Theses of doctoral (PhD) dissertation

Digital image analysis in veterinary pathology

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1. Background and objectives of the doctoral thesis

Digital pathology is frequently used in human medical research and has been widely reported in the veterinary field. Since its release in 2017, QuPath has been the digital image analysis software used in many studies evaluating animal tissues. I used porcine reproductive and respiratory syndrome virus 1 (PRRSV-1) and porcine circovirus type 2 (PCV-2) as models to explore the possibilities of the method. After digitizing the histological sections with a Pannoramic Midi (3D Histech, Budapest, Hungary) slide scanner, we aimed to perform the following tasks using QuPath image analysis software:

- determination of the proportion of virus-infected cells labeled with ISH in lung lesions caused by PRRSV-1 in piglets,
- determination of the number of inflammatory and CD163+ cells in the endometrium of gilts infected with PRRSV-1 strains of different virulence,
- and determination of the number of infected cells labeled by IHC in lymph node tissues of pigs infected with PCV-2 and affected by PCV-2-associated systemic disease (PCV-2-SD).

With objective cell ratios and cell numbers, we aimed to determine through statistical analyses:

- the usability of RNAscope as a semiquantitative method,
- and the effectiveness of the digital method compared to manual procedures in the assessment of PRRS-induced endometritis and the diagnosis of PCV-2-SD.

2. Summary

Digital pathology (DP), also known as virtual microscopy, involves the digitization of traditional histopathological slides, followed by display on a monitor and computer analysis. Despite the challenges and requirements of the technology, it has many advantages in diagnostics, education and research. Slide scanners that perform digitization scan slides stained routinely with hematoxylin and eosin (H&E), special histopathological procedures, and immunohistochemical (IHC) and in situ hybridization (ISH) methods in high resolution. The resulting digital whole slide images (WSIs) can be examined using digital image analysis (DIA) softwares, allowing for the objective and reproducible determination of the number and proportion of cells showing abnormalities (difference in size, shape, staining, and other properties) and cells labeled with various specific staining procedures, as well as other lesions (e.g. fibrosis).

DP could be used in diagnostics to accelerate certain pathological workflows, perform remote work, and replace semi-quantitative procedures. Recently, in addition to quantitative procedures, decision-support digital pathology systems have also been gaining more relevance, which assist pathologists in the preliminary screening of cases and in the identification of regions relevant to the final diagnosis. Several major diagnostic service providers, medical and veterinary universities, companies conducting preclinical studies, and hospitals have already switched to a fully digitalized workflow, and their number is growing. By sharing scanned sections, consultation options are also expanded, meaning that expert assistance requested in connection with a case only requires internet access from anywhere in the world.

The research application of DP is frequent today. The semi-quantitative assessment of morphological changes and tissue labelings visualized by molecular methods is subjective and often difficult to repeat. In addition to objectivity and reproducibility, numerical data obtained with DIA software are statistically easier to handle. The method is suitable for examining numerous parameters/lesions, such as determining the number and ratio of cell types, measuring changes in protein expression, detecting and quantifying cellular changes, and examining the extent of tissue changes (e.g. fibrosis, amyloid plaques). In addition to drug development, it is mostly used for the quantitative determination of tumor and other biomarkers in research investigating potential prognostic markers. In addition to relatively simple cell counting procedures, more publications are appearing in which, using artificial intelligence (AI) and so-called deep learning systems, the softwares are able to more effectively recognize, for example, cancerous or precancerous lesions, and also perform more accurate prognostic classifications based on the tumor pattern.

The use of DP is also becoming more widespread in medical and veterinary education. Educational activities are more interactive, the instructor is able to clearly designate and demonstrate certain lesions to everyone, even by displaying several special staining procedures on one monitor. There is no need for a separate microscope as a student, there is no longer a need to provide for the supply of worn or damaged slides, and students can access digital sections anywhere, anytime. Several publications report the positive reception of digital educational methodology among students. In addition, the system is also suitable for examinations.

QuPath (https://qupath.github.io) is a user-friendly, open-source software designed for the analysis of digitized histological slides. The program is flexible and can be developed to perform the classification of identified cell types with a detailed phenotypic description of each cell in a selected area. A quantitative cellular map of the tissue section is also available, which can be selected, queried and plotted. All this is not immediately visible during traditional microscopic evaluation, but with QuPath software it is available in minutes, without the need for special hardware.

To demonstrate the advantages and broad applicability of DP and QuPath, I used viral swine diseases as models, where I examined inflammatory cells of the endometrium, as well as infected cells labeled with IHC in lymph nodes and with ISH in lungs. Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV-2) are both economically important and worldwide prevalent pathogens, which are still the subject of active research, especially with regard to the pathogenesis of the diseases they cause. Using DP, we can gain new knowledge about the pathology and pathogenesis of reproductive and respiratory disease caused by PRRSV-1, and in the case of PCV-2, the method may bring progress in the diagnosis of virus-associated systemic disease (PCV-2-SD), where visualization and semiquantitative quantification of the virus in the affected tissues is essential, and a digital methodology may make the assessment of cases more objective and reproducible.

In our respiratory PRRSV infection model, 20 7-week-old piglets were infected with a wild, virulent PRRSV-1 strain. We found a moderate positive correlation between the proportion of virus-infected cells labeled by in situ hybridization and the virus copy number determined by quantitative polymerase chain reaction (qPCR) from the same lung lobe. Our experimental results demonstrate that the applied RNAscope ISH combined with digital image analysis can provide valuable semiquantitative data on in situ PRRSV infection of the lung.

Subsequently, in the reproductive model of PRRSV infection, vaccinated and unvaccinated pregnant gilts were infected with a low virulence or virulent PRRSV-1 strain and

inflammatory cell infiltration of the endometrial lamina propria was quantified using image analysis software. We found a high correlation between inflammatory cell counts and manual severity categories. Thus, DIA can be effectively used for objective assessment of endometrial inflammation, making it a suitable method for tissue analysis in addition to or instead of semiquantitative manual scoring in similar research procedures.

In the case of the PCV-2 model, archived lymph node FFPE (formalin fixed, paraffin embedded) blocks from animals affected by PCV-2-SD were subjected to repeated PCV-2 IHC staining, semiquantitative manual scoring, cell counting using the DIA method (determination of infected and uninfected cell numbers), and viral genome copy number was determined from the blocks using qPCR. We found a high correlation between cell counts, manual scoring, and qPCR results. Our results demonstrate the simple and efficient applicability of our digital methodology for objective quantification of IHC signals in PCV-2-SD cases.

3. New scientific results

1. We were the first to identify PRRSV-1 using RNAscope in porcine lung tissue samples and to use digital image analysis to quantify the visualized nucleic acid. RNAscope ISH combined with DIA can provide valuable semiquantitative data on in situ PRRSV infection of the lung.

2. We were the first to use digital image analysis to quantify inflammatory cell counts in the porcine endometrium and compare the method with a manual scoring procedure. DIA may be an effective method for histologic evaluation in research procedures similar to ours, where endometrial inflammation needs to be objectively assessed. Such may include, among others, vaccine development trials, where the degree of lesion reduction is to be quantified, but its methodology may also provide new insights into the reproductive pathology of PRRSV-1.

3. We were the first to develop and apply a digital pathology system for the individual diagnosis of PCV-2-SD. Despite the efficiency of the currently used manual method, a new digital pathology system has the potential to improve the diagnosis of the disease, where visualization of the virus in diseased tissues is essential, and digital quantification makes case assessment more objective and reproducible.

4. Publications

- Dénes, L., Horváth, D.G., Duran, O., Ratkhjen, P.H., Kraft, C., Ács, B., Szász, A.M., Rümenapf, T., Papp, M., Ladinig, A., Balka, G., 2021. In Situ Hybridization of PRRSV-1 Combined with Digital Image Analysis in Lung Tissues of Pigs Challenged with PRRSV-1. Veterinary Science. 8:235. IF: 2,0
- Horváth, D.G., Abonyi-Tóth, Z., Papp, M., Szász, A.M., Rümenapf, T., Knecht, C., Kreutzmann, H., Ladinig, A., Balka, G., 2023. Quantitative Analysis of Inflammatory Uterine Lesions of Pregnant Gilts with Digital Image Analysis Following Experimental PRRSV-1 Infection. Animals (Basel). 13:830. doi: 10.3390/ani13050830. IF: 2,7
- Horváth, D.G., Dénes, L., Igriczi, B., Papp, M., Hidalgo-Martínez, V., Segalés, J., Balka, G., 2025. Digital quantification of porcine circovirus 2 (PCV-2)-infected cells in lymph nodes of pigs with natural PCV-2 systemic disease shows strong association with manual scoring and quantitative PCR. Vet Pathol. doi: 10.1177/03009858251331121. Epub ahead of print. IF: 2,3
- Horváth, D.G., Balka, G., 2025. Digitális metszetelemzés az állatorvosi patológiában. Magyar Allatorvosok Lapja, accepted for publication. IF: 0,3