Szent István University

Doctoral School of Veterinary Science

Epidemiological study of viral pathogens incriminated in enteric disease complexes in Hungarian broiler flocks, with special emphasis on the newly identified parvovirus

Brief version of the doctoral thesis

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Introduction

The newly emerged enteric disease (ED) syndromes, causing serious economic losses throughout the world, have become a challenge for researchers in the field of poultry diseases. Determining their exact etiology, and elucidating their pathogenesis, has the potential of finding treatment and prevention strategies, hence avoiding economic losses and improving the biosecurity of the poultry meat. The purpose of this study was to contribute to the clarifying of the etiology and pathogenesis of the ED syndromes of chickens and turkeys raised under intensive conditions, by providing research data regarding these syndromes, from Hungarian commercial flocks.

Enteric disease complexes in chickens

In broiler chickens the major ED complex is known as malabsorbtion or runting-stunting syndrome (RSS). The RSS is characterized by diarrhea, depression, ingestion of litter, increased vocalization and huddling. Morbidity and mortality are variable, and the economic impact is primarily due to poor production, failure of affected birds to grow, as well as increase in costs of therapy, and poor feed conversion efficiency, but in the severe forms, immune dysfunction and increased mortality have also been reported.

Viruses from numerous families have been identified in the intestinal tracts of poultry with ED: *Astroviridae*, *Coronaviridae*, *Reoviridae*, *Rotaviridae* and more recently *Parvoviridae*. The role of these viruses in the ED is not fully understood but is supported by the syndrome reproducibility with preparations from the intestinal contents of affected birds, which do not contain bacteria or protozoa.

Most enteric viral infections in chicken broilers occur in the first three weeks of life, but it was reported that under certain conditions some could occur later. Since the clinical signs and lesions induced by the different viral agents are similar, it is difficult to attribute a specific viral disease to a given virus. In addition to this difficulty, different combination of the incriminated viruses will result in different presentation of the same disease, making the diagnosis extremely difficult. In general it is considered that a combination of high morbidity and low mortality happens when only one virus is detected, and high mortality combined with various economic losses, takes place when several viruses are detected. No comprehensive treatment or preventive measures have been determined for RSS because of the lack of information regarding the exact etiology of the disease and its pathogenesis, and no commercial vaccines are available for most of these infections.

Enteric disease complexes in turkeys

In turkey poults up to 6 weeks of age, the enteric disease syndrome is referred to as poult enteritis complex (PEC), characterized by diarrhea, depression, ingestion of litter, immunosuppression and increased mortality. In case of high mortality the disease is referred to as poult enteritis and mortality syndrome (PEMS). In the most severe forms of PEMS, up to 100% morbidity and mortality was reported. The etiology of the disease is not completely understood, but is considered multifactorial. Numerous viral and bacterial agents were identified in the intestine of poults with PEC and PEMS. From the bacterial group, enteropathogenic strains of *Escherichia coli* (*E. coli*) are considered to contribute in the manifestation of the disease. As a food animal, the integrity of the gastrointestinal (GI) tract in turkeys is of extreme importance. The efficient utilization of nutrients is primarily dependent on a healthy GI tract, and is considered especially true in case of young animals; hence any damage to the GI tract in the early stages of life will result in irreversible economic damage to the flock.

Frequent coinfections with viral pathogens causing similar clinical signs

Avian nephritis virus (ANV) and infectious bronchitis virus (IBV) can lead to nephropathy, subsequent gout, and enteritis. Infectious bronchitis (IB) is an acute, highly contagious disease that affects the respiratory, renal, intestinal and reproductive systems, causing severe economic loss in the broiler and in the egg layer industry.

Although IBV causes respiratory disease, the virus also replicates in many non-respiratory epithelial surfaces, such as: kidneys, gonads, oviduct, and intestinal tract, where it may cause pathological changes. Infectious bursal disease virus (IBDV) is one of the most important immunosuppressive agents in modern poultry production. Due to the immunosuppression, the flocks become infected with secondary agents that are frequently manifesting with signs of enteritis. The high incidence of cases with similar clinical signs and pathological lesions raises the possibility of simultaneous infection by IBV, IBDV and ANV. As there is a practical need to detect the three pathogens in tissue samples by employing quick, reliable and cost efficient protocols, one of our distinct goals was the development of a rapid and reliable multiplex RT-PCR (mRT-PCR) assay.

Material and methods

Samples collected from chicken farms

The cases considered in this study were collected between January 2007 and March 2010, from flocks experiencing signs of ED. Carcasses of 6 days to 3 weeks old birds from 15 Hungarian chicken broiler flocks, experiencing increased mortality, and/or poor production were sent to the Department of Pathology and Forensic Veterinary Medicine (Szent Isván University, Faculty of Veterinary Science, Budapest, Hungary) for diagnostic purposes.

In order to obtain a better insight into the epidemiology of ED, carcasses from 13 flocks free of ED clinical signs, received for routine assessment of the flock's status were included in this study.

Samples collected from turkey farms

The cases were collected between January 2008 and December 2010 from commercial Hungarian turkey flocks experiencing signs of PEC or PEMS combined with high mortality.

Pooled intestinal tissue (duodenum and ileum) was collected from 49 Hungarian turkey flocks in the Central Agricultural Office, Veterinary Diagnostic Directorate (MgSzH ÁDI) Kaposvár, kindly provided by Dr. Csaba Nemes. One sample corresponds to one flock or house and contains pooled tissue from 5 birds with clinical signs of ED. The age of the birds varied from 6 to 43 days. Two samples were collected in 2010 from the carcasses sent to the Department of Pathology and Forensic Veterinary Medicine (Szent Isván University, Faculty of Veterinary Science, Budapest, Hungary) for diagnostic purposes.

Methods

The examination methods applied in the current study were represented by: (1) macroscopic examination (necropsy), (2) routine bacteriology, (3) histopathology, (4) indirect immunohistochemistry (IHC), (5) electron microscopy (EM), and (6) genetic investigations. Genetic investigations were represented by: purification of the nucleic acid, designing of new primer pairs for diagnostic purpose, amplifications (PCR, RT-PCR, mRT-PCR), RFLP-based techniques (a newly designed *AvaII*-based enzymatic digestion test for the fast differentiation of ChPV, TuPV and TuPV-like ChPV strains), and by nucleic acid sequencing and phylogenetic analysis.

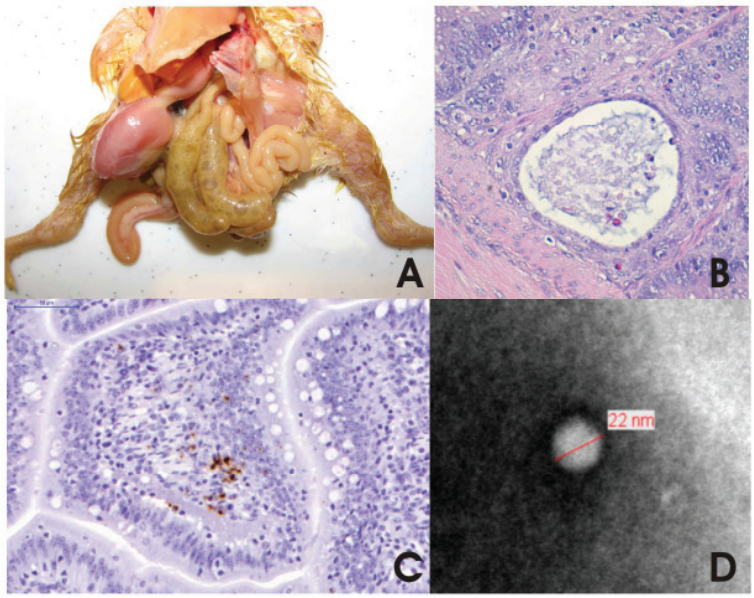
Results

Enteric disease syndrome in chickens

According to the history submitted by the owners and/or treating veterinarians along with the carcasses, the broilers presented slightly higher than normal daily mortality, stunted growth, and diarrhea. At necropsy obvious signs of uneven growth between the members of the same flock and signs of diarrhea were observed. The segments of the small intestine were partially filled with fluid-mucoid content, and large amount of gas (Figure 1 A).

Routine aerobic bacteriological investigations were negative in case of all the chicken samples included in the thesis. The histological examination revealed moderate to severe distention of the intestinal crypts lined with flattened epithelium (Figure 1 B) and containing desquamated cells. Acute catarrhal enteritis with a mixed inflammatory cell population was observed in the jejunum and duodenum, with a low incidence of enterocyte desquamation. Shortening, moderate denudation and fusion of the intestinal villi was observed.

Figure 1



Positive nuclear staining was detected at indirect IHC for chicken parvovirus (ChPV), in the duodenum and jejunum (Figure 1 C). The EM examination of the fractions obtained from the intestinal homogenate revealed the presence of numerous icosahedral, non-enveloped viral particles, which based on their ultrastructural morphology, size and shape, were identified as members of the *Astroviridae*, *Parvoviridae* (Figure 1 D), and *Reoviridae* virus families.

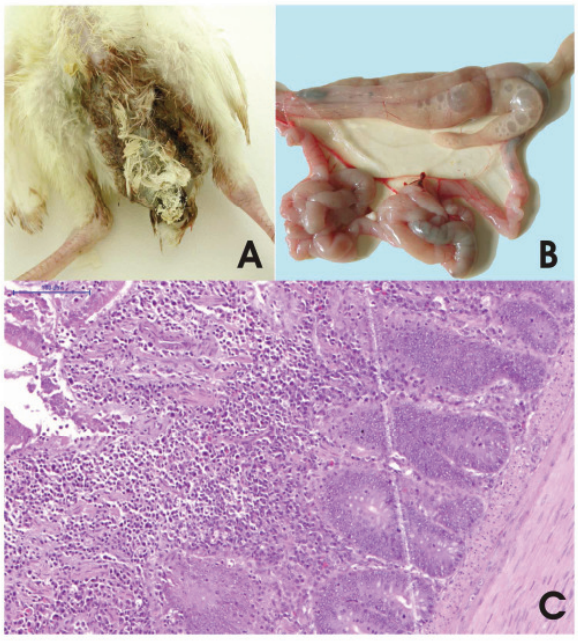
The summarized results of the PCR and RT-PCR applied for the chicken samples are: A high incidence of ChPV: 17 out of 28 samples were positive. Eight chicken flocks, were free from any other enteric viral infection besides ChPV, as determined by PCR. Astroviruses were found to have a high prevalence: 12 out of 28 samples, with ANV being more frequent. In the case of the samples collected from clinically healthy flocks only two chicken flocks were found positive for ChPV, while 6 were positive for ANV and 4 for ARV.

Enteric disease syndrome in turkeys

The poults presented higher than normal daily mortality, stunted growth, diarrhea (Figure 2 A), dehydration and high variation in weight. The small intestine was filled with fluid-mucoid content and gas (Figure 2 B). Dilatation of the intestinal blood vessels and catarrhal enteritis were identified in the jejunum and ileum and atrophy of the bursa Fabricii was observed. Routine aerobic bacteriological investigations were positive for *E. coli* in case of 2 turkey samples.

Shortening, partial denudation and fusion of the intestinal villi was observed at histopathology in the jejunum, with evident inflammation (Figure 2 C). Positive nuclear staining was detected by indirect IHC for turkey parvovirus (TuPV), in the epithelial cells and inflammatory cells from the lamina propria of the duodenum, jejunum, bursa Fabricii, liver and exocrine pancreas.

Figure 2



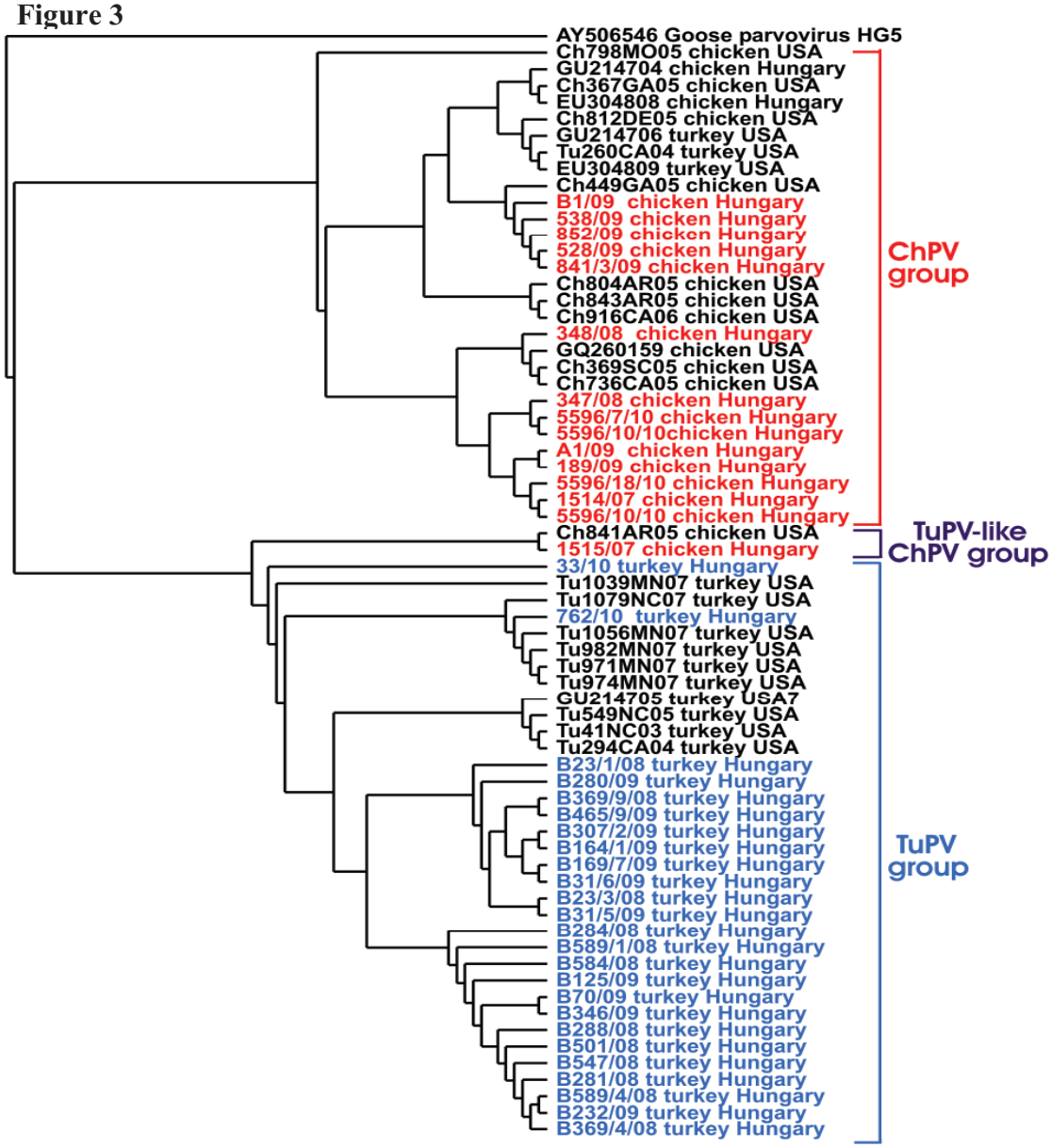
The summarized results of the PCR and RT-PCR amplifications are as follows: turkey astroviruses were found in 83.67% of the cases, and TAstV-2 was found in 26.53% of the cases. Only 14.28% positivity was found for turkey coronavirus (TCV) and avian reovirus (ARV). Two samples were positive for ANV. Due to the unexpected positivity for ANV the results were confirmed by direct sequencing. From a total of 51 samples 25 were found positive for TuPV (49.01%). Singular infections were found in case of 12 flocks (23.52%), from those 6 cases were positive for turkey astrovirus (TAstV) (11.76%), 5 TuPV positive (9.80%) and only one TCV positive (1.9%).

In order to establish whether there is any significant connection and correlation in the incidence of the investigated pathogens, statistical analysis was performed using the correlation testing. The statistical analysis has revealed that with only a few exceptions there are no significant statistical correlations between the incidences of the investigated pathogens. The only statistically significant negative values were observed between the incidence of TAstV-2 and TuPV and TCV and TAstVs, while the only positive, “almost” significant correlation was between the incidence of TAstV-2 and ARV.

Sequence analysis and phylogeny of ChPV and TuPV strains

A total of 15 ChPV and 25 TuPV positive samples were directly sequenced and analyzed. The obtained sequences were 524 bp long for ChPV strains and 527 bp long for TuPV strains respectively. The phylogenetic tree constructed based on the nucleotide sequence of the analyzed NS1 gene segment revealed an evident clustering of the virus strains of different species origin, ChPV group and TuPV group (Figure 3).

Figure 3



Two ChPV strains (1515/07 from Hungary and Ch841AR05 from USA) proved to be more closely related to TuPV strains than to ChPV strains. Samples 1514/07 and 1515/07 were collected from the same flock, but different houses, still they did not cluster together as expected. The same situation was found in case of several samples collected at the same time from the same flock but different houses. With the exception of two samples (33/10 and 762/10) the Hungarian turkey strains clustered separately from the American strains.

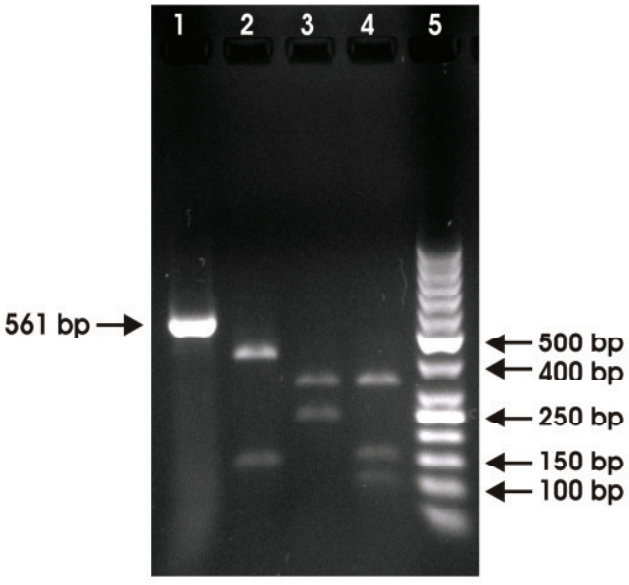
At nucleotide level the identity between the relevant strains considered for comparison, varied from 88.8% to 99.8%, being the lowest when comparing sample 1515/07 with the considered reference strain (EU 304808), and the highest between samples B307/2/09 and B31/6/09. The recombination detection program (RDP) based investigation of the analyzed gene segment did not reveal any recombination events between the examined strains.

The analysis of the deduced amino acid sequence of the ChPV strains resulted in 174 aa long sequences, and 175 aa in case of the TuPV strains. The phylogenetic tree constructed based on the deduced aa sequence of the analyzed NS1 gene segment revealed similar arrangement as described in case of the nucleotide based phylogeny, and the evident clustering of the virus strains of different species origin, ChPV group and TuPV group, was maintained.

Differentiation of ChPV, TuPV and TuPV-like ChPV strains

According to the nucleotide sequences alignement of all existing strains deposited in the GenBank, the sequences of American origin and the Hungarian sequences included in this study, an enzyme site recognition is present on the 561 bp fragment, which has the potential of differentiation of ChPV strains, TuPV strains and TuPV-like ChPV strains that formed a unique subgroup in the TuPV cluster (TuPV-like ChPV), by employing a RFLP-based technique.

Figure 4

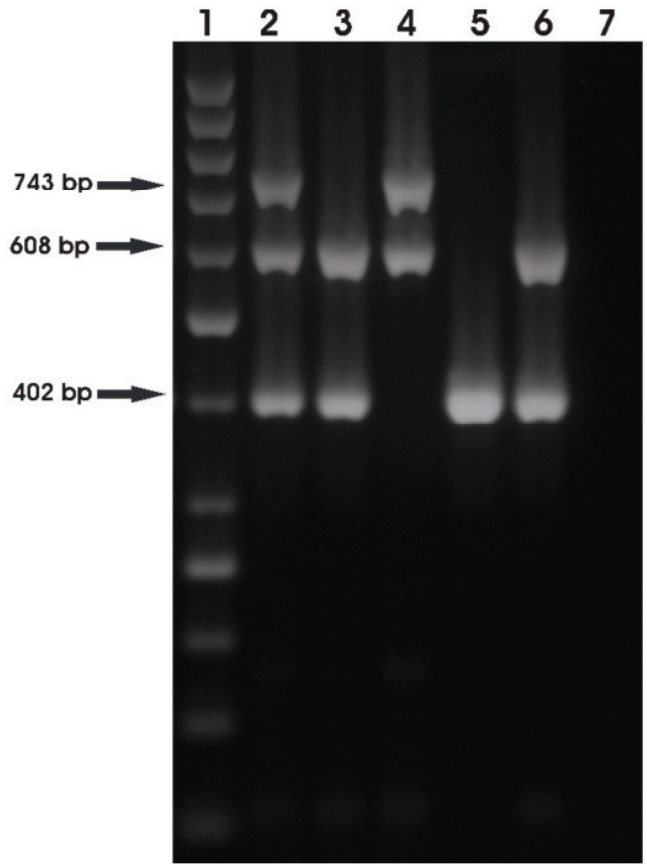


Following the newly developed protocol, in case of the ChPV strains two clearly differentiable bands at the predicted sizes of 415 bp and 146 bp were obtained, in case of the TuPV strains two differentiable bands at 323 bp and 238 bp, and for the amplicons of the two chicken origin samples that clustered in the TuPV-like ChPV group, three clearly differentiable bands were obtained at 323 bp, 146 bp and 92 bp, as expected according to the alignement of all avian parvovirus sequences deposited in the GenBank (Figure 4).

Simultaneous detection of ANV, IBV and IBDV by mRT-PCR

During the trials and optimization of the mRT-PCR the reaction conditions recommended by the commercial kit's manufacturer were modified according to the suggestion in the literature. The critical parameters for the mRT-PCR assay are the primer selection, extension time and annealing temperature.

Figure 5



The primers used in the present study had lengths of 18 to 22 bp and an ideal GC content of 35% to 60%, as previously recommended. As more loci are simultaneously amplified more time is necessary for the polymerase molecule to complete synthesis of all the products.

A longer extension time, 2 min and 30 sec, was used with the purpose of obtaining higher yields of PCR products. Although for the three individual loci the annealing temperature was 53°C for ANV and 55 and 60°C for IBV and IBDV respectively as previously described, the trials demonstrated that by lowering it to 53°C the same loci in multiplex mixtures could be co-amplified successfully.

The sample templates were subjected to mRT-PCR under the previously described optimized thermal profile, and the amplified products were analyzed by agarose gel electrophoresis. The specificity of the primer pairs was proven by the successful amplification of the expected products, without the presence of non-specific bands (Figure 5).

Discussion

Despite the intense research regarding the ED, a major disease complex still seriously threatening the poultry industry, the causative viral pathogens are not accurately identified, and its pathogenesis is not fully understood. Numerous viruses have been incriminated as causing or participating in the etiology of the ED: astroviruses, coronaviruses, reoviruses, and rotaviruses. The most recently accepted causative viral pathogen is a new member of the *Parvoviridae* family that was suspected decades ago as causative agent in RSS.

Epidemiological survey of viral pathogens incriminated in the ED of chickens

A total of 28 chicken broiler flocks were included in the thesis, 15 with clinical signs of RSS, and 13 healthy flocks, included with the purpose of comparison, and selected from the cases received for routine health flock evaluation.

In case of the 15 chicken flocks presenting RSS, the histological examination revealed the moderate to severe distention of the intestinal crypts and acute catarrhal enteritis with a mixed inflammatory cell. Shortening, moderate denudation and fusion of the intestinal villi were observed. All these findings are typical for ED, and similar changes were described by previous studies. In a few cases active regeneration in the small intestine was present, suggestive of the fact that the birds from which the samples were collected probably have passed through the disease and recovered, but the viral pathogens were still present in their tissues as determined by PCR testing. Lympho-histiocytic nodular pancreatitis was also observed in the chicken samples; unfortunately due to the lack of data regarding the pathogenesis of this syndrome the causative agent of this lesion cannot be reliably determined. Reoviruses are known to cause lesions in the pancreas; however, only 3 chicken flocks from the ones presenting clinical signs of RSS were infected with ARV. Routine aerobic bacteriological investigation was negative for all the chicken flocks included in this study, demonstrating that all macroscopic and histopathological changes found, were due to the presence of viral pathogens.

Beside ChPV, astroviruses were found to have the highest prevalence in the chicken flocks, with ANV being the most frequent. This is an expected finding as according to previous studies involving both healthy and diseased chicken flocks, from the enteric viruses the highest prevalence was observed in case of astroviruses. Interestingly ANV was found in 6 out of the 13 chicken flocks included only as routine assessment, with no clinical signs, and only in 3 out of the 15 chicken flocks with clinical signs of RSS. All chicken flocks with

clinical signs of RSS proved to be positive for ChPV, however this pathogen was also found positive in case of two samples with no clinical signs, hence no correlation can be made.

The epidemiologic study attempted in this thesis included a wide range of viral pathogens previously incriminated in ED, such as: astroviruses, coronaviruses, reoviruses, rotaviruses, adenoviruses, but also newly suggested agents as: ChPV. A high incidence of ChPV was determined, 17 out of 28 samples (60.71%) were positive if all chicken flocks are considered. Unfortunately there are only few studies regarding the incidence of ChPV in healthy chicken flocks and to our knowledge no data concerning the prevalence of ChPV in flocks with ED. A low incidence was found in case of ARV. Only 3 cases of positivity were found in the chicken flocks with clinical signs of RSS, and 4 in case of the flocks without clinical signs. This finding was similar with other studies that have reported similar incidence of ARV in healthy and diseased chicken flocks. Chicken astrovirus (CAstV) was found only in case of the flocks with signs of RSS, 4 out of 15 (26.66%), an interesting finding considering that previous studies determined a high incidence of astroviruses in healthy chicken flocks.

Epidemiological survey of viral pathogens incriminated in the ED of turkeys

The viruses identified in the 51 turkey flocks included in this study are also similar with previous reports; however a broader range of pathogens was incorporated, including the scarcely known TuPV, for a better understanding of the epidemiology of ED in turkeys.

Our investigations directly demonstrated the presence of the scarcely known TuPV in 25 Hungarian turkey flocks (49.01%) experiencing clinical signs of ED, making this pathogen the second most identified after astroviruses. The wide distribution of TuPV in American commercial flocks was recently reported. To our knowledge there is no data on the role of TuPV in the ED of turkeys, still the results of the present survey accentuate their potential involvement, as suggested earlier.

Astroviruses were the most identified viral pathogens in the investigated turkey flocks, nevertheless previous reports have shown a high prevalence of astroviruses in healthy turkey flocks, making this finding difficult to interpret. Avian nephritis virus, a pathogen known to infect and cause disease in young chickens and pheasants, was found in 15 flocks but no data is available concerning any possible involvement of this pathogen in PEMS, as it was only recently directly identified for the first time in healthy commercial turkey flocks. Due to the novelty of this infection, positivity for ANV was confirmed by direct nucleotide sequencing.

Singular infections were found in case of 12 flocks (23.52%), from those 6 cases were TAstV positive (11.76%), 5 TuPV positive (9.80%) and only one TCV positive (1.9%). It is

not considered surprising the fact that flocks with singular infections will develop clinical signs of PEC or PEMS, as TAstVs infections are known to cause even severe losses in a flock especially when the TAstV-2 subtype is present. In the early research on PEC and PEMS it was determined that TCV is a pathogen without which the clinical signs cannot appear, being the first viral agent associated with ED, however following studies and experimental infections proved that its presence was not necessary for the disease. The role of reoviruses in PEC and PEMS was intensely debated. Authors have demonstrated that strain ARV CU98 isolated from PEMS poults does cause clinical signs in PEMS.

The statistical analysis results are suggesting a negative correlation between ARV and TuPV, and TCV and TAstVs respectively, hence if one of the viruses is present it is less likely for the second one to appear. The only "almost" positive correlation was found between TAstV-2 and ARV, as they were found most frequently together.

Identification and genetic characterisation of avian parvovirus in the ED complexes

A total of 15 ChPV positive samples and 25 TuPV positive samples were directly sequenced and analyzed in this study. The phylogenetic tree constructed based on the nucleotide sequence of the analyzed NS1 gene segment revealed an evident clustering of the virus strains of different species origin, ChPV group and TuPV group. This finding is consistent with the characteristics of parvoviruses of other species (e. g. canine/feline parvoviruses).

Two ChPV strains (1515/07 from Hungary and 841AR05 from USA) were more closely related to the TuPV group; furthermore they presented unique sequences at several aa sites. This finding could be relevant, as parvoviruses are typically small viruses of approximately 5000 nucleotides long, and minor mutations resulting in only a few key aa changes can lead to drastic changes in their infective behavior, therefore the possibility that ChPV and TuPV could have evolved from a common ancestor cannot be ruled out. Even if according to the RDP based investigation of the analyzed gene segment no recombination events were found between the examined strains, the probability of a recombinant virus should not be excluded, but they could have also evolved separately from other ChPVs.

The chicken and turkey samples analyzed in the present study were collected from geographically isolated regions. However, samples 5596/7/10 HUN, 5596/10/10 HUN, 5596/13/10 HUN, 5596/18/10 HUN were collected at the same time and originated from a single breeder with different age group flocks, still only two of them were 100% identical on the examined region. There are two possible explanations for this finding: either this particular flock got infected with three different stains at the same time, or in the short period of time, dramatic changes occurred with the viral strains, which is an unlikely situation for

parvoviruses. With the exception of two turkey samples (33/10 and 762/10) the Hungarian turkey stains clustered separately from the American strains, in two groups. No time connected clustering was observed throughout the phylogenetic tree; however the Hungarian origin turkey sequences collected in 2008 and 2009 clustered evidently in two groups. On the other hand, in spite of the observed genetic diversity of the analyzed strains, as due to several reasons, no reliable data were available regarding the production performance, no scientifically substantiated conclusions can be drawn regarding the variation in pathogenicity of the Hungarian ChPV and TuPV strains.

Differentiation of ChPV, TuPV, and TuPV-like ChPV strains by RFLP

The differences between the *AvaII* digestion pattern of parvovirus strains belonging to the TuPV, ChPV and TuPV-like ChPV groups seem to provide a quick and reliable differentiation of all these strains without the need for nucleic acid sequencing.

At the moment there are only two chicken origin sequences in the so called TuPV-like ChPV group, with clearly different geographic origin, one American and one Hungarian. Despite their unique nucleotide sequence on the analyzed region, one can only assume that as diagnosis and research will progress, more such sequences will be identified. In the future, the newly described protocol could be used to rapidly obtain valuable epidemiological data, and will turn out to be even more practical in case potential differences in the pathogenicity of these strains should be revealed by future studies.

Simultaneous detection of ANV, IBV, and IBDV by mRT-PCR

These results demonstrate that the newly designed mRT-PCR-based test can be used under the optimized conditions to identify and differentiate the infections caused by the previously mentioned viruses. The mRT-PCR-based test method can be used for differential diagnostic purposes in cases when clinical and pathological changes suggestive of infections with any of these pathogens are present. However one should remain cautious when considering viral pathogens which are controlled by vaccination, as IBV and IBDV. The present test did not have as an objective the strain origin differentiation of the mentioned pathogens.

The newly developed test represents a useful, fast and reliable diagnostic method for the simultaneous detection of ANV, IBDV and IBV from tissue samples, and has great applicability in samples originating from Hungarian chicken flocks, as the similar coinfections have proven to exist in a high prevalence in this geographic region.

In this thesis a total of 28 chicken flocks and 51 turkey flocks from distinct geographical regions of Hungary, were investigated by direct methods for the presence of viral pathogens incriminated in enteric disease, with the goal of determining the epidemiology and pathogenesis of the syndrome. By including a broad range of viral agents in the determination, we are confident that the present study contributes in clarifying the epidemiology of a current economic threat for the poultry industry, the enteric disease syndrome, with the potential of being useful for to the elaboration of comprehensive preventive measures, to avoid in the future the serious losses that the poultry industry is suffering due to this syndrome.

New scientific results

- Epidemiological evaluation of the enteric disease syndromes in Hungarian chicken and turkey commercial flocks.

- Genetic analysis of the newly involved pathogens in enteric disease, chicken parvovirus (ChPV) and turkey parvovirus (TuPV).

- Determining the existence of a new subgroup, the TuPV-like ChPV, besides the two existing ChPV and TuPV groups.

- Fast and reliable differentiation of ChPV, TuPV and TuPV-like ChPV strains by a newly designed *AvaII* based restriction fragment length polymorphism (RFLP) assay.

- Detection of ChPV and TuPV in infected tissues by a newly developed indirect immunohistochemical protocol.

- Simultaneous detection of infectious bronchitis virus (IBV), infectious bursal disease virus (IBDV) and avian nephritis virus (ANV) in coinfected Hungarian field samples, employing a newly developed multiplex RT-PCR (mRT-PCR) protocol.

List of publications

Scientific publications of the thesis

Palade E.A., Demeter Z., Dobos-Kovács M., Rusvai M., Mándoki M.: A fertőző bronchitis vírus, a csirke nephritis vírus és a fertőző bursitis vírus kimutatása multiplex RT-PCR alapú diagnosztikai eljárással (Demonstration of infectious bursal disease virus, chicken nephritis virus and infectious bronchitis virus by multiplex RT-PCR diagnostic technique). *Magyar Állatorvosok Lapja*, 2008, 130: 559-564. (in Hungarian, with English abstract) [IF: 0.155]

Palade E.A., Kisary J., Benyeda Zs., Mándoki M., Balka Gy., Jakab Cs., Végh B., Demeter Z., Rusvai M. Naturally occurring parvoviral infection in Hungarian broiler flocks. *Avian Patholog*y, 2011, 40 (2): 191-197. [IF: 1.654]

Palade E.A., Demeter Z., Hornyák Á., Nemes Cs., Kisary J., Rusvai M. High prevalence of turkey parvovirus in turkey flocks from Hungary experiencing enteric disease syndrome. *Avian Diseases,* in press. [IF: 2.003]

Congress abstracts of the thesis

Palade E.A., Demeter Z., Benyeda Zs., Mándoki M, Rusvai M.: Parvoviral Infection in Hungarian Broiler Flocks and the genetic diversity of the causative agent. Congress of the Hungarian Academy of Sciences and the Doctoral (PhD) School of Veterinary Medicine, January 26, 2011, Budapest, Hungary (in Hungarian)

Palade E.A., Demeter Z., Dobos-Kovács M., Rusvai M., Kisary J., Mándoki M.: Etiological investigations of naturally occurring runting stunting syndrome in hungarian flocks. Congress of the Hungarian Academy of Sciences and the Doctoral (PhD) School of Veterinary Medicine, January 26, 2010, Budapest, Hungary

Palade E.A., Hornyák Á., Demeter Z., Dobos-Kovács M., Benyeda Zs., Rusvai M., Kisary J.: Etiological investigations of naturally occurring runting stunting syndrome in hungarian flocks. *Acta Microbiologica et Immunologica Hungarica*, 2009, 56: 114-263.

Palade E.A., Hornyák Á., Demeter Z., Dobos-Kovács M., Benyeda Zs., Rusvai M., Kisary J.: Genetic characterization of Chicken parvovirus strains from naturally infected Hungarian flocks. 8th International Congress of Veterinary Virology, Budapest, Hungary, 23-26 August, 2009. pp. 204.

Palade E.A., Mándoki M., Dobos-Kovács M., Demeter Z., Rusvai M.: Diagnosis of infectious bronchitis, avian nephritis and infectious bursal disease by multiplex RT-PCR, and the phylogenetical analysis of infectious bursal disease virus strains circulating in Hungary. Congress of the Hungarian Academy of Sciences and the Doctoral (PhD) School of Veterinary Medicine, January 22, 2008

Palade E.A., Mándoki M., Demeter Z., Dobos-Kovács M., Benyeda Zs., Rusvai M.: Development of a multiplex PCR assay for the simultaneous detection of infectious bronchitis virus and avian nephritis virus. Congress of the Hungarian Academy of Sciences and the Doctoral (PhD) School of Veterinary Medicine, January 23, 2007, Budapest, Hungary (in Hungarian)

Other publications in peer-reviewed journals

Palade E.A, Gál J., Mándoki M.: Avian encephalomyelitis vírus okozta megbetegedés magyarországi importált brojlerállományban (Avian encephalomyelitis in imported Hungarian broiler flocks). *Magyar állatorvosok lapja*, 2011, 133, 220-223. (in Hungarian, with English abstract) [IF: 0.155]

Mándoki M., **Palade E.A.**, Kléh Zs., Dobos-Kovács M., Gál J.: Derzsy betegség okozta tömeges elhullás libaállományban (Multitudineuss loss due to Derzsy's disease in goose flocks). *Magyar állatorvosok lapja*, 2011, 133, 13-18. (in Hungarian, with English abstract) [IF: 0.155]

Demeter Z., **Palade E.A.**, Soós T., Farsang A., Jakab Cs., Rusvai M.: Misleading results of the *Mbo*II-based identification of type 2a canine parvovirus strains from Hungary reacting as type 2c strains. *Virus Genes,* 2010, 41, 37-42. [IF: 1.705]

Demeter Z., **Palade E.A.**, Hornyák Á., Rusvai M.: Controversial results of the genetic analysis of a canine distemper vaccine strain. *Veterinary Microbiology,* 2010, 142, 420-426. [IF: 2.073]

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