viruses is not clarified by the results of the prevalence survey, as the rate of virus detection was almost the same in samples of diarrheic (52.3%) and symptomless (49%) animals, neither supports the relevant literature the usage of such data for this purpose. However, positive control samples were detected usually beside positive diarrheic samples of the same group of animals implying that the virus infection and shedding could have been persisted even after the clinical signs ceased. In summary, prevalence data is not applicable to draw conclusions about the pathogenicity of diarrhea associated viruses of swine, still, collection of such data can be useful to determine the direction of protective measures against the enteric disease of swine, which should by all means include the knowledge of the characteristics (hygiene and food management etc.) of each pig farm.

TGEV serology

Diarrhea caused by TGEV became infrequent in the 1990s parallel to the appearance of its deletion mutant PRCV causing usually symptomless respiratory infection, but it still appears sporadically. In Hungary, the virus was found in a clinical case for the last time in 2013 followed by an extended survey, when both TGEV and PRCV was found in seven of 14 farms. In contrast, we did not detect TGEV in either of the examined 17 farms, while PRCV cannot be identified by the PCR we used. In the absence of the detection of the virus we hypothesized that there is high seropositivity in the country with antibodies produced against TGEV or cross-protective antibodies produced against PRCV. Our hypothesis was denied by the result of the

IFT, which showed seropositivity in 15.4% of 908 serum samples. Antibodies against PRCV were dominant in the IFT-positive samples as determined by the differentiating ELISA with only one sample containing antibodies produced against TGEV on a farm, where five other samples tested positive for anti-PRCV antibodies. In this case the animals may have had an inapparent TGEV infection only, as they were protected by antibodies produced against PRCV. The examination of farm 'F' representing more age groups showed similar results to the rate of the country with 17.8% seropositivity of 174 samples and antibodies against PRCV in all positives. The quantitative IFT showed a relatively low titer with an average of 28.4, although it does not necessarily mean the lack of effective protection against TGEV, which is essentially provided by other types of immunoglobulins on the mucous membranes. There were three IFT-positive samples that showed a negative result in ELISA first and only a repeated assay verified the positivity of the samples that may be explained by the low quantity of antibodies, which was not enough to exceed the threshold of the kit. This aspect along with cost-effectiveness contributes to the significance of both diagnostic methods.

In summary, the occurrence of an outbreak caused by TGEV cannot be excluded, as the seroprevalence of anti-TGEV antibodies is low in Hungary. Therefore the detection of this virus remains important and cannot be omitted from the diagnostics of diarrhea in swine.

PEDV cases

Unrelated to the PCR survey, four cases of PEDV occurred in Hungary during the research period. At first a small pig farm was involved in 2016, where an epidemiological inquiry was not conducted, as the owner of the farm rejected further co-operation after the initial diagnosis was made and terminated operation after the clinical signs ceased. Subsequently, no new case occurred in the following two years, neither PEDV was detected till its reappearance in 2018, even though a survey directed to search for the virus was made. As the source of the infection for the new cases the ceased pig farm involved in 2016 was excluded, as well as the boars imported to farm 'A' based on the absence of infection in the exporting Danish pig farm and the PEDV N gene sequence analysis. Farms 'A' and 'B' both transport animals to the same slaughterhouse, which suggested its center role in the infection, but was dismissed, as no other case occurred in other farms in connection with this abattoir. Farm 'C' was not connected to this slaughterhouse and the owners did not approve further examinations. In the end, we were not able to determine the exact introduction routes of PEDV to the farms, although the role of transport vehicles could not be excluded.

In the test of different sample types regarding the PEDV case of 2016, almost half of the rectal swabs tested positive for PEDV, but after the PCR the amount of these samples was not enough for further examinations, therefore an intestinal sample collected from the carcass of a piglet was used afterwards.

In the PEDV cases of 2018 different types of samples could be collected from farms 'A' and 'B' and samples of farm 'A' also varied in age. Fecal and rectal swab samples were collected from the same animals in farm 'A' and in comparison there were more PEDV-positives in fecal samples with a higher virus count. Oral swabs were also collected in farm 'B' with several positive results by PCR, which in contrast remained negative with WITNESS PED-TGE-Rota quick test, probably because of the low viral load of the samples was insufficient to exceed the threshold of the test. Taking all these factors into account, we recommend the collection and examination of fecal samples in the diagnostics of PEDV.

Positive fecal samples were found in all age groups with the exception of sows in farm 'A', while most animals had positive serum samples as well. The virus was also detected in several animals involving all pigsties in farm 'B' along with the seropositivity of all examined pigs. In addition, both farms had positive environmental swabs with a positivity of 47% in farm 'A' and 70% in farm 'B'. All this shows that even though four weeks have passed since the clinical signs appeared and most animals had an immunological reaction during this period, there were still virus shedding animals present responsible for the sustainment of the viral infection with the possibility of re-infection from the environment. These factors emphasize the importance of thorough cleaning and disinfection along with the examination of environmental swabs to supervise their efficiency.

We attempted to detect other viruses in all three PEDV cases of 2018 primarily from fecal samples in order to compare the results with the prevalence survey. Other viruses were detected besides or without PEDV in all farms, but no correlation was found in the prevalence of certain viruses and in comparison of the health status of the animals. Neither the relation of PEDV and other diarrhea associated viruses is clear in the relevant literature.

The isolation of PEDV is not an easily obtained task, it also required an extended time for us to adapt the reported methods to our laboratory environment. The most important modification was the freezing and thawing of the flasks three times instead of only once, in order to free the virus by destructing the cells. This method was processed with an intestinal sample collected from the PEDV case of 2016 and was successful also with three samples of 16 from the cases of 2018. Apart from the productive virus isolation, CPE was observed in a different extent in several cases, but the virus could not be retrieved. We assume that other substances or organisms harmful to cells were present in these samples. According to our knowledge, virus isolation was only performed in Germany from the European PEDV cases of recent years and recombinant PEDV was not isolated, yet. The isolated viruses can contribute to the examination of gene expressions and vaccine development.

The whole genome of the PEDV case of 2016 was assembled by using an intestinal sample directly because of the protracted attempts on virus isolation. The determined PEDV HUN/5031/201 showed a high identity of approximately 99% with European PEDV identified

in recent years. However, the differences mostly found in the S gene were still significant, since a recombination event was detected in it. A Belgian PEDV (Acc. No.: KR003452) and an already recombinant Italian SeCoV (Acc. No.: KR061459) consisting of a TGEV backbone with an S gene from PEDV took part in the recombination.

After the PEDV cases of 2018 the full-length genome was determined by using a successfully isolated virus. Recombination was not evident in the isolated PEDV HUN/S236/2018, even though it showed the highest similarity with the Hungarian PEDV of 2016 and an also recombinant Slovenian PEDV (Acc. No.: KY019623). In a phylogenetic analysis the two Hungarian viruses were the closest with two Slovenian PEDV (Acc. No.: KY019623, KY019624), which were submitted to the GenBank in the end of 2016, but not published, yet. The Hungarian and Slovenian viruses grouped together with low pathogenic European PEDV, although they might should be separated from them considering the distorting effect of recombination. There is no further information available about the two recombinant Slovenian PEDV, therefore it is not known, if these viruses appeared in Hungary or they appeared at the same time in both countries and have a common origin. However, the emergence of these viruses can be expected, since an Italian survey confiding to the examination of the S gene detected similar viruses in 2017. Additionally, since coronaviruses are considerably susceptible to mutations and recombination, the appearance of new variants cannot be excluded, which may lead to changes in pathogenicity as well.

New Scientific Results

1. We determined the prevalence of adeno-, astro-, boca-, calici-, corona-, kobu-, rota- and Torque teno viruses in swine herds of Hungary for the first time within one survey.
2. We assessed with the serological examination of TGEV that the seropositivity is low in Hungary, therefore the possibility of the re-emergence of this pathogen arises.
3. In Hungary we isolated PEDV strains for the first time, at the same time we isolated recombinant PEDV strains first.
4. We determined the whole genome sequence of two Hungarian PEDV strains for the first time.

Scientific Publications

VALKÓ, A., BIKSI, I., CSÁGOLA, A., TUBOLY, T.[†], KISS, K., URSU, K. & Á. DÁN 2017. Porcine epidemic diarrhoea virus with a recombinant S gene detected in Hungary, 2016. *Acta Veterinaria Hungarica* 65 (2): 253-261.

VALKÓ, A., TUBOLY, T.[†] & A. CSÁGOLA 2018. A sertések enterális koronavírussai – Irodalmi összefoglaló. *Magyar Állatorvosok Lapja* 140 (4): 207-216.

VALKÓ, A., MAROSI, A., CSÁGOLA, A., FARKAS, R., RÓNAI, ZS. & Á. DÁN 2019. Frequency of diarrhoea-associated viruses in swine of various ages in Hungary. *Acta Veterinaria Hungarica* 67 (1): 140-150.

VALKÓ, A., ALBERT, E., CSÁGOLA, A., VARGA, T., KISS, K., FARKAS, R., RÓNAI, ZS., BIKSI, I. & Á. DÁN 2019. Isolation and characterization of porcine epidemic diarrhea virus (PEDV) in Hungary. *Acta Veterinaria Hungarica* 67 (2): [recommended for publication]

VALKÓ, A., BÁLINT, Á., BOZSA, Á. & A. CSÁGOLA 2019. Prevalence of antibodies against transmissible gastroenteritis virus (TGEV) in Hungary. *Veterinary and Animal Science* 7:100042.

Conference Presentations

VALKÓ, A., CSÁGOLA, A., TUBOLY, T.[†], DÁN, Á., URSU, K., BIKSI, I. & K. KISS. A sertések enterális megbetegedéseiben szerepet játszó vírusok kimutatása és elterjedtségük felmérése Magyarországon. Akadémiai Beszámolók – előadás, 2017.

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VALKÓ, A. Vírusok a sertések hasmenésének hátterében. Köves napok – előadás, 2019.

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