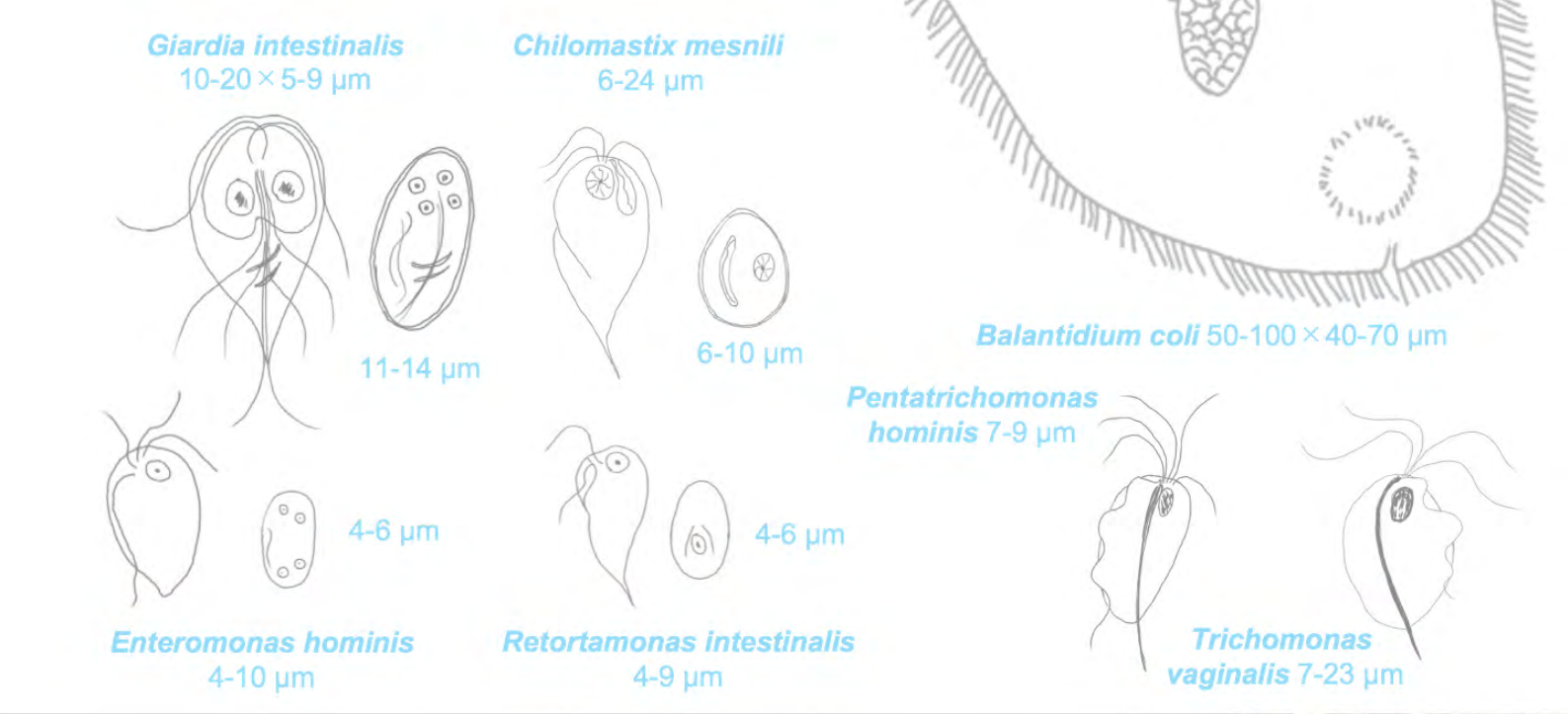

The 17th APCPZ

The 17th Asian Pacific Congress for Parasitic Zoonoses

(Kanazawa Bunka Hall, Kanazawa, JAPAN)

October 18-19, 2025

Preface

On behalf of Japanese parasitologists, and as President of the 17th Asian-Pacific Congress for Parasitic Zoonoses (APCPZ), I am greatly honored to welcome you to this congress. Since its first meeting in 1990, the APCPZ has been carried forward by parasitologists in East Asian countries and their collaborators worldwide, with a sustained focus on zoonotic parasitic diseases. It is a true privilege for me to host the 17th congress here in Kanazawa, Japan, as part of this longstanding tradition.

I believe this congress offers a valuable opportunity to renew collaborations among experts from East Asia and beyond, while also fostering new connections among young researchers. My own commitment to the field of zoonotic diseases is guided by the principles of *One Health*, integrating medical and veterinary parasitology to enhance preparedness for potential future pandemics. I am convinced that such research plays a vital role in strengthening global health security.

I sincerely hope that this congress will provide each of you with meaningful opportunities for scientific exchange, inspiration, and collaboration. Above all, I deeply wish that this distinguished congress will continue to be carried forward and held for many years to come.

October 10, 2025

Masaharu Tokoro

所　正治

President, 17th APCPZ (2025)
Board Member, Japanese Society of Parasitology; Board Member, Japanese Society of Clinical Parasitology; Councilor, Japanese Society of Veterinary Parasitology
Professor, Department of Global Infectious Diseases, Graduate School of Medical Sciences; Vice Director, Environmental Stress Research Center, Kanazawa University, Japan

Organizing Committee for the 17th APCPZ (2025)

☐Taiwan

1. Prof. Chia-Kwung Fan, Department of Molecular Parasitology and Tropical Diseases, Taipei Medical University
2. Prof. Kao-Pin Hwang, School of Medicine, China Medical University
3. Prof. Lian-Chen Wang, Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University
4. Prof. Jyh-Wei Shin, Department of Parasitology, College of Medicine, National Cheng Kung University
5. Prof. Shin-Hong Shiao, Department of Tropical Medicine and Parasitology, College of Medicine, National Taiwan University
6. Prof. Wei-Chen Lin, Department of Parasitology, College of Medicine, National Cheng Kung University
7. Prof. Petrus Tang, Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University
8. Prof. Kwong-Chung Tung, Department of Veterinary Medicine, National Chung Hsing University
9. Prof. Shih-Yi Peng, Department of Biochemistry, School of Medicine, Tzu Chi University
10. Prof. Ting-Wu Chuang, Department of Molecular Parasitology and Tropical Diseases, Taipei Medical University
11. Prof. Po-Ching Cheng, Department of Molecular Parasitology and Tropical Diseases, Taipei Medical University
12. Assoc. Prof. Kuo-Yang Huang, Institute of Pathology and Parasitology, National Defense Medical Center
13. Assoc. Prof. Rong-Jyh Lin, Department of Parasitology, School of Medicine, Kaohsiung Medical University
14. Asst. Prof. Kuang-Yao Chen, Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University

☐Korea

1. Prof. Chai, Jong-Yil, Department of Tropical Medicine and Parasitology, Seoul National University College of Medicine, Seoul, Korea
2. Prof. Sung-Jong Hong, Chung-Ang University College of Medicine, Seoul, Korea
3. Prof. Eun-Hee Shin, Department of Tropical Medicine and Parasitology, Seoul National University College of Medicine, Seoul, Korea
4. Prof. Myeongheon Shin, Department of Tropical Medicine, Yonsei University College of Medicine, Seoul, Korea
5. Prof. Hyun-Hee Kong, Department of Parasitology, Dong-A University College of Medicine, Pusan, Korea
6. Prof. Eun Kyung Moon, Department of Medical Zoology, Kyung Hee University School of Medicine, Seoul, Korea
7. Prof. Bong-Kwang Jung, MediCheck Research Institute, Korea Association of Health Promotion, Seoul, Korea

8. Prof. Eun-Jung Won, Department of Laboratory Medicine, Seoul Asan Medical Center, Seoul, Korea
9. Prof. Hak-Sun Yu, Department of Parasitology and Tropical Medicine, Pusan Nat. Univ. School of Medicine, Yangsan, Korea
10. Prof. Eun-Taek Han, Dept. of Tropical Medicine and Parasitology, Kangwon Nat. Univ. Graduate School of Medicine, Chuncheon, Korea

☐Japan

1. Prof. Haruhiko Maruyama, Division of Parasitology, Department of Infectious diseases, Faculty of Medicine , University of Miyazaki
2. Prof. Mitsuhiro Iyori, Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, Musashino University
3. Prof. Makoto Matsubayashi, Department of Veterinary Immunology, Faculty of Veterinary Medical Sciences, Osaka Metropolitan University
4. Prof. Kensuke Taira, Laboratory of Parasitology, School of Veterinary Medicine, Azabu University
5. Prof. Motoko Nagano-Fujii, Section of Microbiology, Department of Pharmacy, Hyogo Medical University
6. Prof. Daniel Ken Inaoka, School of Tropical Medicine and Global Health, Nagasaki University
7. Prof. Masaharu Tokoro, Department of Global Infectious Diseases, Graduate School of Medical Sciences, Kanazawa University
8. Assoc. Prof. Fumi Murakoshi, Lab. of Veterinary Microbiology, Tokyo University of Agriculture and Technology
9. Asst. Prof. Takahiro Matsumura, Faculty of Health and Medical Sciences Department of Medical Technology and Clinical Engineering, Hokuriku University
10. Asst. Prof. Tetsushi Mizuno, Department of Global Infectious Diseases, Graduate School of Medical Sciences, Kanazawa University

☐ Congress Venue

Venue Name: Kanazawa Bunka Hall (Kanazawa Culture Hall on GoogleMap)

Address: 15-1 Takaoka-machi, Kanazawa, Ishikawa 920-0864, Japan

Access:

- Approximately 10 minutes by taxi or 20 minutes on foot from JR Kanazawa Station.
- From Komatsu Airport: about 50 minutes by airport limousine bus to Kanazawa Station.
- Located near Oyama Shrine（尾山神社）.

Venue Map:

👉 Google Map Link

Facilities:

Meeting Rooms (3F):

The registration desk will be located on the 3rd floor.

Main Hall (Conference Venue), Small Meeting Room 1 (Lounge), Small Meeting Room 2 (Cloakroom)

Wi-Fi:

The network information will be displayed at the reception desk.

Presentation File (PowerPoint) Submission:

Presentation files will be accepted at the registration desk.

Please bring your file well before your scheduled presentation time.

☐ **Banquet Venue (KKR Kanazawa)**

Venue Name: KKR Kanazawa (KKR ホテル金沢)

Banquet Room: 3rd Floor, Kujyaku-no-ma B (孔雀の間 B,)

Address: 2-32 Otemachi, Kanazawa, Ishikawa 920-0912, Japan

Date & Time: October 18 (Saturday), 19:00–21:00

Access:

- About 10 minutes on foot or 3 minutes by taxi from Kanazawa Bunka Hall.
- Located near Kanazawa Castle Park and Kenrokuen Garden.

Style: Standing buffet (casual networking style)
Dress Code: Smart casual
Remarks: Registered participants and accompanying persons are welcome.

Map / Directions:
👉 Google Map Link

Program of the 17th Asian-Pacific Congress for Parasitic Zoonoses (2025)

October 17 (Fri)	Pre-conference banket@ Ichigohan Bunke
19:00~21:00	Invited main members only

☐1st Day – October 18 (Saturday)

October 18 (Sat)	17th APCPZ Venue: Kanazawa Bunka Hall (Kanazawa Culture Hall) , 3rd floor
8:00~	Registration
8:50~9:00	**Opening Remarks** by **Prof. Masaharu Tokoro**, Kanazawa University, Japan (Congress president of the 17th APCPZ) **Prof. Haruhiko Maruyama**, Miyazaki University, Japan （President, Japanese Society of Parasitology）

	Keynote Speech 20 min oral presentation + 10 min discussion
9:00~10:30	**Moderator: Prof. Masaharu Tokoro (Kanazawa University)** 1. **Prof. Chia-Kwung Fan** (Taipei Medical University, Taiwan) Epidemiology and Clinical Perspectives of Human Toxocariasis in Taiwan: A Neglected Indigenous Health Threat. 2. **Prof. Jong-Yil Chai** (Seoul National University College of Medicine, Korea) Anisakidosis in humans, animals, fish, and cephalopods in Korea (1971-2022). 3. **Prof. Makoto Matsubayashi** (Osaka Metropolitan University, Japan) Occurrence of Cryptosporidiosis in Japan and genetic and biological characterizations of zoonotic isolates

10:30~10:45	Coffee Break

10:45~11:45	**Scientific Session: Helminth (1)** 8 min oral presentation + 4 min discussion
	Moderator: Prof. Kensuke Taira (Azabu University) 1. **Maruyama Haruhiko**: Proliferative sparganosis revisited 2. **Ho Yin Pekkle Lam**: Intestinal *Lactobacillus johnsonii* protects against neuroangiostrongyliasis through modulation of immune response 3. **Kota Mochizuki**: Intestinal parasites in wild animal in Ishikawa Prefecture 4. **EUN-MIN KIM**: Secretome Remodeling by *Clonorchis sinensis*–Induced EMT Cholangiocytes Drives Macrophage Polarization 5. **Myung-hee Yi**: Allergenic potential of the cockroach-derived nematode *Leidynema appendiculata* in a murine model

11:45~13:00	Lunch Break

13:00~14:00	**Scientific Session: Helminth (2)** 8 min oral presentation + 4 min discussion
	Moderator: Prof. Haruhiko Maruyama (Miyazaki University) 1. **Kensuke Taira**: Analysis of changes in the habitat area of *Oncomelania hupensis nosophora*, the intermediate snail host of *Schistosoma japonicum*, in the Obitsu River basin in Chiba Prefecture, Japan, using geographic information systems 2. **Anjani Marisa Kartikasari**: First Report of Spirometrosis in a Domestic Cat from Makassar City, Indonesia 3. **Yi-Chen Wang**: A socio-ecological framework for examining liver fluke infection risk 4. **Kuang-Yao Chen**: Identification of novel cross-species microRNAs functions from the excretory-secretory products of *Angiostrongylus cantonensis* fifth-stage larvae 5. **Chitoshi Sato**: Achievements and Challenges in Parasitological examination in the Laboratory division of Japan Disaster Relief

14:00~14:15	Coffee Break

14:15~14:51	**Scientific Session: Protozoa (1)** 8 min oral presentation + 4 min discussion
	Moderator: Prof. Daniel Ken Inaoka (Nagasaki University) 1. **Motoko Nagano-Fujii**: Analysis of Main Antigen Candidate of Kobe-type *Babesia microti* (KoMAC) in the Vicinities of Human Babesiosis Occurrences in Asia 2. **Mitsuhiro Iyori**: The involvement of cellular immune response against the liver-stage *Plasmodium* in a mice model 3. **Jin-Hee Han**: The difference in key molecule interaction for host cell invasion properties in non-Laverania *Plasmodium* species

14:51~15:05	Coffee Break

15:05~15:53	**Scientific Session: Protozoa (2)** 8 min oral presentation + 4 min discussion
	Moderator: Prof. Motoko Nagano-Fujii (Hyogo Medical University) **Asst. Tetsushi Mizuno (Kanazawa University)** 1. **Daniel Ken Inaoka**: High-throughput rate-of-kill (HT-RoK), an innovative approach for prioritization of trypanocidal compounds against intracellular *Trypanosoma cruzi* 2. **Jun Ho Choi**: Screening of Blood-Meal Hosts and Parasites in Tsetse Flies from Tanzania Using Metagenomic Analysis 3. **Fumi Murakoshi**: *Leishmania* RNA Virus 2 Drives Enhanced Pathogenicity in *Leishmania major* 4. **Lon-Fye Lye**: Antileishmanial Drug Discovery

15:53~16:05	Coffee Break

16:05~17:17	**Scientific Session: Protozoa (3)** 8 min oral presentation + 4 min discussion
	Moderator: Prof. Mitsuhiro Iyori (Musashino University) 1. **Young Ah Lee**: *Entamoeba histolytica* Induces Pyroptosis via Caspase-4/Gasdermin D Activation in Colonic Epithelial Cells 2. **Ching-Chun Liu**: Immunomodulatory roles of autophagic flux and IFIT in human ectocervical cells upon *Trichomonas vaginalis* infection 3. **Yuan-Ming Yeh**: Long-read transcriptomics corrects *Trichomonas vaginalis* intron annotations and maps poly(A)/UTR landscapes 4. **Chih-Ming Tsai**: Signal Peptide Variation in Cyst Lectins as a Potential Marker for Pathogenic *Acanthamoeba* spp. 5. **Hyun-Hee Kong**: *Legionella pneumophila* affects encystation mediating gene expression of *Acanthamoeba castellanii* 6. **Chun-Hsien Chen**: The choice of encystation medium determines the quantitative and qualitative outcomes of *Acanthamoeba castellanii* cyst formation

17:17~17:30	Coffee Break

17:30~18:30	**Student Presentation** 6 min oral presentation + 4 min discussion
	Moderator: Asst. Prof. Tetsushi Mizuno (Kanazawa University) 1. **Hsiang-Wei Fan**: The role of intestinal schistosomiasis in colorectal cancer 2. **Hong Zih Bin**: *Trichomonas tenax* exacerbates bacterial infection through immunomodulation and enhanced adhesion 3. **Ting-Ruei Liang**: Naringenin attenuates liver injury in *Schistosoma mansoni*-induced liver fibrosis and oxidative stress 4. **Pei-Yun Chen**: Bifidobacterium breve Enhances Growth and Alters the Transcriptome of *Trichomonas vaginalis* Without Modulating Host Inflammatory Responses 5. **Kaguya Ishihara**: Detection of *Corynosoma cystacanths* from marine fishes caught in Hokkaido and Honshu sold at fish markets in the Kanto region, Japan 6. **Du-Yeol Choi**: Discovery of *Eugregarinida* Associated with House Dust Mites by 18s rRNA Region Nanopore Sequencing

19:00~21:00	The 17th APCPZ Banquet at KKR Hotel Kanazawa

☐2nd Day – October 19 (Sunday)

October 19 (Sun)	17th APCPZ Venue: Kanazawa Bunka Hall (Kanazawa Culture Hall)Venue: Kanazawa Bunka Hall
8:00~	**Registration**
8:30~9:40	**Student Presentation** 6 min oral presentation + 4 min discussion
	Moderator: Assoc. Prof. Fumi Murakoshi (Tokyo University of Agriculture and Technology) **1. Singeun Oh**: Environmental Influences on Eukaryotic Microbiota and Potential Pathogen Prevalence in Domestic Pigeons of Seoul, South Korea **2. Rafarahanta Nirina Norton**: Establishment of metagenomic analysis method for gut protozoal flora **3. Dongjun Kang**: Exploration of gut parasites with anti-inflammatory potential in *Apodemus agrarius* **4. Haruya Yamaguchi**: Molecular detection of *Dientamoeba fragilis* and *Blastocystis* sp. from a diarrheal case **5. Wang Hung-Yang**: Investigating Inflammatory and Immunopathological Differences in the Brains of Blimp-1 Transgenic Mice Infected with Angiostrongylus cantonensis Using 18F-FDG/PET Imaging **6. Shin Hye Park**: IκB kinase 2 and Calcium are involved in ROS production and exocytosis of mast cells stimulated by Trichomonas vaginalis-derived secretory products **7. Michele Lika Furuya**: Integrating Phenotypic Screening and DRUG-seq Transcriptomics to Identify Compounds with Novel Mechanisms of Action Against *Trypanosoma cruzi*

9:40~9:55	Coffee Break

9:55~11:15	**Student Presentation** 6 min oral presentation + 4 min discussion
	Moderator: Asst. Prof. Takahiro Matsumura (Hokuriku University) **1. Arwa Shattaa**: Microbiome and Resistome Profiling of the Synanthropic Blow Fly *Lucilia sericata* Across South Korea **2. Aulia Afriani Mustamir**: Molecular characterization of *Endolimax nana* in humans and animals from eastern Indonesia reveals subtype-level genetic diversity **3. Truc Thi Thanh Tran**: Spatio-temporal patterns of dengue transmission in Tainan city, Taiwan, pre- and post- COVID-19 **4. Wen-Zhen Lee**: Study on mechanisms of microglia in the brain of *Angiostrongylus cantonensis* infected mice **5. Jhen-Wei Syu**: Systematic Analysis of Putative and Novel Microproteome in the *Trichomonas vaginalis* **6. Yu-Tzu Hsu**: *Trichomonas vaginalis* impairs HeLa cell intercellular adhesion, leading to decreased cell density via the MCAM–CREB signaling axis **7. Ng'etich Japheth Kibet**: Integration of AlphaFold Structures with Phenotypic Screening for Target Deconvolution of Antimalarial Hit Compounds **8. Hon-lan Lei**: The molecular effect of recombinant *Toxocara canis* antimicrobial peptides in wound healing

11:15~11:25	**Closing Remarks**

11:30~	**Excursion Tour**
	Visit to the Noto Peninsula Area Affected by the 2024 Earthquake 11:30　Departure from Kanazawa Bunka Hall *Obento lunch will be served on the bus. 12:40–13:20　Break at Noto-Chirihama Resthouse https://www.chirihama.co.jp/ Drive through the disaster-affected area of Wajima Morning Market 15:00–15:40　Visit to Shiroyone Senmaida (Terraced Rice Fields) https://www.ishikawatravel.jp/en/spots/senmaida-rice-terraces/ 16:10–16:30　Visit to Sodegahama Beach https://wajimanavi.jp/tourism/portfolio/sodegahama/ 19:05　Arrival at Kanazawa Station 19:20　Arrival at Kanazawa Bunka Hall *Participants departing from Kanazawa Station may load their luggage onto the bus. The cloakroom at Kanazawa Bunka Hall will remain open until the 20:00.

Presentation Abstracts

[1] Epidemiology and Clinical Perspectives of Human Toxocariasis in Taiwan: A Neglected Indigenous Health Threat

Chia-Kwung Fan[1,2], Chia-Mei Chou[1,2]

[1]*Department of Molecular Parasitology and Tropical Diseases, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[2]*Research Center of International Tropical Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*

Human toxocariasis, primarily caused by *Toxocara canis*, is a globally distributed parasitic zoonosis recognized by the CDC as one of the five neglected parasitic infections. Despite its significance of public health, the disease remains underdiagnosed and underreported in many regions, including Taiwan. This study presents a comprehensive overview of the seroepidemiology and clinical manifestations of human toxocariasis in Taiwanese populations, with emphasis on indigenous communities and emerging neurological cases.

Seroepidemiological surveys have revealed high anti-*T. canis* antibody prevalence rates among Taiwan's indigenous populations (46%) and mountain-dwelling Han residents (30.2%), indicating significant environmental and cultural exposure risks. Among urban college students, a lower but notable seroprevalence (8.4%) was observed, reflecting widespread but often silent exposure. Clinical cases in Taiwan include hepatic, ocular, and increasingly recognized neurotoxocariasis. The first two confirmed Taiwanese neurotoxocariasis cases presented with eosinophilic pleocytosis in cerebrospinal fluid and MRI abnormalities, both resolving after mebendazole treatment. Ocular involvement has also been documented in pediatric patients, with positive serology and resolution following mebendazole-steroid therapy.

Diagnostic challenges persist due to antigenic cross-reactivity and limited access to confirmatory testing. TcES-based ELISA and Western blot remain the most reliable tools for diagnosis. Given the larval affinity for central nervous tissues and potential links to neurodegenerative processes such as Alzheimer's disease, this presentation also explores hypotheses on cerebral toxocariasis as a precursor to neurodegenerative disorders.

We urge broader awareness, improved diagnostic access, and public health interventions targeting high-risk populations to mitigate the neglected burden of toxocariasis in Taiwan and globally.

[2] Anisakidosis in humans, animals, fish, and cephalopods in Korea (1971-2022)

Jong-Yil Chai[1]

[1]*Department of Tropical Medicine and Parasitology, Seoul National University College of Medicine, Seoul 03080, Korea*

Human anisakiasis (or anisakidosis) is a disease caused by ingesting marine fish or cephalopods infected with anisakid nematode larvae from the genera *Anisakis, Pseudoterranova, Contracaecum,* and *Hysterothylacium*. Anisakiasis is a clinically significant disease that often presents as an acute abdominal syndrome requiring emergency medical attention and care. In South Korea (= Korea), at least several thousand clinical cases have been diagnosed to date; however, only a small portion (851 cases) have been reported in the scientific literature (1971-2022). The most common etiological agents were *Anisakis pegreffii* (reported as *Anisakis* sp., *Anisakis* type I, or erroneously *Anisakis simplex*), followed by *Pseudoterranova decipiens, Contracaecum* sp., and *Anisakis simplex* sensu stricto (s.s.). Most cases involve the stomach and small or large intestines, with some affecting the oral cavity (oral mucosa, pharynx, and tonsils), esophagus, omentum, and mesocolic lymph nodes. *Anisakis* allergies and host immune responses have been studied in humans and experimental animals. Marine fish and cephalopods, including sea eel (*Astroconger myriaster*), squid (*Todarodes pacificus*), yellow corvina (*Pseudosciaena manchurica*), Japanese flounder (*Paralichthys olivaceus*), codfish (*Gadus macrocephalus*), yellowtail (*Seriola quinquaradiata*), and rockfish (*Sebastes* spp.), are the primary sources of infection. Surveys on anisakid nematode larvae in marine fish and cephalopods caught in Korea's western, eastern, and southern seas were conducted. Larvae from fish or cephalopods caught in the western and southern seas were predominantly *A. pegreffii*, whereas the larvae from the eastern sea included either *A. pegreffii* (found in chub mackerel, Japanese flounder, and rockfish) or *A. simplex* s.s. (detected in salmon and pollock, which migrate through the northern North Pacific Ocean and Bering Sea to Korea). Public health education emphasizing the avoidance of consuming raw or improperly cooked marine fish and cephalopods, especially viscera, is essential to prevent human anisakidosis in Korea.

[3] Occurrence of Cryptosporidiosis in Japan, and genetic and biological characterizations of zoonotic isolates

Makoto Matsubayashi

[1]*Department of Veterinary Immunology, Faculty of Veterinary Medical Sciences, Osaka Metropolitan University, Osaka, Japan*

The genus *Cryptosporidium*, of phylum Apicomplexa, includes medically important intestinal protozoan parasites that infect both animals and humans worldwide. To date, more than 170 *Cryptosporidium* species and genotypes have been identified, including host-adapted species. Among these, *C. hominis* (anthroponotic) and *C. parvum* (zoonotic species that infects a wide range of hosts and can be often lethal in neonatal calves) are primary causes of human cryptosporidiosis. These parasites cannot be identified based on oocyst morphology alone; thus, molecular analyses (e.g., partial sequencing of conserved gene regions such as the *18S rRNA* gene) are required. *Cryptosporidium* infections are characterized by self-limiting watery diarrhea, but they can be lethal in immunocompromised individuals such as AIDS patients or neonatal animals such as calves. A large number of infective oocysts are typically shed in the feces of infected hosts. These oocysts are highly resistant to commonly used disinfectants and can survive in the environment for long periods. As these small, robust oocysts resist inactivation and removal, outbreaks of cryptosporidiosis via contaminated drinking water are common worldwide. Recent research suggests that cryptosporidiosis can adversely affect growth in children, especially those under five years of age. However, treatments for cryptosporidiosis are limited, with only a single approved drug (nitazoxanide) with questionable efficacy in immunocompromised individuals (HIV patients) and children currently available. Outbreaks caused by *Cryptosporidium*-contaminated drinking water and sporadic cases of human infection were reported in Japan between 1986 and 2018. In addition to human infections, frequent cases of *Cryptosporidium* infections in cattle have been reported in Japan, with high lethality in neonatal calves. In the *Cryptosporidium* lifecycle, the oocysts release sporozoites after oral ingestion, and these sporozoites invade intestinal epithelial cells. Trophozoites formed within the parasitophorous vacuole then develop further via asexual and sexual development to generate new oocysts. Recent whole-genome analysis studies revealed that recombination events involving multiple genetic exchanges can occur in the sexual stage. As Japan is an island country, *C. parvum* strains that infect neonatal calves might have developed independently. Although it is unclear whether Japanese calves are more susceptible to infection or *C. parvum* isolates are more pathogenic, controlling *Cryptosporidium* infections on fattening farms in Japan is challenging. In addition to reporting the occurrence of *Cryptosporidium* infections in Japan, we also discuss here the preliminary results of biological characterization and whole-genome comparative analyses of Japanese *Cryptosporidium* isolates.

[1] Proliferative sparganosis revisited

Tanaka Ryusei[1], Kokubo-Tanaka Mio[1], **Maruyama Haruhiko**[1]

[1] *Department of Infectious Diseases, Division of Parasitology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan*

The first patient of proliferative sparganosis visited the Hospital of the Imperial University of Tokyo in 1904, for the treatment of inguinal hernia. Surgeons at the hospital came to know that her skin harbored numerous parasites, which Ijima Isao, Professor of Zoology, identified as plerocercoids of unknown origin. Professor Ijima considered that the parasite was proliferating, and designated it as *Plerocercoides prolifer*. In 1908, Stiles in the United states reported a similar case in Florida, and he re-designated the parasite as *Sparganum proliferum*, which we use today.

In 2021, we published a draft genome of *S. proliferum*, isolated from a patient in Venezuela in 1981, and compared it with *Spirometra erinaceieuropaei* (which could be *S. mansoni*) genome. We found that *S. proliferum* was closely related to *S. erinaceieuropaei*, but clearly distinct from it. We also found that some genes for the sexual maturation were missing or seemingly not functioning in *S. proliferum*, suggesting that this parasite was lacking the adult stage.

We thought at that time that *Spirometra* tapeworms and *S. proliferum* were clearly distinguishable, with non-proliferating and proliferating plerocercoids, respectively. However, things may not go so simple. Recently, we encountered a strange case of sparganosis. Mitochondrial COX1 sequence indicated it belonging to the Asian clade of *Spirometra*, thus different from Venezuelan *S. prjoliferum*. But the shape and the size of the plerocercoids from the patient were similar to those of *S. proliferum*, and more importantly, the number of plerocercoids were so enormous, even though the patient did not have a chance of massive infection or re-infection.

We now suspect that every clade of *Spirometra* might contain variants with proliferating plerocercoids with variable pathogenicity. Some could cause fatal proliferative sparganosis and others relatively benign one. Careful and detailed molecular and biological study should be required to answer these questions.

[2] Intestinal *Lactobacillus johnsonii* protects against neuroangiostrongyliasis through modulation of immune response

Long Yin Lam[1], Ting-Ruei Liang[2], Wen-Jui Wu[3], **Ho Yin Pekkle Lam**[4,5]

[1]*State Key Laboratory of Chemical Biology and Drug Discovery, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong SAR, China*
[2]*PhD Program in Pharmacology and Toxicology, Tzu Chi University, Hualien, Taiwan,*
[3]*Department of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien, Taiwan*
[4]*Department of Biochemistry, School of Medicine, Tzu Chi University, Hualien, Taiwan*
[5]*Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan*

Neuroangiostrongyliasis is characterized by eosinophilic meningoencephalitis with a robust onset of severe neurological symptoms, by which immunological factors and peripheral metabolites have been postulated to affect the course of the disease. The gut-brain axis provides a bidirectional communication between the gut and the central nervous system, and therefore, understanding the gut microbiome may provide us with a deeper insight into the pathogenesis of angiostrongyliasis. Using 16S rRNA sequencing, we identified an increase in the abundance of different *Lactobacillus* species in *Angiostrongylus cantonensis*-infected mice, which was correlated with the disease severity. However, attempts to inoculate *L. johnsonii* into *A. cantonensis*-infected mice surprisingly revealed an improvement in neuroinflammation and prolonged survival. RNA sequencing suggested an immune-modulatory effect of *L. johnsonii*, which was confirmed by ELISA, showing increased levels of IL-10 and reduced levels of IL-2, IL-4, IL-5, and MCP-1 in the brain. Nevertheless, *L. johnsonii*-associated improvements were not highly associated with microbiome-related metabolites, as UHPLC-MS/MS analysis revealed no change in short-chain fatty acids, tryptophan metabolites, and bile acids. Our results suggest that while intestinal *L. johnsonii* appears to be linked to the progression of neuroangiostrongyliasis, these bacteria are likely attempting to modulate the dysregulated immune response to combat the disease. This is one of the first studies to investigate the gut microbiome in mice with *A. cantonensis* infection, which extends our knowledge from the microbiome-point-of-view of the pathogenesis of angiostrongyliasis and how the body defends against *A. cantonensis*. This work also extends to possible treatment approaches using *L. johnsonii* as probiotics.

[3] Intestinal parasites in wild animal in Ishikawa Prefecture

Kota Mochizuki[1], Takahiro Matsumura[2], Tetsushi Mizuno[3], Masaharu Tokoro[3], Yosaburo Oikawa[1], Manabu Murakami[1]

[1]*Department of Medical Zoology, Kanazawa Medical University, Ishikawa, Japan*
[2]*Faculty of Health and Medical Sciences, Hokuriku University, Ishikawa, Japan*
[3]*Department of Global Infectious Diseases, Kanazawa University, Ishikawa, Japan*

Wild animal can serve as reservoirs for parasitic pathogens. The intestine harbors various parasites, and feces is one of the main sources of environmental contamination. These zoonotic and non-zoonotic parasites can impact both human health and the economy. Although research for pathogens in wild animal is important for public health, reports is limited to date. This study aims to investigate the range of pathogenic parasites harbored by wild animals in the Ishikawa Prefecture, Japan.

Wild deer and boar used in this study were captured by pest control operation. *Oesophagostomum asperum* was detected in the deer intestine and subsequently identified based on morphological characteristics examined by scanning electron microscopy, as well as on nucleotide sequence data. In the Noto peninsula, *Ascaris* sp. was detected in 21% of wild boar, which is comparable to those reported in previous studies. Fecal samples from wild mice captured using Sherman traps were analyzed by 18S rRNA amplicon sequencing, detecting several parasitic species including *Tritrichomonas* sp. and *Cryptosporidium* sp. The release of infective stages of these parasites, such as eggs, cysts, or trophozoites, into the environment contributes to contamination, potentially resulting in infections among humans, livestock, and pets.

These findings highlight the need to recognize the presence of wild animals harboring pathogenic parasites, which are often overlooked in close proximity to human environments. Ongoing monitoring will allow evaluation of the longitudinal prevalence of pathogenic parasites in the region.

[4] Secretome Remodeling by *Clonorchis sinensis*–Induced EMT Cholangiocytes Drives Macrophage Polarization

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Background: *Clonorchis sinensis* infection is a major risk factor for cholangiocarcinoma (CCA). Whether the parasite induces sustained tumor-supportive changes in the host microenvironment remains unclear.

Methods: An EMT-derived cholangiocyte line (CsNH69) was generated by chronic exposure of normal human cholangiocytes (H69) to *C. sinensis* excretory-secretory products (ESPs) and N-nitrosodimethylamine (NDMA). Mitochondrial and lipid alterations were assessed by holotomography. Secretomes from H69 and CsNH69 were analyzed by LC-MS/MS proteomics. Conditioned media were applied to THP-1 monocytes to evaluate macrophage polarizatio

Results: CsNH69 cells exhibited fragmented and aggregated mitochondria with excessive lipid droplet accumulation, reflecting metabolic abnormalities associated with malignant transformation. Proteomic profiling revealed secretome remodeling enriched in cancer-related pathways, including PI3K–AKT, focal adhesion, and cell cycle regulation. THP-1 cells exposed to CsNH69-conditioned media displayed an M2-like phenotype with increased TGF-β expression and reduced pro-inflammatory markers.

Conclusion: This in vitro model demonstrates that *C. sinensis*–induced EMT cholangiocytes undergo metabolic and secretome remodeling that promotes a tumor-supportive microenvironment. Such effects may persist even in the absence of direct parasite presence, warranting further in vivo validation.

[5] Allergenic potential of the cockroach-derived nematode *Leidynema appendiculata* in a murine model

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Cockroach-derived allergens are well-recognized triggers of allergic airway disease, and we show that the parasitic nematode *Leidynema appendiculata* from *Periplaneta fuliginosa* can also independently induce hallmark features of asthma in a murine model. BALB/c mice sensitized and challenged with *L. appendiculata* extract exhibited airway hyperresponsiveness, eosinophilic inflammation, goblet cell hyperplasia, and antigen-specific IgE responses, comparable to those elicited by cockroach fecal extract. These findings demonstrate that cockroach-associated helminths represent an unrecognized allergen source that may interfere with immunotherapy and diagnostic preparations.

[1] Analysis of changes in the habitat area of *Oncomelania hupensis nosophora*, the intermediate snail host of *Schistosoma japonicum*, in the Obitsu River basin in Chiba Prefecture, Japan, using geographic information systems

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Human schistosomiasis is a serious parasitic disease caused by infection with *Schistosoma japonicum*. In order to establish monitoring sites in the *S. japonicum* endemic area in the Obitsu River basin in Chiba Prefecture, Japan, we investigated the habitat of the intermediate snail host, *Oncomelania hupensis nosophora* using geographic information systems (GIS). A series of thematic maps of soil types, land-use, and past wetlands were compared to the distributions of patients and *O. h. nosophora* habitats to identify environmental conditions associated with high risk of the disease. In addition, we divided the period from the 1600s to the present into four sub-periods to estimate how the habitat of *O. h. nosophora* has changed. Our study identified the present risk areas in the Obitsu River basin that should be monitored consecutively, taking into account future global environmental changes that may have the potential to promote re-emergence of the disease.

[2] First Report of Spirometrosis in a Domestic Cat from Makassar City, Indonesia

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Spirometrosis is a zoonotic disease caused by cestodes of the genus *Spirometra*. Felids and canids serve as definitive hosts; however, reports of natural infections in these species remain limited. In this report, we describe a clinical case of spirometrosis in a domestic cat from Makassar City, South Sulawesi, Indonesia. A stray female cat weighing 2.7 kg was rescued and presented with abdominal distension and mucoid watery diarrhea, with no prior history of deworming or vaccination. During hospitalization, the cat vomited adult cestode proglottids measuring approximately 26 × 0.5 cm. Proglottids were processed using carmine staining, which revealed morphological features consistent with *Spirometra*. Fecal examination identified operculated eggs (52,74 × 32,56 μm) with a total of 54 eggs per gram feces. Hematological analysis revealed leukocytosis with eosinophilia, lymphocytosis, macrocytosis with hypochromia, and thrombocytopenia. The cat was treated with a combination of praziquantel and pyrantel, after which diarrhea and vomiting resolved within three days. This case highlights the clinical presentation of spirometrosis in a domestic cat and underscores its potential zoonotic significance in Indonesia.

Keywords: Domestic cat, Indonesia, *Spirometra,* Spirometrosis, neglected disease, zoonosis.

[3] A socio-ecological framework for examining liver fluke infection risk

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Infectious diseases linked to poverty, particularly neglected tropical diseases, have adversely affected the socio-economic development in less wealthy regions. These diseases disproportionally influence people living with limited access to adequate sanitation and basic health infrastructure and services. One of the neglected tropical diseases of concern is human infection of liver fluke *Opisthorchis viverrini* through the consumption of raw freshwater fish. Despite decades of health campaigns, high infection prevalence remains in different areas of the Lower Mekong region. This necessitates the consideration of the infection differences between the human-environment complexities of disease transmission. This study proposed using the socio-ecological model as a framework to examine liver fluke infection risk. Questionnaire surveys were conducted to gather participants' knowledge on liver fluke infection and reasons for raw fish consumption. The findings were analyzed to identify factors influencing liver fluke infection at four socio-ecological levels. At the individual level, gender and age differences in food consumption habits and personal hygiene of open defection presented the behavioral risks. At the interpersonal level, family tradition and social gathering affected the disease risk. At the community level, physical-social-economic environments of land use and modernization and health volunteer support accounted for the varying degree of infection. At the policy level, impacts of regional and national regulations on disease control and health system organization structure were of concerned. The findings provide insights into how infection risks are shaped by people's behavior, social connectedness, interactions with places, and the interplay of these multi-level socio-ecological influences. The framework allows a more comprehensive understanding of liver fluke infection risks to inform a culturally sensitive and sustainable disease control program.

[4] Identification of novel cross-species microRNAs functions from the excretory-secretory products of *Angiostrongylus cantonensis* fifth-stage larvae

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Angiostrongylus cantonensis is a major foodborne zoonotic parasite known to cause severe neuropathological damage and clinical symptoms in humans. Currently, therapeutic approaches for cerebral angiostrongyliasis remain controversial and insufficiently understood. Excretory-secretory products (ESPs) and secreted microRNAs play the pivotal roles in elucidating host-parasite interactions particularly in penetrating host defensive barriers. To date, no studies have elucidated the molecular mechanism driven by *A. cantonensis*-derived microRNAs in helminth-host interactions and drug therapy. In this study, we identified and investigated the function of novel microRNAs from *A. cantonensis* L5 ESPs. We first constructed a comprehensive miRNA dataset from L5 larvae, L5 ESPs, and adult ESPs. Bioinformatic analyses revealed that several novel miRNAs were mainly expressed in L5 ESPs, with significantly higher levels compared to L5 larvae and adult stages. The prediction results indicated that these miRNAs may target key signaling pathways, including Wnt and mTOR. Further experimental validation demonstrated that AcNOVEL55, significantly reduced apoptosis in mouse astrocytes by modulating the RhoA-ROCK signaling pathway following L5 ESP treatment. Additionally, AcNOVEL31 was shown to regulate inflammatory responses and cytokine secretion through the presenilin-1/GSK3B/β-catenin/NF-κB signaling cascade. In conclusion, this study represents the first comprehensive study to elucidate the molecular roles of *A. cantonensis*-secreted novel microRNAs in host-pathogen interactions. The findings not only provide critical insight into host response to helminth infection, but also elevate *A. cantonensis* research to a new level of molecular understanding

Keywords: *Angiostrongylus cantonensis*; neuropathological damage; excretory-secretory products; novel microRNAs; helminth-host interaction; RhoA; Presenilin-1

[5] Achievements and Challenges in Parasitological examination in the Laboratory division of Japan Disaster Relief

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Japan Disaster Relief is one of the medical support teams for overseas disasters. The author is registered with this medical team. According to the WHO's 2021 Classification and Minimum Standards for Emergency Medical Teams, Japan Disaster Relief medical teams are currently classified as EMT Type 2. The clinical laboratory division is required to provide EMT Type 1 basic outpatient testing and rapid diagnostic tests, as well as Type 2 basic inpatient testing. Specifically, this includes endemic disease rapid diagnostic testing appropriate to the context of the deployment and basic diagnostic tools, such as urine pregnancy tests, urine dipstick tests, blood glucose testing, and hemoglobin testing.

As a medical laboratory scientist, the author has participated in four deployments（Pakistan, Nepal,Turkey, and Myanmar）, both before and after 2021. This report focuses on the achievements and challenges of parasitological examination.

[1] Analysis of Main Antigen Candidate of Kobe-type *Babesia microti*（KoMAC） in the Vicinities of Human Babesiosis Occurrences in Asia

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The Kobe strain of *Babesia microti* (Kobe-type), which was isolated from the first human babesiosis patient in Japan, differed for the 18S rRNA genotype and the serotype from the pathogenic strains of *B. microti* (US-type) in human babesiosis endemic areas of the United States. We detected *B. microti* with four genotypes/serotypes (Kobe, Otsu, Nagano and US) in field rodents in Japan. Moreover, we confirmed Kobe-type of *B. microti* in field rodents in the vicinities of human babesiosis occurrences in not only Japan, but also Taiwan and Mainland China. This suggested that Kobe-type of *B. microti* is deeply involved in Asian human babesiosis caused by *B. microti*. To our knowledge, only Kobe-type and US-type are known to cause human babesiosis, which suggests that pathogenicity seems to be vary by genotype/serotype.

In order to develop type-specific diagnostic methods for human babesiosis, we first searched for main antigens of the *B. microti* Kobe strain by screening cDNA expression library with mouse-antisera infected with the Kobe strain. We identified a main antigen candidate (KoMAC), a 363-amino-acid protein, which shows approximately 50% homology with BmSA1 (secreted antigen 1), an antigen of the Gray strain (US-type). An antigen equivalent to KoMAC, 359-amino-acid protein, was also identified by PCR in the Meishan strain (Kobe-type) isolated from a field rodent in Taiwan.

The sequences at position 1 to 26 was very similar and suggested to be signal peptides. The sequences from position 27 to 84 of the Kobe strain and the corresponding part of the Meishan strain with 4-amino-acid deletion showed very low similarity (app. 20%), while the rearward sequences showed high similarity (app. 92%). The rearward sequences of KoMAC of *B. microti* isolates (Kobe-type) deriving from field rodents in Japan and in Mainland China showed more than 99% similarity with that of KoMAC of the Kobe strain and that of the Meishan strain, respectively.

When KoMAC proteins of the Kobe and Meishan strains were expressed with a wheat germ cell-free protein synthesis system, KoMAC proteins reacted only with mouse antiserum against the Kobe and Meishan strains, but not with mouse antiserum against the Hachimaki strain (Otsu-type) or the GI strain (US-type). These results suggest that this antigen can be utilized for the diagnostic method to detect Kobe-type *B. microti* infection. We are now trying to clarify the epitopes of KoMAC.

[2] The involvement of cellular immune response against the liver-stage *Plasmodium* in a mice model

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Acquired immunity against malaria toward specific antigens can prevent malaria deaths directly attributed to the parasites. A key challenge in developing novel vaccines may be identifying the immunological mechanisms that can prevent *Plasmodium* infection during the liver stage. Recently, we have established a malaria treatment model in which intramuscular administration of wild-type baculovirus (BV) induced type I interferon signaling, leading to the elimination of liver-stage *P. berghei*. Interestingly, mice treated with BV after an initial parasite exposure did not become infected upon secondary exposure to sporozoites. In this study, we examined the immune responses in BV-treated mice after sporozoite exposure. We found that humoral immune response against sporozoites were raised in the BV-treated mice, but it might not contribute for the protection from parasites by the result from the challenge infection study using genetically modified parasites. Thus, we next examined the cellular immune response in the mice. The splenocytes from BV-treated mice were dissected and examined for the reaction against multiple epitopes from variety of the liver-stage antigens. ELISpot assay showed several kinds of antigen epitopes induced interferon secretion from T cells. Finally, we have identified potential candidate antigens targeting liver-stage parasites using a multiplex cytotoxicity assay. These findings are expected to lead to important concepts for future vaccine development.

[3] The difference in key molecule interaction for host cell invasion properties in non-*Laverania Plasmodium* species

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Recently, it has been confirmed that the non-*Laverania* malaria species *Plasmodium knowlesi* and *P. cynomolgi* are capable of naturally infecting humans in Southeast Asia. Among these, *P. knowlesi*, originally a simian malaria parasite, has now become established in Malaysia and Indonesia and represents the most prevalent human malaria species in these regions. Understanding the essential interactions between parasite ligands and host cell receptors is therefore crucial for elucidating the mechanisms of non-*Laverania* malaria invasion and adaptation to humans. In this study, we focused on the Duffy Binding Protein (DBP), a key ligand mediating erythrocyte invasion in *Plasmodium* species. We analyzed the interaction properties of DBP region II (DBP-RII) with human and monkey Duffy Antigen Receptor for Chemokines (DARCs). Co-immunoprecipitation assays and biolayer interferometry (Octet) were employed to determine binding affinities and to compare differences in interaction strength among species. Our findings revealed that PvDBP-RII specifically interacted with human DARC in a distinct manner compared to PkDBPα-RII. Interestingly, PcyDBP2-RII did not show detectable binding to either human or monkey DARC, whereas PcyDBP1-RII exhibited strong affinity for human DARC. Moreover, the binding strength of PcyDBP1-RII to monkey DARC was modulated by the presence of glutamic acid at position 23 of the receptor, highlighting the critical role of host genetic variation in parasite–receptor compatibility. Together, these results provide direct evidence that the simian malaria parasites *P. knowlesi* and *P. cynomolgi* possess molecular mechanisms enabling adaptation to human hosts. This study emphasizes the importance of ligand–receptor interactions in zoonotic malaria transmission and offers new insights into how non-*Laverania* parasites may expand their host range to establish sustained human infections.

[1] High-throughput rate-of-kill (HT-RoK), an innovative approach for prioritization of trypanocidal compounds against intracellular *Trypanosoma cruzi*

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Chagas disease, caused by *Trypanosoma cruzi* (*T. cruzi*), remains a major global health concern. Once endemic to Central and South America, its geographic distribution has expanded, with increasing cases in North America, Europe, Oceania, and Asia. An estimated 6–7 million people worldwide are currently infected. Despite its global impact, no vaccines exist, and treatment is limited to two nitroheterocyclic drugs, nifurtimox and benznidazole. Both suffer from severe side effects and depend on parasite bioactivation by type II nitroreductase, creating risks of cross-resistance and treatment failure. Safer, more effective therapies are urgently needed.

A complete parasitological cure is essential to prevent chronic disease. However, many small-molecule candidates fail to eradicate *T. cruzi*, allowing parasite persistence and relapse. The clinical failure of CYP51 inhibitors exemplifies this limitation. Parasite population heterogeneity, including dormant subpopulations, further complicates treatment by reducing susceptibility. Identifying compounds with sterile cure potential has been constrained by in vitro model limitations. While compound washout assays offer improved prediction of regrowth following drug removal, they are low-throughput and require months of incubation, making them impractical for early drug discovery. To overcome this bottleneck, we developed a high-throughput, fast-turnaround screening cascade to prioritize compounds with mechanisms of action suited for sterile cure. Central to this approach is a novel high-throughput rate-of-kill (HT-RoK) assay, which monitors the decline of luciferase signal from intracellular *T. cruzi* amastigotes over five days across varying drug concentrations. This system enables rapid, cost-effective, and scalable evaluation of time- and dose-dependent activity, facilitating early dereplication of suboptimal candidates.

By shifting attrition earlier in the discovery process, HT-RoK profiling reduces late-stage failures and focuses resources on the most promising compounds. This strategy holds strong potential to accelerate Chagas disease drug discovery and improve translational outcomes.

[2] Screening of Blood-Meal Hosts and Parasites in Tsetse Flies from Tanzania Using Metagenomic Analysis

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Tsetse flies are vectors of *Trypanosoma* spp., responsible for transmitting trypanosomiasis in humans, wild animals, and domestic livestock. This study employed amplicon deep sequencing targeting the 12S rRNA gene to identify mammalian blood-meal hosts and the 18S rRNA gene to screen for eukaryotic pathogens, including *Trypanosoma* spp., in tsetse flies collected from Tanzania. A total of 139 tsetse flies were sampled from Serengeti National Park (n=48), Maswa Game Reserve (n=42), and Tarangire National Park (n=49). Analysis of the 12S rRNA gene revealed a diverse range of blood-meal hosts, including humans, common warthogs, African buffaloes, mice, giraffes, African elephants, waterbucks, and lions, with African buffaloes being the most frequent host in Serengeti (P=0.0010). Screening of the 18S rRNA gene identified *Trypanosoma* spp. in six tsetse samples, with subsequent ITS1 gene sequencing confirming the presence of *Trypanosoma godfreyi* and *Trypanosoma simiae.* These findings provide critical insights into the host preferences and parasite prevalence of tsetse flies in Tanzania, contributing valuable data for the development of effective strategies to control African trypanosomiasis.

[3] Leishmania RNA Virus 2 Drives Enhanced Pathogenicity in Leishmania major

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Many protozoan parasites, including *Leishmania,* harbor persistent viruses with double-stranded RNA (dsRNA) genomes. These viruses lack an extracellular infection route and are thought to replicate in parallel with parasite proliferation. Recently, Leishmania RNA virus 1 (LRV1), belonging to the family *Totiviridae* and persisting in Leishmania *guyanensis,* was reported to influence parasite pathogenicity in the host. However, the underlying mechanisms remain largely unclear.

In this study, we focused on Leishmania RNA virus 2 (LRV2), which persistently infects *Leishmania major*, a causative agent of cutaneous leishmaniasis. We generated *L. major* lines that differ only in the presence or absence of LRV2 and performed comparative infection experiments and transcriptome analyses in mice. When injected into the footpads of mice, LRV2-positive parasites induced earlier lesion development and significantly larger lesion sizes than LRV2-negative parasites. Notably, significant footpad swelling was already observed 24 hours post-infection in mice infected with LRV2-positive parasites. Transcriptomic analysis of infected tissues revealed that the largest number of differentially expressed genes occurred within the first week after infection, with upregulation of virus response-related genes specifically in the LRV2-positive group. Moreover, increased expression of multiple genes associated with type I interferon responses was detected.

These findings suggest that dsRNA from LRV2 is recognized by the host, triggering antiviral responses that paradoxically exacerbate disease severity. Thus, the presence of LRV2 enhances the pathogenicity of *L. major,* leading to worsened disease outcomes in infected mice.

[4] Antileishmanial Drug Discovery

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Leishmaniasis is a vector-borne infection caused by kinetoplastid protozoans in the genera *Leishmania* and *Endotrypanum.* Leishmaniasis occurs in more than 98 countries worldwide with more than 1 billion people at risk. About 0.7-1 million new cases are reported. Current chemotherapies present several drawbacks including parasite resistance, and no human vaccine exists. Therefore, there is an urgent need to develop alternative treatments for leishmaniasis. Our project applies a drug repurposing strategy for the discovery of new treatments for leishmaniasis from screening of 1500 FDA-approved compounds. These screening identified 12 pan-active compounds affecting survival of *L. turanica* (caused cutaneous leishmaniasis), and *L. donovani* (caused visceral leishmaniasis) parasites in culture at concentrations less than 10 μ M. Several prioritized compounds are further performing the in vitro induction for drug resistant *Leishmania* lines. Three drug resistant cells were subject to the RNA-seq and whole-genome sequencing for identifying the *Leishmania* drug targeting genes/pathways and drug resistant genes. Currently, our pipeline candidate genes are validating by CRISPR or RNAi technology.

Keywords: *Leishmania*, Leishmaniasis, drug repurposing, drug resistant, drug targets, RNA-seq, CRISPR, RNAi

[1] *Entamoeba histolytica* Induces Pyroptosis via Caspase-4/Gasdermin D Activation in Colonic Epithelial Cells

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Entamoeba histolytica is an enteric protozoan parasite and the causative agent of amoebiasis, a major public health problem that leads to colitis and liver abscess in humans. The inflammasome is an innate immune complex that activates inflammatory caspases, including caspase-4, leading to gasdermin D (GSDMD) cleavage, cytokine release, and pyroptosis. While inflammasome activation by *E. histolytica* has been demonstrated in immune cells, its role in non-immune epithelial cells has not yet been elucidated. Here, we examined the roles of caspase-4 and GSDMD in pyroptosis during colonic epithelial cell death induced by *E. histolytica*. Co-culture of pathogenic amoebae with Caco2 cells induced host cell death, detected more rapidly and sensitively by CellTiter-Glo than by the LDH release assay. Pretreatment of amoebae with D-galactose, the cysteine protease inhibitor E-64, or the calcium chelator EGTA markedly reduced their cytotoxicity. Calpain activity was elevated in Caco2 cells exposed to live amoebae but was suppressed when amoebae were pretreated with D-galactose or E-64. Western blot analysis confirmed the cleavage of caspase-4 and GSDMD in Caco2 cells after amoeba contact, while the pan-caspase inhibitor z-VAD-fmk effectively blocked their activation. These findings suggest that a non-canonical inflammasome, via caspase-4 and GSDMD, mediates pyroptotic cell death in colonic epithelial cells during *E. histolytica* infection.

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[2] Immunomodulatory roles of autophagic flux and IFIT in human ectocervical cells upon *Trichomonas vaginalis* infection

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Trichomonas vaginalis (Tv) is the causative agent of trichomoniasis, the most common non-viral sexually transmitted infection worldwide. Despite its high prevalence, the mechanisms underlying Tv-induced inflammatory responses remain poorly understood. Herein, we investigated the signaling pathways mediating Tv-induced inflammation in ectocervical cells (Ects). We initially measured the production of various cytokines using a multiplex immunoassay, revealing a significant increase in IL-6, IL-8, IP-10, and CXCL1 secretion in Ects upon Tv infection. We then assessed the role of autophagy in regulating Tv-induced inflammation in Ects by using autophagy inhibitors and small interfering RNA targeting LC3B (si-LC3B) to block different stages of autophagy. Our findings indicated that Tv-induced autophagic flux mediates the secretion of proinflammatory cytokines in Ects. Additionally, blocking autophagosome formation via si-LC3B increases IL-6 and IP-10 levels while reducing IL-8 secretion. To further identify novel pathways involved in Tv-induced inflammation in Ects, we conducted a time-series proteomic analysis using 2D-LC–MS/MS. Intriguingly, we noticed robust activation of antiviral-related pathways in Ects after 8 hours of Tv stimulation. Specifically, the most enriched proteins in these pathways were tetratricopeptide repeats (IFIT) family proteins (IFIT1, IFIT2, and IFIT3). Functional validation revealed that IFIT3 positively regulates downstream IL-8 and IP-10 secretion. Furthermore, we proved that si-LC3B enhanced IFIT expression in Ects upon Tv infection, suggesting that autophagy negatively regulates IFIT expression. Collectively, this study demonstrates that Tv infection induces autophagic flux and IFIT overexpression to modulate inflammatory responses in Ects, providing novel insights into the inflammatory mechanisms governing trichomoniasis.

[3] Long-read transcriptomics corrects *Trichomonas vaginalis* intron annotations and maps poly(A)/UTR landscapes

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Trichomonas vaginalis is a prevalent protozoan STI whose large genome remains sparsely and inconsistently annotated for introns. Although the NYU_TvagG3_2 reference (184.2 Mb; 37,794 protein-coding genes) contains few experimentally supported introns, their true coordinates and prevalence remain unsettled. A recent short-read RNA-seq study catalogued 63 “active” introns, but read-length limitations can misplace boundaries and inflate false positives.

Here, we combine Oxford Nanopore direct RNA sequencing (DRS), ONT cDNA long reads, and Illumina RNA-seq to re-annotate introns at full length, delineate 5′/3′ untranslated regions (UTRs), and quantify poly(A) tail lengths on single molecules. Motif-guided searches nominated candidates, while targeted primers and Sanger sequencing provided orthogonal validation; key events were replicated by re-analysis of public SRA datasets.

Long-read evidence reconciled the short-read catalogue, correcting five loci (two false positives, two coordinate mis-annotations, and one gene sequence error) and adding three introns supported by multiple full-length reads and PCR. We release updated coordinates and read-level evidence for each event. DRS precisely mapped transcript end sites and AAUAAA positions relative to poly(A) addition, and we report poly(A) length distributions and UTR boundaries for intron-bearing and intron-free transcripts.

[4] Signal Peptide Variation in Cyst Lectins as a Potential Marker for Pathogenic *Acanthamoeba* spp.

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Acanthamoeba spp. are free-living protists widely distributed in various environments, including kinds of water bodies, soil, and even artificial facilities. The protist can cause opportunistic infections in humans, resulting in sight-threatening disease, Acanthamoeba keratitis (AK), and granulomatous amoebic encephalitis (GAE). *Acanthamoeba spp*. life cycle includes an active trophozoite stage and a dormant cyst stage. The cyst form features a double-layered cellulose wall structured by multiple cyst wall lectins. The cyst form provides resistance against environmental stresses such as drought, pH changes, and drug exposure. The encystation ability and the structure of the cyst are not only crucial for drug resistance but also for environmental adaptability and may contribute to the infectious process. In this study, we examined the phylogenetic analysis of 31 previously identified cyst lectins across 30 *Acanthamoeba spp.* genomes from NCBI databases. Based on the isolation source, the isolates were classified into clinical and environmental groups. Phylogenetic analysis revealed that 8 cyst lectins exhibited clinical isolate-specific clustering, suggesting a potential link to pathogenicity. Notably, 3 of these lectins (ACA1_187760, ACA1_252830, ACA1_287530) lacked signal peptides in the clinical isolate genomes. PCR validation using clinical isolates from National Cheng Kung University Hospital confirmed that these signal peptide deletions were not sequencing artifacts, but were consistently present in pathogenic strains. These results indicate that these lectins may perform alternative functions in clinical isolates, potentially resulting in a distinct cyst wall structure from that of non-clinical strains. Our findings highlight the diversity of cyst wall lectins and their potential association with clinical pathogenicity, thereby providing a molecular basis for predicting the pathogenicity of *Acanthamoeba spp.*

[5] *Legionella pneumophila* affects encystation mediating gene expression of *Acanthamoeba castellanii*

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Abstract

Acanthamoeba is a genus of free-living amoebae commonly found in soil, water, and other habitats. This organism undergoes two distinct stages in its life cycle, the trophozoite and the cyst. Under adverse conditions, trophozoites transform into cysts, which are notably resistant to harsh physical and chemical conditions. Infected by *Legionella pneumophila* has been shown to decrease the number of cysts in its host *Acanthamoeba* species, although the mechanisms responsible for this effect remain poorly understood. In this study, *A. castellanii* was co-cultured with either *L. pneumophila* or *Escherichia coli* to assess the impact on encystation and to explore the genes involved in this process. Following a 72 h encystation induction period, it was observed that *Acanthamoeba* infected with *Legionella* exhibited a 45.8% reduction in cyst formation compared to the control group. In contrast, *Acanthamoeba* that phagocytosed *E. coli* showed a 21.7% decrease. To identify the genes involved in this phenomenon, real-time PCR analysis was conducted on 20 genes known to be upregulated during encystation. This analysis was performed to verify their expression patterns at 24, 48, and 72 h. Notably, eleven genes, including cyst-specific protein 21, RSNARE, encystation-mediating serine proteinase, and cellulose synthase, did not exhibit increased expression in *Legionella*-infected *Acanthamoeba*. However, these genes showed elevated expression levels in both the control group and the bacteria-phagocytosed *Acanthamoeba*. This suggests that several cellular processes, including autophagy and cell wall formation, are inhibited in *Acanthamoeba* infected with *Legionella*, resulting in reduced encystation. These findings are anticipated to offer valuable insights for future research on the encystment mechanism of *Acanthamoeba* and the impact of *Legionella* infection.

[6] The choice of encystation medium determines the quantitative and qualitative outcomes of *Acanthamoeba castellanii* cyst formation

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Acanthamoeba species are widespread, free-living amoebae that cause serious infections such as *Acanthamoeba* Keratitis and Granulomatous Amebic Encephalitis. Their ability to form highly resistant cysts under stress is a key factor in both their pathogenicity and treatment failure. To support drug development, it is important to understand and optimize experimental encystation methods. Our study compared the encystation efficiency of two common liquid media, PBSEB and EB2, across different *Acanthamoeba* cell densities (high, medium, and low) over 24, 48, and 72 hours, using *Acanthamoeba* castellanii strains ATCC_30010, ATCC_50492, ATCC_50370, and the clinical isolate NCKU_D. We observed distinct morphological changes with each medium: PBSEB rapidly induced cell rounding and detachment, while EB2 caused a gradual, time-dependent rounding that progressed to pre-cyst-like and cyst-like forms. Quantitative results showed that PBSEB consistently achieved 80-100% cyst formation across all cell densities. In contrast, EB2's cyst-inducing activity decreased at higher cell densities. While both methods significantly increased CS-I gene expression after 48 hours, electron microscopy images revealed that EB2-induced cysts possessed a more complete structure. Our findings indicate that PBSEB more effectively induces cysts at high cell densities, whereas EB2 excels at producing mature, SDS-resistant cysts, particularly at lower densities. These findings suggest distinct encystation mechanisms and efficacy profiles, offering valuable insights for improving anti-*Acanthamoeba* drug development procedures.

[1] The role of intestinal schistosomiasis in colorectal cancer

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Schistosomiasis is a chronic parasitic disease caused by *Schistosoma*. Among the *Schistosoma* species, S. haematobium is a well-established carcinogen that is associated with squamous cell carcinoma of the bladder. While other species such as S. mansoni and S. japonicum have not been classified as carcinogens, growing evidence has suggested a link between these species and the development of cancer. Colorectal cancer (CRC) is an adenocarcinoma that arises from the abnormal proliferation of glandular epithelium cells in the colon or rectum. In our study, we showed that the coexistence of schistosomiasis and CRC increases both the malignancy and severity of CRC in mouse models induced by azoxymethane (AOM) and dextran sulfate sodium (DSS), and we hypothesized a potential correlation between schistosomiasis and CRC. During *Schistosoma* infection, schistosome eggs accumulate in colonic tissue, leading to chronic inflammation. This prolonged inflammation and tissue damage may therefore facilitate fibrosis but may also induce abnormal proliferation of colonic epithelial cells. While these trapped eggs will continuously release soluble egg antigen (SEA), which affects the host's immune system, we also aimed to explore the role and mechanisms of SEA and its specific protein components (SM14, GST28, and Smp40) in the CRC model. Our results suggest that treatment with SEA, SM14, and GST28 did not significantly alter the disease. However, Smp40 treatment aggravated the symptoms and was accompanied by an increased expression of inflammatory cytokines. Ultimately, we found that Smp40 worsens the progression of CRC. However, in vitro study showed that only SEA directly suppressed the growth of HCT-116 cells, while other egg antigens had no direct effects. Because the tumor microenvironment (TME) is a complex setting where many different cells coordinate the immune response, including cancer cells that secrete cytokines. We hypothesize that this complexity may underlie the observed effects in the in vivo models. Overall, schistosome eggs and their components may have the potential to affect disease progression in CRC. These findings contribute to a better understanding of how intestinal schistosomiasis affects CRC and may offer a new perspective on the connection between parasitic infection and cancers.

[2] *Trichomonas tenax* exacerbates bacterial infection through immunomodulation and enhanced adhesion

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Trichomonas tenax is an anaerobic, flagellated protozoan typically found as a commensal organism in the human oral cavity, specifically in areas with poor oral hygiene like periodontal pockets, gingival crevices, and dental plaque. Recent studies have detected its presence in the bronchoalveolar lavage fluid (BALF) of patients with severe pulmonary conditions, including empyema and acute respiratory distress syndrome (ARDS), suggesting a potential link to respiratory illness. Despite this, the precise role of *T. tenax* in pneumonia has not been fully understood. Our clinical research discovered a notable association between *T. tenax* and clinical indicators of pneumonia, including bacterial infections, high Pneumonia Severity Index (PSI) ratings, and other lung complications. Although *T. tenax* has a high association with bacterial infection in pneumonia, the pathological and immunological effects of *T. tenax* on bacterial infection remain unknown. Notably, we found that *T. tenax* exhibits a higher rate of co-detection with Pseudomonas aeruginosa in BALF. We investigated the pathological role of *T. tenax* on pulmonary cells and mouse lungs infected with P. aeruginosa. Our findings reveal that while *T. tenax* has no impact on host cells and the virulence of P. aeruginosa by itself, it significantly exacerbates the pathology of lung epithelial cells and mouse lungs during a secondary infection. Furthermore, the gene expression of surface receptors that P. aeruginosa adhered to is up-regulated when co-incubated with *T. tenax*. Our investigation into the pathology of co-infection in mice led us to examine the immunological response in human patients. A clinical study revealed that PSI=5 pneumonia patients with both *T. tenax* and pathogenic bacteria had lower IL-1β levels than those infected solely with pathogenic bacteria. Based on this observation, we hypothesized that *T. tenax* modulates the host's inflammatory response. Our subsequent in vitro results supported this, showing that viable *T. tenax*, as well as its total and soluble extracts and cell debris, consistently reduced the production of inflammatory cytokines in LPS-stimulated macrophages. Through size exclusion chromatography, we isolated an immunomodulatory component, Fraction 1, from *T. tenax* soluble extracts that effectively reduced the LPS-induced inflammatory response. In conclusion, our study reveals that *T. tenax* exacerbates lung pathology by damaging the epithelium while simultaneously regulating the immune response through specific immunomodulatory components.

Keywords: T. tenax, P. aeruginosa, Clinical study, Secondary-Infection, Adhesion, Immunomodulation

[3] Naringenin attenuates liver injury in *Schistosoma* mansoni-induced liver fibrosis and oxidative stress

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Abstract

Schistosomiasis, a neglected tropical disease caused by parasitic flukes of the genus *Schistosoma*, affects over 200 million people worldwide and frequently results in severe hepatic complications, including liver fibrosis, hepatosplenomegaly, and portal hypertension. The disease pathology is mainly driven by parasite eggs trapped in hepatic tissue, which elicit granulomatous inflammation, hepatic stellate cell (HSC) activation, and excessive extracellular matrix deposition. In addition to immunopathology, schistosomiasis induces oxidative stress, further accelerating fibrosis. Increased lipid peroxidation and diminished antioxidant enzyme activity create an oxidative imbalance, enhance the deposition of fibrotic proteins, and accelerate liver damage. Currently, praziquantel (PZQ) is the standard treatment and is highly effective at eliminating adult worms. However, it has limited efficacy in clearing deposited eggs in tissues and does not directly resolve established liver fibrosis, suggesting a potential need for adjunctive therapies targeting fibrotic and oxidative mechanisms. In our study, we focused on evaluating the therapeutic efficacy of naringenin against schistosomiasis-induced liver injury. Naringenin, a natural flavonoid abundantly found in citrus fruits, has been reported to exert multiple protective effects, including anti-inflammatory, antifibrotic, and antioxidant activities. Using a *Schistosoma* mansoni-infected BALB/c mouse model, we found that naringenin treatment markedly alleviated hepatosplenomegaly and improved liver function indices. Histopathological evaluation using H&E, Masson's trichrome, and Sirius red staining demonstrated a reduction in granuloma size, immune cell infiltration, and collagen deposition. Western blot analysis further confirmed suppression of fibrosis-related proteins, inflammatory mediators, and oxidative stress markers. In conclusion, these results demonstrate that naringenin significantly attenuates schistosomiasis-induced liver injury by modulating fibrotic, inflammatory, and oxidative pathways, supporting its potential as a promising adjunctive therapeutic strategy for parasite-associated hepatic fibrosis.

[4] Bifidobacterium breve Enhances Growth and Alters the Transcriptome of *Trichomonas vaginalis* Without Modulating Host Inflammatory Responses

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Trichomonas vaginalis is the most prevalent non-viral sexually transmitted parasite, colonizing the vaginal environment alongside commensal bacteria such as *Lactobacillus* and *Bifidobacterium*. A reduction in *Bifidobacterium breve* has been observed in *T. vaginalis*-infected women; however, the interaction between the two species remains poorly characterized.

We investigated the effects of *B. breve* on *T. vaginalis* growth, gene expression, and host-pathogen interactions. HeLa cells were exposed to *T. vaginalis* pretreated with or without *B. breve*, and cytokine responses (IL-6 and IL-8) and cytopathic effects were assessed. Co-culture dynamics were monitored microscopically. Transcriptomic and metabolomic analyses were performed to characterize *T. vaginalis* responses to *B. breve.*

Co-culture for 4 hours significantly increased *T. vaginalis* proliferation and reduced *B. breve* abundance. *B. breve* did not alter *T. vaginalis*-induced IL-6 or IL-8 production in HeLa cells, nor did it reduce cytopathic effects or modify host cell interactions. Microscopy revealed direct physical association between the two organisms. Transcriptomic profiling of *T. vaginalis* showed upregulation of genes involved in fatty acid metabolism and DNA replication. Metabolomic analysis confirmed alterations in long-chain fatty acid content. Virulence-associated genes, including adhesins, were also elevated.

B. breve enhances *T. vaginalis* growth and modulates its transcriptome and lipid metabolism but does not protect host epithelial cells from infection. These findings suggest that *B. breve* may play a community-supportive rather than protective role during *T. vaginalis* infection.

[5] Detection of *Corynosoma cystacanths* from marine fishes caught in Hokkaido and Honshu sold at fish markets in the Kanto region, Japan

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Corynosoma spp. are acanthocephalan parasites that use amphipods as intermediate hosts, fish as paratenic hosts, and pinnipeds or seabirds as definitive hosts. Human infection is rare, but cases have been reported in northern regions such as among Inuit populations in Alaska, suggesting zoonotic potential. In Japan, three human cases have been reported in Hokkaido, but none from mainland Japan. Recently, fresh fish from Hokkaido have been widely distributed in the Kanto region, raising concerns that infections could occur outside Hokkaido. However, no studies have examined the prevalence of *Corynosoma* larvae in fish sold in Kanto. With the global spread of raw fish consumption through Japanese cuisine, *Corynosoma* infection should not be regarded as a strictly regional issue. This study therefore investigated the infection status of *Corynosoma* larvae in fish sold in Kanto and labeled as originating from Hokkaido or mainland Japan.

A total of 473 fish from 24 species labeled as from Hokkaido and 52 fish from 19 species labeled as from mainland Japan were purchased at fish shops and supermarkets in the Kanto region. After dissection, viscera were examined macroscopically and under a stereomicroscope, and encysted larvae were isolated. Larvae were excysted using an artificial digestion solution and identified morphologically. DNA was extracted from selected larvae, and the COX1 gene was amplified by PCR for molecular identification.

Corynosoma larvae were detected in 17 of 24 fish species (70.8%) from Hokkaido, with higher prevalence in benthic fish. No larvae were found in eight species, including *Cololabis saira* and *Pleurogrammus azonus*. From mainland Japan, a single larva was detected in Zeus *faber* from Fukushima Prefecture. Most larvae were located on the serosa of digestive organs. Morphological and molecular analyses identified them as *C. villosum* and *C. strumosum*.

This study revealed a high prevalence of *Corynosoma* larvae in fish sold in Kanto and labeled as from Hokkaido, mainly *C. villosum* and partly *C. strumosum*. Importantly, larvae were also detected for the first time in fish from mainland Japan, indicating that the infection risk is not limited to Hokkaido. Higher infection rates were observed in benthic fish such as flounders, suggesting that human infection could also occur in mainland Japan. Further studies with larger sample sizes are needed to clarify the infection risk. These findings emphasize the need for continuous monitoring of fish-borne parasites to ensure food safety and to better assess zoonotic risk in regions where raw fish is globally consumed.

[6] Discovery of Eugregarinida Associated with House Dust Mites by 18s rRNA Region Nanopore Sequencing

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House dust mites (HDMs) are recognized as sanitary pests because their allergens induce allergic symptoms in humans. Not only allergens but also their microbiome of HDMs has recently been suggested as an additional contributor to allergic diseases. In this study, we investigated the eukaryotic community associated with three HDM species—*Dermatophagoides farinae, D. pteronyssinus*, and *Tyrophagus putrescentiae*. The 1.8Kb length of 18s rRNA gene was amplified by PCR, and sequencing generated a total of 3.6 Gb of bases using the Nanopore MinION platform. Decona analysis revealed eight eukaryotic taxa, among which members of the order Eugregarinida (GenBank accession MG766260.1) were identified from *D. farinae* (85.91%) and *D. pteronyssinus* (86.48%). The two Eugregarinida sequences between *D. farina* and *D. pteronyssinus* exhibited 87.71% homology. The two Eugregarinida sequences from Nanopore data clustered within the Gregarine lineage in Bayesian phylogenetic analysis. Microscopic examination at ×400 magnification further confirmed the presence of Gregarine trophozoites in HDM samples. Overall, these findings provide the molecular and microscopic evidence of *Gregarine* parasites associated with HDMs, highlighting a previously unrecognized component of the HDM eukaryotic microbiome. This discovery expands our understanding of the complex biological interactions within HDMs and raises new questions regarding the ecological role of gregarines and their potential contribution to the allergenic capacity of HDM populations.

[1] Environmental Influences on Eukaryotic Microbiota and Potential Pathogen Prevalence in Domestic Pigeons of Seoul, South Korea

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Feral pigeons, which are prevalent in densely populated areas, may pose significant public health risks due to their potential to carry and transmit zoonotic pathogens. In this study, a total of 149 fecal samples collected from Seoul and surrounding cities were analyzed using metabarcoding targeting the 18S rRNA gene V9 region to investigate the eukaryotic microbiota of pigeons and assess potential zoonotic threats. We hypothesized that environmental factors such as housing density, proximity to water and parks, and the presence of homeless individuals shape parasite communities.

Amplicon sequence variants with five or fewer reads were excluded. Differences in parasite prevalence between groups were assessed using the Yates-corrected chi-square test. To identify environmental predictors of parasite prevalence, we constructed generalized linear models (GLMs) using a logit model (binomial family).

Our findings revealed a high prevalence of protozoan parasites, including *Eimeria, Isospora*, and *Cyclospora*, as well as helminths such as *Tetrameres* and *Baruscapillaria*. *Eimeria* was the most frequently detected parasite (86.58%), followed by *Isospora* (40.94%).

Environmental factors were found to significantly influence parasite prevalence. Proximity to water sources was associated with a higher prevalence of Isospora ($p = 0.0268$, OR = 2.5340), while parks were linked to an increased prevalence of Eimeria ($p = 0.0251$, OR = 5.3015). Additionally, the absence of homeless individuals was positively associated with the prevalence of Isospora ($p = 0.0082$, OR = 3.0496).

This study represents the first empirical investigation into how urban environments influence parasite diversity and wildlife associations in one of the most densely populated cities in the world. By elucidating the complex relationships between environmental factors and pathogen prevalence, our results provide valuable insights for public health interventions and urban planning strategies aimed at reducing zoonotic risks in densely populated cities.

[2] Establishment of metagenomic analysis method for gut protozoal flora

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Protozoa are increasingly recognized as essential components of the gut microbiota. However, their ecological roles within host organisms remain poorly understood, largely due to the lack of appropriate methods for metagenomic analysis of gut protozoal flora. To address this limitation, we developed taxon-specific primers designed to selectively amplify protozoan DNA while reducing the amplification of non-relevant dietary sequences present in fecal samples. This approach minimizes interference from food residues, which often obscure protozoan signals during sequencing, and thereby improves the identification of intestinal protozoan communities through NGS reads. The study is currently ongoing, and recent results obtained from human, and pig fecal samples have revealed a wide diversity of intestinal protozoan communities, which will be presented in this report.

keywords: Intestinal microbiome, Metagenomic analysis, Next-generation sequencing (NGS)

[3] Exploration of gut parasites with anti-inflammatory potential in *Apodemus agrarius*

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Apodemus agrarius, a common wild rodent in Korea, inhabits diverse environments and is frequently exposed to various pathogens, continuously challenging its immune system. This study aimed to identify parasites with potential anti-inflammatory effects by analyzing the gut parasitome and spleen cytokine expression profiles.

Expression levels of cytokines (IL-10, TGF-β, TNF-α), the transcription factor FOXP3, and Toll-like receptors were assessed using RT-qPCR in *A. agrarius* (n = 175). Gut parasitome profiling was conducted via 18S rDNA V9 region-targeted metabarcoding.

Individuals positive for Hymenolepis sp. or *Hypotrichomonas* sp. exhibited significantly elevated TGF-β expression compared to parasite-negative individuals. Specifically, *Hymenolepis*-positive rodents showed a 4.1-fold increase and *Hypotrichomonas*-positive rodents a 2.4-fold increase in TGF-β expression. No significant difference was observed in TNF-α expression.

These findings suggest that specific parasites in *A. agrarius* may contribute to an anti-inflammatory immune environment via TGF-β–mediated mechanisms, offering potential therapeutic applications for inflammatory diseases

[4] Molecular detection of *Dientamoeba fragilis* and *Blastocystis* sp. from a diarrheal case

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[Background] Intestinal parasitic protozoa infecting humans include species whose pathogenicity remains debated, such as Blastocystis sp. and *Dientamoeba fragilis* (*D. fragilis*). Specifically, *Blastocystis* sp. has been reported to exhibit extensive intraspecific polymorphism, including Sub Type 1 (ST1) to ST17 and new subtypes beyond ST40. Furthermore, while at least two intraspecies clusters are believed to exist within *D. fragilis*, genetic references remain limited. Therefore, this study aimed to perform detailed molecular identification of *Blastocystis* sp. and *D. fragilis* detected via metabarcoding analysis using next-generation sequencing (NGS) on clinical samples. This was achieved through long-read PCR sequencing analysis.

[Materials and Methods] Total DNA purified from diarrhea stool samples collected from a 70-year-old Australian male presenting with chronic loose stools was used as a template. Using NGS amplicon analysis targeting intestinal protozoa developed in our laboratory, approximately 200 bp fragments were detected and identified. Using this purified DNA, species-specific long-read PCR amplification was performed for each parasite. The amplified products were directly sequenced, and the obtained DNA sequences were evaluated by phylogenetic analysis.

【Results】The DNA sequence of the amplification product from the *D. fragilis*-specific PCR showed over 100% homology with *the D. fragilis* reference sequence. Phylogenetic analysis using *Trichomonadida* reference sequences placed it within the *D. fragilis* cluster. In contrast, the DNA sequence of the amplification product from the *Blastocystis*-specific PCR was almost identical to the *Blastocystis* subtype 1 (ST1) reference sequence.

【Discussion】In recent years, data have been reported demonstrating the pathogenicity of *D. fragilis,* and dientamoebiasis is now listed on the US CDC website. However, asymptomatic infections are also common, so the accumulation of future epidemiological data is essential to prove its pathogenicity. The situation is similar for *Blastocystis* sp., but our lab's previous evaluations have revealed that among the genotypes detected in humans (ST1-ST4), ST1 in particular may be pathogenic. Therefore, the detection of ST1 in this case is a noteworthy finding. Metabarcoding analysis using NGS is highly effective in clinical testing due to its ability to comprehensively detect and identify a wide range of protozoa. However, as it relies on short DNA fragments, it is unsuitable for intraspecies polymorphism analysis. Therefore, conventional long-read DNA sequencing using Sanger sequencing remains indispensable for detailed intraspecies polymorphism analysis.

[5] Investigating Inflammatory and Immunopathological Differences in the Brains of Blimp-1 Transgenic Mice Infected with *Angiostrongylus cantonensis* Using ^{18}F-FDG/PET Imaging

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Angiostrongylus cantonensis is a common zoonotic parasite in Taiwan and China. Human and mice infections usually occur through ingestion of contaminated snails, slugs, or unwashed produce. As non-permissive hosts, both humans and mice can develop eosinophilic meningitis or meningoencephalitis when L3 larvae breach the blood-brain barrier, potentially leading to severe neurological complications or death. While Th1, Th2, and Th17 cytokines are known to contribute to local inflammation, their precise regulatory pathways remain poorly understood.

This study explores the immunomodulatory role of the transcriptional repressor Blimp-1 during central nervous system (CNS) infection. Blimp-1 influences $CD4^+$ T and B cell differentiation and regulates the activity of T follicular helper (Tfh) and regulatory T (Treg) cells. Although Blimp-1 overexpression is thought to suppress $Foxp3^+$ $ROR\gamma t^+$ Treg-driven inflammation, its function in neuroimmune responses is still not fully defined.

Using Blimp-1 transgenic NOD (Tg^+) and wild-type (Tg^-) mice, we established a CNS infection model and evaluated brain inflammation through ^{18}F-FDG/PET/CT imaging, immunohistochemistry, and protein expression analyses. Tg^+ mice exhibited heightened baseline of immune activity and showed limited radioactivity changes post-infection. Conversely, Tg^- mice showed progressive ^{18}F-FDG uptake during weeks 4 and 5, indicating sustained inflammation. Time-dependent and regional variations in eosinophil and microglial infiltration were also observed. While Tg^- mice displayed prolonged immune cell accumulation, Tg^+ mice showed an early but transient immune activation, suggesting that Blimp-1 may facilitate rapid immune priming followed by resolution.

Although preliminary, our findings highlight Blimp-1 as a potential regulator of CNS inflammation during parasitic infection. The integration of imaging and molecular tools in this study lays the groundwork for future mechanistic study and the development of early immunomodulatory interventions.

[6] IκB kinase 2 and Calcium are involved in ROS production and exocytosis of mast cells stimulated by *Trichomonas vaginalis*-derived secretory products

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Trichomonas vaginalis infection causes vaginitis and cervicitis in women, and asymptomatic urethritis and prostatitis in men. Mast cells are innate immune cells that participate in the inflammatory response against microbial infections. In this study, we investigated the roles of calcium and IKK2 signaling in intracellular reactive oxygen species (ROS) production and exocytic degranulation in the human mast cell line HMC-1 stimulated with *T. vaginalis*-derived secretory products (TvSP).

Western blot analysis revealed that TvSP stimulation induced time-dependent phosphorylation and subsequent degradation of IκB, suggesting activation of the NF-κB signaling pathway. TvSP also promoted a marked increase in intracellular ROS levels and surface expression of CD63, a marker of exocytosis, in HMC-1 cells. Pretreatment with extracellular calcium chelators, EDTA or EGTA, significantly suppressed TvSP-induced ROS production and CD63 expression, indicating a calcium-dependent mechanism. Similarly, pharmacological inhibition of IKK2 with IMD-0354 attenuated both ROS generation and degranulation in a concentration-dependent manner. In addition, TvSP activated ERK1/2, p38 MAPK, and AKT phosphorylation without affecting total protein levels. TvSP-induced degranulation was attenuated by PI3K inhibitors (wortmannin, LY294002) and a PKC inhibitor (Ro-31-8220), indicating involvement of these pathways. TvSP enhanced IL-8 secretion, which was significantly reduced by inhibition of PI3K, ERK, p38 MAPK, and JNK signaling.

These findings suggest that calcium and IKK2 are critical mediators of ROS production and exocytotic degranulation in mast cells activated by *T. vaginalis*-derived secretory products, with additional involvement of diverse signaling processes including MAPK and PI3K pathways, providing insight into the signaling pathways involved in mast cell responses to parasitic infection.

[7] Integrating Phenotypic Screening and DRUG-seq Transcriptomics to Identify Compounds with Novel Mechanisms of Action Against *Trypanosoma cruzi*

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Chagas disease, caused by *Trypanosoma cruzi,* is a neglected tropical disease estimated to affect 6-8 million people worldwide, resulting in over 10,000 deaths annually. Despite being identified in 1909, only two drugs – nifurtimox and benznidazole – are currently available, both with limited efficacy in the chronic stage, and frequent adverse effects that lead to treatment discontinuation.

This research aims to identify and characterize a hit compound with a novel mechanism of action (MoA) against the intracellular amastigote stage of *T. cruzi,* thereby contributing to the development of next-generation therapeutics for Chagas disease. To this end, phenotypic screening of 36,160 compounds from the Nagoya University chemical library was performed, applying a 60% inhibition cutoff (~1% of the library). The resulting 320 hits will undergo further evaluation for efficacy (EC_{50}), cytotoxicity (CC_{50}) in host mammalian cells, selectivity index (SI), activity against four *T. cruzi* strains, and high-throughput rate-of-kill (HT-RoK) profiles.

Compounds with confirmed activity against four *T. cruzi* strains, favorable selectivity, trypanocidal properties, and structural novelty will be prioritized for MoA studies using Digital RNA with Perturbation of Genes (DRUG-seq), a transcriptomic profiling approach based on gene expression changes induced by compound treatment. DRUG-seq enables clustering of compounds by expression signatures to identify shared or unique MoAs (Ye et al., 2018). The inclusion of compounds with known MoAs will help identify hits with novel transcriptomic profiles and potential new MoAs. Our group has already validated this approach for antimalarial drug discovery (Ishii et al., bioRxiv, 2025).

By integrating phenotypic screening with transcriptomics, this study provides a modern strategy to discover stage-specific antitrypanosomal agents with novel MoAs, offering promising leads to overcome the limitations of current treatments.

[1] Microbiome and Resistome Profiling of the Synanthropic Blow Fly *Lucilia sericata* Across South Korea

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Blow flies, including *Lucilia sericata* (Diptera: Calliphoridae), are well recognized for their ecological contributions to organic matter decomposition, yet their close association with decaying substrates also raises concerns regarding their role as carriers of pathogenic microorganisms and antimicrobial resistance genes (ARGs). In this study, we examined the bacterial assemblages and resistome characteristics of *L. sericata* collected from six distinct provinces across South Korea through a combination of 16S rRNA gene metabarcoding and targeted PCR assays. The dominant bacterial genera detected were *Dysgonomonas, Vagococcus, Pseudomonas, Ignatzschineria*, and *Providencia*. Although their relative abundance varied geographically. Microbial diversity was lowest among flies sampled from Chungnam, whereas specimens from Jeonnam and Gyeonggi displayed higher richness and more balanced community structures. Beta diversity further highlighted geographic partitioning, with semi-urban and rural locations supporting more taxonomically diverse microbiomes compared to more developed urban areas. Opportunistic pathogens, notably Proteus mirabilis and Providencia spp., were identified alongside a suite of resistance genes, including *blaTEM, ermB, sul1, aac(6')-Ib-cr, cat,* and *mecA,* as well as integron elements (*intI a*nd *intII*). These results suggest the potential role of *L. sericata* as a reservoir of clinically relevant bacteria and resistance genes, underscoring its significance in public health contexts.

[2] Molecular Characterization of *Endolimax nana* in Humans and Animals from Eastern Indonesia Reveals Subtype-Level Genetic Diversity

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Endolimax nana is a common intestinal amoeba in humans, yet its molecular diversity and host range remain poorly defined. This study aimed to characterize the genetic diversity and host pattern of *Endolimax nana* through a molecular analysis. We analysed 314 stool samples from Wainyapu Village, Sumba Island, Indonesia (humans: n = 143; domestic animals: n = 171). A nested PCR targeting a 1,311bp fragment of the 18S rRNA gene was performed, amplicons were directly sequenced and subcloned when chromatograms were ambiguous. Phylogenetic analyses were constructed using Bayesian inference, neighbor-joining, and maximum parsimony.

Out of 314 samples, *Endolimax nana* was identified in 76 (24%). The parasite was more common in humans (61/143; 43%) than in animals (15/171; 9%). Positive cases among animals included six rats, five pigs, two dogs, one chicken, and one duck. Through subcloning, the number of haplotypes increased from 76 to 127, with 118 confirmed as unique. Considerable variation within individual hosts was evident, particularly in humans, pigs, and rats. Across tree building methods, two well-supported subtypes (ST1, ST2) were consistently resolved, each subdivided into ST1-1, ST1-2, ST2-1, and ST2-2. The co-occurrence of closely related haplotypes in human and animal hosts, together with evidence of mixed infections, suggests possible cross-host transmission.

This represents the first molecular characterization of *E. nana* in Indonesia and demonstrates a broader host spectrum. The subtype framework established here contributes to clarifying taxonomy and provides a basis for future epidemiological studies.

Keywords: *Intestinal protozoa, Endolimax nana, Molecular Taxonomy, Genetic Diversity*

[3] Spatio-temporal patterns of dengue transmission in Tainan city, Taiwan, pre- and post-COVID-19

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Dengue fever remains a significant public health challenge in southern Taiwan, particularly in Tainan City, where favorable climatic conditions and dense urban environments facilitate mosquito proliferation and virus transmission. Given the epidemiological characteristics of dengue in Taiwan, the COVID-19 pandemic may have altered outbreak ecology in the region. This study compared the spatial and temporal patterns of dengue transmission in Tainan before and after the pandemic, focusing on major outbreaks in 2015 (pre-pandemic) and 2023 (post-pandemic). Spatial patterns were analyzed and visualized using emerging hotspot analysis. Land Cover/Land Use (LCLU) data were incorporated to quantify environmental diversity via the Shannon index. A Spatial Lag Model (SLM) was employed to examine associations between dengue incidence, LCLU diversity, and urbanization level. Results revealed distinct outbreak patterns between the two years. Persistent metropolitan hotspots were observed in both periods; however, the 2023 outbreak exhibited greater geographic dispersion, with new hotspots emerging in peri-urban and rural zones. In 2015, hotspots were concentrated in urban southwestern districts, whereas in 2023 they extended into southeastern and northern areas. Across both years, dengue incidence was positively associated with urbanization (2015: coefficient = 3.17, $p = 0.09$; 2023: coefficient = 4.21, $p < 0.01$) and negatively associated with land cover diversity (2015: coefficient = –2.28, $p < 0.01$; 2023: coefficient = –1.99, $p < 0.01$). These findings highlight shifts in dengue spatial patterns following the COVID-19 pandemic, underscoring the need to adapt surveillance and targeted interventions to evolving transmission dynamics.

[4] Study on mechanisms of microglia in the brain of Angiostrongylus cantonensis infected mice

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Angiostrongylus cantonensis infection induces neuroinflammation and cognitive dysfunction by infiltrating the central nervous system (CNS), yet the immune mechanisms linking microglial polarization to neuronal damage remain unclear. In this study, a murine model of infection was established in C57BL/6 mice to evaluate cognitive, synaptic, and immunological alterations, as well as the therapeutic effects of albendazole combined with anti-inflammatory agents. Behavioral assessments revealed significant impairments in spatial learning and long-term memory from the second week post-infection, accompanied by marked reductions in hippocampal dendritic spine density.

Western blotting demonstrated increased TREML2 and NLRP3 expression, particularly in the hippocampus, consistent with M1-associated inflammasome activation, while Arginase-1 increased at later stages, reflecting a compensatory M2 neuroprotective response. Early iNOS suppression suggested an initial Th2-skewed profile. Splenic analysis further revealed that Arginase-1 rose sharply during the first week and declined thereafter, while IL-4 peaked at week two, indicating an early Th2/M2-dominant response. In contrast, iNOS remained unchanged and IFN-γ increased only at week three, reflecting a delayed Th1/M1 activation. This temporal pattern suggests that early peripheral consumption of L-arginine may restrict its availability in the CNS, thereby delaying microglial M2 polarization until the second week and suppressing early M1 activation.

Therapeutically, albendazole alone or combined with resveratrol or minocycline improved behavioral outcomes and restored synaptic structure, with the albendazole–resveratrol combination showing the strongest effects by reducing TREML2 and NLRP3 expression and enhancing dendritic spine density.

Collectively, these findings demonstrate that A. *cantonensis* infection leads to cognitive deficits and synaptic injury through neuroinflammatory mechanisms mediated by microglial polarization, highlight TREML2 as a potential amplifier of M1 activation and inflammasome signaling, and suggest that albendazole combined with anti-inflammatory agents, particularly resveratrol, may provide a promising therapeutic approach with potential relevance to neurodegenerative diseases involving microglial dysregulation.

Keywords*: Angiostrongylus cantonensis*, Microglial polarization, Neuroinflammation, Resveratrol, Minocycline, Cognitive impairment

[5] Systematic Analysis of Putative and Novel Microproteome in the *Trichomonas vaginalis*

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Microproteins (≤ 100 aa) encoded by small open reading frames (sORFs ≤ 300nts), have been increasingly identified with the advancement of genomics and proteomics technologies. Recent studies have demonstrated that microproteins participate in essential biological processes; however, their roles in protozoan parasites remain largely unknown. Here, we use *Trichomonas vaginalis*, as a research model to perform a systematic analysis of the parasite's microproteome. A total of approximately 11,000 putative microproteins were annotated in the reference G3 genome, with a prominent length distribution between 71 and 80 amino acids. However, it is still not clear how many of these putative microproteins are expressed. To clarify this question, we analyzed 73 RNA-seq datasets from 22 *T. vaginalis* isolates to identify expressed microproteins. We observed considerable variation in the number of microproteins detected, ranging from 1,209 to 7,913, which is likely due to batch effects from datasets generated using different sequencing platforms and varying sequencing depths. Based on these findings, we established a microprotein reference database for *T. vaginalis* and revealed a core set of microproteins consistently present across 22 isolates. To determine whether these microprotein transcripts are translated, we are conducting an ongoing proteomics study using LC/MS/MS. Furthermore, we have used ORFfinder to detect over 1,000,000 novels putative sORFs located in the intergenic regions (IRs) of the reference G3 genome. We will examine the expression of these unannotated sORFs by integrating RNA-seq and Nanopore long-read sequencing. As a next step, we will focus on microprotein expression under stress conditions such as iron-depleted, low-glucose, drug-treated conditions, aiming to uncover the potential roles of stress-specific microproteins in *T. vaginalis* pathogenicity.

Keywords: *T. vaginalis*, Microprotein, sORFs, RNA-seq, Stress condition

[6] *Trichomonas vaginalis* impairs HeLa cell intercellular adhesion, leading to decreased cell density via the MCAM–CREB signaling axis

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Human papillomavirus (HPV) is a major cause of cervical cancer, while Trichomonas vaginalis is the most common non-viral sexually transmitted infection (STI). Co-infection with HPV and *T. vaginalis* may increase the risk of cervical carcinoma. Previously, we found that *T. vaginalis* infection induced HeLa cell detachment, although the underlying mechanisms remain unclear.

To investigate the role of *T. vaginalis* in impairing host cell adhesion, we focused on melanoma cell adhesion molecule (MCAM), a protein involved in cell–cell interactions and cancer progression, and regulated by cAMP signaling. HeLa cells were co-cultured with *T. vaginalis* strain 30236 (MOI = 2). After 24 hours, MCAM levels were downregulated, accompanied by reduced CREB and phosphorylated CREB levels, which rebounded at 30 hours. ELISA confirmed a time-dependent decline in intracellular cAMP. These results indicated that *T. vaginalis* reduced the expression of MCAM in host cells by disrupting the cAMP-CREB signal cascade.

To explore upstream regulation, we profiled PDE4 isozymes, the primary cAMP-degrading enzymes, and ADCY isozymes, the major cAMP-producing enzymes. Specifically, PDE4A and PDE4B were downregulated, whereas PDE4C was significantly upregulated after 24 hours of co-culture. In contrast, ADCY1 and ADCY8 were upregulated, whereas ADCY6 was downregulated; these changes were reversed by treatment with a calcium chelator. Subsequent Fluo-4 staining further confirmed elevated intracellular calcium levels in infected cells. Treatment with a PDE4 inhibitor or calcium chelator restored cAMP–CREB–MCAM signaling and cell adhesion, highlighting the roles of PDE4 and calcium-mediated ADCY regulation in *T. vaginalis*–induced detachment. Notably, co-culture with the highly adherent strain 50167 induced stronger MCAM suppression, whereas conditioned medium from either *T. vaginalis* cultures or *T. vaginalis*–HeLa co-cultures had no effect, demonstrating that this response is contact-dependent.

In conclusion, this study demonstrates that T. vaginalis induces detachment of HeLa cells by altering PDE4 isoform expression and disrupting the cAMP–CREB–MCAM signaling axis, thereby impairing cell–cell adhesion. This contact-dependent effect suggests a mechanism by which *T. vaginalis* influences host cell adhesion. Further investigations are warranted to delineate the precise molecular pathways underlying these interactions.

[7] Integration of AlphaFold Structures with Phenotypic Screening for Target Deconvolution of Antimalarial Hit Compounds

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The spread of artemisinin-resistant Plasmodium falciparum threatens global malaria control and underscores the urgent need for new antimalarials with novel mechanisms of action. We screened 36,160 compounds from the Nagoya Chemical Library in a phenotypic assay, prioritizing 441 with sub-6.5 μM efficacy. These compounds were further evaluated by virtual screening against AlphaFold-predicted P. falciparum protein structures, and docking affinities were compared with in vitro growth inhibition data from parasite strains carrying mutations in corresponding target genes. A subset of compounds showed strong concordance between docking scores (≤ –7 kcal/mol) and inhibition profiles, most notably targeting PfPI4K and PfATP4 as potential molecular targets. Several compounds also displayed broad binding promiscuity, emphasizing the importance of downstream biochemical and genetic validation. This study reports novel antiplasmodial activity for a prioritized set of compounds and demonstrates the utility of integrating AlphaFold structural models with phenotypic screening for early-stage target deconvolution. Such an approach offers a practical framework to accelerate the discovery of next-generation antimalarial therapeutics.

[8] The molecular effect of recombinant *Toxocara canis* antimicrobial peptides in wound healing

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Staphylococcus aureus and Methicillin-resistant *Staphylococcus aureus* (MRSA) are common opportunistic and antibiotic-resistant pathogens that pose serious threats to human health. Given the low yield of natural antimicrobial peptides (AMPs) and the high cost of chemical synthesis, recombinant DNA technology provides a promising approach for producing recombinant antimicrobial peptides (rAMPs). In this study, the recombinant *Toxocara canis* AMPs (rTcAMPs) were evaluated for their antibacterial and wound-healing potential.

In the *In vitro* study, three rTcAMPs (No. 9, 10, 11) exhibited potent antibacterial activity against *S. aureus* and MRSA without cytotoxicity toward human keratinocytes or fibroblasts cells. Notably, No. 10 rTcAMP significantly increased collagen I secretion by fibroblasts (154%) and accelerated wound closure in scratch assays. Balb/c mouse excisional wound model demonstrated that rTcAMP treatment enhanced wound closure rates, with histological analysis showing renewed epidermis, hair follicle formation, and abundant collagen deposition in the No. 10 and No. 10+11 treatment groups. Both α-SMA and collagen I expression were markedly upregulated, confirming rTcAMP-mediated wound healing.

Taken together, both *In vitro* and *In vivo* findings indicate that rTcAMPs possess dual functions, with antibacterial activity and promotion of wound repair. These results highlight their strong potential as novel therapeutic candidates for the treatment of *S. aureus*-infected skin wounds.

About APCPZ

The official name of the Asian-Pacific Congress for Parasitic Zoonoses (APCPZ) was established in 1990, when the 1st APCPZ was held in Sendai City, Miyagi Prefecture, Japan. The president of this memorial congress was Prof. Tomio Yamaguchi, Emeritus Professor of Hirosaki University. Since then, the APCPZ has been held biennially, alternating between Taiwan and Japan. In 2010, due to the increasing participation of Korean parasitologists, the congress was held for the first time in a third country — Incheon, Korea.

Subsequent congresses were held as follows:
2012 – Kobe, Japan; 2014 – Taipei, Taiwan; 2016 – Sagamihara, Japan; 2018 – Daegu, Korea; 2023 – Taichung, Taiwan.

Now, the 17th APCPZ is being held on October 18–19, 2025, here in Kanazawa, Japan.

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