Dissertation	Diversity of Eukaryotic Microbes in the Gut of Animals and Humans from Rural and Suburban Areas
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ABSTRACT

Eukaryotic microbiome refers to the microscopic eukaryotes that live in the gastrointestinal (GI) tract. The microbial eukaryotes occupying the intestinal tract of humans and animals are fungi and protists. Previously, the majority of protist species in the gut were considered infectious and therefore harmful. Thus, most previous studies focused on the taxonomy and diversity of gut protists found in the gut of people with gastrointestinal symptoms, mainly diarrhea. However, recent works have shown presence of protists in individuals with healthy guts suggesting that these organisms are at least commensal, rather than parasitic. Recent research results have also shown that the GI tract of residents of rural areas contains more diverse and abundant eukaryotic microbes than that of urban residents. Moreover, specific protists have been associated with increased bacterial community richness and evenness and/or abundance of bacteria considered beneficial for gut health. Therefore, knowledge on the overall diversity of gut protists will contribute towards understanding associations with human gut health and disease states. Hence, this study focused on (1) investigating the diversity, prevalence, and distribution of select protists in the gut of animals and humans living in rural and suburban areas of Thailand, and (2) examining the occurrence, diversity, and transmission dynamics of microbial eukaryotes in residents of this study area.

Stool samples were collected from 238 adult Thai nationals from rural and suburban areas living in Chiang Rai (CR), Phayao (PY) and Chachoengsao (CH) provinces. Human volunteers had no history of gastrointestinal diseases and no gastrointestinal symptoms at the time of sampling. Stool samples from 60 animals, namely six pigs, 34 chickens, four buffalo and 16 ectotherms were also collected. We also acquired 55 environmental samples, seven from soil and 48 from water sources. A small amount from each sample was placed in LYSGM media at 37 °C for three days and observed using a compound microscope. All samples were screened for *Blastocystis*, Entamoebidae, Parabasalia, enteromonads and *Balantidium coli* using the small subunit ribosomal RNA (18S rRNA). Phylogenetic analysis was used to assess the diversity of the microbial eukaryotes identified in the samples.

Herein, a new species of *Entamoeba* was isolated from the gut of Asian swamp eel (*Monopterus albus*), an ectotherm living in northern Thailand. Morphological (performed in live and stained cells) and phylogenetic analysis revealed that the new organism is closely related to *Entamoeba invadens*. The new species is established as *Entamoeba chiangraiensis*. These results add to the *Entamoeba* species that have been isolated from fish and provide the first molecular data of its kind.

Following screening of human stool samples, the green alga *Prototheca bovis* was found in four individuals living in a rural area in Thailand. Pure cultures were established for all four samples. The life cycle of the organism was observed and its stages were characterized in detail over a 36-hour period using the Nikon 80i compound microscopy system. Molecular characterization of all four isolates was also performed using the 18S rRNA and cytochrome b (cytb) genes. The life cycle of *P. bovis* resembles that of zoosporic fungi, with up to eight zoospores per mature sporangium. Phylogenetic analysis showed that *Prototheca* was not monophyletic but split into at least two distinct clades. This is the first report of *P. bovis* in human stool samples of individuals with no gastrointestinal symptoms.

To investigate prevalence and transmission dynamics of *Blastocystis*, a rural community in northern Thailand was used as the study area. Various transmission modes such as human-to-human, animal to human and environment to human were examined. Of these, the role of the environment on transmission of *Blastocystis* is mostly unknown. For this study, humans (n=45), animals (n=44) and the environment (n=35) were sampled. *Blastocystis* was present in 73% of human and 100% of animal hosts, while 91% of environmental samples were positive. Overall, ten subtypes were identified: ST1, ST2, ST3, ST4 ST5, ST6, ST7, ST10, ST23, and ST26. Eight of these were detected in humans: ST1, ST2, ST3, ST4, ST5, ST7, ST10, and ST23. The latter two are reported in Thai adults for the first time. It is also the first instance of ST23 in human hosts worldwide. Three subtypes were detected in other animals (ST6, ST7, and ST23), while seven (ST1, ST3, ST6, ST7, ST10, ST23, and ST26) were found in the environment. Five subtypes of *Blastocystis* were shared between humans and the environment (ST1, ST3, ST7, ST10, ST23), while three (ST7, ST10, ST23) overlapped between the environment and animal hosts. No subtypes were shared between humans living in the same residence suggesting that human-to-human transmission did not occur in the examined households. The results indicate that in the community under study, the major transmission route of *Blastocystis* to humans is the environment. This study identified and proposed soil as a novel transmission route of this eukaryote. Moreover it shows that using the One Health perspective, whereby humans, animals and the environment are considered collectively, can help understand transmission patterns of organisms and therefore contribute in developing effective control strategies against pathogens.

Future studies should focus on investigating larger communities and other areas in Thailand under the One Health umbrella. Moreover, microbial eukaryotes carriage patterns in different communities should be investigated along with their metabolome profiles to better understand their roles in gut health and disease.

Keyword: Microbial Eukaryotes, Transmission Mode, Thailand, 18S rRNA