Programme Booklet

EU-COST-NUS WORKSHOP ON PARASITOLOGY & MICROBIOMES

BLASTOCYSTIS CULTURE & APPLICATIONS

30th May - 1st June 2023

BLASTOCYSTIS UNDER ONE HEALTH

Department of Microbiology and Immunology,
National University of Singapore

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

NUS National University of Singapore | Department of Microbiology and Immunology
Yong Loo Lin School of Medicine

Welcome Message

It gives us great pleasure to welcome our EU-COST colleagues to our inaugural workshop entitled 'Parasitology and Microbiomes: *Blastocystis* Culture and Applications'. The concept of One Health, which highlights the interconnection between humans, animals, and their shared environment, in the emergence of infectious diseases, was soberly illustrated by the COVID-19 pandemic. This principle applies to many organisms, including the ubiquitous gut protist *Blastocystis*. Singapore is proud to be an International Partner Country of the EU-COST initiative '*Blastocystis* under One Health'. One of the major objectives of this project is to foster information sharing on current methodologies, especially in the areas of subtyping, host-*Blastocystis*-microbiome interactions, and *Blastocystis*-omics. Axenic cultures, which are cultures grown in the absence of accompanying bacteria, are critical for advancing our understanding of *Blastocystis*-host interactions and *Blastocystis* molecular and cell biology. The Department of Microbiology and Immunology at the National University of Singapore is delighted to host our EU-COST participants for this training event. We hope to engage and excite you with seminars and laboratory sessions on *Blastocystis* culture techniques, bioimaging, and microbiome-host-parasite interactions. Do also take this opportunity to network and make new friends from Europe and Asia. Finally, we hope you will have some time to visit the beautiful attractions on the sunny island of Singapore!

Kevin SW TAN

A/Prof Kevin SW TAN
Organizing Chairperson

Dr. Anastasios Tsaousis
Co-Organizing Chairperson

Key Details for the Workshop

Event Accommodation

Park Avenue Rochester
31 Rochester Drive Park, Singapore 138637

Getting there via taxi from Changi Airport:
If you are taking a taxi from the airport to Park Avenue Rochester, it will take approximately 45-60 minutes, depending on traffic conditions.

Getting there via MRT from Changi Airport:
From Changi Airport MRT Station (CG2), take the MRT to Tanah Merah MRT Station (EW4) then change to the westbound train to Buona Vista MRT Station (EW21 / CC22). Buona Vista MRT Station (EW21 / CC22) is the interchange station for the East-West Line and Circle Line.

Workshop Venue

National University of Singapore (NUS)
Department of Microbiology and Immunology
5 Science Drive 2, Blk MD4
Singapore 117545

Please refer to the next page on how to get to the workshop venue in National University of Singapore, Department of Microbiology and Immunology (Blk MD4). Talks will be conducted in the morning at MD4, Level 2 while the practical sessions will be conducted in the afternoon at level 4.

Gala Dinner on the 31 May 2023

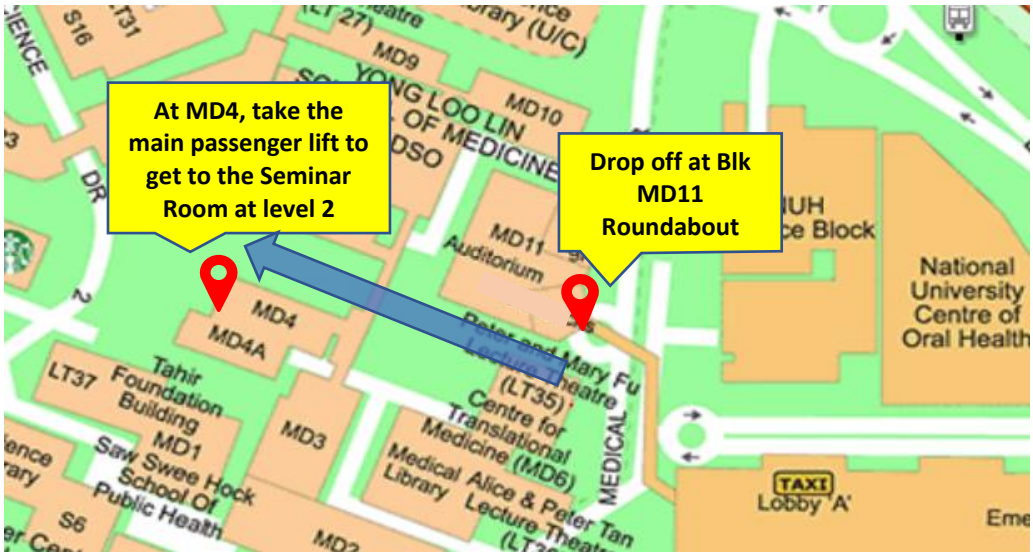
We have arranged a gala dinner for the workshop's speakers and participants at the Asian Market Café at Fairmont Singapore. Asian Market Café is a halal-certified buffet restaurant.

A chartered bus will depart NUS at 6.30pm for the gala dinner. For participants staying at Park Avenue Rochester Hotel, we have also arranged a chartered bus to send you back from Fairmont Singapore after the gala dinner.

Getting to the Department of Microbiology & Immunology:

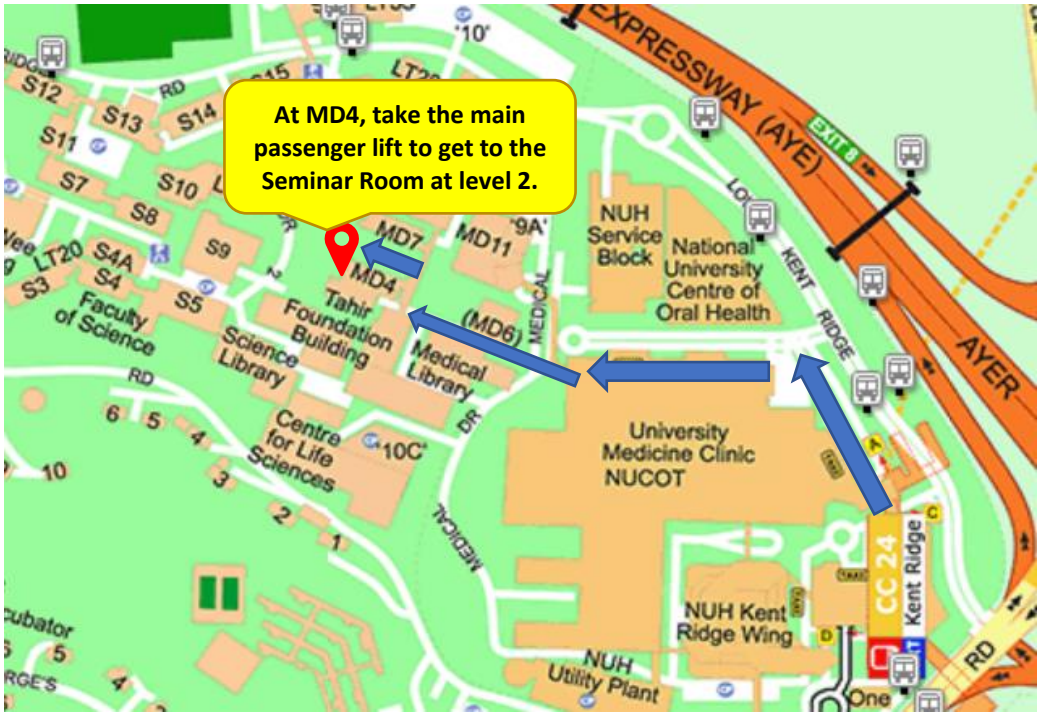
For participants staying in Park Avenue Rochester, there will be a free shuttle bus service from the hotel lobby every morning at 8.30am (for the 3days workshop).

The drop-off drop in National University of Singapore (NUS) is at the Block MD11, roundabout. Please see the direction below to walk from the drop off point to Block MD4.



For participants staying in other hotels, please find below the map and video on getting to Block MD4 from Kent Ridge MRT station.

If you are taking a cab / taxi from your hotel to the workshop venue (in NUS, Blk MD4). You can inform the driver to send you to Block MD11 (10 Medical Drive, Singapore 117597), roundabout. Please refer to the map above for the walking direction to MD4.



(how to walk to MD4 from KR) https://youtu.be/LOe3pyDP4ts

Program Plan

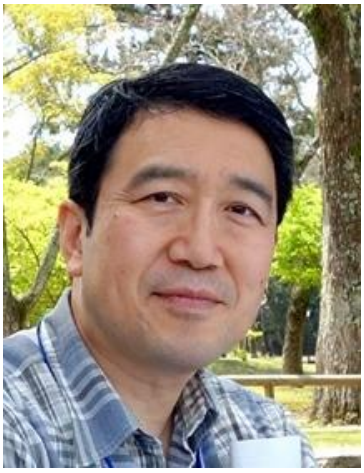
30 May – 1 June 2023

Day 1 Tuesday 30th May Culture and Phenotyping		
Time	Topic	Speaker
9:00 – 9:10	Welcome message by Organizing Chair	Kevin TAN
9:10 – 9:40	Keynote Lecture: *Blastocystis* History and Culture	Hisao YOSHIKAWA
9:40 – 10:10	Panel Discussion: *Blastocystis* Culture - Challenges and Opportunities	Funda DOĞRUMAN AL Kevin TAN Anastasios TSAOUSIS
10:10 – 10:30	Coffee Break	
10:30 – 10:45	*Blastocystis* Microscopy and Phenotyping	Kevin TAN
10:45 – 11:00	*Blastocystis* Genetic Modification	Mark Van Der GIEZEN
11:00 – 11:45	Panel Discussion and Quiz	Mark Van Der GIEZEN Kevin TAN Anastasios TSAOUSIS
11:45 – 12:00	Citation and Award Presentation to Hisao Yoshikawa	Funda DOĞRUMAN AL Kevin TAN
12:00 – 13:30	Talk by Sponsors + Lunch	
13:30 – 13:45	Practical Briefing	Kevin TAN
13:45 – 18:00	P1: Culture • Liquid culture and subculturing • Cryopreservation and thawing • Microscopy of ST1, 4, 7	Geok Choo NG Kevin TAN John YASON
15:45 – 16:00	Coffee Break	
	P2: High-Content Phenotyping • FDA/ PI staining • Amnis demonstration	Trang CHU Kevin TAN John YASON
18:00	End of Day 1	
18:00 – 18:30	Debrief for Instructors	Geok Choo NG Kevin TAN

Day 2 Wednesday 31st May In Vitro and In Vivo Studies		
Time	Topic	Speaker
9:00 – 9:30	Keynote Lecture: *Blastocystis* In Vitro Applications	John YASON
9:30 – 9:45	*Blastocystis* EV Studies	Steven LEONARDI
9:45 – 10:00	*Blastocystis*-Microbiome Interactions #1	Funda DOĞRUMAN AL
10:00 – 10:20	Coffee Break	
10:20 – 10:40	*Blastocystis* In Vivo Applications: Pathology	Chin Wen PNG
10:40 – 10:55	Oral Presentation: Investigation of the effect of *Allium tuncelianum* extract on *Blastocystis* in experimentally infected rats by quantitative real-time PCR	Mehmet AYKUR
11:55 – 11:10	Oral Presentation: Using microfluidic techniques to better understand *Blastocystis*-host microbiome interactions	Daisy SHAW
11:10 – 11:30	*Blastocystis* In Vivo Applications: Immunology	Lukasz WOJCIECH
11:30 – 12:00	Panel Discussion and Quiz	
12:00 – 13:30	Lunch + Poster Session	
13:30 – 13:45	Practical Briefing	Kevin TAN
13:45 – 18:00	P3: In Vitro Studies • Parasite-Microbiota Interactions • Parasite-Host Interactions	Steven LEONARDI Geok Choo NG Kevin TAN John YASON
15:45 – 16:00	Coffee Break	
	P4: In Vivo Studies • Histopathology • Immune Profiling	Chin Wen PNG Lukasz WOJCIECH
18:00	End of Day 2	
18:00 – 18:30	Debrief for Instructors	Geok Choo NG Kevin TAN
18:30	Depart for Gala Dinner	

Day 3 Thursday 1st June Microbiome, Clinical Aspects, Future Directions		
Time	Topic	Speaker
9:00 – 9:30	Keynote Lecture – Microbiomes: From Bench to Bedside	Jonathan LEE
9:30 – 9:45	*Blastocystis*-Microbiome Interactions #2	Kevin TAN
9:45 – 10:00	Oral Presentation: The ecological roles of SCE in the human gut flora	Raul Y. TITO-TADEO
10:00 – 10:20	Coffee Break	
10:20 – 10:50	Microbiome-Brain Axis	Sven PETTERSSON
10:50 – 11:05	Oral Presentation: Occurrence of *Blastocystis* in school children living in Kosovo	Karolina BARANOWICZ
11:05 – 11:20	Oral Presentation: Can *Blastocystis* sp. and potentially pro-carcinogenic bacteria exacerbate colon tumorigenesis in human?	Carolina HERNANDEZ-CASTRO
11:20 – 11:40	Bioimaging Platforms for *Blastocystis* Research	Benoit MALLERET
11:40 – 12:10	Panel Discussion and Quiz	
12:10 – 13:30	Lunch	
13:30 – 13:45	Practical Briefing	Kevin TAN
13:45 – 18:00	P5: Culture – Follow Up • Liquid culture and subculturing • Cryopreservation and thawing • Fluorescence staining	Trang CHU Geok Choo NG Kevin TAN John YASON
15:45 – 16:00	Coffee Break	
	P6: In Vitro Studies – Follow Up • Parasite-Microbiota Interactions	Steven LEONARDI Geok Choo NG Kevin TAN John YASON
18:00 – 18:30	Closing Remarks	Kevin TAN Anastasios TSAOUSIS
18:30	End of Day 3	

Speakers' Profile

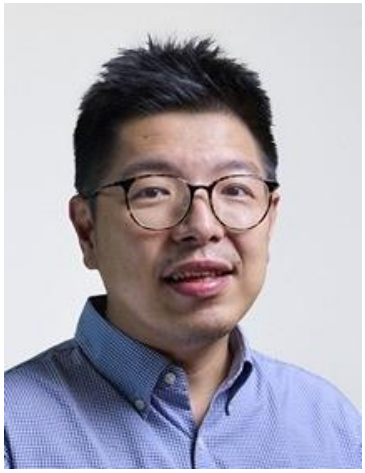


Keynote Speaker – Associate Professor Hisao YOSHIKAWA

Hisao Yoshikawa was born in Osaka. He obtained a bachelor's and master's degree in Biology at Konan University, Kobe and then moved to pursue a Ph.D. in Medical Zoology, equivalent to Parasitology, in Kyoto Prefectural University of Medicine, where he worked on *Pneumocystis carinii*, focusing on ultrastructure of this unclassified microbe at that time. He was skillful in freeze-fracture and immune-electron microscopy, and his expertise contributed to the development of Hitachi's freeze-fracture apparatus FR-7000. After he moved to Nara Women's University, he focused on *Blastocystis* research, especially on morphology, epidemiology, and molecular phylogeny. While there, he temporarily moved to Dr. Don Graves' laboratory, University of Oklahoma, for a collaborative research project on *Pneumocystis carinii* under the NIH grant for one year. He has published about 90 mostly peer-reviewed articles and four book chapters including as editor. He had retired at the end of March this year and moved to Kanazawa University as a Collaborative Researcher, Department of Global Infectious Diseases, Graduate School of Medical Sciences, Kanazawa University, JAPAN.

Keynote Speaker – Dr. John Anthony YASON

Dr. John Anthony YASON finished his PhD from Yong Loo Lin School of Medicine, National University of Singapore. He was also a Postdoctoral Research Fellow at the Laboratory of Molecular and Cellular Parasitology, Department of Microbiology and Immunology in the same University under the supervision of Assoc. Professor Kevin SW TAN. He focused his research on *Blastocystis* and later on studied its interactions with host cells and gut microbiota. He has previously worked on protistan parasites and currently doing epidemiological surveys on pathogens linked to neglected tropical diseases. He was also a consultant for COVID-19 Diagnostic Lab in the Philippines. He is currently the Dean of the Institute of Health Sciences and Nursing and teaches Advanced Microbiology and Research Methods courses at Far Eastern University in Manila, the Philippines.

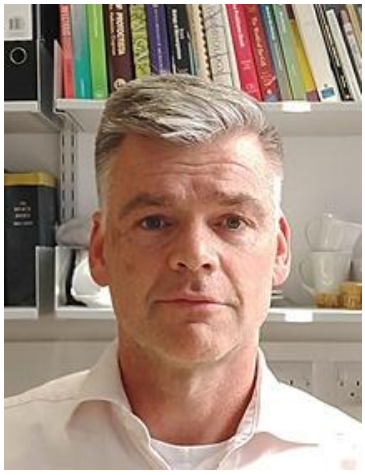


Keynote Speaker – Dr. Jonathan LEE Wei Jie

Dr Jonathan Lee is an early-career clinician scientist at National University of Singapore (NUS) Medicine, a principal investigator at iHealthTech, with a special interest in the field of Microbiome Medicine, as well as consultant Gastroenterology at National University Hospital (NUH), Singapore. He completed his Master of Clinical Investigation, funded by the NMRC-MOH healthcare award to study the mucosal microbiome profiles in patients with non-alcoholic fatty liver disease. He was then awarded the NRMC research fellowship from 2019-2021 to study host-microbiota interactions in gastrointestinal diseases at the Broad Institute MIT-Harvard, under Professor Ramnik Xavier. Dr Lee also currently leads the NUH fecal microbiome transplant programme, and have multiple ongoing studies collecting fecal microbial samples from both healthy patients and patients with gastrointestinal diseases.

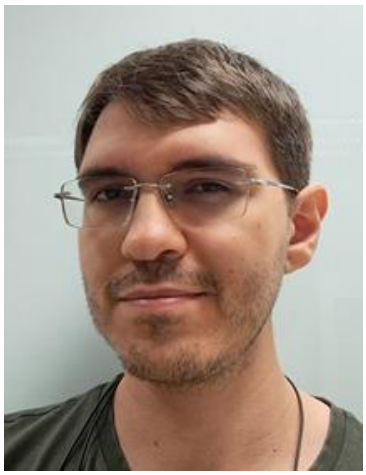
Invited Speaker - Professor Funda DOĞRUMAN AL

Prof. Funda Dogruman-Al (MD, Prof) is a specialist in clinical microbiology in the Department of Medical Microbiology of Medical Faculty, Gazi University, Türkiye (TR). Her scientific interest topics are intestinal protists, especially *Blastocystis*. She has been in Hisao Yoshikawa's laboratory (Nara Women's University, Japan) in 2007 and this experience improved greatly to her studies on *Blastocystis*. Dogruman-Al organized the 1st International *Blastocystis* Symposium in 2015 which provided together experienced scientists and young researchers in this field, and she takes part in the continuation of these events. She is interested in the prevalence and subtypes determination of *Blastocystis* in clinical specimens and relationship symptomatology, and the interaction of *Blastocystis* and the intestinal microbiome. She is also a management member of the COST 21105 OneHealthBlastocystis project and leads the "Mapping *Blastocystis* epidemiology and diagnostics" working group.



Invited Speaker - Professor Mark van der GIEZEN

Prof van der Giezen's research focuses mainly on intestinal microbiology and mitochondrial adaptations to anoxia as found in the animal gut. The topic of his PhD at the University of Groningen (the Netherlands) and his subsequent postdoc at the Natural History Museum (London, UK) was hydrogenosomes (unusual mitochondria) from anaerobic fungi. During his postdoc at Royal Holloway, University of London (UK), where he was involved in the discovery of mitosomes in *Giardia*, the topic shifted to human gut parasites (*Entamoeba* and *Giardia*). During his lectureship (~Assistant Professor) at Queen Mary, University of London (UK), he first worked with *Blastocystis* when he started a collaboration with Graham Clark. In 2007, he moved to the University of Exeter (UK) as a Senior Lecturer and became Associate Professor of Evolutionary Biochemistry. At Exeter, his work shifted partly to animal parasitology and the role of the microbiome in animal health. Since summer 2019, he is the Professor of Biological Chemistry at the University of Stavanger in Norway. Here he continues his research on important parasites for humans and animals and have established close research links with the academic hospital in Stavanger.

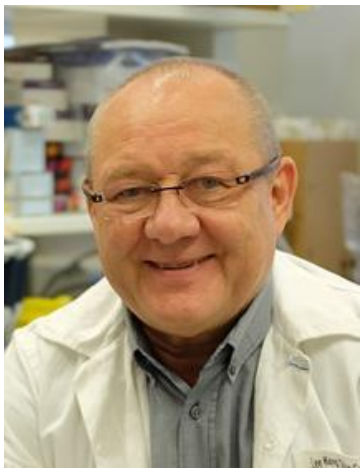


Invited Speaker - Mr. Steven Santino LEONARDI

Mr. Steven Santino LEONARDI is a PhD candidate at the Laboratory of Molecular and Cellular Parasitology (Yong Loo Lin School of Medicine, NUS) under the supervision of Assoc. Prof. Kevin SW Tan. He received his Honour's degree in Biomedical Science from the University of Queensland, Australia, and has interned with A*STAR's Epithelial Biology Lab multiple times under Prof. Birgit Lane. He is experienced in tissue culture, and has previously worked on the STMND1 protein, the p75NTR/TrkA interaction, and Epidermolysis Bullosa Simplex drug screening in an in vitro setting. He is currently investigating the effect of *Blastocystis*'s tryptophan-producing BhTnaA enzyme on downstream neuromodulatory metabolite synthesis by host cells in the gut and liver.

Invited Speaker - Dr. Benoit MALLERET

Dr. Benoit received his PhD in Immunology at the Alternative Energies and Atomic Energy Commission (CEA) in Paris, France. During his PhD, he worked on non-human primate models, and studied the innate response of macaques to Simian Immunodeficiency Virus (SIV) infection. He subsequently moved from Paris to Singapore to take up a post-doctoral research position to study malaria parasites and host cell tropism at the Singapore Immunology Network (SIgN), A*STAR. In 2018, Benoit became an Assistant Professor in the Department of Microbiology and Immunology and his lab focuses on erythrocytic immunobiology and host-microbiome interactions in the gut. He has published over 80 articles with over 5,000 citations. He was awarded the 2020 Yong Loo Lin School of Medicine Young Researcher of the Year Award. He is also Director of Electron Microscopy Unit in NUS Yong Loo Lin School of Medicine and Principal Investigator in SIgN, A*STAR.



Invited Speaker - Professor Sven PETTERSSON

Professor Pettersson, MD. PhD and internationally renowned investigator with a string of high-profile publications in the area of gut microbe body function including how gut microbes and their metabolites regulate brain development and function. Currently, he is appointed at the National Neuroscience Institute (NNI) as Senior PI and affiliated as Professor at NUS. Since 2015 he is elected as a member of the Canadian Institute for Advanced Research (CIFAR) with a focus on the human microbiome and human health. In 2020, the Jeffrey Cheah foundation, Malaysia awarded him a grant to establish an ASEAN Microbiome Nutrition Centre (AMNC) jointly between NNI and Sunway University Malaysia. The focus of the Centre is to study gut-brain communication relevant to neurodegenerative diseases including the role of gut microbes, ageing and Parkinson's disease.

Invited Speaker - Dr. Chin Wen PNG

Dr Png Chin Wen received his PhD in the field of Medicine from the University of Queensland (Australia) and has since focused his research on gut mucosal inflammation, gastrointestinal cancers, and the gut microbiome. Currently, he is a lecturer at NUS and has continued his research to understand the signalling mechanisms involved in colorectal cancer pathogenesis, with a particular emphasis on a group of molecules known as dual specificity phosphatases (DUSPs). At the same time, he has ongoing collaborations with clinicians to investigate the changes in gut microbiome and explore the functional role of gut bacteria that are crucial in disease development.

Invited Speaker – Associate Professor Kevin TAN

Kevin SW Tan is Associate Professor at and Head of Department of Microbiology and Immunology, National University of Singapore. He is also Vice-Dean, Graduate Studies, at the Yong Loo Lin School of Medicine and Head, Innovation in Graduate Studies, at the National University Health System. His curiosity for parasites originated from his graduate student days at NUS and blossomed during his postdoctoral stint at The Rockefeller University, New York City. He is relieved to be awarded tenure in 2011, and can now spend more time on social issues, such as public science education. Kevin's research focuses on understanding how parasites commit suicide and exploiting such knowledge to trigger death mechanisms as an anti-parasite strategy. He is also interested in the problem of drug resistance and his team has developed new ways to find drugs that overcome resistance. More recently, his team has embarked on projects focusing on the role of single cell eukaryotes (SCEs) in the host microbiome. He hopes that the research from his team would accelerate the finding of new cures for parasitic diseases.

Invited Speaker - Dr. Anastasios TSAOUSIS

Dr. Anastasios (Tasos) Tsaousis is the Principal Investigator of the Laboratory of Molecular and Evolutionary Parasitology at the University of Kent. The current research of his laboratory is focused on the investigations of the adaptations of microbial eukaryotic organisms (e.g. *Blastocystis, Cryptosporidium, Naegleria*, Gregarines, ciliates), and their course in parasitic evolution and diversity. To accomplish this, his laboratory is combining detailed bioinformatics analyses of newly generated genomic/transcriptomic/metabolomic results with field, cell biological and biochemical methods to investigate the parasitic and free-living microbial eukaryotes living in diverse and extreme environments. He has numerous publications on the epidemiology, cell biology and biochemical adaptations of *Blastocystis*. He is also the Action Chair of the *Blastocystis* Under One Health EU-COST Action.

Invited Speaker - Dr. Lukasz WOJCIECH

Prior to joining National University of Singapore (NUS), Dr. Lukasz Wojciech completed his PhD in Wroclaw University of Environmental and Life Sciences, Poland in the fields of molecular oncology and postdoctoral training in the United States, where he conducted research in the field of immunology. Currently, Dr. Lukasz Wojciech is a senior research fellow at the National University of Singapore (NUS), where he conducts research on the gut microbiome and its interaction with the host adaptive immune compartment. Specifically, his current work is centered on investigating microbiome-derived metabolites and their role in peripheral T cell polarization in the context of inflammatory gut disorders.

Abstracts – Keynote Speakers

Blastocystis History and Culture

Hisao Yoshikawa

Affiliations
Department of Global Infectious Diseases, Graduate School of Medical Sciences, Kanazawa University, JAPAN

Abstract
Since the genus *Blastocystis* was originally described by Alexieff in 1911 and by Brumpt in 1912 from human stool samples, respectively, similar organisms have been isolated from a variety of animals from mammals to insects. Although, *Blastocystis* had originally reported as a harmless intestinal yeast, this organism was proposed as a protozoan, based on growth and morphological characteristics in protozoan medium, rather than fungal and bacteriological media by Zierdt et al. The taxonomic position of the genus *Blastocystis* was subsequently determined to reside within the heterogenous group of the Stramenopiles, based on SSU rRNA gene phylogeny by Silberman et al. Although the Stramenopile was named from the combination of "Straws" and "Piles" by Patterson, *Blastocystis* lacks the tubular hairs among the Stramenopiles.

The original culture medium and condition for *Blastocystis* growth was the whole egg-slant medium, kept under anaerobic conditions. Although this medium could support growth of *Blastocystis* organisms, many bacterial flora contained in the original stool samples also rapidly grew. Unfortunately, the rapid growth of the bacteria inhibited the proliferation of *Blastocystis* in cultures. Therefore, several other traditional media were also applied for isolation of *Blastocystis* organisms from stool samples. In this lecture, I will talk about the brief history of *Blastocystis* and our applications of laboratory and field cultures for epidemiological research.

Blastocystis Interaction Studies: In Vitro Applications

John Anthony Yason

Affiliations
Institute of Health Sciences and Nursing, Far Eastern University, Manila, PHILIPPINES

Abstract
Blastocystis inhabits the intestines where it interacts with host epithelial cells as well as other members of gut microbiota, most of which are prokaryotic. These interactions shed light on whether a particular subtype of *Blastocystis* may be deemed pathogenic or may be regarded as beneficial to the host. The interactions between different members of the gut microbiota along with their effects on host cells are complex and yet need to be clarified to understand what conditions should exist to promote healthier gut. In this talk, I will introduce in vitro studies involving *Blastocystis* isolates of different subtypes, cultivated together with different representatives of gut bacteria and host epithelial cells. These experiments can serve as starting points for broader studies on gut microbiota which may include in vivo investigations. Determining how each of these organisms are affected when they are co-cultured together will also be discussed.

Microbiome: From Bench to Bedside

Jonathan Lee

Affiliations
National University of Singapore, SINGAPORE
National University Hospital, SINGAPORE
iHealthtech, SINGAPORE
Broad Institute MIT-Harvard, SINGAPORE

Abstract
The human microbiome, composed of trillions of microorganisms residing in and on our bodies, has emerged as a vital component in maintaining human health. Recent advancements in research have uncovered the intricate relationships between the microbiome and various gastrointestinal diseases, paving the way for potential therapeutic interventions. This talk provides an overview of the journey from bench to bedside in understanding and harnessing the power of microbiomes, in relation from gut inflammation to gastrointestinal cancer.

At the bench, we will discuss advanced sequencing technologies to unravel the complexity of microbial communities and their functional roles in the context of gut microbiome inflammation cancer. We will highlight distinct microbial signatures associated with different gastrointestinal diseases, including gastric cancer, colon adenomas, inflammatory bowel disease (IBD), and *Clostridium difficile* infections.

Translating these bench findings to the bedside has shown promise in the development of microbiome-targeted therapies for gut-related cancers and inflammatory conditions. Faecal microbiota transplantation (FMT) has demonstrated efficacy in treating recurrent *Clostridium difficile* infections by restoring a healthy gut microbiome. In IBD, efforts are underway to identify microbial biomarkers for predicting disease progression and response to therapy, enabling personalized treatment approaches. Furthermore, investigations into the role of the gut microbiome in colon adenomas have revealed potential links between specific microbial compositions and the development of precancerous lesions. This knowledge may lead to the development of preventive strategies, such as probiotics or dietary interventions, to modulate the gut microbiome and reduce the risk of colon adenoma progression.

In conclusion, the exploration of gut microbiome, inflammation and cancer from bench to bedside has provided valuable insights into the complex interplay between microbial communities and disease states. By leveraging this knowledge, microbiome-based interventions hold the potential to revolutionize the management of gastrointestinal conditions. Continued research and clinical trials are needed to optimize the safety, efficacy, and personalized nature of these interventions, ultimately improving patient outcomes and shaping the future of precision healthcare.

Abstracts – Invited Speakers

Blastocystis Microscopy and Phenotyping: Seeing the Whole Elephant

Kevin SW Tan

Affiliations
Laboratory of Molecular and Cellular Parasitology, Department of Microbiology and Immunology, Healthy Longevity Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, SINGAPORE

Abstract
Blastocystis is a protistan parasite that colonizes the large intestines of approximately 1-2 billion people globally, making it the most commonly detected gut protist in prevalence studies. The protist is reported to exist in four morphological forms: vacuolar, granular, amoeboid, and cystic. In this short talk, I will highlight the various imaging tools used to characterize *Blastocystis* morphology. Beginning with the humble light microscope, we will observe the gross morphological features that define each form. Next, we will explore how early electron microscopy studies have shed light on *Blastocystis* ultrastructure, including characteristics of unusual organelles. Lastly, the use of a modern high-content imaging tool (Amnis ImageStream) and its power to characterize *Blastocystis* sub-populations, and address a controversy, will be highlighted.

Blastocystis Genetic Modification

MitchellRey Toleco (1), Kevin SW Tan (2), and Mark van der Giezen*(1)

Affiliations
(1) Department of Chemistry, Bioscience, and Environmental Engineering, University of Stavanger, Stavanger, NORWAY.
(2) Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, SINGAPORE

Abstract
Blastocystis is a common single-celled organism that inhabits the human intestinal tract and may be associated with the development of gastrointestinal diseases. Genetic transformation of *Blastocystis* is a relatively new technique in which foreign DNA is introduced into the organism's genome to alter its genetic make-up and study its biological functions.

The transformation of *Blastocystis* has the potential to improve our understanding of its possible pathogenesis and virulence mechanisms. However, genetic manipulation of *Blastocystis* is a difficult process and the development of an efficient transformation protocol has been a major obstacle in this field.

Only one study reports transient transformation of *Blastocystis* using electroporation and a sensitive nano-luciferase (Nluc) reporter system. However, this method is of limited use due to the transient nature of the transfection and the lack of suitable genetic markers for selection. We are currently building on the success of this transient transfection protocol and hope to transform it into a stable genetic transformation method to study the biology of *Blastocystis*.

RNA interference and CRISPR/Cas9 technology has revolutionised the field of genetic transformation, but unfortunately has not yet been established for *Blastocystis*. CRISPR/Cas9 would allow precise

genome editing and has the potential to overcome many of the limitations of traditional transformation methods.

In summary, genetic transformation of *Blastocystis* is still in its infancy but would be promising in improving our understanding of the biology and pathogenesis of this organism.

Extracellular Vesicles: A Budding Branch of *Blastocystis* Research

Steven Santino Leonardi*(1), and Kevin SW Tan (1)(2)

Affiliations
(1) Laboratory of Molecular and Cellular Parasitology, Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, SINGAPORE
(2) Healthy Longevity Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, SINGAPORE
* Presenter

Abstract
Blastocystis is currently relatively poorly understood, with the mechanisms behind its observed effects on other organisms unknown. While suspected for some time, extracellular vesicle synthesis by the parasite was confirmed in a publication by our lab in 2022. Extracellular vesicles (EVs) are a variety of small, lipid-enclosed particles released by cells. They have the ability to store and traffic a range of bioactive molecules, including proteins, nucleic acids, and cytokines. EVs have been implicated in a variety of biological processes, including cell-cell communication, immune response, and disease pathogenesis. In this presentation, I will cover our paper characterising the synthesis of EVs by *Blastocystis*. I will also explore the potential implications of this with regards to host-parasite as well as parasite-microbiome interactions. Further study into *Blastocystis* EV synthesis and cargo may shed light on some of the mysteries surrounding this enigmatic organism.

Blastocystis-Microbiome Interactions

Funda Dogruman Al

Affiliations
Division of Medical Parasitology, Department of Medical Microbiology, Medical Faculty of Gazi University, TÜRKIYE

Abstract
Blastocystis is a globally distributed enteric protist that inhabits the digestive tract of several vertebrates and is the most abundant protist in the human gut. *Blastocystis* has puzzled clinical microbiologists and gastroenterologists for decades. The clinical significance of colonic *Blastocystis* colonisation remains unclear.

The last decade has seen a surge in research into the microbiome and its role in health and disease. The pathogenicity or non-pathogenicity of *Blastocystis* depends on several factors such as the interaction with the gut microbiota, the infecting subtype and the host immune response. Some studies in different settings suggest that *Blastocystis* is part of the normal gut microbiota of humans and other mammals and is able to colonise and persist in the intestinal tract without causing disease. *Blastocystis* is clearly associated with changes in the composition of the microbiota in the human host,

and its colonisation is strongly associated with broad shifts in the gut-dwelling bacterial community and an increase in bacterial diversity.

The behaviour of *Blastocystis* in the host-parasite relationship appears to be that of a beneficial protist in the gut, shaping bacterial population profiles associated with healthy gut microbiota rather than a pathogenic organism.

Blastocystis In Vivo Applications: Pathology

Png Chin Wen

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Abstract
Blastocystis is a protozoan enteric parasite that is commonly found in various vertebrates. Although presence of *Blastocystis* in human may be associated with pathologies such diarrhoea, abdominal pain, constipation, weight loss, and dermatological symptoms, the role of *Blastocystis* in causing diseases is still controversial. The mechanism and extent to which *Blastocystis* infection contributes to the aetiology of gut disorders *in vivo* remain unclear. To better understand the pathogenicity of *Blastocystis*, various animal models have been employed to investigate the significance of *Blastocystis* infection. Recent studies have also shown that the gut microbiome is altered in the presence of *Blastocystis*. This may be an indirect mechanism by which *Blastocystis* influences physiopathology and contributes to disease susceptibility since more than 90% of diseases can be traced back to gut microbiome alteration. For instance, studies have shown that *Blastocystis* and its associated altered gut microbiome can modulate the immune response, which contribute to changes in disease susceptibility. Therefore, understanding the effects of *Blastocystis* on the gut microbiome could provide insights into the pathophysiology of *Blastocystis*-associated diseases. In this talk, we will provide an overview of *Blastocystis*-associated pathology found in human and discuss the applicability of key animal models for characterising the role of *Blastocystis* in disease pathogenesis.

The Modes of *Blastocystis* and Host Immune System Interaction. Why Does This Matter?

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Abstract
Blastocystis is a highly prevalent parasite that can infect the human gut and is the most common among all parasites worldwide. *Blastocystis* infection can either be asymptomatic or present with gastrointestinal and extraintestinal symptoms, and its impact on the host can vary significantly based on the microbiome and the parasite-host interactions. The large intestine is home to an extensive collection of microorganisms that play a crucial and unique role in host physiology. Different *Blastocystis* subtypes (STs) exhibit distinct ways of altering the gut microbiota composition, leading to

unique ST-related reshaping of the metabolomic landscape. The metabolic by-products of the microbiome are essential multimodal transmitters within the host-microbiome interactome, and they contribute to immune homeostasis by fine-tuning the adaptive arm of the immune system. Thus, *Blastocystis*-induced changes in the distribution of microbiome-derived metabolites can significantly impact the balance between specialized regulators and responders within the intestinal immune cell population. It has become apparent that a better understanding of *Blastocystis*-host immune system interactions is critical for advancing our knowledge of gut health and disease. The identification of specific STs associated with a symptomatic or asymptomatic infection and their unique mechanisms of interactions with the host's microbiome and immune system could enable the development of better therapies targeting gut inflammatory diseases.

Recent Advances in *Blastocystis*-Microbiome Interactions

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Abstract
Blastocystis is a protistan parasite that colonizes the large intestines of approximately 1-2 billion people globally, making it the most commonly detected gut protist in prevalence studies. There are increasing reports suggesting that *Blastocystis* may, in reality, be a commensal, or even a beneficial member of the gut microbiome. In this short talk, I will describe several studies from our laboratory that support this idea, but also present recent data to suggest that there may also be subtype and geographical factors that determine *Blastocystis* probiotic or dysbiotic properties.

Gut Microbes, Organ Growth & Function and Biological Ageing

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* Presenter

Abstract:
Biological ageing is associated with organ decline and an increased risk of contracting disease. The speed of organ decline is highly variable between individuals and subject to the regulation of genetic and non-genetic factors, including the indigenous gut microbiota. This revised view of integrating gut microbes and their metabolites into regulating mammalian physiology has dramatically changed our perception of human biology. Following the formation of a new offspring, with its highly specialized organs, an interorgan crosstalk and metabolic homeostasis tuned by the incoming gut microbes and diet is established. It follows, that any age-related change in organ function or interorgan communication, will be sensed by gut microbiota, which reciprocates by alteration in composition,

richness, and secretion of microbe-secreted molecules. The underlying mechanisms by which organ function communicates with gut microbes and how gut microbiota reciprocates to age-related organ decline in organ function is largely unknown. This short presentation will present results showing that gut microbiota and their metabolites can impact body growth and behaviour, especially metabolic organs and the brain. The presentation will also showcase our new ASEAN Microbiome Nutrition Centre (AMNC).

Insights into *Blastocystis* Morphology and Interactions with Host and Gut Microbiome through Bioimaging Platforms

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* Presenter

Abstract
Blastocystis spp. is a widely distributed enteric protist that infects humans and various non-human animals, posing a challenge to establishing a diagnostic method due to limited knowledge of its life cycle. Bioimaging platforms offer promising approaches to gain insights into *Blastocystis* morphology and interactions with the host and gut microbiome. Holotomography imaging enables visualization of internal organelles and cell body structures of the parasite. Scanning Electron Microscopy (SEM) can be used to examine the interaction between the parasite and the gut microbiome in the luminal part of the gut. Additionally, Serial Block Face (SBF) protocol and Transmission Electron Microscopy (TEM) provide high-resolution views of host-parasite interactions in situ. Volume Electron Microscopy (vEM) with array tomography or SBF-SEM allow tri-dimensional reconstruction of these interactions in high resolution.

In summary, bioimaging platforms, including holotomography imaging, SEM, SBF-SEM, and vEM offer powerful tools to investigate *Blastocystis* spp. morphology, behaviour, and interaction with the host and gut microbiome. By integrating bioimaging with multi-omics approaches, a comprehensive understanding of *Blastocystis* spp. biology can be obtained, which can aid in the development of reliable diagnostic method and targeted therapeutics.

Abstracts – Oral Presentation

Investigation of the Effect of *Allium tuncelianum* Extract on *Blastocystis* in Experimentally Infected Rats by Quantitative Real-Time PCR

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* Presenter

Abstract
Blastocystis is a parasite that inhabits the gastrointestinal tract and is commonly found in humans and animals all over the world. It is present in humans both asymptomatically and symptomatically. It commonly causes acute and chronic diarrhoea, gas, abdominal pain, vomiting, and other gastrointestinal symptoms. Metronidazole (MTZ) is recommended as the first drug of choice in treatment. Recently, studies have reported that resistance to MTZ has developed. In addition, this drug has adverse effects including dizziness, headaches, and disrupted intestinal microbiota. Therefore, people prefer treatment options with natural herbal products instead. The project aims to investigate the effect of *A. tuncelianum* extract, a natural plant, on *Blastocystis*-infected animals *in vivo* using microscopic and molecular methods. In the study, six groups were formed: G1 (Control), G2 (*Blastocystis* infected), G3a (Infected + 50 mg/kg/day, *A. tuncelianum* extract), G3b (Infected + 150 mg/kg/day, *A. tuncelianum* extract), G3c (Infected + 250 mg/kg/day, *A. tuncelianum* extract) and G4 (Infected + 10 mg/kg/day, Metronidazole). First, the results of the microscopic parasitological examination were obtained. On day three post-infection, faecal examination of infected rats showed an early reduction in faecal parasite count of 61.9% and 42.9% in the G3c group and G4 group, respectively. While the G3c group had 100% stool parasite reduction on the 12th day, the G4 group had an 84.1% effect on the 15th. In the G3b group, 88.1% of faecal parasites were reduced on the 12th day, and 100% intestinal parasite clearance was achieved on the 15th. Treatments in the G3b and G3c groups (150 mg and 250 mg/kg/day, *A. tuncelianum* extract) provided a better effect than the MTZ treatment in the G4 group. In conclusion, *A. tuncelianum* extract was effective in the earlier period, with a 100% reduction in the stool.

Using Microfluidic Techniques to Better Understand *Blastocystis*-Host Microbiome Interactions

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* Presenter

Abstract
Blastocystis is an anaerobic microbial eukaryote that colonises the gut of humans and a range of other animals. At present, *Blastocystis* can be cultured both xenically and axenically in vitro, with bacteria in xenic culture removing the need to provide anaerobic conditions for this microbe. It is not currently known if certain subtypes of *Blastocystis* maintained in xenic culture are able to be cultured axenically, or whether the presence of bacterially secreted metabolites are necessary for its growth. At present, the in vitro culture of *Blastocystis* is not able to fully recapitulate the environment experienced in vivo, making it difficult to understand its role in the eubiosis of the gut microbiome that it is usually associated with. For example, does it thrive in eubiotic conditions, or is *Blastocystis* itself affecting the diversity of the microbiota? The use of microfluidics has potential as an in vitro tool to enable long-term culture of microbes with other cells (host or other microbes). As such, my aim is to develop a device that will allow investigation and monitor *Blastocystis* behaviour and interactions with the host and gut microbiome in vitro.

The Ecological Roles of SCE in the Human Gut Flora

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* Presenter

Abstract
In recent years, human microbiome research has experienced significant growth, with an emphasis on clinical applications. To maintain appropriate ecological context for biological phenomena, a multidomain approach to study the role of microorganisms in the gut microbiome is crucial. While bacteria and archaea have been the primary focus of microbiome research, the potential impact of single cell eukaryotes (SCEs) is gaining increasing attention. This project aims to characterize the ecological niche of SCEs in the gut microbiome and examine their potential impact on human health. To this end, I will study stool samples, with extensive metadata, representing human lifeways from four continents and will test SCEs impact using in vitro modelling. Through investigation of the interactions between microorganisms and their effects on human health, this study stands to advance our understanding of the gut microbiome and may lead to potential new strategies for diagnostics and therapeutics for a range of human diseases.

Occurrence of *Blastocystis* in School Children Living in Kosovo

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* Presenter

Abstract
Background and Aims: The role of *Blastocystis* as a commensal or parasitic factor has not been clearly defined. Scientific reports link the variable pathogenicity of *Blastocystis* with the presence of as many as 28 protozoan subtypes, showing considerable variability in the gene encoding SSU-r RNA. The incidence of *Blastocystis* in European countries is estimated at 15-25% and up to 50% of the population in developing countries. So far, there are no data on prevalence of *Blastocystis* among Balkan countries. Therefore, the aim of this study was to estimate the degree of the occurrence of *Blastocystis* among children living in Kosovo and to determine its genetic diversity.

Methods: In total, 478 samples fixed in 70% ethyl alcohol were collected from children aged 6-15 from Kaçanik, Kosovo 2018. DNA isolation was performed using A&A Biotechnology's Genomic Mini AX Stool kit. The obtained DNA isolates were then analysed for the presence of the specific SSU-rRNA *Blastocystis* subunit by PCR amplification using primers RD5StenF and BhRDr. Products of amplification were sequenced in order to estimate genotypes of the parasite.

Results: Out of 478 tested trials, 126 (26.4%) positive results were obtained. PCR reaction products from 48 positive samples were sequenced and it was found that the isolated *Blastocystis* show genetic diversity. The most common subtype was *Blastocystis* ST3 (22 trials, 45.7%), followed by *Blastocystis* ST1 (15 trials, 31.3%). The remaining samples tested were classified as *Blastocystis* ST2 (11 trials, 22.9%).

Conclusions: Molecular studies confirmed high level of contamination of investigated children with *Blastocystis* that corresponds most probably with low economic status of Kosovo and poor sanitary conditions.

Can *Blastocystis* sp. and Potentially Pro-Carcinogenic Bacteria Exacerbate Colon Tumorigenesis in Humans?

Carolina Hernández-Castro*(1)(2), Miguel Ángel Toro Londoño (2), Sonia del Pilar Agudelo López (2), Jorge Humberto Botero Garcés (2), Alonso Martínez (2), Maria Victoria Parra Marín (3), Winston Rojas Montoya (2), Rodrigo Castaño Llano (2)(4)(5), Alejandro Munera Duque (2)(6)(7), Juan Camilo Correa Cote (7), Santiago Rojas Restrepo (5)(8), Sergio Sánchez Prieto (1), and David Carmena (1)

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* Presenter

Abstract
Background and Aims: Colorectal cancer (CRC) represents the second leading cause of cancer-related deaths worldwide. A number of enteric bacteria and parasites have been proposed to induce or exacerbate colon tumorigenesis in humans. These include common gut colonizers including the Stramenopile *Blastocystis* sp. and the recently CRC-associated enteric bacteria *Morganella morganii*. This study aims at investigating potential associations among *Blastocystis* sp. and other enteric microorganisms and a higher risk of developing CRC taking into consideration ancestry genetic background and other predisposing factors.

Methods: Stool samples from CRC (*n* = 134) and non-CRC (*n* = 185) patients undergoing colonoscopy screening were collected in five hospital settings in Medellín, Colombia. The presence of parasites and bacteria was investigated by conventional (microscopic examination and culture) and molecular (PCR and Sanger sequencing) techniques. Blood DNA samples were also obtained to investigate ancestry-informative markers (AIM).

Results: Among parasites, *Blastocystis* sp. was the most prevalent species found both in CCR (34.3%, 46/134) and in non-CRC (33.5%, 62/185) patients. Sequence analyses revealed the presence of ST3 (50.0%, 54/108), ST1 (25.0%, 27/108), ST2 (19.5%, 21/108) and ST4 (2.8%, 3/108). No differences in *Blastocystis* prevalence and ST distribution were observed between CCR and non-CCR patients. *Morganella morganii* was more frequently found in CRC patients (7.5%, 10/134) than in non-CRC patients (1.1%, 2/185) (*P*-value 0.0031). AIM and risk factor analyses are currently ongoing.

Conclusions: Regardless of its disputable pathogenicity, no differences in *Blastocystis* sp. occurrence were observed between CRC and non-CRC patients, as opposed to that seen in *M. morganii*.

Abstracts – Poster Presentation

In Vitro Study of *Blastocystis* sp. Detection by Using Small Capped Tube Cultivation and Vital Staining

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* Presenter

Abstract
Blastocystis sp. is an intestinal parasite that has gained social attention in recent years due to its high prevalence in animals and humans. This study aims to detect *Blastocystis* sp. using a small capped tube cultivation and rapid vital staining in medical laboratory. This important potential evidence can be associated with gastrointestinal symptoms and diarrhoea. The idea has become an interest for developing more effective methods for detecting *Blastocystis* in clinical samples. In vitro study has showed a detection of *Blastocystis* infection among healthy individuals. Also, it is an important method that can help research into proteomics and pathogenicity in the future, as well as lead to more sensitive and specific diagnostic tests. Stool samples culture positive for *Blastocystis* sp. were aliquoted for vital staining with Erythrosine B and Trypan blue. Viable *Blastocystis* sp. vacuolar forms were bright and colourless. Dead cells revealed contrasting nuclei and stained cytoplasm. Finally, this is a useful method for cultivation and vital staining that can contribute to the development of *Blastocystis* sp. testing for effective diagnostics in the medical field.

Keywords: *Blastocystis* sp., Small capped tube cultivation, Rapid vital staining, Erythrosine B

Blastocystis spp. Detection Rates in Human Stool Samples in Croatia

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* Presenter

Abstract
Blastocystis spp. is one of the most widespread protists in the gastrointestinal tract. This study was conducted to investigate the prevalence of *Blastocystis spp*. in Croatia. A total of 2560 stool samples were examined microscopically. Of 2560 samples - 1350 were from women and 1210 from men. Of those, 186 samples were positive for *Blastocystis* (7.27% of the overall sample pool) – 90 from women (6.67% of all female samples) and 96 from men (7.93% of all male samples). No statistically significant difference has been observed among these two groups (χ^2=1.521, p=0.218).

When age of tested individuals is concerned, a total of 4.24% samples (38 out of 896) were positive in the 0-18 age group, 6.51% (75 out of 1152) in the 19-64 age group and 14.26% (73 out of 512) in those older than 65 years of age. The positivity in the oldest age group was significantly higher in comparison

to the younger age groups (χ^2=50.292, p<0.001). However, there were no statistically significant differences according to gender in these three age groups (χ^2=3.843, p=0.146).

Basic information on clinical presentation has been available for 82.42% of the tested individuals. Of those with gastrointestinal disturbances, 5.04% had a positive test result for *Blastocystis*, compared to 10.54% without any symptoms, which was a statistically significant difference (χ^2=22.1315, p <0.001). However, only 13.44% of positive samples had a high load of *Blastocystis* which hampers the detailed analysis of clinical-laboratory correlation.

Further studies need to be done to elucidate better importance of *Blastocystis* in human health and disease.

Blastocystis hominis: An Unrecognized Parasite in Need for Deeper Study

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* Presenter

Abstract
Introduction*: Blastocystis* is an anaerobic and genetically diverse unicellular intestinal parasite of humans and a wide range of animals. Due of the high percentage of asymptomatic carriers, and unclear pathogenic potential, its impact on human health is a topic of much discussion. This is why it is important for our study to objectively report the number of people that are infected with this parasite.

Methods: This is a cross-sectional study carried out in the years 2019 to 2022, in the Microbiology laboratory, in Cambridge Clinical Laboratories & ALNET, Albania. Stool samples were examined microscopically in wet direct smear using normal saline and the zinc sulphate flotation technique.

Results: Overall 2541 subjects were included with at least one parasitological examination of the stool, of which 787 examinations were positive, with a simple parasite index of 31%. *Blastocystis hominis* was found in 123 subjects with a specific parasite index of 4.84% including 78 males and 45 females. The paediatric age 5-12 and 30-50 were the most affected age groups by all parasites. The most frequent association was that of *B. hominis* and *Giardia lamblia* with a rate of 21.4% followed by the combination of *B. hominis* and *Entamoeba spp.* (15.0%).

Conclusions: The frequency of *B. hominis* is relatively low in our country. This study revealed that *B. hominis* was the most common protozoon. In Albania, diagnosis is made by detecting characteristic forms of the parasites in faecal samples microscopically. Due to lack of recognition of different morphologic forms of the parasite, several cases are under looked. In this regard, the true prevalence of the infection is not known.

Keywords*: Blastocystis hominis*, prevalence, co-infection

Investigating the Role of the Gut Microbiome in Cypriot Cattle Farming

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* Presenter

Abstract
The gut microbiome is made up of more than 10^3-10^4 microorganisms which encompass 150 times more genes than the host genome and is therefore often considered an organ in itself with a significant influence on the host's body. Minimal research and attempts to manipulate the rumen microbiome of ruminants have been carried out and to the author's knowledge none have been made to influence the lower gut microbiome. The aim of this study is to determine the complexity of the lower gut microbiome of dairy cattle from farms in Cyprus including associations between the microbiome and parasitic infections as well as exploring the risks of potential zoonotic transmission of these parasites to humans. Preliminary results have indicated a significant proportion of farms in Cyprus testing positive for the *Cryptosporidium* parasite. Further research on the association of this parasite with the lower gut microbiome as well as other parasites including *Blastocystis* are part of the future investigations of this project. In addition, the interplay of the gut microbiome, antibiotic use, various environmental factors (e.g. geographical location, pollution), farming practices and milk production in dairy cattle will be investigated. Ultimately, the purpose of this research is to identify primary targets for sustainable and environmentally friendly agricultural practices in order to maximize ruminant production efficiency, reduce antibiotic use and improve animal welfare.

Blastocystis Frequency in Patients with Colonoscopy Examination

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* Presenter

Abstract
Blastocystis is an intestinal anaerobic protozoan, its pathogenicity and genetic diversity are the most controversial issues. *Blastocystis* has a role in the development of colorectal cancer in experimental animals. The aim of this project was to determine the frequency of *Blastocystis* in patients who have colonoscopy examination, and *Blastocystis* subtype distribution and evaluation of colonoscopy findings.

Stool samples will be collected from 150 patients who had colonoscopy examination in the Department of Gastroenterology, Aydin Adnan Menderes University. First of all, stool samples were

tested for *Blastocystis* positivity with direct microscopy of native-Lugol preparations during routine parasitological examination. Of the patients 79 (%52,7) were female and 71 (%47,3) were male. *Blastocystis* was detected 18 (%12) of the patients. All stool samples were subjected to genomic DNA isolation and stored at -20°C for molecular analysis. The molecular analysis and evaluation of colonoscopy findings are in progress.

The study was supported by Aydin Adnan Menderes University BAP Commission (TPF-22031).

The Complexities of *Blastocystis* and Human Health: Unravelling the Mysteries

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* Presenter

Abstract
We propose *Blastocystis* pathogenomics as a means to understanding its pathogenic potential, improve diagnosis, and shed light on the genetic diversity of the organism. We will investigate the association between *Blastocystis* and gastrointestinal symptoms in humans, an area of ongoing research and debate. Answering this research question could help improve our understanding of *Blastocystis* and inform the development of more effective strategies for diagnosing and treating infections.

Our research platform will include epidemiological studies to identify risk factors and the prevalence of *Blastocystis* infection, molecular studies to detect and identify different subtypes of *Blastocystis* and investigate genetic diversity. In vitro studies to investigate the mechanisms by which *Blastocystis* causes disease, and finally, clinical trials to test the efficacy of different treatment strategies.

A multi-disciplinary approach incorporating epidemiological, molecular, and clinical methods could provide a comprehensive understanding of *Blastocystis* and its pathogenic potential.

In conclusion, *Blastocystis* is a complex organism that presents several challenges for researchers and clinicians. The lack of understanding of its pathogenic potential, difficulty in diagnosing infections, and genetic diversity are all factors that contribute to the challenges associated with *Blastocystis*.

Recent advancements in sequencing technologies provide opportunities to access detailed microbiome profiles of patients and improve our understanding of *Blastocystis* diversity and evolution. This knowledge can help to develop more effective strategies for the diagnosis, treatment, and prevention of *Blastocystis* infections and their correlation with other disease aetiologies. Identifying the direct and indirect causes of *Blastocystis* infection is essential for its treatment. Our focus will be the development of multi-omic platforms to better understand pathogenomics and develop more effective strategies for diagnosis and treatment.

Drug Discovery against Protozoal Infections

Ines Sifaoui*(1), María Reyes-Batlle (1)(2), Atteneri López-Arencibia (1)(2), Rubén L. Rodríguez Expósito (1)(2), Iñigo Arberas-Jiménez (1)(2), Javier Chao-Pellicer (1)(2)(3), Patricia Pérez-Pérez (1)(2), Carlos J. Bethencourt-Estrella (1)(2), Desirée San Nicolás-Hernández (1)(2), José E. Piñero (1)(2)(3), and Jacob Lorenzo-Morales (1)(2)(3)

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* Presenter

Abstract
As part of the Laboratory of Chemotherapy against Protozoa Parasite and Free-Living Amoeba, our research focuses on the development of novel, alternative drugs for the treatment of various protozoal infection and the isolation of free-living amoeba from environmental and clinical samples. We work on chemotherapy against various protozoa models including kinetoplastids (*Leishmania spp* and *Trypanosoma cruzi*) and free-living amoebae (*Acanthamoeba spp* and *Naegleria fowleri*). Our research lines focus on the developing of new active antiprotozoal molecules from different origin namely natural, semi-synthetic and synthetic. My first of line of research is based on the isolation of bioactive molecules isolated from different sources such as plant, seaweed, and microorganisms.

Assessment of damage at the cellular level and type of cell death induced by drug, on parasite type *Leishmania spp*, *Trypanosoma cruzi* and free-living amoeba, constitute my second line of investigation. Several strategies are used to elucidate the pathway with which the bioactive molecules could inhibit or eliminate the parasite: until present I have been checking the effect of bioactive molecules on various programmed cell features including ATP levels, mitochondrial membrane potential, plasma membrane permeability, analysis of phosphatidylserine residue exposure, DNA fragmentation by TUNEL and chromatin condensation. Most of those assays are achieved by confocal fluorescence microscopy and image-based system microscopy. Our main objective from participating in the *Blastocystis* Culture & Applications workshop is to add *Blastocystis* as a new model for our antiprotozoal drug development.

List of Speakers and Participants

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