

**Szent István University  
Postgraduate School of Veterinary  
Science**

**Evolution of Swine Circoviruses**

**PhD theses**

**Márta Lőrincz**

**2014**

Supervisor and consultants:

Tamás Tuboly, professor  
Szent István University, Faculty of Veterinary Science  
Department of Microbiology and Infectious Diseases  
Supervisor

István. Kiss, PhD  
CEVA-Phylaxia ZRT.  
Consultant

Imre Biksi, associate professor  
Szent István University, Faculty of Veterinary Science  
Department and Clinic of Food Animal Medicine  
Consultant

Márta Lőrincz

## **INTRODUCTION**

In the past twenty years, diseases caused by porcine circovirus type 2 (PCV2) have been responsible for severe economic damages in pig herds. The virus was shown in the background of multiple diseases; the most important of these is porcine multisystemic wasting syndrome. PCV2 was first identified in Canada, and after a rapid spreading, is now present all over the world.

The virus has a mutation rate uncharacteristic to other DNA viruses, resulting in high genetic diversity. PCV2 is a very heterogenic virus, although the elevated variability observed initially seems to be followed by an increasing genetic uniformity in the past seven-eight years. This observation suggests that PCV evolution is advancing towards the completion of its adaptation to its new host species, the pig. However, vaccinations introduced recently can interfere with this natural process, directing PCV2 evolution towards the generation of variants escaping vaccine induced protection. Virus evolution may also be affected by the infection of wild boar, which is considered a reservoir species for PCV2. This is significant mainly in those pig farms where epidemiologic regulations are loosely followed, meaning that pigs and wild boars may be in direct or indirect contact.

Circoviruses are considered to originate from a common ancestor with the plant pathogen nanoviruses, but they also carry a protein segment displaying strong homology with a protein found in caliciviruses, which are infecting animals. This finding suggests that caliciviruses could have played a role in the genesis of circoviruses. There are several theories to explain how the virus was transmitted from plants to animals, and also how the calicivirus protein part was transferred to circoviruses. However, there are no answers suggested for the questions of circovirus evolution in animals,

regarding its entry to swine species (which were considered the only non-bird hosts for a long time), or which animal species played the role of a connecting agent between birds and pigs. Our aim was to find answers to some fundamental questions regarding porcine circovirus evolution.

The discussed problems are detailed in three main topics:

- *Genetic examinations of PCV2 detected in samples from pigs in Central Europe*

Genetic analyses of PCVs collected from Central Europe and their comparison with virus sequences originating from the same geographic area, but from before 2007, and also with viruses found in other parts of the world. The object of this part of the research was to determine changes in genetic material of PCV2, and to see if there really is a decrease in its variability, and also to assess effects of the introduced vaccinations to virus diversity.

a) Circovirus detection in rodents collected in their natural habitats. We expected to find PCV2 in mice and rats caught in pig farms, and also to detect a circovirus specific to rodents, which would be a transient version towards PCV. To find the hypothesized common ancestor, we also examined rodents originating from sites not connected to pigs.

b) *In vivo* laboratory experiment to assess virus transmission and genetic changes in mice. During these experiments, in addition to virus susceptibility and replication in mice, we evaluated the possibility of pig independent PCV2 spreading, intending to measure possible genetic changes occurring when spreading from one animal to another.

- *Detection of circoviruses in species not yet examined*

Finding a common, hypothetical genetic ancestor was an important one among our objectives. For this, we tried to

determine whole genome sequences of circoviruses possibly found in other host species. Considering that sequences very similar to circoviruses have already been detected in water samples, and that pig feed may contain proteins of fish origin, it seemed obvious to start with fish, amphibians and reptiles.

## MATERIALS AND METHODS

### Sample collection

From Central Europe, we received pig organ samples from 20 farms in Hungary, 13 farms in Romania, 7 farms in Serbia and 28 farms in Croatia. We also received purified DNA samples from 14 pig farms in Poland. From Transylvania, in addition to samples from domestic pigs, 842 wild boar samples were sent, which had been collected in several counties of Transylvania in three different hunting seasons (2006/2007, 2007/2008 and 2010/2011).

We collected 20 mouse (*Mus musculus* and *Mus agrarius*) and 21 brown rat (*Rattus norvegicus*) carcasses from two Hungarian pig farms where PCV2 was present, and the farms were independent from each other. To compare with the viruses detected in rodents, we also analysed parabronchial lymph node and lung tissue samples of 10 pigs from one of the farms. A total of 25 DNA samples from house mice, common voles (*Microtus arvalis*) and yellow-necked mice (*Apodemus flavicollis*) collected from habitats without any connection to a pig farm or PCV2 were also examined.

In the experiment to evaluate transmission and genetic changes of the virus in rodents, we used a total of 30 CRL: NMRI BR, six-week-old female white mice, each weighing 21-24 g.

We examined 74 reptile and amphibian, 63 fish samples and one fish meal sample for the presence of circoviruses, collected from different places and at different times. After having detected circovirus (not PCV2) DNA in common barbel (*Barbus barbus*) fry, 18 further barbels were tested. In 2011, a case of unusually high European catfish (*Silurus glanis*) mortality in lake Balaton suggested the presence of circoviruses in that species, too. The virus was

detected at the Veterinary Diagnostic Directorate (VDD) of the National Food Chain Safety Office and these positive samples were made available to us.

We received blood plasma samples from feeder calves from a farm. In these calves, which were kept together with grower pigs in the same building, mucous-bloody diarrhoea was observed. The samples tested negative for bovine viral diarrhoea virus at VDD.

### **Sample processing, circovirus detection and analysis**

DNA was purified from every sample. We amplified most samples using conventional PCR. In addition, we performed real time PCR for quantification and in cases where there were very low amounts of template DNA in the sample (or to detect a broader spectrum of circoviruses), nested PCR was used. Primers were either adapted from literature or were designed by ourselves. In some cases, to facilitate amplification, we used rolling circle amplification for isothermal circular DNA amplification preceding PCR.

PCR products were verified by sequencing and the resulting sequences were compared with each other and with data available in GenBank. Phylogenetic trees were constructed with programs MEGA and MEGA 5.2 by Lasergene. Full genome sequences were assembled using CAP3, BioEdit Sequence Alignment Editor v 7.0.5.3, and Lasergene MEGA 5 and MEGA 5.2. Amino acid sequences were predicted using the Lasergene package and data available in the GenBank database. Sequence comparisons were performed with applications pairwise alignment and sequence identity matrix of BioEdit Sequence Alignment Editor v 7.0.5.3. To assess genetic distances, distance matrix application of program Lasergene MEGA 5.2 was utilized.

Phylogenetic analyses were generally carried out based on the Neighbour-Joining algorithm, in addition, in case of Central European sequences, Maximum Likelihood and Tamura analyses were also carried out, so that by comparing the results of different methods, we could achieve the most accurate results possible. Pearson's Chi-square test of program package R for Windows 2.12.0 was applied to perform statistical analyses. For recombination detection, RDP v 3.44 program was applied with 6 different algorithms and default settings. Aside from nucleic acid examinations, we attempted virus isolation with propagation on cell cultures, indirect immune fluorescence and immune histochemistry.



## RESULTS

### Genetic changes in PCV2

#### Examinations in Central Europe

Results of analyses of Central European samples are summarized in table 1. We compared the data with full genome sequences from GenBank and from earlier research of Attila Cságola.

**table 1. Prevalence of PCV2 in samples from Central Europe**

	Hungary	Romania	Serbia	Croatia	Poland
total of farms	20	13	7	29	14
PCV2 positive	17	12	6	25	6
sequences	5	3	5	22	2

Farms where at least one positive sample was found were considered positive.

Based on the definition of genotypes, PCV2 can be divided into the following three genotypes: PCV2a, PCV2b and PCV2c. PCV2a and PCV2b genotypes are divided into several subtypes. The classification by Olvera et al. in 2007 is considered the most accepted. According to this, genotype PCV2b consists of three (1A, 1B and 1C) and PCV2a of five (2A, 2B, 2C, 2D and 2E) subtypes. The existence of two additional genotypes, PCV2d and PCV2e, proposed by Wang et al. (2009) is questionable. I refer to these as PCV2b 1C (PCV2d) and PCV2a (PCV2e) in my thesis.

We did not detect any viruses in genotype PCV2c in the Central European samples.

One virus detected in Croatia belonged into subtype 2D in genotype PCV2a. Another two PCV2 samples from

Croatia taken in 2009 could not be grouped into any genotype. The capsid gene sequence and predicted capsid amino acid sequence analyses of these two viruses and one additional virus from China which did not belong into any genotype either, revealed that the C-terminal of the capsid protein is unique in these viruses. Instead of the usual 233, the capsid protein is 234 amino acids long, which is specific to viruses in subtype PCV2b 1C (PCV2d).

In Central Europe, based on the classification by Olvera et al. (2007), viruses in subtype PCV2b 1A are predominant. A total of 28 viruses belonged here. Although two viruses (sequences no. 703. from Croatia and no. 22. from Serbia) were located elsewhere on the phylogenetic tree, based on their genetic relatedness, we decided that they also belong into subtype PCV2b 1A.

One genome from Poland, 2 from Hungary and 2 from Croatia were classified into group PCV2 1B. One porcine circovirus clustered into subtype PCV2b 1C (genotype PCV2d). This group is supposed to consist of viruses with elevated pathogenicity compared to viruses in other subtypes. There are viruses also in this group, where the deletion of the 1035<sup>th</sup> nucleotide results in one addition to the number of amino acids in the capsid protein.

A newly detected group is PCV2a (PCV2e) inside genotype PCV2a. Although according to its definition, this group consists of sequences from China, described before 2006, we detected a few sequences belonging here from after 2009. Genotype PCV2a is branching off from this group, and also based on the distance matrix analysis, this group is part of genotype PCV2a, rather than a separate genotype.

A group not mentioned so far shares a branch with genotype PCV2c. Genome sequences from three distinct subgroups belong here: in the first are ones that are related to group PCV2a 2E, but carry motifs resembling to genotype

PCV2b in their capsid proteins. The second contains sequences coding identical cap proteins to subtype PCV2b 1C (PCV2d). In the third subgroup, there are viruses of 233 amino acid long capsid proteins and 1767 nucleotide genomic lengths.

### **Examinations of PCV in Transylvania**

We took part in a work measuring circovirus prevalence in samples from Transylvania and also in their phylogenetic analyses. The results are demonstrated in table 2.

**table 2. Results of PCV2 sequence analysis in samples from domestic pigs and wild boars from Transylvania.**

	PCV1	PCV2a	PCV2b		
			A	B	C
Wild boar	3	4	12	5	7
Domestic pig	0	0	4	3	1

A, B and C indicate the subtypes according to Olvera et al. (2007).

In Transylvania, PCV2 is present in at least 65 % of pig farms. In case of wild boars, however, a decrease could be detected in the prevalence of the virus.

## **Porcine circoviruses in connection with other species**

### **Examinations of rodents**

PCR testing did not show any positivity for porcine or any other, unknown circoviruses in rodent samples collected independently from pig farms.

In contrast, in rodent samples collected at pig farms, we detected PCV2 in several cases. A total of 13 mice and 5 rats were found to be positive for circovirus, meaning 65.0 % and 23.8 % positivity in mice and rats, respectively. The slaughtered healthy pigs from one of the farms showed 80 % PCV2 positivity.

The primer pair MCV1—MCV2 is specific for porcine circoviruses, and based on or previous experiences, is the part of a very effective and sensitive PCR system. In rodent samples, however, it produced results in such poor quality that made sequence analysis possible in only six cases. The fragments were identified without doubt as pieces of virus genomes in genotype PCV2b, though some differences could be found even in this highly conservative site.

In the experiment with mice, infected animals were PCV2 positive after euthanasia. After the first passage, however, the virus could not be detected using qPCR.

### **Results of bovine blood plasma testing**

All three calf samples and pig samples from the same farm tested positive for PCV2. Full genome sequencing was also successful in case of cattle plasma no. 2. Sites where the sequence was not evident could be identified: we found two locations where two nucleotides occurred in nearly equal amounts. One of the two nucleotides appearing together with the other could always be found in the virus prevalent in pigs of the same farm. Nucleotide differences between viruses

occurring in pigs and in cattle also manifested in amino acid changes. The virus differing from the one considered original and which was shown in the pigs, can also be found in swine living elsewhere and is of subtype PCV2b 1A.

### **Results of examinations of fish, reptiles and amphibians**

Samples of reptile and amphibian origin tested negative. We were not able to detect PCV2 DNA in any of them.

In fish, we detected circovirus (BaCV) in common barbel in eight cases, using the broad-spectrum circovirus PCR system. We verified all eight cases using a diagnostic primer pair designed by ourselves. Circovirus could be detected in three catfish out of six.

In barbel, sequencing resulted in two complete and new virus genome sequences. Both genomes were 1957 bases long, and had 94.7 % identity to each other. Also two full genome sequences were determined from the viruses (CfCV) detected in European catfish. These genomes were 1966 nucleotides long, having 99.4 % identity. The two CfCV and also the two BaCV genomes contained the open reading frame coding the replication protein specific to circoviruses (ORF 1). ORF2, coding the capsid protein, was located on the complementary strand. We identified two additional ORFs in circoviruses from both fishes, although the significance of these is yet to be determined.

Performing the phylogenetical analyses, we compared the newly obtained sequences with representative circo- and cyclovirus genomes in GenBank. The fish viruses were undoubtedly circoviruses, but did not belong to any known circovirus species, forming their very own phylogenetic group. According to the capsid amino acid sequence analysis, the four fish circoviruses (BaCV1, BaCV2, CfCV1 and CfCV2) are

all located together on a separate branch of the phylogenetic tree. This branch forms a distinct group from previously described circoviruses, but clearly inside the genus *Circovirus*.

## **CONCLUSIONS**

### **Genetic changes of PCV2**

Prevalence of genotype PCV2a appears to be decreasing in Central Europe and all over the world. Out of the 278 sequences from after 2007, only 29 belong to this genotype.

The majority of viruses sequenced by us belonged to subtype PCV2b 1A, confirming the hypothesis that the genetic diversity of the virus is decreasing. Previously, this group of PCV2 has been considered to most probably become predominant, and this suggestion seems to be verified in Central Europe. Regarding our whole database of sequences, this dominance appears to be shifting towards subtype PCV2b 1C. We theorize that vaccinations changed natural evolutionary processes, and it is most likely that this was the reason why subtype PCV2b 1C started its spreading, as it overcame vaccine protection more easily. In this group, a deletion on the capsid gene caused an addition to the number of amino acids. This variant became prevalent with the introduction of vaccinations.

Based on phylogenetic analyses, group PCV2a (PCV2e) is branching off from genotype PCV2a. Also, according to the results of the distance matrix analyses, this group is a subgroup of PCV2a, rather than a separate genotype. Subtypes PCV2a 2B and 2C are more closely related, than other subtypes generally, this is confirmed by pairwise comparisons and distance matrices. Taking all these into account, we suggest that, to respect well formed traditions, PCV2a 2B and 2C should remain distinct subtypes, but instead of PCV2a (PCV2e), subtype PCV2a 2F would be a more appropriate classification.

The question of proposed genotype PCV2d generated controversy. According to many, these viruses belong to genotype PCV2b. Neither did our examinations confirm them forming a genotype of their own, as they clustered together with viruses in subtype PCV2b 1C in phylogenetic trees constructed by three different methods. Also, based on distance matrix analyses, it seems clear that they are more closely related, than other genotypes of PCV2 generally, or even subtypes within genotypes. In conclusion, based on these results, the designation “genotype PCV2d” is incorrect.

### **Investigations to identify possible ancestors of PCV2**

We suggested that a possible host for the ancestors of PCV2 might be found among rodents. We could not demonstrate this in the present work. We were not able to question the swine origin in any of the viruses detected in rodents. Sequence analyses of circovirus positive samples proved the viruses to be PCV2b.

Detection of PCV2 in rodents collected at pig farms was successful; we were the first ones in the world to demonstrate natural infection and virus carrying in mice and rats, independently from laboratory environment. It was shown in previous animal experiments that mice are shedding the virus, therefore it is reasonable to suggest that rodents can maintain infection at pig farms, furthermore, they might even re-infect pigs.

All virus sequences from rodent origin belonged to genotype PCV2b, just like viruses from pigs in the same farm. Based on comparisons of sequences obtained from a conservative part of the virus, we determined that the viruses occurring in rodents were very diverse.



Circoviruses specific for cattle have not been described. We successfully detected PCV2 in blood plasma samples of feeder calves. These animals lived on a PCV2 positive pig farm. Sequence analysis showed that the detected virus was PCV2b, which was almost entirely identical to the one found in the pigs on that same farm. However, we identified multiple nucleotide point mutations that strongly suggest virus replication in cattle. Both viruses found in calves belonged into subtype PCV2b 1A.

We extended our search for the suggested ancestor virus to additional vertebrate host species. We detected circoviruses in two fishes (common barbel and European catfish), which, based on their genetic characteristics and phylogenetic properties, are definitely members of the genus *Circovirus*. The virus detected in barbel is the very first circovirus described in fish. Although its epidemiological significance is unexplored as of yet, the first sequences were identified from dead fry in a hatchery, where other pathogens or etiology could not be detected. Circoviruses are known to cause severe immune suppression. In case of catfish, solely the circovirus could be found in the background of the disease, but its role as a causative agent was not established. Although an immune suppressive virus, particularly during the exhausting spawning season, when animals are otherwise weakened in their immune responses, can facilitate the onset and progression of diseases.

The role of fish circoviruses as possible ancestors in evolutionary processes of PCV2 cannot be confirmed, but new information revealed in connection to them bring us one step closer to better understand the underlying mechanisms.

## **NEW SCIENTIFIC RESULTS**

1. We confirmed PCV2b 1A to be the most prevalent subtype in pigs in Central Europe. However, as an effect of immunizations, the group able to overcome vaccine protection, subtype PCV2b 1C, including the proposed PCV2d genotype might become the main direction of virus evolution in the future.
2. Our results showed that the existence of PCV2e as a separate genotype is not established, we propose the formation of a new subtype (2F) within genotype PCV2a instead.
3. We were the very firsts to detect porcine circovirus in rodents from their natural habitats. Presumably, rodents living in pig farms play a role as reservoirs in PCV2 epidemiology.
4. The PCV2b identified in cattle displayed variability compared to circoviruses of pigs in the same farm. Having detected this, we showed that the virus is able to replicate in this species.
5. We detected circoviruses in fish, which was the first description of the virus aside from the usual bird and pig host species.

## **ACKNOWLEDGEMENTS**

I would like to thank the huge amount of help I received from my supervisor, Tamás Tuboly, and also to thank my colleagues in the lab: Attila Cságola, Kata Tombácz, Irén Herbákné and Daniel Cadar.

I am very thankful for their assistance in sample collection to Sándor Kecskeméti, Imre Biksi, István Kiss, Emőke Horváth, Attila Stanczel, Miklós Gyuranecz, Szentimrei Mezőgazdasági KFT and the veterinarians working there: Márta Balogh, József Lőrincz, Fiorács KFT and István Szentirmay, Pigomix KFT and its veterinarians: Adél Orosz and Mihály Dániel. Samples from abroad were collected by Dániel Cadar, Dinko Novosel, Tomasz Stadejek and Zsolt Becskei.

For the help I received during sample preparation and procession, I am grateful to Ádám Dán and the employees of the Molecular Biology Department in VDD (Ádám Tóth, Ernőné Ottinger, Ágnes Juhász), György Csaba, Mária Láng, and Zsuzsanna Rónai, and from the ÁOTKI to Szilvia Farkas, Csaba Székely, Kálmán Molnár, and their assistant Györgyi Ostorosné Pataki.

I am thanking Levente Szeredi and Ágnes Ráczné Mészáros for training me in sample preparation for IHK and its evaluation.

I thank my students in the Scientific Student's Association, Simon Andersson, Adrienn Falus, Janka Petrilla and Julie Nielsen, for the part they took in this work.

## PUBLICATIONS

*Lőrincz M.*, Cságola A., Biksi I., Szeredi L., Dán A., Tuboly T. (2010) Detection of porcine circovirus in rodents - short communication. *Acta Vet Hung.* 58. 265-268. IF: 1,264

Cadar D., Cságola A., Spînu M., Dán A., Ursu K., *Lőrincz M.*, Tuboly T. (2010) Prevalence of porcine circoviruses in Transylvanian wild boars, detected by real-time PCR —short communication. *Acta Vet Hung.* 58. 475—481. IF: 1,264

*Lőrincz M.*, Cságola A., Farkas S.L., Székely C., Tuboly T. (2011) First detection and analysis of a fish circovirus. *J Gen Virol.* 92. 1817—1821. IF: 3,363

*Lőrincz M.*, Dán A., Láng M., Csaba G., Tóth A.G., Székely C., Cságola A., Tuboly T. (2012) Novel circovirus in European catfish (*Silurus glanis*). *Arch Virol.* 157. 1173—1176. IF: 2,030

Cadar D., Cságola A., *Lőrincz M.*, Tombácz K., Spînu M., Tuboly T. (2012) Detection of natural inter- and intra-genotype recombination events revealed by cap gene analysis and decreasing prevalence of PCV2 in wild boars. *Infect Genet Evol.* 12. 420—427. IF: 2,768

*Lőrincz M.*, Tombácz K. (2014) Fish circoviruses. *MÁL.* 136. 123—127. IF: 0,146

Novosel D, Tuboly T, Csagola A, *Lorincz M*, Cubric-Curik V, Jungic A, Curik I, Segalés J, Cortey M, Lipej Z. (2014) Origin of porcine circovirus type 2 (PCV2) from swine affected by PCV2-associated diseases in Croatia. *Vet Rec.* 174. 431. doi: 10.1136/vr.102064. IF: 1,803

Cadar D., *Lőrincz M.*, Kiss T., Novosel D., Podgorska K., Becskei Z., Tuboly T., Cságola A. (2013) Emerging novel porcine parvoviruses in Europe: origin, evolution,

phylodynamics and phylogeography. *J Gen Virol.* *94.* 2330—2337. IF: 3,127

Fehér E., Székely C., *Lőrincz M.*, Cech G., Tuboly T., Singh H.S., Bányai K., Farkas S.L. (2013) Integrated circoviral rep-like sequences in the genome of cyprinid fish. *Virus Genes.* *47.* 374—377. IF: 1,769

*Lőrincz M.*, Biksi I., Andersson S., Cságola A., Tuboly T. (2013) Sporadic re-emergence of enzootic porcine transmissible gastroenteritis in Hungary. *Acta Vet Hung.* DOI: 10.1556/AVet.2013.043. IF: 1,173

Cságola A., *Lőrincz M.*, Cadar D., Tombácz K., Biksi I., Tuboly T. (2012) Detection, prevalence and analysis of emerging porcine parvovirus infections. *Arch Virol.* *157.* 1003—1010. IF: 2,030

Cadar D., Dán Á., Tombácz K., *Lőrincz M.*, Kiss T., Becskei Z., Spînu M., Tuboly T., Cságola A. (2012) Phylogeny and evolutionary genetics of porcine parvovirus in wild boars. *Infect Genet Evol.* *12.* 1163—1171. IF: 2,768

Cságola A., *Lőrincz M.*, Tombácz K., Wladár Z., Kovács E., Tuboly T. (2012) Genetic diversity of pigeon circovirus in Hungary. *Virus Genes.* *44.* 75—79. IF: 1,769

Cadar D., Cságola A., *Lőrincz M.*, Tombácz K., Kiss T., Spînu M., Tuboly T. (2011) Genetic detection and analysis of porcine bocavirus type 1 (PoBoV1) in European wild boar (*Sus scrofa*). *Virus Genes.* *43.* 376—379. IF: 1,845

Cadar D., Cságola A., *Lőrincz M.*, Tombácz K., Spînu M., Tuboly T. (2011) Distribution and genetic diversity of porcine hokovirus in wild boars. *Arch Virol.* *156.* 2233—2239. IF: 2,111

Cságola A., Cadar D., *Lőrincz M.*, Tuboly T. (2010) Replication and transmission of porcine circovirus type 2 in mice. Short secondary communication. *MÁL.* *131.* 456—458. IF: 0,300

Forgach P., Boncz A., Erdélyi K., *Lőrincz M.*, Molnár B., Zentai J., Szűcs Gy., Reuter G., Bakonyi T. (2010) Hepatitis E virus — literature review and situation in Hungary from veterinary point of view. *MÁL.* 132. 237—248. IF.: 0,300

Cságola A., *Lőrincz M.*, Tombácz K., Biksi I., Balka Gy., Tuboly T. (2009) Detection of human parvovirus 4 related porcine hokoviruses in Hungary. *Acta Microbiologica et Immunologica Hungarica* 56.135.

Biksi I., *Lőrincz M.*, Molnár B., Kecskés T., Takács N., Mirt D., Cizek A., Pejsak Z., Martineau G.P., Sevin J.L., Szenci O. (2007) Prevalence of selected enteropathogenic bacteria in Hungarian finishing pigs. *Acta Vet Hung.* 55. 219—27. IF.: 0,474