IWOP 2021

OnLine Conference

15th International Workshops on Opportunistic Protists
15-17 June, 2021

České Budějovice, Czech Republic

Program and Abstract book

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Welcome to the 15th International Workshop on Opportunistic Protists (IWOP 2021).

Dear participants, let us welcome you to IWOP 2021. For the first time in our 33 year history, this year's meeting will be held as an online conference. We are not holding the online conference as a move towards modern technology or out of laziness to hold a traditional conference, but as a result of the current epidemic situation in the world. It has been four years since we last met face to face. Since that time we have all moved forward in science, and we have certainly added many new results that we would like to share. Despite the challenges of the online format, we have tried to maintain the open and friendly tradition of the IWOP meeting, where anyone can present their work.

We welcome all international and national participants who have found the desire, time and courage to participate in our e-conference. Unfortunately, you cannot attend any of the social programs we had originally prepared for you. You cannot take a walk in one of the most beautiful landscapes of our country, you cannot visit one of the most visited castles in the Czech Republic (Hluboká nad Vltavou) and you cannot attend a unique revolving theatre in the garden of an ancient castle in one of the most beautiful UNESCO towns (Český Krumlov). We are very sorry that you will miss out on these experiences and that is why we have prepared virtual tours for you on the conference website. We are aware that it cannot replace the personal experience, but maybe it will attract you to visit our country and maybe, who knows, another IWOP will be organized here in the future.

The Biology Centre CAS (BC) is one of the largest non-university institutes worldwide, acting in the field of organismic biology. The institute is primarily active in basic research, but results of BC research are used in agriculture, forestry, fisheries, human and veterinary medicine, public and state administration, and other social sectors. The BC is a nationally leading research institution and provides a research platform for the University of South Bohemia in České Budějovice, with which BC shares a campus. The BC publishes the European Journal of Entomology and Folia Parasitologica for the benefit of the global scientific community. Several international scientific meetings with hundreds of participants are hosted by the BC in České Budějovice each year.

České Budějovice, the South Bohemian capital with a population of 90,000, was founded by the Bohemian King Přemysl Otakar II in 1265 on the confluence of the Rivers Malše and Vltava (Moldau) as a royal city so the king could strengthen his position of power in South Bohemia. The strong fortifications made the city a strategically important place during the Hussite Wars, the later estates uprising and the subsequent Thirty Years' War. The sixteenth century brought the city unprecedented prosperity and considerable profits flowing into the city coffers, particularly from mining of local silver, but also from beer brewing, fish farming and the salt trade. The Theresian reforms after the middle of 18th century transformed České Budějovice into the seat of the newly created region. A number of historical monuments have also survived to this day when the city became a political, cultural and educational centre.

We would like to thank all partners and sponsors who have supported this workshop.

On behalf of all the members of the organising committee, we wish you lots of fun, opportunities, experience and education.

Organising committee: Martin Kváč, Bohumil Sak, Nikola Holubová, Lenka Hlásková, Dana Květoňová

History of International Workshop on Opportunistic Protists

- 1st International Workshop on Opportunistic Protists, Bristol, U.K. 1988
- 2nd International Workshops on Opportunistic Protists, Bozeman, MT, USA, 1991
- 3rd International Workshops on Opportunistic Protists, Cleveland, OH, USA, 1994
- 4th International Workshops on Opportunistic Protists, Tuscon, AR, USA 1996
- 5th International Workshops on Opportunistic Protists, Lille, France, 1997
- 6th International Workshops on Opportunistic Protists, Raleigh, NC, USA, 1999
- 7th International Workshops on Opportunistic Protists, Cincinnati, OH, USA , 2001
- 8th International Workshops on Opportunistic Protists, Hilo, HI, USA, 2003
- 9th International Workshops on Opportunistic Protists, Lisbon, Portugal, 2006
- 10th International Workshop on Opportunistic Protists, Boston, MA, USA, 2008
- 11th International Workshops on Opportunistic Protists, Hilo, HI, USA, 2010
- 12th International Workshops on Opportunistic Protists, Tarrytown, NY, USA, 2012
- 13th International Workshops on Opportunistic Protists, Seville, Spain, 2014
- 14th International Workshops on Opportunistic Protists, Cincinnati, OH, USA, 2017
- 15th International Workshops on Opportunistic Protists, České Budějovice, Czech Republic, 2021 (online)

15th International Workshops on Opportunistic Protists would not be possible without the warm support of our partners and sponsors, thank you!

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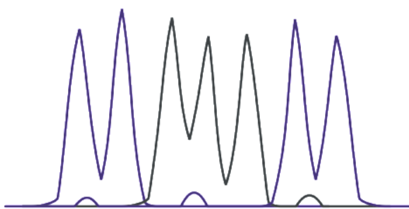

GenSeq s.r.o.

Conference schedule

Day 1 (June 15, Tuesday)		
CET Time	**Presenter**	**Title of the lecture**
11:00-12:00	**Access to the website**	
12:00-12:10	**Welcome and opening of the conference**	
***Pneumocystis* section 1 and *Toxoplasma* section**		
12:10-12:30	Caroline Meier	**O1:** Characterization of the expressed and non-expressed gene repertoires of the most abundant *Pneumocystis* major surface glycoprotein present in patients
12:30-12:50	Philippe Hauser	**O2:** A model for the sexual cycle of *Pneumocystis* species
12:50-13:10	De-Hua Lai	**O3:** Inducible nitric oxide synthase knockout in SD rat results complete against *Toxoplasma gondii* infection
13:10-13:15	**Advertising break**	
***Tritrichomonas* section**		
13:15-13:35	Maciej Kochanowski	**O4:** Comparative evaluation of different molecular assays for diagnosis of feline tritrichomonosis
13:35-13:55	Joanna Dąbrowska	**O5:** Prevalence of *Tritrichomonas foetus* infection in animal hosts in Poland
13:55-14:15	Joanna Dąbrowska	**O6:** Whole genome sequencing of a feline strain of *Tritrichomonas foetus* reveals massive genetic differences to bovine and porcine isolates
14:15-14:25	**Advertising break**	
***Cryptosporidium* section 1**		
14:25-14:45	Na Li	**O7:** Subtyping *Cryptosporidium xiaoi*, a common pathogen in sheep and goats
14:45-15:05	Yaoyu Feng	**O8:** Concentrated animal feeding operations and transmission of *Cryptosporidium parvum*
15:05-15:25	Lihua Xiao	**O9:** Advances in comparative genomics of *Cryptosporidium* spp.
15:25-15:35	**Advertising break**	
***Pneumocystis* section 2**		
15:35-15:55	Lisa Bishop	**O10:** Cxcr6 positive CD4+ T cells accumulate in the lungs during *Pneumocystis* infection but Cxcr6 is not required for clearance
15:55-16:15	Shelly Curran	**O11:** Memory-like innate immunity following infection with *Pneumocystis murina*
16:15-16:35	Liang Ma	**O12:** Molecular epidemiological investigation of an outbreak of *Pneumocystis jirovecii* pneumonia among renal transplant recipients in the United States
16:35-16:45	**Advertising break**	
***Cryptosporidium* section 2**		
16:45-17:05	Jan Mead	**O13:** The effect of short chain fatty acids on the inhibition of *Cryptosporidium parvum* growth *in vitro*
17:05-17:25	Martin Kváč	**O14:** Adaptation of gastric *Cryptosporidium* to excystation - preliminary data
17:25-17:45	Żaneta Zajączkowska	**O15:** Alternative factors potentially predisposing patients for *Cryptosporidium* infection
17:45-18:00	**Advertising break**	
Poster section 18:00-19:00		
18:00-19:00	Joanna Dąbrowska	**P1:** Unexpected cross-reaction with honigbergiella-like DNA in a PCR for detection of bovine *Tritrichomonas foetus*
18:00-19:00	Jiřina Marková	**P2:** Selected Carnivora species from the Czech Republic as a potential source of food-borne pathogens
18:00-19:00	Ofélia Nhambirre	**P3:** Detection of enteroparasites, namely *Cryptosporidium* spp. and *Giardia duodenalis* in children up to 14 years old, with diarrhea, in Mozambique
18:00-19:00	Barbora Zalewska	**P4:** Detection of parasitic DNA in irrigation water using molecular method

Day 2 (June 16, Wednesday)		
CET Time	**Presenter**	**Title of the lecture**
11:00-12:00	**Access to the website**	
12:00-12:10	**Welcome by the Director of the Biological Centre**	
***Pneumocystis* section 3**		
12:10-12:30	Marta Kicia	**O16:** Bronchopulmonary dysplasia can be associated with *Pneumocystis jirovecii* colonization in preterm infants
12:30-12:50	Enrique Calderón	**O17:** *Pneumocystis jirovecii* and fungal microbiota in preterm newborn infants with and without respiratory distress syndrome
12:50-13:10	Barbara Blasi	**O18:** *Pneumocystis suis* in swine farms: a co-infection study
13:10-13:15	**Advertising break**	
***Cryptosporidium* section 3**		
13:15-13:35	Miranda Procter	**O19:** Genetic diversity of *Cryptosporidium* spp. from ungulates in the United Arab Emirates
13:35-13:55	Anastasios Tsaousis	**O20:** Prevalence of *Cryptosporidium* species in dairy cows' farms from the Netherlands, Belgium and France
13:55-14:15	Sumaiya Hoque	**O21:** Investigating the 2019-2021 prevalence of *Cryptosporidium* spp. across European dairy farms
14:15-14:25	**Advertising break**	
***Pneumocystis* 4 and Diplomonads 1 section**		
14:25-14:45	Magdalena Szydlowicz	**O22:** Shelter animals as a reservoir of zoonotic pathogens
14:45-15:05	Pragya Tripathi	**O23:** Tagging of pyruvate dehydrogenase candidates in *Diplonema papillatum*
15:05-15:25	Alexey Porollo	**O24:** Gene expression profiling of *Pneumocystis murina* response to copper
15:25-15:35	**Advertising break**	
Microsporidia section		
15:35-15:55	Marta Kicia	**O25:** Human extraintestinal microsporidiosis caused by zoonotically transmitted *Encephalitozoon cuniculi*
15:55-16:15	Bohumil Sak	**O26:** The Trojan Horse of the immune system: Does *Encephalitozoon cuniculi* exploit migrating immune cells for their own dispersal in the host body?
16:15-16:35	Louis Weiss	**O27:** Development of Novel MetAP2 Inhibitors for the treatment of microsporidiosis
16:35-16:45	**Advertising break**	
***Pneumocystis* section 5**		
16:45-17:05	Christiane Weissenbacher-Lang	**O28:** Comparison of prevalence, histopathology, and genetic relationship of *Pneumocystis* spp. in eleven mammal families
17:05-17:25	Ousmane Cissé	**O29:** Genomic insights into the host specific adaptation of the *Pneumocystis* genus
17:25-17:45	Sandra Rebholz	**O30:** A novel compound against *Pneumocystis*
17:45-18:00	**Advertising break**	
Poster section– 18:00-19:00		
18:00-19:00	Maciej Kochanowski	**P5:** Development of a novel loop-mediated isothermal amplification (LAMP) assays for the detection of *Tritrichomonas foetus* in the feces of domestic cats
18:00-19:00	Nikola Holubová	**P6:** Molecular identification of *Cryptosporidium* spp. and *Encephalitozoon* spp. in wild and farmed pigeons in the Czech Republic
18:00-19:00	Diego Macedo	**P7:** New viral discoveries in *Leptomonas pyrrohcorris*, a rising RNA virus hotbed and model organism
18:00-19:00	Cátia Silva	**P8:** Fresh fruits and vegetables contamination by intestinal parasites in Maputo markets and supermarkets, Mozambique
18:00-19:00	Maria Wesołowska	**P9:** *Blastocystis* infection in children undergoing allogeneic hematopoietic cell transplantation in Poland. Preliminary results

Day 3 (June 17, Thursday)		
CET Time	**Presenter**	**Title of the lecture**
11:00-12:00	**Access to the website**	
12:00-12:10	**Organizational issues**	
***Cryptosporidium* section 4**		
12:10-12:30	Jana Ježková	**O31:** Prevalence and diversity of *Cryptosporidium* of red squirrels and European ground squirrel in the Czech Republic and Slovakia
12:30-12:50	Manasi Sawant	**O32:** First study to characterize the role of epigenetics in the biology of the Apicomplexan parasite *Cryptosporidium parvum*
12:50-13:10	Lenka Tůmová	**O33:** Biology of *Cryptosporidium* sp. chipmunk genotype I
13:10-13:15	**Advertising break**	
***Pneumocystis* section 6**		
13:15-13:35	Melanie Cushion	**O34:** Extended treatment with the long-acting echinocandin, rezafungin, eliminates *Pneumocystis* pneumonia in a murine model
13:35-13:55	Steven Sayson	**O35:** Proteome of extracellular vesicles from *Pneumocystis*-infected rat lungs
13:55-14:15	Alan Ashbaugh	**O36:** The formation of asci is necessary for growth and transmission of *Pneumocystis murina* infection
14:15-14:25	**Advertising break**	
Selection of the IWOP 16th organizing team		
14:25-15:25	Meeting of all conference participants. Presentation of the new organizing team	
15:25-15:35	**Advertising break**	
Diplomonads section 2		
15:35-15:55	Martin Kolísko	**O37:** Phylogenomics and comparative transcriptomics of secondarily free-living diplomonads
15:55-16:15	Jenny Maloney	**O38:** A hybrid sequencing strategy to produce whole genomes of *Giardia duodenalis* using cysts purified directly from fecal samples
16:15-16:35	Monica Santin	**O39:** Next generation amplicon sequencing of the *Giardia* beta-giardin gene for detection of mixed assemblage infections
16:35-16:45	**Advertising break**	
Closing ceremony		
16:45	Farewell to the conference participants.	

Abstracts

Abstract O1 – Day 1 (12:10-12:30 CET)

Characterization of the expressed and non-expressed gene repertoires of the most abundant *Pneumocystis major* surface glycoprotein present in patients

<u>Caroline Meier</u>, Marco Pagni, Philippe M. Hauser

Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Switzerland

The major surface glycoproteins (Msg) of *Pneumocystis jirovecii* constitute potential virulence factors located at the cell surface. They are classified into six to seven families (Msg-I to VI, or A1-3 to E), Msg-I (A1) being the most abundant. One of their function would be to create antigenic variation through their hypervariability in order to evade the immune system of the host. Antigenic variation is thought to be generated by (i) gene mosaicism created by recombinations between the genes of each family thanks to their location at the subtelomeres, and (ii) mutually exclusive expression in family msg-I (A1), i.e. the expression of only one gene out of ca. 80 present in each genome. Mutually exclusive expression would result from the presence of a single promoter in the genome and the exchange of the expressed gene through recombination. The latter is believed to occur between a short sequence that is present at the end of the promoter as well as at the start of each msg-I gene. Thus, a single *P. jirovecii* population would be a mixture of different subpopulations expressing each a different msg-I gene.

The aim of the present work was to characterize the repertoires of expressed and non-expressed msg-I genes present in five Swiss patients. Full-length genes were amplified using generic PCRs, followed by deep sequencing. A dedicated bioinformatics pipeline enabled the accurate identification of the different msg-I alleles present in each repertoire, as well as their abundance. The data revealed highly diverse and dynamic msg-I repertoires. The non-expressed repertoire present in each patient was specific, but a number of the alleles were also present in the repertoire of other patients. Our observations are compatible with the mechanisms of antigenic variation believed to be in action in the msg-I system, as described above.

Abstract O2 – Day 1 (12:30-12:50 CET)

A model for the sexual cycle of *Pneumocystis* species

Philippe M. Hauser

Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Switzerland

Sequencing genomes followed by comparative genomics allowed a better understanding of the mechanisms involved in the sexuality of *Pneumocystis* organisms. The structure of their mating-type locus corresponding to a fusion of two loci, Plus and Minus, and the concomitant expression of the three mating-type genes revealed that their mode of sexual reproduction is primary homothallism. This mode is favored by microbial pathogens and involves a single self-compatible mating-type that can enter into the sexual cycle on its own. Reverse transcriptase-PCR amplification showed that *Pneumocystis* sexuality is obligatory within host's lungs during pneumonia in adults. Observation of asci and epidemiological evidence suggested that this sexuality is also obligatory during primary infection in children as well as during colonization. Pneumocystis sexuality participates in cell proliferation, airborne transmission to new hosts, and probably antigenic variation, processes that are crucial to ensure the survival of the fungus. Thus, sexuality is central in *Pneumocystis* life cycle. I propose here a model for the sexual cycle of *Pneumocystis* species derived from the observations made so far. The obligate biotrophic parasitism with obligate sexuality of *Pneumocystis* is unique among fungi pathogenic to humans. *Pneumocystis* organisms are similar to the plant fungal obligate biotrophs that complete their entire life cycle within their hosts including sex, and that are also reluctant to growth *in vitro*.

Abstract O3 – Day 1 (12:50-13:10 CET)

Inducible nitric oxide synthase knockout in SD rat results complete protection against *Toxoplasma gondii* infection

Zheng-Jie Wang, Zhao-Rong Lun, **De-Hua Lai**

Sun Yat-Sen University, Guangzhou, China

Nitric oxide (NO) is an important immune molecule which acts against extra- and intracellular pathogens in most hosts. However, after knockout of inducible nitric oxide synthase ($iNOS^{-/-}$) in Sprague Dawley (SD) rats, these $iNOS^{-/-}$ rats were found to be completely resistant to *Toxoplasma gondii* infection. Once the $iNOS^{-/-}$ rat peritoneal macrophages (PM) were infected with *T. gondii*, they produced high levels of reactive oxygen species (ROS) triggered by GRA43 secreted by *T. gondii*, which damaged the parasitophorous vacuole membrane and PM mitochondrial membranes within a few hours post infection. Further evidence indicated that the high levels of ROS caused mitochondrial superoxide dismutase 2 depletion and induced PM pyroptosis and cell death. This discovery of complete resistance to *T. gondii* infection, in the $iNOS^{-/-}$-SD rat, demonstrates a strong link between NO and ROS in immunity to *T. gondii* infection and showcases a novel and effective backup innate immunity system.

Funding: National Key R&D Program of China (2017YFD0500400), National Natural Science Foundation of China (31772445)

Abstract O4 – Day 1 (13:15-13:35 CET)

Comparative evaluation of different molecular assays for diagnosis of feline tritrichomonosis

Maciej Kochanowski, Joanna Dąbrowska, Mirosław Różycki, Jacek Karamon, Jacek Sroka, Tomasz Cencek

Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Puławy, Poland

Tritrichomonas foetus is a protozoan parasite that has been traditionally identified as a cause of reproductive tract disease in cattle. However, several year ago it was discovered that *T. foetus* may cause severe diarrhea in cats all over the world. In order to evaluate the methodology in coprological molecular diagnosis of feline tritrichomonosis, we compared previously published ("old") and newly developed ("novel") loop-mediated isothermal amplification (LAMP) (targeted to the *T. foetus* β-tubulin and the elf1α 1 gene, respectively) as well as an old conventional and an old and novel real-time PCR (all targeted to overlapping regions of *T. foetus* rDNA) assays regarding their diagnostic sensitivities and specificities. Here, the novel real-time PCR yielded the best methodical performance in that a sensitivity with a detection limit of < 0.1 trophozoites (corresponding to ca. < 0.13 trophozoites per mg feces) and a maximal specificity for diagnosis of *Tritrichomonas* spp. was achieved. The other test systems exhibited either an approximately 10-times lower sensitivity (< 1 trophozoite corresponding to ca. < 1.3 trophozoites per mg feces) (conventional PCR and both LAMP assays) or a lower specificity (old real-time PCR). Conversely, the diagnostic performance assessed with clinical fecal samples from cats demonstrated identical sensitivities (8 of 20 samples tested were positive) for the novel PCR and both LAMP assays. Diagnostic sensitivities were significantly higher than those found for the old real-time (5 positive samples) and conventional PCR (6 positive samples), respectively. Accordingly, our data suggested the novel PCR and both LAMP assays to be well suited molecular tools for direct (i.e. without including an *in vitro* cultivation step) coprological diagnosis of tritrichomonosis in cats. Interestingly, relative high (novel LAMP, 7 positive samples) to at least moderate (old LAMP, 6 positive samples and 1 sample with equivocal score) diagnostic sensitivities were also achieved by testing clinical samples upon simple visual inspection of colorimetric changes during the LAMP amplification reactions. Accordingly, both LAMP assays may serve as practical molecular tools to perform epidemiological studies on feline (and bovine as well as porcine) tritrichomonosis under simple laboratory conditions.

Funding: Statutory funds of the National Veterinary Research Institute in Puławy, Poland (S/224)

Abstract O5 – Day 1 (13:35-13:55 CET)

Prevalence of *Tritrichomonas foetus* infection in animal hosts in Poland

Joanna Dąbrowska, Jacek Karamon, Maciej Kochanowski, Tomasz Cencek

Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Puławy, Poland

Tritrichomonas foetus is an intriguing protozoan parasite which is pathogenic in cattle and cats and is a commensal organism of pigs. *T. foetus* is the causative agent of the sexually transmitted disease in cattle called tritrichomonosis. In Poland, no cases of bovine tritrichomonosis have been found since 1997. However, strict regulations exist to prevent the reintroduction of the disease to dairy herds. Moreover, bovine tritrichomonosis is a notifiable disease on the OIE list of notifiable animal diseases. Feline tritrichomonosis has a worldwide occurrence and *T. foetus* mainly causes large bowel diarrhea. *T. foetus* was also identified in the nasal cavity, stomach, and intestines of pigs as a commensal. There is no obligation to examine and report porcine tritrichomonosis, so the real prevalence of parasites in Poland is unknown. Molecular methods were adapted for the *T. foetus* identification, among them, conventional PCR, and also new sensitive methods like loop-mediated isothermal amplification (LAMP). The aim of this study was to determine the prevalence of *T. foetus* infection in selected populations of cats, pigs and cattle in Poland using the molecular tools: conventional PCR and own techniques- loop-mediated isothermal amplification (LAMP). A total of 117 freshly voided feline fecal samples from cats, 172 swabs from the nasal cavities of pigs and 180 bovine specimens from the area of Poland were collected for the study. All samples were examined by PCR and own method LAMP. The statistical analysis was performed using Statistica v10 (StatSoft Inc., Tulsa, OK, USA).

The prevalence of feline tritrichomonosis was 20.51%, and statistically significant differences were obtained between groups of animals regarding age, breed, the number of cats, diarrhea, and place of living. Positive PCR and LAMP results for *T. foetus* were estimated for 16.28% of pigs, and the obtained data were significantly correlated with age. Conversely, no significant differences were observed concerning the farm size factor. In our survey, no cases of bovine tritrichomonosis were found, which is consistent with the data from the other countries of the European Union.

In conclusion, our survey demonstrates the presence of *T. foetus* in cat and pig populations from Poland. Therefore, despite Poland being considered free of bovine tritrichomonosis, and the occurrence of *T. foetus* in pigs may increase the risk of *T. foetus* transmission to cattle.

Funding: Statutory funds of the National Veterinary Research Institute in Puławy, Poland (S/224)

Abstract O6 – Day 1 (13:55-14:15 CET)

Whole genome sequencing of a feline strain of *Tritrichomonas foetus* reveals massive genetic differences to bovine and porcine isolates

Joanna Dąbrowska, Jacek Karamon, Maciej Kochanowski, Tomasz Cencek

Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Puławy, Poland

Tritrichomonas foetus is a protozoan parasite that colonizes the reproductive tract of cattle as well as the gastrointestinal tract of cats. Bovine tritrichomonosis is a sexually transmitted disease whereas feline tritrichomonosis is thought to be transmitted by the fecal-oral route. Furthermore, *T. foetus* is known as an essentially apathogenic commensal located in the nasal cavity of pigs. Transmission of *T. foetus* between the different hosts has to be considered a realistic scenario that may have important implications for the epidemiology of infections and disease. In our study, we generated whole genome sequencing (WGS) data from bovine, feline and porcine *T. foetus* strains to investigate the genetic (dis)similarities among these diverse strains. As a reference, we used a previously released draft assembly from a bovine *T. foetus* strain K isolated from an infected bull in Brazil. In particular, we identified single nucleotide polymorphisms (SNPs) and the insertion-deletion (indel) variations within the genomes of the different strains. Interestingly, only a low degree of polymorphism (68 SNPs and indels) was found between the bovine and the porcine strains in terms of variants with a predicted impact of moderate or high and where one species is homozygous for one allele and the other homozygous for the other allele. Conversely, however, a 964 times higher number of such differences was detected by comparing the feline with either the bovine (65,569) or the porcine (65,615) strain. These data clearly indicated a close phylogenetic relationship between bovine and porcine *T. foetus* but a remarkable genetic distinctness of these two strains from the feline strain. The latter observation was confirmed by PCR-based sequencing of 20 in silico selected indel markers and five in silico-selected SNP markers that uniformly demonstrated a relatively distant phylogenetic relationship of three independent feline *T. foetus* isolates in comparison to the bovine and porcine strains investigated.

In summary, our comparative genome sequencing approach provided further insights into the genetic diversity of *T. foetus* in relation to the different host origins of the parasite. Furthermore, our study identified a large number of SNP- and indel-containing sequences that may be useful molecular markers for future epidemiological studies aimed at the elucidation of the transmission patterns of *T. foetus* with in different host species.

Funding: Statutory funds of the National Veterinary Research Institute in Puławy, Poland (S/376)

Abstract O7 – Day 1 (14:25-14:45 CET)

Subtyping *Cryptosporidium xiaoi*, a common pathogen in sheep and goats

Yingying Fan[1,2], Xitong Huang[1], Xin Yang[3], Yaqiong Guo[1], Yaoyu Feng[1,2], Lihua Xiao[1,2], **Na Li**[1,2]

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[3]College of Veterinary Medicine, Northwest A&F University, Yangling, China

Cryptosporidiosis is a significant cause of diarrhea in sheep and goats. *Cryptosporidium xiaoi* is one of the dominant species infecting ovine and caprine animals, but the lack of subtyping tools makes it impossible to examine the transmission of this pathogen. In the present study, we identified and characterized the 60-kDa glycoprotein (gp60) gene by analysing the whole genome sequences of *C. xiaoi*. The GP60 protein of *C. xiaoi* had a signal peptide, a furin cleavage site of RSRR, a glycosylphosphatidylinositol anchor and multiple O-glycosylation sites. Based on the gp60 sequence, a subtyping tool was developed and used in characterizing *C. xiaoi* in 355 positive samples from sheep and goats in China. A high sequence heterogeneity was observed in the gp60 gene, with 94 sequence types in 12 subtype families, namely XXIIIa to XXIIIl. Co-infections with multiple subtypes were common in these animals, suggesting genetic recombination might be responsible for the high diversity within *C. xiaoi*. This was supported by the mosaic sequence patterns among the subtype families. In addition, potential host adaptation was identified within this species, reflected by the exclusive occurrence of XXIIIa, XXIIIc, XXIIIg and XXIIIj in goats. This subtyping tool should be useful in the characterizations of the genetic diversity and transmission dynamics of *C. xiaoi*.

Funding: National Natural Science Foundation of China (U1901208 and 32030109)

Abstract O8 – Day 1 (14:45-15:05 CET)

Concentrated Animal Feeding Operations and Transmission of *Cryptosporidium parvum*

Yaoyu Feng and Lihua Xiao

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Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou, China

The congregation of susceptible animals associated with concentrated animal feeding operations (CAFOs) can lead to heavy environmental contamination with pathogens, promoting the spread of hyper-transmissible and virulent pathogens such as *Cryptosporidium parvum*. As a result, CAFOs have been associated with outbreaks of diseases in farm animals and increased transmission of zoonotic pathogens in humans. This is exemplified by the wide occurrence of some *C. parvum* subtypes in both humans and dairy calves in industrialized nations, while they are largely absent in most developing countries where traditional animal farming is practiced. They are also rare on free-ranging beef, sheep, and goat farms in many industrialized nations. In these areas, bovine and ovine animals are mostly infected host-adapted, less pathogenic *Cryptosporidium* species with low human infectivity. *Cryptosporidium parvum*, however, has begun to spill over from dairy calves to free-ranging farm animals in European countries, where zoonotic *C. parvum* is now responsible for more human cryptosporidiosis cases than anthroponotic *C. hominis*. In industrialized nations with intensive farm faming, contact with calves and lambs is a risk factor in cryptosporidiosis epidemiology, humans *C. parvum* infections are common in rural areas, and outbreaks of cryptosporidiosis are common in veterinary students and children attending agricultural camps and fairs. One Health measures should be developed to minimize the impact of *C. parvum* infections in farm animals and prevent the occurrence of zoonotic infections in humans in areas practicing CAFOs.

Funding: National Natural Science Foundation of China (U1901208)

Abstract O9 – Day 1 (15:05-15:25 CET)

Advances in comparative genomics of *Cryptosporidium* spp.

Lihua Xiao

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Whole genomic sequencing (WGS) and comparative genomics are increasingly used in the characterization of *Cryptosporidium* spp. They are facilitated by the establishment of procedures for WGS analysis of clinical specimens without laboratory propagation of pathogens. Results of recent comparative genomics analysis suggest that gene duplication might be associated with broad host ranges of some zoonotic *Cryptosporidium* species and subtypes, while genetic recombination could be involved in the emergence of virulent subtypes. The availability of WGS data has further facilitated the development of advanced molecular typing tools. The use of these tools together with comparative genomics analyses has begun to improve the investigations of outbreaks in industrialized nations. More WGS data, however, are needed from both industrialized nations and developing countries before we can have in-depth understanding of the population genetics and evolution of *Cryptosporidium* spp. and genetic determinants of various phenotypic traits in human-pathogenic subtypes.

Funding: National Natural Science Foundation of China (31820103014)

Abstract O10 – Day 1 (15:35-15:55 CET)

Cxcr6 positive CD4$^+$ T cells accumulate in the lungs during *Pneumocystis* infection but Cxcr6 is not required for clearance

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CD4 T cells have been shown to be critical for controlling *Pneumocystis* infection, and low CD4$^+$ T cell counts in HIV positive patients has been predictive of *Pneumocystis* since the early days of the epidemic. With an increase in organ transplantations and the use of biological drugs which often target immune cell populations, it is important to understand the role of critical immune cell populations in controlling *Pneumocystis* infections, including CD4$^+$ T cells. Previous studies in our laboratory showed that the chemokine receptor Cxcr6 and its only known ligand, Cxcl16 were significantly upregulated in lung CD4$^+$ T cells during *Pneumocystis* infection in healthy mice. We examined *Pneumocystis* infection, using a co-housing model, in homozygous Cxcr6$^{eGFP/eGFP}$, where the coding region of both Cxcr6 alleles were replaced with eGFP (and thus do not express Cxcr6), and heterozygous Cxcr6$^{+/eGFP}$ mice, which express a functional Cxcr6 on one allele and eGFP on the other allele. Using flow cytometry, we phenotyped eGFP+ cells in the lungs of *Pneumocystis* infected mice. In both homozygous and heterozygous mice, GFP$^+$ CD4$^+$ T cells accumulated in the lungs near the peak of infection and then decreased but remained above controls following clearance. This increase in percent positive cells was accompanied by increased GFP expression per CD4$^+$ T cell. While GFP expression was detected in pulmonary CD8$^+$ T cells of infected mice, their percentage did not increase during infection, and GFP expression per cell was low compared to that of CD4 T$^+$ cells. There was also an increase in GFP$^+$CD4$^-$CD8$^-$ double negative T cells, though with overall low numbers. GFP positive cells were present but did not accumulate in the spleens of these mice. Both Cxcr6$^{eGFP/eGFP}$ and Cxcr6$^{+/eGFP}$ mice cleared *Pneumocystis* infection with similar kinetics as wild-type mice, demonstrating that Cxcr6$^+$expression is not required for clearance of infection.

Abstract O11 – Day 1 (15:55-16:15 CET)

Memory-like innate immunity following infection with *Pneumocystis murina*

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Pneumocystis pneumonia (PCP) continues to be a life-threatening infection in HIV-infected patients, renal transplant patients, and other immunocompromised individuals. Dendritic cells are key antigen presenters to $CD4^+$ T cells, which are critical to controlling *Pneumocystis* infection. β-1,3 glucans are found in the cell wall of the cyst form of *Pneumocystis* and are a known activator of innate immune cells via the dectin-1 receptor. β-glucans derived from other fungi have been shown to induce an enhanced "memory-like" immune response, termed trained immunity, in innate cells, including monocytes, macrophages, and dendritic cells. Therefore, we explored if *Pneumocystis* infection can induce an enhanced innate immune response in mice following exposure via a natural route of infection. We conducted mixing experiments using purified $CD4^+$ T cells and dendritic cells from unexposed C57BL/6 mice and mice that spontaneously cleared infection. We found that $CD4^+$ T cells from naïve mice exhibited a significantly greater proliferation after 5-day cultures when a crude *Pneumocystis* antigen was presented by $CD11c^+$ dendritic cells from exposed mice compared to unexposed mice. In support of this, $CD4^+$ T cells from C57BL/6 mice that were immunized with Freund's adjuvant alone exhibited significantly greater proliferation when a crude *Pneumocystis* antigen was presented by either $CD11c^+$ dendritic cells or $CD11b^+$ macrophages from mice immunized with *Pneumocystis* antigen mixed with Freund's adjuvant vs. adjuvant alone. These results suggest that *Pneumocystis* infection stimulates a memory-like innate immune response which may be important in controlling subsequent exposure to *Pneumocystis*, as well as exposure to other pulmonary pathogens.

Funding: The Intramural Programs at the National Institutes of Health

Abstract O12 – Day 1 (16:15-16:35 CET)

Molecular epidemiological investigation of an outbreak of *Pneumocystis jirovecii* pneumonia among renal transplant recipients in the United States

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Despite increasing reports of outbreaks of *Pneumocystis jirovecii* pneumonia (PCP) in renal transplant recipients worldwide, the molecular epidemiology of these outbreaks remains not well understood. Although the United States was the first country to document an outbreak in the early 1980s, no further outbreaks were reported over the next 4 decades. Here we report a recent outbreak of PCP in a single hospital in the USA, and present the results of molecular typing of strains involved in this outbreak.

Between July 1, 2019 and May 18, 2020, 19 cases of PCP were reported from the Yale renal transplantation clinic. Respiratory samples were collected from 9 cases and subjected to strain typing by restriction fragment length polymorphism (RFLP) analysis and multilocus sequence typing (MLST) in combination with next-generation sequencing (NGS) and multiplexity of infection prediction.

Initial MLST based on Sanger sequencing of a dozen genetic loci identified homogenous sequences in most (7/9) cases, resulting in 6 distinct *P. jirovecii* strains. Subsequent NGS identified extensive variations within and between all cases and revealed co-infection with up to 7 *P. jirovecii* strains in all patients. Combination of MLST and RFLP profiles allowed identification of five clusters. The identity of selected strains within clusters was further supported by analysis of the complete or large portions of the *P. jirovecii* mitogenome and nuclear rRNA operon sequences. Remarkably, one patient was likely co-infected with 7 *P. jirovecii* strains, 4 of which were shared with 7 other patients.

Although we were unable to construct haplotypes for minor strains from short NGS reads, we examined NGS data for a ~100-bp polymorphic non-coding (PNC) region of the *P. jirovecii* mitogenome from all 9 patients, and identified 22 unique genotypes. Four patients showed coinfection with 6-7 different PNC types. Every patient isolate contained at least one PNC type shared with at least one other patient, and one patient contained 3 PNC types shared among 7 patients. These observations imply an interhuman transmission as supported by overlaps in clinic visits between patients.

Our investigation suggests the possibility of interhuman transmission of multiple strains in a PCP outbreak in renal transplant recipients, and highlights the critical role of NGS and the potential usefulness of PNS in better characterizing the epidemiology of these outbreaks.

Funding: Intramural research funds from the U.S. NIH Clinical Center

Abstract O13 – Day 1 (16:45-17:15 CET)

The effect of short chain fatty acids on the inhibition of *Cryptosporidium parvum* growth *in vitro*

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Atlanta VA Medical Center, Decatur, GA, USA

In previous study, we observed significant increase in severity of cryptosporidial infection and increase in gut permeability in mice treated with cloxacillin, which mainly targets Gram positive bacteria. Concurrent with decreases in Gram positive bacteria in cloxacillin treated mice, we observed significantly decreased levels of short chain fatty acids (SCFAs). These include butyrate, propionate, and acetate, the main SCFAs produced in the gut. SCFAs regulate the activity of regulatory T cells but also have a direct effect on the host epithelial cells.

In this study we examined the effect of short chain fatty acids on *Cryptosporidium* growth in infected human ileocecal adenocarcinoma (HTC-8) cells. HTC-8 cells were infected with 2×10^5 *C. parvum* oocysts. After 3 hours, different short chain fatty acids were then incubated with the infected cells. After 48 hours of incubation with the different SCFAs, cells were fixed and labelled with monoclonal antibody directed to all intracellular stages and the number of parasites were quantitated using a fluorescent microscope.

Acetate, butyrate, propionic acid and valproic acid significantly inhibited growth, with an EC50 between 4 and 10 mM. Additionally, we examined short chain fatty acids in combination. When used together, certain short chain fatty acids (e.g. butyrate, acetate and propionic acid) showed increased efficacy. Butyrate also inhibited growth when incubated with sporozoites prior to infection of cell monolayer. We conclude that acetate, propionic acid and butyrate have direct inhibitory activities on host cells against *C. parvum* while butyrate can also affect sporozoite infectivity. Future studies will help determine the mechanism of the inhibition.

Funding: NIAID (AI157730) and the Foundation for Atlanta Veterans and Research

Abstract O14 – Day 1 (17:05-17:25 CET)

Adaptation of gastric *Cryptosporidium* to excystation - preliminary data

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The *Cryptosporidium* life cycle is s completed within a single host and involves excystation, attachment and invasion, asexual and sexual reproduction and sporogony. The infection is acquired through the ingestion of sporulated oocysts. *Cryptosporidium* spp. do not parasitize the initial site of entry, but the development of life cycle of each species is localized in different parts of the digestive tract - stomach, small intestine, caecum, colon. This localization does not change in typical hosts without immunodeficiency. *Cryptosporidium* oocysts have evolved to protect sporozoites and maximize their survival from the *moment* they are released from the epithelial cell until they are ingested by a new host. Since the infectious dose can be lower than 10 oocysts, the mechanism controlling oocyst excystation must be under strong selection to maximize the probability of infection. *Cryptosporidium* oocysts with different predilection sites of infection have been shown to excyst under different conditions. Has been showed that temperature is an important excystation trigger in the gastric species, but not the intestinal species. The question is whether the temperature required for excystation is the same for all species of gastric *Cryptosporidium* or whether individual species are adapted to their hosts. However, if temperature was the only trigger required for excystation, then newly formed oocysts of gastric species would excyst during passage through the gut of infected hosts.

We experimentally studied the infectivity of oocysts of gastric *Cryptosporidium* spp. originating from different parts of the gastrointestinal tract and the effect of temperature on excystation. Preliminary data have shown that oocysts from different parts of the digestive tract are capable of inducing infection in a susceptible host. If oocysts are maintained at a temperature equal to the host body temperature after excretion from the host, there is no significant change in the number of oocysts in the sample/excystation within 3 hours after excretion. The temperature to which oocysts are cooled after excretion from the host affects the number of oocysts excysted in the new host. The minimum temperature to which oocysts need to be cooled for most oocysts to excyst is 27°C. The excystation temperature correlates with the body temperature of the typical host at which most oocysts are excysted. While rodent gastric cryptosporidia (*C. proliferans*) excysted best at 38°C, *C. andersoni* parasitizing ruminants at 39°C. We did not achieve 100% excystation in any of the tests.

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Abstract O15 – Day 1 (17:25-17:45 CET)

Alternative factors potentially predisposing patients for *Cryptosporidium* infection

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In humans, *Cryptosporidium* is known mainly as the etiological agent of severe diarrhea and, less often, the cause of infection of the respiratory system among high-risk individuals, such as children from developing countries and immunosuppressed patients, particularly those with HIV infection. Currently, patients with pharmacologically induced immunosuppression, especially transplant recipients and oncologically treated, are considered as a new group at risk of cryptosporidiosis. Since in immunocompetent people *Cryptosporidium* infection is usually limited to the gastrointestinal tract causing a self-limiting diarrhea or persists asymptomatic, this group of patients is rarely in the field of interest.

Here we compare *Cryptosporidium* occurrence in different groups of patients, both those receiving and not receiving immunosuppressive treatment: (i) renal transplant recipients, (ii) cancer patients, (iii) patients with various pulmonary diseases, (iv) children with inflammatory bowel diseases (IBD), (v) pre-school children. Samples from intestinal tract (stool or colon tissue) or aspiratory specimens were screened. Molecular detection was performed by nested-PCR amplifying *Cryptosporidium* SSU rRNA and 60 kDa glycoprotein genes, followed by sequencing and phylogenetic analyses. Microscopic methods were used to confirm the presence of pathogen. *Cryptosporidium* was identified in children with IBD (stool) and immunocompetent patients with neoplastic lesions: one suffering from colon adenocarcinoma (cancer tissue), and the second with lung hamartoma (bronchial washings). Microscopic observations revealed the presence of *Cryptosporidium* in all infected patients.

Our results emphasize that other groups of patients, not only being under life-long immunosuppression, might be at risk of *Cryptosporidium* infections. Finding of pathogens in cancer patients and children with chronic inflammatory process suggests that pathologically changed tissue might be more susceptible for *Cryptosporidium* infection.

Funding: Polish Ministry of Health (STM.A060.17.038, STM.A060.20.093, STM.A060.20.105) from the IT Simple system of Wroclaw Medical University

Abstract P1 – Day 1 (18:00-19:00 CET)

Unexpected cross-reaction with *Honigbergiella*-like DNA in a PCR for detection of bovine *Tritrichimonas foetus*

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The prevalence of bovine *Tritrichomonas foetus* infection has decreased almost to zero in most European countries, such as Poland, which has been *Tritrichomonas foetus*-free since 1997. Nevertheless, trichomonosis is a notifiable disease and is one of the diseases listed by the World Organization for Animal Health (OIE). Due to the unspecified symptoms, it is very difficult to diagnose bovine trichomonosis based on clinical signs only. Therefore, it is necessary to use laboratory techniques. The standard diagnostic methods are microscopic observation (with or without prior cultivation on media) and molecular tests. However, correct *T. foetus* microscopic identification based only on the morphology of the parasite and its characteristic “rolling motion” has its limitations, such as relatively low sensitivity and accuracy. The major disadvantages of molecular tests are cross-contamination and carry over of amplicons which can lead to the misinterpretation of results. Moreover, PCR can also amplify fragments from other microorganisms, and thus false-positive results have been noted in diagnoses of bovine trichomonosis. In this context, here we present a case of an unspecific reaction with *Honigbergiella*-like DNA identified during a routine microscopic examination and identified using molecular methods. The bovine sample was submitted to the Department of Parasitology National Veterinary Research Institute in Puławy (NVRI) for confirmatory testing after having been examined at the Regional Veterinary Laboratory, during a routine *T. foetus* diagnosis. In our study we used conventional PCR according to Felleisen which amplified the fragment specific to *T. foetus* (347 bp). Positive results from microscopic observation and cultures were confirmed. Noteworthy, parasites grew on Diamond’s medium only after seven days of incubation, while optimal growth of trichomonads is generally observed after two to four days for this medium. Moreover, by using PCR we obtained positive results for the presence of *T. foetus*. However, sequencing of the amplification product revealed 99.62% identity with *Honigbergiella* sp. Our data suggest that false-positive results may occur in commonly used PCR tests. Thus, unexpected results should be carefully interpreted. Therefore, it is important to share the research involved in the diagnosis of trichomonosis, especially in relation to the possible use of non-specific PCR products.

Funding: Statutory funds of the National Veterinary Research Institute in Puławy, Poland (S/224)

Abstract P2 – Day 1 (18:00-19:00 CET)

Selected Carnivora species from the Czech Republic as a potential source of food-borne pathogens

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Wildlife is the subject of a wide range of scientific studies, from ecological, through nature conservation to epidemiological. In our study, we examined representatives of selected species of small mammals from order Carnivora that can serve as a potential reservoir of pathogens capable of causing food-borne infections in humans. The study included faecal samples from 309 animals hunted in the years 2015-2020 in 11 regions of the Czech Republic. The examined animal species included 108 northern raccoons (*Procyon lotor*), 96 Eurasian badgers (*Meles meles*), 62 red foxes (*Vulpes vulpes*) and 43 raccoon dogs (*Nyctereutes procyonoides*). Samples were analyzed for the presence of DNA of selected zoonotic bacteria and parasites (*Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella enterica*, *Yersinia enterocolitica*, *Escherichia coli* (serotype O26), *Giardia* spp., *Cryptosporidium* spp., *Echinococcus multilocularis*) using multiplex oligonucleotide ligation – polymerase chain reaction (MOL-PCR) with an adaptation to xMAP detection system. This provided a qualitative analysis of different nucleic acids from one biological sample simultaneously in one reaction. The most abundant pathogen in the faeces of wild animals was enterohemorrhagic *E. coli* O26, the least represented were bacteria *Salmonella enterica* and *Listeria monocytogenes*. Parasitic DNA was found only in the faeces of canine and *E. multilocularis* was detected only in samples from the red foxes. DNA of *Cryptosporidium* spp. was detected in the samples of ten animals (in five raccoon dogs and five red foxes), DNA of *Giardia* spp. only in faeces from six red foxes. Small mammals of the order Carnivora from the Czech Republic could be reservoir of selected food-borne pathogens and excrete them in faeces. Because the route of human infection is oral, situations that increase the risk of these infections should be avoided.

Funding: Security Research of Ministry of the Interior of the Czech Republic (VI20152020044) and Ministry of Agriculture of the Czech Republic (QK1810212)

Abstract P3 – Day 1 (18:00-19:00 CET)

Detection of enteroparasites, namely *Cryptosporidium* spp. and *Giardia duodenalis* in children up to 14 years old, with diarrhea, in Mozambique

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Diarrhea remains a major public health problem in developing countries, including Mozambique, even with the control strategies in place. This study aimed to determine the frequency of intestinal parasites, namely *Cryptosporidium* and *Giardia duodenalis* in children from the southern, central and northern regions of Mozambique. From May 2014 to December 2019, diarrheal stool samples of 2,420 children (0-14 years old) were collected and 1,424 stools examined by optical microscopy for identification of intestinal parasites. In order to evaluate potential risk factors associated with parasites infection, sociodemographic, clinical and environmental data of the children studied were obtained by questionnaires. The occurrence of parasites in the dry and rainy seasons were evaluated. For data analysis, cross tabulation were applied (Chi-square or Fisher\'s and Mann-Whitney-U tests) to identify factors associated to parasitic infections. P-values <0.05 were considered significant. Molecular diagnosis was performed on positive samples identified by microscopy and/or ELISA technique, by nested-PCR targeting SSUrRNA and gp60 loci for 69 *Cryptosporidium* spp. samples, and the β-giardin locus for 79 *G. duodenalis* samples. A single intestinal parasitic infection were detected in 19.2% [(95% CI: 17.2– 21.3); 273/1424], of the stools monitored. *Cryptosporidium* spp. was the most common parasite (8.1%; 115/1424), followed by *Trichuris trichiura* (3.8%; 54/1424). Multiple intestinal parasites infections were observed in 26.0% (71/273) of the children, being the combination of *Ascaris lumbricoides* and *T. trichiura* (26.8%; 19/71) the most common. Children aged 5 years and over and those from Maputo had higher rates of intestinal parasitic infections (30.8%-20/65; 23.9% 178/745; respectively; p-value <0.05). The highest occurrence of parasites was observed in the rainy season 23.1% (191/828) compared to the dry season 13.6% (80/588) (p-value <0.001). Regarding the molecular diagnosis, 26% (18/69) and 28.9% (22/69) were positive for the *Cryptosporidium* SSU-rRNA and gp60 genes, respectively. Among the 79 isolates of *G. duodenalis*, 26.5% (21/79) of the samples were positive for the β-giardin gene. These preliminary results suggest that intestinal parasites are widespread among the population of children studied, with diarrhea, appearing to represent an important public health problem in Mozambique. The epidemiological and molecular analysis of the data is in progress.

Funding: PhD Grant, Camões – Instituto da Cooperação e da Língua, I.P. scholarship programme, Portugal

Abstract P4 – Day 1 (18:00-19:00 CET)

Detection of parasitic DNA in irrigation water using molecular method

Barbora Zalewska, Barbora Bartošová, Petra Vašíčková, Iva Slaná

Veterinary Research Institute, Brno, Czech Republic

Fruit and vegetables irrigated with polluted surface water can be cause of parasitical infection. Several studies have confirmed the irrigation water as a possible source of contamination to the produce. We inspected water from rivers intended for irrigation of greengrocery for pathogens including *Giardia intestinalis*, *Cryptosporidium parvum* and *Toxoplasma gondii*. Out of 15 tested river locations in the Czech Republic, we detected parasites in less than half of tested catchments. The results are not alarming; however, the regular monitoring and testing of the water quality is necessary and recommended.

Funding: Ministry of Agriculture of the Czech Republic (QK1810212)

Abstract O16 – Day 2 (12:10-12:30 CET)

Bronchopulmonary dysplasia can be associated with *Pneumocystis jirovecii* colonization in preterm infants

Marta Kicia[1], Magdalena Szydłowicz[1], Barbara Królak-Olejnik[2], Żaneta Zajączkowska[1], Dorota Paluszyńska[2], Anna Szczygieł[2], Olga Matos[3], Andrzej B. Hendrich[1], Sergio L. Vargas[4]

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Pneumocystis jirovecii, a parasitic fungus residing in human lungs, is a causative agent of *Pneumocystis* pneumonia (PcP), but can also persist as a colonization. The primary *Pneumocystis* infection is typically mild or even asymptomatic and therefore can often be misdiagnosed. However, it may have a pathological influence on the lung tissue and increase the risk of respiratory failure. The primary route of infection for *P. jirovecii* is airborne, but according to the previous reports, vertical transmission can also occur. Considering relatively high prevalence of *P. jirovecii* among pregnant women in the third trimester, such risk of transplacental transmission is likely. To date, a significant increase of respiratory distress syndrome has been documented in *Pneumocystis*-colonized preterm infants.

In our study we investigated the prevalence of *P. jirovecii* among newborns with respiratory distress syndrome, born prematurely, and/or with very low birth weight. Nasopharyngeal aspirates were obtained from preterm newborns (n=56) immediately after birth (approximately 10-15 minutes), and oral washes were collected from mothers (n=34) shortly after labour. Molecular detection was performed by nested-PCR amplifying partial sequence of *P. jirovecii* mtLSU rRNA gene, followed by sequencing and phylogenetic analyses. Microscopic examination was performed using direct immunofluorescence assay (DFA). *Pneumocystis* DNA was detected in eight (14%) of 56 newborns and in seven (21%) of 34 mothers. DFA confirmed cysts in 75% (6/8) infants and 57% (4/7) mothers with positive PCR result and all of them were considered colonized. Comparing to non-colonized infants, more frequent occurrence of bronchopulmonary dysplasia was seen in colonized ones (P=0.02). Moreover, extended duration of oxygen therapy, without statistical significance in *Pneumocystis*-colonized infants was observed.

Our study documents that blood-borne transmission via the transplacental route may be an alternative for *Pneumocystis* transmission. Correlation of colonization with bronchopulmonary dysplasia suggests a potential clinical importance of this pathogen in abnormal lung development, thus it should be considered in the etiologic workup of newborns suffering from respiratory disorders, especially in infants born prematurely.

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Abstract O17 – Day 2 (12:30-12:50 CET)

Pneumocystis jirovecii and fungal microbiota in preterm newborn infants with and without respiratory distress syndrome

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Recently, we have shown the high prevalence of *Pneumocystis jirovecii* colonization in preterm infants and its association with neonatal respiratory distress syndrome. Changes in the bacterial microbiota airway in preterm infants have related to respiratory disorders, such as bronchopulmonary dysplasia. However, the he airway fungal microbiota in infants has not been described. The aim of the present study was to investigate the airway fungal microbiota and its relation with *Pneumocystis* infection in preterm infants and the possible difference between preterms with and without respiratory distress syndrome.

Twenty-six infants, thirteen with respiratory distress syndrome and thirteen without it, were enrolled. Identification of *P. jirovecii* colonization was done analyzing nasopharyngeal aspirate samples by means of a 2-step protocol for nested polymerase chain reaction (PCR) assay that amplifies a portion of the gene encoding the mitochondrial large-subunit ribosomal RNA. Identification of fungal microbiota was performed by molecular techniques amplifying the fungal ITS gene by nested-PCR, and sequencing of positive samples.

Ascomycota phylum was identified in 92% of the preterms, while basidiomycota phylum was found in 38%. *Cladosporium* was the predominant genus in the airway of preterm infants at birth, increased in respiratory distress group (84% vs 61%). *Candida* was the second genus in frequency. There was a case of *Candida parapsilosis* in a preterm with respiratory distress syndrome. The prevalence of *Pneumocystis jirovecii* was double in respiratory distress group. *Malassezia* was the predominant genus in Basidiomycota phylum. There were also major taxonomic groups identified in respiratory distress group.

This is the first report that describes fungal microbiota in preterm infants and shows differences relates with neonatal respiratory distress syndrome. High prevalence of *P. jirovecii* in preterm infants with neonatal respiratory distress syndrome observed in this study sustain the role of *Pneumocystis* in this syndrome. However, future studies are needed to further define the role of mycobiota in general and *Pneumcoystis* in particular in respiratory diseases of neonates.

Funding: Instituto de Salud Carlos III, FIS PI19/01845

Abstract O18 – Day 2 (12:50-13:10 CET)

*Pneumocystis sui*s in swine farms: a co-infection study

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Pneumocystis is a genus of opportunistic fungal pathogens that reside exclusively in the lungs of their host. Although the fungus is responsible for severe and potentially lethal pneumonia in immunocompromised humans, rats and mice, its presence in swine has never been directly linked to a specific lung condition. The porcine respiratory disease complex (PRDC) is the term used to describe the polymicrobial nature of pig´s respiratory disease, which represents one of the main economic losses in swine industry. The current study was undertaken to investigate the contribution of *Pneumocystis* to this scenario.

We serially sampled serum and bronchoalveolar lavage (BAL) of 10 pigs/farm from 5 Austrian farms at 5 time-points (suckling piglets, 3 weeks old; weaned piglets, 2 and 3 months old; fattening pigs, 4 months old). DNA and RNA were isolated and qPCRs were run in order to quantify the presence of *P. suis* and other pulmonary pathogens including *Mycoplasma* spp., *Bordetella* spp., *Pasteurella multocida*, *Glaesserella parasuis*, *Actinobacillus pleuropneumoniae*, *Streptococcus suis*, Swine Influenza Virus (SIV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), and Porcine Circovirus 2 (PCV2).

Pneumocystis suis was detected in swine´s early life stages including the first and second weeks of life in 2 and 3 out of 5 farms, respectively, similar to what has been reported in human and rat newborns. By the second month of life, the organism was present in more than 50% of pigs in all 5 farms.

Pneumocystis was the most abundant organism in BAL (up to 1010 copies/ml), followed by *G. parasuis*, *M. hyorhinis* and *S. suis* (up to 108, 106 and 105 copies/ml, respectively). In four out of five farms, moreover, at the time points with the highest prevalence of the fungus we observed the highest prevalence of coinfection with 6 to 7 other pathogens. Notably, *G. parasuis* switched to pathogenic serovars when the bacteria was most abundant.

Surprisingly, the two viruses mainly responsible for inducing immunosuppression in swine, PCV2 and PRRSV, were not detected in any of the farms, questioning whether immunosuppression is required for *Pneumocystis* proliferation in swine.

While more studies are required to define the pathogenic role of *P. suis* in PRDC, its high prevalence in swine from an early age suggests a potentially important involvement in this scenario, even though having been largely neglected.

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Abstract O19 – Day 2 (13:15-13:35 CET)

Genetic diversity of *Cryptosporidium* spp. from ungulates in the United Arab Emirates

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Species of *Cryptosporidium* are parasitic apicomplexans, which cause the disease cryptosporidiosis in humans and a wide variety of animals worldwide. Infection occurs via the faecal-oral route and usually results in diarrhea, nausea, and vomiting. Symptoms can range from mild to severe and may result in high morbidity and/or death for young children and animals. There is currently no cure for the disease, and only prophylactic measures can help to prevent infection. In the United Arab Emirates (UAE) there have been sporadic, mostly genus level, reports of *Cryptosporidium* infections over the last few decades, in both humans and animals. To determine the genetic diversity of *Cryptosporidium* species in the UAE, we obtained samples from ungulates confirmed to be infected with *Cryptosporidium* species via microscopic analyses, through collaboration with the Central Veterinary Research Laboratory (CVRL) in Dubai. DNA sequences were obtained for two gene regions (SSU and gp60), which revealed the occurrence of five distinct *Cryptosporidium* species. This has been the first study to genetically identify *Cryptosporidium* species occurrence in the UAE, to our knowledge.

Funding: Survey of Gastrointestinal Parasites of Camels from Farms and Slaughterhouses in Al Ain (G00002877)

Abstract O20 – Day 2 (13:35-13:55 CET)

Prevalence of *Cryptosporidium* species in dairy cows' farms from the Netherlands, Belgium and France

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Cryptosporidium genus is comprised of protozoan parasites, which infect a wide range of hosts, causing a disease called cryptosporidiosis. In cattle-farms, the incidence of cryptosporidiosis results in high mortality and, consequently, is a source of considerable economic loss in the livestock industry. Infected animals also might act as major reservoir of *Cryptosporidium* spp., in particularly *C. parvum*, the most common cause of cryptosporidiosis in cattle, and thus pose a significant risk to other farms via breeding centers, to the trading of livestock, and to human health. This study, funded by the Interreg-2-seas, aims to assess *C. parvum* prevalence across dairy farms in the Netherlands, Belgium, and France, and further investigate the zoonotic potential of the circulating *C. parvum* subtypes. To accomplish this, 1084 cow stool samples, corresponding to 57 dairy-farms from all three countries, were analysed. Well-established protocols amplifying the 18S-rRNA and gp60 genes fragments, followed by DNA sequencing, were used for the detection and subtyping *C. parvum*; the DNA sequences obtained were further characterised using a combination of bioinformatics and phylogenetics methods. Our results show 20.8%, 25.67% and 24.86% prevalence of *Cryptosporidium* spp. in the Netherlands, Belgium, and France respectively. The GP60 subtyping carried out demonstrated a significant number of the *C. parvum*-positives belong to the IIa allelic family, which has been also detected in humans. Consequently, this study highlights how widespread is *C. parvum* in dairy-farms and endorses cattle as a major carrier of zoonotic *C. parvum* subtypes, which subsequently pose a significant threat to human health.

Funding: EU Interreg-2-Seas

Abstract O21 – Day 2 (13:55-14:15 CET)

Investigating the 2019-2021 prevalence of *Cryptosporidium* spp. across European dairy farms

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Cryptosporidiosis is a major diarrheal disease with a high global burden, caused by the apicomplexan parasite *Cryptosporidium*. *Cryptosporidium* spp. have a wide host range and geographical distribution, having been found to infect humans, livestock and wildlife worldwide. In particular, its presence in livestock poses a significant threat to human health due to the close proximity between them. *Cryptosporidium parvum* is a typically pathogenic strain, most commonly found in cattle, that has been found to infect humans too. Therefore, it is important to identify key sources of *C. parvum* infection to establish effective control measures and curb the potential public health risk. Between 2019 to 2021, 945 faecal samples were collected from 46 dairy farms across Belgium, Cyprus and the Netherlands and screened for the presence of *Cryptosporidium*. Overall prevalence was found to be 29.3%, 17.8% and 25.2%, respectively. All farms investigated in each country had various *Cryptosporidium* species present. In Cyprus, only *C. parvum* and *C. bovis* were found, with the former being the predominant species present. Further investigation is currently underway to identify the species present in the Dutch and Belgian farms. Our findings demonstrate that *Cryptosporidium* is widespread in the dairy farms in these countries, which might have a wider impact in the wellbeing of the animals. Additionally, high prevalence of *C. parvum* is consistent with previous studies on cattle and supports their role as a major zoonotic reservoir. Further investigations will be required to identify specific transmission routes to implement disease control strategies.

Funding: EU Interreg-2-Seas

Abstract O22 – Day 2 (14:25-14:45 CET)

Shelter animals as a reservoir of zoonotic pathogens

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Cryptosporidium spp., *Giardia intestinalis*, and microsporidia are ubiquitous intestinal pathogens occurring in broad range of vertebrate hosts. Individuals with impaired immunity are particularly susceptible to infection by these opportunistic microorganisms. Domesticated animals constitute important reservoir of these pathogens in the environment. As they excrete the dispersive forms with feces, they may pose a direct risk of infection or indirectly contribute to spread of these pathogens in human population. The aim of the present study was to investigate the occurrence of *Cryptosporidium* spp., *G. intestinalis*, and microsporidia (*Enterocytozoon bieneusi* and *Encephalitozoon* spp.) in dogs living in different animal shelters in Poland by using molecular methods. Fecal samples were collected from 101 dogs living in five animal shelters in Lower Silesia, Poland. Genomic DNA was extracted from samples and genus-specific nested PCR protocols were used to detect pathogens' DNA. Amplification of SSU (small subunit) rRNA and TPI (triosephosphate isomerase) loci was used for detection of *Cryptosporidium* spp. and *G. intestinalis*, respectively. For microsporidia, the partial sequence of the 16S rRNA gene, the entire ITS (internal transcribed spacer) region, and a partial sequence of the 5.8S rRNA gene amplification protocols were used. General pathogens' prevalence was 29% (29/101), with the highest reported for *G. intestinalis* – 20.8% (21/101). *Enterocytozoon bieneusi* was observed in 5.9% animals (6/101), while *Cryptosporidium* spp. and *Encephalitozoon* spp. in 2% (2/101) each. No co-infection was found. The high observed pathogens' prevalence in tested dogs, especially of *G. intestinalis*, confirms that domesticated animals are a significant source of zoonotic infection for their owners and other people or animals from the environment. This should be borne in mind by people at risk of development of symptoms of these opportunistic diseases who consider adoption of homeless animals.

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Abstract O23 – Day 2 (14:45-15:05 CET)

Tagging of pyruvate dehydrogenase candidates in *Diplonema papillatum*

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Diplonemids are a group of highly abundant and diverse marine microeukaryotes that belong to the phylum Euglenozoa and a sister clade to the well-studied, mostly parasitic kinetoplastids. Not much is known about the biology of diplonemids, as few species have been formally described and just one, *Diplonema papillatum*, has been studied at the molecular level. *Diplonema papillatum*, the type species of diplonemids, was recently established as a model organism, which can be genetically modified with ease. Homologous recombination is achieved by using long overlaps (about 1.5 kb) and can be utilised to endogenous tagging or knocking out specific genes. Pyruvate dehydrogenase (PDH) is a multienzyme complex which catalyses the decarboxylation of pyruvate and generates acyl-coenzyme A (CoA) and NADH. Since diplonemids have a fully active aerobic mitochondrion with a complete respiratory chain, the presence of a PDH activity to convert pyruvate into acetyl-CoA would be advantageous. At the genome level it seems that all diplonemids have lost the genes for the PDH complex E1 and E2 components (pdhA, pdhB and pdhC), but have retained pdhD (dihydrolipoamide dehydrogenase or E3). The E3 subunit of PDH is however also shared with other α-keto acid dehydrogenase (KADH) complexes, so it might function exclusively as a subunit of these other complexes in diplonemids. An alternative hypothesis proposes that a bacterial pyruvate decarboxylase (aceE), which is also present, may replace the missing E1 enzyme in a novel PDH complex in these organisms. Using endogenous gene tagging of pdhD and aceE and proteomics, we aimed to answer the following question: Is the diplonemid mitochondrial pyruvate dehydrogenase (PDH) complex homologous to the canonical complex found in mitochondria of other eukaryotes, or is it a hybrid enzyme also consisting of a bacterial pyruvate decarboxylase (aceE)? Results of these experiments will be presented.

Funding: Centre for research of pathogenicity and virulence of parasites (CZ.02.1.01/0.0/0.0/16_019/0000759)

Abstract O24 – Day 2 (15:05-15:25 CET)

Gene expression profiling of *Pneumocystis murina* response to copper

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Pneumocystis species are pathogenic fungi that cause *Pneumocystis* pneumonia (PCP) in mammals with weakened immune systems. These fungi demonstrate a parasitic life style adapted to colonize and infect the host lungs while maintaining an extracellular life cycle in the lung alveoli. Mammalian lungs are a high copper (Cu) environment being part of innate immunity. Of the essential metals, Cu is unique as it is required in trace amounts but quickly becomes highly toxic at elevated levels. Mammalian hosts have used both copper limitation, as in the case of intracellular fungi, and copper overload, which disrupts Fe–S clusters and displaces other metals from their sites in proteins, as defense mechanisms. In this work, we sought to reveal what molecular mechanisms the pathogen employs to cope with excessive Cu produced by host defense. Using a rodent model of PCP, we challenged the freshly extracted *Pneumocystis murina* (mouse infecting species) organisms with Cu^{2+} at 3 different concentrations for up to 5 hours. Sampling for RNA-seq was conducted at 3 time points. Depending on the comparison groups, we found 11 to 104 significant differentially expressed genes ($FC > 2$, $q < 0.05$). Among these, we found heavy metal ion transporters and thioredoxin known to be involved in Cu detoxification in other fungi. The other differentially expressed genes require further investigation. The understanding of molecular mechanisms employed by these pathogens to tolerate excessive Cu will enable the identification of virulence factors and new potential drug targets in these fungi.

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Abstract O25 – Day 2 (15:35-15:55 CET)

Human extraintestinal microsporidiosis caused by zoonotically transmitted *Encephalitozoon cuniculi*

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Encephalitozoon spp. (*E. cuniculi*, *E. hellem* and *E. intestinalis*) are one of the most clinically important microsporidia infecting wide spectrum of animal species. With their low host specificity and the fact that spores can be widely released to the environment via hosts' stool, urine and respiratory secretions, they pose a risk of zoonotic transmission. The primary site of Encephalitozoons infection is the epithelium of the small intestine but they are known to disseminate causing central nervous system, respiratory and urinary tract infections. As opportunistic pathogens, they cause symptomatic extraintestinal and disseminated infections mainly in immunocompromised hosts. When it comes to immunocompetent people, microsporidiosis has been described in children, the elderly, patients with malignant disease undergoing chemotherapy and a diabetics. Here, we describe the case of two immunocompetent bird owners suffering from *E. cuniculi*-caused microsporidiosis acquired from their infected pet birds. Two 41-year-old woman and man suffered from non-specific symptoms including frequent colds, night palpitations, headaches, strong fatigue, joint and muscle pain along with symptoms similar to bronchitis. Symptoms emerged after two years of breeding exotic birds. Over the period of two years more than half of the birds died due to various reasons including infectious diseases. *E. hellem* and *E. cuniculi* has been confirmed in birds' stool specimens and tissue samples in some of them. *E. cuniculi* was also confirmed in both patients' urine samples. In all tested samples microsporidia were confirmed by using both microscopic examination and genus-specific nested PCR followed by genotyping. Treatment with albendazole was administrated. Patients gradually improved and in follow-up examination three months after the treatment, their urine was negative for *E. cuniculi*. Patients' stool remained negative during the entire diagnostic process. Considering that birds are important source of Encephalitozoons, the risk of avian–human transmission of *E. cuniculi* documented in our study indicates the need for special awareness for people from risk group of opportunistic infections while breeding exotic birds.

Funding: Grant Agency of the Czech Republic (20-10706S)

Abstract O26 – Day 2 (15:55-16:15 CET)

The Trojan Horse of the immune system: Does *Encephalitozoon cuniculi* exploit migrating immune cells for their own dispersal in the host body?

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Microsporidia of the genus *Encephalitozoon* are generally connected with severe infections with lethal outcome in immunodeficient hosts. In immunocompetent hosts microsporidiosis typically establish a balanced host-parasite relationship that produces minimal clinically overt disease. Although the alimentary tract represents one of the main primary target tissue, the mechanisms of reaching other tissues during systemic microsporidian infections remain unclear.

In the present study we tested the relation between inflammation induction in immunodeficient and immunocompetent mice and presence of spores of *E. cuniculi* in inflammation induced and non-induced tissues by using molecular methods.

We reported the positive connection between inflammation induction and significant increase of *E. cuniculi* occurrence in inflammation foci in both immunodeficient Severe Combined Immuno Deficient (SCID) and immunocompetent BALB/c mice in the acute phase of infection. Moreover, the particular *E. cuniculi* genotypes differed in the speed and persistence of their predominance in induced loci compared to non-induced parts.

The results imply possible involvement of immune cells serving as vehicles transporting *E. cuniculi* purposefully across the whole host body towards inflammation. With increasing number of records of infections it is necessary to reconsider microsporidia as agents responsible for various pathologies. The elucidation of possible connection with pro-inflammatory immune responses represents an important challenge with consequences for human health and development of therapeutic strategies.

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Abstract O27 – Day 2 (16:15-16:35 CET)

Development of novel MetAP2 inhibitors for the treatment of microsporidiosis

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Current therapies for human microsporidiosis are suboptimal. We undertook a rational drug design approach to the development of medications for the treatment of microsporidiosis using structure activity relationships (SAR) for agents that inhibit methionine aminopeptidase type 2 (MetAP2). The initial choice in drug design is the selection of the target among the dozens of potential targets. MetAP2 is an extremely logical therapeutic target for these pathogens. Microsporidia lack methionine aminopeptidase type 1 (MetAP1) making MetAP2 an essential enzyme. Among eukaryotes this makes them highly susceptible to MetAP2 inhibitors and limits the toxicity of these compounds in their hosts as humans have both MetAP1 and MetAP2. Use of fumagillin and its derivatives, which are non-competitive inhibitors that covalently bind to and inhibit MetAP2 (but not MetAP1), has confirmed that inhibition of MetAP2 is an effective *in vitro* and *in vivo* therapeutic target for many species of microsporidia. In fact, fumagillin has been demonstrated to have efficacy in humans infected with *Enterocytozoon bieneusi*; however, its use has been limited by bone marrow toxicity. Our research group has cloned, expressed and determined the crystal structure of the MetAP2 of the microsporidian *Encephalitozoon cuniculi* (i.e. EcMetAP2) as well as developed yeast dependent on EcMetAP2 for growth. We have identified and cloned *Enterocytozoon bieneusi* MetAP2 (EbMetAP2). Exploiting differences in the structure of MetAP2 between host and pathogen should permit the design of selective therapeutic competitive inhibitors of MetAP2 with decreased host toxicity. These new inhibitors are tested *in vitro* and *in vivo* for efficacy and in an iterative process we use this information to refine our models and improve inhibitor design. Using this Limited Rational Design (LDR) approach we have generated new libraries based on our lead compounds that we plan to further modify to generate new libraries with improved selectivity and pharmacologic properties. Two lead compounds, BL6 and D63, from our initial LDR/SAR studies have shown increased selectivity for microsporidian MetAP2 and have efficacy in *in vitro* and *in vivo* models of microsporidiosis. Since MetAP2 is important in other pathogenic organisms, as well as microsporidia, these compound libraries can also lead to the identification of additional classes of drugs that could prove useful in the treatment of other infections.

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Abstract O28 – Day 2 (16:45-17:05 CET)

Comparison of prevalence, histopathology, and genetic relationship of *Pneumocystis* spp. in eleven mammal families

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Pneumocystis spp. are a group of highly diversified opportunistic fungi, which are adapted to the lungs of potentially all mammals. Because of its high impact on human health, research has concentrated on humans and rodent models. This study aimed to investigate the prevalence, histopathology, and genetic relationship of *Pneumocystis* in various livestock, pet and wild mammals.

Formalin-fixed and paraffin-embedded lung tissue samples of 240 Suidae, 104 Muridae, 96 Bovidae, 76 Felidae, 47 Mustelidae, 36 Equidae, 31 Canidae, 23 Camelidae, 21 Cervidae, 18 Pteropodidae and 14 Leporidae animals were screened by *in situ* hybridization (ISH) using a universal 18S rRNA probe for *Pneumocystis*, followed by H&E stain for determining histopathological lesions. Selected samples from 12 animal species were used to evaluate their genetic relationships.

The highest *Pneumocystis* prevalence was detected in Canidae (61%) followed by Mustelidae (55%), Suidae (48%), Bovidae (25%), Leporidae (21%), Cervidae (14%), Equidae (14%), Pteropodidae (11%), Camelidae (9%), Felidae (7%), and Muridae (7%). In all species, the number of cases with high ISH signal density was low (Leporidae 7% [rabbit]; Suidae: 6% [domestic pig, wild boar], Canidae: 3% [dog]; Bovidae: 2% [sheep, goat]; Muridae 2% [rat]; Mustelidae: 2% [badger]). *Pneumocystis* could be described for the first time in the following mammalian species: bison, blackbuck, chamois, water buffalo, alpaca, Bactrian camel, badger, marten, otter, skunk, and wolf. For most of the samples, microscopic exam of H&E-stained lung tissues showed minor lesions consistent with an interstitial or granulomatous pneumonia; *Pneumocystis* organisms were primarily located within the alveoli by ISH. In a small number of samples with severe infection, *Pneumocystis* organisms were also detected on the ciliated respiratory epithelium of bronchioles and bronchi. In only one sample from a sheep, a significantly higher *Pneumocystis* organism load was observed within the larger respiratory airways than within the alveoli. The extent of phagocytosis of the fungus by alveolar macrophages varied substantially. The sequences clustered according to the phylogenetic evolution of their host species.

The results of the present study underlined the wide distribution of *Pneumocystis* within the Mammal class. Prevalence varied substantially, with a low organism load in all samples, suggesting a colonization role in these animals. More comprehensive studies are required to better understand the epidemiology, host specificity and pathogenesis of *Pneumocystis.*

Abstract O29 – Day 2 (17:05-17:25 CET)

Genomic insights into the host specific adaptation of the *Pneumocystis* genus

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Pneumocystis jirovecii, the fungal agent of human *Pneumocystis* pneumonia, is closely related to macaque *Pneumocystis*. Little is known about other *Pneumocystis* species in distantly related mammals, none of which can establish infection in humans. The molecular basis of host specificity in *Pneumocystis* remains unknown as experiments are limited due to an inability to culture any species *in vitro*. To explore *Pneumocystis* evolutionary adaptations, we recently published a comparative genomics analysis of seven *Pneumocystis* species infecting different mammals (Cissé, O.H., Ma, L., Dekker, J.P. et al. Commun Biol 4, 305 (2021). https://doi.org/10.1038/s42003-021-01799-7). We have reconstructed the evolutionary history of *Pneumocystis* genus and highlighted genetic features such as the msg superfamily as potentially playing a role in host adaptation. Although our study and those of others have provided valuable insights, the details of host specificity remain elusive. Here I'll highlight challenges in investigating the host specificity of *Pneumocystis*, discuss complementary biologic methods focused on lineage specific gene families and present a roadmap of future collaborative genome sequencing projects.

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Abstract O30 – Day 2 (17:25-17:45 CET)

A novel compound against *Pneumocystis*

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This study investigates the effectiveness of an investigational compound A31S against *Pneumocystis carinii* and *Pneumocystis murina*. A31S is a bio-engineered reactive oxygen delivery system that replicates antimicrobial components found in honey. This patented technology provides a pool of hydrogen peroxide with sustained and prolonged therapeutic 72-hour release of hydrogen peroxide. The 72-hour 50% Inhibitory Concentration for A31S had very marked activity against both *P. carinii* and *P. murina in vitro*. Nektr Technologies plans to further develop this compound for different usages and delivery mechanisms such as for microbial infections of the skin and nails as well as inhalation formulation for respiratory tract infections.

Funding: National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, Department of Health and Human Services, under Contract No. 75N93019D00022, Task Order A04

Development of a novel loop-mediated isothermal amplification (LAMP) assays for the detection of *Tritrichomonas foetus* in the feces of domestic cats

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In the field of parasitology, loop-mediated isothermal amplification (LAMP) of DNA was tested as a molecular diagnostic alternative for PCR and was already successfully applied for detection of protozoan in humans or different animal hosts. LAMP assay is a simple, rapid, and cost-effective method that basically can be executed without sophisticated equipment needed for DNA amplification. In the present study, a novel LAMP assay for detection of *Tritrichomonas foetus* in the feces of domestic cats was developed. The designed LAMP primer set includes two outer primers (TF-βtub-F3: 5'-CCTTGTTCCATTCCCACGT-3'; TF-βtub-B3: 5'-TCTTTGGATGACATGCGTCC-3') and two inner primers (TF-βtub-FIP: 5'-AGAACATGATGGCTGCCTGCGAAGTGGGCGGAAACTGTG-3'; TF-βtub-BIP: 5'-GCGGTATTGTTGGCTTCCGCCTCCACTTCTTCATCGTCGG-3'). The following protocol for the LAMP assay was applied: the total reaction volume was 15 µl and contained 7.5 µl of Isothermal Mastermix (OptiGene), 1µl of each primer (20 pmol TF-βtub-FIB/TF-βtub-BIP and 5 pmol TF-βtub-F3/TF-βtub-B3), 1.5 µl of PCR-grade H2O and 2 µl of DNA template. Reactions were run for 90 min at 65 °C in the LightCycler 2.0 Instrument (Roche Diagnostics). Fluorescence was measured every 30 s with the channel setting F1/1. Methodical sensitivity evaluated by testing of serial 1:10 dilutions of genomic DNA from *T. foetus* (bovine genotype) allowed detection of DNA equivalent to 1 parasite. Specificity was evaluated by testing the amplification of DNAs (10 ng DNA per reaction) from various trichomonads and different animal pathogens. LAMP assay scored negative for *T. vaginalis*, *Trichomonas gallinae*, *Tetratrichomonas gallinarum*, and *Pentatrichomonas hominis* DNA. Overall negativity was also observed for the protozoan enteropathogen *Giardia duodenalis* and the enterobacterium *Escherichia coli*. As expected, assay amplified DNA from the *Tritrichomonas* genus, namely *Tritrichomonas mobilensis* and the porcine genotype of *T. foetus*. In conclusion, novel LAMP assay is basically suited to perform the specific and sensitive diagnosis of tritrichomonosis in different hosts.

Funding: Statutory funds of the National Veterinary Research Institute in Puławy, Poland (S/224)

Abstract P6 – Day 2 (18:00-19:00 CET)

Molecular identification of *Cryptosporidium* spp. and *Encephalitozoon* spp. in wild and farmed pigeons in the Czech Republic

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Cryptosporidium spp. and microsporidia are zoonotic parasites causing predominantly intestinal diseases. They are single-celled epicelullar/intracellular parasites which occur in several animals, both vertebrates and invertebrates.

Fecal samples from farmed (168) and wild (376) pigeons were collected in the Czech Republic during the period 2019–2021 and examined for the presence of specific DNA of *Cryptosporidium* spp. and *Encephalitozoon* spp. by PCR/sequencing targeting the small subunit rDNA (SSU) and 60 kDa glycoprotein (gp60) of *Cryptosporidium* and the internal transcribed spacer (ITS) of microsporidia. Moreover, the number of spores in samples was quantified using quantitative realtime PCR (qRT-PCR) targeting SSU. Tissue specimens from experimentally infected animals including the stomach, small and large intestine were sampled and processed for histopathological, electron microscopical and PCR examinations.

Out of the total of 544 animals, 17 and 38 were positive for *Cryptosporidium* spp. and *Encephalitozoon* spp., respectively. Sequence analysis of SSU gene revealed presence of *Cryptosporidium baileyi* (n=2), *C. meleagridis* (n=6), *C. parvum* (n=4), *C. muris* (n=2), *C. galli* (n=1) and *C. ornithophilus* (n=2). At the gp 60 locus, novel gp60 subtype of *C. meleagridis* (IIIk) was identified in all 6 positive animals. All isolates of *C. parvum* belonged to subtype IIa. Out of *Encephalitozoon*-positive pigeons, 31 were positive for *E. hellem* (genotypes 1A (n=13) and 2B (n=18)) and seven for *E. cuniculi* genotype I. Mixed infections, *E. hellem* and *C. ornithophilus* and *E. hellem* and *C. meleagridis* were detected in pigeons.

The novel isolate of *C. meleagridis* subtype IIIk obtained from naturally infected pigeons was experimentally infectious for chickens (*Gallus gallus* f. *domesticus*) with prepatent period 4 days, but not for SCID mice (*Mus musculus*). The infection was detected in the duodenum, ileum and colon.

Results of this study show that pigeons infected by cryptosporidia and microsporidia have the potential to transmit human-pathogenic parasites and could represent health risk for humans and domestic animals.

Funding: Grant Agency of the University of South Bohemia (007/2021/Z)

Abstract P7 – Day 2 (18:00-19:00 CET)

New viral discoveries in *Leptomonas pyrrochorris*, a rising RNA virus hotbed and model organism

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RNA viruses represent an immense and diverse group inhabiting a wide spectrum of living organisms. Viral associations with protists have been studied relatively poorly, with the exception of those in trypanosomatids. The discovery of the Leishmania RNA virus (LRV1) and the mechanisms of increased pathogenicity of LRV1-bearing *Leishmania guyanensis* attracted attention to the study of viruses in these parasitic flagellates. Here, we studied the presence and diversity of RNA viruses in a monoxenous relative of *Leishmania*, *Leptomonas pyrrhocoris*, which parasitizes a widely distributed firebug *Pyrrhocorris apterus*. We screened 98 axenic cultures of this trypanosomatid isolated from across Europe for the presence of dsRNA and obtained NGS data from positive samples. In addition to the two viral species previously described in *L. pyrrhocoris*, namely a Tombus-like virus (Leppyr-TLV) and Ostravirus, we documented and characterized numerous members of the families Narnaviridae and trypanosomatid-specific Leishbunyaviridae. Our phylogenetic analyses demonstrated that within these two latter groups viral associations with *L. pyrrhocoris* originated independently several times. This trypanosomatid species seems to be a hotbed for viruses and that can be associated with the omnivorous nature of its insect host.

Funding: Czech Science Foundation (20-22689S)

Abstract P8 – Day 2 (18:00-19:00 CET)

Fresh fruits and vegetables contamination by intestinal parasites in Maputo markets and supermarkets, Mozambique

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Intestinal parasites transmission by food, especially fresh fruits and vegetables raises great concern. This study aims to understand the occurrence of intestinal parasites in general and *Giardia duodenalis*, *Cryptosporidium* spp. and *Enterocytozon bieneusi* specifically in fresh fruits/vegetables sold in the main municipal markets of Maputo city, Mozambique. A total of 321 samples of fresh horticultural products were collected (carrot, coriander, green pepper, lettuce, parsley, pointed white and Portuguese cabbage and tomato) in the rainy and dry seasons of 2019, in Maputo markets, and in Infulene Valley, a production zone. The samples were studied by parasitological and molecular methods. Among the horticultural products monitored by microscopy and/or PCR, the species most frequently detected were from the *Entamoeba* group: cysts of *Entamoeba* spp. (6.5%; 21/321), *Entamoeba coli* (6.2%; 20/321), *Entamoeba histolytica*/*E. dispar* complex (2.8%; 9/321), *Entamoeba hartmani* (0.3%; 1/321) and *Iodamoeba bustchlii* (0.3%; 1/321). The following most frequent protozoans were *Giardia duodenalis* with 3.7% (12/321), *Balantidium coli* with 3.1% (10/321) and *Endolimax nana*, *Chilomastix mesnili* and *Blastocystis hominis* with 1.2% (4/321), each. The microsporidian *Enterocytozoon bieneusi* was identified in 1.2% (4/321) of the samples. Eggs of helminths and nematodes were both found with similar percentage 1.2% (4/321) and *Strongyloides stercoralis* was detected in 0.9% (3/321) of the samples. A high level of intestinal parasites contamination of fresh horticultural products (24.9%) was observed. A higher number of positive cases was observed in the dry season than in the rainy season (29.2% vs 20.3%), being this difference statistically significant ($p=0.000$). The leafy vegetables presented the highest values of contamination. Supermarkets and markets have a similar likelihood of contamination. The presence of important pathogenic intestinal parasites were identified in the horticultural products, from the markets and supermarkets, even in those with better hygiene and sanitary facilities. This fact must be taken into account when planning their management, in order to reduce the risk of fresh food contamination by parasites, and when training for good practices in handling fresh food, to prevent foodborne diseases outbreaks.

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Abstract P9 – Day 2 (18:00-19:00 CET)

Blastocystis **infection in children undergoing allogeneic hematopoietic cell transplantation in Poland. Preliminary results**

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Parasitic infections are rarely documented in children undergoing allogeneic hematopoietic cell transplantation and they are the most understudied. In addition, medical advances such as allogeneic hematopoietic cell transplantation compromise the immune system of patients, which render them vulnerable to previously rare opportunistic organisms or to the exacerbation of latent parasitic infections. *Blastocystis* is the most common unicellular parasite found in human intestine and a variety of animal hosts. The human *Blastocystis* infection rates range from 10% in developed countries up to 100% in developing countries. The clinical symptoms of *B. hominis* infection are nonspecific and include abdominal pain, nausea, cramps and diarrhea.

The aim of this study was to determine the incidence of *Blastocystis* infection in the pediatric patients undergoing allogeneic hematopoietic cell transplantation.

A total of 37 faecal samples from children undergoing allo-HSCT, were analyzed using xenic in vitro culture (XIVC) with a modified Jones' medium and molecular methods (PCR). Gene fragment of SSU-rRNA was amplified with forward primer RD5 (5′-ATCTGGTTGATCCTGCCAGT-3′) and reverse primer BhRDr (5′-GAGCTTTTTAACTGCAACAACG-3′). PCR products were sequenced and the resulting sequences were compared to the *Blastocystis* sequences deposited in the GenBank database. Phylogenetic analysis using Bayesian inference was performed, taking into account 24 reference sequences representing the *Blastocystis* ST1–ST9 subtypes.

Blastocystis was found in 3 (8%) study participants. Preliminary molecular results evidenced *Blastocystis* ST3 which is typical anthroponotic subtype.

The close monitoring of parasitic infection, including *Blastocystis,* in pediatric patients with severe compromise the immune system is critical to reduce the risk of complications during intensive medical therapy.

Funding: UMW A060.21.048

Abstract O31 – Day 3 (12:10-12:30 CET)

Prevalence and diversity of *Cryptosporidium* of red squirrels and European ground squirrel in the Czech Republic and Slovakia

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A total of 330 synanthropic rodents, including 173 European ground squirrel (*Spermophilus citellus*) and 157 red squirrels (*Sciurus vulgaris*) from the Czech Republic and Slovakia, were examined as a part of the *Cryptosporidium* prevalence study. The ground squirrel droppings were collected from their natural habitat. In total, samples were obtained from 42 localities in the Czech Republic. Red squirrel droppings were collected from the wild, from rescue stations in the Czech Republic (12 sites) and Slovakia (15 sites) and from carcasses found by roadsides. None of the faecal samples had a consistency that would indicate diarrhoea. Out of the 330 animals examined, 8.2% (n=27) were positive for the presence of *Cryptosporidium*-specific DNA at the SSU, actin and gp60 loci. Microscopic methods showed the presence of oocysts in only five juvenile red squirrels (1.5%). The intensity of infection in microscopically positive individuals ranged from 22,000 to 72,000 oocysts per gram of faeces (OPG). Of the total number of examined squirrels positive for *Cryptosporidium*, 8% (n=21) originated from the wild and 7.6% (n=5) from rescue stations. Phylogenetic analyses based on partial sequences of genes encoding SSU and actin revealed the presence of *Cryptosporidium* sp. ferret genotype in all positive red squirrel samples. A tree constructed from partial sequences of the gene encoding gp60 showed the presence of three different *Cryptosporidium* sp. ferret genotype groups clustering to two previously detected subtypes of this genotype (VIIIb and VIIIc) and one new subtype group (VIIIe). None of the SSU and actin sequences recovered from the ground squirrels were identical to sequences deposited in GenBank. Phylogenetic analyses revealed the presence of five previously undescribed genotypes, which we have been named *Cryptosporidium* sp. ground squirrel genotype IV–VIII.

Funding: Grant Agency of the University of South Bohemia (006/2021/Z) and Czech Science Foundation (21-23773S)

Abstract O32 – Day 3 (12:30-12:50 CET)

First study to characterize the role of epigenetics in the biology of the Apicomplexan parasite *Cryptosporidium parvum*

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The role of epigenetic mechanisms known to be targeted by other pathogens in order to hijack cellular host functions, are unexplored concerning *Cryptosporidium* infection. The objective of the study was to explore the role of epigenetics and in particular of histone lysine methylation in *Cryptosporidium* and in the host.

In silico analysis for the identification of epigenetic players was performed. Phylogenetic analysis allowed the identification of putative substrate specificities of the lysine methylation regulators. Analysis of the gene expression of these regulators was performed by RT-PCR during *Cryptosporidium* infection *in vitro*. Immunofluorescence analysis of *Cryptosporidium*-infected cells with antibodies recognizing specific methylated-Lysine modifications in the parasite as well as in the host was performed.

In silico, 11 putative lysine methyltransferases (KMTs) were identified. Further, alignment of the SET-domain sequences of the putative KMTs with the representatives of the SET domain subfamilies classified the predicted *C. parvum* KMTs into 5 subfamilies: CP SET1, CP_SET2, CP_SET8, CP_KMTox and CP_AKMT. CP_SET1, CP_SET2 and CP_SET8 are predicted as histone lysine methyltransferases (HKMTs) while CP_KMTox and CP_AKMT have been identified as KMTs, found exclusively in the phylum Apicomplexa. We found no evidence of histones lysine demethylases. Phylogenetic analysis confirmed the classification of the 5 subfamilies of *C. parvum* KMTs and their associated putative substrate specificities. Site specific methylation at lysine 4 (K4) and K36 of histone H3 and K20 of histone H4 in sporozoite stage of *C. parvum* confirmed substrate specificities of the identified HKMTs. We compared the gene expression profile of these putative KMTs during different stages of the parasite development, to observe HKMTs (CP_SET1, CP_SET2) to be highly expressed during the trophozoite stage of the parasite. Consistently, the specific histone lysine marks also displayed dynamic changes during the parasite development. Furthermore, we showed that the infection induces global downregulation of the histone lysine methyl marks in the host cell.

This study indicates the importance of epigenetic mechanisms in gene regulation of virulence factors of the enteric parasite *Cryptosporidium* and the potential to exploit host epigenetic regulation to its advantage.

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Collaborator: Dr J Weitzman, University Paris Diderot, France

Abstract O33 – Day 3 (12:50-13:10 CET)

Biology of *Cryptosporidium* sp. chipmunk genotype I

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Infections caused by *Cryptosporidium* sp. chipmunk genotype I were previously described in various hosts such as humans, chipmunks, squirrels and several species of rodents. In this study an isolate of *Cryptosporidium* sp. chipmunk genotype I originally obtained from naturally infected wild eastern grey squirrel (*Sciurus carolinensis*) trapped in Northern Italy, was used for study of its biological characteristic including oocyst size, host specificity, course and location of infection, and pathogenicity. To determine the susceptibility to *Cryptosporidium* sp. chipmunk genotype I red squirrels (*Sciurus vulgaris*) and mice (*Mus musculus*) of various strains, gerbils (*Meriones unguiculatus*) and guinea pigs (*Cavia porcelus*) were used for experimental infections. While red squirrels, mice and gerbils were susceptible to infection, guinea pigs did not produce detectable infection by microscopy and PCR. SCID mice and red squirrels positive for *Cryptosporidium* sp. chipmunk genotype I shed microscopically detectable oocysts, which measured 4.8–5.3 × 4.7–5.0 μm with a prepatent period of 9–11 days. The infection was exclusively localised in caecum and colon. Mucosal smears and tissue from *Cryptosporidium*-affected intestinal parts were stained with Wright methods and prepared for histology and scanning and transmission electron microscopy, respectively, to visualize the characteristic morphological structures of developmental stages. Using Wright staining and transmission electron microscopy we were able to identified trophozoites, Type I and Type II meronts, merozoites, macrogamonts, microgamonts, zygotes and oocysts.

Funding: Czech Science Foundation (21-23773S) and Grant Agency of the University of South Bohemia (017/2020/Z)

Abstract O34 – Day 3 (13:15-13:35 CET)

Extended treatment with the long-acting Echinocandin, Rezafungin, eliminates *Pneumocystis* pneumonia in a murine model

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Rezafungin is a novel, next generation echinocandin in Phase 3 development for prevention of invasive fungal disease caused by *Candida* spp., *Aspergillus* spp., and *Pneumocystis jirovecii* in blood and marrow transplantation patients. Typically, these patients receive standard antifungal prophylaxis comprised of an azole for *Candida* and *Aspergillus*, plus trimethoprim-sulfamethoxazole (TMP-SMX) for *P. jirovecii* pneumonia, despite drug-drug-interactions and intolerability that may limit their use. Previous studies with commercially available echinocandins in the same immunosuppressed model demonstrated depletion of asci after 3 weeks of treatment, with large populations lacking asci remaining in the lungs. In the present study, the *in vivo* effects of rezafungin against *P. murina* were evaluated in immunosuppressed mouse models of prophylaxis and treatment using microscopic and qPCR detection methods. In the prophylaxis model, immunosuppressed mice inoculated with *P. murina* were administered TMP-SMX (50/250 mg/kg 1x/week or 3×/week), caspofungin (5 mg/kg 3×/week), rezafungin (20 mg/kg, 3×/week, or 1×/week; 5 mg/kg, 3×/week) intraperitoneally for 2, 4, 6, and 8 weeks. Rezafungin administered for 4 weeks prevented *P. murina* from developing infection after rezafungin was discontinued. In the treatment model, immunosuppressed mice infected with a moderate *P. murina* pneumonia (5–6 wks) were treated with rezafungin 20 mg/kg 3×/week intraperitoneally for 2, 4, 6, and 8 weeks. Treatment with rezafungin for 8 weeks resulted in eradication of all *P. murina*, indicating that the remaining organisms cannot be sustained by asexual reproduction in the absence of asci during extended rezafungin treatment. Collectively, these studies showed that prophylaxis and treatment with rezafungin could prevent and eradicate *P. murina* pneumonia. These findings support the obligate role of sexual reproduction for survival and growth of *Pneumocystis* spp. and warrant further investigation for treatment of *P. jirovecii* pneumonia in humans.

Funding: Cidara Therapeutics, Inc., San Diego, CA

Abstract O35 – Day 3 (13:35-13:55 CET)

Proteome of extracellular vesicles from Pneumocystis-infected rat lungs

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Prevalence of the lethal pneumonia caused by the host-obligate fungal pathogen *Pneumocystis jirovecii* is rising with increased use of immunosuppressive therapies. Fungi in this genus reside in the lung alveoli of immunocompromised mammals (including humans) where they preferentially attach to the alveolar type I (ATI) cells, block gas exchange, and cause *Pneumocystis* pneumonia (PCP). *Pneumocystis* spp. have a highly compact genome with considerable loss of many biological pathways. Notably, *Pneumocystis* is unable to synthesize all amino acids and ergosterol, the major sterol in most fungi. Instead, cholesterol is the major sterol found in *Pneumocystis*. However, the mechanism of cholesterol and nutrient acquisition remains unclear. Extracellular vesicles (EVs) have long been observed in *Pneumocystis*-infected lungs, but their function has never been explored, due in part to a lack of facilitative technology. Alveolar type I cells, the cells responsible for gas exchange, have been shown to increase EV release in response to antigenic stimuli. These EVs have been shown to be enriched in various biological components. Specifically, EVs contain cholesterol and free amino acids. Additionally, previous studies in our lab have shown the presence of rat Podoplanin (PDPN), an ATI cell protein, contained within *P. carinii*. Since EVs contain PDPN, cholesterol, and free amino acids, this observation may suggest that *P. carinii* can uptake extracellular EVs to supplement its metabolic needs. Using mass spectrometry of *P. carinii*-infected bronchial alveolar lavage fluid (BALF) from immunosuppressed rats, we identified that EVs contain proteins with functional classifications in response to stimuli, biological adhesion, locomotion, and immune system processes. Additionally, homologs of *P. carinii* proteins associated with molecular chaperones, EV secretion, meiosis, and metabolic processes were identified. Collectively, these data suggest that both the host and *Pneumocystis* secrete EVs during infection. Completing the *Pneumocystis* EV proteome will aid our understanding of *Pneumocystis* survival in the lungs, help to develop an in vitro cell culture, and accelerate the discovery of therapeutic options.

Funding: NIH R01HL146266, VA I01BX004441, GSG Research Fellowship

Abstract O36 – Day 3 (13:55-14:55 CET)

The formation of asci is necessary for growth and transmission of *Pneumocystis murina* infection

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The life cycle of *Pneumocystis* is not well understood but is thought to utilize both asexual and sexual replication strategies. We have reported that treatment of *Pneumocystis murina*-infected mice with echinocandins (ECH) deplete the asci containing β-1,3-D-glucan (BG) and do not allow the mice to transmit the infection. However, large numbers of life cycle forms that do not express BG remain in the infected lungs. In these studies, we continue to interrogate the hypothesis that asci are required for completion of the *Pneumocystis* life cycle.

In Study 1, the replication competency of *P. murina* from ECH treated mice was investigated. *Pneumocystis murina*-infected mice were treated with the ECH for 3 weeks and sacrificed. The remaining organisms from these mice were then inoculated into immunosuppressed donor mice, and these mice were continued on the same anidulafungin treatment for up to 6 weeks.

In Study 2, we sought to identify the mode(s) of replication in mice prophylactically treated with anidulafungin. Mice were infected with *P. murina* and started on the ECH at the same time. Mice were then sacrificed weekly for 6 weeks. At all time points, the organisms were evaluated by qPCR, β-1,3-D-glucan assay (Glucatell™) and light microscopy.

In *P. murina*-infected mice treated with anidulafungin for 3 weeks, no asci were observed, but large numbers of other life cycle forms were observed as expected. However, when donor mice were inoculated with anidulafungin-treated *Pneumocystis murina* and the ECH treatment was continued, no organisms were seen at weeks 2-6 by microscopy. Also, β-1,3-D-glucan levels in these mice remained low, indicating asci were not formed in the lungs of ECH treated mice. In mice prophylactically treated with anidulafungin for up to 6 weeks, small numbers of life cycle forms were observed at weeks 5 and 6 but BG levels in these mice also remained very low. Genes mediating BG synthesis, meiosis, and cell remodeling were found to be upregulated in the treated mice by qPCR indicating that *Pneumocystis murina* is attempting to undergo sexual replication but cannot due to a lack of BG.

Collectively, these data suggest that *Pneumocystis* is unable to complete its life cycle in the absence of asci and therefore, sexual replication is necessary for proliferation to infection. Studies are underway to track the emergence of the sexual cycle by identifying gene signals and biomarkers and determining when the infection can be transmitted after cessation of ECH treatment.

Funding: NIH NHLBI RO1 and Veterans Affairs Merit grants

Abstract O37 – Day 3 (15:35-15:55 CET)

Phylogenomics and comparative transcriptomics of secondarily free-living diplomonads

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Diplomonads are anaerobic flagellated microbial eukaryotes that lack conventional mitochondria. They belong within Metamonada and their representatives are primarily host-associated commensals or parasites that reside in the intestinal tract of animals, including humans (e.g., *Giardia intestinalis*). In addition, a number of free-living diplomonads inhabiting freshwater and marine anoxic sediments have been described (e.g., *Hexamita inflata*). Of particular interest, current molecular phylogenetic trees show that the free-living taxa appear to be nested within a clade of host-associated species, suggesting a reversal from host-dependence to become secondarily free-living. However, all available phylogenetic studies suffer from low taxon and gene sampling, especially among the free-living diplomonads. Secondary reversion to a free-living life style is rare and thought to be an evolutionary difficult process. Endobiotic diplomonads have adapted to their environment by scavenging nutrients and metabolites from the host and streamlining metabolic pathways by the loss of genes that are often essential for a free-living life-strategy. If free-living diplomonads arose from host-associated ancestors, they would be required to replenish their metabolic capacity by re-acquiring essential gene functions lost in their ancestors. Indeed, a previous transcriptomic investigation of the putatively secondarily free-living diplomonad *Trepomonas* sp. PC1 suggested that it acquired several genes by horizontal gene transfer (HGT), increasing its metabolic capacity and facilitating the reversal back to a free-living lifestyle.

In this study we sequenced the transcriptomes of an additional 12 free-living and 2 endobiotic diplomonad isolates for phylogenomic and comparative analyses. We have robustly resolved evolutionary history of diplomonads to create a solid framework for subsequent comparative gene analyses. The 14 newly sequenced transcriptomes were investigated for genes originating from HGT, especially those that may be necessary to live in free-living environments. Comparative analyses exploring the genomic basis enabling the transition from parasitism to a free-living life strategy is ongoing.

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Abstract O38 – Day 3 (15:55-16:15 CET)

A hybrid sequencing strategy to produce whole genomes of *Giardia duodenalis* using cysts purified directly from fecal samples

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The pathogenic intestinal protozoan *Giardia duodenalis* is a common parasite of humans and many other animals. Transmission occurs via the fecal-oral route with contaminated food or water being common modes of infection. *Giardia duodenalis* is a species complex divided into groups called assemblages based on the genetic characterization. Assemblages display varying degrees of host specificity. However, the genetic basis of host specificity and zoonotic potential of *Giardia* assemblages remains undetermined. Whole genome sequencing and comparative genomics of multiple *Giardia* assemblages and isolates can help to elucidate these traits, but such analyses require data from the genomes of many *Giardia* isolates obtained from multiple hosts. Comparative genomics for *Giardia* is currently hindered by a lack of genomes from diverse isolates due in part to the difficulties associated with obtaining quality genetic material from complex matrixes such as feces. To address this need, whole genome sequencing using cysts purified directly from two isolates obtained from naturally infected hosts was performed. The first isolate came from a heavily infected cat presenting with diarrhea and was determined to be assemblage A. The second isolate came from a heavily infected diarrheic dog which continued to shed cysts following metronidazole treatment and was determined to be assemblage D. Cysts from both samples were cleaned and concentrated using a cesium chloride gradient and immunomagnetic separation. Initial assemblage identification was performed by PCR and Sanger sequencing of the triosephosphate isomerase, beta-giardin, and glutamate dehydrogenase genes. Whole genome sequencing was performed using an Illumina MiSeq and an Oxford Nanopore MinION to obtain both short and long reads to facilitate a hybrid assembly strategy. We successfully generated MinION and Illumina sequence data for both isolates, and analyses of hybrid assemblies demonstrated that it is possible to achieve good coverage of the *Giardia* genome as a whole and for genes of interest from fecal isolates. Assembly strategies and comparisons between genomes obtained from fecal isolates in this study and reference genomes from culture will be discussed. Further comparisons of protein coding regions and potential virulence factors from these genomes may assist in understanding how strain and assemblage level genetic differences could influence the pathogenicity and zoonotic potential of *Giardia*.

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Abstract O39 – Day 3 (16:15-16:35 CET)

Next generation amplicon sequencing of the *Giardia* beta-giardin gene for detection of mixed assemblage infections

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Giardia duodenalis is an enteric protozoan parasite which infects humans and many other animals around the world. *Giardia duodenalis* is a species complex that is divided into eight subgroups called assemblages. Assemblages are morphologically indistinguishable but show consistent genetic uniqueness and display varying degrees of host specificity. *Giardia* mixed assemblage infections with multiple assemblages observed in the same host have been documented, however the frequency and importance of mixed infections have not been fully characterized. This field has been hindered by the lack of sequencing technologies which can readily detect mixed infections. To address this problem and explore mixed assemblage infections in multiple hosts, we have developed a next generation amplicon sequencing (NGS) protocol and analysis pipeline for detecting *Giardia* assemblages using the beta-giardin gene. The NGS protocol was validated using 37 isolates that included *Giardia muris* and six assemblages (A–F) of *Giardia duodenalis* obtained from seven different hosts. Comparisons between NGS and traditional PCR and direct Sanger sequencing were performed to determine the ability of NGS to detect *Giardia* species, assemblages, and mixed assemblage infections. The same assemblage was observed in all samples by both methods demonstrating that NGS works as well as PCR and Sanger sequencing for assemblage detection. Furthermore, NGS has the added benefit of detecting mixed assemblage infections, low abundance assemblages, and intra-assemblage variation in samples which would have been missed using direct Sanger sequencing alone. Together these findings indicate that NGS is a powerful tool for exploring *Giardia* assemblage diversity in infected hosts which may aide in understanding *Giardia* epidemiology and zoonotic transmission.

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