Theses of doctoral (PhD) dissertation

Infection dynamics and comparative genetic analysis of porcine circo- and parvoviruses in large-scale pig herds

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1 Background and objectives of the doctoral thesis

Over the past decades, pig farming has undergone significant transformations, during which smaller, traditional farms have been gradually replaced by large-scale production systems. This development has enabled increased production efficiency but has also created conditions favorable for the faster spread of infectious agents, the emergence of new, more virulent pathogens, as well as frequent co-infections and various multifactorial diseases. The rapid transmission of pathogens and the frequent subclinical infections pose new challenges in terms of prevention, eradication and the developement of effective control strategies. During our research we investigated two groups of viruses that are widespread in pig populations and have significant economic importance. These are the porcine circo- and parvoviruses, whose most important members, PCV2 and PPV1, have been recognized as major pathogens for decades. Since the early 2000s, with the rapid advancement of molecular biology and bioinformatics methods, two new circoviruses (PCV3 and PCV4) and seven new parvoviruses (PPV2–PPV8) have been identified. Although the presence of these newly identified viruses has been reported globally, their pathogenicity and impact on swine health remain largely unknown.

During our research, we aimed to assess the presence and prevalence of PPV1–8 and PCV2–4 in Hungarian swine herds. By analyzing the genetic diversity of these viruses, we intented to provide a clearer picture of the genetic variability and phylogenetic relations of the Hungariam viral strains. Our goals were the

(i) detection of PPV1–8 és PCV2–4 in serum, oral fluid and processing fluid samples from various pig farms in Hungary,

(ii) assessment of the prevalence of PPV1–8 és PCV2–4 in Hungarian pig population,

(iii) identification of the within-herd infection dynamins and the involvment of different age,

(iv) partial or full genome sequencing and phylogenetic analysis of the collected PPV- and PCV-strains.

2 Summary

Porcine circoviruses (PCV) and parvoviruses (PPV) are both small DNA viruses that are endemic in swine populations worldwide. Their associated infections can cause significant economic losses worldwide, requiring continuous control measures including regular vaccinations.

PCV2 was first identified when the systemic, wasting disease was shown to be caused by this virus and it was first reported in Hungary in 1999. PCV2-SD is clinically characterized by respiratory distress, wasting, diarrhea, jaudice and enlarged subcutaneous lymph nodes. PCV2 infection can be responsible for the development of various systemic, reproductive, respiratory, and enteric diseases, which are collectively referred to as PCV2 diseases (PCVD). Eight PCV2 genotypes have since been identified (PCV2a–h), but most of the currently available vaccines are based on the PCV2a genotype. In 2016, a third circovirus species, PCV3 has been identified in the United States in tissues of pigs suffering from PDNS, reproductive failure, myocarditis or multisystemic inflammation. Recently the presence of PCV3 has also been confirmed in Hungary. In 2020, Chinese researchers described a novel circovirus species from severe respiratory disease and diarrhea cases and named it PCV4.

The first known porcine parvovirus, the PPV1, has been the most important cause of reproductive disorders worldwide. Infection of the sows or gilts with PPV1 leads to stillbirth, mummification, embryonic death and infertility. In the last two decades seven novel PPVs (PPV2–PPV8) were identified in swine in various countries. Besides PPV1, the presence of PPV2, PPV3 and PPV4 have been confirmed in Hungarian pig herds also.

Between 2020 and 2023 we collected serum, oral fluid and processing fluid samples from 27 Hungarian and two Slovakian swine farms, using a comprehensive cross-sectional sampling protocol. The samples were tested by specific qPCR methods optimized for the detection of PCV2–4 and PPV1–8. At least one virus was detected on each farm. For serum samples, an average of 100 samples per farm were collected from pigs of 2, 4, 6, 8, 10, 12, 14, and 18 weeks of age, as well as from gilts and sows of two and four parities, which were examined in pools of 5. Oral fluid samples were collected from 8–12 and 18–20 weeks old animals. At least one PCV was detected in 93% (25/27) and at least one PPV in 96% (26/27) of the Hungarian farms included in our study. Ono ne farm, only PPV8 was found, while on the remaining farms, multiple viruses were present simultaneously. In four herds, the co-circulation of all tested viruses was identified, with the exception of PCV4.

Significant differences in virus detection rates were observed across the different diagnostic matrices, and the infection dynamics of PCVs and PPVs also varied between age

groups. For PPVs, serum samples of the 14–18-week-old pigs proved to be the most suitable for virus detection, while for PCVs, serum samples of the 4–6-week-old pigs were more appropriate. The oral fluids were diagnostically reliable and well-suited for PPV detection, while processing fluid samples were primarily useful for PCV3 screening.

Based on our results, the presence of certain PPVs may be associated with the below-average production parameters of the herds included in our study. Our statistical analysis indicated that, in addition to PPV1, PPV5 and PPV8 may also have a negativ impact on certain reproductive parameters, such as farrowing and conception rates and the number of piglets born.

Phylogenetic analysis of the detected PCV2 strains showed that all of our sequences belonged to the PCV2d genotype, which might be associated with earlier onset of viraemia and increased virulence compared to PCV2b. The Hungarian PCV3 strains were classified within the PCV3a genotype and showed high nucleotide identity with sequences originating from diverse geographical regions. This indicates that PCV3 has a relatively low evolutionary rate compared to PCV2.

The Hungarian PPV1 strains were classified into the PPV1a and PPV1b genotypes, which include the highly virulent 27a and 143a strains, respectively. Some of our sequences from one PPV1-positive herd may represent an evolutionary intermediate between the primarily Asian PPV1c and the European PPV1b genotypes. Currently there are no official classification for the novel PPVs. The sequences included in our phylogenetic analysis clustered into two–four distinct clades, which successfully reflected both the characteristic amino acid mutations and the geographical origin of the strains. Interestingly, for PPV2 and PPV7, we observed that our sequences show closer relationships to strains from China or America than to each other, indicating their affiliation with distinct genetic lineages and suggesting a prolonged evolutionary history in Europe. Conversely, for the other four viruses, our results indicate a slower evolutionary rate.

3 New scientific results

1. We were the first to identify PPV5, PPV6, PPV7 and PPV8 in Hungary, while PPV8 was detected for the first time outside of China.

2. We determined that serum samples of 14–18-weeks-old fattening pigs are the most suitable for PPV detection, while serum samples of 4–6-weeks-old weaned pigs are the most suitable for deceting PCVs. Additionally, we found that oral fluid samples are primarily useful for screening PPVs, while processing fluids are most suitable for PCV3 detection. We were the first to detect all eight PPVs (PPV1–PPV8) in processing fluid samples.

3. For the first time, we determined the NS1 protein-coding sequences of the PPV5, PPV6 and PPV7 strains, the partial capsid protein-coding region of PPV8 strains identified in Hungary. We were the first the determine partial capsid protein-coding regions of Slovakian PPV2 and PPV8 strains. For PPV2–PPV8, we also identified several variable regions and amino acid mutations that may potentially influence the antigenic properties of these viruses.

4 Publications

- Igriczi, B., Dénes, L., Schönhardt, K., Woźniak, A., Stadejek, T., Balka, G., 2024. Comparative Prevalence Estimation and Phylogenetic Analysis of Novel Porcine Parvoviruses (PPV2–7) in Hungarian Pig Herds, Transboundary and Emerging Diseases. 5117884, 11. doi:10.1155/2024/5117884, IF: 3.595
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- Igriczi, B., Dénes, L., Biksi, I., Albert, E., Révész, T., Balka, G., 2022. High Prevalence of Porcine Circovirus 3 in Hungarian Pig Herds: Results of a Systematic Sampling Protocol. Viruses. 14(6):1219. doi:10.3390/v14061219, IF: 4.870
- Igriczi, B., Dénes, L., Balka, G., 2025. A sertéscircovírusok kórtani jelentősége és genetikai jellemzői. Magyar Állatorvosok Lapja. 147./131-145. doi:10.56385/magyallorv.2025.03.131-145, IF: 0.314