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Slovakia

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INFECTIOUS DISEASES

IN THE CONTEXT OF

ONE HEALTH

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SEPTEMBER

9–10 2026

KOŠICE, SLOVAKIA

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UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE, SLOVAKIA

AND

COLLABORATING ORGANISATIONS

INFECTIOUS DISEASES IN THE CONTEXT OF ONE HEALTH

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of scientific abstracts and contributions

September 9-10, 2026

Košice, Slovakia

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Anna Ondrejková

University of Veterinary Medicine and Pharmacy in Košice, Slovakia

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- One Health Perspectives on Viral, Bacterial, and Parasitic Diseases
- Pathogen Ecology and Epidemiological Trends
- Host-Pathogen Interactions and Virulence Mechanisms
- Antimicrobial Immunity and Therapeutics

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INFECTIOUS DISEASES IN THE CONTEXT OF ONE HEALTH

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P R O C E E D I N G S
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PROGRAMME

EPTEMBER 9, 2026	
11:00 – 17:00	Registration
12:00 – 12:15	Opening ceremony Nagy Jozef Mojžišová Vačšinec Jana
Session chairs	Kočišová Alica Loziakova Peňazziová Katarína
12:15 – 12:55	Plenary Lecture **A FOCUS ON THE NEUROBORRELIOSIS: FROM INFECTION TO THERAPEUTIC ADVANCES** Bhide Ramesh Mangesh
12:55 – 13:35	Plenary Lecture **HIDDEN THREATS: HANTAVIRUSES IN EUROPE AND BEYOND** Klempa Boris
13:35 – 13:55	Lecture **STIMULATION OF IMMUNE RESPONSE IN MICE INFECTED WITH TAPEWORM *ECHINOCOCCUS MULTILOCULARIS* AFTER COMBINED TREATMENT WITH ALBENDAZOLE AND LEUKOCYTE EXTRACT** Hrčková Gabriela
13:55 – 14:15	Lecture **MAPPING THE NEGLECTED: *IXODES ARBORICOLA* ON BIRD SPECIES IN THE KOŠICE AREA** Korytár Ľuboš
14:15 – 14:45	Coffee Break
Session chairs	Csank Tomáš Schusterová Petra
14:45 – 15:25	Plenary Lecture **SEQUENCING AS A ONE HEALTH TOOLKIT TO UNDERSTAND MOSQUITO VIRUS INTERACTIONS AND PREPARE FOR ARBOVIRUS EPIDEMICS IN EUROPE** Tóth Gábor Endre
15:25 – 16:05	Plenary Lecture **TICK-BORNE ENCEPHALITIS: EPIDEMIOLOGY AND DIAGNOSTICS IN A CHANGING WORLD** Ličková Martina
16:05 – 16:25	Lecture **SURVEILLANCE OF WEST NILE AND USUTU VIRUSES IN *CULEX* MOSQUITOES IN SLOVAKIA (2024–2025)** Loziaková Peňazziová Katarína
16:25 – 16:45	Lecture **ANALYSIS OF BACTERIOPHAGES AND THEIR INTERACTIONS WITH HOSTS BASED ON BACTERIAL CRISPR LOCI DATA** Nagy Máté
16:45 – 18:00	Poster Exhibition
19:00	Gala Dinner

SEPTEMBER 10, 2026	
09:00 – 11:30	Registration
Session chairs	Pavlová Alica Pivka Soňa
09:00 – 09:40	Plenary Lecture **BEYOND THE BITE: MOSQUITOES IN A CHANGING WORLD** Kurucz Kornélia
09:40 – 10:20	Plenary Lecture **VALIDATION OF MOLECULAR DETECTION METHODS: FROM PRINCIPLES TO PRACTICE** Drzewnioková Petra
10:20 – 10:35	Coffee Break
Session chairs	Korytár Ľuboš Kočíková Božena
10:35 – 10:55	Lecture **10 YEARS OF ANIMAL HEALTH LAW ACHIEVEMENTS AND VIEW FROM THE MEMBER STATE PERSPECTIVE** Chudý Martin
10:55 – 11:15	Lecture **FECAL MICROBIOTA TRANSPLANTATION (FMT) ALLEVIATES HISTOPATHOLOGICAL CHANGES IN EXPERIMENTAL COLITIS IN PSEUDO GERM-FREE MICE** Lauko Stanislav
11:15 – 11:35	Lecture **ORTHOFLAVIVIRUS INFECTIONS IN HORSES IN NORTHEASTERN HUNGARY - CLINICAL FINDINGS AND VECTOR SURVEILLANCE OF WEST NILE VIRUS AND TICK-BORNE ENCEPHALITIS VIRUS** Kiss Vivien
11:35	Closing Ceremony Ondrejková Anna

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PLENARY LECTURES

A FOCUS ON THE NEUROBORRELIOSIS: FROM INFECTION TO THERAPEUTIC ADVANCES

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ABSTRACT

Organisms that have crossed the species barrier from animals to humans include several pathogens like viruses (such as Hendra, Nipah, SARS, and influenza), as well as bacteria (*Borrelia*, *Streptococcus*, etc.) and others. It is still unclear why new pathogens like SARS-CoV-2, which appears to cause respiratory symptoms, manage to cross the blood-brain barrier (BBB) and enter the brain. Do these pathogens circulate among a variety of animal species? And what exact mechanisms do they use to get over the BBB, after all? Here, *Borrelia*, as a most fascinating model of a neuroinvasive pathogen is presented and its journey from the skin to the brain is illustrated. Since infectious diseases of the central nervous system (CNS) are very hard to treat, they generally continue to be a significant cause of morbidity and mortality. A major obstacle to curing brain diseases is the blood-brain barrier (BBB), which impedes therapeutic agents from reaching the brain and targeting the pathogens. The second part of the talk will provide an overview of our efforts in developing a proof-of-concept drug delivery system consisting of pathogen-specific nanobodies and peptides to target brain infections. The delivery system is equipped with BBB-homing moiety, which can enhance its translocation across BBB via receptor-mediated transcytosis. Finally, some exciting results will be presented to demonstrate how delivery systems are non-toxic to cells, how they effectively neutralize, and how they achieve better biodistribution in the CNS. We anticipate attracting an audience in the fascinating field of developing drug delivery systems.

ACKNOWLEDGEMENT

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TICK-BORNE ENCEPHALITIS: EPIDEMIOLOGY AND DIAGNOSTICS IN A CHANGING WORLD

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ABSTRACT

Tick-borne encephalitis (TBE) is one of the most important tick-borne viral neuroinfections in Europe. The epidemiology of TBE is undergoing significant changes driven by climate change, environmental factors, and increasing human activity in natural transmission foci. This presentation provides an overview of the biology of tick-borne encephalitis virus (TBEV), its natural transmission cycle, and the routes of human infection, with particular emphasis on alimentary transmission. Current approaches to TBEV surveillance will be discussed, including virus detection in ticks, monitoring of viral RNA in milk, virus isolation from dairy products, and serological surveillance in animals and exposed human populations. A One Health approach integrating epidemiological surveillance, environmental monitoring, and vaccination strategies is essential for improving TBE prevention and control in an evolving world.

ACKNOWLEDGEMENT

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BEYOND THE BITE: MOSQUITOES IN A CHANGING WORLD

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ABSTRACT

Mosquito-borne diseases emerge from complex interactions among vectors, pathogens, vertebrate hosts and their environment. Consequently, understanding mosquito vectors requires considerably more than documenting their presence or testing them for pathogens. This is particularly relevant for invasive mosquito species, which are rapidly expanding their geographic ranges, colonising human-dominated environments and creating novel interfaces among wildlife, domestic animals and humans. This presentation will provide a One Health perspective on the study of invasive mosquitoes, highlighting the importance of integrating vector ecology, behaviour, host interactions and pathogen surveillance. Particular attention will be given to the ecological processes that determine where mosquitoes occur, how they exploit urban and peri-urban habitats, which hosts they interact with, and how these characteristics may ultimately influence their role in pathogen transmission.

Using examples from our research on invasive *Aedes* mosquitoes and other disease vectors, I will illustrate how studies ranging from species distribution and habitat use to blood-feeding patterns, microbial and pathogen screening, and environmental drivers can contribute complementary pieces to understanding vector-borne disease systems. Such an integrated approach is especially important in a rapidly changing world, where biological invasions, urbanisation, climate change and altered human–animal interactions may reshape both vector communities and transmission risks.

Rather than viewing mosquitoes simply as carriers of pathogens, a One Health framework considers them as components of dynamic ecological networks connecting the environment, animals and humans. Understanding these connections is essential for anticipating emerging vector-borne disease risks and for developing more effective, evidence-based surveillance and vector management strategies.

LECTURES

STIMULATION OF IMMUNE RESPONSE IN MICE INFECTED WITH TAPEWORM *ECHINOCOCCUS MULTILOCULARIS* AFTER COMBINED TREATMENT WITH ALBENDAZOLE AND LEUKOCYTE EXTRACT

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ABSTRACT

Alveolar echinococcosis (AE) caused by the larval stage of *Echinococcus multilocularis* is a serious parasitic diseases associated with the host´s immunosuppression. In the present study we investigated the effects of albendazole (ABZ), human leukocyte extract (HLE) and their combination on murine alveolar echinococcosis, developing in the peritoneal cavities, by monitoring cyst weight, levels of circulating parasitic miRNAs, cytokine levels and the proportions of lymphoid cells in spleens. HLE potentiated antiparasitic effect of ABZ, markedly decreased circulating parasite-specific microRNAs, expression of Treg- associated transcription factors, IL-10 and TGF-β cytokines whereas elevated production of IFN-γ and proportions of CD4 T cells. The present study demonstrated that co-administration of HLE can enhance the antiparasitic effect of albendazole and alleviate immunosuppression on humoral and cellular immunity.

INTRODUCTION

Alveolar echinococcosis (AE) is a life-threatening parasitic disease caused by the cestode *Echinococcus multilocularis*. Current treatment relies almost entirely on the drug albendazole, which inhibits parasite growth. Effectiveness of therapy varies widely among patients. The parasites induce suppression of both innate and adaptive immunity, characterised predominantly by increased numbers of myeloid-derived suppressor cells (MDSCs), M2 macrophages, $Foxp3^+$ regulatory T lymphocytes, and elevated levels of the cytokines IL-10 and TGF-β (Vuitton & Gottstein 2010; Wang and Gottstein 2016). Host immunity is considered pivotal in determining the clinical outcome of AE, yet the interactions between immune responses and benzimidazole treatment remain insufficiently understood (Wang et al. 2020). Immunotherapy appears to be a highly promising treatment strategy, particularly when used as an adjunct to chemotherapy. Low-molecular-weight leukocyte extracts represent heterogeneous mixtures of substances below 10–12 kDa, from mononuclear white blood cells or tissue leukocytes from non-immune human or animal donors and contains 4 groups of proteins with different functions (Zuniga-Navarrete et al. 2021). MiRNAs are small (19–24 nucleotides) non-coding RNAs that regulate gene expression post-transcriptionally by inhibiting protein translation or destabilising target transcripts (O'Brien et al. 2018). The most abundant parasite-derived miRNAs are secreted either free or within extracellular vesicles (Ding et al. 2019). In present study we investigated

the effects of HLE, ABZ, and their combination administered during sub-chronic stage of infection on cyst mass, parasitic miRNAs and immune responses during murine alveolar echinococcosis.

METHODS

Infection with metacestode *E. multilocularis* was maintained by intraperitoneal passage through gerbils (*Meriones ungulatus*) at animal facilities of the Institute of Parasitology. Suspensions of protoscoleces, tissue were prepared from the peritoneal cysts isolated from gerbil. Mice were infected with dose of approximately 2000 protoscoleces intraperitoneally and were allocated into four groups: infected (INF), treated with albendazole (ABZ), treated with human leukocyte extract (HLE) and treated with the combination of albendazole and human leukocyte extract (ABZ+HLE). Treatments were administered daily for 14 consecutive days from week 4 post-infection. ABZ was resuspended in 1.0% cremophor oil in 0.9% NaCl for oral application and HLE was manufactured by Aumed, Ltd., pharmaceutical company (Prague, Czech Republic). miRNAs expression was detected by qPCR and cytokine concentrations by ELISA in serum of mice. Splenocyte suspensions were used to evaluate the proportions of CD3 T cell, CD19 B cells, Cd4 Thelper and CD8 Tcytotoxic T lymphocytes by flow cytometry, Tbet, GATA3 and Foxp3 transcription factors mRNA expression and proteins concentrations by immunoblots and in vitro proliferation.

RESULTS

ABZ treatment resulted in a significantly lower cyst mass after 14 days of administration compared with untreated controls ($p < 0.05$; Fig 1A). HLE moderately reduced cyst mass and the most pronounced decrease was observed after combination therapy ($p < 0.01$). Serum levels of IFN-γ (Th1), IL-4 (Th2), IL-17 (Th17) and IL-10, TGF- (Tregulatory) cytokines are on shown Fig 1B. ABZ treatment resulted in moderate increase of Th1 and Th2 cytokines and significant reduction of IL-10 and TGF-β. HLE administration alone and in combination with ABZ markedly elevated IFN-γ and IL-4 cytokines and decreased Treg´s immunity-linked cytokines. We next quantified four the most abundant *E. multilocularis* miRNAs: miR-71a-5p, let-7-5p, miR-10a-5p and miR-4989-3p (Guo and Zheng, 2017) in serum of mice to assess

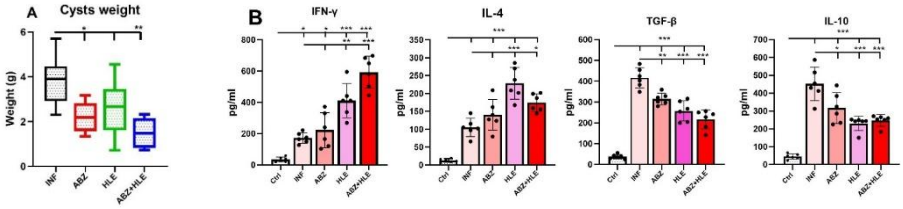


Figure1. A.The effects of therapies on parasitic cysts weight. B, serum levels of cytokines of Th1/Th2 and Treg´s type of immunity.

association between their levels and reduction of cyst mass. All four parasite-derived miRNAs were profoundly and significantly reduced in mice receiving HLE alone or in combination with ABZ (Fig.2), however ABZ therapy resulted in decrease of only miR-10a-5p.

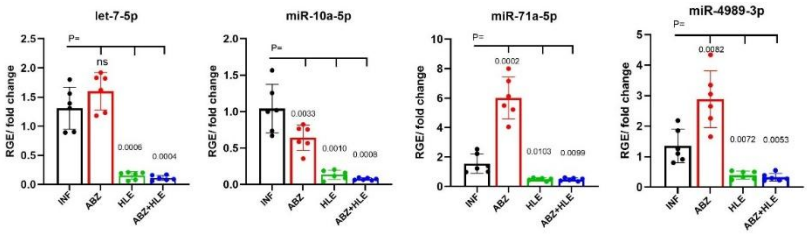


Figure 2. Serum levels of parasite derived miRNAs detected by qPCR

In ABZ+HLE treated group mRNA expression and protein levels of transcription factor T-bet were upregulated, whereas GATA3 and Foxp3 were significantly reduced (Fig.3A,B,C). Responses to ABZ therapy were consistent with cytokine levels. Flow cytometry analysis revealed that all therapies elevated the proportions of T and B cells (Fig.3D).

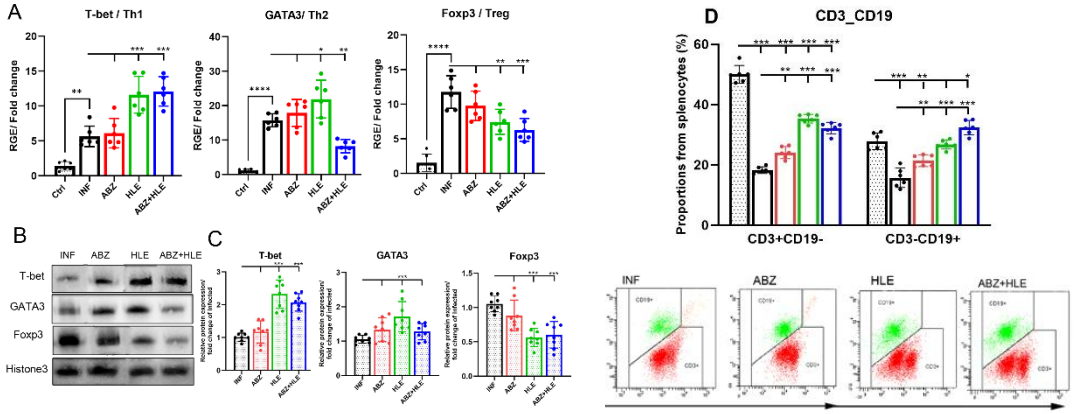


Figure 3. A, relative gene expression of mRNA of transcription factors and proteins T-bet, GATA3, and Foxp3 in splenocytes, B-C, protein expression; D, The proportions of CD3 (T cells), CD19 (B cells).

DISCUSSION AND CONCLUSIONS

Our findings demonstrate that human low molecular weight leukocyte extract (HLE) exerts profound immunomodulatory effects during sub-chronic murine *Echinococcus multilocularis* infection and, when administered with ABZ, enhanced its antiparasitic efficacy. Albendazole therapy increased serum levels of miR-71a-5p, let-7-5p and miR-4989-3p and suppressed miR-10a-5p compared with untreated mice. HLE given alone or in combination with ABZ, resulted in consistently low serum levels of all four parasite-derived miRNAs, indicating regulatory mechanisms distinct from those triggered by albendazole. Host immune responses to *E. multilocularis* involve a T cell-dependent process that includes sequential and overlapping Th1, Th2, Th17 and Treg involvement. Changes in parasite-derived miRNAs were accompanied by modulation of cytokine levels and cell populations in spleens. HLE alone and as adjuvant to ABZ increased serum levels of IFN-γ and IL-4, while

significantly reducing cytokines IL-10 and TGF-β. Alterations in cytokines secretion were supported by changes in gene and protein expression of corresponding T lymphocyte transcription factors T-bet, GATA3 and Foxp3. The combined therapy resulted the most pronounced normalization of immune response. Collectively, these results propose HLE as a promising adjunct immunotherapeutic candidate capable of counteracting parasite-induced immunosuppression and potentiating benzimidazole chemotherapy.

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MAPPING THE NEGLECTED: *IXODES ARBORICOLA* ON BIRD SPECIES IN THE KOŠICE AREA

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ABSTRACT

This paper focuses on studying the host preferences of the ornithophilic (bird-associated) tick *Ixodes arboricola* and detecting transmitted pathogens in collected samples. The survey was conducted between 2024 and 2025 at selected sites in the Košice region. A total of 145 tick samples collected from 10 species of wild passerines were examined. *I. arboricola* accounted for 11% of the total ticks collected, confirming its strict preference for cavity-nesting birds, specifically the great tit (*Parus major*) and the blue tit (*Cyanistes caeruleus*). All tick samples underwent molecular diagnostic testing for the presence of *Borrelia* spp. and tick-borne encephalitis virus (TBEV). The overall positivity rate for *Borrelia* spp. reached 55.16%. No tick-borne encephalitis virus was detected in the analyzed samples. These results highlight the significant role of wild birds as reservoirs and vectors of Lyme borreliosis.

INTRODUCTION

Ticks are widely distributed across almost all geographical zones, and their distribution is primarily limited by environmental humidity and temperature. Due to climate change and global warming, these arthropods are expanding their range into higher altitudes where they were previously absent. The pathogenic potential of ticks is both direct, through host blood-feeding, and indirect, by acting as vectors for various pathogens causing tick-borne diseases. While public awareness often reduces the role of ticks to human diseases such as tick-borne encephalitis or Lyme borreliosis, their impact on animal health can lead to severe veterinary, environmental, and economic consequences (Estrada-Peña et al. 2017).

In Central Europe, wild birds serve as important hosts for several specialized ornithophilic tick species, including *Ixodes arboricola*, *Ixodes frontalis*, and *Ixodes lividus* (Estrada-Peña et al. 2017). Unlike exophilic species that actively seek hosts in the open environment, nidicolous ticks like *I. arboricola* prefer the stable microclimate inside nests, tree cavities, or artificial nest boxes (Otranto and Wall 2024). Migratory birds play a crucial role in the long-distance transport of these ektoparazites across geographical barriers, contributing to the circulation of viral, bacterial, and protozoan zoonoses (Buczek et al. 2020). Despite this ecological link, *I. arboricola* remains a relatively understudied species. The main objective of this study was to monitor the occurrence of *I.*

arboricola on wild birds in eastern Slovakia and to evaluate its vector capacity for *Borrelia* spp. and TBEV.

METHODS

The field monitoring was carried out from March 25, 2024, to October 23, 2025, in the Košice region, specifically in the municipalities of Drienovec, Rozhanovce, and the Horný Bankov recreational area in Košice. Wild passerines were captured using mist nets with a 16 mm mesh size, which is suitable for small birds. To attract birds to the capture sites, feeders filled with sunflower, wheat, millet seeds, and apple pieces were utilized.

Attached ticks were removed using forceps or a parasitological spoon and placed into plastic tubes containing a blade of grass to prevent desiccation during transport. In the laboratory, ticks were identified down to the species and developmental stage under a binocular magnifier and a stereozoom microscope based on standard morphological keys (Estrada-Peña et al. 2017). Morphometric analysis was performed using the Promicra QuickPHOTO software. Verified samples were stored at -80 °C to preserve nucleic acids.

Total DNA and RNA were isolated after sample homogenization using the SpeedMill device. Extraction was performed with the commercial DNeasy Blood and Tissue kit for DNA and the Indispin kit for RNA. Nucleic acid concentration was verified using the NanoDrop One spectrophotometer. For *Borrelia* spp. detection, a polymerase chain reaction (PCR) targeting the IgS region was performed using the Takara Master Mix. The PCR products were visualized by agarose gel electrophoresis (ELFO) under UV light. Positive samples were subsequently sent to a commercial laboratory for sequencing to determine the exact pathogen species.

RESULTS

During the monitoring period, a total of 145 tick samples were collected from 10 different species of small passerines. The most frequently captured hosts were the great tit (*Parus major*, n=41), the European robin (*Erithacus rubecula*, n=39), and the common blackbird (*Turdus merula*, n=18).

Morphological identification revealed the presence of four tick species. The dominant species was *Ixodes ricinus*, accounting for 86% (125 samples; 50 larvae and 75 nymphs). The target ornithophilic species, *Ixodes arboricola*, represented 11% (16 samples). The remaining proportion consisted of *Haemaphysalis concinna* (2%, 3 samples) and *Ixodes frontalis* (1%, 1 sample). Notably, *I. arboricola* was recorded exclusively on two host species: the great tit (11 samples) and the blue tit (5 samples). All collected *I. arboricola* individuals were either larvae (6 specimens) or nymphs (10 specimens).

Tick Species	**Total Samples**	**Larvae (Total)**	**Positive Larvae for *Borrelia***	**Nymphs (Total)**	**Positive Nymphs for *Borrelia***
Ixodes ricinus	125	50	30 (24.0%)	75	39 (31.2%)
Ixodes arboricola	16	6	3 (18.75%)	10	6 (37.5%)
Haemaphysalis concinna	3	1	0 (0.0%)	2	2 (66.7%)
Ixodes frontalis	1	0	0 (0.0%)	1	0 (0.0%)
Total	**145**	**57**	**33 (22.75%)**	**88**	**47 (32.41%)**

Table 1. Distribution of tick samples and their positivity for Borrelia spp.

Molecular screening showed a high prevalence of *Borrelia* spp. spirochetes, which were detected in 80 out of 145 samples, yielding an overall positivity rate of 55.16%. In *I. arboricola,* 18.75% of larvae and 37.5% of nymphs tested positive. All 145 samples tested negative for the presence of tick-borne encephalitis virus (TBEV).

DISCUSSION

Our epizootiological survey confirmed the strict ecological specialization of *Ixodes arboricola*, which was found exclusively on great tits and blue tits. This finding aligns with its known status as a nidicolous species adapted to tree holes and artificial nest boxes (Estrada-Peña et al. 2017). Nest boxes offer a highly favorable microclimate with optimal humidity, protecting the ticks from desiccation and supporting their multi-host life cycle (Otranto and Wall 2024). In contrast, *Ixodes ricinus* dominated on open-nesting birds or ground-foraging species like robins and blackbirds. This pattern is consistent with the co-infestation dynamics described in Central Europe, where a seasonal overlap between exophilic and nidicolous ticks creates pathways for cross-species pathogen transmission (Kocianová et al. 2017).

The overall *Borrelia* spp. positivity rate of 55.16% is remarkably high. Because transovarial transmission does not typically occur in *Borrelia* species, larval stages must acquire the bacteria directly from an infectious host during blood-feeding (Stevenson et al. 2022). The fact that 18.75% of *I. arboricola* larvae and 24.0% of *I. ricinus* larvae were positive suggests that passerines serve as highly competent reservoir hosts. Wild birds play a significant role in maintaining the natural foci of Lyme borreliosis, as their immune systems display varying degrees of serum resistance to different *Borrelia* genotypes (Sürth et al. 2021). Although *I. arboricola* does not bite humans (Estrada-Peña et al. 2017), its role in maintaining pathogen circulation within bird populations indirectly amplifies the infection risk for bridging vectors like *I. ricinus*. Meanwhile, the complete absence of TBEV support the idea that small rodents, rather than birds, remain the primary reservoirs for this virus in Central European foci (Wimbauer et al. 2022).

CONCLUSION

This study provides valuable insights into the circulation of tick-borne pathogens among wild bird populations in eastern Slovakia. We confirmed that *Ixodes arboricola* is a well-established ektoparazite of cavity-nesting tits in the Košice region. The high prevalence of *Borrelia* spp. in both larval and nymphal stages demonstrates that bird nesting habitats function as active reservoirs for Lyme borreliosis. Even though *I. arboricola* lacks a direct link to human infestation, its co-occurrence with *I. ricinus* on the same hosts facilitates the maintenance and spread of zoonotic pathogens, maintaining infection loops that ultimately affect public health.

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SURVEILLANCE OF WEST NILE AND USUTU VIRUSES IN *CULEX* MOSQUITOES IN SLOVAKIA (2024–2025)

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ABSTRACT

West Nile virus (WNV) and Usutu virus (USUV) are zoonotic flaviviruses maintained in bird–mosquito cycles. This study compared their circulation among Culex mosquitoes collected in Slovakia during 2024 and 2025 as part of the OH SURVector surveillance project. Fourteen localities were sampled from June to mid-October. In total, 41,533 *Culex* mosquitoes were tested in 2024 and 8,814 in 2025. WNV RNA was detected only in 2024 (8 positive pools; MIR 0.19/1,000), whereas USUV RNA was detected in both seasons (61 and 13 positive pools; MIR 1.47 and 1.48/1,000). The findings indicate wider and potentially persistent USUV circulation.

INTRODUCTION

West Nile virus (WNV) and Usutu virus (USUV) are mosquito-borne flaviviruses of veterinary and public-health importance in Europe. Their enzootic cycle is maintained primarily between birds, which serve as amplifying hosts, and *Culex* mosquitoes, which act as vectors, while humans and horses typically serve as incidental hosts. Most infections remain asymptomatic, but both viruses may cause febrile disease and, less frequently, neuroinvasive disease (ECDC & EFSA, 2023; Cadar & Simonin, 2023). In Slovakia, WNV has been detected in mosquitoes, birds, horses, wildlife, and humans, whereas knowledge of USUV remains more limited (Čabanová et al., 2019; Korytár et al., 2020). Because vector-based surveillance can detect viral circulation before clinical cases emerge, systematic mosquito monitoring is a useful component of One Health risk assessment. The aim of this study was to compare the occurrence and distribution of WNV and USUV RNA in *Culex* mosquito populations collected in Slovakia during the 2024 and 2025 seasons.

METHODS

Mosquito monitoring was conducted as part of the OH SURVector project. For a direct year-to-year comparison, 14 Slovak localities sampled in both years were included. Trapping was performed continuously from June to mid-October using BG-Sentinel traps with CO_2 as an attractant. The selected sites represented different ecological settings, including wetlands, urban and peri-urban areas, farms and localities with previous evidence of WNV or USUV circulation. Trap nets were stored locally at -20 °C and transported to UVMP, where mosquitoes were morphologically identified on ice, following Becker et al. (2010); after identification, the mosquitoes were stored at -80 °C until further processing. *Culex* mosquitoes were pooled by locality, date, sex and species, with 10-50 individuals per pool. Viral RNA was extracted and screened by WNV- and USUV-specific real-time PCR (Kolodziejek et al., 2014; Weissenböck et al., 2013). Positive samples were further processed by generic flavivirus PCR and sequencing when sufficient material was available (Scaramozzino et al., 2001). The minimum infection rate (MIR) was calculated as the number of positive pools per 1,000 tested mosquitoes.

RESULTS

A marked interannual difference in mosquito abundance was observed. The 2024 season yielded 41,533 tested *Culex* mosquitoes from the 14 shared localities, grouped into 976 pools, whereas 8,814 *Culex* mosquitoes, grouped into 311 pools, were tested in 2025. The material consisted predominantly of *Cx. pipiens complex* mosquitoes, with 39,096 individuals in 2024 and 8,748 in 2025; *Cx. modestus* accounted for 2262 and 66 individuals, respectively. Temporal dynamics showed two main abundance peaks: the highest mosquito catches were recorded in mid-July (weeks 28-29), followed by another increase at the end of August (weeks 33-35) in both seasons.

WNV RNA was detected only in 2024. Eight WNV-positive pools were found across four localities, and all were *Cx. pipiens* complex females. The overall MIR for WNV was 0.19/1,000 tested mosquitoes in 2024, while no WNV-positive pools were detected in 2025. Temporally, WNV-positive pools appeared from the end of July to the beginning of September (approximately weeks 31-36). In contrast, USUV RNA was detected in both seasons and showed a broader geographical distribution. Overall, 74 USUV-positive pools were detected across 13 localities: 61 in 2024 and 13 in 2025. In 2024, USUV was detected throughout most of the trapping season, with the highest number of positive pools at the end of August and beginning of September (approximately weeks 35-36); in 2025, only USUV was detected, and its occurrence fluctuated during the season. Most USUV-positive pools consisted of *Cx. pipiens complex* mosquitoes, with one *Cx. modestus*-positive pool recorded in 2024. Despite substantially lower mosquito abundance in 2025, the USUV MIR remained

unchanged between seasons (1.47/1,000 in 2024 and 1.48/1,000 in 2025). Repeated USUV detection at several localities in both years suggests stable or recurrent circulation in different parts of Slovakia. Sequencing and phylogenetic analysis of the partial NS5 gene provided additional information on the genetic background of the detected viruses. WNV-positive mosquito samples were assigned to WNV lineage 2 and clustered with European strains, supporting the continued circulation of this lineage in Slovakia. USUV-positive mosquito samples were grouped within the Europe 2 lineage, consistent with previous findings from Slovakia.

DISCUSSION

The results indicate different epidemiological patterns for the two flaviviruses. WNV detections were restricted to the warmer southern localities and occurred only during the high-abundance 2024 season. This pattern is consistent with the strong influence of temperature on *Culex* development, vector activity and WNV transmission efficiency (Fros et al., 2015; Vogels et al., 2016). The exceptionally warm 2024 season, together with above-average precipitation in June and early July, probably supported mosquito population growth and created favourable conditions for virus amplification. The absence of WNV RNA in 2025 may reflect lower vector abundance, less favourable climatic conditions, stochastic local transmission dynamics, or a combination of these factors.

USUV was detected more widely than WNV and remained detectable in 2025 despite a much smaller number of mosquitoes. The identical MIR values across seasons suggest that USUV circulation may be less dependent on overall mosquito abundance than raw positive-pool counts imply. Repeated positivity at several localities raises the question of local persistence, possibly through overwintering infected mosquitoes, recurrent seasonal amplification in resident birds, or repeated introduction by migratory birds.

Preliminary genetic analyses are consistent with the presence of WNV lineage 2 and USUV Europe 2 in Slovakia, while neighbouring countries report a mosaic of USUV lineages. These findings support the need to integrate vector screening with bird, sentinel animal, equine, and human surveillance to clarify endemic distribution and public health relevance.

CONCLUSION

The 2024-2025 surveillance confirmed circulation of WNV and USUV in *Culex* mosquitoes in Slovakia, with WNV detected only in 2024 and USUV detected in both seasons. USUV showed broader geographical distribution and stable MIR values despite lower mosquito abundance in 2025. Continuous One Health surveillance, combining entomological, molecular, veterinary, and human health data, is needed to identify areas of persistent circulation, evaluate seasonal risk, and support early warning for emerging mosquito-borne infections.

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ANALYSIS OF BACTERIOPHAGES AND THEIR INTERACTIONS WITH HOSTS BASED ON BACTERIAL CRISPR LOCI DATA

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ABSTRACT

The CRISPR-Cas adaptive immune systems embed short spacer sequences derived from past phage infections directly into the bacterial genome, providing a molecular record of past phage exposures. We present an automated pipeline that detects CRISPR arrays in bacterial genomes, queries extracted spacer sequences against a curated phage sequence database, and maps sequence matches to annotated phage open reading frames retrieving the original function of these sequences. By enabling the reconstruction of host-phage interaction histories, this pipeline may support host prediction for uncharacterized phages, may aid investigations into phage-host coevolution, and can help elucidate the roles of uncharacterized phage genes that CRISPR spacers target.

INTRODUCTION

Although CRISPR-Cas systems have become widely known as powerful genome-editing tools, their natural role is to provide adaptive immunity in bacteria and archaea. During infection, fragments of foreign DNA are incorporated into CRISPR arrays as spacer sequences, creating a heritable record of previous encounters with pathogens (Barrangou et al. 2007; Jinek et al. 2012). Upon subsequent infections, these spacers guide the recognition and destruction of invading genetic material. As a result, CRISPR loci preserve information not only about past infections but also about the specific genomic regions targeted by host immunity, making them valuable resources for reconstructing the evolutionary history of phage–host interactions (Stern et al. 2010).

Bacteriophages are the most abundant biological entities on Earth (Comeau et al. 2008). They are major drivers of bacterial mortality, horizontal gene transfer, and they play a key role in shaping the composition of microbial communities across virtually every environment (Brüssow and Hendrix 2002). Beyond their ecological importance, phages have attracted increasing attention as potential therapeutic agents in response to the global rise of antibiotic resistance.

As a result of the enormous diversity and abundance of bacteriophages, our understanding of their biology remains incomplete. Recent advances in metagenomics and high-throughput sequencing have uncovered vast numbers of previously unknown viral genomes, many of which cannot be confidently classified or linked to a host organism. This knowledge gap has led to the concept of "viral dark matter," referring to the large fraction of viral sequence diversity that remains poorly characterized (Dion et al. 2021). One of the major challenges in phage biology is therefore the identification of phage hosts and the characterization of the molecular interactions that determine host range.

CRISPR spacer sequences provide a unique opportunity to address these challenges. Because spacers originate directly from past infections, they can be used to infer phage–host relationships and have recently become the basis of bioinformatic approaches for host prediction that do not rely solely on sequence similarity or taxonomic classification (Dion et al. 2021).

We therefore aimed to detect CRISPR loci in bacterial genomes and subsequently identify bacteriophage genome fragments in said CRISPR arrays. Using Escherichia coli as a model host, we demonstrate a reproducible framework for investigating phage–host interactions and exploring functional patterns in phage sequences targeted by CRISPR systems.

METHODS

The pipeline, developed to carry out the end-to-end analysis, is implemented in Nextflow, a workflow management framework that enables scalable, reproducible bioinformatic analyses (Di Tommaso et al. 2017).

Complete and chromosome-level bacterial genome assemblies are downloaded from NCBI RefSeq for a user-specified host species using the NCBI datasets command-line tool (O'Leary et al. 2024). Phage genome sequences, associated annotation files (GFF3), and metadata are retrieved via the PhageScope platform (Wang et al. 2024). PhageScope aggregates specialized phage databases from diverse environments, but because these independently curated resources vary in annotation standards and metadata quality and often contain redundant records, sequences are deduplicated by sequence level identity checks, retaining a single representative per unique sequence. The consolidated phage metadata is searched by bacterial genus to identify phages annotated as infecting the target host, and to rule out potential sequencing or assembly artefacts the host-specific phage set is clustered using MMseqs2 (Steinegger and Söding 2017) at 98% nucleotide sequence identity.

CRISPR arrays are detected using PILER-CR (Edgar 2007), and spacer sequences from each genome are searched against the non-redundant clustered phage genome database using MMseqs2 (Steinegger and Söding 2017). Hits with a minimum sequence identity of 80% are intersected with the consolidated phage GFF3 annotation file, identifying which annotated phage open reading frames

overlap with each spacer match. Nucleotide sequences of the targeted phage proteins are retrieved from the clustered phage genomes at the matching coordinates and gene IDs are transferred from the phage genome annotation.

RESULTS

The pipeline was applied to *E. coli* as a model host organism. All phages identified by the pipeline as containing sequences matching *E. coli* spacer regions were found to belong to the class *Caudoviricetes*. Intersection of spacer-match coordinates with phage GFF3 annotations revealed that most spacer-targeted regions correspond to genes annotated as hypothetical proteins (9842/24833) or proteins of unknown function (5684/24833). Among the annotated targets, genes with lytic activity, specifically N-acetylmuramoyl-L-alanine amidase activity gene (2238/24833), and structural genes, particularly phage portal protein, SPP1 Gp6-like (700/24833) and major capsid protein E (634/24833), were among the most frequently identified gene products.

DISCUSSION

The pipeline presented here provides an automated computational framework for the systematic analysis of CRISPR-mediated phage immunity at genomic scale. By integrating genome download, multi-database phage aggregation, redundancy removal, CRISPR detection, sequence alignment, genomic feature intersection, and annotation transfer into a single workflow, it removes the substantial manual effort previously required to conduct such analyses.

The observed predominance of *Caudoviricetes* among spacer-matching phages likely reflects a genuine biological signal but also introduces a potential database sampling bias that should be considered when interpreting results.

The observed pattern, in which most spacers match phage proteins of unknown function (62.5%) while muralytic genes and structural genes account for most of the functionally annotated targets, is concordant with prior works showing that CRISPR spacers target phage genomes non-randomly and frequently match structural genes such as those encoding capsid and tail proteins (Nasko et al. 2019). As sequencing efforts generate ever-growing amounts of genomic and metagenomic phage data, the diversity of spacer matches is expected to increase correspondingly, which the workflow is readily able to incorporate. The high proportion of spacer-targeted genes annotated as hypothetical proteins warrants further investigation, as it may reflect incomplete phage genome annotation in current databases.

CONCLUSION

We present an automated pipeline that integrates CRISPR array detection and spacer-to-phage sequence matching into a single reproducible workflow. The pipeline is directly applicable to any bacterial species as long as reference genomes for the species and phage sequence data are available. This framework provides a scalable resource for advancing our understanding of CRISPR-mediated adaptive immunity, phage-host coevolution, and the functional genomics of bacteriophages.

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FECAL MICROBIOTA TRANSPLANTATION (FMT) ALLEVIATES HISTOPATHOLOGICAL CHANGES IN EXPERIMENTAL COLITIS IN PSEUDO GERM-FREE MICE

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ABSTRACT

The aim of this study was to characterize the clinical and histopathological responses across different models of acute ulcerative colitis (UC) induction and to evaluate the therapeutic effect of fecal microbiota transplantation (FMT) in a pseudo germ-free (PGF) mouse model. The therapeutic effect of FMT from a healthy donor was evaluated using the Disease Activity Index (DAI) and Histology Activity Index (HAI), together with a qualitative assessment of histopathological changes in the colonic mucosa following UC induction across the different acute UC models.

INTRODUCTION

The histopathological features of acute UC are a cornerstone of diagnosis, reflecting the dynamics of the inflammatory process and enabling differentiation from other inflammatory bowel diseases. Experimental models of acute UC are essential tools for elucidating disease pathogenesis, characterizing disease-associated histopathological changes, and evaluating novel therapeutic approaches. However, the clinical and histopathological manifestations of acute UC may vary substantially depending on the experimental model used, potentially influencing the interpretation of disease severity and therapeutic efficacy. Therefore, comprehensive characterization of these models is essential for the reliable assessment of novel therapeutic interventions.

METHODS

Two scoring systems, the HAI and the DAI, were used for the quantitative assessment of experimental ulcerative colitis activity. The scoring criteria and point values for individual parameters were adopted from Lauko et al. (2023), enabling objective evaluation of histopathological and clinical

changes during disease progression. HAI is a quantitative scoring system for assessing histopathological UC activity based on the evaluation of changes in colonic tissue. The assessed parameters included the presence or absence of inflammatory cell infiltration, distortion or loss of intestinal crypts, reduction of the mucin-positive area of goblet cells, and epithelial erosions. DAI was used for the quantitative assessment of clinical disease activity. Three main parameters were evaluated: stool consistency, rectal bleeding, and body weight loss. The combined assessment of these parameters enabled objective determination of disease severity and comparison of clinical changes between experimental groups. The experimental design comprised three groups: DSS (n = 24), with acute UC induced by 5% DSS; UC (n = 36), with acute UC induced by FMT from a patient with UC; and DSS-UC (n = 36), with acute UC induced by combined administration of DSS and FMT from a patient with UC. Following the induction period, mice in the UC and DSS-UC groups received FMT from a healthy donor at a dose of 0.2 mL/mouse/day for 5 days. Based on individual differences in DSS intake and the development of the clinical features of acute UC, characterized primarily by rectal bleeding and body weight loss, animals in the DSS and DSS-UC groups were further stratified according to disease severity. Both groups were divided into two clinical subgroups representing moderate disease (DSS/S and DSS-UC/S) and severe disease (DSS/T and DSS-UC/T). In the UC group, animals were classified into mild (UC/M) and moderate (UC/S) disease subgroups. All procedures were performed at an accredited Laboratory of Gnotobiology (SK U 10021). Specific pathogen-free (SPF) mice were obtained from a certified breeding facility in the Czech Republic (Velaz s.r.o., Prague). Following antibiotic decontamination, the mice were maintained as PGF animals and used for the experimental procedures. The experimental protocol (No. 4073/18-221/3) was approved by the relevant ethics committee. Human fecal samples were obtained under an approved clinical research project (No. 14/2018/OVaV).

RESULTS AND DISCUSSION

Experimental models of acute UC are used to understand disease pathogenesis and to evaluate alternative therapeutic approaches, including FMT. The therapeutic potential of FMT varied across the different experimental models of colitis (Chieppa et al. 2025; Mishra et al. 2025). The results demonstrated a differential response depending on the nature of the induced intestinal injury. After 5 days of FMT administration (Fig. 1), evaluation of the individual DAI parameters showed that in the DSS groups (DSS and DSS-UC), bleeding in severe forms of the disease remained at a level comparable to that observed after induction of ulcerative colitis (DSS/T: 2.7–2.8 vs. 2.8; DSS-UC/T: 2.7 vs. 2.5). In the UC group, FMT administration did not result in the expected improvement in clinical status; rather, an unfavorable trend toward disease progression was observed. Despite the absence of hemorrhagic enteritis and persistently low body weight loss, rectal bleeding scores

increased across all disease severity categories, and a severe disease phenotype (UC/T) emerged that had not been present during the induction phase. This was reflected by an increase in the overall DAI scores (UC/M: 3.3; UC/S: 3.6; UC/T: 3.7), indicating persistence and potential worsening of clinical disease activity (Fig. 1).

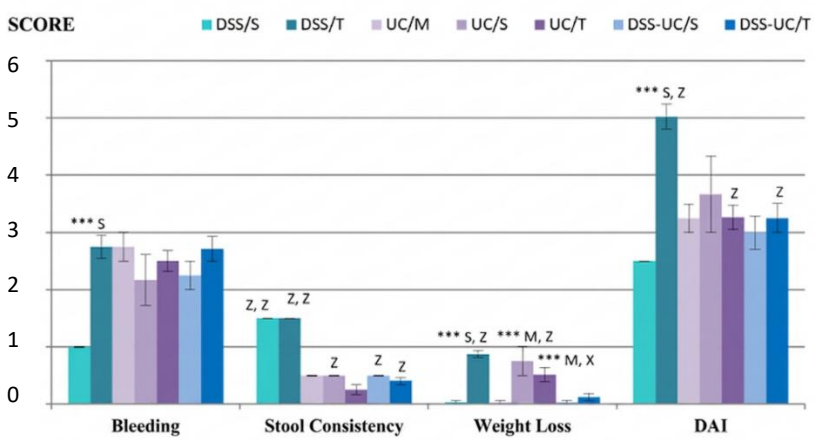


*Figure 1. Comparison of DAI between models and severity grades of acute UC after FMT therapy. DSS group – induction of acute UC by DSS exposure; DSS/S – moderate UC (n=3), DSS/T – severe UC (n=10); UC – induction of UC using microbiota from a patient with UC; UC/M – mild UC (n=2), UC/S – moderate UC (n=3), UC/T – severe UC (n=11); DSS-UC – combined induction of UC (chemically and using microbiota from a patient with UC); DSS-UC/S – moderate UC (n=3), DSS-UC/T – severe UC (n=13). Results are presented as mean ± SD. **p < 0.001 (statistically significant difference between UC severity forms); X p < 0.05; Z p < 0.001 (statistically significant difference between groups).*

In the DSS-UC group, a marked reduction in the epithelial erosion score was observed following FMT administration. In the moderate form (DSS-UC/S), the values decreased from 1.152 after induction to 0.48 after treatment. A similar trend was observed in the severe form (DSS-UC/T), with a decrease from 1.481 to 0.631; these values were significantly lower compared with the DSS group ($p < 0.01$). In the context of the overall histological assessment, it can be concluded that FMT administration resulted in a reduction in the severity of tissue damage, with the most pronounced effect observed in severe forms of colitis. This trend was supported by a significant decrease in the total HAI score, which was most pronounced in the DSS-UC/T ($p < 0.01$) and UC/T ($p < 0.001$) groups compared with the untreated DSS group (Fig. 2). In the DSS-UC group, the decrease in the total score was largely determined by a reduction in the inflammatory component, as evidenced by a marked attenuation of the inflammatory infiltrate following FMT. In contrast, parameters reflecting deformative damage to the intestinal crypts were only minimally affected. This discrepancy was reflected in the persistence of relatively higher total scores (1.6–1.75; Fig. 2), despite their significant reduction, and indicates a different response dynamics of inflammatory and structural changes to therapy. In contrast to the DSS-UC group, the UC group exhibited a different response profile to FMT treatment, without a marked reduction in the inflammatory component. The decrease in the total HAI score was primarily associated with a reduction in intestinal crypt damage, which was observed in approximately 40% of the evaluated animals.

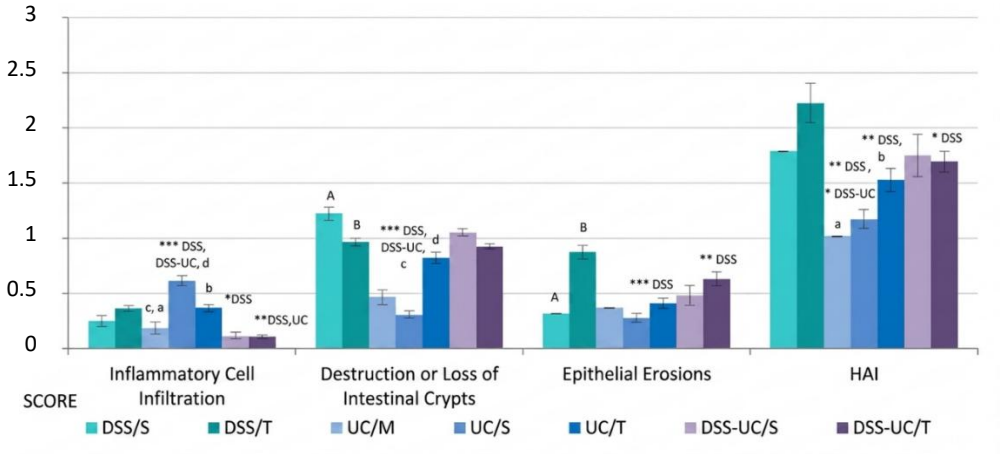


*Figure 2. Comparison of HAI between models and severity grades of acute UC after FMT therapy. DSS/S – moderate UC (n=1), DSS/T – severe UC (n=7); UC/M – mild UC (n=1), UC/S – moderate UC (n=3), UC/T – severe UC (n=6); DSS-UC/S – moderate UC (n=2), DSS-UC/T – severe UC (n=7). Results are presented as mean ± SD. *p < 0.05; **p < 0.01; ***p < 0.001 (statistically significant difference between UC severity forms); a,b p < 0.05; A,B p < 0.01; c,d p < 0.001 (statistically significant difference between severity forms within the group); ***p < 0.001 (statistically significant difference between UC severity forms across groups).*

CONCLUSION

These findings indicate a discrepancy between improvement in the inflammatory component and the persistence of structural mucosal damage, which was observed in both colitis models. While the effect of FMT in the UC group was limited and primarily reflected structural changes, a more pronounced therapeutic effect was observed in the combined DSS-UC model, as evidenced by a significant reduction in both total DAI and HAI, particularly in severe disease. Despite this improvement, damage to the intestinal mucosal architecture persisted in both models, suggesting that restoration of epithelial barrier integrity may lag behind modulation of the inflammatory response. These findings highlight the limitations of FMT as a standalone therapeutic approach in severe forms of colitis, which, although capable of modulating the inflammatory component of the disease, appears to have only a partial and delayed effect on mucosal repair.

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ORTHOFLAVIVIRUS INFECTIONS IN HORSES IN NORTHEASTERN HUNGARY - CLINICAL FINDINGS AND VECTOR SURVEILLANCE OF WEST NILE VIRUS AND TICK-BORNE ENCEPHALITIS VIRUS

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ABSTRACT

Orthoflaviviruses, particularly West Nile virus (WNV) and Tick-borne encephalitis virus (TBEV), pose a growing concern for animal and public health in Europe. In 2025, blood samples were collected from 175 autochthonous asymptomatic horses. Serum samples were screened using a virus neutralization test to differentials for WNV, TBEV and Usutu virus (USUV). Concurrently, mosquito surveillance was conducted around horse stables at three sites using CO_2 -baited BG-sentinel 2 traps, followed by morphological species identification and RT-PCR screening for orthoflavivirus RNA. Specific neutralizing antibodies were detected in equine sera with seroprevalence of 4.59% for WNV (titers 1:14-1:101) and 3.40% for TBEV (titers 1:28-1:806), with zero cross-reactivity. No neutralizing antibodies against USUV were detected. Mosquito screening did not contained WNV or USUV RNA (only mosquitoe-specific orthoflaviviruses were found – data not shown); however, vector diversity was notable, with Szirmabesenyő exhibiting the highest species diversity (7 different species) and Kulcsár valley demonstrating a high abundance of *Culex* spp. vectors. The presence of neutralizing antibodies confirms the circulation of WNV and TBEV in healthy horses, reinforcing their role as effective sentinel animals for arbovirus monitoring. The co-occurrence of competent vectors underscores the necessity of continuous integrated surveillance, vector control, and horse vaccination programs within a One Health framework.

INTRODUCTION

Equine infections caused by orthoflaviviruses, particularly West Nile virus (WNV) and tick-borne encephalitis virus (TBEV), represent an increasing concern for veterinary and public health in Europe. In Hungary, WNV has been an endemic pathogen since 2007, with recurrent outbreaks affecting both humans and horses, while TBEV persists in natural foci in the northeastern regions. Our study aimed

to provide a serological cross-sectional study on the prevalence of WNV and TBEV infections in horses living in Northeastern Hungary, in combination with mosquito and tick targeted surveillance.

METHODS

Animal origin and blood collection

In 2025 autochthonous asymptomatic horses were screened by virus neutralization test (VNT) for WNV and, TBEV antibodies. Orthoflaviviruses exhibit a high degree of serological cross-reactivity. Horses were chosen by the differential geographical areas where the previous mapping suspected high abundance of the mosquito population. During the collection year the horses have no symptoms for diseases, the health status was observed during the collection dates. The chosen places for blood collection were in Csemer, Gergés, Szilvásvárad, and Tard. The lead breed of horses was Hucul and Lipizzaner, beside Hungarian Sport horses and ponies.

Serological examination

Hence, we included to the differential serodiagnostics Virus Neutralization Test (VNT) for Usutu virus (USUV), West-Nile Virus (WNV), Tick-borne Encephalitis Virus (TBEV) which are known to be endemic in Central Europe from the blood specimens of horses. Mosquito targeted entomological surveillance was carried out using CO2-baited BG Sentinel 2 traps from and/or around horse stables at three sites (Kulcsár valley, Szuhakálló, Szirmabesenyő). The mosquitoes were identified morphologically, then orthoflavivirus RNA was screened using RT-PCR.

RESULTS

Equine sera (n=174) showed a seroprevalence of 4.59% for WNV and 3.40% for TBEV, with no cross-reactivity. Neutralizing antibodies against USUV were not detected in any of the tested animals. The NAab titer of WNV of positive results was between 1:14 and 1:101; TBEV was measured between 1:28 and 1:806. The positive samples geographically coming from Gergés and Csemer. However, we did not detect WNV in local mosquitoes either. Only mosquitoe specific orthoflaviviruses had been screened. According to the mosquitoe screening the most diverse area was in Szirmabesenyő where 7 different species were identified, however in Kulcsár valley had the highest number of Culex species. Our research wants to highlight the importance of continuous monitoring, vector control strategies, and vaccination programs in equine populations.

DISCUSSION

Entomological and serological surveillance data underscores the endemic and evolving nature of mosquito- and tick-borne orthoflavivirus infections in the region, highlighting hidden transmission even when vector screening is negative. Improved diagnostic awareness, vaccination strategies, and

coordinated One Health surveillance are essential to mitigate the impact of emerging and re-emerging arboviral diseases.

The detection of neutralizing antibodies against TBEV and WNV confirms the continued circulation of both orthoflaviviruses in Northeastern Hungary, despite the lack of clinical signs in the sampled horses. As horses are dead-end-hosts, as humans, their serological status provides valuable information on local virus circulation and supports their role as sentinel animals. Although, WNV RNA was not detected in mosquito pools, this does not exclude local virus circulation, as mosquitoe infection rates are tipically low and depend on the timing of sampling. The detection of mosquitoe-specific orthoflaviviruses demonstrates the suitability of the surveillance approach. The high abundance of *Culex spp*. In Kulcsár Valley may indicate an increased potential for WNV transmission, while the presence of *Ixodes, Dermacentor* and *Haemaphysalis* ticks supports the possibility of continues TBEV circulation.

Overall, our findings emphasise the importance of integrated serological and entomological surveillance for monitoring arbovirus activity. Continued vector surveillance and WNV vaccination of horses in endemic areas remain essential to reduce the risk of future outbreaks in horses.

CONCLUSION

Our research study confirms the sporadic circulation of West Nile virus and Tick-borne encephalitis virus among horses in Northeast Hungary, as demonstrated by the detection of virus-specific neutralising antibodies in otherwise clinically healthy horses. Although no WNV RNA was detected in the collected mosquitoes, the presence of abundant Culex spp. Populations and competent tick vectors indicates that environmental conditions remain favourable for the maintenance and transmission of the examined orthoflaviviruses. The absence of USUV seropositivity suggests that its circulation in the investigated region is currently limited. These findings highlighted the value of horses as sentinel animals for arbovirus surveillance and underlines the importance of integrating serological and entomological monitoring within a One Health framework. Continuous surveillance, preventive measuring (including vaccination of horses against WNV in endemic areas), vector monitoring is essential to mitigate the risk of future outbreaks affecting both animal and human health.

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POSTER EXHIBITION

OPTIMIZATION OF A PSEUDO GERM-FREE ANIMAL MODEL OF ACUTE ULCERATIVE COLITIS: A MULTI-OMICS PERSPECTIVE

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ABSTRACT

The aim of this study was to compare the effects of chemical, microbiota-based, and combined induction of ulcerative colitis (UC) in a pseudo germ-free (PGF) mouse model and to identify the experimental approach that best reproduces the pathogenesis of the disease. Combined induction resulted in the most pronounced alterations in gut microbiota, accompanied by a significant reduction in bacterial viability, extensive metabolic changes, and the highest Histological Activity Index (HAI). In contrast, dysbiotic microbiota alone induced only mild to moderate inflammatory changes. These findings demonstrate that the combined chemical and microbiota-based induction represents a suitable experimental model for investigating UC pathogenesis and evaluating novel therapeutic strategies.

INTRODUCTION

The dextran sodium sulfate (DSS)-induced model of ulcerative colitis (UC) is among the most widely used experimental models, as it reproduces many of the clinical, histopathological, and molecular characteristics of human UC and represents a robust and reproducible model for investigating its pathogenesis and evaluating novel therapeutic approaches (Yang et al., 2024). However, this model primarily reflects chemically induced epithelial injury and does not fully capture the contribution of intestinal dysbiosis to UC pathogenesis. In the present study, a PGF mouse model with reduced intestinal microbiota was used to investigate acute UC. To better mimic the intestinal ecosystem associated with the disease, chemical induction by DSS was complemented by fecal microbiota transplantation (FMT) from a patient with active UC, thereby incorporating the dysbiotic component of the disease. Since it remains unclear whether dysbiotic microbiota plays a causal role in UC pathogenesis or merely reflects inflammation-associated changes, the aim of this study was to evaluate the effects of UC-derived

microbiota on gut microbial composition, bacterial viability, cecal metabolomic profile, and histopathological changes in a PGF mouse model of acute UC.

METHODS

The experiment was conducted at the accredited Laboratory of Gnotobiology (SK U 10021) using specific pathogen-free (SPF) mice obtained from a certified breeder (Velaz s.r.o., Prague, Czech Republic). Experimental procedures (No. 1381/2020-220) and UC patient-derived fecal material (No. 14/2018/OVaV) were obtained under the respective approved protocols.

A pseudo germ-free model was established by targeted depletion of the intestinal microbiota using an antibiotic protocol described previously (Gancarčíková et al., 2018). Mice were assigned to three groups: DSS (n = 12), receiving 5% DSS in drinking water for 5 days; UC (n = 15), receiving FMT from a UC patient (0.2 mL/mouse/day); and DSS-UC (n = 15), combining both interventions. Bacterial viability was assessed by flow cytometry using cFDA/PI staining (Gancarčíková et al., 2018). Fecal samples were collected for microbiome sequencing and untargeted metabolomic profiling. DNA isolation, library preparation, sequencing, and bioinformatic analyses were performed by Novogene Europe (Cambridge, UK). Untargeted LC–MS metabolomic profiling and KEGG pathway enrichment analysis of differential metabolites (VIP > 1.0, $p < 0.05$) were performed by BMKGENE (Münster, Germany). Histopathological changes in the caudal colon were evaluated on hematoxylin and eosin (H&E)-stained sections using the Histological Activity Index.

RESULTS AND DISCUSSION

Flow cytometric analysis demonstrated a significant reduction in cecal bacterial viability in the DSS group (44.7%; $p < 0.0001$) compared with the UC and DSS-UC groups (Fig. 1).

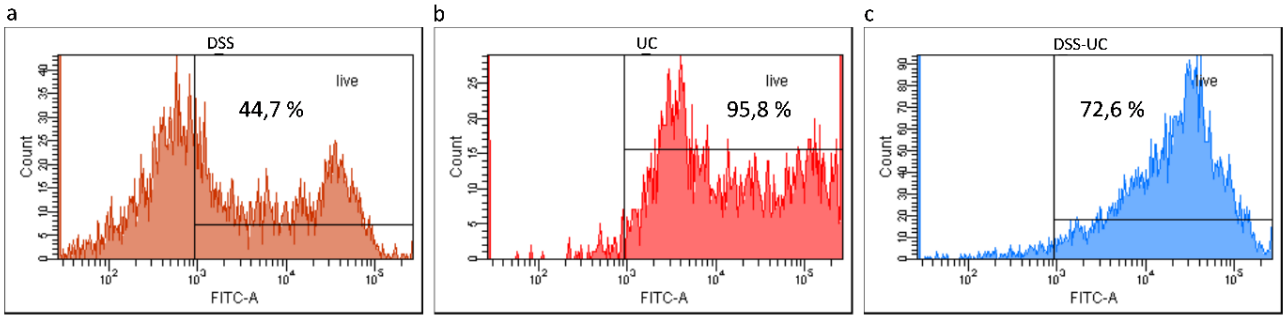


Figure 1. Representative histograms of bacterial viability in cecal samples from the experimental groups (a–c), determined by flow cytometry following UC induction. (a) DSS group; (b) UC group; (c) DSS-UC group.

The highest bacterial viability was observed in the UC group (95.4%; $p < 0.0001$), whereas the combined DSS and FMT treatment significantly reduced bacterial viability (72.5%; $p < 0.0001$), suggesting an inhibitory effect of DSS on the intestinal microbiota. NGS analysis demonstrated that FMT from a patient with UC significantly reshaped the cecal microbiota of PGF mice. Compared with the C, DSS, and DSS-UC groups, the UC group was characterized by a marked increase in the relative abundance of Firmicutes

(87%) and a 4–5-fold reduction in Bacteroidota (8%). At the species level, the UC group exhibited significantly higher relative abundances of the potential pathogens *Clostridioides difficile*, *Providencia* spp., and *Erysipelatoclostridium ramosum* ($p < 0.05$), accompanied by a significant reduction in *Bifidobacterium longum*. Similar differences in species composition were also observed between the UC and DSS-UC groups.

Given the pivotal role of the gut microbiota in the production of bioactive metabolites that contribute to host metabolic homeostasis (Turroni et al., 2018), changes in the cecal metabolomic profile were subsequently analyzed. Venn diagram analysis identified 39 shared differential metabolites in the C vs. UC and C vs. DSS comparisons (Fig. 2A), whereas 50 shared differential metabolites were identified in the DSS-UC vs. DSS and DSS-UC vs. UC comparisons (Fig. 2B), indicating more extensive metabolic alterations associated with the combined chemical and microbiota-mediated induction of ulcerative colitis.

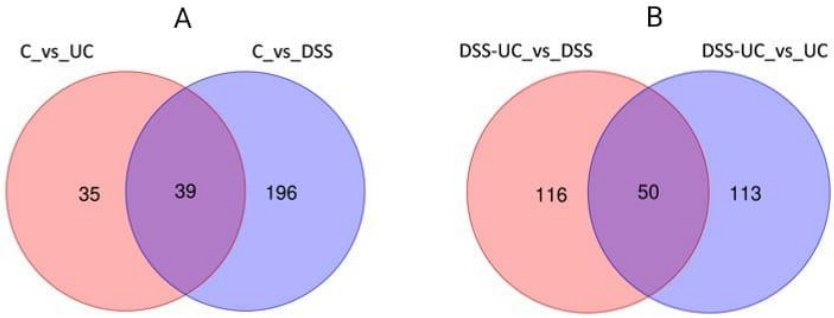


Figure 2. Venn diagrams of cecal metabolites showing the overlap of differential metabolites among the C, UC, DSS, and DSS-UC groups.

A more detailed analysis of differential metabolites using a volcano plot revealed statistically significant differences between the compared groups. The C vs. UC comparison identified 30 significantly up-regulated and 44 down-regulated metabolites. The UC vs. DSS comparison identified 127 up-regulated and 57 down-regulated metabolites. The most pronounced differences were observed in the C vs. DSS-UC comparison, with 136 up-regulated and 101 down-regulated metabolites. Analysis of the top 10 down-regulated differential metabolites in the C vs. UC comparison revealed marked disruption of metabolic pathways associated with intestinal homeostasis. Reduced levels of 3-hexenal and chlorogenate suggested impaired antioxidant and anti-inflammatory defense, whereas down-regulation of 5-ureido-4-imidazole carboxylate and 5′-S-methyl-5′-thioinosine indicated disturbances in purine and nucleotide metabolism. In addition, decreased levels of UDP-N-acetylmuramoyl-L-alanyl-D-glutamate suggested impaired peptidoglycan metabolism and compromised intestinal barrier integrity. Collectively, these alterations indicate impaired mucosal regeneration, persistent inflammation, and dysregulation of microbiota-associated metabolic functions in ulcerative colitis. Compared with the UC group, the DSS-UC group exhibited more pronounced metabolic alterations, characterized by remodeling of lipid metabolism (PI(12:0/16:0), 4β-methylzymosterol-4α-carboxylic acid), increased nucleotide turnover (deoxyribose, formamidopyrimidine nucleoside triphosphate), impaired folate metabolism (5-methyltetrahydrofolate),

and further disruption of microbiota-associated metabolic functions (2-amino-2-deoxyglucitol-6-phosphate, 12-methyltetradecanoic acid). These alterations indicate a deeper metabolic dysregulation of the intestinal environment induced by the combined chemical and microbiota-based induction of ulcerative colitis.

Based on the severity of clinical manifestations, animals were classified into mild (UC/M), moderate (UC/S, DSS/S, DSS-UC/S), and severe (DSS/T, DSS-UC/T) forms of UC, enabling subsequent histopathological evaluation of the colon according to disease severity. Quantitative assessment using the HAI demonstrated significantly higher epithelial injury scores in the DSS/T group than in the DSS-UC/T and DSS/S groups ($p < 0.001$). Inflammatory infiltration was mild to moderate in the UC/M, UC/S, and DSS-UC/S groups (scores 0.35–0.63), whereas in the DSS/S, DSS/T, and DSS-UC/T groups it extended into the muscularis mucosae (score 1). Overall, the HAI confirmed that DSS-induced chemical injury was essential for the development of severe UC, whereas dysbiotic microbiota from a patient with UC alone induced only mild to moderate inflammatory changes.

CONCLUSION

The obtained results demonstrated that the combined chemical and microbiota-based induction of ulcerative colitis using DSS and FMT from a patient with UC represents a biologically relevant experimental model that reproduces the complex microbial, metabolic, and histopathological features of the disease. This model provides a valuable platform for future studies investigating ulcerative colitis pathogenesis and evaluating novel therapeutic approaches.

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GIARDIASIS IN LIVESTOCK IN THE CONTEXT OF ONE HEALTH

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ABSTRACT

Giardia duodenalis is a zoonotic gastrointestinal protozoan with worldwide distribution. Prevalence of this pathogen in livestock has not been sufficiently studied despite the fact that these animals represent an important reservoir. In this study, we focused on the occurrence of giardiasis in calves, sheep and lambs. The prevalence in calves was 23.3 %. *Giardia* cysts were also detected in lambs in two out of nine farms. In these samples, molecular analysis comfirmed the presence of *G. duodenalis* assemblage E. This assemblage was confirmed to cause human giardiasis and thus represent a risk to public health. In addition to its zoonotic importance, giardiasis may negatively affect livestock productivity, manifesting as reduced weight gain and impaired growth in calves and lambs, potentially leading to decreased production efficiency and economic losses.

INTRODUCTION

Giardia duodenalis is a protozoan pathogen with a worldwide distribution. It is one of the most common causes of parasitic diarrhoea in animals and humans (Adam 2021). Giardiasis is characterized by enteritis, malabsorption, and maldigestion. In lambs and calves, it adversely affects growth and development, leading to reduced feed conversion efficiency and decreased weight gain (Jacobson et al. 2016; Santin 2020). Clinical disease is observed predominantly in young individuals, although asymptomatic infections are also common. (Benhassine et al. 2020). Giardiasis represents a major public health concern because of its zoonotic potential, high prevalence and global distribution (Feng and Xiao 2011).

Based on molecular methods and genotyping, eight assemblages, designated A–H, have been identified. These assemblages differ in their host range. Within the species complex only assemblages A and B are considered to be zoonotic. Giardiasis in livestock is predominantly caused by assemblage E (Xiao and Feng 2017). Despite its presumed host specificity, human cases of giardiasis caused by assemblage E have also been reported (Abdel-Moein and Saeed 2016; Zahedi et al. 2017).

G. duodenalis is transmitted via the faecal–oral route. Its simple direct life cycle, broad host range, low infectious dose (approximately 10 cysts), and environmentally resistant cysts facilitate the transmission of this pathogen (Ryan et al. 2019). *G. duodenalis* may enter the food chain via contaminated water or soil. Fresh fruits and vegetables contaminated with *Giardia* cysts represent the main source of human giardiasis (FAO/WHO 2014). Infective cysts have also been detected in other

food products, including dairy products, meat, and processed foods such as canned salmon, salads, and sandwiches (Ryan et al. 2019).

METHODS

A total of 73 faecal samples collected from calves and pooled faecal samples from lambs and adult sheep from nine farms in eastern Slovakia, were examined microscopically using the Faust flotation method (33% zinc sulfate ($ZnSO_4$) flotation solution).

DNA was extracted from microscopically *Giardia*-positive faecal samples using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, USA) according to the manufacturer's instructions. The extracted DNA was used as a template for nested PCR targeting the glutamate dehydrogenase (*gdh*) gene following the protocol described by Cacciò et al. (2008). The primary PCR reaction was performed using the primers Gdh1 and Gdh2. The secondary PCR reaction employed the primer pair Gdh3 and Gdh4. For both PCR reactions were used identical conditions: an initial hot start at 94°C for 2 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. PCR products (530 bp) were analysed by electrophoresis on a 1.5% agarose gel and visualized under UV transilluminator. Positive secondary PCR amplicons were sent to the Microsynth Seqlab (Vienna, Austria) for purification and Sanger sequencing.

RESULTS

Of the 73 faecal samples collected from calves, *Giardia* cysts were microscopically detected in 17 animals (23.3%). However, successful amplification of the *gdh* gene fragment was only in 3 samples. The efficiency of molecular detection may be influenced by the quality of the extracted DNA as well as by the presence of faecal PCR inhibitors (Safitri et al. 2026).

No *Giardia* cysts were microscopically detected in faecal samples collected from ewes. However, *Giardia* cysts were identified by coprological examination in faecal samples from lambs on two farms. Molecular analysis confirmed the presence of *Giardia duodenalis* assemblage E, which is commonly reported in this host group (Geng et al. 2023).

DISCUSSION

Giardiasis in livestock is a common yet neglected disease. The prevalence of *Giardia* in cattle in Europe is 35.1% (Mateusa et al. 2023). Although assemblage E predominates in livestock, infections with assemblages A and B have also been reported (Zajaczkowski et al. 2021; Geng et al. 2023). All three mentioned assemblages have the potential to infect humans and therefore represent a zoonotic risk (Fantinatti et al. 2016).

During acute infection these animals excrete large numbers of *Giardia* cysts (up to 10^7 cysts per day). Together with the prolonged shedding of cysts, this makes cattle an important reservoir of *Giardia duodenalis* and one of the main sources of environmental contamination with infective cysts. (Budu-Amoako et al. 2011; Mateusa et al. 2023; Bulumulla et al. 2025).

Food contamination with *Giardia* cysts may occur through multiple routes at different stages of the food production chain (Budu-Amoako et al. 2011). Pre-harvest crop contamination with *Giardia* cysts may occur through the contaminated irrigation water, agricultiural runoff, application of organic fertilizers, pasturing livestock near the arable land, where animals may serve as reservoirs of *G. duodenalis* and other pathogens (Smith et al. 2007). Effective prevention requires the identification of critical control points and the implementation of good agricultural practices and appropriate personal hygiene among food handlers (Siwila 2023).

CONCLUSION

The prevalence of *Giardia duodenalis* in livestock has not yet been sufficiently investigated. Beside its veterinary importance, *G. duodenalis* has zoonotic potential (Wang et al. 2016) and may therefore represent a health risk to people working with livestock, food handlers, and consumers.

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SURVEILLANCE OF TICK-BORNE ENCEPHALITIS VIRUS IN SLOVAKIA

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ABSTRACT

Tick-borne encephalitis (TBE) is an important zoonotic disease in Slovakia, where the number of human cases has shown an increasing trend since 2000. The aim of this study was to determine the prevalence of tick-borne encephalitis virus (TBEV) in tick populations during nationwide surveillance conducted in 2024 – 2025. Ticks were collected at 16 localities using the standard flagging method and tested for TBEV by two-step RT-qPCR. 2,372 ticks were analysed in 2024, and no TBEV-positive specimens were detected; 2,443 ticks were examined in 2025, and TBEV was detected in seven individuals (0.29%). The results confirm the low prevalence of TBEV in tick populations in Slovakia.

INTRODUCTION

Tick-borne encephalitis (TBE) is one of the most important tick-borne viral diseases in Europe and Asia. It is caused by tick-borne encephalitis virus (TBEV), a member of the genus *Orthoflavivirus*, which is endemic in more than 25 European countries. Approximately 5,000 – 10,000 human cases are reported annually, with the highest incidence occurring in the Baltic States and Central Europe (Hills et al. 2022; Pustijanac et al. 2023).

Three major TBEV subtypes have been described, of which the European subtype (TBEV-Eu) predominates in Europe (Kunze et al. 2022). During the last decade, the geographical distribution of TBE has expanded beyond traditionally endemic areas, and increasing numbers of cases have also been reported from regions previously considered non-endemic (Parfut et al. 2023). Slovakia follows this trend, with a gradual increase in TBE incidence since 2000 and the highest incidence recorded in 2020 (3.4 cases per 100,000 inhabitants) (Stanko et al. 2022).

METHODS

Tick collection

As part of the national TBEV surveillance programme, tick collections were conducted during 2024 and 2025 at 16 localities across Slovakia selected based on the high incidence of human TBE cases reported between 2019 and 2023. Questing ticks were collected from vegetation during the spring and autumn activity peaks using the standard flagging method. At each locality, sampling followed a

standardized protocol consisting of either a 100 m transect or a 15-minute collection period. Collected ticks were transported to the laboratory, morphologically identified to species, developmental stage, and sex using standard taxonomic keys, and individually stored at -80 °C until laboratory analysis.

Molecular analysis

Adult ticks and nymphs were processed individually. Total RNA was extracted using the innuPREP Tick DNA/RNA Kit (IST Innuscreen, Germany) according to the manufacturer's instructions. RNA concentration was determined spectrophotometrically using a Thermo Scientific™ NanoDrop™ One spectrophotometer (Thermo Fisher Scientific, USA), followed by reverse transcription with the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). After cDNA synthesis, samples were pooled in groups of five individuals of the same species and developmental category (nymphs, males or females). TBEV RNA was detected using a two-step RT-qPCR assay targeting the 3′ non-coding region of the viral genome. Amplification was performed using TBEV-specific primers TBEV-F1(5′-TCCTCCTATACAAAATTCCCCCTC-3′) and TBEV-R1 (5′-CCGAGTCACACATCACCTCC-3′) and a TaqMan probe TBEV-P (5′FAM-TGAGCCACCATCACCCAGACACA-3′BHQ). All reactions were performed in duplicate and included positive and negative controls. RT-qPCR amplification was carried out using the Real-time Thermal Cycler (qPCR) (Analytik Jena, Germany) and Luna® Universal Probe qPCR Master Mix (New England Biolabs, USA). Positive and negative controls yielded the expected results, confirming the reliability of the assay.

RESULTS

During 2024, a total of 2,372 ticks were examined as part of the national TBEV surveillance programme in Slovakia. The predominant species was *Ixodes ricinus* (2,265 specimens; 95.49%), followed by *Dermacentor reticulatus* (n = 31), *Dermacentor marginatus* (n = 45), and *Haemaphysalis concinna* (n = 31). Samples were analysed in 476 pools; no TBEV-positive samples were detected.

A total of 2,443 ticks were examined in 2025, of which 2,299 (94.11%) were identified as *Ixodes ricinus*. The remaining specimens included *Dermacentor reticulatus* (n = 72), *Dermacentor marginatus* (n = 39), *Haemaphysalis concinna* (n = 31), and *Ixodes frontalis* (n = 2).

Screening identified TBEV RNA in three of the 494 analysed pools. Subsequent individual testing of cDNA from all ticks comprising the positive pools identified seven TBEV-positive ticks, corresponding to an overall prevalence of 0.29% (7/2,443). Positive specimens included two nymphs, one male, and four females. Two TBEV-positive ticks originated from the Bratislava Region, whereas five were collected in the Žilina Region.

DISCUSSION

Tick-borne encephalitis remains an important zoonotic disease in Slovakia, with human cases reported regularly throughout the country. Between 2019 and 2023, the highest incidence was recorded in the Banská Bystrica, Žilina, and Trenčín Regions. In 2024, TBE cases were reported from all Slovak regions except the Trnava Region, with the highest incidence again observed in the Banská Bystrica and Žilina Regions (MPRV SR, 2024).

Despite the continuous occurrence of human TBE cases, the present surveillance study demonstrated a low TBEV prevalence (0.29%) in tick populations. This finding is consistent with previous European surveillance studies reporting a persistently low but stable prevalence of TBEV in questing ticks. In Lithuania, a minimum infection rate (MIR) of 0.4% was reported for *Ixodes ricinus* and *Dermacentor reticulatus* (Sidorenko et al. 2021), while Pettersson et al. (2014) reported an average TBEV prevalence of 0.28% based on the analysis of 39,358 ticks collected in Nordic countries.

One possible explanation for the low prevalence is the highly focal distribution of TBEV in nature. Ličková et al. (2025) demonstrated considerable spatial variation in TBEV prevalence in Slovakia, ranging from 0% to 8.72% between sampling sites separated by only 150–180 m. Active virus circulation is restricted to small natural foci (microfoci) of approximately 2,500 m², the establishment and persistence of which are determined by local ecological condition.

The high proportion of nymphs among the examined ticks may also have contributed to the low detection rate. During metamorphosis, TBEV undergoes an eclipse phase characterized by the temporary absence of detectable viral activity, and viral RNA becomes detectable again approximately two months after ecdysis (Chitimia-Dobler 2024).

CONCLUSION

The surveillance conducted in Slovakia during 2024 – 2025 confirmed a low prevalence of TBEV in tick populations. The findings are consistent with previous European surveillance studies and support the concept of active and stable TBEV circulation within natural microfoci. Furthermore, they highlight the importance of investigating ecological factors involved in the establishment and maintenance of these microfoci. The eclipse phase of TBEV should also be considered when interpreting molecular surveillance results, as it may temporarily affect the detectability of viral RNA in infected ticks.

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MULTIOMIC ANALYSIS OF THE EFFECTS OF FECAL MICROBIOTA TRANSPLANTATION IN EXPERIMENTAL ACUTE ULCERATIVE COLITIS

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ABSTRACT

The aim of this study was to evaluate the effects of fecal microbiota transplantation (FMT) in experimental models of acute UC induced by chemical exposure to dextran sulfate sodium (DSS), FMT from a patient with UC, or a combination of both, using an integrated multi-omics approach. FMT induced more pronounced changes in microbial composition, metabolic pathways, and intestinal gene expression in the UC model, consistent with the restoration of intestinal homeostasis, whereas inflammation and gut ecosystem dysregulation persisted in the DSS-UC model.

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease associated with disruption of intestinal homeostasis and gut microbial dysbiosis. The therapeutic efficacy of FMT depends not only on restoring the taxonomic composition of the gut microbiota but also on restoring its functional and metabolic potential. The aim of this study was to evaluate the effects of FMT in an experimental model of acute UC using an integrated multi-omics approach, including 16S rRNA gene sequencing of cecal microbiota, metabolomic profiling, and transcriptomic analysis of intestinal tissue.

METHODS

The study was conducted at the accredited Laboratory of Gnotobiology (SK U 10021) using specific pathogen-free (SPF) mice obtained from a certified breeding facility (Velaz s.r.o., Prague, Czech Republic). Experimental procedures (No. 1381/2020-220) and donors-derived fecal material (No. 14/2018/OVaV) were obtained under the respective approved protocols. A pseudo germ-free (PGF) model was established by targeted antibiotic depletion of the intestinal microbiota as previously described (Gancarčíková et al., 2018). Mice were assigned to three groups: DSS (n = 12), receiving

5% DSS in drinking water for 5 days; UC (n = 15), receiving FMT from a UC patient (0.2 mL/mouse/day); and DSS-UC (n = 15), combining both interventions. Subsequently, mice in the UC and DSS-UC groups received FMT from a healthy donor (0.2 mL/mouse/day) for 5 days. Fecal samples were collected for microbiome sequencing and untargeted metabolomic profiling, whereas colonic tissue samples were collected for transcriptome analysis. DNA and RNA isolation, library preparation, sequencing, and bioinformatic analyses were performed by Novogene Europe (Cambridge, UK). Untargeted LC–MS metabolomic profiling and KEGG pathway enrichment analysis of differential metabolites (VIP > 1.0, $p < 0.05$) were performed by BMKGENE (Germany).

RESULTS AND DISCUSSION

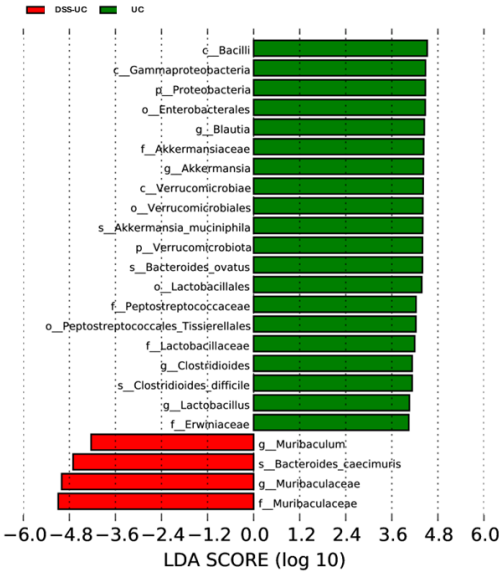


Figure 1. LEfSe analysis of microbial biomarkers comparing the DSS-UC and UC groups. Only taxa with a log_{10} LDA score > 2 are shown.

The therapeutic effect of FMT in ulcerative colitis depends not only on the successful colonization of the transplanted microbiota but also on the restoration of its functional and metabolic potential (Poppe et al., 2024). In the present study, these processes were reflected by distinct responses of the UC and DSS-UC models to FMT, as demonstrated at the levels of the gut microbiota, metabolome, and intestinal transcriptome. The Venn diagram of alpha diversity showed a marked increase in unique bacterial taxa after FMT in the UC group (28.6% vs. 11.3%), whereas only minor changes were observed in the DSS-UC (10.6% vs. 9.5%) and DSS (5.5%) groups. These differences in microbiota restructuring were further supported by LEfSe analysis of microbial biomarkers (Fig. 1), which revealed significant enrichment of the taxa Bacilli, Gammaproteobacteria, Proteobacteria, Enterobacterales, the genera *Blautia*, *Akkermansia* (including *Akkermansia muciniphila*), *Lactobacillus*, and *Clostridioides*, the families Peptostreptococcaceae and Erwinaceae, as well as members of the phylum Verrucomicrobiota in the UC group.

Species-level relative abundance analysis showed that FMT in the UC model resulted in a significant increase in taxa assigned to *Bacteroides ovatus* (p = 0.029) and *Bacteroides caccae* (p = 0.045), accompanied by a decrease in *Clostridioides difficile* (p = 0.004) and members of the genus *Providencia* (p = 0.009). These changes indicate a more pronounced shift of the gut microbial ecosystem toward a physiological state, characterized by the predominance of strictly anaerobic, metabolically beneficial bacteria and a reduction in taxa associated with dysbiosis. In contrast, the DSS-UC model showed a significant increase in *Bacteroides uniformis* (p = 0.049) and *Parabacteroides merdae* (p = 0.019) following FMT; however, the relative abundance of *Erysipelatoclostridium ramosum* also increased significantly (p = 0.035), suggesting the persistence of microbiota alterations associated with the DSS-induced inflammatory environment. These differences suggest that the healthy donor microbiota became established more effectively in the UC model, whereas DSS-induced disruption of the intestinal environment likely limited engraftment of the transplanted microbiota and the complete restoration of the gut microbial ecosystem. Taxonomic changes alone do not provide a complete picture of gut ecosystem restoration following FMT, as the therapeutic efficacy of transplantation also depends on the recovery of the metabolic functions of the gut microbiota, including the production of microbial metabolites involved in maintaining intestinal barrier integrity, colonocyte energy metabolism, and immune regulation (Sinha et al. 2022; Ikeda et al. 2024). In this context, principal component analysis (PCA) of the metabolomic profiles revealed a clear separation of the DSS, UC, and DSS-UC groups. While the DSS group formed a compact cluster, the UC and DSS-UC groups exhibited greater sample dispersion, with the most pronounced differences observed between the DSS group and the FMT-treated groups.

Univariate analysis identified 669 differentially regulated metabolites in the DSS versus UC comparison (172 upregulated and 497 downregulated), 247 metabolites in the DSS versus DSS-UC comparison (21 upregulated and 226 downregulated), and 405 metabolites in the DSS-UC versus UC comparison (70 upregulated and 335 downregulated), with downregulated metabolites predominating in all comparisons. These metabolomic differences were further reflected in KEGG pathway enrichment analysis (Fig.2), which identified significant differences between the UC and DSS-UC groups in butanoate metabolism.

Together, these findings suggest that the response to FMT differed between the two models not only in bacterial composition but also in the functional reorganization of gut microbial metabolic pathways. Differences in the functional potential of the gut microbiota were also reflected in the gene expression profiles of colonic tissue. In the UC group, increased activity of the PPAR signaling pathway, Wnt signaling pathway, and oxidative phosphorylation was observed, whereas the DSS-UC group exhibited persistently higher expression of genes associated with hematopoietic cell lineage, cytokine–cytokine receptor interaction, cell adhesion molecules (CAMs), Th1 and Th2 cell

differentiation, the intestinal immune network for IgA production, and the inflammatory bowel disease signaling pathway.

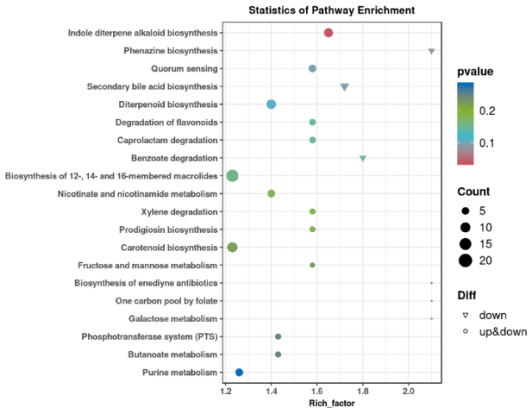


Figure 2. Metabolic pathway enrichment analysis comparing the DSS-UC and UC groups. Bubble size indicates the number of differential metabolites, color represents the p-value, and the Rich factor is shown on the x-axis.

These findings suggest that, despite FMT administration, the combined DSS-UC model retained a more pronounced inflammatory transcriptomic profile, whereas the UC model displayed transcriptional changes consistent with the restoration of intestinal homeostasis.

CONCLUSION

Our results demonstrated the efficacy of a 5-day FMT therapy in the UC model, whereas in the combined DSS-UC model, FMT alone did not achieve complete restoration of intestinal homeostasis. These findings suggest the need for further research focused on promoting intestinal epithelial regeneration as part of FMT-based therapeutic strategies.

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SYNBIOTIC INTERVENTION MODULATES THE SEX-SPECIFIC DYSBIOTIC GUT MICROENVIRONMENT ACCOMPANYING AUTISM SPECTRUM DISORDER

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ABSTRACT

The aim of this study was to evaluate the effects of synbiotic intervention in pseudo germ-free (PGF) mice following fecal microbiota transplantation from girls with autism spectrum disorder (ASD). Synbiotic intervention remodeled the gut microbiota by increasing the abundance of bacteria associated with short-chain fatty acid production. These changes were accompanied by systematic remodeling of the metabolomic profile, particularly in pathways related to aromatic amino acid and lipid metabolism, as well as significant improvement in colonic histopathology. Our findings support the potential of targeted synbiotic modulation of dysbiotic gut microbiota to restore intestinal homeostasis and alleviate gastrointestinal comorbidities accompanying autism spectrum disorder.

INTRODUCTION

Autism spectrum disorder is a multifactorial, highly heritable neurodevelopmental disorder characterized by impairments in social interaction, communication, stereotyped behaviors, restricted interests, and food selectivity. Individuals with ASD frequently present with gastrointestinal comorbidities such as constipation, diarrhea, or abdominal discomfort, which are often associated with alterations in the composition and abundance of the gut microbiota. Synbiotic intervention represents a potential approach to modulating the gut microbiota and its metabolic functions. The aim of this study was to evaluate whether targeted modulation of the gut microbiota by synbiotic intervention could alleviate dysbiotic alterations and restore the metabolic profile disrupted by fecal microbiota transplantation from children with ASD in a PGF model.

METHODS

The experiment was performed using 106 specific pathogen-free (SPF) female BALB/c mice (6 weeks old) housed in the accredited Laboratory of Gnotobiology (SK U 10021) with *ad libitum* access to sterilized feed and water. A pseudo-germ-free model was established by depletion of the intestinal

microbiota using an antibiotic decontamination protocol as previously described (Gancarcíkova et al. 2018). Subsequently, mice were randomly assigned to four experimental groups: control (C; n = 30), NT (n = 30), ASD (n = 23), and ASD+pro/pre (n = 23). In the first phase, the NT group received fecal microbiota transplantation (FMT) from neurotypical girls, whereas the ASD and ASD+pro/pre groups received FMT from girls with ASD. FMT was administered orally at 0.2 mL/mouse/day for 3 days. In the second phase, only the ASD+pro/pre group received probiotic/prebiotic intervention. The probiotic strains *Lactiplantibacillus plantarum* CCM 7512 and *Limosilactobacillus reuteri* CCM 8617 were administered orally at 0.1 mL/strain/mouse/day (1×10^9 CFU/mL) for 5 days, while the diet was supplemented with 4% irradiated ground flaxseed of the LIBRA cultivar was characterized by a high content of omega-3 fatty acids, predominantly α-linolenic acid (ALA; 54.9%), and omega-6 fatty acids, mainly linoleic acid (17.1%).

Animal experiments were approved by the State Veterinary and Food Administration of the Slovak Republic (No. 4356/2022-220), and human fecal sample collection was approved by the Ethics Committee of the Faculty of Medicine, Comenius University and the University Hospital Bratislava (Project No. 79/2021). Psychological and psychiatric assessments were performed at the Academic Research Center for Autism, Institute of Physiology, Faculty of Medicine, Comenius University in Bratislava. ASD-FMT donors were girls diagnosed with ASD using the gold-standard diagnostic tools ADOS-2 and ADI-R. NT-FMT donors were neurotypical girls recruited from the local population. The bacterial profile of fecal samples was analyzed by next-generation sequencing (NGS) targeting the V3–V4 region of the 16S rRNA gene. Library preparation, sequencing, and primary bioinformatic processing were performed by Novogene (Munich, Germany). The fecal metabolome was analyzed using untargeted LC-MS metabolomics performed by BMKGENE (Münster, Germany). Differential metabolites were identified using the criteria VIP > 1.0 and $p < 0.05$, and their biological relevance was interpreted by KEGG pathway analysis. Histopathological examination of the caudal colon was performed on hematoxylin and eosin (H&E)-stained sections. Histological changes were evaluated using the Histological Activity Index (HAI), including epithelial damage, crypt damage, and inflammatory cell infiltration. All evaluations were performed in a blinded manner by two independent experienced pathologists.

RESULTS AND DISCUSSION

Probiotics and prebiotics influence the structure and function of the gut microbiota through interconnected metabolic and immunoregulatory mechanisms. Their combined administration as synbiotics enables simultaneous modulation of gut microbiota composition and metabolic activity, including the production of short-chain fatty acids (SCFAs), thereby supporting intestinal epithelial integrity and immune homeostasis (Silva et al. 2020). In our study, Metastat analysis demonstrated that synbiotic intervention significantly remodeled the gut microbiota in the ASD+pro/pre group compared with the ASD group, as reflected by a reduced relative abundance of the genus *Bacteroides* and increased relative abundances of the genera *Akkermansia*, *Phascolarctobacterium*, and *Blautia*. The observed increase in bacteria

associated with fermentative metabolism and mucin metabolism suggests a functional shift of the gut microbiota toward a profile supporting SCFA production and intestinal epithelial integrity (Singh et al. 2023). PCA demonstrated a clear separation between the metabolomic profiles of the ASD and ASD+pro/pre groups without overlap, indicating a pronounced shift in the metabolome following synbiotic intervention (Fig.1a). Hierarchical clustering analysis confirmed this finding by grouping samples according to the experimental groups (Fig. 1b). Volcano plot analysis identified 148 metabolites with increased and 127 metabolites with decreased levels in the ASD+pro/pre group, whereas 810 metabolites remained unchanged, indicating a systematic remodeling of the metabolic profile.

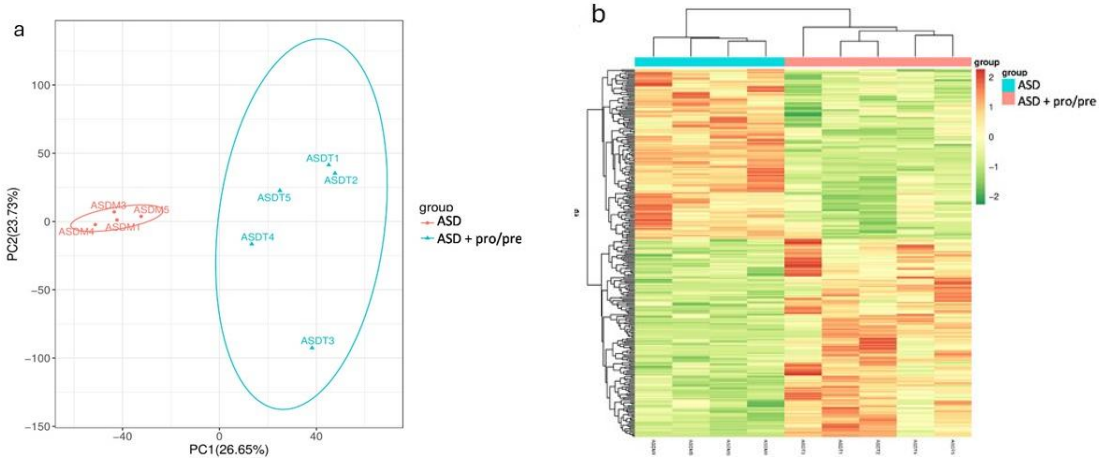


Figure 1 (a) Principal component analysis (PCA) of metabolomic profiles in the ASD and ASD+pro/pre groups. PC1 and PC2 explained 26.65% and 23.73% of the total variance, respectively. (b) Heatmap of standardized metabolite abundance (z-scores) in the ASD and ASD+pro/pre groups. Colors indicate relative metabolite abundance, ranging from low (green) to high (red).

Metabolite set enrichment analysis (MSEA) following correction for multiple testing (FDR < 0.05) revealed significant enrichment of pathways related to aromatic amino acid and lipid metabolism. The most prominently enriched pathways included tyrosine metabolism, tryptophan metabolism, ether lipid metabolism, and α-linolenic acid metabolism. Since alterations in aromatic amino acid metabolism have been repeatedly reported as characteristic metabolic features of autism spectrum disorder (Needham et al. 2021), these findings suggest that synbiotic intervention modulated metabolic pathways representing an important link between the gut microbiota and host metabolism (Gao et al. 2020). Although butanoate metabolism did not remain statistically significant after correction for multiple testing, its biological relevance is supported by the concurrent increase in bacteria associated with fermentative metabolism and short-chain fatty acid production (*Akkermansia*, *Phascolarctobacterium*, and *Blautia*) (Liu et al. 2023).

At the level of individual metabolites, the ASD+pro/pre group showed higher levels of homovanillic acid, the final product of dopamine degradation within the tyrosine metabolic pathway, further supporting the observed alterations in aromatic amino acid metabolism. Significant differences were also observed in lipid metabolism. Although flaxseed supplementation may have directly influenced α-linolenic acid metabolism, alterations across multiple lipid metabolic pathways suggest a broader reorganization of lipid metabolism beyond the direct nutritional effect.

Histological evaluation demonstrated that synbiotic intervention significantly reduced inflammatory cell infiltration, crypt damage, and epithelial damage compared with the ASD group ($p < 0.001$). Consequently, the overall histological activity index was significantly lower in the ASD+pro/pre group than in the untreated ASD group ($p < 0.01$). Notably, epithelial damage scores in the ASD+pro/pre group were also significantly lower than those in the NT group ($p < 0.05$). These histopathological findings were consistent with the microbiota remodeling and metabolic changes observed following synbiotic intervention.

CONCLUSION

Our findings support the hypothesis that targeted synbiotic modulation of dysbiotic gut microbiota following fecal microbiota transplantation from girls with autism spectrum disorder not only remodels the gut microbiota but also restores its metabolic functions and improves the histopathological status of the colon. These findings highlight the importance of functional modulation of the gut microenvironment as a potential therapeutic strategy for gastrointestinal comorbidities accompanying ASD.

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PREVALENCE OF *BORRELIA* SPP. IN QUESTING TICKS FROM URBAN AND PERI-URBAN HABITATS IN SLOVAKIA

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ABSTRACT

Questing ticks collected from urban and peri-urban green spaces across Slovakia were screened for *Borrelia* spp. using PCR. A total of 777 ticks were examined, of which 222 (28.6%) tested positive. Infection was detected in all studied regions, with marked spatial variability among localities. Six *Borrelia* genospecies were identified, with *B. afzelii*, *B. garinii*, and *B. valaisiana* being the most frequent. These findings demonstrate considerable genetic diversity of *Borrelia* spp. in urban tick populations and highlight the epidemiological importance of urban and peri-urban environments as potential sites of human exposure to tick-borne pathogens.

INTRODUCTION

Species of the genus *Borrelia* are among the most important tick-borne pathogens in Europe. They circulate in complex enzootic cycles involving ixodid ticks and vertebrate reservoir hosts, particularly small mammals and birds. The principal vector of *Borrelia* spp. in Europe is *Ixodes ricinus*, which transmits several genospecies associated with human disease. Among them, members of the *Borrelia burgdorferi* sensu lato complex are responsible for Lyme borreliosis, the most common tick-borne disease in Europe (Stanek et al. 2012; Estrada-Peña et al. 2018).

Urbanization has substantially altered natural ecosystems, creating environments where humans, wildlife, and tick populations increasingly overlap. Urban and peri-urban green spaces can support diverse vertebrate host communities and provide suitable habitats for ticks, thereby contributing to the maintenance and circulation of tick-borne pathogens (Heylen et al. 2019).

The prevalence and diversity of *Borrelia* spp. in questing ticks may vary considerably between geographical regions and habitat types. Long-term surveillance is therefore essential for understanding local epidemiological risks and monitoring changes in pathogen circulation (Nuttall 2022; Rusňáková Tarageľová et al. 2024).

The aim of this study was to determine the prevalence and species diversity of *Borrelia* spp. in questing ticks collected from urban and peri-urban green spaces across Slovakia.

METHODS

Ticks were collected from vegetation in urban and recreational sites as part of *Borrelia* spp. surveillance during spring and autumn seasons using the flagging method. Sampling was performed by dragging a 1 × 1 m cotton cloth over vegetation along a defined 100 m transect, with a standardized collection time of 15 minutes per sampling event. Tick species identification was performed using standard morphological keys for Ixodidae (Slovák 2010; Estrada-Peña et al. 2017). Samples were stored at −80 °C until nucleic acid extraction. DNA was individually extracted from each tick using the innuPREP Tick DNA/RNA Kit (IST Innuscreen, Germany) following the manufacturer's instructions.

PCR amplification was performed using primers targeting the *rrfA–rrlB* intergenic spacer, enabling the detection of *Borrelia* spp. (Derdáková *et al.* 2003). The reaction mixture contained template DNA, Takara HS Master Mix (Takara Bio, Japan), primers (F: 5'-CGACCTTCTTCGCCTTAAAGC-3'; R: 5'-AGCTCTTATTCGCTGATGGTA-3'), and nuclease-free water. Thermal cycling conditions consisted of an initial denaturation followed by 35 cycles of denaturation at 94 °C for 5 s, annealing at 52 °C for 1 s, and extension at 68 °C for 10 s. Amplified products were visualized by electrophoresis on a 1% agarose gel stained with GelRed Nucleic Acid Stain (Biotium, USA). The expected amplicon size was 250 bp. Each PCR run included a positive control (*Borrelia garinii* DNA) and a negative control (nuclease-free water).

RESULTS

A total of 777 questing ticks collected from urban parks across eight regions of Slovakia were examined for the presence of *Borrelia* spp. DNA. The majority of specimens were identified as *Ixodes ricinus*, while *Dermacentor reticulatus*, *Dermacentor marginatus*, and *Haemaphysalis concinna* were recorded sporadically. All *Borrelia*-positive samples originated from *I. ricinus* ticks.

Overall, 222 ticks tested positive for *Borrelia* spp., corresponding to a prevalence of 28.6%. *Borrelia* DNA was detected in all investigated regions. Considerable variation was observed among localities, with prevalence ranging from 5.7% to 50%. The highest prevalence was recorded in Trnava and Považská Bystrica, while the lowest values were observed in Svätý Jur and Čadca.

Molecular analysis identified six genospecies within the *Borrelia burgdorferi* sensu lato complex: *B. afzelii*, *B. garinii*, *B. valaisiana*, *B. burgdorferi* sensu stricto, *B. lusitaniae* and *B. spielmanii*. The most frequently detected species were *B. afzelii*, *B. garinii*, and *B. valaisiana*, which were present across most sampling sites, whereas other genospecies occurred sporadically.

DISCUSSION

The prevalence of *Borrelia* spp. detected in the present study (28.6%) was higher than that reported by Rusňáková Tarageľová et al. (2024), who recorded an overall prevalence of 18.8% in 17,249 *Ixodes ricinus* ticks collected from 16 Slovak localities during 1999–2019. Similar spatial variability in *Borrelia* prevalence has previously been documented across different habitat types and localities in Slovakia, suggesting that local environmental conditions and host communities may play an important role in shaping transmission dynamics (Vaculová et al. 2019, Kazimírová et al. 2023).

The predominance of *B. afzelii*, *B. garinii*, and *B. valaisiana* observed in this study corresponds with previous findings from Slovakia, where these genospecies were consistently identified among the most common members of the *Borrelia burgdorferi* sensu lato complex (Rusňáková Tarageľová et al. 2024). The detection of six *Borrelia* genospecies further confirms the considerable diversity of *Borrelia* circulating in Slovak tick populations.

The results highlight urban and peri-urban green spaces as environments capable of maintaining diverse *Borrelia* communities and underline their epidemiological importance as potential sites of human exposure to tick-borne pathogens.

CONCLUSION

The present study confirmed the circulation of *Borrelia* spp. in questing ticks from urban and peri-urban green spaces across Slovakia. Considerable variation in prevalence and species diversity was observed among localities, with six *Borrelia* genospecies detected. These findings highlight the ecological complexity of *Borrelia* circulation in urban environments and support the importance of continued surveillance of tick-borne pathogens in Slovakia.

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OCCURRENCE OF *ANAPLASMA* SPP. IN TICKS AND SELECTED FREE-RANGING WILDLIFE SPECIES IN THE KOŠICE REGION

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ABSTRACT

Anaplasmosis is an emerging tick-borne bacterial disease, that has received increasing attention in Europe in recent years. The aim of this study was to determine the occurrence of *Anaplasma* spp. in ticks and selected species of free-ranging wild animals in the Košice Region. A total of 180 ticks and 36 samples of parenchymatous organs collected from free-ranging wild animals were examined. The detection of *Anaplasma* spp. was performed using nested PCR targeting the *16S rRNA* gene. The detection of *Anaplasma* spp. in both ticks and free-ranging wild animals confirms the circulation of *Anaplasma* spp. in the natural ecosystems of Slovakia and highlights the importance of continuous surveillance of tick populations and wildlife.

INTRODUCTION

Tick-borne diseases represent a growing concern for both public health and veterinary medicine throughout Europe. Species of the genus *Anaplasma* are among the most important tick-borne bacterial pathogens, infecting a wide range of mammalian hosts, including wildlife, livestock, and humans. Some species, including certain strains of *Anaplasma phagocytophilum*, have zoonotic potential and pose significant public health and economic concerns, particularly for livestock production (Stuen et al. 2013).

In Europe, the tick *Ixodes ricinus* is considered the principal vector of *A. phagocytophilum*. The natural transmission cycle of the pathogen is maintained between ticks and reservoir hosts, primarily free-ranging wild ruminants (Rizzoli et al. 2014). Monitoring the occurrence of *Anaplasma* spp. in tick populations and free-ranging animals is essential for improving our understanding of the ecology and epidemiology of anaplasmosis and represents an important component of the One Health approach.

The aim of this study was to determine the occurrence of *Anaplasma* spp. in ticks and selected free-ranging wild animal species, thereby providing additional data on their circulation in the Košice Region of eastern Slovakia.

METHODS

A total of 180 questing ticks belonging to the species *Ixodes ricinus*, *Dermacentor reticulatus*, and *Dermacentor marginatus* were examined for the presence of *Anaplasma* spp. Tick collection was

carried out by the flagging method during the spring and autumn of 2024. All 180 ticks originated from the Košice Region in eastern Slovakia. Each tick was processed individually, and total DNA was extracted using the commercial BlackPrep Tick DNA/RNA Kit (Analytik Jena, Germany) according to the manufacturer's instructions. The quality and concentration of the extracted DNA were subsequently assessed using a NanoDrop™ spectrophotometer.

Considering the expected low prevalence of *Anaplasma* spp. in tick populations, individual DNA samples were subsequently pooled into groups of five. All positive pools were then re-analysed individually to identify the actual number of positive ticks.

Detection of *Anaplasma* spp. DNA was performed using nested PCR targeting the *16S rRNA* gene. The first amplification reaction employed the primer pair ACn-16S-F1 and ACn-16S-R1, while the second reaction used the primer pair ACn-16S-F2 and ACn-16S-R2 (Table 1).

Primer	**Sequence (5′–3′)**
ACn-16S-F1	CACATGCAAGTCGAACGGATTATTC
ACn-16S-R1	TTCCGTTAAGAAGGATCTAATCTCC
ACn-16S-F2	AACGGATTATTCTTTATAGCTTGCT
ACn-16S-R2	GGCAGTATTAAAAGCAGCTCCAGG

Table 1 Primers used for nested PCR amplification of the 16S rRNA gene

In addition, 36 samples of parenchymatous organs from free-ranging wildlife species collected in the Košice-okolie District, specifically from the localities of Makovica and Rozhanovce, were examined. The analysed material comprised 25 samples from red deer (*Cervus elaphus*), 4 from red foxe (*Vulpes vulpes*), 6 from European badger (*Meles meles*), and 1 from a mouflon (*Ovis musimon*). The spleen was used for DNA extraction, while liver tissue was analysed when spleen samples were unavailable. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The presence of *Anaplasma* spp. in wildlife samples was detected using the same nested PCR protocol as that applied to ticks collected from vegetation.

RESULTS

The presence of *Anaplasma* spp. DNA was confirmed in 7 of the 180 examined ticks (3.89%), including three nymphs, three males, and one female. All positive ticks were identified as *Ixodes ricinus*. Among the free-ranging wildlife examined, *Anaplasma* spp. DNA was detected only in red deer (*Cervus elaphus*). Of the 25 samples analysed, 18 (72%) were positive. All positive samples were collected from animals originating from the Makovica locality. In contrast, no *Anaplasma* spp. DNA was detected in samples obtained from red foxes (*Vulpes vulpes*), European badgers (*Meles meles*), or mouflon (*Ovis musimon*).

DISCUSSION

The results of the present study confirmed the presence of *Anaplasma* spp. in *Ixodes ricinus* tick populations in the Košice Region, indicating the continued circulation of these pathogens in this geographical area of Slovakia. Similar findings have been reported in previous studies conducted in Slovakia, where the presence of *A. phagocytophilum* in *I. ricinus* ticks was confirmed using molecular methods (Derdáková et al. 2003).

According to Stuen et al. (2013), the circulation of *A. phagocytophilum* in natural foci is strongly influenced by the presence of suitable reservoir hosts and by environmental factors affecting tick survival and activity.

Molecular analysis of free-ranging wildlife species samples demonstrated a high prevalence of *Anaplasma* spp. DNA in red deer (*Cervus elaphus*). As an important host of *I. ricinus*, this species may play a significant role in maintaining the natural transmission cycle of *Anaplasma* spp. Similar observations have been reported from eastern Slovakia, where Štefanidesová et al. (2011) confirmed the occurrence of *A. phagocytophilum* in free-ranging wild ruminants.

From the One Health perspective, free-ranging wildlife represents an important host for ticks and contributes to the maintenance of pathogen circulation in natural ecosystems. Ticks subsequently facilitate pathogen transmission among wildlife, livestock, and humans. Therefore, monitoring *Anaplasma* spp. in tick populations and free-ranging wildlife is an important tool for assessing the epizootiological and epidemiological situation and for evaluating the risk of tick-borne diseases in Slovakia.

CONCLUSION

This study confirmed the occurrence of *Anaplasma* spp. in questing ticks collected in the Košice Region and demonstrated the presence of the pathogen in free-ranging wildlife from the Košice-okolie District. Positive findings were detected exclusively in red deer (*Cervus elaphus*).

These results further support the important role of free-ranging wildlife in maintaining the natural transmission cycle of *Anaplasma* spp. and highlight their significance as hosts of ticks and potential reservoirs of the pathogen. Continued surveillance of tick populations and free-ranging wildlife, together with molecular characterisation using additional genetic markers, is essential for improving our understanding of epidemiology of anaplasmosis, enabling accurate species identification, and assessing the zoonotic risk of circulating *Anaplasma* strains within the One Health framework.

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SEASONAL DYNAMICS OF MOSQUITO ABUNDANCE AND SPECIES COMPOSITION IN RELATION TO ENVIRONMENTAL CONDITIONS IN SLOVAKIA

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ABSTRACT

Mosquito abundance is strongly influenced by environmental conditions. This study evaluated seasonal mosquito dynamics in relation to temperature and precipitation at four localities in Slovakia during 2025. Adult mosquitoes were collected using BG-Sentinel 2 traps from June to October, and meteorological data were analysed on a weekly basis. Mosquito abundance showed spatial and temporal variation, with the highest numbers generally recorded during warmer periods and declining towards autumn. In contrast, the relationship with precipitation was less consistent. These findings highlight the importance of integrating entomological and meteorological surveillance to better understand seasonal mosquito population dynamics.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are important vectors of pathogens affecting both humans and animals. Their abundance and seasonal dynamics are strongly influenced by environmental conditions, particularly temperature and precipitation, which affect mosquito development, survival and the availability of breeding habitats. However, these relationships may vary among species and local environmental conditions (Krol et al. 2023). Despite the increasing importance of mosquito surveillance in Europe, information on seasonal mosquito dynamics in relation to meteorological conditions in Slovakia remains limited. Therefore, this study aimed to evaluate seasonal variation in mosquito abundance at selected localities in Slovakia in relation to temperature and precipitation.

METHODS

Mosquitoes were collected as part of a nationwide monitoring programme conducted at 16 localities across Slovakia. Sampling was performed using BG-Sentinel 2 mosquito traps (Biogents AG, Regensburg, Germany) baited with CO_2 and operated continuously from June to October 2025. Mosquitoes were morphologically identified according to Becker et al. (2010).

For a detailed assessment of seasonal mosquito dynamics in relation to meteorological conditions, four localities (Podunajské Biskupice – SYNOP station Bratislava–Ivanka, Nitra - SYNOP station Nitra, Mojšova Lúčka - SYNOP station Žilina–Hričov and Senianske rybníky - SYNOP station Trebišov - Milhostov) were selected based on high mosquito abundance and the proximity of SYNOP meteorological stations providing corresponding meteorological data.

Meteorological data were obtained from publicly available records of the SYNOP meteorological station network and included daily mean, maximum, and minimum air temperatures and daily precipitation totals (OGIMET, 2026). To correspond with the mosquito sampling intervals, temperature data were aggregated into weekly means, while daily precipitation amounts were summed to obtain weekly precipitation totals.

RESULTS

Mosquito abundance showed pronounced temporal and spatial variation among the four monitored localities (Fig. 1). The highest abundances were generally recorded during the first half of the monitoring period, with distinct peaks occurring between weeks 24 and 29, although their timing and magnitude differed among sites. The most pronounced peak was observed in Nitra, followed by Bratislava–Ivanka, Trebišov–Milhostov and Žilina–Hričov. After the main seasonal peaks, mosquito abundance generally declined towards the end of the monitoring period and remained low during the final weeks. Abundance did not consistently coincide with weekly precipitation, as several major rainfall events occurred during periods of relatively low mosquito activity. In contrast, the main abundance peaks occurred during periods of relatively high temperatures, while the late-season decline in mosquito numbers coincided with decreasing mean, minimum and maximum temperatures.

Weekly mosquito abundance is shown together with weekly precipitation and temperature data for Bratislava–Ivanka, Nitra Žilina–Hričov and Trebišov–Milhostov. In the upper panels, green lines represent total mosquito abundance and blue bars represent weekly precipitation totals (mm). In the lower panels, green bars represent total mosquito abundance, while orange, blue and yellow lines indicate weekly maximum (Tmax), minimum (Tmin) and mean (Tavg) temperatures (°C), respectively.



Figure 1. Seasonal dynamics of mosquito abundance in relation to temperature and precipitation at four monitoring localities in Slovakia during 2025: Weekly mosquito abundance is shown together with weekly precipitation and temperature data for Bratislava–Ivanka, Nitra, Žilina–Hričov and Trebišov–Milhostov. In the upper panels, green lines represent total mosquito abundance and blue bars represent weekly precipitation totals (mm). In the lower panels, green bars represent total mosquito abundance, while orange, blue and yellow lines indicate weekly maximum (Tmax), minimum (Tmin) and mean (Tavg) temperatures (°C), respectively.

DISCUSSION

The seasonal dynamics observed in our study were generally consistent with previous mosquito monitoring studies, with the highest abundances occurring during the warmer part of the season and declining towards autumn as temperatures decreased. Temperature is an important driver of mosquito development and survival and consequently of seasonal population dynamics (Marini et al. 2016). The relationship with precipitation was less immediate, as high weekly precipitation totals often

coincided with relatively low mosquito catches. However, rainfall may affect mosquito abundance with a time lag by creating or replenishing larval habitats, with increased adult abundance becoming apparent in subsequent weeks; therefore, considering precipitation and mosquito catches within the same week may underestimate its effect (Blanco-Sierra et al. 2024). Differences among localities may further reflect local environmental conditions and species composition, highlighting the complex interaction of climatic and ecological factors shaping mosquito phenology (Campbell et al. 2023).

CONCLUSION

The present study demonstrates clear spatial and seasonal variation in mosquito abundance across the monitored localities in Slovakia. Mosquito populations reached their highest abundances during the warmer part of the season and gradually declined with decreasing temperatures, while the relationship with precipitation was less consistent. These findings highlight the importance of continuous mosquito surveillance combined with meteorological monitoring for a better understanding of seasonal population dynamics.

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THE EFFECTS OF PROBIOTIC BACTERIA ON CYTOKINES AND TRANSCRIPTION FACTORS IN SPLEENS OF MICE INFECTED WITH TAPEWORM *ECHINOCOCCUS MULTILOCULARIS*

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ABSTRACT

Probiotic bacteria produce antimicrobials - bacteriocins, and other compounds with antiparasitic and immunomodulatory effects, which involve maintaining Th1/Th2 balance. In present study mice were infected with *Echinococcus multilocularis* protoscoleces and the strains *Enterococcus faecium* AL41 (CCM8558), *Enterococcus faecium* 2019 (CCM7420), *Enterococcus durans* ED26E/7, and *Enterococcus mundtii* EM41/3 were administered daily in dose of 10^9 CFU/ml in 100 µl *per os* during the first month of the infection. Effect of individual strain application was monitored by development of cysts and cytokines in spleens up to day 120 p.i. The highest reduction of cysts was obtained using strains *E. faecium* CCM7420 (85.9%) and *E. faecium* CCM8558 (68.2%). mRNA levels for cytokines IFN-γ, IL-4 and IL-10 were upregulated during application of bacteria with a gradual decrease in the chronic stage. Individual probiotic strains induced different dynamics of cytokine mRNA levels and their concentrations in accordance with the lineage-specific transcription factors: T-bet, GATA3 and Foxp3. Study revealed that various *Enterococcus* strains can influence the host's immune microenvironment and represent a promising tool in the complementary therapy of alveolar echinococcosis in humans and animals.

INTRODUCTION

Alveolar echinococcosis (AE) is a zoonotic disease and in humans representing accidental hosts is characterized by progressive hepatic damage caused by the continuous tumor-like proliferation of the larval stage (metacestode) of the fox tapeworm *Echinococcus multilocularis*. Treatment strategy includes surgical intervention and the use of parasitostatic benzimidazoles. Experimental murine AE is immunologically characterized by an initial Th1 polarization response during the early stage of infection that gradually switches to a more dominant Th2 polarization during the chronic phase of AE (2–4 months p.i.) showing mixed Th1/Th2 profile (Vuitton and Gottstein 2010; Nono et al. 2014). The variable clinical outcomes of AE development depend on the immunological status of humans, and susceptible cases are characterised by the immunosuppression during chronic stage (Jebbawi et al. 2021). Probiotics are defined as live microorganisms, which, when consumed in adequate amounts, confer a health benefit to the host. (Saracino et al. 2021). The ability to promote health

benefits by these bacteria resides in the direct effects on other microorganisms in the gut lumen and on the mucosa as well as in the modulation of immune and non-immune cells by their products on local and systemic level (Saracino et al. 2021; Plaza- Diaz et al. 2019). In the present study we investigated the effects of an early administration of four probiotic strains on cysts development in the peritoneal cavity and modulation of mRNA expression for key cytokines and lineage-specific transcription factors in T lymphocytes.

METHODS

Mice of Balb/c strain were infected intraperitoneally with approximately 3000 *E. multilocularis* protoscoleces and were divided into five groups: infected untreated (INF) and four groups receiving daily probiotic strains of (1) *Enterococcus faecium* AL41 (CCM8558), (2) *Enterococcus faecium* 2019 (CCM7420), (3) *Enterococcus durans* ED26E/7, and (4) *Enterococcus mundtii* EM41/3 in dose of 10^9 CFU/ml in 100 µl *per os* during the first month of infection. Healthy mice (Ctrl) were included as control for each analysis. Spleens were isolated from mice on day 15, 30, 45, 60, 90 and 120 post infection and were used to obtain single cell suspensions for in vitro cytokine production by ELISA assays and RNA isolation by Trizol reagent. The prepared cDNAs served as a templates in qPCR aimed in detection of mRNA levels of cytokines IL-4, IFN-γ and IL-10 as well as T lymphocytes transcription factors T-bet, GATA3 and Foxp3. The development of parasitic infection was assessed by the cyst mass isolated from mice on indicated days.

RESULTS

In present study we monitored changes in gene expression on mRNA level of key cytokines of Th1 (IFN-γ), Th2 (IL-4) and Treg (IL-10) type of immunity in spleens of *E. multilocularis* infected untreated mice and after administration of probiotic bacteria within 120 days p.i. Expression of IL-4 was markedly upregulated by all four bacterial strains within 45 days p.i. and during this period the levels were higher in comparison with untreated group, except of day 30 p.i. Expression of IFN-γ peaked on day 30 p.i., the most after administration of *E. durans* ED26E/7. In comparison with untreated group elevated expression persisted up to day 90 p.i. in group treated with *E. faecium* CCM7420. Infection resulted in strong activation of gene for IL-10 already on day 15 p.i. followed by drop on day 30 and moderate elevation from day 90 p.i. The most potent persisting upregulation of IL-10 was found in infected mice treated with *E. faecium* CCM7420.

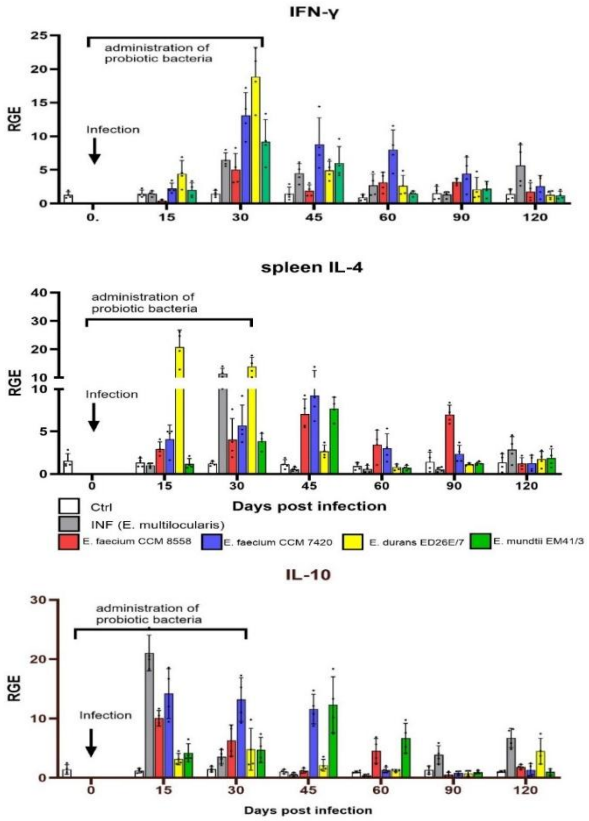


Figure 1. Relative gene expression (RGE) of cytokines in spleens of healthy, infected untreated mice and infected and treated with four Enterococcus probiotic strains.

mRNA levels of T-cell lineage commitment transcription factors *T-bet* (Th1), *GATA3* (Th2), and *Foxp3* (Tregs) by qPCR showed the similar trend in modulation of T cell specific immunity by individual bacteria within monitored period. Administration of bacteria resulted in parasitostatic effect persisting within 3 months after termination of therapy confirmed by slower growth of *E. multilocularis* cysts compared to the untreated group of mice. The highest reduction effect was shown by strains *E. faecium* CCM7420 (85.9%) and *E. faecium* CCM8558 (68.2%).

DISCUSSION

Study showed the ability of selected strains of probiotic bacteria to significantly suppress the growth of cysts in the peritoneal cavity of mice, with the strongest effect being achieved with application of *E. faecium* CCM7420. We assume that probiotic bacteria alleviated cyst growth with their specific products, which entered the bloodstream from the intestine and influenced the local and systemic anti-parasitic immune responses. Indeed, it was shown that products secreted by strains in gut may have also anthelmintic effects and can reduce the virulence of many parasites and for this reason probiotics may be an integral part of helminth parasite control strategies (Berrilli et al. 2012). Analysis of gene expression for cytokines IFN-γ, IL-4 and IL-10 in spleens revealed a significant effect of probiotic strains on T cells during their application (first month p.i.) with a gradual reduction in the chronic stage. Individual probiotic strains induced different dynamics of cytokine expression and

corresponding transcription factors, especially in the case of IFN-γ and IL-4. The reduction in cyst growth in the chronic phase was probably initiated by stimulation of T lymphocytes producing the aforementioned cytokines already in the early phase of infection. In case of murine experimental trichinellosis, application of probiotic strains and their products bacteriocins stimulated phagocytosis and metabolic activity of blood phagocytic cells (Vargová et al. 2023) and reduced parasitic burden due to lowering their reproductive capacity (Petrová et al. 2024).

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SERUM PROTEIN ELECTROPHORETIC COMPARISON IN DOGS WITH *BABESIA GIBSONI* AND *BABESIA CANIS* INFECTION

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ABSTRACT

Canine babesiosis varies widely in clinical presentation and immune response depending on the infecting *Babesia* species, complicating diagnosis and prognosis. The aim of the study was to compare serum protein fraction profiles in dogs naturally infected with *B. gibsoni* versus *B. canis.* Seventeen dogs with *B. gibsoni* and forty with *B. canis* infection were diagnosed by PCR. Serum protein electrophoresis measured albumin, α_1, α_2, β_1, β_2, and γ-globulins; albumin/globulin ratio and total protein were determined. *B. gibsoni*-infected dogs showed significantly lower relative albumin, α_1, α_2 and A/G ratio, and markedly higher relative and absolute γ-globulins and TP, whereas β-fractions showed no consistent overall difference. *B. gibsoni* infection produces a distinct, more chronic inflammatory serum protein pattern than *B. canis*, characterized by hypergammaglobulinemia and reduced A/G ratio.

INTRODUCTION

Canine babesiosis is an important parasitic disease with variable clinical severity depending on the *Babesia* species involved. *B. canis* typically causes an acute disease with marked hemolytic anemia, hemoglobinuria, fever, and icterus, whereas *B. gibsoni* often leads to milder, chronic, or subclinical infection, although severe disease may also occur (Karasová et al. 2022a).

The aim of the study was to evaluate differences in serum protein fractions in dogs naturally infected with *B. gibsoni* and to compare them with dogs infected with *B. canis*.

METHODS

The study included 17 client-owned dogs naturally infected with *B. gibsoni* and 40 dogs naturally infected with *B. canis*. Blood was collected for PCR confirmation of babesiosis, and serum protein electrophoresis.

DNA was first extracted from EDTA blood and screened by PCR targeting the 18S rRNA gene to detect *Babesia* spp. and positive samples were then tested with *B. gibsoni*-specific primers. The PCR products were visualized on agarose gels, purified, sequenced, and compared with GenBank references to confirm species identity (Karasová et al. 2022b).

Serum protein fractions were measured as relative and absolute values, including albumin, α_1-, α_2-, β_1-, β_2-, and γ-globulins, and the albumin/globulin ratio (A/G) was calculated. The total protein concentrations (TP, g/l) were determined using an automated biochemical analyser Alizé (Lisabio, Poully en Auxois, France) according to the biuret method with commercially available diagnostic kits (Randox, Crumlin, United Kingdom).

Statistical comparisons were made between the *B. gibsoni* and *B. canis* groups, using unpaired t-test, carried out by using the program GraphPad Prism V5.02 (GraphPad Software Inc., California, USA).

RESULTS

Dogs infected with *B. gibsoni* showed a distinctly different serum protein profile from dogs infected with *B. canis*. Relative albumin, α_1-globulins, α_2-globulins, and A/G ratio were significantly lower in the *B. gibsoni* group, while relative γ-globulins were markedly higher. The relative β_1- and β_2-globulin fractions were not significantly different overall (Table 1).

Parameter	***B. gibsoni***	***B. canis***
Albumin	34.9 ± 6.6	44.8 ± 6.2*
α_1-Globulins	3.5 ± 0.6	5.1 ± 1.2*
α_2-Globulins	11.5 ± 1.8	16.6 ± 4.0*
β_1-Globulins	11.2 ± 2.7	12.9 ± 4.1
β_2-Globulins	11.1 ± 2.0	11.1 ± 2.7
γ-Globulins	27.8 ± 5.9	9.4 ± 2.5*
A/G	0.55 ± 0.16	0.83 ± 0.21*

*Table 1. Differences in the relative concentrations of serum protein fractions (%) and albumin/globulin ratio (A/G) between dogs infected with B. gibsoni and B. canis (mean ± SD). A/G, albumin/globulin ratio; *Significance of the differences between B. gibsoni and B. canis infected dogs at $P < 0.001$.*

In absolute terms, *B. gibsoni* dogs had significantly higher total serum protein and higher β_1-, β_2-, and γ-globulin concentrations than dogs infected with *B. canis*. The increase in γ-globulins was especially pronounced and was described as roughly threefold higher in *B. gibsoni* infection (Table 2). A subset of *B. gibsoni* infected dogs also showed a double α_2-zone or β-γ bridging on electrophoretograms, suggesting marked alterations in the protein pattern (Figure 1).

Parameter	*B. gibsoni*	*B. canis*
TP	77.2 ± 6.7	56.2 ± 7.6†
Albumin	26.7 ± 3.9	25.3 ± 5.1
α_1-Globulins	2.7 ± 0.4	2.8 ± 0.5
α_2-Globulins	8.8 ± 1.4	9.4 ± 2.7
β_1-Globulins	8.7 ± 2.6	7.2 ± 2.3*
β_2-Globulins	8.6 ± 1.7	6.3 ± 1.8†
γ-Globulins	21.7 ± 6.3	5.3 ± 1.6†

*Table 2. Differences in the concentrations of total serum proteins (TP, g/l) and absolute values of protein fractions (g/l) between dogs infected with B. gibsoni and B. canis (mean ± SD). TP, total proteins; *,†Significance of differences between B. gibsoni and B. canis infected dogs: *P < 0.05, †P < 001.*

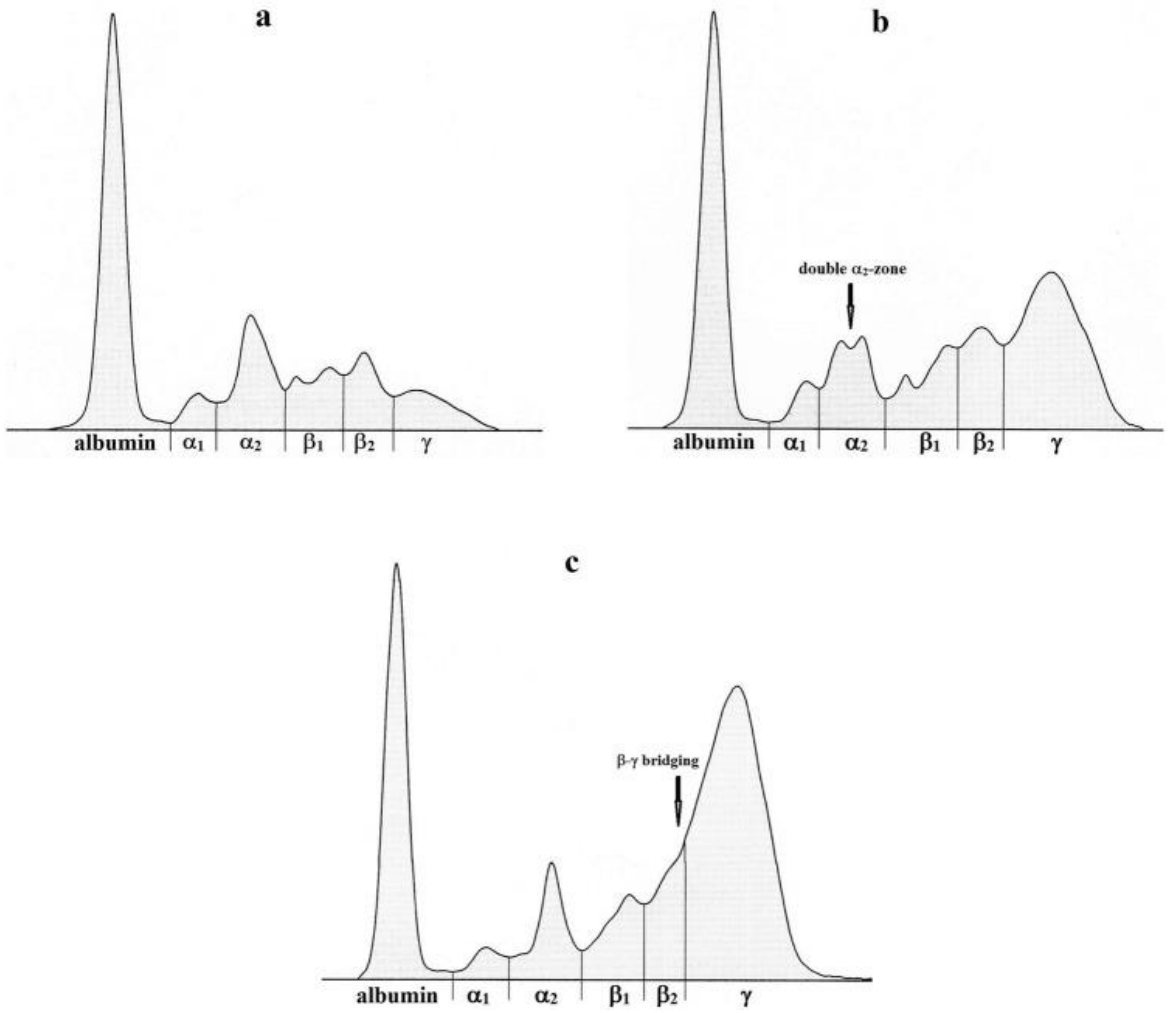


*Figure 1. Representative electrophoretograms characterizing the differences in serum protein fractions in dogs infected with B. canis (**a**) and B. gibsoni (**b**, **c**).*

DISCUSSION

The results suggest that *B. gibsoni* and *B. canis* cause different types and magnitudes of host response. The low albumin and low A/G ratio in *B. gibsoni* infected dogs likely reflect severe inflammatory activity and increased globulin production rather than simple protein loss alone. The markedly increased γ-globulins are consistent with chronic antigenic stimulation and strong humoral immune response.

The lower α-globulin fractions in *B. gibsoni* dogs contrast with the higher α-fractions seen in *B. canis* infection, which may indicate differences in acute-phase response between the two species. A double α_2-zone and β–γ bridging may reflect more severe intravascular hemolysis or altered immunoglobulin distribution. Overall, the study supports the view that *B. gibsoni* infection may induce a more chronic inflammatory protein pattern than *B. canis*.

CONCLUSION

This study shows that serum protein electrophoresis can distinguish the laboratory response of dogs infected with *B. gibsoni* from those infected with *B. canis*. *B. gibsoni* was associated with higher total proteins and γ-globulins, and lower albumin and A/G ratio. These findings may help improve interpretation of serum protein profiles in canine babesiosis and support further research into the metabolic and inflammatory effects of *Babesia* spp. infections.

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SUCCESSFUL STABILIZATION OF A THERAPEUTICALLY CHALLENGING FIV-POSITIVE CAT

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ABSTRACT

Feline immunodeficiency virus (FIV) predisposes cats to chronic inflammatory conditions that may contribute to insulin resistance (IR) and complicate the management of diabetes mellitus (DM). A 5-year-old rescue cat was diagnosed with FIV, DM and severe gingivitis. Histopathological examination of gingival tissue confirmed eosinophilic granuloma complex (EGC). Because corticosteroid therapy could further impair glycaemic control, premolars with grade 2 – 3 mobility were extracted instead, and treatment with a diabetic diet and Caninsulin (2 U/cat BID) was initiated. Resolution of oral inflammation resulted in improved insulin sensitivity and successful diabetic stabilization without corticosteroid administration. This case highlights the role of chronic inflammation as a reversible cause of IR and demonstrates that targeted management of the underlying inflammatory condition may facilitate glycaemic control in FIV-positive diabetic cats.

INTRODUCTION

Feline immunodeficiency virus is a lentivirus of the *Retroviridae* family that causes lifelong infection in domestic cats through integration of proviral DNA into the host genome. The course of infection consists of three stages: an acute phase characterized by transient viraemia and lymphadenopathy, a prolonged asymptomatic phase during which infected cats may remain clinically healthy for several years, and a terminal phase associated with immunosuppression and opportunistic infections (Akhtar et al. 2025, McDonnel et al. 2013). Although many infected cats remain clinically asymptomatic for years and may have a normal life expectancy under appropriate management, progressive immunosuppression predisposes them to opportunistic infections, neoplasia and other secondary diseases (Akhtar et al. 2025, Westman et al. 2019).

Diabetes mellitus is associated with impaired immune function, predisposing affected cats to secondary infections through abnormalities in cell-mediated immunity and impaired phagocyte function (Greco et al. 1994). Consequently, infections involving the skin, urinary tract, hepatobiliary system, and other tissues occur more frequently and are often more severe in diabetic patients, with urinary tract infections representing the most common secondary infection in diabetic cats (Chair et al. 2025, Hartmann et al. 2022). Conversely, concurrent infections and chronic inflammatory diseases are important contributors to IR and diabetic decompensation through the endogenous release of

counter-regulatory hormones, including cortisol. Therefore, identification and treatment of concurrent inflammatory conditions are essential for improving insulin sensitivity and achieving optimal glycaemic control in cats with DM (Hartmann et al. 2022).

However, despite the well-recognized association between chronic inflammation and insulin resistance, reports describing cats with concurrent FIV and DM remain scarce, and evidence-based recommendations for their clinical management are lacking. In a retrospective case-control study of 127 FIV-positive cats, only 9 (7.1%) were diagnosed with concurrent DM (Sykes 2013). Nevertheless, the coexistence of both disorders presents a therapeutic challenge because FIV-associated immunosuppression predisposes affected cats to chronic inflammatory disease, while persistent inflammation further aggravates IR and impairs glycaemic control. Therefore, recognition and resolution of concurrent inflammatory conditions may play a pivotal role in achieving diabetic stabilization in such patients.

METHODS

A 5-year-old male rescue cat was presented with a history of polyuria, polydipsia, progressive weight loss, and severe oral disease associated with reduced food intake due to gingival pain. On physical examination, the cat was moderately dehydrated and exhibited severe generalized gingivitis with advanced periodontal disease. Diagnostic interventions included a complete blood count, serum biochemistry profile, measurement of blood glucose concentration, and serological testing for FIV and feline leukaemia virus using a SNAP FIV/FeLV Combo Plus assay (IDEXX). Initial stabilization consisted of the introduction of a commercially available diabetic diet and insulin therapy with Caninsulin® (40 IU/ml, MSD) at a starting dose of 2 U/cat every 12 hours. Following stabilization, a comprehensive oral examination was performed under general anaesthesia, during which gingival biopsy specimens were obtained for histopathological examination. Teeth with grade 2 – 3 mobility were subsequently extracted.

RESULTS

Histopathological examination of the gingival tissue confirmed ECG as the underlying cause of the severe oral inflammation. Following extraction of the premolars, progressive resolution of the gingival inflammation was observed during follow-up, resulting in marked improvement in the overall clinical appearance of the oral cavity. The reduction of the chronic inflammatory burden was accompanied by improved glycaemic control, allowing successful stabilization of DM without the need for corticosteroid therapy.

DISCUSSION

Reported therapeutic approaches primarily rely on system immunomodulatory agents targeting T-cell-mediated inflammation, particularly glucocorticoids (Moon et al. 2017). Although glucocorticoids are considered the treatment of choice because of their rapid and effective anti-inflammatory action, their use in diabetic cats is problematic because they promote insulin resistance and may impair glycaemic control (Lowe et al. 2008). Moreover, glucocorticoid therapy has been identified as a predisposing factor for the development of DM in cats, with one study reporting that high-dose prednisolone administration (≥ 1.9 mg/kg) once daily for more than 3 weeks resulted in DM in 9.7% of treated cats (Nerhagen et al. 2020). Consequently, extraction of the affected premolars was performed to remove the source of persistent oral inflammation, thereby avoiding systemic corticosteroid administration.

Oral disorders, particularly gingivitis and periodontal diseases, have a bidirectional relationship with DM, where chronic oral inflammation may impair glycaemic control, while persistent hyperglycaemia further promotes the development and progression of oral disease. Furthermore, DM alters the oral environment by reducing salivary flow, modifying salivary pH, and increasing salivary glucose concentrations, thereby promoting bacterial proliferation and progression of periodontal disease. However, evidence of the precise relationship remains limited, and the impact of oral health interventions on diabetic regulation in cats require further investigation (Hrițcu et al. 2025). An additional challenge in the present case was that severe oral disease reduced voluntary food intake, thereby further complicating glycaemic stabilization and insulin therapy.

Similar to the bidirectional relationship between oral inflammatory disease and DM, FIV infection and DM may interact to create a vicious cycle of immune dysfunction and chronic inflammation. FIV-associated immunosuppression predisposes affected cats to persistent inflammatory and infectious diseases, which may exacerbate insulin resistance and impair glycaemic control. Conversely, DM further compromises immune function and delays resolution of inflammatory processes, potentially perpetuating chronic disease. Although neither condition is curable, appropriate recognition and management of concurrent inflammatory disorders may interrupt this cycle and facilitate successful stabilization of concurrent disorders.

CONCLUSION

Successful diabetic stabilization in this FIV-positive cat with concurrent DM and severe oral disease was achieved through resolution of the underlying oral inflammation rather than conventional immunosuppressive therapy. This case demonstrates that identification and management of concurrent inflammatory disease may represent a crucial component of diabetic treatment and should be considered before intensifying insulin therapy in cats with poor glycaemic control.

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HEALTH OUTCOMES OF TICK-BORNE ENCEPHALITIS IN THE BANSKÁ BYSTRICA REGION (2023–2024)

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ABSTRACT

The incidence of tick-borne encephalitis (TBE) in Slovakia has an increasing trend. The aim of the study was to analyze the occurrence of long-term health outcomes one year after TBE in patients from the Banská Bystrica region and to identify risk factors for their development. The sample consisted of 93 patients with confirmed TBE from 2023–2024, of whom 88 were evaluated after a year through a telephone survey as part of epidemiological surveillance. Difficulties were coded into subjective and objective. Relationships were analyzed using the McNemar test and multivariate Poisson regression analysis ($\alpha = 0.05$). Complete recovery was reported by 59.1% of patients, while 40.9% (n = 36) suffered from long-term sequelae (subjective: 21.6%; objective: 19.3%) with zero overlap between categories. Subjective manifestations were dominated by fatigue and headache, while objective findings were dominated by paresis. The regression model identified age as the only predictor of subjective sequelae ($p = 0.0094$), with persons over 45 years of age paradoxically having a 67.6% lower risk. For objective sequelae, the only predictor was the severity of the acute phase ($p = 0.0196$; RR = 3.121). TBE in the Banská Bystrica region represents a serious public health concern, as post-encephalitic syndrome affects up to 41% of patients. Younger adults exhibit a higher risk of subjective complications, whereas objective neurological deficits are conditioned by a severe acute course. The zero vaccination rate in the cohort highlights the urgent need to modify the current immunization strategy and introduce full vaccine reimbursement in endemic areas.

INTRODUCTION

In the European region, tick-borne encephalitis (TBE) and Lyme borreliosis represent the two most clinically severe tick-borne neuroinfections. Despite the availability of effective specific prevention in the form of vaccination, the incidence of TBE in Slovakia has exhibited a long-term upward trend (ÚVZ SR, 2024). While the Trenčín region historically recorded the highest incidence of TBE, the Banská Bystrica and Žilina regions have dominated since 2015, a geographic shift attributed to the migration of ticks to higher altitudes driven by global warming (Peťko et al. 2014). While the acute course of the infection typically requires inpatient treatment and intensive therapy, health complications persisting after the resolution of the acute phase are increasingly becoming a focal point of medical research. Overcoming TBE leaves long-term health outcomes in many patients, which negatively impact their social, personal, and professional lives, while concurrently burdening

the healthcare system. The aim of this study was to analyse the long-term health outcomes in patients from the Banská Bystrica region one year after overcoming TBE and to identify selected risk factors that statistically significantly influence the probability of their development.

METHODS

The sample consisted of 88 patients from the Banská Bystrica region with confirmed TBE in 2023 and 2024, classified in the Epidemiological Information System (EPIS) according to ECDC criteria (EU, 2018). In order to eliminate selection bias, febrile forms were also included in the sample. As part of the descriptive analysis, the sample was monitored for a spectrum of selected demographic, clinical, and epidemiological parameters. After one year from the acute phase (± 1 month), the patients were contacted by telephone by the same epidemiology worker as part of standard epidemiological surveillance (informed consent was not required). During the interview, a standardized question was asked: "Has your health status or quality of life changed after overcoming TBE compared to the period before the disease?" The reported complaints were coded as subjective and objective (measurable neurological findings). The data were processed using MS Excel and R (version 4.6.1, RStudio). McNemar's exact test was used to compare the prevalence of subjective and objective outcomes. The influence of risk factors on the occurrence of complications was assessed by multivariate Poisson regression analysis, with the expression of relative risk (RR) and a 95% confidence interval (95% CI). The significance level was set at $\alpha = 0.05$.

RESULTS

Analysis of the cohort of 88 patients with a one-year interval since overcoming TBE confirmed complete recovery in 59.1% (n = 52) of cases. The identified long-term outcomes were divided into subjective (21.6%; n = 19) and objective (19.3%; n = 17) categories. The dominant subjective manifestations were fatigue and headache, while objective findings were dominated by paresis (13.6%; n = 12). Additionally, 2 deaths were recorded (2.3%). Although a higher percentage of subjective complaints was recorded (21.6%) compared to objective ones (19.3%), McNemar's exact test did not confirm this difference as statistically significant ($p = 0.868$). Multivariate Poisson regression analysis identified risk factors: subjective outcomes affected patients over 45 years of age less frequently (RR = 0.324), whereas the occurrence of objective outcomes was significantly increased by the severity of the acute phase of the disease (RR = 3.121).

DISCUSSION

The prevalence of sequelae one year after the acute phase of TBE reached 41%, which confirms the high morbidity of the disease in accordance with international data (Bogovič et al. 2018). Although the occurrence of subjective complaints exceeded objective findings, McNemar's exact test did not

confirm this difference as statistically significant. Both forms of PES are therefore quantitatively comparable, which partially contradicts studies where subjective complaints significantly dominated (Mickienė et al., 2002). Multivariate Poisson regression analysis revealed diametrically different profiles of independent predictors. For subjective outcomes, the only significant predictor was age ($p = 0.0094$), with a paradoxical trend of higher risk in younger individuals, which is in contradiction with the favorable effect of young age reported in some studies (Grosa et al. 2026). This phenomenon may reflect a distinct immunopathological profile of the younger organism with a tendency towards subclinical neuroinflammation (Grygorczuk a spol. 2018). For objective outcomes, the only independent predictor was the severity of the acute phase of the disease ($p = 0.0196$), which correlates with the results of several studies (Slížek a spol. 2026; Mickienė a spol. 2002).

CONCLUSION

The presented study confirms the significant public health dimension of TBE in the Banská Bystrica region, where up to 41% of patients exhibit long-term health outcomes even one year after overcoming the acute phase. A comparable occurrence of subjective symptoms and objective findings was recorded, with both forms fundamentally impacting the personal and professional lives of patients. The complete absence of vaccination in the monitored cohort appears to be the primary modifiable factor responsible for the unnecessary clinical burden on patients and the enormous economic pressure on the healthcare system. This critical situation underscores the urgent need to revise the national public health strategy. The new concept should focus on introducing full vaccine reimbursement by health insurance companies for residents of the highest-risk endemic areas, regardless of age, such as the Banská Bystrica region. Such a measure would fundamentally reduce not only the incidence of severe neuroinvasive forms but also the long-term outcomes of TBE.

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EXPANDING THE GEOGRAPHIC RANGE OF KEMEROVO VIRUS: SEROLOGICAL EVIDENCE FROM CENTRAL EUROPEAN AUTOCHTHONOUS HORSES

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ABSTRACT

Kemerovo (KEMV) and Tribeč (TRBV) viruses are neurotropic, tick-borne orbiviruses. To investigate their geographic occurrence, surveillance for KEMV and TRBV antibodies was conducted using serum from autochthonous horses living in northeastern Hungary. Initial virus neutralization testing revealed 32% orbivirus seropositivity. No serum samples reacted exclusively to TRBV; nine were positive for KEMV (18%), while seven cross-reacted (14%) with TRBV and KEMV. $PRNT_{90}$ confirmed KEMV seroconversion in three other double-positive samples. This first detection of KEMV infection in autochthonous horses indicates a broad geographic range from Western Siberia to Central Europe.

INTRODUCTION

Kemerovo virus (KEMV) and Tribeč virus (TRBV) are tick-borne orbiviruses (genus *Orbivirus*; family *Sedoreoviridae*). KEMV was isolated from *Ixodes persulcatus* in the 1960s in the Western Russia (Chumakov et al. 1963). TRBV was isolated from *I. ricinus* ticks in Czechoslovakia soon after (Libíková et al., 1964). Despite research from the 1960s–1980s showing their potential as neurotropic agents in humans (Libíková et al. 1978), these viruses have been left off encephalitis diagnostic differential lists, and current knowledge of their occurrence in humans and animals in Central Europe is limited. Results have demonstrated both seropositivity (Peňazziová et al., 2022) and viral isolation (Schmidt & Shope, 1971) in migrating birds on and off the European continent. Surveillance of neglected viruses is ever more important as disease dynamics alter in a rapidly changing global climate. By conducting surveillance for KEMV and TRBV antibodies using neutralization assays on blood serum samples from autochthonous horses living in northeastern Hungary, we aim to investigate the level of seropositivity to these viruses and expand our understanding of their regional spread.

METHODS

Fifty serum samples from clinically healthy autochthonous horses from northeastern Hungary were tested for TRBV and KEMV antibodies by virus neutralisation test (VNT) at final dilution 1:10. Samples showing cross-reactivity were further tested by plaque reduction neutralization test (PRNT). Heat-inactivated (56 ◦C for 30 min) and diluted serum samples were mixed with equal volume of virus culture of KEMV (kindly provided by Professor Gerhard Dobler, Germany) and TRBV strain 16.C/16 (Loziaková Peňazziová et al. 2026). Vero E6 cells were added to each well and plates were incubated for three days.

Previous studies indicate cross-serological reactions among TRBV and KEMV viruses co-circulating in Central Europe. Six-well plates with Vero E6 cells were prepared one day before. The cultivation medium was discarded, and each serum dilution-virus mixture was used for the infection of one well. After adsorption, the serum-virus mixture was discarded and replaced with semisolid medium. Plates were further incubated and plaques were counted in each serum dilution after staining with crystal violet. PRNT end-point titres are the reciprocal of the last serum dilution that shows a desired percent reduction in plaque counts.

RESULTS

Due to insufficient data regarding the criteria for distinguishing tick-borne orbivirus infections and potential cross-reactivity, we initially conducted a PRNT using serum samples TRBV (Institute of Virology, Biomedical Research Center of the SAS) and KEMV-specific mouse sera to establish a cut-off percentage for plaque reduction.

Analysing the percentage reduction across the entire serial dilution range, it was observed that using TRBV antigen with homologous serum exhibited plaque reduction exceeding 66%, reaching the $PRNT_{90}$ at dilution 1:160. Conversely, the anti-KEMV serum showed less than 50% reduction in each dilution tested. In the case of the KEMV antigen, homologous mouse serum reached $PRNT_{90}$ at dilution 1:640 and the anti-TRBV serum reached $PRNT_{90}$ at dilution 1:40. However, in subsequent dilutions, the PRNT fell below 90%, but in neither case below 50%. These findings indicated that a more rigorous $PRNT_{90}$ expression for the endpoint is effective in distinguishing infections in animals exposed to antigenically similar TRBV and KEMV.

In total, sixteen (32%) serum samples showed positivity either from KEMV or TRBV antibodies in the screening VNT. Interestingly, no sample reacted only with TRBV antigen. Each sample which showed neutralization was KEMV or KEMV+TRBV positive. Out of the seropositive samples, nine reacted only with KEMV, and seven cross-reacted with KEMV and TRBV, corresponding to 18% KEMV and 14% KEMV+TRBV seropositivity. The KEMV+TRBV positive samples were further tested by endpoint $PRNT_{90}$. Further differentiation between KEMV and TRBV seropositivity was

based on at least a 4-fold difference of the $PRNT_{90}$ titer. Due to limited volume of one KEMV+TRBV positive sample, only six horse serum samples were subjected for $PRNT_{90}$ titration. Three samples cross-reacted with both antigens, out of which two have $PRNT_{90}$ titer in favor of KEMV, and one in favour of TRBV. However, none of the $PRNT_{90}$ titer reach a four-fold difference. Therefore, orbivirus infections in these animals could not be differentiated. In three other samples the difference was at least 4-fold and the KEMV $PRNT_{90}$ reached 1:80 and 1:320 titer values.

DISCUSSION

Up to now, the presence of KEMV in natural environments has been documented in Western Siberia and the Ural Mountains (Chumakov et al., 1963; Dedkov et al., 2014; Tkachev et al., 2017).

A serological investigation conducted on wild birds in Slovakia indicates its detection in a previously unreported geographic region (Peňazziová et al., 2022). In our research, we found autochthonous horses living in northeastern Hungary seropositive for KEMV infection. These findings raise questions how horses in Central Europe get infected. The main vector of the KEMV is *I. persulcatus* tick, yet according to tick distribution maps from the European Centre for Disease Prevention and Control, there is no evidence of this tick species being present in Slovakia, Poland, or Hungary. Conversely, I. *persulcatus* has been reported in northern Ukraine (ECDC, 2022). A plausible hypothesis is that KEMV may utilize an alternative vector capable of replication and transmission to horses. In Central Europe, *I. ricinus* represents the principal medically significant tick species capable of transmitting TRBV. Recent research demonstrated that an artificial feeding system enabled *I. ricinus* to acquire KEMV and subsequently transmit it transstadially; however, these ticks were unable to transmit the virus to IFNAR-/- or BALB/c mice (Migné et al., 2022, 2024).

CONCLUSION

This is the first study investigating KEMV infection in autochthonous horses in northeastern Hungary. Results indicate a wide geographic occurrence spanning from Western Siberia to Central Europe. Although the role of horses as tick-borne orbivirus reservoirs remains unclear, targeted monitoring effectively surveys zoonotic arbovirus introductions into new geographic areas.

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CURRENT OVERVIEW OF MOSQUITO'S SPECIES IN THE KOŠICE METROPOLITAN AREA IN EASTERN SLOVAKIA AND RISK THEIR SPREADING

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ABSTRACT

For the past 25 years, we have been monitoring the occurrence of mosquitoes in Košice. Increasing urbanization and global changes are contributing to the spread of several mosquito species of great importance to public health. Every year, we record the species composition of mosquitoes at eight locations in Košice. While we collected 6 species in 2000, by 2025 that number had risen to 16 species, including the invasive *Aedes j. japonicus*. This relatively large mosquito feeds on blood during the day and is a confirmed vector and potential vector of viruses of the genus *Flavivirus* as well as parasitic filariae. Underestimating the spread of mosquitoes in urban environments could have a negative impact on the health of both humans and animals.

INTRODUCTION

Due to changes in environmental conditions, selective pressure, and human activity, new and emerging (so-called "emerging and re-emerging") mosquito-borne pathogens are being diagnosed with increasing frequency, with dramatic consequences for both humans and animals. Over the past century, anthropogenic activity has significantly influenced the global climate. Climate change is likely to dramatically affect ecosystems, particularly by altering the distribution and abundance of ecothermic vector species. The physiology, life cycle, behaviour, and ability to transmit pathogens in mosquitoes are highly dependent on ambient temperature (Cator et al. 2020). Temperature can also alter the life cycles of pathogens in individual mosquito species, in humans, and in other vertebrate hosts, and is typically a key factor limiting the establishment of invasive species in temperate regions and determining their seasonal abundance and distribution (Brugueras et al., 2020). Since 2000, we have observed an increasingly frequent rise in the number of mosquito species in cities (Medlock et al., 2012), and Košice is no exception. This risk is reflected in the increasing prevalence of canine heartworm disease (Miterpáková et al. 2023). In this context, it is abundantly clear and research in Slovakia confirms this (Čabanová et al. 2018; 2021) that many mosquito species have adapted to the climatic conditions in new regions and are colonizing non-endemic areas (Couper et al. 2021). The goal of our research is to monitor the occurrence and changes in species composition in Košice, Slovakia's second-largest city, and to highlight the associated public health risks.

METHODS

To determine the species composition of mosquitoes in the city of Košice, traps were set up on a regular and occasional basis at the following locations: the Ťahanovce housing area, the Nad Jazerom housing area, the Public Cemetery, Riding area of UMVP in Košice, the Botanical Garden, the Zoo, Anička Park, and the UVMP campus in Košice.

We trapped mosquitoes using an entomological net with a diameter of about 35 cm and a lightweight, sturdy handle, or an electric insect exhauster, usually in the early evening (18:00-21:00). We transferred the captured individuals to labelled plastic containers containing a small amount of paper wadding moistened with ethyl acetate. In addition, at stable sites, we used CDC Miniature Light Traps for collection; in the early years, these were baited with dry ice, and starting in 2017, for easier handling, we used a pressurized canister containing carbon dioxide (CO_2) as an attractant. We identified the captured mosquitoes under a Zeiss-Stemi DV-4 microscope based on morphological characteristics according to available diagnostic keys (Kramář 1958; Becker et al. 2010). Mosquitoes that were damaged and difficult to identify morphologically were identified using PCR over the past 6 years. Molecular identification was based on sequencing partial sequences of the mitochondrial cytochrome c oxidase subunit I gene (cox-1), which serves as a DNA barcode for the species. DNA extraction was performed using the commercial DNeasy Blood and Tissue Kit (QIAGEN, GmbH, Hilden, Germany) according to the manufacturer's instructions. We visualized the amplified products on a 1% agarose gel under UV light. We sent the positive PCR products to the commercial laboratory SEQme (Dobříš, Czech Republic) for purification and sequencing. The resulting sequences were analysed and edited using MEGA X software, version 10.1.5, build 10191107 (Kumar et al., 2018). We compared the resulting sequences with those stored in GenBank using the BLAST nucleotide algorithm at the National Centre for Biotechnology Information (NCBI).

RESULTS

Between 2000 and 2010, we collected 9 species in the city, namely: *Aedes vexans*, the *Anopheles maculipennis* complex, *An. plumbeus*, *Culex pipiens*/*torrentium*, *Ochlerotatus cinereus, Oc. cantans/annulipes, Oc. caspius, Oc. geniculatus*, and *Oc. sticticus*. We recorded the highest prevalence in: *Culex pipiens/torrentium* (43.5%) and *Aedes vexans* (21.5%). In individual years, abundance and species composition were significantly influenced by adverse climatic conditions, high temperatures during the summer season, and low humidity. In the subsequent years, 2011–2020, we captured an additional 5 species in the urban environment, namely: *Culiseta annulata*, *Culex modestus*, *Oc. cantans/annulipes*, *Oc. geniculatus*, and *Oc. cataphylla*. The results indicate the dominant presence of 3 species: *Aedes vexans* (57.9%), *Culex pipiens/torrentium* (23.9%), and *Oc. sticticus* (11.5%) - using molecular diagnostics, we confirmed a 100% match for this mosquito with

PP941679.1 (Italy). In addition, using PCR, we confirmed a 99.6% match of *An. maculipennis* with AF491687.1, Ioannina, Greece. In 2024, we collected *Anopheles messeae* on the University campus with a 99.4% match to *An. messeae* HE659641.1, Russia: Tomsk area; and *Aedes japonicus japonicus* with a 99.8% match to *Ae. j. japonicus* OR668894.1, Italy: Friuli-Venezia-Giulia.

DISCUSSION

Research on mosquitoes in Slovakia has a long tradition dating back more than 200 years, but the first significant scientific studies on mosquitoes of the genus *Anopheles* were not published until the first half of the 20th century (Korbela 1952). Kramář (1952) made a significant contribution to expanding knowledge about mosquitoes and their ability to transmit pathogens in Slovakia, as did later researchers Trpiš, Vostál, Országh, Danielová, Labuda, Jalili, and Halgoš. In eastern Slovakia, Bocková (2013) updated the research on mosquitoes in an extensive dissertation. Currently, studies by research teams led by Čabanová (2018; 2021) are highly valuable. The occurrence of invasive mosquitoes such as *Ae. albopictus*, *Ae. j. japonicus*, and *Ae. koreicus* raises legitimate concerns. Together with *Ae. vexans* (Bocková, 2013), they are among the major vectors of viruses and parasites in Slovakia. The presence and spread of many mosquito species in urban environments particularly invasive ones constitute a serious public health problem. Given their opportunistic behaviour and high vector competence, they pose a potential risk of transmitting pathogens to humans even in urban environments.

CONCLUSION

The present results revealed an increase of the mosquito fauna of Košice in a total number of 16 species during the last 25 years confirming the presence of the invasive species *Ae. j. japonicus*.

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TRENDS IN MEDICINAL CONSUMPTION AND PHARMACEUTICAL WASTE MANAGEMENT IN THE SLOVAK REPUBLIC: ENVIRONMENTAL IMPLICATIONS AND PUBLIC AWARENESS

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ABSTRACT

The irrational handling of unused medicines poses a serious environmental threat to water, soil, and air, while simultaneously endangering public health. This study analyzes medicinal consumption trends in the Slovak Republic (SR) from 2015 to 2024 and evaluates public disposal habits through a survey of 88 respondents. Results indicate a significant increase in both pharmaceutical consumption and waste tonnage, with costs for disposal rising from approximately 332 000 € in 2015 to over 971 000 € in 2024. While 85.23% of respondents return medicines to pharmacies, a concerning 17.05% still utilize municipal waste, and awareness of the actual disposal process remains low at 23.9%.

INTRODUCTION

The incorrect disposal of unused and expired medicines represents a potential risk to the environment and the health of both humans and animals. As medicinal consumption grows globally, so does the penetration of pharmaceutical active substances into the ecosystem (Snopková et al. 2021). In modern medicine, over 3 000 different active substances are utilized, and more than 200 compounds have already been identified in the environment (Mackuľak et al. 2017). These substances, classified as hazardous waste, often enter the environment through municipal wastewater treatment plants, which are frequently incapable of fully extracting these micropollutants (Castiglioni et al. 2006). The presence of pharmaceutical residues including psychoactive drugs, antibiotics, and analgesics leads to increased bioavailability, bioaccumulation, and biomagnification across the food chain. For instance, residues can enter the soil through compost or irrigation with reclaimed water, eventually contaminating agricultural crops consumed by humans. This contamination can trigger allergic reactions, toxicity, or bacterial resistance to antibiotics (Alabi et. al. 2016). Despite these risks, the Slovak Republic currently lacks legislation mandating regular monitoring of pharmaceutical contaminants or endocrine disruptors in its water systems (Miarov et al. 2020).

METHODS

This study utilized a dual-method approach to assess the state of pharmaceutical waste in Slovakia:

Comparative Analysis

Data regarding medicinal consumption and waste tonnage were extracted from the National Center for Health Information (NCZI) and the annual reports of the State Institute for Drug Control (ŠÚKL) for the period between 2015 and 2024.

Public Survey

A primary survey was conducted with 88 randomly selected respondents (71.6% female, 28.4% male). The survey focused on identifying what citizens consider pharmaceutical waste, their disposal methods, and their level of awareness regarding the legal and technical aspects of waste destruction.

RESULTS

Consumption and Waste Trends (2015–2024)

Pharmaceutical consumption in the SR has remained high, reaching 163.5 million packages in 2024, it´s 1.6% increase from the previous year. Notably, 2024 saw a historical peak in prescription medications, with 93.5 million packages issued, in average 17 packages per citizen. Cardiovascular medications represent the highest volume of consumed drugs reimbursed through public health insurance.

The volume of pharmaceutical waste collected by ŠÚKL has seen a dramatic upward trend:

- 2015: 107 tons collected, costing 332 371 €.
- 2020: 161 tons collected.
- 2024: 291 tons collected, costing 971 145 €.

Regionally, in the baseline year of 2015 the Bratislava region produced the highest amount of waste (24 tons), while the Nitra region produced the least (10 tons).

Public Awareness and Disposal Habits

The survey revealed that while most citizens recognize human (82 respondents) and veterinary medicines (75 respondents) as pharmaceutical waste, very few recognize nutritional supplements (14) or outer packaging as part of this category.

Disposal habits among the 88 respondents were as follows:

- 85.23% (75 respondents): Return medicines to a public pharmacy.
- 17.05% (15 respondents): Dispose of medicines in municipal household waste.
- 1.14% (1 respondent): Flush medications down the toilet.

Despite the high percentage of people using pharmacies, 76.1% of respondents admitted they do not know how the destruction of this waste is legally or technically secured.

DISCUSSION

The accumulation of unused medicines in households is often driven by factors such as overstocking, expiration, or inappropriate package sizes, pointing toward irrational drug use (Kusturica et al. 2017). When compared internationally, Slovakia's disposal habits show significant room for improvement. In Sweden, where pharmacies provide specialized bags for waste and the state funds high-temperature incineration, only 3% of citizens use municipal waste for disposal. Conversely, in Poland, up to 68% of respondents reported using municipal waste or toilets for disposal (Persson et al. 2009; Rogowska et al. 2022). In Slovakia, public pharmacies are the primary collection point for waste from physical persons, as mandated by Act No. 362/2011 Coll. on Medicines and Medical Devices. However, the system efficiency relies on consumer behavior. The presence of drugs like tramadol, diclofenac, and various antibiotics in Slovak surface waters indicates that improper disposal and inadequate treatment plant technology remain critical issues (Mackuľak et al. 2020). Pharmaceutical substances can migrate into the soil through compost and subsequently penetrate to plant, which are consumed by animals or human. Research suggests that the presence of antibiotic residues in fruits and vegetables may adversely affect human health, as contaminated food products can trigger allergic reactions, toxicity, or even promote bacterial resistance to antibiotics (Kruopienė and Dvarionienė 2010). Pharmacists, as highly accessible healthcare professionals positioned to be the primary educators in improving public awareness regarding rational pharmacotherapy and the environmental impact of medicinal waste (Snopkova et al. 2021).

CONCLUSION

Pharmaceutical waste remains a significant threat to public health and the environment in the Slovak Republic. While the tonnage of waste collected from pharmacies has nearly tripled since 2015 substantial portion of the population still utilizes improper disposal methods. The lack of a mandatory monitoring framework for pharmaceuticals in water systems, combined with low public awareness of disposal processes, necessitates a stronger focus on education. Public pharmacies must continue to serve as the "front line" for both waste collection and patient counseling to mitigate the risks of environmental contamination and antibiotic resistance.

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FIRST RECORD OF *IXODES ARIADNAE* (ACARI: IXODIDAE) IN SLOVAKIA

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ABSTRACT

Ixodes ariadnae is a bat-associated tick species with a relatively limited documented distribution in Europe. In this study, we present the first confirmed record of *I. ariadnae* in Slovakia. A single engorged female was collected from a greater mouse-eared bat (*Myotis myotis*) during spring 2025. Species identification was confirmed using morphological and molecular analyses. Sequence comparison revealed 99.7–100% identity with previously published *I. ariadnae* isolates from Europe. Phylogenetic analyses placed the Slovak specimen within the *I. ariadnae* lineage This record broadens the known geographic range of the species and emphasizes the need for further research on bat-associated ticks and their distribution in Slovakia.

INTRODUCTION

Bats represent one of the most diverse groups of mammals and play important ecological roles in natural ecosystems (Moreno Santillán et al. 2021; Kunz et al. 2011). They host a wide range of ectoparasites, including ticks, which may participate in the circulation of various pathogens (Socolovschi et al. 2012, Szentiványi et al. 2024). Among bat-associated hard ticks in Europe, *Ixodes simplex* and *Ixodes vespertilionis* have been known for decades (Arthur 1956; Nosek & Sixl 1972). In 2014, a third bat-associated species, *Ixodes ariadnae*, was described from caves in Hungary (Hornok et al. 2014). Since its description, *I. ariadnae* has been reported only sporadically from several European countries, including Hungary, Germany, Belgium and Turkey (Hornok et al. 2015; Hornok & Krawczyk 2016; Hornok 2017). Despite extensive studies on ticks in Slovakia, *I. ariadnae* has not previously been reported. The aim of this study was to document the first occurrence of *I. ariadnae* in Slovakia and confirm its identity using morphological and molecular methods.

METHODS

A greater mouse-eared bat (*Myotis myotis*) was admitted to the Clinic of Birds, Exotic and Free-Living Animals of the University of Veterinary Medicine and Pharmacy in Košice in April 2025. The animal was found exhausted and unable to fly in an urban area of eastern Slovakia. During the clinical

examination, one engorged female tick was collected and preserved for further analysis. Morphological identification was performed using stereomicroscopy and published identification keys for bat-associated Ixodes species (Hornok et al. 2014). Diagnostic morphological characters were evaluated and compared with descriptions available in the literature. Genomic DNA was extracted from the tick using a commercial extraction kit. Partial mitochondrial cytochrome c oxidase subunit I (COI) and mitochondrial 16S rDNA genes were amplified by conventional PCR and subsequently sequenced using the Sanger method. Obtained sequences were compared with reference sequences available in the GenBank database using BLAST analysis. Phylogenetic analyses were performed using Maximum Likelihood methods based on COI and 16S rDNA sequences. Reference sequences representing *I. ariadnae* and related Ixodes species were included to verify the taxonomic position of the Slovak isolate.

RESULTS

Morphological examination revealed characters consistent with *Ixodes ariadnae*. The specimen possessed a broad scutum, relatively short palps, a characteristic basis capituli and a well-developed hypostome. These features corresponded to previously published descriptions of *I. ariadnae* and allowed its preliminary identification. Molecular analyses further confirmed the species identity. Partial COI and 16S rDNA sequences were successfully obtained and deposited in GenBank. BLAST analysis demonstrated a very high level of similarity with previously reported *I. ariadnae* isolates from Europe. The COI sequence showed 99.7–100% identity with isolates from Hungary, Germany and Turkey, while the 16S rDNA sequence exhibited 99.8–100% identity with available reference sequences. Phylogenetic analyses based on both mitochondrial markers consistently placed the Slovak isolate within a well-supported *I. ariadnae* clade. The obtained sequence clustered with previously published *I. ariadnae* isolates and was clearly separated from other bat-associated tick species. These findings provide both morphological and molecular evidence confirming the first occurrence of *I. ariadnae* in Slovakia.

DISCUSSION

The present study documents the first record of *Ixodes ariadnae* in Slovakia and extends the known geographical distribution of this bat-associated tick species. Until now, the species had been reported only from a limited number of European countries and from Japan (Hornok et al. 2014; Hornok et al. 2015; Hornok et al. 2016). The collected specimen was found on a greater mouse-eared bat (*Myotis myotis*), one of the host species previously associated with *I. ariadnae*. Because *M. myotis* populations are capable of seasonal movements between Slovakia and neighbouring countries, including Hungary, it is possible that the tick was acquired outside Slovakia (Hutterer et al. 2005). However, the possibility of local occurrence cannot be excluded.

Morphological identification corresponded closely with published descriptions of *I. ariadnae* (Hornok et al. 2014). Nevertheless, because several bat-associated Ixodes species may occur in Central Europe, molecular confirmation is particularly important. In the present study, both mitochondrial markers provided clear evidence supporting species identification. The obtained sequences showed almost complete identity with previously published *I. ariadnae* isolates and formed a distinct phylogenetic cluster separated from related species (Lv et al. 2014). The discovery of *I. ariadnae* in Slovakia highlights the importance of monitoring bat ectoparasites and their distribution. Future surveys focused on bat colonies and roosting sites will be necessary to determine whether *I. ariadnae* represents an occasional introduction or an established component of the Slovak tick fauna. Although the vector competence of *Ixodes ariadnae* remains poorly understood, bat-associated ticks have been shown to harbour a variety of bacterial and other microorganisms of potential veterinary and public health importance.

CONCLUSION

This study presents the first confirmed record of *Ixodes ariadnae* in Slovakia. The identification was supported by both morphological examination and molecular analysis of mitochondrial COI and 16S rDNA markers. The obtained sequences showed high similarity with previously reported *I. ariadnae* isolates and clustered within the corresponding phylogenetic lineage. The finding expands the known distribution of this bat-associated tick species and underlines the need for further studies on bat ectoparasites and their epizootological significance in Slovakia.

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ONE HEALTH SURVEILLANCE AND ZOONOTIC RISK ASSESSMENT OF *BRUCELLA* SPP. AND *TOXOPLASMA GONDII* IN SMALL RUMINANTS AND CALVES FROM KAJIADO COUNTY, KENYA

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ABSTRACT

Brucellosis and toxoplasmosis are important livestock-associated zoonoses causing reproductive losses and posing significant public health risks. This study assessed seroprevalence of *Brucella* spp. and *Toxoplasma gondii* in livestock from two East African farms and evaluated potential zoonotic risks. Antibodies against *Brucella* spp. were detected in 7.86% of animals, exclusively on one farm, while *T. gondii* antibodies were found in 19% of examined animals, with higher prevalence in goats and sheep. Identified risk factors included consumption of raw animal products, close human–animal contact, shared grazing areas, and limited surveillance. The findings highlight the need for integrated One Health surveillance and targeted risk management strategies.

INTRODUCTION

Zoonotic diseases remain a major challenge for animal production, food safety, and public health worldwide. Among the most important livestock-associated zoonoses are brucellosis and toxoplasmosis, both responsible for reproductive losses in livestock and significant health risks for humans. In pastoral production systems of East Africa, close interactions among livestock, wildlife, and humans create favorable conditions for pathogen transmission.

The project VAF37956, supported by Zoetis, investigates selected parasitic and infectious zoonoses in wild and farmed animals used for human consumption and promotes scientific collaboration between the University of Veterinary Medicine and Pharmacy in Košice and the University of Nairobi. The aim of this study was to investigate the occurrence of antibodies against *Brucella* spp. and *Toxoplasma gondii* in livestock from Kajiado County, Kenya, and evaluate their implications for animal and public health within a One Health framework.

METHODS

A total of 89 animals were examined, including 33 sheep, 36 goats and 20 calves from two farms located in Kajiado County, Kenya. Blood was collected from the jugular vein and sera were stored at −20 °C until analysis. Antibodies against *Brucella* spp. were detected using a commercial indirect ELISA (ID Vet Brucellosis Serum Indirect Multispecies, REF MS 5P). Antibodies against

Toxoplasma gondii were detected using ID Screen Toxoplasmosis Indirect Multispecies ELISA (ID Vet, France). Samples with S/P values ≥ 50% were considered positive.

RESULTS

Antibodies against *Brucella* spp. were detected in 7.86% (7/89) of animals. All seropositive animals originated from Farm 2, including 30% (6/20) of calves and 4.54% (1/22) of goats. No seropositive sheep were identified. Antibodies against *T. gondii* were detected in 19% (17/89) of examined animals. Seroprevalence reached 26% (9/35) in goats and 22% (8/37) in sheep, whereas all calves were negative. Farm 1 showed substantially higher seroprevalence (39%) than Farm 2 (12%). On Farm 1, six animals exhibited S/P values above 200%, suggesting recent or intensive exposure to *T. gondii*.

Risk Assessment

The detected seropositivity for both pathogens warrants a structured zoonotic risk assessment. Several factors increase the probability of transmission in the study area: consumption of raw milk and fresh animal blood; close daily contact between livestock owners and animals; shared grazing and watering points; limited veterinary and public health surveillance; interaction between livestock and wildlife populations. From a qualitative risk assessment perspective, the risk of human exposure to *Brucella* spp. may be considered moderate to high in affected herds due to direct contact with infected animals and animal products. Similarly, the risk of *T. gondii* transmission through meat consumption should not be underestimated, particularly where food hygiene practices are insufficient. Human brucellosis and toxoplasmosis remain underdiagnosed in many rural regions of East Africa, highlighting the need for closer collaboration between veterinary and medical sectors in disease surveillance, diagnosis, and outbreak investigation. Future risk assessment activities should include molecular characterization of circulating strains, evaluation of food-chain contamination pathways, assessment of occupational exposure among farmers and slaughterhouse workers, and investigation of seroprevalence in local human populations. Such data would support evidence-based risk management strategies and targeted public health interventions.

DISCUSSION

The detection of antibodies against *Brucella* spp. and *T. gondii* confirms the circulation of important zoonotic pathogens in livestock populations of Kajiado County. The occurrence of Brucella antibodies exclusively on Farm 2 suggests localized transmission, which is consistent with previous reports from Kenyan pastoral systems where communal grazing and livestock movement contribute to pathogen persistence (Akoko et al. 2021; Akoko et al. 2023). The relatively high seroprevalence of *T. gondii* on Farm 1 indicates substantial environmental exposure, potentially associated with contamination of feed or water by infected felids. Sheep and goats are recognized intermediate hosts

and may contribute to foodborne transmission to humans (Vilela et al. 2024; Fereig et al. 2024). Differences observed between farms suggest that local management practices and environmental factors influence pathogen circulation. Similar observations have been reported from wildlife–livestock interface ecosystems in Kenya, where ecological conditions affect the maintenance and transmission of zoonotic pathogens (Nthiwa et al. 2019). Based on the detected seropositivity and local production practices, the risk of human exposure to *Brucella* spp. can be considered moderate to high in affected herds, particularly among farmers, herders, veterinarians, and slaughterhouse workers. The presence of *T. gondii* antibodies in sheep and goats also indicates a potential foodborne risk associated with the consumption of undercooked meat (Vilela et al. 2024).

One Health Implications

The results should be interpreted within the One Health framework, which recognizes the interconnectedness of human, animal, and environmental health (Destoumieux-Garzón et al. 2018; Mackenzie and Jeggo 2019). In pastoral ecosystems such as Kajiado County, livestock, wildlife, and humans frequently share grazing areas and water resources, creating favorable conditions for pathogen maintenance and transmission (Nthiwa et al. 2019). Brucellosis and toxoplasmosis are exemplary One Health diseases because their epidemiology depends on interactions among animal hosts, environmental reservoirs, food production systems, and human behavior. Human brucellosis is commonly associated with direct contact with infected animals and consumption of unpasteurized milk or fresh blood, while human toxoplasmosis is primarily linked to contaminated food, water, or undercooked meat (Muturi et al. 2018; Wainaina et al. 2020; Vilela et al. 2024). The findings emphasize the need for integrated surveillance systems linking veterinary and human health sectors. Such integrated risk-based approaches encompassing animal, human, and environmental health sectors are considered essential for effective zoonotic disease prevention and control (Lee et al. 2013; Mackenzie & Jeggo 2019). Simultaneous monitoring of livestock, wildlife, environmental contamination, food products, and exposed human populations would enable a more comprehensive assessment of zoonotic disease burden and transmission pathways. Such an approach is consistent with the objectives of the One Health Joint Plan of Action (2022–2026) promoted by WHO, FAO, UNEP, and WOAH (WHO et al. 2022). Integrated surveillance systems may facilitate earlier detection of zoonotic threats and improve preparedness for emerging infectious diseases at the livestock–human–environment interface.

CONCLUSION

This study confirmed the presence of antibodies against *Brucella* spp. and *Toxoplasma gondii* in livestock from Kajiado County, Kenya, demonstrating ongoing circulation of important zoonotic pathogens at the livestock–human interface. The results contribute to the implementation of the One Health Joint Plan of Action (2022–2026) by providing baseline epidemiological data from East Africa

and emphasize the need for integrated surveillance, molecular diagnostics, and interdisciplinary risk assessment. Strengthening collaboration between veterinary and human health sectors will be essential for improving zoonotic disease prevention and control in pastoral production systems.

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DETECTION AND PARTIAL SEQUENCING OF *ORTHOFLAVIVIRUS USUTENSE* FROM MOSQUITOES IN SLOVAKIA

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ABSTRACT

Orthoflavivirus usutense (USUV) is a mosquito-borne arbovirus belonging to the *Flaviviridae* family and the genus *Orthoflavivirus*. To date, eight genetic lineages of USUV have been described, comprising three African and five European lineages. The aim of this study was to investigate the circulation of USUV in mosquito populations collected from several localities across Slovakia and to expand current knowledge of the genetic diversity of circulating strains. Partial sequencing of the viral NS5 gene revealed genetic relatedness to the Europe 2 lineage. These findings support the growing evidence that this lineage is established in Central Europe and is capable of long-term local persistence.

INTRODUCTION

Orthoflavivirus usutense (USUV) is an enveloped virus with a diameter of approximately 40–60 nm, containing a single-stranded, positive-sense RNA consisting of approximately 11,000 nucleotides (Smit et al., 2011). USUV shares the same vectors and host bird species as West Nile virus (WNV) they often circulate in the same environment (Zannoli, 2019). Phylogenetic studies based on sequencing of the NS5 gene of viral RNA have shown that USUV strains isolated in various regions of the world can be divided into eight lineages: three African and five European, and all except African lineage 1 circulate in Europe (Cadar et al. 2016). USUV was first confirmed in Slovakia in 2018 through the detection of antibodies in birds and reptiles, with co-circulation of USUV and WNV also detected in mosquitoes (Csank et al., 2018, 2019; Čabanová et al., 2019). In 2024, USUV was detected in mosquitoes collected from several districts of Slovakia, and the first laboratory-confirmed human cases of USUV infection were reported (UVZSR, 2024).

METHODS

Mosquito pools samples consisted of female mosquitoes, which were grouped according to species, collection site, and collection date. Detection of viral RNA in pooled mosquito samples involved several steps, including pool homogenization, RNA extraction, cDNA synthesis, and end-point PCR screening. Positive qPCR samples were subsequently subjected to hemi-nested PCR targeting a 360 bp fragment of the NS5 gene. Amplified products were sequenced by Sanger sequencing. The obtained nucleotide sequences were compared with reference sequences available in the GenBank database and aligned using the MAFFT algorithm. Phylogenetic relationships were inferred using the FastTree 2.1.11 implemented in Geneious v.9.1.8. Sequences identical to the reference Europe 2 lineage (Usutu virus strain Bologna 2009, HM569263) were excluded from the dataset to reduce redundancy. Phylogenetic tree was built using only unique Slovak USUV sequences.

RESULTS

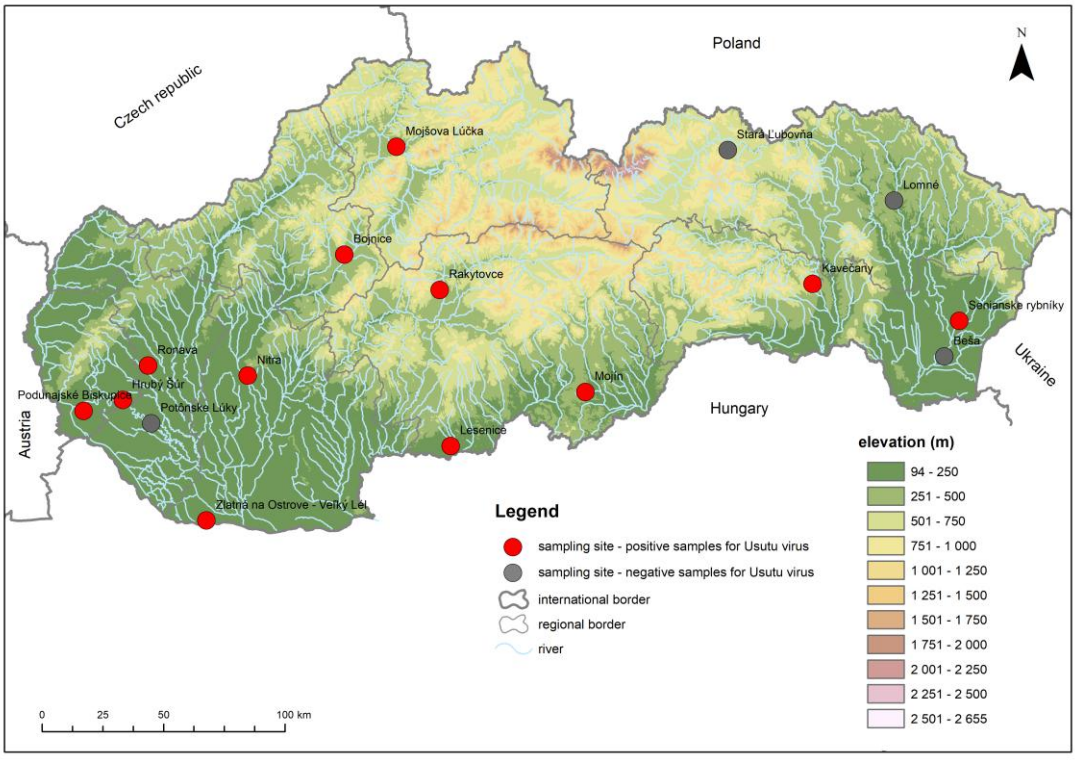


Figure 1 Map of Slovakia with the mosquito trapping sites and the occurrence of USUV

The presence of USUV RNA was confirmed in 62 positive mosquito pools. A total of 38 positive pools from 12 locations shown in Figure 1 were selected for phylogenetic analysis. These pools consisted of mosquitoes belonging to the *Culex pipiens complex*, except for pool 739.C/2024/V. Lel-KN/SVK, which consisted of *Culex modestus* mosquitoes. Most USUV-positive pools were concentrated in western and southwestern Slovakia, suggesting that these regions represent important areas of virus circulation. In contrast, positive pools were detected less frequently in central and

eastern Slovakia. Phylogenetic analysis showed that all Slovak USUV isolates clustered within the Europe 2 lineage. The Slovak sequences were grouped together with previously described Europe 2 reference strains, including reference sequences HM569263, JF266698 from Italy and PV523802 from Hungary. Minor genetic variation was observed among individual Slovak isolates, indicating the circulation of a genetically related viral population in Slovakia.

DISCUSSION

The detection of Europe 2 in Slovakia agrees with reports from neighbouring countries, including Austria (Weidinger *et al*., 2020), Hungary (Szabó et al., 2025), the Czech Republic (Hönig *et al*., 2019) and Germany (Ziegler et al., 2016), where this lineage has also been documented. The low pairwise genetic distances observed among Slovak isolates further indicate limited genetic diversity and support the hypothesis of local circulation of closely related viral strains. These findings suggest that the Slovak isolates form part of the broader Central European circulation pattern of USUV lineage Europe 2. It is important to emphasise that this study analysed only a partial segment of the NS5 gene. A whole-genome analysis is currently underway, which will provide more accurate and comprehensive data on the circulation of USUV strains in Central Europe.

CONCLUSION

The presence of USUV Europe 2 in Slovakia further supports the growing evidence that this lineage is established in Central Europe and is capable of long-term local persistence. Its repeated detection in the region is consistent with the broader European dynamics of USUV, where the virus has undergone multiple introductions followed by local evolution and maintenance within enzootic transmission cycles.

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LATEST VIEW ON ROLE OF CYTOKINES IN HOST-VIRUS INTERACTION AND VIRULENCE MECHANISM IN CANINE PARVOVIROSIS

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ABSTRACT

Canine parvovirosis is highly contagious disease caused by Canine parvovirus type 2 (CPV-2), responsible for haemorrhagic enteritis, myocarditis and depletion of bone marrow with high morbidity and mortality especially in young unvaccinated dogs. The aim of this study was to review the mechanisms of CPV-2 interaction with host innate and adaptive immune system. We focused on TNF-α and IL-6, pro-inflammatory cytokines that promote acute innate immune responses by inducing inflammation and supporting recruitment and activation of phagocytic cells. Furthermore, we also reviewed activity of IL-4 and IFN-γ regulating differentiation and effector functions of B and T lymphocytes, including plasma cells and helper T-cell subsets and therefore mediate adaptive immune response.

INTRODUCTION

Canine parvovirosis is highly contagious viral disease primarily affecting young dogs between 6 weeks and 6 months of age. The virus is characterised by tropism to rapidly proliferating tissues including intestinal crypt epithelium, myocardium or lymphoid tissue such as one of bone marrow. Although considerable progress has been made in understanding the disease, no specific antiviral treatment is currently available, leaving supportive therapy and host immune modulation as the primary therapeutic approaches (Asil et al. 2023; Li et al. 2025).

METHODS

Cytokines are soluble signalling molecules responsible for regulation of key pathways involved in non-specific (innate) and specific (adaptive) immune responses. In this report we reviewed several clinical studies to determine most effective way of measuring IL-6 and TNF-α to assess the inflammatory response and therefore the ability of innate immune system to respond to antigen stimulation. Additionally, role of other cytokines such as IL-4 and INF-γ was also evaluated to assess their connection to adaptive immunity and therefore determine their function in host-virus interaction (Gruys et al. 2005; Liu et al. 2017; Jakobsen et al. 2024).

RESULTS

As a part of the early innate response, TNF-α is among the first cytokines released during the inflammatory cascade, being secreted by activated macrophages and mast cells in first few hours after stimulation. Although both TNF-α and IL-6 increase rapidly following the onset of inflammation, TNF-α reaches its peak concentration early and typically begins to decline within approximately 6 hours, whereas IL-6 remains elevated for a considerably longer period, often for 24 hours or more. For this reason, serological measurements of IL-6 show more value, with higher serum concentrations being associated with greater disease severity, clinical manifestation, and increased mortality (Gruys et al. 2005; Rau et al. 2007; Song et al. 2012; Ulas et al. 2023; Jakobsen et al. 2024).

IL-2 and IFN-γ are closely associated with T-cell activation, clonal expansion, and cellular immunity. IL-2 promotes the cytotoxic T-cell response against virus-infected cells, whereas IFN-γ enhances antiviral defence by inducing antiviral proteins in surrounding cells, thereby limiting viral replication. In contrast, IL-4, produced mainly by helper (Th) cells, promotes B-cell activation and antibody production while suppressing cellular immune responses (Liu et al. 2017). However, Dik et al. recently reported little significant difference in serum IL-2, IL-4 and IFN-γ concentrations in CPV-2 positive dogs compared to healthy controls, suggesting measurement of these cytokines may have limited standalone value compared to TNF-α or IL-6 (Dik et al. 2024).

DISCUSSION

Upregulation of pro-inflammatory cytokines has also been confirmed in other viral diseases such as Canine distemper, where RT-PCR (reverse transcription PCR) and ELISA analyses demonstrated significantly increased concentration of TNF-α and IL-6 in cerebrospinal fluid of infected dogs compared to healthy controls (Mahmoodabadi et al. 2025). Similar findings have been reported in swine infected with Vesicular stomatitis virus or murine models experimentally infected with Influenza, where elevated serum IL-6 concentrations were observed during the acute phase of infection (Velazquez-Salinas et al. 2019). The involvement of these cytokines extends beyond viral infections, as their serum concentrations have been shown to increase during parasitic infestations in canine heartworm disease caused by *Dirofilaria immitis* and Canine visceral leishmaniosis (*Leishmania chagasi*) (de Lima et al. 2006; Kosić et al. 2024). Moreover, elevated systemic levels of IL-1β, IL-6, and TNF-α have also been associated with chronic inflammatory conditions, including epilepsy, chronic kidney disease or dilated cardiomyopathy in dogs (Merbl et al. 2014; Maia et al. 2024; Jyothsna et al. 2025).

Beside the IL-6 and TNF- α, canine and feline leishmaniosis can also serve as an example of the involvement of IFN- γ and IL-4 in both cellular and humoral part of adaptive immunity, as their expression was detected by flow cytometry or real time quantitative PCR in $CD4^+$ and $CD8^+$ T

lymphocytes in the peripheral blood of dogs and cats with clinical leishmaniosis (Matralis et al. 2023; Louzada-Flores et al. 2026). Similarly, increased expression of IL-2, IFN- γ and IL-4 and several other cytokines has been reported in blood of cattle infected with bovine tuberculosis or enzootic bovine leukosis (Amills et al. 2002; Blanco et al. 2014).

CONCLUSION

This review indicates that cytokine profiling represents a valuable approach for assessing the host immune response during canine parvovirus infection. Among the cytokines evaluated, IL-6 appears to be the most promising biomarker due to its prolonged persistence in circulation and its consistent association with disease severity, clinical outcome, and mortality. Although TNF-α plays a key role in initiating the early inflammatory response, its rapid and transient release limits its diagnostic value when used alone.

In contrast, IL-2, IFN-γ, and IL-4 are essential mediators of adaptive immunity and contribute to adaptive antiviral cellular and humoral immune responses; however, current evidence suggests that their serum concentrations provide limited prognostic or diagnostic value in dogs with CPV-2 infection and therefore this topic should be studied more thoroughly.

Importantly, the upregulation of IL-6 and TNF-α has also been demonstrated in numerous viral, parasitic, and chronic inflammatory diseases, indicating that these cytokines reflect the magnitude of systemic inflammation rather than being disease-specific biomarkers. Therefore, cytokine measurements, particularly IL-6, should be interpreted alongside clinical findings and conventional laboratory diagnostics to improve the assessment of disease progression and prognosis in canine parvovirosis.

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OCCURRENCE OF *ROCAHEPEVIRUS RATTI* IN RODENTS FROM SLOVAKIA

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ABSTRACT

Rocahepevirus ratti (originally rat hepatitis E virus, RHEV) has recently been shown to cause zoonotic infections and symptomatic disease – viral hepatitis E in human population worldwide. Our study was based on molecular-genetic detection of RHEV RNA in 40 liver samples from: brown rat (*Rattus norvegicus*) (n = 16), house mouse (*Mus musculus*) (n = 14), and common vole (*Microtus arvalis*) (n = 10) from Slovakia. Presence of RHEV RNA was confirmed by molecular-genetic methods in the liver samples from wild living brown rats (11/16, 68.8%). None sample from house mouse and common vole was found to be positive. The obtained Slovak rat RHEV sequences were closest to rat and human RHEV sequences from different European countries. The circulation of *Rocahepevirus* in wildlife population raises the question of the possible zoonotic transmission to human in Slovakia, but the further research of this infectious agent in animal and human population is necessary.

INTRODUCTION

Rocahepevirus ratti (RHEV) is an emerging zoonotic pathogen that belongs to the family *Hepeviridae*. Members of the genus *Rocahepevirus* are phylogenetically distinct from other viruses in the family with a different host range, being found mainly in rodents and carnivores (Rivero-Juarez et al. 2026). The genus is divided into two species: *Rocahepevirus ratti* (rat hepatitis E virus, RHEV) and *Rocahepevirus eothenomi* (vole hepatitis E virus, VHEV) (Erdin et al. 2025). In 2017, zoonotic potential of *Rocahepevirus ratti* has been confirmed in imunocompromised patient in Hong Kong, later with increasing number of human cases reported worldwide – in immunocompromised patients, immunocompetent persons but also in children (Sridhar et al. 2018; Andonov et al. 2019; Caballero-Gómez et al. 2025). Presence of RHEV RNA was also confirmed in wastewater in Sweden and France (Churqui et al. 2024; Rouba et al., 2024). Direct contact with rats or with rat droppings, together with possible contamination of environment are considered to be the main transmission routes, but many exposure factors remains unknown (Sridhar et al., 2021). The aim of this study was to determine the presence of genus *Rocahepevirus* in rodents from Slovakia.

METHODS

A total of 40 liver samples were collected from three different rodent species: brown rat (*Rattus norvegicus*) (n = 16), house mouse (*Mus musculus*) (n = 14), and common vole (*Microtus arvalis*) (n = 10). Total RNA was extracted from 140 µL of 10 % (w/v) liver homogenates prepared with 0.9 % isotonic saline solution (B. Braun GmbH, Germany) using the QIAamp Viral RNA Mini Kit (QIAGEN GmbH, Germany) by robotic station QIAcube (QIAGEN GmbH, Germany) according to the manufacturer's instructions. RNA was eluted in 50 µl of elution buffer and stored at -80°C until analysis.

All samples were screened individually using a nested broad-spectrum RT-PCR targeting the 332 bp fragment in ORF1 according to Johne et al. (2010). The size of amplicons were checked by electrophoresis in a 2% agarose gel after staining with GelRed™ (Biotium, Inc., USA) and visualized by GelDoc Go Gel Imaging System (BioRad, Inc., USA). For the phylogenetic analysis, five PCR products were selected based on DNA quantity and electrophoretic quality. They were purified and sequenced by the Sanger method in a commercial company (Microsynth Seqlab GmbH, Germany). The sequence and phylogenetic analyses were assessed using SeqMan, EditSeq and MegAlign (Lasergene, DNASTAR Inc., USA). Molecular evolution model test and the phylogenetic tree were created in MEGAX (Kumar et al. 2018). The maximum likelihood phylogenetic tree was built with a Tamura-Nei (TN93+G+I) substitution model, and a bootstrap resampling process (1000 replications) was used to assess node support.

RESULTS

A total of 11 rat liver samples (11/16, 68.8%) were tested positive, with the amplification products of the expected size. Liver samples from other species were negative for the presence of viral RNA. Positive rats originated from seven different locations within Slovakia. Phylogenetic analysis was performed with five rat positive samples, based on their PCR products quality. The obtained sequences were checked against the NCBI GenBank database using nucleotide BLAST, which confirmed all sequences belonged to species *Rocahepevirus ratti*. The comparisons with all HEV sequences in GenBank showed that the nucleotide identity of the obtained sequences were closest to rat RHEV from Hungary and Denmark (GenBank Acc.Nos. MW795567 and KX774646) and human RHEV from Spain (GenBank Acc.Nos. OK082152).

DISCUSSION

In Europe, the first report of *Rocahepevirus ratti* was in 2010, from wild Norway rats (*Rattus norvegicus*) in Germany (Johne et al. 2010). Currently, presence of this species has been confirmed in different animal species, humans and even in wastewater, which highlights its importance in the context of One Health. High prevalence of RHEV RNA in Slovak rat population (68.8%) confirms circulation of this virus in Slovakia. The obtained Slovak rat RHEV sequences were closest to rat and

human sequences from different European countries. Further surveillance of rocahepeviruses in more animal species is needed to assess their threat to public health and to understand their diversity.

CONCLUSION

This study provides the first molecular-genetic evidence of *Rocahepevirus ratti* in rats from Slovakia. Phylogenetic analysis confirmed that the detected strains are closest to rat RHEV and human RHEV sequences from European countries. These findings indicate the importance of the studies for this infectious agent in animal and human population in Slovakia.

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ASIAN-LIKE CPV-2B IN SLOVAKIA: A FATAL CASE IN A VACCINATED DOG AND THE POTENTIAL ROLE OF CLIMATE CHANGE IN VIRAL INTRODUCTION

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ABSTRACT

This study reports the fatal clinical outcome of canine parvovirus type 2 (CPV-2) infection in a vaccinated dachshund puppy and documents the first detection of an Asian-related CPV-2 variant in Slovakia based on *VP2* gene analysis. CPV-2 infection was confirmed by PCR and sequencing of a rectal swab specimen. The characterized isolate exhibited amino acid signatures typical of Asian CPV-2c lineages (5G, 267Y, 324I, 370R, and 440T) and clustered phylogenetically within these clades, while remaining distinct from European CPV-2b strains. The virus is presumed to derive from an Asian-like CPV-2c-like lineage harboring the D426 substitution. These findings represent the first evidence of an Asian-like CPV-2b strain circulating in Slovakia.

INTRODUCTION

Canine parvovirus type 2 causes highly contagious acute enteritis associated with high morbidity and mortality, particularly in puppies, and may also affect immunocompromised adult dogs (Tuteja et al. 2022). The virus has undergone evolutionary changes in the VP2 protein, leading to the emergence of CPV-2a, CPV-2b, and CPV-2c variants, with certain mutations being associated with increased virulence and immune evasion (Miranda and Thompson 2016). Despite vaccination, CPV-2 infections still occur in vaccinated individuals, most often due to maternal antibody interference and inappropriate vaccination timing (Altman et al. 2017). CPV-2 is a globally distributed pathogen, with circulating variants reported across all continents (Mira et al. 2019). Studies have confirmed the introduction of Asian CPV-2 strains into Europe through imported dogs, highlighting the role of animal movement in viral spread (Mira et al. 2018). These strains are characterized by a specific set of mutations in structural and non-structural proteins and exhibit close phylogenetic relatedness (Canuti et al. 2022). This study describes a fatal case of CPV-2 infection in a vaccinated puppy and confirms the presence of a novel CPV-2 variant in Slovakia based on molecular analysis of the VP2 gene.

METHODS

Molecular detection of CPV-2 DNA and sequencing

DNA was extracted from a rectal swab and CPV-2 was confirmed by PCR amplification of a VP2 gene fragment. The full-length VP2 gene was then amplified in overlapping fragments, sequenced by the Sanger method, and compared with a reference sequence after assembly.

Analysis of amino acid residues of the VP2 protein, Phylogenetic analysis

The VP2 nucleotide sequence was translated into an amino acid sequence to identify the CPV-2 antigenic variant based on residue 426 and selected amino acid substitutions. Analysis was performed using Geneious v9.1.8 and compared with reference strains from GenBank. Phylogenetic analysis of 61 reference VP2 sequences was conducted in MEGA v11 using ClustalW alignment and the maximum likelihood method under the Tamura-3 (T92+G+I) model. Branch support was evaluated by bootstrap analysis with 1,000 replicates, and values >50% were displayed at the corresponding nodes.

Cell culture and virus isolation

For the isolation of CPV from the fecal sample, the Madin–Darby Canine Kidney (MDCK) cell line was used and cultured to confluence in MEM supplemented with 10% FBS and 1% antibiotic–antimycotic solution at 37 °C in 5% CO_2 and 95% humidity. Virus isolation was performed according to Hirayama et al. (2005), and the presence of the virus in culture supernatants was subsequently confirmed by PCR using CPV-2–specific primers.

RESULTS

Molecular Detection of CPV-2 DNA and Sequencing

The VP2 gene sequence was deposited in GenBank (OR825360) and analyzed using BLASTn. It showed highest identity with CPV-2b and CPV-2c strains, particularly from Asian lineages, and no close similarity to CPV-2a or the original CPV-2 strain.

Analysis of VP2 Amino Acid Residues, Phylogenetic Analysis

The isolate Slovakia/Kosice/47/2022 was classified as CPV-2b based on Asp426 (D426) and the S297A substitution, a marker of “new” CPV-2a/2b variants. It also showed an amino acid profile typical of Asian CPV-2c strains (5G, 267Y, 324I, 370R, 440T). Phylogenetic analysis grouped the isolate with Asian CPV-2b strains from Italy (2022) and Hungary (2021), with high bootstrap support, and indicated close relatedness to Asian CPV-2c lineages, including an Italian strain.

Cell Culture and Virus Isolation

A cytopathic effect (CPE), characterized by plaque formation and partial lysis of the cell monolayer, was observed on day 4 post-inoculation during the first passage. No morphological changes were

detected in the negative control cultures. The presence of CPV in the culture supernatant was confirmed by PCR amplification of a 573-bp fragment of the VP2 gene using universal CPV primers.

DISCUSSION

PCR coupled with sequencing represents a standard approach for pathogen detection (Khatri 2017). In the present case, CPV-2 infection in the puppy was confirmed by multiple diagnostic methods, including a rapid antigen test, PCR assay, and the identification of VP2 amino acid substitutions (K80R, K93N, V103A, and D323N) known to differentiate CPV from feline parvovirus (FPV) (Zhou et al. 2017). Based on VP2 residue 426, the isolate Slovakia/Kosice/47/2022 was classified as CPV-2b (D426). Additionally, the S297A substitution, a molecular marker associated with the emergence of the "new" CPV-2a/2b variants, was detected (Ohshima et al. 2008). The isolate also exhibited an amino acid profile (A5G, F267Y, Y324I, and Q370R) characteristic of Asian CPV-2c strains, suggesting a close evolutionary relationship with this lineage. Similar molecular signatures have been reported in CPV-2b isolates from Hungary (Boros et al. 2022) and Italy, collectively referred to as the "Asian CPV-2b" lineage (Schirò et al. 2022b), which has been hypothesized to have arisen through convergent evolution. Phylogenetic analysis demonstrated that the Slovakia/Kosice/47/2022 isolate clustered closely with Asian CPV-2b and CPV-2c lineages, supporting a shared evolutionary origin. Although the geographic origin of these strains remains uncertain, their circulation within Europe has been documented (Franzo et al. 2023). The amino acid substitutions A5G, Q370R, F267Y, and Y324I have been associated with antigenic properties, host range adaptation, and receptor-binding capacity (Sarabandi and Pourtaghi 2023). In particular, the Q370R substitution may influence the structural conformation of the VP2 capsid protein and its interaction with host cell receptors (Hao et al. 2022). The widespread global distribution of these mutations indicates that they are no longer restricted to Asian CPV lineages, highlighting the ongoing evolution of CPV-2 and its potential implications for viral antigenicity and vaccine efficacy.

CONCLUSION

The Slovakia/Kosice/47/2022 isolate is antigenically classified as CPV-2b but phylogenetically belongs to the Asian CPV-2c clade, sharing several VP2 capsid mutations. These mutations may affect vaccine efficacy and immune evasion, potentially contributing to insufficient immune response and the rapid fatal outcome in the vaccinated puppy.

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WHEN VACCINATION IS NOT ENOUGH: ACUTE PARVOVIRUS INFECTION CAUSED BY AN "ASIAN" CPV-2C VARIANT IN A DOMESTIC DOG

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ABSTRACT

An 8-month-old vaccinated English Cocker Spaniel was presented with hemorrhagic diarrhea, vomiting, and lethargy. Canine parvovirus (CPV-2) infection was indicated by a weakly positive rapid antigen test and confirmed by serological, molecular, and virological methods. The virus was successfully isolated in A-72 cells. Serology showed high IgM titer (1:2,700), absence of IgG, and protective level of functional antibodies (≥1:640). *VP2* sequencing identified a CPV-2c variant with amino acid signatures typical of the "Asian" lineage (5G, 267Y, 324I, 370R, and 440T). After intensive supportive therapy, the dog recovered. This report provides the first evidence of "Asian" CPV-2c circulation in Central Europe.

INTRODUCTION

Canine parvovirus type 2 (CPV-2) remains one of the most important viral pathogens of dogs, causing acute gastroenteritis with high morbidity and mortality, particularly in puppies (Tuteja et al. 2022). Continuous genetic evolution of the *VP2* gene has led to the emergence and global spread of antigenic variants CPV-2a, CPV-2b, and CPV-2c (Li et al. 2025). The VP2 capsid protein is crucial for host–receptor binding, antigenicity, and virus–host interactions (Hueffer et al. 2003). In recent years, increasing attention has been given to the "Asian" CPV-2c lineage, characterized by specific VP2 amino acid residues (5G, 267Y, 324I, 370R, and 440T), which have been reported in Asia, Europe, and Africa (Wang et al. 2016; Mira et al. 2019). Vaccination is the cornerstone of CPV-2 prevention, and current attenuated vaccines are generally considered effective against circulating variants (Larson et al. 2008). Nevertheless, sporadic CPV-2 infections in vaccinated dogs continue to be reported (Decaro et al. 2020), emphasizing the need for careful diagnostic interpretation and molecular surveillance. This report describes acute hemorrhagic gastroenteritis in a vaccinated 8-month-old English Cocker Spaniel, in which infection with an "Asian" CPV-2c variant was confirmed by serological, molecular, and virological methods. The case highlights that CPV-2 infection should remain a differential diagnosis in clinically compatible cases regardless of vaccination status, and that molecular characterization is important for monitoring emerging CPV-2 variants in vaccinated dogs.

METHODS

Clinical examinations

An 8-month-old vaccinated male English Cocker Spaniel with acute gastrointestinal disease was examined at the Clinical Workplace of Infectious and Parasitic Animal Diseases, University of Veterinary Medicine and Pharmacy in Košice. Clinical examination was performed according to a standard protocol. On the day of admission (November 17, 2025), CPV infection was assessed using a rapid CPV/CCV/Giardia antigen test. Peripheral blood was collected for hematological and biochemical examination, and a rectal swab was subjected to bacteriological and mycological cultivation.

Molecular, virological and serological examinations

Viral DNA was extracted from the rectal swab (DNeasy Blood & Tissue kit; Qiagen, Germany) and used for PCR amplification and Sanger sequencing of the full-length *VP2* gene (1,755 bp). The obtained sequence was assembled (Geneious Prime v2025.2), compared with GenBank sequences, and analyzed at amino acid positions relevant for CPV-2 characterization. Phylogenetic analysis was performed using full-length *VP2* sequences by the Maximum Likelihood method with 1,000 bootstrap replicates (MEGA11). Virus isolation was performed in A-72 cell culture (CRL-1542; ATCC, USA), and viral replication was confirmed by PCR and *VP2* sequencing of the culture supernatant. The infectious virus titer was determined by the $TCID_{50}$ assay. Patient serum was tested for CPV-specific IgM and IgG antibodies, while functional antibodies were assessed by hemagglutination inhibition (HI) (Cavalli et al. 2021) and serum neutralization tests (SNT) (Cavalli et al. 2008) using the isolated virus.

RESULTS

Clinical findings

The rapid antigen test showed a weakly positive result for CPV. Hematological examination revealed leukopenia with severe neutropenia, while serum biochemistry showed hypoproteinemia, hyponatremia, and hypochloremia. Bacteriological examination of the rectal swab revealed normal intestinal microbiota, and mycological culture and selective cultivation for *Campylobacter* spp. were negative. After intensive supportive therapy, the dog recovered and was discharged after seven days of hospitalization.

Molecular, virological and serological findings

Full-length *VP2* sequencing confirmed CPV-2 infection. Based on glutamic acid at VP2 residue 426, the virus was identified as CPV-2c. Further amino acid analysis revealed the pattern 5G, 267Y, 324I, 370R, and 440T, corresponding to the “Asian” CPV-2c lineage. Phylogenetic analysis confirmed clustering of the Slovak isolate with related “Asian” CPV-2c strains. The virus was successfully

isolated in A-72 cell culture, and PCR and sequencing of the culture supernatant confirmed the same CPV-2c genotype as detected in the clinical sample. The infectious virus titer was $10^{4.67}$ $TCID_{50}$/mL. Serology showed a high CPV-specific IgM titer (1:2,700) with the absence of IgG antibodies. HI and SNT revealed functional antibody titers of 1:640 and 1:1,280, respectively, both exceeding levels generally considered protective (Cavalli et al. 2008; Schultz et al. 2002).

DISCUSSION

This report describes acute hemorrhagic gastroenteritis in a vaccinated young dog caused by an "Asian" CPV-2c variant. The weakly positive antigen test result emphasizes that equivocal rapid test findings should be interpreted cautiously and confirmed by molecular methods in clinically compatible cases. Full-length *VP2* sequencing identified the virus as CPV-2c and revealed the amino acid pattern 5G, 267Y, 324I, 370R, and 440T, corresponding to the "Asian" CPV-2c lineage (Wang et al. 2016). Phylogenetic analysis further supported clustering of the Slovak isolate with related "Asian" CPV-2c strains (>99.94%), indicating the epidemiological relevance of this finding in Central Europe. Although vaccination remains the most effective measure for CPV-2 prevention (Squires et al. 2024), sporadic infections in vaccinated dogs may occur (Decaro et al. 2020). In this case, clinical disease may have been influenced by incomplete or insufficiently developed protective immunity, possible maternally derived antibody interference, individual immune variability, or infection with an emerging CPV-2c variant. The high IgM titer with absence of detectable IgG antibodies was more consistent with an acute infection-associated humoral response than with established post-vaccination immunity. However, HI and SNT revealed functional antibody titers above levels generally considered protective (Cavalli et al. 2008; Schultz et al. 2002), suggesting that an antibody response was developing during infection. This case highlights the need for comprehensive diagnostics and continued molecular surveillance of CPV-2 variants in vaccinated dogs.

CONCLUSION

CPV-2 infection should remain a differential diagnosis in dogs with compatible clinical signs regardless of vaccination status, and molecular surveillance is essential for monitoring the emergence and spread of new CPV-2 variants.

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PARTIAL PHYLOGENETIC ANALYSIS OF WEST NILE VIRUS STRAINS OBTAINED FROM CULEX MOSQUITOES CAPTURED IN SLOVAKIA (2024-2025)

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ABSTRACT

West Nile virus (WNV) is a mosquito-borne arbovirus posing a significant public health threat. From June to mid-October 2024 and 2025, 52 605 mosquitoes (1 378 pools) were collected across Slovakia and screened using qPCR. WNV RNA was detected in seven *Culex pipiens* complex pools in 2024, while all 2025 samples were negative. Sequence analysis revealed 95.9–99.9% identity among Slovak strains, clustering them closely with WNV lineage 2 from Austria and the Czech Republic. These findings confirm active WNV circulation in Slovakia, emphasizing the critical need for systematic vector surveillance and integrated "One Health" approaches.

INTRODUCTION

West Nile virus (WNV, genus Orthoflavivirus) is maintained in an enzootic cycle between birds (reservoirs) and *Culex* mosquitoes (vectors), while humans and horses are dead-end hosts (Peňazziová et al., 2023). In Slovakia, WNV was first isolated in the 1970s from *Aedes cantans* and wild birds (Labuda et al. 1974; Grešíková et al. 1975). After a long hiatus, its circulation in *Culex modestus* and *Cx. pipiens* was confirmed in 2018 (Čabanová et al. 2019). The first modern autochthonous human case in Slovakia was diagnosed in 2019, followed by a neuroinvasive case in 2022 and 9 confirmed cases in 2024 (Čabanová et al. 2021; EPIS 2022; Report on zoonoses 2024). Serological studies also confirm WNV circulation in wildlife, captive birds, horses, and bears (Hubálek et al. 2013; Csank et al. 2018; Vitásková et al. 2019). Given this active transmission, an integrated "One Health" approach—linking human, veterinary, and environmental surveillance—is crucial for early pathogen detection.

METHODS

Mosquito collection was performed continuously from June to mid-October in 2024 and 2025 using BG-Sentinel 2 traps (Biogents AG, Germany) with CO_2 as an attractant at 16 localities across

Slovakia. Morphological identification followed Becker et al. (2010). Female mosquitos were pooled (10–50 individuals/pool) by species, sex, locality, and date and homogenized in 10% EMEM. Following RNA extraction, reverse transcription and qPCR were performed according to Kolodziejek et al. (2014). Pools tested positive for WNV RNA were sequenced via Sanger sequencing. Phylogenetic analyses were performed in MEGA11 using the Maximum Likelihood method and the Tamura-Nei model with 1,000 bootstrap replicates (Tamura & Nei 1993; Tamura et al. 2021).

RESULTS

A total of 1 378 mosquito pools representing 52 605 individuals were screened: 1 046 pools (43 533 individuals) in 2024 and 332 pools (9 072 individuals) in 2025. WNV RNA was confirmed in seven 2024 pools consisting of *Culex pipiens* females. No positive pools were detected in 2025. The WNV-positive pools originated from four distinct localities: Potônské Lúky (Dunajská Streda district), Zlatná na Ostrove – Veľký Lél (Komárno district), Lesenice (Veľký Krtíš district), and Senianske rybníky (Michalovce district). Partial phylogenetic analysis of five Slovak strains showed a high sequence identity (95.9% to 99.9%). The 2024 strains from Veľký Lél and Lesenice formed a monophyletic cluster (bootstrap 84–85) closely related to WNV lineage 2 strains from Austria (Cx. pipiens/Vienna/2014) and the Czech Republic (Cz 13-502, Cz 13-104) indicating the regional spread of WNV within Central Europe. While sharing a common ancestor with Italian strains, they remained distinct from West European, Greek, and Balkan lineages, confirming a stable Central European evolutionary branch.

DISCUSSION

Historical and current data confirm that West Nile virus (WNV) is well-established in Slovakia, circulating long-term within local natural foci. The primary vector, the *Culex pipiens* complex, is widespread across the country, inhabiting both natural ecosystems and densely urbanized areas. The ability of these mosquitoes to adapt to different types of environments significantly increases the risk of virus transmission to humans (Jalili et al., 2000; Strelková & Halgoš, 2012; Blažejová et al., 2018; Oboňa et al., 2020). Our current phylogenetic analysis indicates that the Slovak WNV strains belong to the Central European branch, which is clearly separated from the Balkan branch. Furthermore, the high degree of sequence similarity among our strains suggests a common origin and long-term local circulation of the virus in the studied area (Labuda et al. 1974; Csank et al. 2016; Čabanová et al. 2019; Drzewnioková et al. 2019; Peňazziová et al. 2021).

CONCLUSION

This study confirms active West Nile virus (lineage 2) circulation across Slovak urban and natural ecosystems. To address this threat, the OH SURVector project contributed to establishing vector

surveillance in Slovakia, enhancing intersectoral One Health cooperation. In the context of climate change and invasive species, strengthening national capacities for molecular detection and genetic typing remains vital for public health risk management.

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TICK-BORNE ARBOVIRUSES AND THE INNATE IMMUNE RESPONSE IN BOVINE MACROPHAGES

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ABSTRACT

The aim of our *in vitro* study was to compare the induction of type I interferons (IFN-I, including IFN-α and IFN-β) by tick-borne arboviruses in bovine macrophages. Macrophages differentiated from blood monocytes (MDMs) were infected with the tick-borne encephalitis virus (TBEV; *Flavivirus*), the Kemerovo virus (KEMV; *Orbivirus*) or the Tribeč virus (TRBV; *Orbivirus*). Significantly increased mRNA levels of type I interferons (IFN-I) were found for both tick-borne orbiviruses (TBOs) at 24 hours post infection (hpi). However, a robust cytopathic effect (CPE) was observed only in TRBV-infected MDMs. Conversely, TBEV caused no CPE and slightly downregulated the IFN-β response in bovine MDMs. In conclusion, this study provides unique insights into the innate immune responses to these arboviruses in ruminants, an area of research that is currently very limited.

INTRODUCTION

In general, tick-borne arboviruses can initially replicate in keratinocytes, dendritic cells (DCs), macrophages and neutrophils (Wikel et al. 1994; Labuda et al. 1996; Ho et al. 2001). Macrophages are professional antigen-presenting cells (APCs) that play a key role in the pathogenesis and dissemination of many viruses. This is associated with a reduced maturation and decreased production of cytokines and presentation molecules. However, these viruses can also promote the migration of APCs, induce evasion of apoptosis or delay this process (Mercer & Greber, 2013).

In Slovakia, tick-borne encephalitis virus (TBEV; *Flavivirus*) and some tick-borne orbiviruses (TBOs) are endemic zoonotic arboviruses with neurotropic potential. In this study, two TBOs were used, namely the Kemerovo virus (KEMV) and the Tribeč virus (TRBV). While TBEV is a well known and a significant public health concern with clear clinical manifestations in humans, KEMV and TRBV are frequently overlooked despite their potential to cause neurological disease. Goats, sheep and cows do not show any clinical signs of disease during TBEV, KEMV or TRBV infection.

In endemic areas, the prevalence of these viruses in ruminants is relatively high which posing a risk of alimentary TBEV infection to consumers of raw dairy products.

METHODS

Bovine macrophages (derived from blood monocytes, or MDMs) were infected with TBEV, KEMV or TRBV at a low multiplicity of infection (MOI = 0.1). After a one-hour adsorption period, the inoculum was discarded and the cells were washed with an excess volume of culture medium (RPMI supplemented with 10% horse serum). The infected cells were then incubated for 24 hours at 37°C in a CO_2 atmosphere. For gene expression analysis, the cells were lysed and the lysates stored at -20°C. Total RNA was isolated using TRIzol™ (ThermoFisher Scientific, USA) according to the manufacturer's instructions and stored at -80°C. Prior to reverse transcription, the RNA samples were treated with recombinant DNase I (Takara Bio Europe, France). First-strand complementary DNA (cDNA) was synthesised using RevertAid Premium Reverse Transcriptase and random hexamer primers (all ThermoFisher Scientific, USA). The cDNA was then stored at -20 °C until qPCR. Real-time PCR was performed using an iCycler CFX96 (Bio-Rad, USA), and the expression of type I interferons (IFN-I) was evaluated using the $2^{-\Delta\Delta Ct}$ method and β-actin as the reference gene (CFX96 Manager software).

RESULTS AND DISCUSSION

To evaluate the *in vitro* response of bovine MDMs to tick-borne arboviruses, we assessed the induction of CPE (Figure 1) and IFN-I gene expression (Figure 2). For IFN-I response, a significantly increased mRNA levels was observed for both TBOs at 24 hpi. However, a robust CPE was found only in TRBV-infected MDMs. Conversely, TBEV caused no CPE and slightly downregulate IFN-β response in bovine MDMs. Our results are consistent with a recent study by Adjadj et al. (2026) which aimed to analyse the mRNA expression profiles of selected cytokines and chemokines in the prescapular lymph nodes of sheep. This study revealed a weak to moderate innate antiviral immune response in sheep infected with TBEV. TBEV is well known for its immune evasion mechanisms, such as the suppression of the IFN-I response, which allow it to persist within the host's immune cells without triggering strong inflammatory or antiviral defences. In general, flavivirus-induced antagonism with IFN response also contributes to the productive infection of APCs. Langat virus (LGTV) is a tick-borne flavivirus that is genetically and antigenically similar to TBEV. In the early stages of LGTV infection of murine dendritic cells, transcription factor activation is suppressed, leading to delayed IFN production (Robertson et al. 2014). In the case of the Zika virus, STAT-dependent inhibition of IFN-stimulated gene activation occurs in human DCs differentiated from monocytes (MoDCs) but not in MDMs from the same donor (Yang et al. 2021). The knowledge of

the effect of TBEV on ruminant immune cells is very limited and completely absent in the case of the studied TBOs.

CONCLUSION

In conclusion, this study reveals that bovine macrophages exhibit distinct responses to arbovirus infection. While tick-borne orbiviruses (TBOs) elicit a robust IFN-I response, tick-borne encephalitis virus (TBEV) exhibits immune-evasive characteristics. These findings provide novel, critical insights into antiviral mechanisms specific to ruminants, expanding our limited knowledge in this research area.

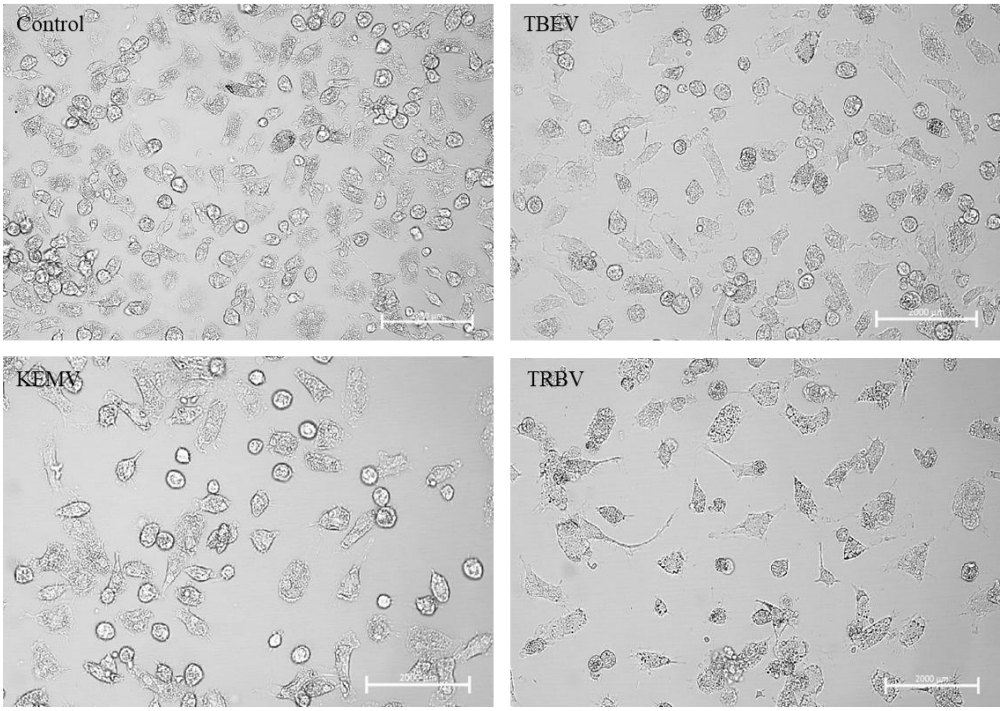


Figure 1 Morphological changes in bovine macrophages (derived from monocytes, MDMs) infected with tick-borne encephalitis virus (TBEV), Kemerovo virus (KEMV) or Tribeč virus (TRBV). The MDMs were infected with viruses at multiplicity of infection 0.1 for 24 hours.

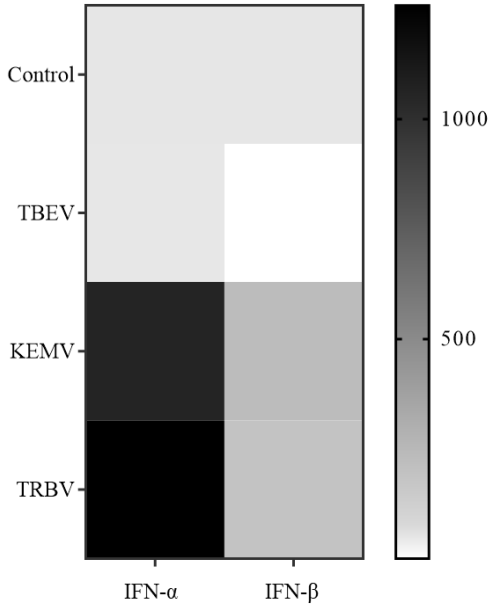


Figure 2 Gene expression of interferon (IFN)-α and IFN-β in bovine macrophages (MDMs) infected with tick-borne arboviruses in vitro. The MDMs were infected with viruses at multiplicity of infection 0.1 for 24 hours. Results are clearly displayed in the heat map prepared using GraphPad Prism 9.0.0. Legend: TBEV – tick-borne encephalitis virus, KEMV – Kemerovo virus, TRBV – Tribeč virus

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EXPERIMENTAL MODEL OF A HUMAN SEPTIC KIDNEY GRAFT DONOR IN PIGS INFECTED WITH THE MULTIDRUG-RESISTANT STRAIN *KLEBSIELLA PNEUMONIAE* ATCC BAA-1705

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ABSTRACT

The aim of this study was to simulate a human septic kidney graft donor using an animal model infected with the multidrug-resistant strain *Klebsiella pneumoniae* ATCC BAA-1705. Compared with the normothermic model, mild hypothermia enabled maintenance of a controllable course of experimental sepsis during general anesthesia, slowed the progression of organ dysfunction as assessed by the modified SOFA score, and resulted in a sufficient bacterial load in renal tissue. The obtained results represent an optimized experimental model of a human septic organ donor suitable for future experimental research in kidney transplantation.

INTRODUCTION

Experimental animal models represent an integral part of transplantation research, as they enable the evaluation of novel surgical procedures, organ perfusion technologies, and therapeutic strategies under conditions closely resembling clinical practice. Among large animal models, pigs are considered the preferred experimental species owing to their anatomical, physiological, and immunological similarity to humans. An additional advantage is the similarity of their gut bacteriome (Tang et al., 2025) and mycobiome (Prisnee et al. 2023), which enhances the translational relevance of experimental findings. The aim of this study was to establish an experimental porcine model simulating a human septic kidney graft donor by infection with the multidrug-resistant strain *Klebsiella pneumoniae* ATCC BAA-1705 and to optimize its course for subsequent transplantation experiments.

METHODS

Animal experiments were approved by the State Veterinary and Food Administration of the Slovak Republic (No. 7788/2025-220). The study was conducted in the accredited Laboratory of Gnotobiology (SK U 10021) at the University of Veterinary Medicine and Pharmacy (UVMP) in Kosice, Slovakia. Three female crossbred pig [(Landrace × Large White) × Pietrain], weighing 55–65 kg (K1-K3), originating from the same litter and bred at the Clinic of Swine, University Veterinary Hospital, UVMP in Kosice, Slovakia (farm registration No. 112 AUMK6), were included in the study. Animals that had received antibiotics or anti-inflammatory drugs within the previous three months or had undergone anesthesia or sedation during the preceding 30 days were excluded from the study. Animals were maintained under general anesthesia throughout the experiment. Anesthesia was induced using butorphanol, medetomidine, and tiletamine-zolazepam, and maintained with sevoflurane in 100% oxygen using a semi-closed breathing system. Sepsis was induced by *i.v* administration of 20 mL of carbapenem-resistant *Klebsiella pneumoniae* ATCC BAA-1705 (5×10^9 CFU/mL) through an *i.v.* catheter at a rate of 40 mL/h. During the experiment, body temperature, mean arterial pressure (MAP), central venous pressure (CVP), peripheral oxygen saturation (SpO_2), and hourly urine output were continuously monitored. Hematological, biochemical (urea, creatinine, bilirubin, transaminases), acute-phase inflammatory, blood gas, and acid-base parameters were evaluated every 2–3 h. Organ dysfunction was assessed using modified Sequential Organ Failure Assessment (SOFA) and quick SOFA, including three-domain (respiratory parameters, MAP, urine output) and five-domain (including bilirubin and platelet count) models. The duration of anesthesia and monitoring was adjusted according to the animals' clinical condition and SOFA scores.

RESULTS AND DISCUSSION

The physiological body temperature of pigs ranges from 38.0 to 39.3 °C. Hypothermia reduces host metabolic activity, attenuates the inflammatory response, and simultaneously slows the growth and expression of virulence factors of *Klebsiella pneumoniae* (Müller et al. 2024). Based on these observations, the experimental design was established to compare the progression of experimentally induced sepsis under hypothermic and normothermic conditions.

In pig K1, sepsis was induced for 9 h under hypothermic conditions (33.9–36.0 °C), whereas pig K2 was maintained under normothermia (38–39 °C) for 24 h. Hematological analyses demonstrated distinct systemic responses. In K1, the initial endotoxemic phase was followed by a compensated immune response characterized by leukocyte recovery and partial stabilization of platelet counts without progression to septic shock. In contrast, K2 developed septic shock within 3–5 h, accompanied by marked leukopenia, neutropenia, thrombocytopenia, and persistent coagulopathy. These differences corresponded with the extent of *Klebsiella pneumoniae* dissemination. In K1, the

pathogen was detected in the liver (4.60 $\log_{10}$ CFU/g), spleen (4.13 $\log_{10}$ CFU/g), and only the central kidney region (3.40 $\log_{10}$ CFU/g), whereas bronchoalveolar lavage remained culture-negative. In contrast, K2 exhibited markedly higher bacterial loads, particularly in the spleen (6.92 $\log_{10}$ CFU/g) and central kidney region (5.77 $\log_{10}$ CFU/g), with diffuse renal dissemination. Progressive renal and hepatic dysfunction in K2 was reflected by increasing urea, creatinine, bilirubin, and liver enzyme concentrations. Following a brief period of oliguria, serum creatinine increased progressively to 318 µmol/L, consistent with acute kidney injury (Fig. 1a,b), whereas creatinine remained within the physiological range throughout the experiment in K1**.** Overall, normothermia accelerated the progression of systemic infection and septic shock, whereas hypothermia maintained a compensated course of sepsis.

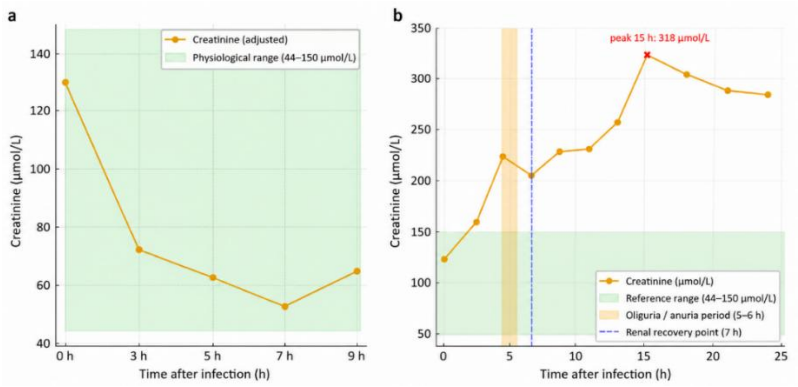


Figure 1 Time-course of serum creatinine concentration following infection with Klebsiella pneumoniae ATCC BAA-1705 in pigs ***(a)*** *K1 and* ***(b)*** *K2.*

Based on these findings, the experimental protocol for pig K3 was modified to achieve a more slowly progressing septic state while maintaining systemic dissemination of the pathogen. Sepsis was induced for 10 h under mild hypothermia (35–36 °C). The hematological profile resembled that of K1 and was characterized by transient leukopenia (WBC 9.59 → 2.34 × 10^9/L) and neutropenia (NEU 3.52 → 0.80 × 10^9/L) during the first 3 h after infection, followed by reactive leukocytosis during hours 9–10 (WBC 13.17–16.33 × 10^9/L; NEU 7.76–8.77 × 10^9/L). Thrombocytopenia persisted throughout the experiment (388 → 131 × 10^9/L), corresponding to coagulation dysfunction (SOFA score 2), without progression to septic shock. Renal function remained preserved without evidence of acute kidney injury; serum creatinine showed only a mild increase without a progressive trend, urine output fluctuated from transiently reduced values to polyuria, and blood urea remained within the physiological range. Hepatic dysfunction was reflected by a continuous increase in ALT, AST, and GLDH activities together with a 4–5-fold increase in bilirubin concentration (20–21 µmol/L; hepatic SOFA score 2). A marked increase in serum amyloid A confirmed the development of a systemic inflammatory response, reaching 139.8–159.9 mg/L during hours 9–10 of the experiment.

Overall, pig K3 represented a model of compensated acute sepsis with preserved renal function and without progression to multiple organ failure.

Bacterial dissemination was evaluated in pig K3 after 10 h of sepsis induction. Microbiological culture analyses confirmed multiorgan dissemination of the carbapenem-resistant strain *Klebsiella pneumoniae* ATCC BAA-1705. The highest bacterial loads (Fig. 2) were detected in the liver (6.48 $\log_{10}$ CFU/g), spleen (6.38 $\log_{10}$ CFU/g), and lungs (6.32 $\log_{10}$ CFU/g). The pathogen was also detected in bronchoalveolar lavage (3.85 $\log_{10}$ CFU/mL), the pancreas (5.00 $\log_{10}$ CFU/g), myocardium (4.18 $\log_{10}$ CFU/g), and kidneys, which exhibited a mild to moderate bacterial burden (2.48–3.30 $\log_{10}$ CFU/g) without pronounced regional predominance.

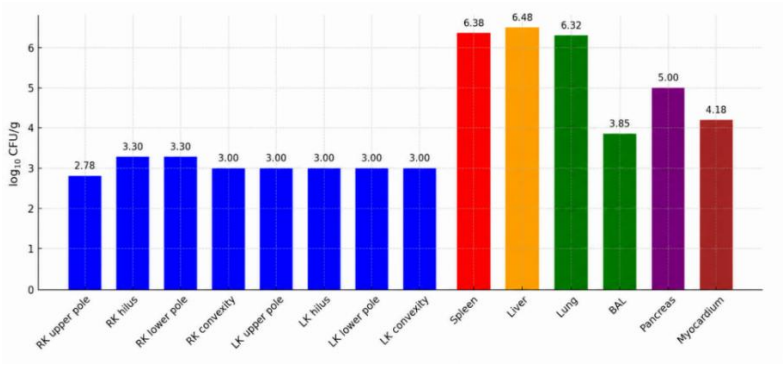


Figure 2 Bacterial load ($\log_{10}$ CFU/g) in tissues of pig K3 10 h after i.v. infection with Klebsiella pneumoniae ATCC BAA-1705. RK, right kidney; LK, left kidney; BAL, bronchoalveolar lavage.

CONCLUSION

The obtained results demonstrate the successful optimization of an experimental sepsis model designed to simulate a human septic organ donor. The introduction of mild hypothermia enabled controlled bacterial dissemination to the kidneys while maintaining a stable course of sepsis, thereby providing an experimental model suitable for future research in kidney transplantation.

ACKNOWLEDGEMENT

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CROSS-BORDER MOLECULAR SURVEILLANCE OF EQUINE VECTOR-BORNE PATHOGENS IN CENTRAL AND EASTERN EUROPE: A DECADE OF MOLECULAR SURVEILLANCE

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ABSTRACT

Equine tick-borne diseases represent an emerging veterinary problem, and climate change, wildlife dynamics, and the expanding distribution of tick vectors strongly influence their epidemiology. Horses sharing grazing areas with wildlife may serve as sentinels of pathogen circulation. In 2016, the first molecular survey of equine tick-borne pathogens in Ukraine, Poland, and Slovakia established baseline data on the occurrence of *Theileria equi* and *Anaplasma phagocytophilum*. A decade later, these data provide a valuable reference for long-term epidemiological surveillance. These findings demonstrate the silent circulation of vector-borne pathogens in horses and highlight the importance of integrated One Health surveillance involving horses, wildlife, and vectors.

INTRODUCTION

Equine vector-borne diseases represent an emerging veterinary concern, with their epidemiology being strongly influenced by climate change, wildlife dynamics, and the expanding distribution of blood-sucking vectors (Almazán et al. 2022). Horses sharing grazing areas with wildlife may serve as sentinels of pathogen circulation within the One Health framework. In 2016, the first molecular survey of equine tick-borne pathogens in Ukraine, Poland, and Slovakia established baseline data on the occurrence of *Theileria equi* and *Anaplasma phagocytophilum* (Slivinska et al. 2016). A decade later, these data provide a valuable reference for long-term epidemiological surveillance.

METHODS

In 2016, blood samples from 215 clinically healthy horses originating from Ukraine, Poland, and Slovakia were collected from multiple farms (Figure 1). Genomic DNA was extracted from EDTA-treated blood samples using a commercial extraction kit. Molecular detection of *Theileria equi* and *Anaplasma phagocytophilum* was performed using conventional and nested PCR targeting the 18S and 16S rRNA genes, respectively (Casati et al. 2006; Hornok et al. 2014; Massung et al. 1998). Selected amplicons were confirmed by DNA sequencing and compared with GenBank reference sequences. Associations between pathogen occurrence and host characteristics (age and sex) were evaluated using the χ^2 test, with statistical significance set at $P < 0.05$.

In 2026, blood samples from more than 460 clinically healthy horses originating from Ukraine, Poland, and Slovakia were examined using nested PCR assays targeting *Trypanosoma* spp., *Babesia/Theileria* spp., *Anaplasma phagocytophilum*, hemotropic *Mycoplasma* spp., and *Rickettsia* spp. Associations between pathogen occurrence and selected epidemiological variables, including country, season, age, and sex, were evaluated using statistical analyses.



Figure 1. Location of investigated farms from 2016. The white dots represent the places where no animal was found to be positive using the PCR method. The black dots represent the locations where Theileria equi-positive animals were detected by PCR. The grey dots represent the locations where Anaplasma phagocytophilum-positive animals were detected by PCR. 1—Kyiv Region, North Ukraine (50°27'N; 30°30'E); 2—Chernobyl Exclusion Zone, North Ukraine (51°16'N; 30°13'E); 3—Odessa Region, South Ukraine (47°132N; 30°02'E); 4—Khersonskyi Region, South Ukraine (47°27'N; 33°52'E); 5—West Crimea, South Ukraine (45°22'N; 32°32'E); 6—Chernivtsi Region, West Ukraine (48°17'N; 25°55'E); 7—Biebrza National Park, Podlasie District North-East Poland (53°28'N; 22°39'E); 8—Warmia-Masurian District, North-East Poland (53°46'N; 21°45'E); 9—Prešov, North-Eastern Slovakia (48°59'N; 21°14'E); 10—Košice, Eastern Slovakia (48°42'N; 21°15'E); 11—Luba, South-West Slovakia (47°51'N; 18°36'E).

RESULTS

In a previous study, *T. equi* DNA was detected in 30/215 horses (13.9%), with the highest prevalence in Ukraine (29.0%) and the lowest in Poland (1.3%), while no positive horses were identified in Slovakia. Sequence analysis confirmed the identity of all examined isolates as *T. equi*. *Anaplasma phagocytophilum* DNA was detected in three clinically healthy horses (1.4%), originating from Poland and Slovakia. No co-infections were observed. *T. equi* occurrence differed significantly among countries ($P < 0.001$), whereas no association with horse age or sex was found. Likewise, *the occurrence of A. phagocytophilum* was not associated with host age, sex, or geographic origin. Nucleotide sequences obtained in the previous study were submitted to the GenBank database under the following accession numbers: T. equi 18S rRNA (KP868757, KP868758, and KP868759); A. phagocytophilum msp4 (KP868760, KP868761).

In 2026, protozoal and bacterial vector-borne pathogens were detected in horses from all three countries. *Babesia/Theileria* spp. represented the most prevalent group, followed by *Rickettsia* spp., whereas *A. phagocytophilum*, *Trypanosoma* spp., and hemotropic *Mycoplasma* spp. were detected less frequently. Detected *Trypanosoma* isolates clustered within the *Trypanosoma theileri* complex. The occurrence of *Babesia/Theileria* spp. and *Rickettsia* spp. differed significantly among countries and sampling seasons, reflecting regional ecological conditions and vector activity. Coinfections with two or three pathogens were also identified in the examined horses.

DISCUSSION

Horses may serve as valuable sentinels for monitoring vector-borne pathogens circulating at the wildlife–livestock interface. The 2016 study was the first molecular detection of *T. equi* and *A. phagocytophilum* in horses from Slovakia, Poland, and Ukraine, providing baseline epidemiological data for the region. The prevalence of both pathogens varied considerably among countries, reflecting differences in vector distribution, environmental conditions, and diagnostic approaches. Similar geographic variation has been reported across Europe and Asia, where the prevalence of *T. equi* and *A. phagocytophilum* ranges widely depending on the study area and the diagnostic methods employed (Leblond et al. 2005; Passamonti et al. 2010; Abedi et al. 2014). These findings highlight the importance of standardized molecular surveillance for reliable comparison of pathogen occurrence over time and between regions.

A decade later, these data provide a valuable reference for long-term epidemiological surveillance. The current survey, conducted ten years later, demonstrates a broader spectrum of vector-borne pathogens in horses, indicating increased detection capacity and/or changing epidemiological patterns in the region.

CONCLUSIONS

The findings demonstrate that horses in Central and Eastern Europe are exposed to a diverse range of vector-borne pathogens, often without clinical signs. The decade-long comparison underscores the value of long-term surveillance, revealing an expansion in the spectrum of detected pathogens. These results underscore the importance of a One Health approach that integrates data from horses, vectors, wildlife, and the environment. Coordinated cross-border surveillance is essential to improve disease monitoring and to develop effective strategies for the prevention and control of emerging vector-borne infections.

ACKNOWLEDGEMENT

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COMPARISON OF PATHOGEN PREVALENCE IN HOST-ASSOCIATED AND HOST-SEEKING *LIPOPTENA* SPP. (DIPTERA: HIPPOBOSCIDAE) FROM SLOVAKIA

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ABSTRACT

Deer keds (*Lipoptena* spp.) are ectoparasites increasingly recognised as potential vectors of pathogens. This study investigated the occurrence of selected vector-borne pathogens in host-associated deer keds collected from hunted cervids and host-seeking deer keds from the environment in Slovakia. A total of 782 specimens were analysed using molecular methods. Host-associated deer keds showed a high prevalence of *Bartonella* spp. (88,33%), followed by *Babesia/Theileria* spp. (60,83%), *Anaplasma phagocytophilum* (24,58%), Kinetoplastida (20,42%), and haemotropic *Mycoplasma* spp. (4,56%). *Setaria cervi* was detected in 2,5% of eighty tested specimens. In contrast, host-seeking deer keds were positive for *Bartonella* spp. (7,38%), *Rickettsia* spp. (0,92%), and haemotropic *Mycoplasma* spp. (0,37%), whereas *Borrelia* spp. were not detected in either group.

INTRODUCTION

Deer keds of the genus *Lipoptena* (Diptera: Hippoboscidae) are obligate blood-feeding ectoparasites of mammals, primarily members of the family Cervidae. Six species of the genus *Lipoptena* have been reported from Europe to date (Salvetti et al. 2020; González et al. 2024), while only two occur in Slovakia: *Lipoptena cervi* (Linnaeus, 1758) and *Lipoptena fortisetosa* Maa, 1965 (Oboňa et al. 2019). There are studies on the role of hippoboscids as vectors of numerous vector-borne pathogens; however, vector competence has not yet been confirmed in any of them (Bruin et al. 2015; Werszko et al. 2020; Dibo et al. 2023; Peña-Espinoza et al. 2023).

METHODS

During 2023-2024, deer keds were collected from wildlife hosts (13 red deer and 2 fallow deer) and from the environment (via flagging or directly from clothing) at multiple localities across Slovakia (Figure 1). Specimens were identified morphologically using a key of Oboňa et al. (2022). Genomic DNA was extracted individually by alkaline hydrolysis (host-seeking deer keds) (Guy and Stanek, 1991) or using the NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany). Extracted DNA was screened for selected vector-borne pathogens by PCR according to published protocols (references with author). Individual specimens were analysed for *Bartonella* spp., *Babesia/Theileria* spp., and members of the order Kinetoplastida. A subset of the samples (n=80) was additionally tested for filarial DNA. Haemotropic *Mycoplasma* spp., *Rickettsia* spp., *Borrelia* spp., and *A. phagocytophilum* were initially analysed in pools of 10 specimens, with positive pools subsequently tested individually. Selected positive PCR products were purified using the NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany) and subjected to DNA sequencing.

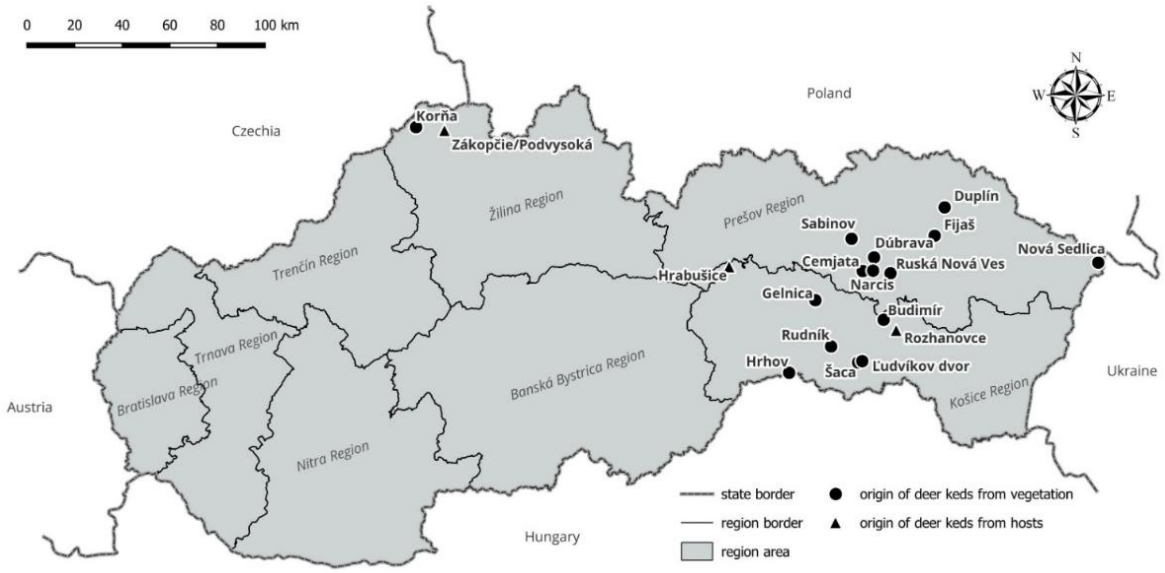


Figure 2 Origin of Lipoptena spp.

RESULTS

A total of 782 deer keds were collected, including 437 *L. fortisetosa* and 345 *L. cervi*. The host-associated group comprised 240 wingless specimens collected from cervids (230 *L. cervi* and 10 *L. fortisetosa*), whereas the host-seeking group consisted of 542 winged specimens collected from the environment (427 *L. fortisetosa* and 115 *L. cervi*).

Molecular analyses revealed a markedly higher pathogen prevalence in host-associated deer keds. *Bartonella* spp. were the most frequently detected pathogen (88,3%), followed by piroplasmids *Babesia/Theileria* spp. (60,83%), *A. phagocytophilum* (24,58%), Kinetoplastida (20,42%), and

haemotropic *Mycoplasma* spp. (4,56%). In addition, *Setaria cervi* DNA was detected in 2 of 80 examined *L. cervi* specimens (2,5%).

In contrast, pathogen prevalence was substantially lower in host-seeking deer keds. *Bartonella* spp. remained the most prevalent pathogen (7,38%). Unlike the previous group, *Rickettsia* spp. were detected in host-seeking deer keds, with a prevalence of 0,92%. Haemotropic *Mycoplasma* spp. were also detected at a low prevalence (0,37%). *Borrelia* spp. were not detected in either group.

DISCUSSION

This study shows clear differences in pathogen prevalence between host-associated and host-seeking deer keds. Few studies have compared deer keds collected from hosts and from the environment (Bruin et al. 2015; Gałęcki et al. 2021; Vogt et al. 2024; Shimizu et al. 2025). The prevalence of *Bartonella* spp., piroplasmids, *A. phagocytophilum*, *Trypanosoma* spp., and filarial DNA in host-associated deer keds indicates that cervids serve as reservoirs of these pathogens, although the evaluation of the deer keds as potential vectors of these pathogens requires further investigation. The detection of *Bartonella* spp., *Rickettsia* spp., and haemotropic *Mycoplasma* spp. in host-seeking deer keds may suggest transstadial transmission; however, this requires further confirmation (Bruin et al. 2015; Gałęcki et al. 2021). In Slovakia, only one study has confirmed the presence of *A. phagocytophilum* in *L. cervi* collected from cervids (Víchová et al. 2011).

CONCLUSION

Our results demonstrate a broad spectrum of vector-borne pathogens in deer keds, with markedly higher prevalence in host-associated compared to host-seeking individuals. In addition, a previously undocumented association between *L. cervi* and the filarial nematode *S. cervi* was revealed. These findings highlight the potential role of deer keds in pathogen circulation within wildlife and the need for further studies combining molecular, microscopic, and experimental approaches to clarify their vector competence. Given the increasing interaction between wildlife, livestock, and humans, a One Health approach is essential for evaluating and reducing emerging parasitic risks.

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MOLECULAR GENETIC DETECTION OF NOROVIRUS ON A SELECTED FARM IN SLOVAKIA

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ABSTRACT

Noroviruses are important enteric pathogens of humans and animals. No information about their occurrence in livestock in Slovakia are available. This study investigated the presence of noroviruses in fecal samples collected from 20 calves and 20 pigs using nested RT-PCR targeting the RNA-dependent RNA polymerase (RdRp) gene, followed by sequencing and phylogenetic analysis. Norovirus RNA was detected in 5/20 (25%) bovine and 1/20 (5%) porcine samples. Phylogenetic analysis classified the bovine strains within genogroup GIII (GIII.1 and GIII.2) and the porcine strain within genogroup GII (GII.18). The detected sequences clustered with host-associated animal norovirus lineages and showed no close phylogenetic relationship to human norovirus strains. This study provides the first molecular evidence of bovine and porcine noroviruses in livestock in Slovakia.

INTRODUCTION

Noroviruses are highly infectious, genetically diverse, non-enveloped, positive-sense RNA viruses belonging to the family *Caliciviridae* and are important causes of acute gastroenteritis in humans and animals (Karst et al. 2014). Based on genetic diversity, noroviruses are classified into multiple genogroups and genotypes with distinct host associations; human infections are mainly associated with GI, GII, and GIV, while animal noroviruses have been identified in several species, including cattle (GIII) and pigs (GII) (Chhabra et al. 2019). Although direct animal-to-human transmission has not been confirmed yet, serological evidence of antibodies against bovine and canine noroviruses in humans and the detection of human norovirus in bovine feces suggest that interspecies transmission may occur (Widdowson et al. 2005; Mattison et al. 2007). In livestock, norovirus infections may contribute to enteric disease and economic losses, although asymptomatic infections are also observed. The aim of this study was to detect the presence of norovirus in pigs and cattle on a farm in eastern Slovakia and to evaluate the genetic relationship of detected strains with known animal and human norovirus lineages.

METHODS

Samples

A total of 40 fecal samples were collected from randomly selected calves aged up to two weeks, most of which had diarrhea (n = 20), and pigs of different ages (n = 20) with no clinical signs, from different production units within the same farm in eastern Slovakia. Samples were transported to the laboratory at 4 °C within 24 h of collection and processed immediately upon arrival.

RNA extraction

Fecal suspensions were prepared by diluting each sample 1:10 (w/v) in isotonic saline solution (Sigma Aldrich), followed by centrifugation at 14,000 × g for 5 min at 4 °C. Viral RNA was extracted from the supernatants using the QIAamp Viral RNA Mini Kit (QIAGEN GmbH) on a QIAcube automated robotic station (QIAGEN GmbH) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized using RevertAid Reverse Transcriptase (Thermo Fisher Scientific) and random hexamer primers (Invitrogen). RNA were stored at −80 °C until further analysis.

Nested RT-PCR, sequencing and phylogenetic analysis

Bovine and porcine noroviruses were detected by nested RT-PCR targeting the RNA-dependent RNA polymerase (RdRp) gene. Bovine norovirus (BoNoV) was amplified using the outer primer pair CBECU-F/CBECU-R (Smiley et al. 2003), followed by the internal primer pair CBECU-nF/CBECU-nR (Park et al. 2006), yielding a 326 bp amplicon. Porcine norovirus (PoNoV) was detected using primer pairs designed in the present study (NOROF5/NOROR6 and NOROF6/NOROR6), amplified a 599 bp amplicon. PCR products were separated on 2% agarose gels stained with GelRed and visualized by GelDoc Go System (Bio-Rad). Previously sequenced and BLAST-confirmed BoNoV- and PoNoV-positive samples were used as positive controls and isotonic saline solution was used as the negative control. Amplicons of the expected size were purified and sequenced by the Sanger method (Microsynth Austria GmbH). Sequence assembly and analysis were performed using SeqMan, EditSeq, and MegAlign (Lasergene, DNASTAR Inc). Molecular evolutionary model selection and phylogenetic tree construction were carried out using MEGA XII (Kumar et al. 2024). Reference strains representing various subtypes of genotypes GII and GIII were included in the phylogenetic analysis.

RESULTS

A total of 5 bovine samples (5/20, 25%) and 1 porcine sample (1/20, 5%) yielded amplification products of the expected size after nested RT-PCR. Positive bovine norovirus samples were identified as ZT3T, ZT7T, ZT9T, ZT13T, and ZT16T, while the positive porcine norovirus sample was identified as ZT5V.

Maximum likelihood phylogenetic analysis of partial RdRp gene (284 bp) using the Kimura-2 parameter model with Gamma distribution (K2 + G) was employed. Phylogenetic analysis showed that one partial RdRp sequence (ZT5V) was most closely related to genotype GII.18, whereas the five bovine partial sequences were most closely related to GIII bovine norovirus sequences. The sequence ZT3T was most closely related to GIII.1 viruses, whereas the sequences of ZT7T, ZT9T, ZT13T and ZT16T were most closely related to GIII.2 viruses.

DISCUSSION

Animal noroviruses are not routinely monitored or subject to mandatory reporting, therefore, the absence of previous data does not necessarily indicate the absence of viral circulation in the country. Phylogenetic analysis of PCR products confirmed the host-associated clustering of the detected strains. The bovine sequences grouped within genogroup GIII, whereas the porcine sequences clustered within genogroup GII, in agreement with the current classification of the genus *Norovirus* (Chhabra et al. 2019). Although porcine noroviruses belong to the same genogroup as the majority of human noroviruses, they represent distinct evolutionary lineages and remain genetically separated from human GII strains (Scipioni et al. 2008). Bovine noroviruses constitute a separate genogroup (GIII) which has been consistently associated with cattle (Oliver et al. 2006; Mauroy et al. 2014). The phylogenetic relationships observed in the present study provide no evidence supporting zoonotic transmission between livestock and humans. No clustering of the detected animal strains (ZT3T, ZT7T, ZT9T, ZT13T, ZT16T, ZT5V) with human norovirus lineages was observed, suggesting that the detected viruses represent host-associated lineages. To date, a direct link of norovirus infection from animal to human has not been confirmed (Mattison et al. 2007).

CONCLUSION

This study provides the first molecular evidence of bovine and porcine noroviruses circulating in livestock in Slovakia. Phylogenetic analysis confirmed that the detected strains belong to host-associated norovirus lineages, with bovine strains classified within genogroup GIII and the porcine strain within genogroup GII. These findings emphasize the need for further surveillance of animal noroviruses to better understand their distribution and genetic diversity.

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MOLECULAR SURVEY OF HAEMOTROPIC *MYCOPLASMA* SPP. AND VECTOR-BORNE PATHOGENS IN HORSES, DOMESTIC RUMINANTS, AND RED DEER IN SLOVAKIA

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ABSTRACT

Haemotropic *Mycoplasma* spp. and selected vector-borne pathogens were investigated in domestic animals, red deer, and *Dermacentor reticulatus* ticks in Slovakia. Haemoplasmas were detected in cattle, sheep, and goats, while *Theileria capreoli* occurred frequently in red deer. Horses tested positive for *Anaplasma phagocytophilum* and *Bartonella* spp. Ticks harboured several bacterial, protozoan, and nematode pathogens, providing updated data on pathogen distribution in Slovakia.

INTRODUCTION

Vector-borne pathogens represent a major threat to the health and productivity of domestic animals and wildlife. Tick-borne bacteria and protozoa, together with haemotropic mycoplasmas (haemoplasmas), contribute to economic losses in livestock and reflect pathogen circulation within natural ecosystems. Ongoing changes in climate, wildlife populations, and land use have altered the distribution of tick vectors across Europe, increasing the need for surveillance of vector-borne pathogens in domestic and wild animals.

Despite the growing importance of vector-borne diseases in Central Europe, information on haemoplasmas and tick-borne pathogens in livestock, horses, wildlife, and their associated vectors remains scarce in Slovakia. Therefore, this study investigated the occurrence of haemotropic *Mycoplasma* spp. and selected vector-borne pathogens in domestic and wild nimals in Slovakia.

METHODS

EDTA-treated blood samples were collected from horses, cattle, goats, and sheep on 17 livestock farms located in Slovakia between 2008 and 2026. A total of 589 blood samples were analysed, comprising 232 from cattle, 167 from goats, 150 from sheep, and 40 from horses. *Dermacentor reticulatus* ticks (n=60) feeding on horses were also collected. In total, 43 liver samples from red deer were obtained during the 2022 and 2023 hunting seasons. Sample collection was performed in accordance with the ethical standards and applicable legislation of the Slovak Republic. Blood and liver samples were screened for haemotropic *Mycoplasma* spp. (Varanat et al., 2011) and piroplasmids (*Babesia/Theileria* spp.) (Casati et al., 2006) using PCR-based molecular methods. Additionally, blood samples from horses (n = 40) and attached *D. reticulatus* ticks collected from the same animals (n = 60) were screened for *A. phagocytophilum* (Courtney et al. 2004), *Rickettsia* spp. (Stenos et al. 2005), *Borrelia* spp. (Derdáková et al. 2003), *Bartonella* spp. (Diaz et al. 2012), kinetoplastids (Votýpka et al. 2015; Maslov et al. 1996), and filarial nematodes (Casiraghi et al. 2001) by PCR or real-time PCR.

RESULTS

The prevalence of detected pathogens in animal samples and *D. reticulatus* ticks is shown in Tables 1 and 2. Sequencing of the 16S rRNA gene identified "*Candidatus* M. haematobovis" (PQ728987, PQ728988) and/or *M. wenyonii* (PQ738962-PQ738967) in cattle samples, while *M. ovis* (PQ776912-PQ776915) was detected in sheep and goat samples. Sequencing of the 18S rRNA gene revealed the presence of *Theileria* spp. (PQ682422-PQ682427) in tested red deer. Horses were also positive for *A. phagocytophilum*, and *Bartonella* spp. and negative for other tested pathogens. *Dermacentor reticulatus* ticks were positive for DNA of *A. phagocytophilum*, *Rickettsia* spp., *Bartonella* spp., Kinetoplastida spp., and for filarial nematodes. Sequencing of selected amplicons showed the highest similarity to *Babesia canis*, and *Rickettsia raoultii* in engorged *D. reticulatus* ticks.

DISCUSSION

Haemotropic *Mycoplasma* infections in cattle have been reported worldwide, including in several European countries, where prevalence typically ranges from 50% to 97% (de Souza Ferreira et al., 2024). In the present study, haemoplasmas were detected on eight of 11 cattle farms, with an overall prevalence of 53.5%, confirming that these infections are widespread in Slovakia. Sequence analysis identified *Mycoplasma wenyonii* and *Candidatus* Mycoplasma haematobovis, the two haemoplasma species most commonly reported in cattle. Interestingly, three farms were negative, suggesting that management practices or environmental factors may influence transmission. Haemotropic mycoplasmas were not detected in horses, and our result aligns with other reports of very low prevalence in horses (Dieckmann et al. 2010; Konstantinović et al. 2026). No piroplasmids were

detected in cattle, irrespective of haemoplasma infection status. This finding agrees with the hypothesis of pathogen interference proposed by Tagawa et al. (2013), who observed reduced susceptibility of haemoplasma-infected cattle to *Theileria orientalis*. Similar observations were reported in Hungarian water buffaloes, in which high haemoplasma prevalence coincided with the absence of *Theileria*, *Babesia*, and *A. phagocytophilum* (Hornok et al. 2017). However, environmental conditions, vector abundance, or low circulation of piroplasmids in the study areas may also explain their absence in Slovak cattle. Red deer showed an opposite infection pattern. Haemoplasmas were not detected, whereas 65.1% of liver samples tested positive for *Theileria* spp.

Animal	**Tested**	**Sample type**	***Mycoplasma* spp.** **+/%**	***Babesia/Theileria* spp.** **+/%**
Horses	40	blood	0	0
Cattle	232	blood	124/53.5	0
Goats	167	blood	7/4.2	3/1.8
Sheep	150	blood	90/60	0
Red deer	43	liver	0	28/65.1

Table 1 Prevalence of haemotropic Mycoplasma spp. and Babesia/Theileria spp. in farm animals and wild ruminants in Slovakia.

Sample type	**Tested**	***Anaplasma phagocytophilum*** **+/%**	***Rickettsia* spp.** **+/%**	***Borrelia* spp.** **+/%**	***Bartonella* spp.** **+/%**	**Kinetoplastida** **+/%**	**Filaria** **+/%**
Horses	40	4/10	0	0	3/7.5	0	0
Dermacentor reticulatus	60	10/16.7	30/50	0	5/8.3	6/10	2/3.3

Table 2 Detection of vector-borne pathogens in horses and D. reticulatus ticks.

CONCLUSION

Overall, the present study demonstrates distinct host-specific patterns of pathogen occurrence: haemoplasmas predominated in domestic ruminants, whereas *Theileria* spp. were mainly associated with red deer.

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