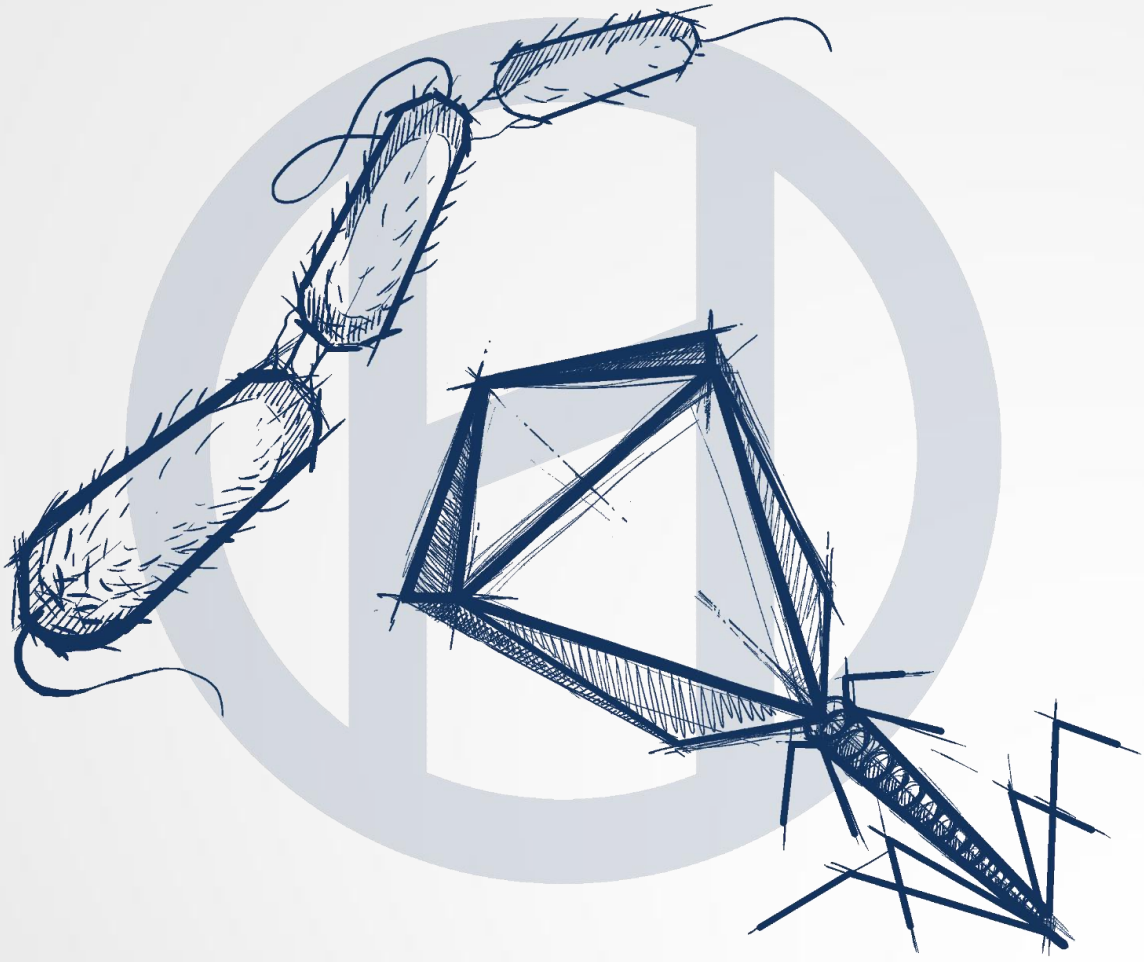
Book of abstracts

1st Debrecen Online Conference on Infectious Diseases in a One Health context (DOCIDOH)

27th-28th March, 2024 - Online



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Preface

It is now widely acknowledged that infectious diseases need to be studied, monitored and contained using the One Health approach. Human and veterinary pathogens can spread among multiple hosts and the environment creating complicated and frequently poorly understood transmission networks or triggering evolutionary events leading to emergence or reemergence of novel or neglected infectious diseases. These infections include food-, water- and vector-borne diseases, as well as the hidden epidemic of antimicrobial resistance, all representing major healthcare, economic and societal burden demanding attention. The recent and still ongoing pandemic drew attention to the importance of preparedness, which yet again need the close and intensive cooperation of physicians, veterinarians, environmentalists, epidemiologists, molecular biologists, ecologists, and various other experts in order to learn the key factors in disease dynamics and to plan countermeasures.

To promote discussion and knowledge exchange, the One Health Institute of the Faculty of Health Sciences, the Faculty of Agricultural and Food Sciences and Environmental Management and the Department of Metagenomics at the University of Debrecen and the Veterinary Medical Research Institute and the Centre for Ecological Research of the Hungarian Research Network announces the first Debrecen Online Conference on Infectious Diseases in a One Health context (DOCIDOH) taking place 27-28 March 2024.

The conference aims at attracting researchers in the topics

- **antimicrobial resistance in a One Health perspective**
- **alternatives to antimicrobials in human and veterinary medicine**
- **genomic epidemiology of pathogens across sectors**
- **emerging diseases and vectors**
- **ecology of infections, environmental DNA and environmental health**

On the conference website (https://konferencia.unideb.hu/en/node/1244), participants will find up-to-date information during the event, such as the detailed program and online access links to the sessions and breakout rooms.

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Organizing and scientific committee

- **Dr. Gábor Kardos** – One Health Institute, Faculty of Health Sciences, University of Debrecen
- **Dr. Csongor Freytag** – One Health Institute, Faculty of Health Sciences, University of Debrecen
- **Dr. Dalma Papp** – One Health Institute, Faculty of Health Sciences, University of Debrecen
- **Dr. Enikő Fehér** – HUN–REN Veterinary Medical Research Institute
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- **Dr. Levente Laczkó** – One Health Institute, Faculty of Health Sciences, University of Debrecen
- **Dr. Pálfyné Dr. Nóra Vass** – Department of Animal Husbandry, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen
- **Dr. Renáta Knop** – Department of Animal Husbandry, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen
- **Flórián Sipos** – Institute of Metagenomics, University of Debrecen

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Program at a glance

March 27.

9:30:00 (CET)	10:00:00 (CET)	Opening
10:00:00 (CET)	10:45:00 (CET)	Section I
10:45:00 (CET)	12:30:00 (CET)	Section II
13:00:00 (CET)	14:00:00 (CET)	Breakout rooms
14:00:00 (CET)	15:45:00 (CET)	Section III
15:45:00 (CET)	17:00:00 (CET)	Breakout rooms

March 28.

10:00:00 (CET)	11:45:00 (CET)	Section IV
12:00:00 (CET)	13:00:00 (CET)	Breakout rooms
13:00:00 (CET)	15:45:00 (CET)	Section V
15:45:00 (CET)	16:15:00 (CET)	Concluding remarks
17:00:00 (CET)	19:00:00 (CET)	Breakout rooms

For the detailed program see the conference website:
https://konferencia.unideb.hu/en/program-1st-debrecen-online-conference-infectious-diseases-one-health-context-docidoh

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Abstracts

Invited talks

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What wastewater can tell you – environmental surveillance in public health decision-making

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Environmental surveillance in wastewater (WES) is a well-established tool to monitor poliovirus circulation, but it only gained wider recognition during the COVID-19 pandemic. Wastewater collects all pathogens and other substances which are shed in excreta or body fluids, providing a community-level composite sample. WES is a complementary tool to other surveillance systems: it represents the bottom part of the epidemic pyramid as it captures both asymptomatic and symptomatic infections. In the past few years, many countries have set up formalised WES system for tracking SARS-CoV-2 and its variants. Evidence suggests that WES can predict outbreak trends with a lag time of one-two weeks. Other use-cases include early warning of (re)emergence in non-outbreak scenarios or monitoring transportation hubs to identify transboundary transmission. The potential scope of WES extends well beyond COVID and expands rapidly. Targets include other respiratory and enteric pathogens, antimicrobial resistance, chemical substances including illicit drugs, non-communicable disease and lifestyle markers. Its efficiency in detecting and localising emerging diseases such as measles or monkeypox was already demonstrated. On-going regional and global initiatives address harmonisation of methods to ensure comparability of results and explore options of data sharing to exploit the full potential of WES for public health decision-making.

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Antimicrobial resistance (AMR) problems in companion animals

Els Broens[1]

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Antimicrobial resistance (AMR) is one of the major public health threats for modern medicine and public health. Several pathogens responsible for the majority of untreatable infections due to AMR are identified, among them Methicillin-Resistant Staphylococci (MRS), Extended-Spectrum-Beta-Lactamase producing Enterobacterales (ESBLs), Carbapenemase producing Enterobacterales (CPE), *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. These pathogens are increasingly associated with nosocomial infections in human and animal hospitals and may lead to increased morbidity, mortality, prolonged length of stay and an economic burden. The direct and close contact between companion animals and humans facilitates transfer of microorganisms, including resistant bacteria. Veterinarians appear to have a higher risk of colonization with multidrug resistant pathogens and colonization with ESBLs and MRS in companion animals and their owners as well as transmission between them has been reported. This presentation addresses the emergence of multi-drug resistant pathogens in companion animals, emphasizing their zoonotic potential.

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Foodborne transmission of AMR in low and middle-income countries

Maya L. Nadimpalli[1]

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Antimicrobial resistance (AMR) disproportionately affects low and especially middle- income countries, but new evidence indicates that these patterns cannot be explained by differences in human antibiotic consumption alone. Here, I will present evidence that food animals and the environment likely play a role in the spread of AMR in some low-resource settings. I will describe case studies from southeast Asia and east Africa that suggest that food value chains – and in particular, wet markets – are likely important settings to implement new interventions to reduce community-level exposures to AMR.

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The transformative effect of the mNGS on virus discovery, research, and outbreak prevention/management in the context of climate change and anthropogenic impact: A One Health approach

Alexandru Tomazatos[1], Marike Petersen[1], Balázs Horváth[1], Alexandra Bialonski[1], Heike Baum[1], Marc Lütgehetmann[2], Alexander Schlaphof[1], Daniel Camprubí[3], Jose Muñoz[3], Stephanie Jansen[4], Renke Lühken[1], Gábor Endre Tóth[1], Jonas Schmidt-Chanasit[1,4], Dániel Cadar[1]

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Metagenomic next-generation sequencing (mNGS) has revolutionized the field of virology by offering unprecedented insights into the diversity of viral populations within various ecological niches. Real-time genomic surveillance using mNGS has proven invaluable in tracking virus introductions, monitoring viral evolution, and identifying potential changes in pathogenicity and transmission dynamics. Additionally, mNGS facilitates a "One Health" approach by uncovering novel host-virus associations in wildlife and domestic animals, contributing to a deeper understanding of the ecological drivers of viral emergence. Viral unbiased mNGS, unlike targeted approaches, offers a powerful tool for tracking zoonotic infections, identifying novel pathogens ("Disease X"), and conducting broad explorations of the viral world. We present a universal, cost-effective, and time-efficient mNGS method adaptable for various sequencing platforms, including portable options. This method allows for the detection and characterization of diverse RNA and DNA viruses, enhancing preparedness for future outbreaks. We successfully tested and validated the protocol using the portable Illumina iSeq100 platform, demonstrating its potential for mobile deployment and broad applicability, especially in resource-limited settings. This novel mNGS method holds significant potential to accelerate our understanding of zoonotic potential viruses, their transmission dynamics, and ecological factors influencing their emergence. By responsibly harnessing the power of mNGS, we can significantly improve our preparedness and response strategies to safeguard public health from emerging viral threats.

keywords: zoonoses, metagenomics, next-generation sequencing, One Health, outbreaks.

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Phage therapy as an alternative for antibiotics: current obstacles and future strategies

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Bacteriophages possess very attractive features enabling targeted killing of bacteria and are relatively easy to obtain. Therefore, phage therapy (PT) offers hopes in the fight against antimicrobial resistance. In the past years there has been a substantial emergence of new PT centers in the world and facilities dedicated to manufacturing phage products. This should be driven by arising regulatory frameworks dedicated to phage production. Although widespread optimism considering reintroduction of PT as a standard treatment, evidence-based medicine data confirming the efficacy of PT are still scarce. Uncritical belief in efficacy of phages has to be faced with their pharmacokinetics and pharmacodynamics which are more complex than those of antibiotics.

In 2005 we have opened a Phage Therapy Unit – the first such center in European Union, where we admit patients with different chronic bacterial infections not responding to antibiotic treatment on an out-patient basis under the rules of a therapeutic experiment. Since that time we have treated over 750 patients. Our clinical results are promising and they confirm general and remote safety of the phage use. Besides we also initiated studies on phage interaction with the immune system, phage use in treatment of autoimmune diseases, as well as formulation of hypothesis on possible phage application beyond their antibacterial action (phage repurposing). Further research is needed to secure the place of phages in modern medicine.

This work has been supported by funds from the National Science Centre, Poland for project No. 2018/31/B/NZ6/03999.

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Contributed talks

Section I – Environmental DNA and environmental health

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Survey on antibiotic resistant bacteria in commercial fish ponds

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Fish is a valuable source of proteins and commercial fish farms help to satisfy the demand. Hungarian farms produce a large amount of fresh fish every year, in many lakes countrywide. Due to the fish farming activities (the use of antibiotics for curing fish with bacterial infections) and the migrating birds (reservoir of pathogenic microbes), these lakes may be sources of pathogenic bacteria that confer antibiotic resistance. These bacteria may pose a threat not only to the fish but employees and consumers, as well. A joint project of the Institute of Metagenomics and the microbiology laboratory of the Institute of Food Sciences is to perform regular surveys on the microbiological quality of fish farm lake water, with emphasis on the detection of possible AMR bacteria, in accordance with the Farm to Fork Strategy. The survey started in 2023 and 10-10 lakes were sampled in two fish farms, respectively. After filtration of water samples, bacteria were isolated on vancomycine and Eosine Methylene Blue agar plates, then identified by MALDI-TOF. Though, numerous bacterial species were found, no traces of vancomycine resistant Enterococcus isolates were found yet.

We thank the helpful attitude and of the Bocskai Halászati Kft. and the Biharugrai Halgazdaság Kft.

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Section II – Antimicrobial resistance and pathogens across sectors in a One Health perspective I.

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Occurrence and characteristics of ESBL-producing Enterobacterales in free-ranging Hooded crows (*Corvus cornix*) in Hungary

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[2]**Institute of Metagenomics, University of Debrecen, 4032 Debrecen, Hungary**

Recently, WHO has integrated wildlife into their One Health concept, recognising its significance in the interconnection between animal and human health. Here, we study the presence of ESBL-producing Enterobacterales in hooded crows (*Corvus cornix).* In Hungary, crows live sedentary lives in both rural and urban areas. In 2020, we collected 264 faecal samples from wild crows from both areas and tested the carriage of ESBL-producers. We identified ESBL-encoding genes using PCR and characterised our *E. coli* isolates using WGS. Four sampled rural crows and 125 urban ones (7% vs. 60%, $p<0.0001$) yielded ESBL-producers. The CTX-M-1 group was the most predominant. Genes encoding resistance to other antimicrobials were also detected, and various virulence genes, in association with several plasmids, were predicted. Most isolates belonged to B1 and A phylogenetic groups, suggesting that hooded crows carry ESBL-producers and might serve as reservoirs of ESBL-encoding genes. Overall, 22 sequence types and 33 core-genome MLSTs were defined. The most prevalent STs were previously described in human and animal isolates, including wildlife. Our research provides insights on an understudied part of the ecosystem that warrants further attention in AMR studies. The higher frequency of carriers in the urban population points to the potential role of anthropogenic sources in the origin of ESBL-producers and to that of hooded crows living in proximity to humans in the spread of AMR-encoding genes.

The Stipendium Hungaricum Program of the Tempus Public Foundation provided funding for this study under grant number SH-00355-004/2019.

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Conjugation kinetics of different ESBL plasmids with and without antibiotic exposure – an in vitro study

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In this study we aimed to test the conjugation potential and kinetics of different ESBL plasmids of animal originating strains and study the effects of different beta-lactam antibiotics for the plasmid transfer. We tested 11 ESBL producing *E. coli* strains of wild rook origin as potential donor strains in conjugation experiments using human originating strain (J53) as recipient; every experience was repeated after antibiotic incubation with cefotaxime, ceftazidime or ceftriaxone. Whole genome sequencing was performed with Illumina MiSeq and Oxford Nanopore. Donors belonged to different STs, carried different plasmids and ESBL genes. 6/11 isolate showed conjugation; IncN plasmids of four ST162 and one ST744 isolates and IncI1 plasmid of one ST162 isolate were transmissible. Conjugation frequency were higher with IncN carriers than with IncI1 carrier. The ST744 isolate with significantly more transfer genes showed higher conjugation frequency than ST162 isolates. The effect of antibiotics was different for the kinetics, however both cefotaxime, ceftazidime and ceftriaxone decreased the frequency of plasmid transfer. Antibiotics induced the conjugation in case of one ST24 isolate with IncN plasmid, which showed no conjugation in the absence of antibiotics. IncF type plasmids of ST24 and ST131 isolates never showed conjugation. Different plasmids have different conjugation potential, that can influence the spread of the carried AMR genes, which can be affected by antibiotics.

This project was partially supported by „New National Excellence Programme" granted by the Ministry of Innovation and Technology. Application number: ÚNKP-22-2-I-DE-276 (2022-2023) On behalf of the "Harmadik generációs szekvenálási adatok bioinformatikai elemzése" (Bioinformatic analysis of third generation sequencing data) project we are grateful for the possibility to use ELKH Cloud (see Héder et al., 2022; https://science-cloud.hu/) which helped us achieve the results published in this paper.

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Interspecies transmission of β-lactamase genes carrying plasmids in dense mixed breeding colonies used by gulls and terns

Balázs Koleszár[1,2], Bálint Timmer[2,3], Renáta Bőkényné Tóth[1], Dalma Papp[1], Dóra Boros-Pál[2], Réka Bubán[1], László Miló[1], Pál Tóth[4], Ádám Kiss[4], Péter Balázsi[5], Ádám Lovas-Kiss[1,6], Levente Laczkó[1], Gábor Kardos[1,7]

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Landfill feeding Black-headed gulls (*Chroicocephalus ridibundus*), Mediterranean gulls (*Ichthyaetus melanocephalus*) and fish-eating Common terns (*Sterna hirundo*) cohabit in breeding colonies. We investigated interspecies transmission of third-generation cephalosporin-resistant (3GCR) Enterobacterales among chicks. Faecal samples from 64 Black-headed gull, 56 Mediterranean gull, and 82 Common tern chicks were collected across three locations from 2020-2022. Selective culturing yielded 108 3GCR Enterobacterales, with 69 ESBL or AmpC-producing E. coli genomes sequenced using the Oxford Nanopore MinION platform. We identified 37 sequence types (STs) and 8 unclassifiable isolates, including human and avian pathogenic STs (ST648, ST155, ST23). While some STs shared plasmids and genes within the same species and site (e.g., ST88 with $bla_{CTX\text{-}M\text{-}1}$ or ST1718 with $bla_{CMY\text{-}59}$), STs varied across species and breeding colonies. Notably, IncI1-alpha plasmids predominated (n=28), carrying $bla_{CMY\text{-}59}$ (n=10) or $bla_{CTX\text{-}M\text{-}1}$ (n=14). Highly similar IncI1-alpha plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ suggested plasmid transfer among isolates from different species. In conclusion, 3GCR E. coli was diverse and prevalent among chicks, with IncI1-alpha plasmids facilitating interspecies transmission, potentially spread by bird migration, emphasizing the role of horizontal gene transfer in global resistance gene epidemiology.

We are grateful to the coworkers of the Institute of Metagenomics, the One Health Institute and Birdlife Hungary for helping us to achieve these results.

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The spread of antibiotic resistance in farm animals and food in Estonia

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The aim of current study was to analyse the use of antibiotics in farm animals in order to determine current trends in AMR in farm animals and detect possible AMR transmission pathways and mechanisms from agricultural sector to humans. To achieve this goal, target organisms *E.coli* (including ESBL and AmpC producing *E.coli*), *Enterococcus faecalis*, *E. faecium*, *Staphylococcus aureus* and *Klebsiella pneumonia* were isolated from samples of dairy cows, fattening pigs, broilers, horses and aquacultures (N=780). Additionally, 752 food samples from fresh meat (pork, beef and broiler) and milk were included to the analyses. No recent (i. e. taken place during last 5 to 10 years) transfers between human-animal-food-environment segments were observed.

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Livestock-associated MRSA in Hungarian pig farms – high prevalence and genomic evidence for the spillover to humans

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Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) strains of clonal complex (CC) 398 are widely disseminated in pigs and are considered emerging pathogens in human medicine. To investigate the prevalence, genetic characteristics, and zoonotic potential of the pathogen in pig production settings, dust samples were collected from 40 pig operations in Hungary, along with nasal swabs of attending veterinarians and other swine professionals (n = 27) in 2019. MRSA isolates were further characterized by performing whole-genome sequencing and susceptibility testing. The whole-genome sequences of 14 human-derived LA-MRSA clinical isolates from the same year were also included in the study. The proportion of positive farms was 83% (33/40), which is a dramatic increase, compared with the 2% prevalence reported by the European Food Safety Authority baseline study in 2008. Of the swine professionals, 70% (19/27) carried the pathogen. Genomic analysis suggested recent transmission events between the farm environment and humans, both asymptomatic carriers and diseased. Half of the swine-related strains showed decreased susceptibility to eight or more antimicrobials, and along with human isolates, they carried eight different types of multidrug-resistance genes, including cfr. The wide range of antimicrobial resistance of the strains, accompanied by the emergence of the pathogen in humans call for revision of the risk posed by LA-MRSA to the public health.

The authors thank Bernadett Kelemen, Sára Heinik, and Alexandra Collaud for their technical assistance; Krisztián Kiss for his valuable comments on swine industry-related matters; and all veterinarians and farm managers who contributed to the study by submitting samples.

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Section III – Antimicrobial resistance and pathogens across sectors in a One Health perspective II.

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Investigation and characterization of staphylococci strains isolated from cow's milk and sheep milk

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Over the past three decades, global milk production had increased from 530 million tons in 1988 to 852 million tons in 2019 by more than 60 percent. As the population of the world rises rapidly, the consumption of milk and milk products also increases with an expected per capita consumption of 125.2 kg by 2025. To improve food and nutrition security, milk products are becoming a regular requirement. Though, more than 80% of world milk is from dairy cows, approximately 1.3% of milk comes from sheep. In addition to harmless and beneficial microbes, contaminant/pathogenic microorganisms enter into milk from several sources, predominantly from the farm environment and animal body. The use of antibiotics in veterinary triggered the appearance of resistant microbes, including methicillin resistant *Staphylococcus aureus* (MRSA), which is one of the most common causes of severe bacterial infections in Europe. The development of new instrumental and molecular investigation methods help research and public health institutes in the identification and characterization of *S. aureus* isolates that may pose a threat to the human and animal health. In our experiments the aim was the identification, furthermore the phenotypic (tellurite production, lecithinase activity, coagulase test, hemolysis, catalase test, oxidase test and antibiotics resistance) and genotypic (enterotoxin gene presence) characterizations of staphylococci isolates of raw sheep and cow milk origin.

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Occurrence of multidrug resistant Enterobacteriaceae and Enterococcaceae strains in pets and their owners

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This pilot study acknowledges the dynamics of antibiotic resistance in the context of the animal-human relationship through the One Health approach. Our aim was to investigate the prevalence of resistant bacteria in human and animal individuals living in the same household. We focused on extended-spectrum β-lactamases, AmpC β-lactamases, carbapenemase-producing members of the *Enterobacteriaceae* family and vancomycin-resistant enterococci. Faecal samples were collected from 19 study groups of animal-owners, including 25 human and 31 animal samples. Of the 128 bacterial strains identified, only 12 contained phenotypic resistance mechanisms. Based on the results of the third-generation sequencing, we could not establish genetic identity or similarity between the isolates and the common resistance elements of animal and human individuals from the groups studied, but we confirmed the results of the antimicrobial susceptibility test. We found that 50% of the resistant strains derived from animals had a resistance-encoding genetic element on a plasmid. In summary, 16% of humans and 23% of animals carried a resistant bacterial strain and only a small phenotypic overlap was found between faecal bacterial isolates from pets and their owners from the same households. Our future plan to involve more participants to demonstrate significant correlations between the type of animal housing, antibiotic use and the prevalence of antibiotic resistance.

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Cefiderocol susceptibility of carbapenemase producing *K. pneumoniae* high-risk clones in Hungary

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Carbapenemase-producing *Klebsiella pneumoniae* (CPKP) strains pose a severe global health threat due to limited treatment options. We analyzed the structure of the CPKP population in Hungary and investigated their in vitro susceptibility to cefiderocol – a new siderophore cephalosporin. The study was performed on 420 CPKP strains from 32 Hungarian healthcare institutes submitted to the National Reference Laboratory for Antimicrobial Resistance (01/01/2021–01/04/2023). The molecular investigation used Illumina Miseq sequencing and PFGE to confirm eight high-risk clones of CPKP: ST395, ST147, ST258, ST15, ST307, ST11, ST17 and ST383. The CPKP clones showed a very clear regional distribution. The $bla_{\text{NDM-1}}$ was predominantly found in ST147, while the $bla_{\text{NDM-5}}$ was prevalent in ST395. Cefiderocol resistance rates varied by carbapenemase type, with bla_{VIM}-carrying strains at 20%, $bla_{\text{OXA-48-like}}$-carrying strains at 44%, bla_{KPC}-carrying strains at 70% and $bla_{\text{NDM-}}$ and $bla_{\text{OXA-48-like}}$+bla_{NDM}-carrying strains at 75%. Overall, the cefiderocol resistance rate was 65%, with bla_{NDM}-carrying strains showing the highest resistance, especially in the ST147 (CT7378) strain carrying $bla_{\text{NDM-1}}$. High-risk CPKP clones, particularly those harbouring bla_{NDM} and $bla_{\text{OXA-48-like}}$+bla_{NDM}, were prevalent in Hungary, indicating therapeutic challenges due to varying susceptibility to cefiderocol among different CPKP strains.

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Genetic characterization of *Collyriclum faba* from Slovakia

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On 24th May 2023, during Constant Effort Site Bird Ringing on Drienovska Wetland, Slovakia, a specimen of *Acrocephalus arundinaceus* infested by trematodes *Collyriclum faba* was recorded. Dermal cysts of *C. faba* were located on ventral parts of *A. arundinaceus*. The DNA from ethanol-fixed trematode specimens was isolated using the DNeasy® Blood & Tissue kit (Qiagen). Trematode species were identified by amplifying two nuclear DNA loci: a fragment of 18S rDNA and a full-length internal transcribed spacer 2 (ITS2) with the flanking conserved 5.8S and 28S rDNA region sequences. Amplicons of the expected product size of 18S rDNA (508 bp) and ITS2 (663 bp) were purified and sequenced by the Sanger method. BLASTn analysis for 18S rDNA showed 100% pairwise identity with partial 18S rRNA gene sequence of *C. faba* (JQ231122) for both specimens. Sequencing of ITS2 suggested that both specimens share a similarity ranging from 100% to 92.5% with *C. faba* isolates deposited in the GenBank database. Sequence data of partial 18S rDNA and ITS2 of both *C. faba* isolates from this study were uploaded to the GenBank database under accession numbers PP316965, PP3169666 and PP317288, PP317289 respectively.

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Isolation of *Clostridium perfringens* bacteriophages from poultry faeces

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Clostridium perfringens, a Gram-positive spore-forming anaerobic bacteria, causes necrotic enteritis, necrotic dermatitis, and cholangiohepatitis in chicken, resulting in significant economic losses for the industry. Moreover, *Clostridium perfringens* has been linked to food poisoning in humans. Bacteriophages are an effective alternative to antibiotics for controlling *Clostridium perfringens*. The discovery of *Clostridium perfringens* in healthy chicken birds indicates that they may be a reservoir for clostridial infection, potentially causing significant losses to the poultry industry. The study aimed to isolate and characterize *Clostridium perfringens*-specific phages in order to determine the most effective phage cocktail for biocontrol applications. A study was conducted to isolate and characterize *Clostridium perfringens* from different poultry farms in Hungary. In this study, we isolated two bacteriophages (P3, P4), showing antimicrobial effect against *Clostridium perfringens*, from poultry faeces by Drop method. The selected bacterial strain was identified as *Clostridium perfringens* by nanopore sequencing. The bacteriophages from the isolated strain exhibited strong inhibitory activity against the same strain of *Clostridium perfringens*, producing clear plaques on solid (RCA) medium.

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Isolation of bacteriophages infecting *Stenotrophomonas maltophilia*

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Stenotrophomonas maltophilia, a Gram-negative multidrug-resistant nosocomial pathogen, has a natural resistance to most antibiotics; therefore, alternative therapeutic agents such as bacteriophages may be of particular importance. We screened 19 water samples from a wastewater treatment plant in Budapest for phages against different strains of *S. maltophilia*. We performed phage detection using the spot method by placing a drop of phage suspension on the bacterial lawn, where the appearance of a clear plaque indicated an effective interaction between phages and bacteria defined by phage multiplication and bacterial lysis. We isolated and sequenced genomic DNA using Oxford Nanopore sequencing. After careful filtering and *de novo* assembly, we selected the high-quality genome assemblies for further bioinformatic analyses, including predictions of gene function, host and lifestyle, and taxonomic classification. As a result, we present 26 bacteriophages capable of infecting *S. maltophilia*. The isolated phages, which were all virulent, had a dsDNA genome with a length of 40 to 60 kb. We could not detect any bacterial resistance genes or virulence factors in the phage genomes. Taxonomic classification revealed that 15 phages belonged to the family *Mesyanzhinoviridae*, 8 to the family *Autographiviridae* and 3 to the family *Drexlerviridae*. Our study presents several new phages infecting *S. maltophilia* that could be considered for therapeutic intervention.

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Acetone extract of *Cladonia foliacea* (Huds.) Willd., a potential biopesticide as oral toxic sugar bait against Anopheles gambiae

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Despite the application of intervention methods against *A. gambiae*, malaria remains high in Sub-Saharan African countries and accounts for 90% of malaria deaths. Lichen secondary metabolites (LSMs) exhibit biological and insecticidal properties. This study aimed to explore the potential of *Cladonia foliacea* extract that contains (-)-usnic acid and fumarprotocetraric LSMs toxin as attractive toxic sugar bait (ATSB) against adult *A. gambiae*. Soxhlet apparatus and rotary evaporator were used to obtain extract and diluted to 10, 20, 30, 40, 50,70, 80, and 90 mg/ml and mixed with 10% sugar solution and 2% red dye. Ten Newly emerged starved *A. gambiae* Kisumu strains were offered 5ml of the toxic extract in three replicates and negative control. Analysis of variance (ANOVA; at $p \leq 0.05$) was performed on post-exposure mortality at 4, 24, 48, and 72 hours using the R software version 4.3.2. Compared to control, mortality was significantly associated with the concentration at 50mg/ml; 24 and 48 h post-exposure. At 90mg/ml and 72 h, there was no significant difference in mortality between males and females. This study for the first time has confirmed that *C. foliacea* extract is a potential oral biological insecticide against *A. gambiae*. This study was supported by the National Research Development and Innovation Fund NKFI K 124341 and the Stipendium Hungaricum Scholarship (2020–2024).

Keywords: *Anopheles gambiae*, bioassay, lichen extract, malaria, toxic sugar bait, (-)-usnic acid.

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Chitosan as a basic substance inhibited sour cherry postharvest pathogens bud did not affected its shelf life

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Controlling fruit pathogens after harvest is one of the most important challenges in the fruit sector. Moulds cause significant economic losses. Pesticides remain the most effective means of controlling postharvest diseases of fruits and vegetables. One of the main goals of the European Green Deal is to reduce the use of pesticides by 50% to 2050. Therefore, we must find environmentally friendly alternative to prevent post-harvest diseases of the fruits and vegetables. Chitosan is a natural linear polysaccharide. It is produced by deamination of chitin, using NaOH solutions or chitinase enzyme. The European Union was the first to allow the use of chitosan as a plant protection agent in organic farms and in integrated plant protection. Two chitosan products were tested on different moulds, isolated from the surface of sour cherries in in vitro tests. 1% chitosan solution was also used in the orchard as pre-harvest treatment, and harvested fruits were dipped in a 1% chitosan solution. All tested chitosan concentration inhibited the mycelial growth of the studied moulds (*Alternaria alternate*, *Botrytis cinerea*, *Cladosporium herbarum*, *Colletotrichum clavatum*, *Epicoccum nigrum*, *Fusarium solani*, *Fusarium solani*, *Penicillium expansum*), except *Aspergillus niger*. However neither pre- no postharvest chitosan treatment had significant effect on the shelf life of sour cherrys.

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Testing alternative plant protection methods against walnut pathogen fungi

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Reducing chemical pesticides is important both from an ecological and economic point of view. The prudent use of pesticides is essential for sustainability and health protection. Environmentally friendly control methods deserve priority given the biodiversity, beneficial microorganisms, soil health, food quality, and consequently the negative effects on human health. One way to reduce chemical agents in plant protection is the use of biocontrol agents. *Trichoderma* sp. and *Epicoccum* species have an antagonistic effect, and they improve the condition and growth of the host plant as well. Fungal infection of walnut trees is a complex disease caused by *Botryosphaeria dothidea*, *Diaporthe eres* and *Diplodia seriata*. In the last decade, walnut rot has become serious worldwide, causing significant yield losses. In our study, *Epicoccum nigrum* isolated from walnut bud, *Trichoderma afroharzianum* (TR04), *Trichoderma simmonsii* (TR05) and *Trichoderma gamsii* (TR08) stains were tested against the walnut pathogens in vitro. The antagonistic effect of *E. nigrum* was moderate (Biocontrol-Index=47-53%), however, the three *Trichoderma* stains indicated promising alternative to pesticides in the control of walnut fungal infections (BI=100%). The antagonist grew on pathogen colonies with hyperparasitism, inhibit their growth. We can conclude that the tested Trichoderma strains are suitable for controlling the walnut pathogen species in vitro.

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Endophytic fungal biostimulants starins with biocontrol portential: new possibility to decrease pesticid usage in crop managemen

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Reducing pesticide usage in plant production is one of the key element of sustainable agriculture. *Trichoderma* strains have been described to be effective biocontrol agents as well as plant biostimulants. Moreover, the application of endophytic strains provide sustained effect with reduced environmental risk. Ten *Trichoderma* strains were isolated from healthy plants in Hungarian vineyard with serious grapevine trunk disease symptoms. They were identified as *Trichoderma gamsii*, *T. orientale*, *T. simmonsii*, *T. afroharzianum*, *T.atrobrunneum* and *T. harzianum* sensu stricto on the basis of their ITS1 and ITS2 and tef1 sequences. The growth potential of the strains was assessed at different temperatures. *T. orientale* was characterized as potential human pathogen, based on its temperature preferences and taxonomic characteristics. Biocontrol potential of the selected strains was tested against plant pathogens and were also tested in commercial vineyards. Three of the tested *Trichoderma* species were re-isolated up to four years after rootstock soaking treatment with conidiospores, performed before planting. The treatments decreased the overall percentage of lost plants of about 30%. Both plant growth and harvested grape quantity and quality increased for several years after the treatment, reflecting usefulness of the endophytic *Trichoderma* strains in sustainable agriculture in general and integrated plant production in particular.

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Section V – Zoonotic viruses and vectors

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Detection of arboviruses in ticks collected from vegetation in eastern Slovakia

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In the Slovak Republic, ticks act as vectors for tick-borne encephalitis virus (TBEV), Tribeč virus (TRBV), Lipovník virus (LIPV) and Uukuniemi virus (UUKV). This study aimed to evaluate the presence of flaviviruses, phleboviruses, and orbiviruses within ticks collected from vegetation in eastern Slovakia (Drienovec and Dúbrava regions) from 2019 to 2021. Examination of 502 pools (2966 ticks), was conducted. Viral identification involved molecular techniques and classical virological methods. Phlebovirus RNA was confirmed in five tested pools, with subsequent sequence analysis revealing the presence of UUKV. Two strains were isolated from ticks in Dúbrava (2019) and three from Drienovec (2020). The positive ticks were identified as *Ixodes ricinus*. Comparative partial nucleotide sequence analyses (Segments S, M, and L) of four of our isolates with other strains of UUKV and the Chize virus available in GenBank were conducted, facilitating the construction of phylograms. A high degree of similarity between our isolates and UUKV strains from Slovakia, Czech Republic, and the prototype UUKV strain from Finland was demonstrated (92.5% to 99.3%). Conversely, UUKV strains from the United Kingdom and Norway and Chize virus from France exhibited lower sequence similarity to our strains (68.1% to 77.2%). All tested pools were negative for orbivirus and flavivirus RNA. Nevertheless, this study significantly contributes to expanding the UUKV sequence repository.

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Prevalence of zoonotic arboviruses in ticks collected from birds in Drienovská wetland

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To date, three tick-borne zoonotic arboviruses have been described in Slovakia: the tick-borne encephalitis virus (TBEV, +ssRNA, *Flaviviridae*), and two orbiviruses of the Great Island virus serogroup (dsRNA, *Sedoreoviridae*) - Tribeč (TRBV) and Lipovník (LIPV) viruses. These arboviruses primarily replicate in small forest mammals, while the role of avian species in their transmission cycles remains unclear. This study aimed to detect TBEV, TRBV, and LIPV in ticks collected from birds in the Drienovská wetland. A total of 68 pools (211 ticks), were analyzed using molecular and classical virological methods. TRBV was identified in two of these pools, positive *Ixodes ricinus* ticks were collected from a dunnock and a black thrush, designated as isolates 283.C/19 and 248.C/19, respectively. Comparative analysis of AA sequences for VP1 and VP5 proteins with other tick-borne orbivirus strains was conducted to determine sequence identity and to construct phylograms. The VP1 protein of the isolates showed a high sequence identity (97.8-98.9%) with TRBV and LIPV strains from Slovakia, Ukraine, and Romania. In the case of the VP5 protein, isolate 283.C/19 was most closely related to Muko virus strains from Japan (95.2-95.3%). None of the tick pools tested positive for TBEV or LIPV. These findings highlight the different origins of our isolates, and the importance of avian monitoring for understanding the spread of tick-borne viruses, which may not typically be associated with birds.

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Detection, isolation of Tribeč virus (*Orbivirus*) and its replication kinetics in human, rodent, and bovine cells

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Tribeč virus (TRBV) is a serotype of the Great Island virus (GIV) belonging to the family *Sedoreoviridae* (*Orbivirus* genus). TRBV is transmitted by *Ixodes* species ticks, and its zoonotic potential supports seroconversion in humans with or without neurological disorders. The knowledge about members of the GIV serogroup is limited. *I. ricinus* ticks were collected from grazing goats were examined by RT-PCR and one pool of ticks was positive. Sequence analysis showed 93-94 % pairwise identity with the prototype TRBV from Slovakia and two Ukrainian TRBVs. Immunofluorescence with hyperimmune serum showed virus antigen in Vero E6 cells showing CPE. The isolate was used to study its replication kinetics in BHK-21 (rodent), HFF-1 (human), and BT (bovine) cell lines. The eclipse took 6 hpi in the HFF-1 and BT cells. In HFF-1 cells, virus load peaked 12 hpi (6.83×10^3 PFU/ml). In the BT cells, the virus reached the peak after 48 hours (5.00×10^4 PFU/ml). In the BHK-21 the eclipse took 8 hours and the replication gradually increase until the end of the study (72 hpi) and reached 5.75×10^5 PFU/ml. CPE developed in each cell line, but during 24 hpi, the human cells showed little changes in cell morphology and the integrity of the monolayer. Results of this work suggests, that TRBV have distinct replication kinetics, which may be due to the innate antiviral response in host cells of different origin. Study of the antiviral response after tick-borne orbivirus infection are in progress.

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West Nile virus in Hungary: current situation of the human infections and phylogenetic analysis of the virus strains

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Following the identification of the first neuroinvasive cases of West Nile virus (WNV) in 2004, WNV has shortly become one of the most important human arbovirus infections in Hungary. The study aimed to summarize the current epidemiological situation in Hungary and sequence the WNV-positive clinical specimens and virus isolates by next-generation whole genome sequencing (NGS) to obtain a detailed phylogenetic analysis of the WNV strains. Altogether 533 human infections were diagnosed between 2004 and 2023. Between 2015 and 2022, 15 WNV isolates, and 10 PCR-positive clinical specimens were analysed by NGS. In 2023, a further 6 WNV complete genomes were characterized, obtained directly from human urine samples. Phylogenetic analysis revealed that both of the major European WNV lineage 2 clades (the Eastern European and the Central European) are presented in Hungary. Strains of the Balkan and other smaller clusters within the Central European clade are co-circulating in Hungary, following a characteristic geographical distribution. The co-circulation of multiple lineage 2 WNV strains could be identified in the last few years, which can presumably be associated with bird migration routes and nesting sites. In light of the 2018 WNV outbreak, sequence-based typing of the currently circulating WNV strains could highly support outbreak investigations.

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Citizen science informed prediction of the distribution of three invasive mosquito species in Hungary

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Due to their potential role in pathogen transmission, invasive mosquitos pose considerable threats to human and animal health. Several studies have identified the most important ecological drivers mediating the spread of the key species, and predicted their range expansion in the future. We evaluated the effect of an exhaustive list of environmental predictors on distribution of three invasive species in Hungary (*Ae. albopictus*, *Ae. japonicus*, and *Ae. koreicus*) based on citizen science observations. Current distribution maps of these species were generated from a 5-year survey, then were compared with various predictor maps reflecting climate, habitat type, food supply, traffic, and interspecific competition by using a Boosted Regression Trees approach that resulted in a subset of variables with the strongest impact. The best predictor sets were used to predict the probability of occurrence of the focal species on regions, where no citizen science reports were available. These predictions were evaluated against the results of a recent field surveillance focusing on previously unsampled areas. Different predictor sets were selected for the three different species, and only predictions for *Ae. albopictus* could be validated with direct trapping data. Therefore, citizen science informed distribution maps can be used to identify ecological predictors that determine the spread of invasive mosquitos, and also to draw risk map based on the predicted distribution.

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Rickettsial infections in Hungary

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Rickettsial infections are one of the most important emerging diseases in Europe. In majority of the cases the symptoms are non-specific. For isolation, BSL3 laboratory is required and serology is still the gold standard technique, but genomic approaches, including real-time polymerase chain reaction (qPCR) and whole genome sequencing are the most powerful tools in the identification and always recommended. At the NNGYK, all diagnostic methods are available since 2016. Until now, 786 human specimens were admitted to our laboratory. Human sera were tested by indirect immunofluorescence assay (Focus), samples were considered to be positive if IgM/IgG titers were higher than 1:128 and/or fourfold increase of IgG antibodies. 31 samples were positive by serology, 26 out 31 samples were reactive to spotted fever group and 5 samples were reactive to typhus group. qPCR were performed in 520 cases and only 5 samples (whole blood and lesion samples) were positive, two samples were suitable for whole genomic sequencing. The genomic analysis showed that *R. africae* and *R. typhi* were the aetiological agents. Acute infections are often difficult to diagnose due to mild and non-specific symptoms, transient bacteraemia phase of illness, poor sensitivity of serology in the early stage, and the use of effective empirical antibiotics. Our data suggest that continued and improved *Rickettsia* spp. diagnostics is required to determine the travel-related or possible autochton infections in Hungary.

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Investigation of diversity and host spectrum of coronaviruses

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Coronaviruses infect a wide variety of avian and mammalian species. Spillover events may result zoonotic and reverse zoonotic infections among humans and mammals. Thus, early recognition of the infections is of particular impact for both the human and animal health care. However, overall detection is challenging due to the genomic diversity of coronaviruses. In our recent study we aim to develop a universal pan-coronavirus detection system specific virtually for the known coronaviruses. Accordingly, multiple novel oligonucleotides have been designed or redesigned to construct a broad-spectrum nested RT-PCR system. The method has been successfully tested on human and animal coronavirus isolates and samples diagnosed positive for coronaviruses previously (SARS-CoV-2, NL63, OC43, infectious bronchitis virus, feline coronavirus, transmissible gastroenteritis coronavirus, porcine epidemic diarrhea virus, bat coronavirus). Collection of field samples has started from camelids and swine to survey coronaviruses in Hungary that will be continued later with sampling of further domestic, companion and wild animals, as well as wastewater.

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Evidence of CPV-2 in dogs with acute parvovirus infection by PCR and cultivation on MDCK cells

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Canine parvovirus type 2 (CPV-2) with its respective variants is one of the main enteropathogens causing gastroenteritis with acute hemorrhagic diarrhea. This study focused on the demonstration of CPV-2 in hospitalized dogs with confirmed parvovirus disease by a commercial SNAP test. In dog patients, targeted supportive and symptomatic therapy was performed. The study group consisted of 16 dogs (1 dose of vaccine – 5 dogs, 2 doses – 2 dogs, 3 doses – 2 dogs, and absence of vaccination – 7 dogs). The samples used for the study were faeces and serum from infected animals. The virus DNA was isolated using the DNeasy® Blood & Tissue kit (Qiagen). The presence of CPV-2 in tested samples was confirmed by PCR amplification of a full-length VP2 gene (1755 bp) and by infection of the Madin-Darby Canine Kidney (MDCK) cell culture. Molecular CPV-2 detection revealed 93.75% positivity (15/16) of fecal samples. Cytopathic effect (CPE) in the form of rounding, granulation, and cell aggregation was detected in 3 serum samples on the 4th day and in 13 samples on the 5th day post-infection. This study reports confirmation of CPV-2 by commercial SNAP test, molecular analysis, and cultivation on cell culture including in fully vaccinated dogs. Therefore, additional assays such as a virus neutralization test to detect antibody titer as a part of vaccination effectiveness monitoring, are required. Moreover, sequencing and phylogenetic analysis to detect possible amino acid substitutions in the VP2 protein sequence which may result in vaccine efficacy failure are also crucial.

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