VIIth International Giardia and Cryptosporidium Conference

Favennec Loïc

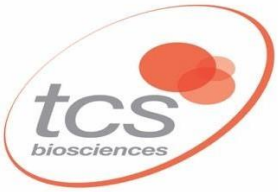
VIIth International *Giardia* and *Cryptosporidium* Conference

Conference Abstracts on USB Key

June 23-26, 2019

UFR Santé, University of Rouen, France

Acknowledgments



Sunday Presentations

9h00-12h30 **REGISTRATION ON SITE**
12h30-13h30 **Lunch on site**

		ORAL SESSION AMPHI 350 : «OMES» 1	13h30 – 16h00 Chair : SVARD S - LALLE M
13h30-13h50	Su-O1	Omics as a tool to understand giardiasis	Svard S
13h50-14h02	Su-O2	The new *Giardia intestinalis* and *Giardia muris* genomes	Xu, F, Jerlström-Hultqvist J, Jiménez-González A, Einarsson, E, Ástvaldsson, Á, Peirasmaki, D, Eckmann, L, Andersson, O. J, Svärd, G. S.
14h02- 14h14	Su-O3	Re-discovery of *Giardiavirus:* genomic and functional in deep analysis of different *Giardiavirus* strain in naturally infected *Giardia duodenalis* isolates	Marucci G., Bertuccini L., Cecchetti S., Zullino I., Dayaram A., McEwen G., Greenwood A.D., Lalle M.
14h14-14h26	Su-O4	Annotation of the *Giardia* proteome through structure-based homology and machine learning	Ansell BRE, Pope BJ, Georgeson P, Emery-Corbin SJ, Jex AR
14h26-14h38	Su-O5	Exploring genomic variation in *Giardia duodenalis* using well characterised reference isolates	Jex AR, Fang X, Xu F, Wiesz F, Tichkule S, Ansell B, Emery S, Müller N, Lalle M, Cacciò S, Svärd , Gasser RB
14h38-14h50	Su- O6	Investigation of a waterborne outbreak of giardiasis in Italy by comparative genomics	Sannella AR, Zuccaro V, Bruno A, Novati S, Grande R, Maserati R, Lalle M, Cacciò SM
14h50-15h00		**10-minute Break**	
15h00-15h12	Su- O7	Whole genome sequencing of assemblage C and D of *Giardia intestinalis* from the dog	Kooyman F, Wagenaar J, Zomer A
15h12-15h24	Su- O8	Characterization of flavohemoprotein in *Giardia* isolates	Saghaug CS, Klotz C, Kallio JP, Pedersen TOE, Rafferty S, Aebischer A, Langeland N and Hanevik K
15h24-15h36	Su- O9	Mitosomal dynamics in *Giardia intestinalis*	Voleman L. Tůmová P. Wanner G. Doležal P
15h36 -15h48	So- O10	Whole Genome Sequencing of *Giardia duodenalis* Isolates from a Cat and a Dog	Maloney J., Molokin A., Santin M
15h48-16h00	Su- O11	Unusual localization and potential functions of three paralogs of a heme protein in *Giardia intestinalis*	Yee J, Dayer G, Sajer B, Pyrih J,Tachezy J,Rafferty S
16h00-16h24		**Coffee break**	16h00–18h22 Chair: ORTEGA PIERREZ G BALLET JJ
16h24-16h36	Su- O12	Crypto and *Giardia*DB.org: free, online resources bringing Omics to every scientist.	Warrenfeltz S, Kissinger JC
16h36-16h48	Su- O13	Allele Sequence Heterozigosity and recombination patterns detected by Multiplex PCR amplification and Massive Parallel Sequencing of single cysts of *Giardia duodenalis* assemblage B.	Gabín-García L.B. Bartolomé C. Llovo J. Méndez S. Sobrino, B. Maside, X
16h48-17h00	Su- O14	Variation in Promoter Sequences Drives Differential Expression of GLORF-C4 in the Human Infective Assemblages of *Giardia duodenalis*	Black, A. Mousley, C; Paget, T. Steuart, R
17h00-17h12	Su- O15	The two nuclei of *Giardia intestinalis* during mitosis.	Tůmová, P, Wanner, G, and Nohýnková, E
17h12-17h24	Su- O16	The protein methylation network in the early-branching protozoan parasite, *Giardia duodenalis*	Emery-Corbin SJ, Tichkule S, Balan B, Strohlein A, Cooper C, Ansell BRE, Baker L, Vuong D, Lacey E, Svärd SG, Jex AR
17h24-17h36	Su- O17	High-resolution, quantitative proteome of *Giardia duodenalis* during encystation	Balan B, Emery-Corbin S , Sandow J. Webb A , Jex A
17h36-17h46		**10-minute break**	
17h46-17h58	Su- O18	Impact of early-life exposure to *Cryptosporidium parvum* infection on intestinal homeostasis at adulthood.	Lacroix-Lamandé S, Ménard S, Baillou A, Virologeux-Payant I, Sallé G, Olier M, Nicolosi A, Pezier T, Laurent F.
17h58-18h10	Su- O19	Role of Paneth cells during infection of neonatal mice by *Cryptosporidium parvum*.	Nicolosi A, Pezier T, Guesdon W, Pedron T, Laurent F, Lacroix-Lamandé S.
18h10-18h22	Su- O20	Identification of heparin-binding proteins in *Cryptosporidium parvum*	Yin J, Wang D, Zhang T, Gao X,Jiao X,Zhang N, Lu H

Monday Presentations

		ORAL SESSION AMPHI 350- HOST PARASITE INTERACTIONS	8h00-9h20 :Chair : LAURENT F. CANDON S.
8h00-8h20	Mo-O1	How innate immune responses shape *Cryptosporidium* infection	Laurent F.
8h20-8h32	Mo- O2	Shifts in Treg/Th17 balance correlate with differential susceptibility to infection with *Giardia muris*	Yordanova, A. I., Heimesaat, M. M, Hartmann, S., Rausch S.
8h32-8h44	Mo- O3	Variability in giardiasis: roles for immune responses and microbiota	Singer, S.M, Li, E, Keselman, A, Maloney, J, Zachary, E, Coelho, C, da Silva Lanna, M. C Fink, M
8h44-8h56	Mo- O4	Temporal and spatial analysis of the intestinal IL-17A response following a *Giardia* infection	Paerewijck O, Gagnaire A, Maertens B Geldhof P.
8h56-9h08	Mo- O5	Transcriptional Profiling of Differentiated CaCo-2 Intestinal Epithelial Cells Response to *Giardia Intestinalis* during Early Onset of in vitro Interactions: Insights into the Pathways of Cytokine Production and Regulation	Maayeh S, Knörr L, Sköld K, Hoeppner Mp, Grabherr M, Stadelmann B, Svärd Sg
9h08-9h20	Mo- O6	Human small intestinal organoids - a new model to investigate *Giardia* sp. infection	Kraft MR, Klotz C, Aebischer T
9h20-9h40		**OPENING CEREMONY**	9h40-10h30 Chair : WIDMER G. GARGALA G.
9h40-9h52	Mo- O7	*In vivo and in vitro* studies of the host-parasite interactions of *Spironucleus salmonicida* and the Atlantic salmon	Ástvaldsson A, Stairs C, Xu F, Haag L, Alfjorden A, Janson E, Svärd, SG
9h52-10h04	Mo- O8	Deprivation of dietary fiber enhances susceptibility of mice to cryptosporidiosis	Oliveira, B.C.M, Bresciani, K.D.S, Widmer, G.
10h04-10h16	Mo- O9	Domesticating a parasite: developing a genetically tractable natural mouse model of cryptosporidiosis	Sateriale A, Slapeta J, Baptista RP, Engiles JB, Jodi A. Gullicksrud GT, Herbert GT, Brooks CF, Kugler EM, KissingerJC, Hunter CA, and Striepen B.
10h16-10h30	Mo- O10	The sporozoite-trophozoite transition in *Cryptosporidium* – the role of sugar metabolism, calcium signalling and redox potential	Paziewska-Harris, A, Thomas, JR, Lugonja, B, Cable, J.
10h30-10h50		**Coffee break with croissants**	10h50-12h22 Chair : BURET A.G STRIEPEN B.
10h50-11h10	Mo-O11	Symptom variability in giardiasis : Why ?	Buret A.
11h10-11h22	Mo- O12	Prolonged duodenal mucosal lymphocyte alterations in patients with and without post-giardiasis functional gastrointestinal disorders	Dizdar V, Hausken T , Laerum OD , Langeland N, Hanevik K.
11h22-11h34	Mo- O13	Impact of *Giardia* on the intestinal lipid metabolism.	Maertens B.
11h34-11h46	Mo- O14	Heterogeneity of *Giardia lamblia* Peripheral Endocytic Compartments revealed by Super Resolution Microscopy	Santos, R, Faso, C, Hehl, AB.
11h46-11h58	Mo- O15	Disc-o-fever: getting down with *Giardia's* groovy microtubule using new molecular genetic tools	Hagen K.D., Williams J.A., Hilton N.A., C. Nosala, Dawson S.C.
11h58-12h10	Mo- O16	Enolase of *Giardia duodenalis*: a moonlighting protein secreted as monomer by trophozoites activates host cell plasminogen and induces a necroptotic-like damage in epithelial Cells	Barroeta-Echegaray E, Fonseca-Liñán R, Argüello-García R, Rodríguez Muñoz R, Bermúdez RM and Ortega-Pierres G
12h10-12h22	Mo- O17	Molecular analysis of the *Cryptosporidium* life cycle progression	Striepen B.
		POSTER SESSIONS	
12h40-13h00	**Amphi 100 A**	***GIARDIA* AND *CRYPTOSPORIDIUM* ENVIRONMENTAL STUDIES (1)**	Chair : FOURNIER M. RAZAKANDRAINIBE R.
12h40-12h45	MoA- P1	Monitoring of *Cryptosporidium oocysts* and *Giardia* cysts in the Nakdong River in Korea	Cho PY, Yang HW, Nam SW, Jang SW.
12h45-12h50	MoA- P2	Detection of *Cryptosporidium and Giardia* in Oysters by Nested-PCR and Sequence Analysis in Portugal	Santos A, Ruano F, Grade A, Meireles AS, Gomes G, Lindo M, Faria CP, Sousa MC
12h50-12h55	MoA- P3	Diagnostics of *Cryptosporidium* spp. From water basins in košice region using artemia franciscana metanauplii	Hatalová, E., Valenčáková, A., Kalinová J., Špalková M.
12h55-13h00	MoA- P4	Novel Water Treatments for the Zoonotic Waterborne Pathogen *Cryptosporidium*	Lugonja B, Paziewska-Harris A, Williams CF,Cable J

	Amphi 100 B	***GIARDIA* : CELL BIOLOGY - 1**	12h40-14h00 Chair : BURET A.G. SVARD S.
12h40-12h45	MoB- P1	The immunological interplay between *Giardia duodenalis* and *Toxoplasma gondii* during murine co-infection	Coelho C.H.
12h45-12h50	MoB- P2	*Giardia lamblia* modulates LPS-induced pro-inflammatory response in macrophages through cleavage of NF-κB p65RelA by proteases	Faria CP, Neves BM, Lourenço A, Cruz MT, Martins JD, Silva A, Pereira S, Sousa MC
12h50-12h55	MoB- P3	Biochemical characterization of *Giardia* heme proteins	Rafferty S. Nezamololama N. Crowley E. Gordzevich R. Yee J. Deol H. Meiering E.
12h55-13h00	MoB- P4	*Giardia intestinalis* peripheral vesicles harbor multivesicular bodies over the parasite life cycle	Midlej V, De Souza W and Benchimol M
13h00-14h00		**Lunch on site**	
		POSTER SESSIONS	
14h00-15h00	**Amphi 100 A**	***GIARDIA* AND *CRYPTOSPORIDIUM* ENVIRONMENTAL STUDIES (2)**	Chair : FOURNIER M. RAZAKANDRAINIBE R.
14h00-14h05	MoA- P5	First detection of zoonotic *Cryptosporidium parvum* in red-eared slider turtle of invasive alien species in water environment in poland	Kaupke A , Rzeżutka A,
14h05-14h10	MoA- P6	*Cryptosporidium* spp. (Apicomplexa: Cryptosporidiidae) in Passeriformes birds and biology of novel *Cryptosporidium* great-tit genotype and *Cryptosporidium* swallow genotype	Holubová N, Limpouchová Z, Sak B, Veselý P, Halajian A, Moriarty E, Kváč M,
14h10-14h15	MoA- P7	Diversity of *Cryptosporidium* spp. in Rattus norvegicus in the Czech Republic	Kváč M, Ježková J, Horčičková H, Holubová N, McEvoy J, Sak B.
14h15-14h20	MoA- P8	Diversity of *Cryptosporidium* spp. in East- and West-European house mice	Kváč M, Ježková J, Rašková V, McEvoy J, Piálek J, Sak B.
14h20-14h25	MoA- P9	Novel *Cryptosporidium* genotypes in wild and cultured trout	Couso-Pérez, S, Ares-Mazás, E and Gómez-Couso, H
14h25-14h30	MoA- P10	First report of *Cryptosporidium parvum* GP60 genotypes (IIaA15G2R1 and IIaA16G3R1) in wild ponies from northern Iberian Peninsula	Couso-Pérez, S, Bárcena-Varela de Limia, F, Ares-Mazás, E and Gómez-Couso, H.
14h30-14h35	MoA- P11	First report of *Cryptosporidium parvum* subtype IIaA16G3R1 in cervids	Pires TeixeiraWF, Leite de Oliveira M, De Faria Peres PH, Bertequini Nagata W, Nicoleti Santana B, Oliveira BCM, Barbanti Duarte JM, Vasconcelos Meireles M, Zanetti Lopes WD, Saraiva Bresciani KD
14h35-14h40	MoA- P12	First report of parasites of the *Cryptosporidium genus* in *Mazama americana, Mazama nana* and *Blastocerus dichotomus*	Pires TeixeiraWF, Leite de Oliveira M, De Faria Peres PH, Bertequini Nagata W, Nicoleti Santana B, Oliveira BCM, Barbanti Duarte JM, Meireles VM, Zanetti Lopes WD, Saraiva Bresciani KD
14h40-14h45	MoA- P13	Prevalence, molecular identification and risk factors for *Cryptosporidium infection* in edible marine fish: a survey across sea areas surrounding France	Gantois, N; Follet, J; Hammouma-Ghelboun, O.; Guyot, K.; Benamrouz-Vanneste, S; Fréalle, E; Seesao, Y; Delaire, B; Creusy, C; Even, G; Verrez-Bagnis, V; Ryan, U; Gay, M; Aliouat-Denis, C.M; Viscogliosi, E; Certad, G.
14h45-14h50	MoA- P14	*Giardia* spp. infection in a population of crested porcupine *(Hystrix cristata* L., 1758) from central Italy	Perrucci S., Berrill F., Coppola F., Maestrini M., Felicioli A.
14h50-14h55	MoA- P15	Molecular identification of *Giardia microti* in captive vole *Microtus guentheri* (Mammalia: Rodentia) from Italy	De Liberato C., Montalbano Di Filippo M., Sagrafoli D., Ferraro D., Berrilli F.
14h55-15h00	MoA- P16	Molecular epidemiology of giardiosis in Cuban paediatric population and its association with clinical data.	Jerez Puebla, LE, Núñez FA ,Rojas LR, Martínez IS, Ayllón LV, Atencio IM, Müller N

	Amphi 100 B	*GIARDIA :* CELL BIOLOGY (2)	14h00-15h00 Chair : LETELLIER M. BURET A.G.
14h00-14h05	MoB- P5	Functional polymorphism of arginine deiminase, a putative *Giardia duodenalis* virulence factor	Klotz, C. Marek, S. Schramm, S. Ewald, C. Aebischer, T.
14h05-14h10	MoB- P6	A new model based on human intestinal organoids to investigate *Giardia duodenalis* intestinal barrier interaction: Characterization of differentiation and cell type composition	Holthaus, D. Kraft, MR. Schulzke, JD. Aebischer, T Klotz, C.
14h10-14h15	MoB- P7	Novel insights in the structural organization of the *Giardia intestinalis* cytoskeleton	Ana Paula Rocha Gadelha AP, Benchimol M, De Souza W.
14h15-14h20	MoB- P8	The role of the Microribbon-Crossbridge Complex in the Structure and Function of *Giardia*'s Ventral Disc	Hilton, NA, Dawson, SC.
14h20-14h25	MoB- P9	Defining in vivo *Giardia* physiology and commensal microbiota dysbiosis associated with parasite density during infection in mice	Starcevich, H, Nosala, C, Dawson, S.C.
14h25-14h30	MoB- P0	Comparison of mucin expression in non-mucinogenic cells infected with *Giardia lamblia*	Tsantarlis, K; Tonelli, R.R .
14h30-14h35	MoB- P11	Metabolic stress in *Giardia*-infections: in vivo importance of arginine for parasite and host	Ehret T, Klotz C, Aebischer T.
14h35-14h40	MoB- P12	Phosphoinositide-binding proteins mark, shape and functionally modulate highly-diverged endocytic compartments in the parasitic protist *Giardia lamblia*	Faso, C, Cernikova, L, Hehl AB.
14h40-14h45	MoB- P13	Structural inheritance of microtubule organelles in *Giardia*	Williams, Joseph A., McInally, Shane G., Dawson, Scott C.
14h45-14h50	MoB- P14	Functional polymorphism of arginine deiminase, a putative *Giardia duodenalis* virulence factor	Klotz, C. Marek, S; Schramm, S; Ewald, C. Aebischer, T.
14h50-14h55	MoB- P15	High Cysteine Proteins play a major role during *Giardia intestinalis* interactions with host intestinal epithelial cells.	Peirasmaki D, Ferella M, Höppner M, Campos S, Ankarklev J, Stadelmann B, Grabherr M, Svärd SG.
14h55-15h00	MoB- P16	HuTu-80 cells as a model for *Giardia lamblia* infection	Souza, J. B. ,Tonelli, R. R.
		ORAL SESSION AMPHI 350 : «OMES» 2	15h00-16h08 Chair : KAPEL N. WIDMER G.
15h00-15h20	Mo- O18	Towards the development of nutritional interventions for cryptosporidiosis	Widmer G
15h20-15h32	Mo- O19	Genome evolution in *Cryptosporidium parvum*	Wang, T, Zhang, Z, Wang, Y, Roellig, D, Guo, Y, Feng, Y, Xiao, L.
15h32-15h44	Mo- O20	Developing in vitro tools for investigating host-parasite interactions in *Cryptosporidium* spp.	Josse, L, Bones A. J, Miller C.N, Purton T.J, Kváč M, Tsaousis A.D
15h44-15h56	Mo- O21	Genetic basis for virulence differences of various *Cryptosporidium parvum* carcinogenic isolates	Audebert C, Bonardi F, Caboche S, Guyot K, Touzet H, Merlin S, Gantois N, Creusy C, Meloni D, Mouray A, Viscogliosi E, Certad G, Benamrouz-Vanneste S, Chabé M.
15h56-16h08	Mo- O22	Comparative genomics of *C. hominis* IbA10G2 isolates from Europe	Alako BTF, Liu X, Sannella AR, Chalmers RM, Robinson G, Morris A, Harrison PW, Cochrane G, Cacciò SM
16h10-16h30		**Coffee break**	16h30- 17h50 Chair : TOURNIER I. CACCIO S.
16h30-16h50	Mo- O23	Comparative genomics of *Cryptosporidium* : the current picture	Caccio S.
16h50-17h02	Mo- O24	Strand-specific RNA Sequencing in *Cryptosporidium parvum* Suggests Widespread and Developmentally Regulated Long Noncoding RNA Transcription and Intron encoded Small RNAs	Li, Y, Baptista, R.P, Sateriale, A, Striepen, B and Kissinger, J.C.
17h02-17h14	Mo- O25	Development of SureSelect target enrichment for whole genome sequencing of *Cryptosporidium directly* from stool samples	Asis Khan, Eliza Carneiro Alves Ferreira, and Michael E. Grigg
17h14-17H26	Mo-026	*Cryptosporidium parvum* exports proteins into the cytoplasm of the epithelial host cell	Dumaine J , Sateriale, A, Reddy, A , Striepen, B.
17h30-17h50	Mo- O27	*Cryptosporidium* and colon cancer: is there a causal link?	Certad G.
19h30		**Reception at the Rouen City Hall (Hotel de Ville)**	

Tuesday Presentations

		ORAL SESSION – AMPHI 350 –EPIDEMIOLOGY – 1	8h00-9h20 Chair : CHALMERS R.M. COSTA D.
8h00-8h20	Tu-O1	A UK perspective on tools for identifying, investigating and preventing *Cryptosporidium* outbreaks	Chalmers R
8h20-8h32	Tu- O2	Epidemiology and distribution of *Giardia duodenalis* Genotypes in humans in Metropolitan Sydney, Australia	Zajaczkowski, P. Mazumdar, S. Conaty, S. Ellis, J. T. Fletcher-Lartey, S. M.
8h32-8h44	Tu- O3	Occurrence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in a large asymptomatic school children population in the Madrid area. Central Spain	Muadica AS, Reh L, Köster PC, Hernández de Mingo M, Bailo B, Esther Chércoles R, Balasegaram S, Verlander N, Carmena D
8h44-9h56	Tu- O4	Novel multi-locus genotypes of *Giardia duodenalis* isolates among children in rural communities in southern palawan, philippines	Briones, J.G., Rivera, P.T, Kawazu, S., , Bascos, D.M. , Rivera, W.L., Cochon, K.L., Bertuso, A.G., Reyes, J.
8h56-9h08	Tu- O5	Recirculation of *Giardia duodenalis* genotype A in children after treatment with metronidazole: reinfection or parasitic resistance?	Fantinatti M, Oliveira LAPL, Cascais T, Austriaco-Teixeira P, Verrissimo E, Bello AR, Da-Cruz AM.
9h08-9h20	Tu- O6	*C. hominis* waterborne outbreak in a french military camp, 2017	Costa D, Razakandrainibe R, Tong C, Watier S, Holterbach L Merens A, . Petit C, V. Pommier de Santi V, Gargala G, Favennec L.
9h20-9h30		**10-minute break**	**9h30-10h18 Chair : CHALMERS R.M. XIAO L**
9h30-9h42	Tu- O7	*Cryptosporidium* spp. Infection and carriage in rural Madagascar: cluster detection among humans and animals	Krumkamp R; Melhem S, Rakotozandrindrainy R, May J, Eibach D
9h42-9h54	Tu- O8	MCS6-7 sequences as markers of the bovine origin of *Cryptosporidium parvum* isolates from infected bovines and humans	Razakandrainibe R; Costa D; Diawara I E; Lecomte M; Gargala G; Favennec L
9h54-10h06	Tu- O9	Transmission networks of *Cryptosporidium* spp. in rural sub-Saharan Africa: a multi-country study	Eibach D, Kramkamp R, Caccio S, Adegnika A, Amuasi J, Lusingu J, Rakotozandrindrainy R, May J,
10h06-10h18	Tu- O10	Cryptosporidiosis outbreak within a middle school in western France, november 2017	Loury P, Favennec L, Razakandrainibe R, Gross L, Dugast F, Dalle F, De Rougemont A, Polack B, Giraudeau D, Valot S, Costa D, Hubert B,
10h20-10h40		**Coffee break with croissants**	**10h40-11h36 Chair : KORTBEEK L.M. SAVOYE G.**
10h40-11h00	Tu- O11	*Giardia* and *Cryptosporidium* : should we always consider both?	Kortbeek L.M.
11h00-11h12	Tu- O12	Health Sequelae of Human Cryptosporidiosis – a 12 month prospective follow-up study	Carter B, Stiff RE, Elwin K, Hutchings HA, Mason B, Davies AP, Chalmers RC,
11h12-11h24	Tu- O13	Three year population based *Cryptosporidium* studyin the Nederlands :: risk factor and long term sequela	Kortbeek L.M, Igloí Z, Nic Lochlainn L , Roelfsema J, Schimmer B, Mooij S, van Pelt W, Franz E
11h24-11h36	Tu- O14	Intestinal epithelial cell damage and plasma cytokines levels can be related to giardiasis in Brazilian pre-school children	Cascais T, Austriaco-Teixeira P, Fantinatti M, Silva-Freitas ML, Santos-Oliveira JR, Coelho CH, Singer SM, Da-Cruz AM,
11h36-11h46		**10-minute break**	**10h46-12h46 Chair : ROBERTSON L. FAVENNEC L**
11h46-11h58	Tu- O15	Validation of a multilocus variable number tandem repeat scheme for *Cryptosporidium parvum* subtyping during outbreak investigations.	Pérez-Cordón G, Robinson G, Chalmers RM.
11h58-12h10	Tu- O16	Global population structure and genetic diversity of *Cryptosporidium hominis*	Tichkule S, Cacciò S, Mueller I, Bahlo M, Eibach D, Jex A
12h10-12h22	Tu- O17	Human microsporidiosis in France in 2018 : Data from the French microsporidiosis network.	Nourrisson C, Moniot M, Bonnin V, Delbac F, Dalle F, Favennec L, Poirier P ; French Microsporidiosis Network
12h22-12h34	Tu- O18	Using biofilms to monitor *Cryptosporidium* contamination in surface water	Jellison K, Cannistraci D, Fortunato J, Hall L
12h34-12h46	Tu- O19	Evolution of anthroponosis in *Cryptosporidium*	Nader JL, Mathers TC, Ward BJ, Pachebat JA, Swain MT, Robinson G, Chalmers RM, Hunter PR, Oosterhout CV, Tyler KM

13h00-14h00		Lunch on site	14h00-15h08 Chair : ORTEGA Y CACCIO S
14h00-14h20	Tu-O20	The detection and persistence of *Cyclospora cayetanensis*	Ortega Y.
14h20-14h32	Tu- O21	Opportunistic intestinal protozoan and coccidian infection among various groups of immunocompromised patients in Nepal	Sherchand JB, Shah S, Shrestha, Gautam R, Sherchan
14h32- 14h44	Tu- O22	Profiling the diversity of *Cryptosporidium* species and genotypes in wastewater treatment plants in Australia using next generation sequencing	Zahedi, A, Gofton, A, Greay, T, Monis, P, Oskam, C, Ball, A, Bath A, Watkinson, A, Robertson, I, Ryan, U.
14h44-14h56	Tu- O23	Development and Application of Bioinformatics Tools: Automation of Species/Genotype Identification and Genetic Subtyping of *Cryptosporidium*	Yanta CA, Bessonov K, Robinson G, Guy RA.
14h56-15h08	Tu- O24	A New Protocol for Molecular Detection of *Cyclospora cayetanensis* as contaminants of Berry Fruits	Temesgen T, Tysnes K, Robertson L.
15h08-15h28		Coffee break	15h28-16h28 Chair : CHALMERS R.M VILLENA I
15h28-15h40	Tu- O25	Prevalence of *Giardia* and *Cryptosporidium* in the Viennese urban water bodies: a first overview	Cervero-Aragó, S. Sommer, R. Lindner, G. Hafeli, .; Knetsch, S. Lettl, A. Derx, J. Walochnik, J.
15h40-15h52	Tu- O26	The impact on water quality from improved land management decisions for *Cryptosporidium* control in a catchment with a history of public water supply contamination	Wells B, Shaw H, Hotchkiss E, Gilray J, Green J, Katzer F, Wells A and Innes E,
15h52-16h04	Tu- O27	*Cryptosporidium* and *Giardia* in Dairy Calves--30 years of observation	Ongerth, J.E.
16h04-16h16	Tu- O29	*Cryptosporidium* in water: what makes a good method for genotyping?	Elwin, K, Robinson, G. Chalmers R.
		POSTER SESSIONS	
16h30-17h15	Amphi 100 A	EPIDEMIOLOGY - 2	16h30-17h15 Chair : LA CARBONA S. ORTEGA Y.
16h30-16h35	TuA- P1	Prevalence of *Cryptosporidium* spp, *Giardia duodenalis* and *Toxoplasma gondii* in three leafy green vegetables usually consumed in Marrakech region, Morocco.	Berrouch S, Amraouza Y, Escotte-Binet S, Razakandrainibe R, Favennec L, Hafid J, Villena I
16h35-16h40	TuA- P2	Monitoring of *Cryptosporidium parvum* in fruits, vegetables, herbs and sprout seeds	Bartosova B, Slana I, Moravkova M, Slany M.
16h40-16h45	TuA- P3	Presence of *Giardia intestinalis* in ready to eat foods of plant origin	Slana I, Bartosova B, Moravkova M, Slany M
16h45-16h50	TuA- P4	Investigation on *Cyclospora cayetanensis* in fresh produce in Italy	Barlaam, A., Tefera, T, Sannella, A.R, Marangi, M., Cacciò, S.M, Tysnes, K., Robertson, L., Iangaspero, A.
16h50-16h55	TuA- P5	Presence of *Giardia intestinalis* in ready to eat foodstuff	Slana I, Bartosova B, Moravkova M, Slany M,
16h55-17h00	TuA- P6	*Cyclospora cayetanensis* infections in Sweden – underdiagnosed or uncommon?	Beser, J and Bergstrand, T
17h00-17h05	TuA- P7	Multi-locus genotyping provides epidemiological insights about a potential common source of infection with *Enterocytozoon bieneusi microsporidia* in a hematological unit.	Desoubeaux G, Nourrisson C, Moniot M, De Kyvon M, Bonnin V, Ertault De La Bretonnière M, Morange V, Bailly E, Lemaignen A, Morio F, Poirier P
17h05-17h10	TUA- P8	Evaluation of a ceramic candle filter to remove *Cryptosporidium* oocysts in drinking water	Abeledo-Lameiro, M.J; Cañizo-Outeiriño, A; Couso-Pérez, S; Ares-Mazás, E; Gómez-Couso, H.
17h10-17h15	TuA- P9	Molecular characterization of *Cryptosporidium* in Algerian HIV/AIDS patients	Malika S; Costa D; Fatiha R; Favennec F; Hamoudi Haiet A; Razakandrainibe R
17h15- 17h20	TuA-P10	Investigating the Presence of Multiple sub-Populations within Read Archives Generated from Individual Clinical Isolates of Cryptosporidium parvum	Arthur Morris, Justin Pachebat, Guy Robinson, Rachel Chalmers, Martin Swain
17h20-17h25	TuA-P11	Asymptomatic Cryptosporidium infections in ewes and lambs are a source of environmental contamination with zoonotic genotypes of Cryptosporidium parvum	Bordes L.1, Houert P.1, Costa D.', Vial-Nou\/ella C.', Fidelle F.3, Grisez C.1, Prevot F.1, Razakandrainibe R ; Jacquiet P.1, Favennec L.2

	Amphi 100 B	CELL BIOLOGY - 3	16h30-17h25 Chair : CERTAD G. LAURENT F.
16h30-16h35	TuB- P1	Investigations on co-infections with Bovine corona virus and *Cryptosporidium parvum* using in vitro methods: experimental setup and preliminary results	Shakya R, Myrmel M, Makvandi-Nejad S, Markussen T, Robertson LJ
16h35-16h40	TuB- P2	The challenge of inferring host shifting in *Cryptosporidium* parasites with complex life cycles and population strategies	Juan C. Garcia-R, Murray P. David T. S. Hayman
16h40-16h45	TuB- P4	*Cryptosporidium parvum* can subvert the host immune response through manipulation of CRAMP expression during neonatal infection.	Guesdon W, Pezier T, Diana J, Tottey J, Laurent F Lacroix-Lamandé S
16h45-16h50	TuB- P5	Influence of prior excystation procedures on *Cryptosporidium parvum* proliferation in HCT-8 host cell culture	Kubina S, Razakandrainibe R, Costa D, Diawara E.H.I, La Carbona S, Villena I. Favennec L.
16h50-16h55	TuB- P6	Identification and localization of the *Cryptosporidium parvum* Gigantic Extracellular protein (CpGE)	Akira Nakamuran A, Passerò I, Bertuccini L, Barca S and Tosini F
16h55-17h00	TuB- P7	Challenge accepted: consistent, comparative and evidence-based genome assembly and annotation for the three closely-related species, *Cryptosporidium parvum, C. hominis* and *C. tyzzeri*	Baptista, R.P, Li, Y, Sateriale, A, Ansell, B, Jex, A, Sanders, M, Brooks, K, Tracey, A, Berriman, M, Striepen, B, Cotton, J.A and Kissinger, J.C.
17h05-17h10	TuB-P9	Epidemiology of human cryptosporidiosis cases in immunocompromised patients in France, 2015-2018	Costa D, Cryptosporidiosis french national network Razakandrainibe R, Villier V, Dalle F, Gargala G, Favennec L,
17h10-17h15	TuB-P10	*Giardia:* an under-reported foodborne parasite	Ryan U, Hijjawi N, Feng Y, Xiao L
17h15-17h20	TuB-P11	Characterization of the proteome and dynamics of encystation specific vesicles of *Giardia intestinalis*.	Markova L, Vinopalova M, Najdrova V, Dolezal P.
17h30-18h00	**Amphi 350**	**ROUND TABLE ON FOODBORNE ASPECTS**	Robertson L. La Carbona S. Hijjawi S.
20h00		**Gala Dinner, Halle aux Toiles, Rouen**	

Wednesday Presentations

		ORAL SESSION – AMPHI 350 - ANIMAL INFECTION EPIDEMIOLOGY - 1	8h00-8h56 Chair : POLACK B. ONGERTH J.E.
8h00-8h20	We- O1	Giardiosis in domestic mammals: clinical importance and public health consequence	Polack B.
8h20-8h32	We- O2	Long-term monitoring of *Cryptosporidium* in animals inhabiting drinking water catchments in three states across Australia	Zahedi A, Monis P, Gofton A, Oskam C, Ball A, Bath A, Bartkow M, Robertson I, Ryan U,
8h44-8h56	We- O3	Genetic diversity of *Cryptosporidium* in fish	Ryan U, Palermo U, Boland,S , Zahedi,A , Lymbery A, Okam C.
8h44-8h56	We- O4	Propagation of *Cryptosporidium baileyi* and *C. parvum* in chickens following in ovo inoculation	Holubová N, Sak B, Kváč M,
8h56-9h18		**20-minute break**	8h18- 9h06 Chair : XIAO L. RAZAKANDRAINIBE R.
9h18-9h30	We- O5	*Cryptosporidium* spp. (Apicomplexa: Cryptosporidiidae) in Psittaciformes birds and biology of *Cryptosporidium* avian genotype III	Kváč M, Zikmundová V, Sak B, McEvoy J, Holubová N.
9h30-9h42	We- O6	What is the source of *Cryptosporidium parvum* infection for beef and dairy calves?	Shaw H, Thomson S, Innes EA, Katzer F,
9h42-9h54	We- O7	Occurrence and zoonotic potential of *Cryptosporidium* in horses from the Netherlands	Uiterwijk M, Dam C, Roelfsema J, Broens E, Hordijk J, Cuperus T, van der Giessen J
9h54-10h06	We- O8	Wildlife vectors of *Cryptosporidium parvum* as contributors to parasite transmission between farms and to water sources	Wells B, Paton C, Bacchetti R, Shaw H, Gilray, J, Katzer F and Innes E
10h10-10h30		**Coffee break with croissants**	10h30-11h18 Chair : CHALMERS R.M. POLACK B.
10h30-10h42	We- O9	Identification of *Cryptosporidium parvum* subtype diversity using next generation sequencing in pre-weaned calves in Argentina	Santin M., George N. , Molokin A, Maloney J, Lombardelli J, Encinas M, M Lujan TomazicM M, Garro C , Tiranti K, Schnittger L
10h42-10h54	We- O10	Molecular identification, genotyping, and subtyping of individual *Cryptosporidium* oocysts isolated from bovine faeces with special reference to zoonotic significance	Gharieb R M. A, Bowman D D, Liotta J L , Xiao L
10h54-11h06	We- O11	*Cryptosporidium* and *Giardia* in Dairy Calves--30 years of observation	Ongerth, J.E
11h06-11h18	We- O12	MCS6-7 sequences as markers of the bovine origin of *Cryptosporidium parvum* isolates from infected bovines and humans	Razakandrainibe R; Costa D; Diawara I E; Lecomte M; Gargala G; Favennec L
		POSTER SESSIONS	
11h30-12h50	**Amphi 100 A**	**ANIMAL INFECTION EPIDEMIOLOGY - 2**	11H30-12h50 Chair : KORTBEEK L.M., KVÁČ M.
11h30-11h35	WeA- P1	Molecular characterization of *Crypstosporidium* isolates from diarrheal dairy calves in France	Mammeri M, Chevillot A, Chenafi I, Julien C, Vallé I, Polack B, Follet J, Adjou, K.T.
11h35-11h40	WeA- P2	Molecular characterization of zoonotic *Cryptosporidium* and *Giardia duodenalis* pathogens in Algerian sheep	Sahraoui L , Thomas M, Chevillot A, Mammeri M, Polack B, Vallée I, Follet J, Ain-Baaziz H Adjou, K.T.
11h40-11h45	WeA- P3	Prevalence of potentially zoonotic assemblages of *Giardia duodenalis* in domestic and wild animals in Scotland.	Bartley PM, Chia SL, Roehe BK, Bacchetti R, Wells B, Innes EA and Katzer F.
11h45-12h50	WeA- P4	Zoonotic species *Cryptosporidium parvum* in cattle in Slovakia	Mravcová K, Štrkolcová G, Goldová M, Rastislav M.
11h50-11h55	WeA- P5	Occurrence of *Cryptosporidium* suis in Italian pig farms	Cervone M, Cacciò S, Fichi G, Perrucci S.
11h55-12h00	WeA- P6	A snapshot of *Cryptosporidium* spp infection in livestock in France: Public health risk concern	Razakandrainibe R, Costa D, Leméteil D Camuset P, Berthet H, Gargala G, Favennec L
12h00-12h05	WeA- P7	Evaluation for associations amongst *Giardia duodenalis* Assemblages and fecal score of dogs.	Scorza AV, Buch J,Franco P, Mc Donald C, Lappin M R..
12h05-12h10	WeA- P8	The assemblages of *Giardia duodenalis* circulating among humans and animals in Slovakia	Štrkolcová G, Mravcová K, Goldová M, Mucha R
12h10-12h15	WeA- P9	Investigations on zoonotic cryptosporidiosis in Northern Ethiopia	Tesama KYT, Tysnes KR, Debenham JJ, Nødtvedt A, Terefe G, Robertson LJ.
12h15-12h20	WeA- P10	Frequency and molecular diversity of *Giardia duodenalis* and *Cryptosporidium* spp. in captive non-human primates and their keepers in Spain and rescued wild chimpanzees (*Pan troglodytes*) in Sierra Leone	Köster PC, Alameda A, Salimo Muadica A, Hernández de Mingo M, Bailo B, Lapuente J, Calero-Bernal R, Carmena D.

12h20-12h25	WeA- P11	Transmission of *Cryptosporidium parvum* from cattle to a veterinary student in Slovakia	Mravcová K , Štrkolcová G, Goldová M, Mucha R.
12h25-12h30	WeA- P12	Multilocus sequence typing strategies for the characterization of *Giardia duodenalis* Assemblage A isolates from human and animals in Italy	Montalbano Di Filippo M, De Liberato C, Di Cave D, Berrilli F.
12h30-12h35	WeA- P13	Cryptosporidiosis in non-human primate in Ethiopia a Public health problem	Wortea Hailu A; Costa D; Petros B; Adamu H; Favennec L; Razakandrainibe R
12h35-12h40	WeA- P14	Interreg 2 seas Project : Health For Dairy Cows, H4DC.	Tsaousis A.D, Vlandas A, Vuylsteke I, Canniere E, Dellevoet M, Roemen J, Barbier-Bourgeois A, Leruste H, Roblin M, Windal F, Benabiles H, Hammouma O.Deweer C.Tyler K.Hunter P.Follet J
12h40-12h45	WeA- P15	*Cryptosporidium* horse genotype infection in immunocompromised child with Crohn's disease.	Kopacz Ż., Kicia M., Akutko K., Iwańczak B., Sak B., Hendrich A.B., Kváč M.
12h45-12h50	WeA- P16	Tools for understanding the public health risks of *Cryptosporidium* in swimming pools	Chalmers RM. Johnston R . Luxford M . Miller R
	Amphi 100B	**DIAGNOSIS AND THERAPY - 1**	11h40–12H55 Chair : STRIEPEN B. FAVENNEC L.
11h40-11h45	WeB- P1	The enteric syndrome in calves, the consequence of infections with *Cryptosporidium parvum*, *Giardia duodenalis* and *Eimeria* spp. under production conditions, and the therapeutic efficacy of Azithromycin	Iacob O., Nica V., Nica E., Miron L.D, Roman C,. Mîndru R, Lupu A.
11h45-11h50	WeB- P2	Drug Susceptibility Testing in *Giardia lamblia:* Cysteine strongly affects the effectivities of Metronidazole and Auranofin, a novel and promising antimicrobial	Leitsch D
11h50-11h55	WeB- P3	Anti-*Giardia duodenalis* activity of nicotinamide	Lagunas-Rangel F. A., Bazán-Tejeda M. L., García-Villa E., Bermúdez-Cruz R. M.
11h55-12h00	WeB- P4	Cell-penetrating peptide dramatically improves the efficacy of Nitazoxanide in the inhibition of *Cryptosporidium parvum* growth	Nguyen-Ho-Bao T, Daugschies A , Kamena F
12h00-12h05	WeB- P5	Histone deacetylase inhibitors affect growth and cell organization of of *Giardia intestinalis* trophozoites	Gadelha APR; Oliveira RVF ; Huber K Bracher F Benchimol M de Souza W
12h05-12h10	WeB- P6	Efficacy of anti-diarrheal traditional plant used in Côte d'Ivoire against *Cryptosporidium parvum* in both in vitro and in vivo model	Tuo K, Chevillot A, Mammeri M, Ndocho T, Bolou E, Vallee I, Adjou K, Toure A, Polack B, Jambou
12h10-12h15	WeB- P7	Is *Cryptosporidium parvum* able to induce intestinal neoplasia in vitro?	Sawant M , Baydoun M, Creusy C , Gantois N , Chabé M, Mouray A , Viscogliosi E , Certad G , Benamrouz-Vanneste S
12h15-12h20	WeB- P8	Standardization of single-tube nested real-time PCR and genetic sequencing for detection and species characterization of avian *Cryptosporidium* spp.	Santana, BN.; Nascimento, IG; Meireles, MV
12h20-12h25	WeB- P9	a four-plex qpcr-hrm assay for the detection and quantification of human diarrhea parasites	Lamien-Meda A, Leitsch D, Schneider R, Walochnik J, Auer H, Wiedermann U
12h25-12h30	WeB- P10	Validation and application of a salivary antibody assay for measuring exposure to *Cryptosporidium*	Elwin K. Puleston R. Chalmers R.M.
12h30-12h35	WeB- P11	Evaluation of LAMP detection of *Giardia* DNA for microfluidic environment	Hartdégen M. Farkasvölgyi K. Iván K. Laki A.J. Plutzer J
12h35-12h40	WeB- P12	Validation of a semi-quantitative real-time PCR assay for the diagnosis and the monitoring of *Giardia intestinalis* infection in canine faeces samples according to the French standard NFU47-600-2	Thomas M., Va F, Polack B., Ciancia C., Klubkova V., Pelletier C., Sellal E., Bellier S.
12h40-12h45	WeB- P13	Effect of potassium dichromate medium on preservation of *Giardia duodenalis* cysts in faecal specimens of three different livestock species	Benhassine, S, Baroudi, D, Hadjoudja, M, Hakem, A, Köster, C. P, Bailo, B.Carmena, D.
12h45-12h50	WeB- P14	Evaluation of the FTD stool parasites Fast track diagnostic kit	Costa D, Razakandrainibe R, Villier V, Dalle F, Gargala G, Favennec L
12h55-13h10		**HUW-SMITH AWARD CEREMONY**	

13h10-14h00		Lunch on site	
		POSTER SESSIONS	
14h00-15h00	**Amphi 100 A**	**EPIDEMIOLOGY - 3**	14h00-15h00 Chair : KORTBEEK L.M. TROELL K.
14h00-14h05	WeA- P17	Cohort study of associations between intestinal protozoa infection and intestinal barrier function, nutritional status, and neurodevelopment in infants in São Tomé.	Garzón M
14h05-14h10	WeA- P18	Prevalence of intestinal parasites and associated risk factors in primary school children in lahore, Pakistan	Latif A. A, Butt A, Mansha M,Fatima S, Ateeq A and Farooq M.A
14h10-14h15	WeA- P19	Retrospective analysis of *Cryptosporidium species* in Western Australian human patients (2015-2018), and emergence of the *C. hominis* IfA12G1R5 subtype	Braima K, Zahedi A, Oskam C, Reid S, Pingault N, Xiao L, Ryan U.
14h15-14h20	WeA- P20	*Cryptosporidium chipmunk* genotype I – an emerging cause of human *cryptosporidiosis* in Sweden	Beser J, Ydring E, Killander G, Bujila I, Nordahl M, Troell K and Lebbad M
14h20-14h25	WeA- P21	Presence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in asymptomatic schoolchildren and symptomatic subjects seeking medical attention in the province of Zambezia, Mozambique	Salimo Muadica A, Köster PC, Hernández de Mingo M, Bailo B, Paulos S, Fuentes I, Carmena D
14h25-14h30	WeA- P22	Genetic diversity of *Giardia duodenalis* and *Cryptosporidium* spp. in symptomatic individuals attending public hospitals in Spain. A multicentre study	Köster PC, Pérez-Ayala A, Jiménez AB, Molina A, Trelis M, Ruiz G, García-Hortelano M, Mellado MJ, Cuadros J, Martínez Ruiz R, Guerrero M, Azcona Gutiérrez JM, Merino FJ, Paulos S, Hernández de Mingo M, Bailo B, Salimo Muadica A, Fuentes I, Carmena D
14h30-14h35	WeA- P23	The epiCrypt study protocol: investigating household transmission of *Cryptosporidium* in England and Wales	McKerr C, Chalmers RM, Elwin K, Vivancos R, O'Brien SJ, Christley RM,
14h35-14h40	WeA- P24	Cryptosporidiosis in French nationals traveling abroad (2015-2018)	Costa D Cryptosporidiosis french national network, Gargala G,Dalle F, Razakandrainibe R, Favennec L
14h40-14h45	WeA- P25	Epidemiology of human giardiasis. A 14 years survey	Codrean GA, Gorcea AM, Negrut MN, Csep NA, Indries FM, Cozma PA, Dumitrascu LD, Junie LM,
14h45-14h50	WeA- P26	Genetic Diversity of *C. hominis* in an Urban Slum Population in Southern India	Rao Ajjampur SS, Ward H and Kang G
14h50-14h55	WeA- P27	Epidemiological characteristics of cryptosporidiosis cases in France, 2015-2018	Costa D Cryptosporidiosis french national network, Gargala G,Dalle F, Razakandrainibe R, Favennec L
14h55-15h00	WeA-P28	Evaluation of the immunochromatographic tests Crois *Giardia*-Strip and Crypto-Strip for the detection of *Giardia Lamblia* and *Cryptosporidium parvum* in stools	Kaoutar O T, Hind es, Sanaa I, Maha SA
	Amphi 100 B	**CELL BIOLOGY - 4**	14h00-15h00 Chair :**STRIEPEN B. GARGALA G.**
14h00-14h05	WeB- P16	Understanding metronidazole resistance in *Giardia duodenalis*: Identifying patterns by transcriptomics combined with biochemical analysis of two oxygen-insensitive nitroreductases	Krakovka S; Svärd SG
14h05-14h10	WeB- P17	*Giardia* 14-3-3 protein post-translational modifications: Assemblage specificity and correlation with cell growth.	Lalle M., Camerini S., Salzano A.M., Cecchetti S., Marucci G., I. Zullino
14h10-14h15	WeB- P18	Epigenetic control of gene expression during differentiation of *Giardia intestinalis*	Rojas-Lopez L, Einarsson E, Ulf Ribacke , and Svärd SG.
14h15-14h20	WeB- P19	High-resolution, quantitative proteome of *Giardia duodenalis* during encystation	Balan B, Emery-Corbin S, Sandow J, Webb A, Jex A
14h20-14h25	WeB- P20	Characterization of the secreted cysteine protease CP17516 as a potential virulence factor	Grüttner J, Liu J, Svärd SG
14h25-14h30	WeB- P21	How does ISC system work in the mitosomes of *Giardia intestinalis*?	Motyckova A, Stairs C, Najdrova V, Horackova V, Dolezal P.
14h30-14h35	WeB- P22	Characterization of the proteome and dynamics of encystation specific vesicles of *Giardia intestinalis*.	Markova L, Vinopalova M, Najdrova V, Dolezal P.
14h35-14h40	WeB- P23	Novel protein of *Giardia intestinalis* links the function of the mitosomes and the encystation specific vesicles.	Vinopalová M , Voleman L, Pelc J, Marková L, Najdrová V. Doležal P

14h40-14h45	WeB- P24	Multicentric evaluation of the real-time PCR assay Amplidiag Stool Parasites for *Cryptosporidium* spp. detection in human stools *Cryptosporidium* National Network	Razakandrainibe R., Frealle E. Nourrisson C., Valot S, Dutoit E, , Kapel N., Poirier P. Dalle F., Favennec L., Cryptoanofel *Cryptosporidium* National Network.
14h45-14h50	WeB- P25	Is oxygen a crucial factor for the survival of *Cryptosporidium* during solar water disinfection?	Abeledo-Lameiro, M.J; Campos-Ramos, R; Ares-Mazás, E; Gómez-Couso, H,
14h50-14h55	WeB- P26	Study of DNA repair protein recruitment and chromatin remodeling during DNA Double-Strand Break repair process in *Giardia duodenalis*	Espinoza-Corona, S., García-Huerta, E, Bermúdez-Cruz, R.M
		ORAL SESSION AMPHI 350 - DIAGNOSIS AND THERAPY - 2	15h00-15h56 Chair : DALLE F. FAVENNEC L.
15h00-15h20	We-O13	Detection of *Giardia duodenalis and Cryptosporidium spp.* in stools : Is microcopy still accurate ?	Favennec L
15h20-15h32	We- O14	The CerTest VIASURETM PCR simplex and multiplex assays for the detection of *Giardia intestinalis*, *Entamoeba sp* and *Cryptosporidium sp.*: Comparative evaluation with two commercial multiplex PCR kits and routine in-house simplex PCR assay	Basmaciyan L, Francois A, Vincent A, Valot S, Costa D, Morio F, Favennec L and Dalle F.
15h32-15h44	We- O15	Auramine-phenol staining and light-emitting diode fluorescence microscopy and a rapid dipstick test for detection of *Cryptosporidium infection*: a diagnostic accuracy study in a university hospital and a rural health centre in Ethiopia	Johansen Ø H., Abdissa A, Zangenberg M, Mekonne Z, Eshetu B, Bjørang O, Alemu Y, Sharew B, Langeland N, Robertson L, Hanevik K.
15h44-15h56	We- O16	Integrated selective *Cryptosporidium* EWOD concentrator	Lejard-Malki R, Follet J. Vlandas A. Senez V
15h56-16h10		**Coffee break**	16h10-17h30 Chair : STRIEPEN B. GARGALA G.
16h10-16h30	We-O17	Therapeutic aspects of cryptosporidiosis	Gargala G
16h42-16h54	We- O19	New potent and selective anti-*Giardia* compound series	Hart CJS, Riches A, Ryan J, Abraham S, Abraham R and Skinner-Adams TS
16h54-17h06	We- O20	Metronidazole drug-resistance in *Giardia*: emerging roles of epigenetic and post-translational modifications and sub-species variation	Emery-Corbin SJ, Louise Baker L, Ansell BRE, Mirzaei M, Haynes PA, Lacey E, McConville MJ, Svärd SG, Jex AR
17h06-17h18	We- O21	Developing Therapeutics to Reduce *Cryptosporidium Morbidity* and Mortality Among Children in Low-Resource Settings	De Hostos, E. L.
17h18-17h30	We- O22	Parenteral aminoxanide as a novel route to treat cryptosporidiosis	Diawara HI Razakandrainibe R, Rossignol JF. Stachulski AW, Le Goff L, François A, Favennec L. Gargala G.

Sunday, 23rd June, 2019

ORAL PRESENTATIONS

Su-O1-13h30/13h50

Omics as a tool to understand giardiasis

Svärd G S

Department of Cell and Molecular Biology, Uppsala University, Sweden

Giardia intestinalis is a non-invasive protozoan parasite that causes giardiasis, a common cause of diarrhea in humans. Until recently the molecular pathogenesis of giardiasis was poorly defined. The parasite has a tetraploid genome divided into two nuclei, which makes classical genetic approaches problematic as a tool to study pathogenesis but different types of omics techniques have contributed to a better understanding of the disease.

The sequence of the first *Giardia* genome (WB, assemblage A) was published in 2007 and genomics has had a huge impact on the *Giardia* research field. This has been followed by other genomes and now new types of questions can be asked with a larger number and more complete *Giardia* genomes from different assemblages. To better understand the crosstalk between *G. intestinalis* and human intestinal epithelial cells (IECs) simple in vitro interaction systems have been developed. The cross-talk between parasites and host cells has been studied via differential gene expression analyses on the RNA level (transcriptomics) using cDNA microarrays, subtractive hybridization, serial analyses of gene expression and lately RNA sequencing on both the parasites and host cells. Transcriptomics has also been used to study gene expression in parasites from the small intestine of mice. RNA-based studies have been complemented by proteomic studies using 2D gels and LC/MS/MS analyses. Proteomics has also been used to study changes in the secretomes during host-parasite interactions.

Our omics analyses have shown that chemokines are highly up-regulated on the RNA level early during host-parasite interaction but this do not correspond to the level of protein expression. The parasites up-regulate a large number of genes in response to the host cells, among them a family of cysteine rich membrane proteins (HCMPs) and immunolocalization showed that they localize to the parasite surface. Major parts of the IEC and parasite secretomes are constitutively secreted proteins but several proteins are released in response to host-parasite interaction. The human secretome contain many metabolic and immunomodulatory proteins whereas that of *Giardia* contain immunodominant and glycosylated proteins, as well as new candidate virulence factors. Several proteins involved in arginine metabolism are secreted and this affect nitric oxide (NO) production of the IECs. The three main secreted cysteine proteases cut or re-localize the intercellular junctional complex proteins (claudin-1 and -4, occludin, JAM-1, β-catenin and E-cadherin) of IECs. They can also degrade several of the chemokines expressed in IECs during *Giardia*-host cell interactions, as well as *IgA, mucins and defensins.*

Su-O2-13h50/14h02

The new *Giardia intestinalis* and *Giardia muris* genomes

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The first draft genome of *Giardia intestinalis* was published in 2007 in Science. The genome was sequenced in the era of Sanger sequencing, and was assembled into 306 contigs on 92 scaffolds, leaving 137 gaps of 1.64 Mbp in size. We resequenced the genome with PacBio technology, and the draft HGAP assembly was then scaffolded with the optical maps. The new draft genome consists of 35 scaffolds, with the five biggest scaffolds corresponding to the five chromosomes. There are only 4 gaps of 0.87 Mbp in size in the new near complete genome, all falling into the repetitive regions. Besides the new reference for *G. intestinalis*, we have also a new reference genome for *Giardia muris* in five near complete chromosomes. *G. muris* has been used as a natural infective model of *Giardia* infection to study the immunological response to infection in host for many years. It infects mice and causes similar symptoms as *G. intestinalis* infection in humans. However, very little is known about the genome and biology of this parasite since it is recalcitrant to axenization. *G. muris* genome shows high levels of streamlining, even beyond that of *G. intestinalis*. Although conserved in metabolic pathways, *G. muris* is highly diverged from *G. intestinalis* when it comes to genome synteny. For example, *G. muris* also harbors a large family of candidate variant-specific surface proteins (VSPs) that are likely to be involved in antigenic variation. Interestingly, the predicted surface-antigen repertoire is mostly maintained as arrays of pseudogenized VSPs close to the telomeres with functional VSPs encoded away from a telomere-proximal position. The new near complete draft genomes of *G. muris* and *G. intestinalis* really enable comparative genomic studies at the chromosomal level, like difference in chromosomal gene distribution and structure variation. We hope the two new genomes could not only provide new reference genomes for the *Giardia* community, but also promote comparative studies of *Giardia* biology.

Su-O3-14h02/14h14

Re-discovery of *Giardia*virus: genomic and functional in deep analysis of different *Giardia*virus strain in naturally infected *Giardia duodenalis* isolates

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giardiasis, caused by the protozoan parasite *Giardia duodenalis*, is an intestinal disease affecting almost one billion people worldwide. Infection can be asymptomatic or cause an acute and/or chronic diarrheal disease. Although clearly multifactorial, the exact pathogenic mechanisms and the factors associated with isolates virulence have not been completely identified. A small dsRNA cytoplasmic viruses comprising 2 ORFs (capsid protein and capsid protein-RNA dependent RNA polymerase fusion protein), referred to as GLV (*G. lamblia* virus), family Totiviridae, has been reported in association with many human and animal isolates of *G. duodenalis*. Only two and almost identical GLV genomes have been fully sequenced, whereas extensive studies were done in 80s and 90s with the original GLV isolate. Although increased virus:parasite ratio can decrease trophozoites growth rate, correlation between the presence of GLV and *Giardia* virulence has not been investigated. In the perspective to better define the role of GLV, the characterization of three GLV strains from naturally infected *G. duodenalis* trophozoites were studied, GLV infected *G. duodenalis* isolates, from a human animals, and the GLV-free WBC6 isolate were used. A mouse polyclonal antibody, raised against a 6XHIS tagged N-terminal portion of the GLV Capsid Protein (CP), was used for western blot and CLSM immunofluorescence. Intracellular localization of GLV was further investigated by TEM. Nucleotide polymorphism among the three GLV genomes was observed by RT-PCR amplification and sequencing. Similarly, a difference in the encoded CP size occurs between the GLV isolates, supporting the occurrence of alternative translation starting codons. These genomes and protein variants also correlate with a differential distribution of the viral particles in the trophozoites three parasite isolates, as observed by CLSM and TEM. Different approaches were used to sequence the complete GLV genomes, i.e. conventional RT-PCR and cloning and specific inverse PCR primers (SIP) and PacBio sequencing. The existence of different slippage sequences regulating frameshift mechanism to allow CP-RdRP fusion protein translation is suggested. In conclusion our studies clearly highlight the existence of GLV strains that differ from those actually reported and suggest that such differences might impact virus infection biology. Complete sequencing of other GLV strains, controlled infection experiments in GLV-free *Giardia* strains and the effect of the virus in parasite-host interactions models are the next steps to evaluate GLV among factors affecting *Giardia* virulence.

Su-O4-14h14/14h26

Annotation of the *Giardia* proteome through structure-based homology and machine learning

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The *Giardia duodenalis* WB genome encodes ~5,500 protein coding genes. Understanding the function of each of these genes provides important insight into *Giardia*'s molecular biology, and mechanisms underpinning its metabolism, host-parasite interactions, infection biology and resistance to antiparasitic drugs. Direct functional study of *Giardia* has proven difficult. Advances in protein localization, over-expression and targeted gene knockdown (most recently using CRISPr technology) provide major tools to improve understanding of this important parasite. However, with ~1/3rd of *Giardia*'s coding genes having an unknown function and lacking recognizable protein domains or homologs in other organisms, prioritization of gene targets for further functional study is needed. In silico methods can help to guide this prioritization. A key example of this is through the large-scale prediction of protein structural homology, which observes that while protein sequences can diverge dramatically over evolutionary time, their underlying structure and subsequent function can remain highly conserved. Using this approach, 3D protein structure is modelled based on its underlying inferred amino acid sequence. These structural models are compared with solved structures for proteins of known function in public repositories. Highly conserved protein structures are then used to infer functional similarity. Here, we describe our recent publication of a comprehensive protein structural homology-based analysis of the complete *Giardia duodenalis* WB proteome. We described the development of a novel, machine-learning pipeline to filter these predictions and categorize them into low through high-confidence categories. Using this method, we provide new, high confidence predicted structures for more than 1,000 *Giardia* proteins, which can now be further explored through functional study. We discuss examples of how these data provide new insights into key molecular mechanisms used in redox defense, genetic regulation and cell signalling.

Su-O5-14h26/14h38

Exploring genomic variation in *Giardia duodenalis* using well characterised reference isolates

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Draft genomes for reference isolates of *Giardia duodenalis* have been available for more than a decade. However, little resequencing exploring the genomic variability within or among the assemblages, particularly in relation to phenotypic characteristics, such as virulence or drug resistance, has been undertaken. Extensive work over the last few decades, spearheaded in large part by the Upcrofts, has generated a substantial base of knowledge of the biology, phenotypic behavior and genomic plasticity of this parasite. This work, however, was all completed pre-genome and prior to the advent of high-throughput sequencing. Using updated and essentially 'finished' versions of the *G. duodenalis* WB (PacBIO), GS (PacBIO + KpnI optical map) and P15 (PacBIO) genomes, we have undertaken detailed comparative study of 47 *Giardia duodenalis* genomes (34 A, 12 B and 1 E), including 40 isolates have represented reference culture stocks for the field since the 1980s (e.g., *G. duodenalis* 713 and *G. duodenalis* 106 isolates). We report on this work here, with a focus on Assemblage A parasites. The data provide sequence barcodes for each major isolate to ensure their accurate identification among research labs and support further transcriptomic, proteomic and functional studies. We further explore the overall variability among these genomes, identifying key gene families (e.g. variant surface proteins and NEK kinases) under restrictive or diversifying selection and explore genetic variation linked to key phenotypes, including host-specificity, virulence/pathogenicity and/or resistance to anti-*Giardia*l drug classes, as well as the effects of prolonged in vitro passage.

Su-O6-14h38/14h50

Investigation of a waterborne outbreak of giardiasis in Italy by comparative genomics

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The protozoan *Giardia duodenalis* is a flagellated unicellular parasite with a simple life cycle that includes the production of highly resistant cysts. Molecular investigations have demonstrated the occurrence of large genetic variability within the *G. duodenalis* species complex, with the recognition of at least eight genetic groups (Assemblages A to H), of which two (A and B) are able to infect humans. Further genetic variability exists within each Assemblage. Transmission occurs by both direct and indirect routes, with water playing a major role. In fact, many waterborne outbreaks have been reported worldwide.

Here, we report on the investigation of a waterborne outbreak that occurred in a small village in a mountainous region (1421m above sea level) in the North of Italy (Bergamo province). At the end of 2017, about a hundred people spent their Christmas holidays in the village. On January 2018, the University Hospital of Pavia and the Sacco Hospital of Milan registered an unexpected number of patients with enteric symptoms. Following detection of *Giardia* cysts in stool samples and epidemiological investigations, the Health Authorities recognized an outbreak of giardiasis. The outbreak involved 75 individuals, of which 35, from eight families, were available for this study. Diarrhoea was reported in almost all cases (97%), followed by fever (41%), nausea (41%) vomiting (37%) and abdominal cramps (24%). Genomic DNA was extracted from the 35 stool samples, and tested for *Giardia* by PCR and sequencing of the beta-giardin gene. This showed that all patients were infected with Assemblage A, sub-assemblage A2, which is a genetic variant commonly found in humans but not in animals. To gain further insights, cysts from six stool samples were purified by immune-magnetic separation, followed by DNA extraction, a generic 16S rDNA PCR to check bacterial contamination, and Whole Genome Amplification. An illumina Hi-Seq platform was used to sequence libraries (2x150 bp, paired-ends), and to generate 13-19 million reads, of which 17-96% (average 65%) mapped to the reference WB genome. We focused on Single Nucleotide Polymorphisms (SNPs), and found that 357-1039 SNPs were specific for each isolate (i.e., singletons). Thus, the genomes of the six isolates are extremely similar, although not identical. We will discuss the distribution of SNPs and their impact on protein-coding genes, and present a comparative analysis of all A2 genomes currently available.

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Su-O7-15h/15h12

Whole genome sequencing of assemblage C and D of *Giardia intestinalis* from the dog

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Background: Of the 8 assemblages of *Giardia intestinalis*, only assemblages A, B and E can be cultured. The unculturable assemblages have so far resisted genome sequencing efforts. Aim of this study is to sequence assemblage C and D from dogs using single cell approaches and compared them with genomes of assemblages A and B from humans and assemblage E from ungulates. We investigated the allelic sequence heterozygosity (ASH), the phylogeny of the assemblages and the relationship between presence or absence of certain genes and host specificity. Methods: Cysts were isolated from feces of infected dogs by centrifugation on sucrose cushion and FACS. The genomes of individual and pooled cysts were amplified with multiple displacement amplification (MDA) and subsequently the genomes were sequenced on Illumina MiSeq platform with 250 bp paired ends.

Results: The genomes from the individual and pooled cysts were sequenced with approximately 230 fold coverage and assembled. All the genomes were considered complete (>99% single copy genes observed). The phylogenetic distance based on the whole genome between assemblage C and D was about the same as between assemblage A and B. The ASH for assemblage C and D (0.89 % and 0.74%, respectively) was higher than for assemblage B (0.5 %) and much higher than for assemblages A and E (>0.04%). Genes coding for flavohemoglobin and 4Fe-4S binding domain family protein were present in all the genomes of assemblages A, B and E, but were not found in the genomes of assemblage C and D. Many orthologs of cathepsin-B genes were identified in all genomes from all assemblages. Six cathepsin-B genes clades contained orthologs in all genomes, but 3 clades of cathepsin-B genes were very diverse. These diverse clades contained many genes from assemblage B and the only gene from assemblage C and D had no orthologs in assemblage A and E, suggesting a role in host specificity.

Conclusions: Whole genome sequencing from a single *Giardia* cyst is possible and makes the genomes of unculturable *Giardia* assemblages accessible. Assemblage C and D are closer related to human assemblage B, than the other human assemblage A. A low ASH, resulting from increased recombination, seems to be a derived feature, present only in the branch containing assemblages A and E. The majority of cathepsin-B genes is well conserved and may have similar function in all assemblages, while the smaller, diverse group of cathepsins is possibly associated with host specificity.

Su-O8-15h12-15h24

Characterization of flavohemoprotein in *Giardia* isolates

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Introduction

Metronidazole (MTZ) is used for treatment of *Giardia lamblia* and is thought to cause oxidative- and nitrosative stress, once metabolized. The nitrosative stress detoxification protein, flavohemoprotein (gFlHb) has previously been found to have high genetic diversity in *Giardia*. The aim of this study was to analyze gFlHb haplotype diversity in addition to structural examination of gFlHb in the two assemblages A and B. Methods and Materials

Twenty clinical isolates of *Giardia* were whole genome sequenced (WGS) by illumina, and the respective gFlHb genes from eighteen isolates were sequenced individually after PCR amplification and cloning.

The gFlHb of *Giardia* assemblage A (E2RTZ4) and B (6U182) genes were cloned for protein expression in *E. coli*. Two constructs, one assemblage A and one assemblage B, were expressed and purified to homogeneity. Structural analysis was done using multi-angle light scattering (SEC-MALS), circular dichroism (CD), small-angle X-ray scattering (SAXS) and crystallization trials were started.

Results

gFlHb gene read coverage varied between WGS data of the isolates and was from 1 to 5 times higher than average gene coverage. 116 gFlHb sequences from eleven assemblage A2 isolates harbored 56 unique haplotypes, while of 45 sequences in four B isolates, 31 unique haplotypes were found. Further analysis showed that six A2 and three B isolates had negative Tajima's D values, and one significant. Adding heme and FAD to the proteins during purification was a key factor for obtaining a pure and homogeneous sample. CD predicted a folded secondary structure, and Tm between 40-45 °C. SAXS analysis predicted a monomeric and globular shape. Ongoing crystallization attempts have yielded small crystals.

Discussion

High genetic variation in gFlHb is due to both gene copy number variation and many unique haplotypes/sequence polymorphisms among *Giardia* isolates. The multicopy findings will be verified using digital PCR. The negative Tajima's D values found in nine isolates could indicate bottleneck selective pressure events during culturing. However, the remaining isolates had positive Tajima D values.

Purified gFlHb was prone to precipitation on storage and freezing. High genetic variability in gFlHb may allow adaption to nitrosative stress in *Giardia* and could play a role in MTZ susceptibility.

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Su-O9-15H24/15H36

Mitosomal dynamics in *Giardia intestinalis*

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Mitosomes are the smallest evolutionary forms of mitochondria that evolved in eukaryotes adapted to anaerobic environments. This adaptation manifests as the absence of the mitochondrial genome and vast majority of the mitochondrial proteome, including the components of the mitochondrial division machinery. Here, we studied the dynamics of mitosomes in *Giardia intestinalis* using FIB-SEM technique. This method showed that the mitosomes are actually asymmetrical. They are partially flattened on the side where the two membranes surrounding the organelle get closer to each other. We also found that mitosomal division is restricted to mitosis, when both central and peripheral organelles divide in a unique and synchronized manner. During the segregation of the divided mitosomes, the subset of the organelles between two *G. intestinalis* nuclei has a prominent role. These central mitosomes are physically connected to the flagella via specialized fibril during the whole process, thus ensuring proper inheritance of these prominent organelles to the daughter cells. Moreover, despite the absence of the ERMES components, mitosomal division involves association with the endoplasmic reticulum, a relationship commonly seen during the division of mammalian and fungal mitochondria.

Su-O10-15h36/15h48

Whole Genome Sequencing of *Giardia duodenalis* Isolates from a Cat and a Dog

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Giardia duodenalis is a pathogenic intestinal parasite of humans and many other animals with a global distribution. It is transmitted via the fecal-oral route, and infection is often associated with consumption of contaminated food or water. Whole genome sequencing and comparative genomics of multiple *Giardia* assemblages and isolates allows for the identification of traits that are either shared by all *Giardia* assemblages or unique to an individual assemblage or strain. These types of analyses require data from the genomes of many *Giardia* isolates obtained from multiple hosts. However, published genomes are currently available for only three of the eight *Giardia* assemblages, A, B, and E. To better understand how both strain level and assemblage level genetic differences could influence the pathogenicity and zoonotic potential of *Giardia*, whole genome sequencing of two isolates from naturally infected hosts was performed. The first isolate came from a heavily infected cat presenting with diarrhea. The second isolate came from a heavily infected diarrheic dog which continued to shed cysts following metronidazole treatment. Cysts from both samples were cleaned and concentrated using a CsCl gradient and immunomagnetic separation. Whole genome sequencing was performed using an Illumina MiSeq with paired end reads. Preliminary analysis of these genomes found that 96.7% of the reads from the 10.4 Mb genome of the cat isolate mapped to the WB_A1 genome (assemblage A), and that the genome from the cat isolate was more similar to the P15_E genome (Assemblage E) than to the GS_B genome (Assemblage B). The genome from the dog isolate appears to be assemblage D at multiple loci. Intriguingly, it had the greatest similarity to P15_E with 6.2% of reads mapping to this genome. However, overall it appears quite different from any other *Giardia* genomes currently available. The genome from the dog isolate was also smaller than any other published *Giardia* genomes at 9.9 Mb. Future comparisons of protein coding regions and potential virulence factors from these genomes may reveal important differences related to their pathogenicity and host specificity.

ORAL PRESENTATION

Su-O11-15h48-16h00

Unusual localization and potential functions of three paralogs of a heme protein in *Giardia intestinalis*

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Giardia intestinalis is a microaerotolerant protist that possesses a highly reduced form of mitochondria called the mitosome that lacks the capacity for oxidative phosphorylation and heme biosynthesis. However, its genome encodes five heme proteins, including four isotypes of cytochrome b5. These cytochromes are small electron transfer proteins found in all kingdoms of life. The archetypical members (Class I) are the mammalian cytochrome b5s with a C-terminal hydrophobic domain that anchors the protein to the endoplasmic reticulum or mitochondrial outer membrane, and which participate in well-characterized electron transfer pathways. In contrast, the four *Giardia* isotypes are soluble, do not reside within *Giardia* mitosomes, and belong to Class II. Multiple isotypes of soluble cytochrome b5 (Class II) also exist in other protozoan parasites including *Plasmodium, Toxoplasma, Leishmania* and *Trichomonas*. However, despite the widespread distribution of Class II CYTB5s and their high prevalence in anaerobic protozoa, the functions of these proteins are unknown.

We focussed our initial studies on the three smallest *Giardia* cytochrome b5s (gCYTB5-I, II and III) since they have similar sizes (14.5 to 15.5 kDa) while isotype IV is much larger (29 kDa), owing to an N-terminal domain of unknown function. We examined the cellular localization of the *Giardia* cytochromes with custom peptide antibodies that uniquely recognize each isotype. Immunofluorescence microscopy showed that gCYTB5-I is in the nucleolus, gCYTB5-II is associated with the peripheral vesicles; and gCYTB5-III is in the nucleoplasm. Next, we examined the response of the gCYTB5s to three nitrosative stressors (nitrite, GSNO, and DETA NONOate). Upon exposure to all three stressors, gCYTB5-I moves out of the nucleolus into the nucleoplasm and cytosol. In contrast, the cellular localizations of the other two isotypes (gCYTB5-II and III) remain unchanged under the same conditions. Differential responses of the three gCYTb5 isotypes at the protein level to the different nitrosative stressors are also observed. We have also begun co-immunoprecipitation (coIP) experiments to identify the interacting partners of the *Giardia* cytochromes. These analyses will give insights into the role and function of these enigmatic proteins in *Giardia* and other anaerobic protists.

Su-O12-16h24/16h36

Crypto and *Giardia*DB.org: free, online resources bringing Omics to every scientist

Warrenfeltz S [1], Kissinger JC [1,2,3]

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CryptoDB and *Giardia*DB (http://cryptodb.org, http://*Giardia*db.org) are components of the Eukaryotic Pathogen Database Resources (EuPathDB, http://eupathdb.org), free, online data mining resources that support over 190 organisms within Amoebazoa, Apicomplexa, Chromerida, Diplomadida, Trichomonadida, Kinetoplastida and numerous phyla of oomycetes and fungi. These resources facilitate the discovery of meaningful biological relationships from large volumes of data by integrating pre-analyzed Omics data with advanced search capabilities, data visualization and analysis tools. CryptoDB currently contains data from 14 different *Cryptosporidium* species and strains including C. *parvum*, and *Giardia*DB contains data from 5 assemblages and Spironucleus. The intuitive graphic interface allows users to take full advantage of the data without the need for computational training. Data types range from genome sequence and annotation to transcriptomics, proteomics, epigenomics, metabolomics, population resequencing, clinical data, and host-pathogen interactions. Data are analyzed using standard bioinformatics workflows. Also, an in-house analysis pipeline generates data including domain predictions and orthology profiles across all genomes which permit inferences from data-rich organisms to organisms with limited or missing data. These resources offer several perspectives for data mining – record pages which compile all data for genes, pathways, etc; a genome browser for visualizing sequence data aligned to a reference genome; a search strategy system for querying pre-analyzed data to find genes or features that share biological characteristics; and a private Galaxy workspace tools to map RNA-Seq data and call SNPs (among others) and viewing it in context with public data already integrated into EuPathDB. Since IGCC 6, we have added new genomes and annotation, isolate whole genome sequences, RNA sequence data and GO Slim annotations, as well as several new gene page, Galaxy and functional enrichment tools. These free, comprehensive data mining resources easily merge evidence from diverse data types and across organisms to place the power of bioinformatics with the entire scientific community. Our active user support offers an email help desk, social media, a You Tube channel and a worldwide program of workshops. Please stop by our booth at the poster sessions for a demonstration, to suggest a data set or ask a question.

Su-O13-16h36/16h48

Allele Sequence Heterozigosity and recombination patterns detected by multiplex PCR amplification and Massive Parallel Sequencing of single cysts of *Giardia duodenalis* assemblage B.

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Giardia duodenalis infections can present high levels of genetic heterogeneity as has been observed in numerous genotyping studies (especially in assemblage B isolates) by the presence of overlapping peaks in direct sequencing of PCR products or of different haplotypes when PCR products were previously cloned. This variation can be caused by differences among (mixed infections), within individuals (allelic sequence heterozygosity, ASH) or a combination of both.
To determine the contribution of the different sources of genetic variation in five assemblage B isolates, a multilocus genotyping analysis of single cysts based on micromanipulation, direct multiplex PCR of seven single copy loci (beta-giardin, caltractin, ferredoxin, glutamate dehydrogenase, mlh1-like protein, ribosomal protein L7a, triose phosphate isomerase) and massive parallel sequencing (MPS) using Ion Torrent Personal Genome MachineTM (Ion PGM) was conducted. Isolate controls consisting of pools of >100 cysts were subjected to the same protocol.
A total of 745845 clonal reads that covered >95% length of the amplicon were obtained, although they were unevenly distributed among cysts and loci: ferredoxin and caltractin were underrepresented (Avg.: 244.7 reads per cyst; S.E.: 13.3) compared to the other five loci (Avg.: 3425.6; S.E.: 242.7), which prevented the unambiguous identification of genotypes in some cases.
Allele calling was performed with specialized software (Amplian and Amplisas) and a custom filtering pipeline (USEQ). Two isolates were monomorphic at all loci, another one showed low variability at two loci and two other isolates showed two to four alleles per loci except in one monomorphic locus. Considering the five loci with the highest coverage, all single cysts presented essentially the same Multi-Locus Genotypes (MLGs) that their respective isolate controls, with only 11 single-locus discrepancies involving nine out of 40 cysts. These differences were limited to the failure to detect one allele or to the detection of one extra allele (usually present in other isolates) and did not involve the presence of exclusive MLGs. These results confirm that the main source of variation was ASH rather than coinfection with different lineages. Differences between single cyst MLGs from distinct isolates usually corresponded to alternative combinations of the same alleles, which were observed both in homozygous and in heterozygous states. These findings go in line with previous evidence for genetic recombination in *Giardia*'s life cycle.

Finally, alleles identical to sequences used to designate subassemblages BIII and BIV were observed simultaneously in single cysts, questioning that these are true separate taxonomical entities.

Su-O14-16h48/17h00

Variation in Promoter Sequences Drives Differential Expression of GLORF-C4 in the Human Infective Assemblages of *Giardia duodenalis*

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Giardia duodenalis, an important human pathogen, has a two-stage lifecycle; the cyst and trophozoite. There are eight distinct genetic groups, two of which, assemblages A and B infect humans. Encystation is the encasement of the trophozoite by cyst wall material and is a major virulence determinant. Previous proteomic work from our lab has found GLORF-C4 protein exclusively in assemblage B trophozoites. Therefore, we wished to determine the differential expression of GLORF-C4 between assemblages A and B of G. *duodenalis* and the basis for this expression. Glorf-c4 expression was measured using qPCR in trophozoites and during encystation for assemblages A and B. There was no significant increase (~1.3-fold) in the level of glorf-c4 present in assemblage B isolates, however assemblage A isolates showed a 4 to 10-fold increase during the first 8 hours post-induction of encystation depending on the isolate. When compared to their endogenous controls, assemblage B had significantly higher levels of GLORF-C4 mRNA than assemblage A, and the assemblage A levels, even after induction, were still below those of assemblage B. We concluded that GLORF-C4 in assemblage B isolates is constitutively expressed whereas its expression is significantly increased upon induction of encystation in assemblage A. Examination of the upstream region of both the assemblage A and B GLORF-C4 genomic sequences identified a region where there is a 14bp insertion/deletion. In assemblage B, this region is homologous to known prokaryotic promoter motifs, which is disrupted by a 14bp deletion in assemblage A.

Given the putative role of GLORF-C4 in encystation, we induced assemblage A and B isolates to encyst and sampled cells every 6 hours for 72 hours, to determine if there was variance in encystation kinetics. We found that the assemblage B isolate reached maximum cyst numbers 12 hours sooner than assemblage A, although the percentage of encysting cells was equivalent for all time points between both assemblages. Therefore, it would appear that assemblage B cells are able to rapidly convert encysting cells to cysts whereas assemblage A cells are held in encystation.

GLORF-C4 has structural homology to small heat shock proteins (sHSPs). sHSPs are induced during stress conditions in other organisms however no obvious sHSP has been identified in *Giardia*. Encystation can be deemed a stress response. Therefore GLORF-C4 has a possible role as a sHSP in *Giardia* during encystation. Additionally, the constitutive expression of GLORF-C4 in assemblage B cells may be beneficial for cyst production during encystation.

Su-O15-17h00/17h12

The two nuclei of *Giardia intestinalis* during mitosis

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Alike other diplomonads, *Giardia* has two equally sized nuclei in a trophozoite cell. They divide each by its own spindle apparatus during a semi-open mitosis. The tiny chromosomes (300 nm-1.5 µm) condense on mitotic onset and their anaphase poleward movement can be followed. The genome segregation is however prone to missegregation errors due to absence of the spindle checkpoint. The nuclei have been considered identical. We have however previously shown they contain different chromosomal sets, display various levels of aneuploidy and proceed through the cell cycle with some asynchrony. We demonstrated by fluorescence in situ hybridization that several genes from chromosome 5 are lost in one of the two nuclei of the WBc6 *Giardia* line. The lacking segment stretches over at least 50 kb near the 5´ chromosome end. In both WB and WBc6 *Giardia* cell lines, chromosome 5 is trisomic in one nucleus and monosomic in the other nucleus. *Giardia* is thus capable of carrying out gene expression from only one nucleus. The two nuclei display a certain level of diversity, making each of them irreplaceable. Fine structural features of the two dividing nuclei will be shown by FIB/SEM tomography and 3D reconstruction, including spindle apparatus and kinetochores.

Su-O16-17h12-17h24

The protein methylation network in the early-branching protozoan parasite, *Giardia duodenalis*

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Giardia duodenalis has a unique cell biology shaped by early-branching origins in the eukaryotic phylogeny. In particular, protein methylation enzymes in *Giardia* are minimised, with no annotated demethylase or protein arginine methyltransferase (PRMT) domain- containing proteins, and only six SET-domain-containing proteins in support of reduced lysine methylation (K-Me) machinery. However, despite a lack of methylation data, there is sufficient evidence for K-Me regulation in surface antigen switching, differentiation and drug resistance.

We have comprehensively demonstrated a conserved, functionally essential K-Me network in *Giardia*. Using *in silico* structural modelling, domain homology and phylogenetics, we have consolidated known Class V SET-domain methyltransferases and annotated new Class I seven- beta-strand lysine methyltransferases. We have demonstrated that PRMTs are lost in the diplomonad lineage, and further verified minimal arginine methylation in *Giardia* using immunoblotting, amino acid analysis, and control immunoaffinity enrichment (IAP), which coincides with a lack of PRMT inhibitor activity in chemical screens. IAP of lysine methylation in the infective (trophozoite) and transmission (cyst) life-stages has identified 524 methylation sites on 322 proteins. Cytoskeletal proteins are significantly enriched, and we detected methylated RNA helicases and ribosomal/ribonucleoproteins in support of a role of K-Me in gene regulation, as well as on histone H2 and H3 variants. Additional mass spectrometry of histone-enriched fractions has allowed us to detect over 50 acetylation, methylation and phosphorylation sites on *Giardia* histone variants for the first time, including conserved, canonical H3 methyl marks for chromatin state. Indeed, only inhibitors of histone lysine methyltransferases (HKMTs) have detectable activity in *Giardia*, and we have explored their activity during *in vitro* log-phase and encysting cultures, complimented by quantitative proteomics of over 2000 proteins, and scanning electron microscopy. Collectively, these data provide first, key links between gene regulation, phenotype and inhibitor pharmacology, and confirms K-Me plays a dynamic, essential role in *Giardia* biology.

Su-O17-17h24/17h36

High-resolution, quantitative proteome of *Giardia duodenalis* during encystation

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Encystation in *Giardia duodenalis* is a seemingly simple, yet critically important and highly complex developmental process that is essential for the parasite's transmission. Large-scale studies of the major changes induced in *Giardia* during the encystation process have greatly improved our understanding. However, although RNA-seq methods have achieved high-coverage characterization of the encystation process at a transcriptional level, significant limitations in the sensitivity of proteomic methods have limited a similar depth of understanding of the protein expressional changes. Currently, proteomic data are available for just ~16% of predicted protein coding genes during encystation. By contrast, 97% of these genes are identifiable as transcripts in this stage. Many of these transcripts may indeed be under translational repression in the cyst stage. Understanding this provides important insights into the mechanisms and regulation of encystation and excystation, but requires a more comprehensive characterization of the cyst proteome.

Using cutting-edge, mass spectrometry methods, we have generated the first high-resolution proteome for *Giardia duodenalis* across encystation, identifying over 4,000 proteins and reproducibly quantifying ~3,600 proteins across biological replicates, effectively tripling the currently published proteome coverage. We use this approach to comprehensively explore proteomic changes during trophozoite growth, low-bile encystation priming, mid-encystation and in mature cysts. Our analysis is the first quantifying changes in trophozoites during low bile priming prior to in vitro induction of encystation, documenting events that may provide crucial insights into early signalling in trophozoites pre-empting encystation. Our dataset also identifies 43 of the 45 lipid metabolic enzymes at protein level and demonstrates a concerted upregulation of a set of lipid metabolic enzymes during the progression of encystation. These include ~10 lipid metabolic enzymes, including an unannotated phospholipase B like enzyme which we have verified using HMM and structural modelling, and which is upregulated almost 4-fold in the cyst, and may indicate new roles of lipid metabolism in mediating encystation.

This new high-resolution proteome dataset will be of value for large scale transcriptomic-proteomic correlation studies, verifying protein expression, as well as providing insights into parasite transmission for developing strategies to block transmission blocking drugs.

Su-O18-17h46/17h58

Impact of early-life exposure to *Cryptosporidium parvum* infection on intestinal homeostasis at adulthood.

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Numerous studies recently describe the relationship between influences during early-life period and later-life health and disease. *Cryptosporidium parvum* is a zoonotic parasite responsible for a diarrheal disease that affects mostly children under 5 and immunocompromised patients (AIDS or organ transplant patients). Epidemiological studies have reported that after resolution of C. *parvum* infection, patients still suffer for abdominal pain. In this context, by using a neonatal mouse model of cryptosporidiosis, we tried to decipher the intestinal consequences at adulthood of the neonatal infection by analyzing the composition of the microbiota, the composition of immune cells in the intestine but also consequences on visceral sensitivity and the susceptibility to an unrelated intestinal infection.

We observed that adult mice infected by C. *parvum* during the neonatal period display a modification of microbiota and of the composition of immune cells. These intestinal modifications were associated with an increased viscero-sensitivity and with a higher sensitivity to Salmonella infection. Altogether these results clearly demonstrate that an infection by C. *parvum* during the neonatal period induces intestinal imprinting that can be responsible for abdominal pains and increased susceptibility to another intestinal infection, way after the resolution of C. *parvum* infection.

Su-O19-17h58/18h10

Role of Paneth cells during infection of neonatal mice by *Cryptosporidium parvum*

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Cryptosporidium parvum is a zoonotic apicomplexan parasite responsible for a diarrheal disease named cryptosporidiosis. This protozoan parasite is found worldwide and is transmitted by contaminated water. The immature intestinal immune system in very young animals and children under 5 places them at high risk of developing severe cryptosporidiosis. Paneth cells (PC) are specialized intestinal epithelial cells located at the base of intestinal crypts producing antimicrobial peptides (AMPs) that develop and mature after birth. We and others have already described in vitro that antimicrobial peptides such as CRAMP and CCL20 can alter the viability of sporozoites of C. *parvum* (1).

We therefore wondered whether PCs and the AMPs that they produce can participate in the protective innate immune response against the parasite. By using a mouse model of neonatal cryptosporidiosis, we investigated the role of Paneth cells in the innate immune response against C. *parvum*. We first compared the susceptibility to C. *parvum* of mice genetically modified to be depleted of PCs (Sox9flox/flox-vil-Cre mice) and observed an increased level of infection when PCs are absent, associated with a reduced expression of AMPs. We also determined the effect of *Cryptosporidium parvum* infection on PC development and activity. By immunofluorescence, we observed on intestinal sections that C. *parvum* infection decreases the number of granule-positive-PCs and lysozyme-positive-PCs in neonatal mice. Altogether, these first results clearly demonstrate that PCs are important contributors of the innate protective immune response in mice and that lyzozyme, already described to be efficient in vitro on C. *parvum* sporozoite viability, may be involved in this effect.

(1) Guesdon W, Auray G, Pezier T, Bussière FI, Drouet F, Le Vern Y, Marquis M, Potiron L, Rabot S, Bruneau A, Werts C, Laurent F, Lacroix-Lamandé S. CCL20 Displays Antimicrobial Activity Against *Cryptosporidium parvum*, but Its Expression Is Reduced During Infection in the Intestine of Neonatal Mice. J Infect Dis. 2015 .

Su-O20-18h10/18h22

Identification of heparin-binding proteins in *Cryptosporidium parvum*

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It has been reported that heparin could mediate *Cryptosporidium parvum* invasion in vitro, but the precise role of heparin or heparin sulfate during C. *parvum* infection and mechanisms underlying these interactions are poorly understood. In this study, Proteins containing thrombospondin type 1 (TSP1) domains were selected to study their binding properties to HCT-8 cells and heparin sepharose. At first, specific McAbs or polyclonal antibodies against 10-14aa peptides from these proteins were prepared, then subcellular localization of these proteins on sporozoites and merozoites were identified by IFA using these antibodies. After that, the extracellular regions of genes encoding these proteins were cloned into pGEX-4T-1 and expressed in E. coli BL21(DE3). Then Cell-binding ELISA and FACS were used to evaluate the binding properties of these recombinant proteins to HCT-8 cells. After that, pull down assay were used to characterized the binding of these recombinant proteins to heparin sepharose. Finally, the neutralization effects of McAbs against peptides from TSP4 and TSP7 on C. *parvum* infection were evaluated by QRT-PCR. Three TSP1 domains containing proteins, named TSP3, TSP4 and TSP7 respectively, were localized on the surface of sporozoites and merozoites. All of these recombinant proteins bind to HCT-8 cells in dose-dependent and saturable manner. Also, these proteins could bind to heparin sepharose and the binding could be inhibited by free heparin salt, but not by chondroitin sulfate A. Both McAbs could significantly reduced the infection rates. As adhesion to host cells are necessary for successful invasion, these TSP1 domains proteins may mediate C. *parvum* infection by binding heparan sulfate on host cells.
Key words: *Cryptosporidium parvum*, TSP-1, Adhesion, Heparin.

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Monday, 24th June, 2019

ORAL PRESENTATIONS

Mo-O1-8h00/8h20

How innate immune responses shape *Cryptosporidium* infection

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Immune status of the host is of critical importance for controlling *Cryptosporidium* infection. Logically cryptosporidiosis primarily affects young ruminants, infants, and immunocompromised individuals. The infection is transient in immunocompetent individuals but severe in young mammals in particular when exacerbated by malnutrition and persistent in adults with immune deficiency. Cryptosporidiosis is still poorly controlled by chemotherapy and available FDA approved molecule Nitazoxanide (NTZ) require for its efficacy the presence of a certain level of host immunity. Indeed, oral suspension and Nitazoxanide tablets have not been shown to be superior to placebo for the treatment of diarrhea caused by *Cryptosporidium* in HIV-infected or immunodeficient patients. Therefore, understanding immune mechanisms controlling parasite replication and host pathology is a prerequisite for developing host-directed therapies for controlling cryptosporidiosis alone or in combination with chemotherapy.

Cryptosporidium is minimally invasive and its development is restricted to the apical side of epithelial cells *in vivo.* Intestinal epithelial cells (IECs) are therefore central in the mechanisms of protection representing the watchdog of the immune system, signaling the infection to the immune system by releasing soluble mediators such as chemokines but also *Cryptosporidium* antigens via small vesicles, and apoptotic bodies. Murine models allowing deep investigation of immune mechanism demonstrate that induction of an adaptive immune response is required for a definitive clearance of the infection but innate immunity plays the main role in controlling the acute phase of the disease. Proportions of the subsets evolve in young animals the days following birth and are also deeply modified by the infection. Among the four conventional dendritic cell (cDC) subsets present in the neonatal intestine we identified CD103+ Batf3+ DC (cDC1) as the key subset by their ability to produce large amount of IL12 which in turn can activate T lymphocytes, NK cells and ILC1 to produce IFNγ a cytokine known to restrict *C. parvum* growth in intestinal epithelial cells. We are currently characterizing the cDC1 cells in lymphoid and non-lymphoid intestinal tissues in ruminants to validate our findings in a target species. A strong recruitment of immune cells in close contact to infected IEC is not necessarily linked to protection. We have indeed observed that the large influx of Ly6-C inflammatory monocytes during infection has no effect on parasite replication rate but was linked to intestinal permeability with a possible link with physiopathology. This highlight the importance of being able to transform observations and correlations into functional demonstrations. When immunomodulatory treatments affecting mononuclear phagocytes are investigated, it is therefore important to identify their role on both cDC1 and inflammatory monocytes and to evaluate the general benefit for the host. Once effectors cells are activated, they in turn produce immune mediators acting on IEC. Another way to strengthen the efficacy of immune responses is to identify evasion strategies implemented by the parasite. Several mechanisms have been identified to date including parasite immune evasion through miRNA-mediated mechanisms induced in IECs. During infection alterations in miRNA expression profiles affect epithelial cell immune and inflammatory response and release of apical and basolateral exosomal vesicles.

We need to continue our efforts on preventive and control measures to fight the disease. The combination of host immune modulation with new chemotherapeutic solutions currently in active development in many laboratories represents a very attractive way for efficiently control the disease.

Mo-O2-8h20/8h32

Shifts in Treg/Th17 balance correlate with differential susceptibility to infection with *Giardia muris*

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Infections with *Giardia* lamblia remain a common cause of food and water-borne diarrhoeal disease worldwide. Most patients display few symptoms and clear the parasite rapidly, however infection may also lead to chronic diarrhoea, abdominal pain, fatigue or malabsorption. Effective host protection primarily relies on intestinal IgA and Th17 responses. The high prevalence of asymptomatic infections raises the question if immunoregulatory circuits are at play during giardiasis. Furthermore, *Giardia* infections lead to alterations in the composition of the hosts intestinal microbiota and the structure of the gut microbiome is associated with differential susceptibility to experimental giardiasis. Hence, we asked whether shifts in the balance between Th17 and regulatory T cells (Treg) influence on the control of infection with *Giardia* and if gut microbiome signatures can be linked to high/low susceptibility.

Confirming previous reports, we observed higher faecal cyst numbers in G. muris-infected BALB/c mice than in C57BL/6 mice. RORγT+ Th17 cell frequencies and numbers were comparable between the two mouse strains during infection. However, more resistant C57BL/6 mice displayed elevated Th17 cell proliferation and IL-17A production in Peyer's patches (PP) and small intestinal lamina propria (siLP) compared with BALB/c mice. In contrast, more susceptible infected BALB/c mice displayed significantly higher frequencies and absolute numbers of Foxp3+ Tregs and RORγ-expressing Foxp3+ Tregs in siLP compared to C57BL/6 mice. Consequently, Treg/Th17 cell ratios were elevated in G. muris-infected BALB/c, but not C57BL/6 mice and Treg/Th17 ratios correlated positively with cyst excretion rates. In addition, 16s rRNA-gene based quantitative PCR intestinal microbiota analysis revealed the expansion of Enterobacteria in G. muris-infected BALB/c mice and constitutively higher Enterobacteria loads in Th17-prone C57BL/6 mice. In contrast, Bifidobacteria loads decreased notably during infection in both mouse lines. Importantly, cohousing of BALB/c and C57BL/6 mice did not alter the observed immunological phenotype during infection, indicating that the observed Treg/Th17 shifts during murine giardiasis and their effect on the control of infection are independent of the intestinal microbiota. In conclusion, our study therefore demonstrates that localized shifts in the balance between Th17 cells and Tregs in the siLP of G. muris-infected mice does indeed correlate with susceptibility to infection.

Mo-O3-8h32-8h44

Variability in giardiasis: roles for immune responses and microbiota

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Giardia duodenalis infects hundreds of millions of people around the world each year. During the acute stages of infection, some individuals develop diarrhea cramps and other overt signs of disease. However, the majority of infected individuals do not display overt symptoms, although they may still exhibit reduced levels of lactose utilization and nutrient absorption. Furthermore, it is becoming clear that following infection with *Giardia* some individuals can go on to develop post-infectious irritable bowel syndrome (IBS). Work in our lab has focused on how immune responses develop and contribute to the development of symptoms. In a mouse model, we have shown that CD8+ T cells do not contribute to control of infection, but are necessary for development of lactase deficiency. Activation of this CD8 response is lacking in mice treated with broad-spectrum antibiotics, as is lactase deficiency. Treatment with antibiotics also reduces the accumulation of macrophages in the intestinal lamina propria normally observed during infections. Stimulation of peritoneal macrophages with live trophozoites ± LPS in vitro indicate that *Giardia* initiates a minimal response on its own, but synergizes with LPS to induce production of both IL-10 and TNF. Analysis of macrophage function in vivo suggests that they participate in the development of regulatory responses, but are not needed to for control of the infection. We have also begun analyzing patients with symptomatic and asymptomatic *Giardia* infections. Analysis of sera has identified IFNγ as potentially being associated with development of overt symptoms. Like the CD8 response, IFNγ is not needed for control of infection in the mouse model. Metabolomic analyses have also revealed that patients with both symptomatic and sub-clinical infections have elevated levels of serotonin, a molecule important for regulation of intestinal motility and a potential link to IBS. Sequencing of fecal samples from these same patients are underway to determine if infection outcomes correlate to differences in the microbiome.

Mo-O4-8h44/8h56

Temporal and spatial analysis of the intestinal IL-17A response following a *Giardia* infection

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The intestinal protozoan *Giardia* has a wide vertebrate host range. The infection can pass without acute symptoms, but can also lead to a condition called giardiasis characterized by an intestinal distress such as diarrhea, abdominal pain, nausea and weight loss. Most of the time, symptoms rapidly disappear, but some host develop long-term sequalae which can extend for several months. Previous studies have highlighted the critical role of IL-17A in orchestrating the protective immune response against the parasite. However, the initiation site of the immune response as well as the cellular source of the IL-17A in the intestine is still unclear. As such, our goal was to determine more precisely which cell types were involved in the IL-17A response and where the response was initiated.

To this end, the Peyer's patches (PP), the mesenteric lymph nodes (MLN) and the small intestine were sampled. The relative expression levels of mRNA coding for IL-17A and for the IL-17A-related genes RORγt and IL-6 were measured by qPCR analysis in the different tissues obtained from uninfected control mice and from *Giardia*-infected mice at day 7 and day 14 p.i. Flow cytometry was then used to characterize IL-17A producing cells in the different organs at day 14 p.i.

From qPCR analysis we confirmed previous observations made in the small intestine showing increased expression of IL-17A both at 7 and 14 days p.i and a decreased expression of RORγt. The same trend was observed in the PP although not significant for the IL-17A. In the MLN no differential expression was detected compared to control animals for any of the gene tested. Flow cytometry demonstrated that CD45+ IL-17A+ cells were increased in the PP and the intraepithelial compartment of the small intestine when mice were infected. Interestingly, the opposite phenomenon was described in the MLN with a decreased in the infected animals. Phenotypic characterization of these cells unrevealed different source of IL-17A by CD4+ T cells and TCR- cells.

This study demonstrates that the IL-17A response in the Peyer's patch tissue is not different from the one observed in the small intestine. Moreover, cells present in different parts of the intestinal immune system, contribute to the IL-17A response following G. muris infection.

Mo-O5--8h56-9h08

Transcriptional Profiling of Differentiated CaCo-2 Intestinal Epithelial Cells Response to *Giardia Intestinalis* during Early Onset of in vitro Interactions: Insights into the Pathways of Cytokine Production and Regulation

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Introduction: *Giardia* Intestinalis is a protozoan parasite that causes diarrhea in humans. Despite the manifestation of diarrheal disease, little inflammation is seen during infections indicating that *Giardia* might exert immunomodulatory responses in the host. Objective: Therefore, we aimed to study whether *Giardia* attenuates inflammation using an in vitro model of interaction. Method: We used transcriptomics to identify upregulated genes at early hours of interaction (1.5, 3 and 4h) between WB isolate and differentiated Caco2 cells (DCCs). Results: A transcriptional peak at 1.5h was seen for genes encoding the cytokines/chemokines IL-8, CCL2, CCL20, CXCL1, CXCL2 and CXCL3 together with nuclear recruitment of proinflammatory genes transcription factors, nuclear factor kappa B (NF B) and activator protein-1 (AP-1), declining afterwards. Despite the increased phosphorylation of Erk1/2 and P38, cytokines concentrations were low (< 200 pg/ml) and discordant with their transcriptional levels, indicating a post-transcriptional regulation of cytokine production. A gene encoding zinc finger protein 36 (i.e. tristetraprolin, TTP) was upregulated (1.5h) whose product is known to bind 3'UTR of cytokines transcripts inducing their decay. We show that *Giardia* induces TTP expression in DCCs and when incubated with inflamed DCCs the increase in TTP expression coincided with an inhibition of ERK1/2 and P38 phosphorylation. A reduction in Luciferase activity in DDCs transfected with a reporter plasmid with 3'UTR of IL8, CCL20 and CXCL1, confirmed the role of TTP in regulating cytokine production post-transcriptionally. Conclusion: This study highlight a role for *Giardia*-induced expression of anti-inflammatory factors in DCCs to control inflammation and enhance parasite persistence during infection.

Mo-O6-9h08/9h20

Human small intestinal organoids - a new model to investigate *Giardia* sp. infection

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Giardia duodenalis is one of the most abundant parasites, responsible for over 280 million cases of the enteric disease “giardiasis” every year, worldwide. One proposed pathomechanism is the induction of epithelial barrier dysfunction by apoptosis or tight junctional alterations, which increase epithelial permeability, may impact nutrient uptake and normal gut function, or even lead to invasion by luminal bacteria. In vitro studies using a popular Caco-2 cell line model of the gut epithelium reproduced contradicting results often indicating no barrier destructing effects upon *Giardia*-infection. As a new approach, human small intestinal organoids were established here. By generating epithelial monolayers from stem cell enriched organoid cultures we were able to set up a model which reproduces more aspects of the in vivo epithelium than current cancer cell line-based systems. Infection of such organoid-derived epithelial monolayers allowed conditions in which the parasite is reproducibly able to destroy the integrity of monolayers, leading to barrier dysfunction past 24 h after infection. This destruction is correlated with increased apoptosis (~5%) and higher numbers of ClCa1-positive cells (~15%), an indicative of goblet cell differentiation, which could be a response to tissue damage. Thus, with this highly promising new model, the parasite’s pathomechanism can be investigated in more detail and subtle effects, conventional models fail to detect, may be revealed.

Mo-O7-9h40/9h52

In vivo and in vitro studies of the host-parasite interactions of *Spironucleus salmonicida* and the Atlantic salmon

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The diplomonads are a diverse group of flagellated eukaryotic microbes adapted to life in oxygen depleted environments. An understudied member of the diplomonads, Spironucleus salmonicida, is known to be able to cause a systemic infection in salmonids (e.g. Salmo salar), resulting in economic losses in farmed fish during outbreaks of the parasite. Little is known about its life cycle and research is needed to give the ability to control for transmission and outbreaks.
To shed a light on the life cycle we have infected juvenile Atlantic salmons with S. salmonicida and followed the course of infection up to 10 weeks in 5 individual trials. During these trials we have collected infected fish tissues for histological analyses and RNA sequencing. During two of the trials we intubated salmons with transfected parasites stably expressing the Firefly luciferase reporter, upon genomic integration, to monitor the infection closely using a Xenogen Ivis camera system.
To dissect the host-parasites interaction further, we used an in vitro infection model including the Atlantic salmon kidney cell line (ASK) in a time course experiment for 24 hrs. RNA samples were collected at 6 different time points and we make an attempt to identify important transcriptional changes associated with the early stages of infection. Additionally, we have collected SEM and TEM images during the course of the experiment.

These are the first molecular studies to look at the host-parasite interaction of S. salmonicida and salmon and the results give highly valuable information on the parasites behavior and are vital in the completion of the life cycle of S. salmoncidia.

Mo-O8-9h52109h04

Deprivation of dietary fiber enhances susceptibility of mice to cryptosporidiosis

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The benefits to intestinal health of diets rich in plant fibers are well known. Mechanisms by which fiber deprivation affects the intestinal microbiota, the gut epithelium and host physiology are being investigated. Research on the interaction between the microbiota and the intestinal epithelium has revealed the importance of bacterial metabolites, such as short-chain fatty acids (SCFAs) originating from the breakdown of plant polysaccharides. In contrast to what is known about the effect of diet and bacterial metabolites on the intestine, little research has been conducted to elucidate to what extent diet affects enteric infections. This deficiency is particularly true for cryptosporidiosis. In light of the lack of effective anti-cryptosporidial drugs, we investigated whether parasite proliferation can be mitigated with dietary interventions. Based on our initial observation showing that mice consuming a probiotics product develop more severe cryptosporidiosis (doi: 10.1128/AEM.01408-18), we investigated the impact of dietary fibers on the proliferation of *Cryptosporidium parvum* and *Cryptosporidium* tyzzeri in the mouse. Mice were orally infected with oocysts and parasite multiplication measured by quantifying fecal oocyst output. High-throughput sequencing of 16S ribosomal RNA amplicons was used to characterize the impact of the infection and diet on the intestinal microbiota. On average, mice fed a diet without fiber (cellulose, pectin and inulin) developed more severe infections. A diet without fibers also significantly altered the fecal microbiota. Consistent with this observation, mice fed either a prebiotic product sold for human consumption or SCFAs excreted significantly fewer oocysts in the feces. The fecal microbiota of mice consuming no plant polysaccharides was characterized by a decreased relative abundance of Bacteroidetes. These results indicate that that dietary fiber deprivation increases the susceptibility to severe cryptosporidiosis and suggest that this effect is mediated by the metabolic activity of the intestinal microbiota and is reversible with prebiotics or SCFA. These observations underscore the potential of the bacterial metabolism to control the severity of cryptosporidiosis and raise the possibility of developing low-cost and simple interventions to protect infants and neonatal ruminants at risk of severe cryptosporidiosis.

Mo-O9-10h04/10h16

Domesticating a parasite: developing a genetically tractable natural mouse model of cryptosporidiosis

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Cryptosporidiosis is a leading cause of diarrheal disease and an important contributor to global infant mortality. There are no efficacious drugs or vaccines for this apicomplexan parasite, and basic research to drive their development has stagnated from a lack of genetic tools and facile animal models. To expand the capabilities of the field we isolated a species of the parasite that naturally infects wild mice, *Cryptosporidium tyzzeri*, from poultry farms in close vicinity to the University of Georgia. We then used this parasite to develop a genetically tractable mouse model that closely mirrors human cryptosporidiosis. We performed de novo assembly *C. tyzzeri* genome, then adapted CRISPR-driven homologous repair to genetically manipulate the parasite. Using this CRISPR based approach, we have created several reporter strains that allow us to non-invasively track parasite tissue burden throughout the gastrointestinal tract. In healthy laboratory mice, infection is self-limiting and clearance is dependent on the host T-cell response. Mice that have resolved infection show significant immunity to subsequent challenge, and an attenuated vaccine provides robust protection. In summary, we now have a mouse model for cryptosporidiosis where both host and parasite are genetically tractable, allowing for mechanistic investigation of virulence and host-pathogen interactions in the context of a working immune system.

Mo-O10 – 10h16/10h30

The sporozoite-trophozoite transition in *Cryptosporidium* – the role of sugar metabolism, calcium signalling and redox potential

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The transition from sporozoite to trophozoite is arguably the most critical phase in the life cycle of *Cryptosporidium*; the sporozoite must emerge from the oocyst following exposure to acidic conditions in the stomach and to bile salts in the upper duodenum, and must then attach to and establish a feeding relationship with a host epithelial cell. Sporozoites are analogous to the numerically more important merozoites which undergo a similar transition later in the life cycle but are more amenable to study; and a study of the sporozoite-trophozoite transition also sheds light on the claims made for development in axenic culture by *Cryptosporidium* trophozoites. Using evidence from microscopy, FACS, excystment, invasion and viability assays and transcriptomics we have experimentally dissected the sporozoite-trophozoite transition to identify key triggers for differentiation of *Cryptosporidium parvum* IOWA sporozoites. Sporozoites appear to lack endogenous energy stores and require exogenous carbon for survival; less than 50% survive more than 1 hour after excystment from the oocyst, and of these many have rounded up and have commenced differentiation. Ethanol (200mM) and caffeine (1mM) have opposite effects on differentiation, suggesting differences from the differentiation pathways seen in Toxoplasma. However, this situation is complicated by the presence of reductants in the medium. Dithiothreitol (DTT) at sub-millimolar concentrations (10 and 100 µM) enhances excystment and can reverse the age-related decline in viability of oocysts stored at 4°C (50% survival c. 3 months). Similar excystment enhancement follows treatment with NADPH, but not 2-mercaptoethanol, and it is not clear whether this is a general property of reductants or due to specific effects of DTT and NADP. Transcriptome changes in sporozoites during transformation will be presented, but clearly environmental conditions, especially redox potential, can have major effects on the differentiation pathway and must be controlled for and explicitly stated in published methodologies. We identify especially in this respect a protein of unknown function, cgd3_1410, transcription of which is strongly elevated in our studies but which has not been previously identified in the literature, presumably because of differences in the post-excystment environment.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 663830 (grant number CU118) and from the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) (grant number NC/R000913/1).

Mo-O11-10h50/11h10

Symptom variability in giardiasis **: Why ?**

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During the acute phase of infection, *Giardia* actively interacts with the intestinal surface, causing epithelial alterations, mucus layer disruptions, and gut microbiota dysbiosis. These effects occur at the site of infection and beyond, and result at least in part from the actions of *Giardia* excretory and secretory products and the host immune response. *Giardia* is a common cause of diarrheal disease, and may cause post-infectious complications, including Irritable Bowel Syndrome. However, giardiasis can also occur in the absence of symptoms, and it appears to protect children against diarrhea in countries with poor sanitation. Hosts may be co-infected with multiple diarrheal-disease causing pathogens, and final disease outcome results from the complex interactions between the host and this polymicrobial cross-talk. The mechanisms remain obscure. Recent studies using “-omics platforms”, human tissues and gut microbiota, live rodent models of co-infection reproducing co-infections with Escherichia-coli and *Giardia* in humans, or co-infected human enterocytes, are starting to shed light on why the production of symptoms in giardiasis may be so variable.

The various genotypes of *Giardia* isolates have different metabolomic profiles, and have been shown to differentially affect host intestinal epithelia. Recent studies lend support to the hypothesis that the immunomodulatory effects of *Giardia* may attenuate the pathophysiological responses induced by gastrointestinal pathogens causing disease via severe inflammation. The effects include cysteine protease-dependent cleavage of pro-inflammatory mediators such as CXCL8 (IL-8), which in turn avoids the local accumulation of neutrophils. While recent experiments suggest that in a model of malnourished mice, co-infection with *Giardia* and entero-aggregative E. coli may worsen disease, others clearly demonstrate that co-infection with *Giardia* protects against intestinal disease induced by an attaching-effacing enteropathogen. In this co-infection, the mechanisms appear to include activation of beta-defensin and intestinal trefoil factor-3 epithelial anti-microbial peptides, via an activation of the NLRP3 inflammasome. Other reports demonstrate that high fat versus low fat diets may facilitate pathogenic factors in giardiasis, offering further support to the hypothesis that diet also plays a role in symptom variability.

Together, these observations shed new light on the biology of polymicrobial infections in the gastrointestinal tract, and underscore the multifactorial basis of symptom variability in giardiasis. The findings point to new research directions in our attempts at the developing novel strategies to control enteric disease.

Mo-O12-11h10/11h22

Prolonged duodenal mucosal lymphocyte alterations in patients with and without post-giardiasis functional gastrointestinal disorders

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Objective: Persisting low grade inflammation is suggested to play a role in post-infectious functional gastrointestinal disorders (PI-FGID). The present study examined alterations in duodenal mucosal lymphocytes during and after *Giardia* gastroenteritis in patients who did, or did not, develop PI-FGID.

Design: Duodenal mucosal intraepithelial lymphocytes (IELs) and lamina propria CD3, CD4, CD8 and CD20 lymphocytes were quantified in 28 chronic giardiasis (CG) patients, 66 patients with persistent abdominal symptoms after acute *Giardia* infection (PI-FGID), 19 recovered controls (RC) and 16 healthy volunteers (HC). Patients were examined from 3 months to 19 months after initial onset of giardiasis. Associations with illness duration, abdominal symptoms and histology grade were assessed.

Results: Duodenal CD4 IEL were significantly elevated in CG, then decreased, followed by an upwards trend after one year in both the PI-FGID and RC groups. Duodenal lamina propria crypt CD4 T cells were decreased in CG, and stayed low for about 14 months before normalizing in both PI-FGID and RC groups. Lamina propria CD20 cells were persistently elevated in all three *Giardia* exposed groups. Biopsies with microscopic inflammation showed increased lamina propria CD20 levels. Alteration in lamina propria villi normalised earlier than alterations in lamina propria crypts.

Conclusions: Duodenal mucosa lymphocyte alterations were prolonged after *Giardia* infection. Patients who developed PI-FGID and recovered asymptomatic controls had similar and equally slow normalization of lymphocyte alterations. A study design with pertinent control groups were important for correct interpretation of study results.

Mo-O13-11h22/11h34

Impact of *Giardia* on the intestinal lipid metabolism

Maertens B.

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Giardia duodenalis is one of the most commonly found intestinal parasites in mammalian hosts. Infections can generally be cleared by the development of an adequate protective immune response. However, a significant proportion of infected hosts develop a chronic infection lasting for several months. giardiasis often leads to a fatty diarrhea, vomiting and poor absorption of nutrients. Previous studies have shown that *Giardia* lacks genes that are responsible for de novo synthesis of lipids. Indicating that the parasite depends on its host for the uptake of lipids, which might lead to changes in the lipid metabolism of the host. In order to investigate the influence of *Giardia* infections on lipid metabolism of its hosts, we made use of both *Giardia muris* and *Giardia duodenalis* infection models. Lipid extractions, of feces collected at different time points during infection, were separated by liquid chromatography and subsequently detected by high-resolution full-scan Q-Exactive Orbitrap mass spectrometry. For G. muris we could show a parasite-mediated shift in lipid composition. Interestingly this shift changed significantly when G. muris infected mice received an antibiotic treatment, indicating the involvement of the gut microflora in this response. In addition, gene expression analysis indicated the transcriptional downregulation of several intestinal lipid transporters such as CD36, FATP4 and ABCG5 following a *G. muris* infection. Overall, this study showed that *Giardia* affects the lipid metabolism of its host in a direct manner by affecting the expression by lipid transporters and in an indirect manner by changing the intestinal flora. Interestingly, such changes were not observed following a *G. duodenalis* infection in mice.

Mo-O14-11h34/11h46

Heterogeneity of *Giardia lamblia* Peripheral Endocytic Compartments revealed by Super Resolution Microscopy

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The protist parasite *Giardia lamblia* is highly adapted to the environment in the intestinal lumen of its mammalian host. Plasma membrane invaginations mediate bulk fluid phase material uptake to the endolysosomal compartments just beneath it - the peripheral vacuoles (PV) – where digestion and sorting is thought it occur. This is the only documented entry route for bulk uptake of nutrients in the parasite. For this reason, PV organelle homeostasis is clearly essential for parasite survival.

Despite their essentiality, little is known about how PV morphology is maintained. Due to their minute dimensions bellow light's diffraction limit, conventional light microscopy is not suitable for a detailed ultrastructural morphological study of these organelles. Furthermore, most reported PV-focused ultrastructure studies are based on 2D Transmission Electron Microscopy. Indeed, a complete ultrastructural analysis of these organelles is essential to comprehend endocytosis and nutrient uptake in G. lamblia.

To address this question, we employed the resolving power of both 3D Super Resolution Light Microscopy based on Stimulated Emission Depletion (STED) and Single Molecule Localization Microscopy (SMLM) based on Stochastic Image Reconstruction Analysis (STORM). Single organelles can now be imaged at resolutions of 20nm in the axial plane. These techniques allowed for an appreciation of the morphological heterogeneity of the PV population and for a quantification of organelle volume and interconnectivity. Furthermore, we also employed 3D Focus Ion Beam Scanning Electron Microscopy (FIB-SEM) for a complete reconstruction of almost all G. lamblia endomembrane compartments including the endoplasmic reticulum, PVs and unknown vesicular carriers.

Given the observed diversity in PV organelle morphology and volumes, we propose the new term Peripheral Endocytic Compartments (PEC), to better reflect the highly divergent nature of this endomembrane system in G. lamblia.

Mo-O15-11h46/11h58

Disc-o-fever: getting down with *Giardia's* groovy microtubule using new molecular genetic tools

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Giardia is a widespread zoonotic intestinal parasite that causes diarrheal disease in more than 280 million people each year. Motile trophozoites colonize and attach to the small intestine with the ventral disc, a complex microtubule organelle. Attachment is required for infection as it allows *Giardia* to resist peristalsis. During early stages of attachment, we discovered that the disc margin and ventral groove regions of the disc undergo specific conformational changes. These changes, along with the presence of a curved disc, likely create a "seal" that enables attachment by limiting fluid flow underneath the disc. Our pioneering work on disc architecture and composition, combined with our development of stable CRISPR-interference (CRISPRi)-mediated knockdowns and bioluminescent imaging of infection dynamics in animals, enable us to genetically test the structural and/or functional roles of the 90 disc-associated proteins (DAPs) we have identified. Currently, we have evaluated structural and attachment defects of over 10 CRISPR-interference (CRISPRi) DAP knockdowns using high-resolution live imaging, electron microscopy, and biophysical assays. We have observed several structural classes of DAP mutants, including those with discs that lack the overlap zone region, lack overall curvature, or lack proper organization of the MT array. Functional classes of DAP mutants include those that are unable to resist shear forces or lack a lateral crest seal. We predict that DAP knockdown mutants have overall defects in disc biogenesis or disc stability and flexibility, resulting in aberrant disc structures that have limited capabilities in attachment. Using our in vivo bioluminescent imaging animal model we are testing the ability of DAP mutants with significant attachment defects to colonization the host. We anticipate that therapies targeting DAPs required for parasite attachment would limit host colonization and limit the dissemination of infectious cysts.

Mo-O16-11h58/12h10

Enolase of *Giardia duodenalis*: a moonlighting protein secreted as monomer by trophozoites activates host cell plasminogen and induces a necroptotic-like damage in epithelial Cells

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Enolase is a key glycolytic enzyme in the cytoplasm of cells and it is considered a multifunctional protein. Besides its essential role in the cell cytoplasm, enolase mediates mechanisms of host-pathogen interactions, as the plasminogen system. This is a proenzyme of the fibrinolytic system and after its activation acquires proteolytic activity cleaving fibrin, fibronectin and laminin activating other proteolytic enzymes that results in the cleavage of collagen, elastin, and proteoglycans. This interaction represents a mechanism to enhance virulence of various microorganisms by capturing surface-associated proteolytic activity.

In this work, enolase one of several enzymes secreted by G. *duodenalis* trophozoites upon interaction with epithelial cells was analyzed on its effect on epithelial cells. This was approached by cloning G. *duodenalis* enolase (Gd-Eno) and the recombinant protein was used to analyze its role in the interaction with epithelial cells and for the production polyclonal antibodies (anti-rGd-Eno-Abs) to determine its localization in trophozoites.

The expression pattern of enolase by immunofluorescence assays showed that it is localized in small cytoplasmic vesicles and in the surface of trophozoites with a higher abundance on the ventral disk. Interestingly, anti-rGd-Eno-Abs significantly inhibited trophozoite attachment in co-culture with intestinal IEC-6 cell monolayers. Gd-Eno was detected as a dimer in trophozoite lysates while it was secreted as monomer in co-culture supernatants.

rGd-Eno was able to bind human plasminogen and to enhance plasmin activity in vitro when added to trophozoites using the intrinsic plasminogen activators of epithelial cells. Further rGd-Eno caused damage to IEC-6 monolayers characterized by profuse vacuolization, separation between the cells that detached from the substrate. This damage was effectively inhibited by either anti-rGd-Eno Abs or the plasmin inhibitor 6-aminocaproic acid and it was associated with secretion of tumor necrosis factor α (TNFα) and activation of the apoptosis inducing factor (AIF) suggesting that a necroptosis like cell dead is induced by rGd-Eno.

Consistent with these experimental data, protein modeling and molecular docking studies predicted a striking structural similarity of Gd-Eno with its human homolog and the influence of Mg^{2+} binding for the folding of Gd-Eno monomer and its expected mode of interaction with the serine protease, Kringle-3 and -4 domains of HsPls.

All together these results suggest that *Giardia* enolase is a moonlighting protein with a novel role by causing damage to epithelial cells through a necroptotic-like pathway involving TNFα and AIF activities.

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MO-O17-12h10/12h22

Molecular analysis of the *Cryptosporidium* life cycle progression

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The protozoan parasite *Cryptosporidium* is a leading cause of severe diarrhea in young children and an important contributor to early childhood mortality. Fully effective drugs and vaccines to treat or prevent cryptosporidiosis are lacking. A main roadblock for their development has the overall poor tractability of this parasite. We established a powerful molecular genetic model to overcome this hurdle. Here we use the new-found ability to genetically engineer *Cryptosporidium* to make life cycle progression and parasite sex tractable. We derive reporter strains to follow parasite development in culture and infected mice and define the genes that orchestrate sex and oocyst formation through mRNA sequencing of sorted cells. After two days, parasites in cell culture show pronounced sexualization, but productive fertilization does not occur and infection falters. In contrast in infected mice, male gametes successfully fertilize females, leading to meiotic division and sporulation. To rigorously test for fertilization, we devised a two-component genetic crossing assay employing a Cre recombinase activated reporter. Our findings suggest obligate developmental progression towards sex in *Cryptosporidium,* which has important implications for the treatment and prevention of the infection.

POSTER SESSION MORNING

Amphi 100A

MoA-P1-12h40/12h45

Monitoring of *Cryptosporidium* oocysts and *Giardia* cysts in the Nakdong River in Korea

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The most frequently identified source of infestation is water, and exposure involves either drinking water or recreation in swimming pools or natural bodies of water. The major protozoa causing waterborne diseases in Korea are *Cryptosporidium parvum*, *Giardia* lamblia. C. *parvum* and G. lamblia inhabit the intestinal tracts of vertebrates and provoke watery diarrhea and steatorrhea. Many waterborne disease outbreaks were reported in worldwide by contamination of these protozoa into water supply because they are not eliminated by water purification system using chlorine and fluorine.

In this study, we screened *Cryptosporidium* oocysts and *Giardia* cysts in three points of Nakdong River from January to December 2018, monthly. Point 1 is tributary possibly highly contaminated by cattle wastes, point 2 is tributary near livestock wastewater treatment plant, and point 3 is tributary near water resource. Microscopic examination using rapid immunoassay kit was performed according to the manufacturer's instructions. A total of 36 water samples of 20 L were examined. *Cryptosporidium* oocysts were detected in 28% (10/36) of samples and *Giardia* cysts in 58% (21/36). The maximum numbers of *Cryptosporidium* oocysts and *Giardia* cysts is 3 and 14 per 10 L of water sample, respectively. All three points had similar prevalence of *Cryptosporidium* oocysts and *Giardia* cysts. Thus, effective monitoring plans must take into account the spatial and temporal parameters of contamination because they affect the prevalence and distribution of these protozoan cysts in local water resources.

MoA-P2-12h45/12h50

Detection of *Cryptosporidium* and *Giardia* in Oysters by Nested-PCR and Sequence Analysis in Portugal

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Introduction: *Giardia* and *Cryptosporidium* are two protozoan parasites that infect man and animals and have been detected with frequency in shellfish mollusc. Both parasites have adequate life cycles to be transmitted by water and food. *Giardia* cysts and *Cryptosporidium* oocysts are the infective forms and are very resistant to environmental factors as well as to chemical treatments applied to water for human consumption. In Portugal, the research of these protozoa in food is scarce. Thus, the present work had as objective the detect *Giardia* and *Cryptosporidium* in oysters applying techniques of molecular biology to study the level frequency of contamination in these products and to make a food risk analysis associated with the human mollusc consumption.
Material and methods: Oysters (n=190) of three different species, European oyster, Ostrea edulis, Pacific oyster, Crassostrea gigas and Portuguese oyster, Crassostrea angulata, were collected in different areas of Portugal (North, Center and South) by Portuguese Institute of the Sea and the Atmosphere (IPMA) between 2011 and 2017. Oysters gills DNA was extracted by DNeasy Blood & Tissue kit (Qiagen) and the amplification of *Cryptosporidium* and *Giardia* small-subunit ribosomal RNA (ssu rRNA) was performed by nested-PCR. The species/genotypes identification was performed by DNA sequencing.

Results: The locus of the *Giardia* ssu rRNA gene was amplified by nested-PCR in 29 oyster samples (15.3%). The DNA sequencing identifies *Giardia* lamblia in 21 nested-PCR positive samples and the assemblage A in ten samples. *Cryptosporidium* sp. was not identified in any sample.
Conclusion: The high detection of G. lamblia assemblage A in the oyster samples is potentially alarming since this assemblage is usually associated with human infections worldwide. The use of prevention and control methods for parasites in the production of shellfish mollusc must be implemented to reduce the risk of food contamination.

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MoA-P3-12h50/12h55

Diagnostics of *Cryptosporidium* spp. from water basins in košice region using artemia franciscana metanauplii

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This study was aimed at using Artemia franciscana metanauplii and molecular methods for the diagnostics of *Cryptosporidium* spp. in water basins used for recreational purposes in Košice region, Slovakia. Previous experiments conducted at our department confirmed the capability of Artemia metanauplii to catch *Cryptosporidium* oocyst by water filtration. We have decided to apply this method to samples from the environment.
Material and methods. Sample collection. Samples of water were collected from five locations in Kosice region: Hornád river in Košice, Ružín water reservoir in Kosice district, Jazero lake in Košice and lakes Čaňa and Geča in Košice district. The river Hornád flows through all of the mentioned basins, from Ružín north of Kosice, through Jazero lake in Košice, continuing to lakes Geča and Čaňa south of Košice. Samples were taken to 5-liter sterile glass flasks and transported to the laboratory for immediate processing. Samples were poured to sterile plates. To each 5 liters sample, 2 500 (± 200) Artemia metanauplii were added.

DNA isolation. After 24 hours at laboratory temperature, metanauplii were removed from the samples and washed with distilled water. Metanauplii were placed in micro-tubes with 1.0 mm zirconium beads, 0.5 mm glass beads, 300 µl of lysis solution and were homogenized at 6 500 rpm. Genomic DNA was isolated using DNA Sorb-AM nucleic acid extraction kit according to the manufacturer's instructions. Molecular diagnostic. Samples were used in nested PCR using primers GP60 F1/R1 and GP60 F2/R2, targeting the GP60 gene of C. *parvum* and C. *hominis*. Final products were visualized by electrophoresis in agarose gel. Samples positive on the gel were sent for DNA sequencing. Final sequences were compared with homologous sequences from GenBank using BLAST program. Results and discussion. We have confirmed the presence of *Cryptosporidium* spp. in two samples. In Jazero lake, we have identified *Cryptosporidium parvum* genotype IIaA18G1R1. *Cryptosporidium hominis* genotype IeA12G3T3 was identified in Geča lake. Our method of diagnosing *Cryptosporidium* spp. from water using Artemia metanauplii has proven to be reliable not only in artificially contaminated water but for environmental samples as well. This method does not require complex and expensive laboratory apparatus (vacuum pumps) and it is suitable for smaller volumes of water. This is the first report of the occurrence of *Cryptosporidium* spp. in recreational waters in eastern Slovakia.

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MoA-P4-12h55/h13h00

Novel Water Treatments for the Zoonotic Waterborne Pathogen *Cryptosporidium*

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Cryptosporidium, cause of the gastrointestinal illness cryptosporidiosis, is a waterborne, apicomplexan parasite of global importance. Claiming hundreds of thousands of lives annually, it is the second most important pathogen responsible for deaths due to diarrhoea. It is a particularly devastating disease for children under 5 years old and those with impaired immune systems. With anti-rotavirus vaccinations implemented recently, *Cryptosporidium* is predicted to become the leading cause of death due to diarrhoea globally. As a waterborne parasite, *Cryptosporidium* can cause mass outbreaks, is a danger to not only human but also animal health and may significantly impact the economies of affected communities. The *Cryptosporidium* oocyst is highly infectious, with just one oocyst capable of causing disease. Due to the robust nature of the oocyst wall ultrastructure, chlorine treatment is largely ineffective and currently UV is the gold standard for water treatment. However, due to expense it is not present in all water treatment plants; particularly in developing countries. This, coupled with a lack of therapeutics, puts transmission prevention at the centre of *Cryptosporidium* research, however the challenge remains as to how to prevent transmission by efficiently removing the parasite from our water systems. Novel microwave technology may provide an effective solution. Our interdisciplinary project, in collaboration with Cardiff University's Centre for High Frequency Engineering and Water Research Institute, is assessing the effect of cutting-edge patented microwave technologies on the viability and infectivity of *Cryptosporidium* parasites present in water. In addition, we are investigating whether exposure of *Cryptosporidium* to microwave frequencies in conjunction with UV may improve current treatment systems.
The ultimate aim of our work is to enable the development of a novel water treatment system that can be adaptable for use in industrial, commercial and domestic settings to prevent *Cryptosporidium* transmission.

POSTER SESSION MORNING

Amphi 100B

MoB-P1-12h40/12h45

The immunological interplay between *Giardia* duodenalis and *Toxoplasma* gondii during murine co-infection

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Enteric infections in the developing world are typically poly-microbial rather than due to a single pathogen. Data from the Global Enteric Multicenter Study (GEMS) suggested that co-infection with *Giardia duodenalis* reduces the incidence of severe diarrhea due to other pathogens. We have used oral infection with Toxoplasma gondii in mice with prior G. *duodenalis* infection as a model to understand how these parasites interact with each other and their host. We infected C57BL/6 mice with 1 million G. *duodenalis* trophozoites (strain GS, assemblage B) three or seven days prior to oral infection with 10 cysts of T. gondii (type II strain 76K GFP-Luciferase). After five days of T. gondii infection, we assessed parasite burden daily by in vivo imaging and qPCR for 18S gene. Nine days post-infection we collected tissue from the duodenum and ileum for analyses. Our preliminary results showed that co-infected mice presented a significant reduction in the number of CD8+ and CD4+ T cells in lamina propria and an elevated number of neutrophils, as verified by flow cytometry. We also observed an increased burden of T. gondii in mice previously infected with *Giardia*. In the duodenum, qPCR revealed reduced levels of IL-6, TNF-⟨, IL-10 and Arginase-1 in the co-infected groups compared to the controls. Ongoing analyses include qPCR in the jejunum, luminex measurements of cytokines from intestinal samples and in sera, histology and weight gain. In conclusion, our preliminary results suggest that prior *Giardia* infection modulates rodent immune response during acute toxoplasmosis.

MoB-P2-12h45/12h50

Giardia lamblia modulates LPS-induced pro-inflammatory response in macrophages through cleavage of NF-κB p65RelA by proteases

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The protozoan *Giardia* lamblia is the most common cause of parasitic gastrointestinal infection worldwide. The parasite developed sophisticated, yet not completely disclosed, mechanisms to escape immune system and maintain a favorable habitat for gastrointestinal growth. To further understand the interaction of G. lamblia with host immune cells, we investigated the ability of parasites to modulate the canonical activation of macrophages (Raw 264.7 cells) triggered by the TLR4 agonist, lipopolysaccharide (LPS). We observed that G. lamblia impairs LPS-evoked pro-inflammatory status in macrophages through inhibition of cyclooxygenase-2 and inducible nitric oxide synthase expression and subsequent NO production. This effect was in part due to the activity of three G. lamblia proteases, a 135kDa metalloprotease and two cysteine proteases with 75 and 63kDa, that cleave the p65RelA subunit of the nuclear factor-kappa B (NF-κB). Moreover, TNF-α and CCL4 transcription was increased in the presence of the parasite. Overall, our data indicates that, in order to successfully colonize small intestine, *G. lamblia* could modulate macrophages inflammatory response through impairment of the NF-κB, thus silencing a crucial signaling pathway of the host innate immune response.

The knowledge of the intracellular signaling profile modulated by *Giardia* parasites in host immune cells highlight putative molecular targets for further development of new therapeutic strategies against giardiasis.

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MoB-P3-12h50/12h55

Biochemical characterization of *Giardia* heme proteins

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Giardia lacks a pathway for heme biosynthesis yet it is known to express five heme proteins: a flavohemoglobin (gFlHb) and four members of the cytochrome b5 family of electron transfer proteins (gCYTB5-I-IV). Here, we focus on gFlHb as the sole heme-containing enzyme of *Giardia*, and on gCYTB5-III for its unusual nuclear localization.

gFlHb possesses nitric oxide dioxygenase activity, and its inhibition through binding of imidazole-based ligands to its open coordination site may interfere with the ability of *Giardia* trophozoites to counter nitrosative stress. While flavohemoglobins share many invariant residues, notably among those involved in O2 and NO binding, the binding affinity of imidazole-based agents can vary several thousand-fold. Such large differences in responses may be attributed to differences within the active site ligand binding pocket of residues that are not directly involved in ligand binding, but may influence the pocket size or the conformational changes that occur on ligand binding. Using optical titrations and isothermal calorimetry we studied the ligand binding properties of gFlHb towards imidazoles and compare these to Hmp, the flavohemoglobin of the commensal gut microorganism E. coli. Of the imidazoles examined, the bulky imidazole miconazole has the highest affinity for both gFlHb and Hmp (Kd = 3-5 µM). We are also developing a method that would permit rapid screening of compounds for their ability to inhibit flavohemoglobins, which would also be useful to identify those that would show selectivity towards gFlHb over Hmp.

With respect to gCYTB5-III, our goal is to determine its structure, which may provide clues as to why this protein associates with the nuclei of *Giardia*. Heme proteins are especially interesting targets for NMR studies; in addition to multidimensional NMR experiments, 1H-1-dimensional NMR can provide useful information on the local heme environment owing to the significant effects on the chemical shifts of nearby protons caused by the strong aromatic ring current of the porphyrin and the paramagnetism of the iron in the ferric state. For comparison purposes, we also studied bovine microsomal cytochrome b5 (CYTB5A). Both cytochromes were expressed in E. coli; optimal expression in unlabeled rich media yielded 160 mg / L culture, while the yield in 15N-labelled minimal media was four-fold lower. The 1H-1D and 1H-15N HSQC NMR spectra of CYTB5A compared well to those reported previously, while those of gCYTB5-III reveal significant differences in its heme-binding pocket, despite sharing the same coordination environment provided by a pair of histidine residues.

MoB-P4-12h55/13h00

Giardia intestinalis **peripheral vesicles harbor multivesicular bodies over the parasite life cycle**

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G. *intestinalis* have an intriguing endomembrane system, basically composed by four organelles: the nucleus, endoplasmic reticulum (ER), mitosomes and peripheral vesicles (PVs). The PVs comprise a set of ~ 150 nm vesicles, distributed underlying the plasma membrane on dorsal side and in a specific region near the ventral disc. These vesicles are part of *Giardia*'s endossomal-lysosomal system, mainly due some characteristics such as: its acidic nature, presence of hydrolase activity inside this organelle and the capacity to uptake exogenous particles or molecules. Extracellular vesicles, collectively known as microvesicles (MVs), comprehend two sets of different sized vesicles with diverse origins: (1) exosomes, with a diameter ranging from 30-100 nm, are released during fusion of multivesicular bodies with the cell surface, and (2) ectosomes, ranging from 100-1000 nm, are directly formed from the plasma membrane. Recently, it was shown G. intestinalis releases microvesicles, which contribute to parasite pathogenesis. However, from where these microvesicles are originated and how they are released remains unclear. In the present study we analyzed the origin of multivesicular bodies (MVB) in the PVs and its profile during encystation. To investigate the behavior of PVs during the cell cycle, G. intestinalis were grown in conventional medium and induced to encyst and excyst in vitro. We used advanced microscopy techniques; such as Transmition electron microscopy (TEM), High Resolution Scanning Electron Microsocpy (HR-SEM), Electron Tomography (ET) and Dual-Beam microscopy (DBM) to better understand the biogenesis and behaviour of MVBs during G. intestinalis life cycle. A cytochemical localization of acid phosphatase was performed in both vegetative and encysting cells. ET and DBM showed MBV inside the PVs. Moreover, during the encystations process, a membrane fusion of MVBs characterized an exocytosis event. We observed an exocytosis of microvesicles by TEM and HR-SEM. During the excystation process a number of MVBs were seen near the cyst wall. In conclusion, a change of PVs behavior occurs during parasite differentiation. We observed a partial acid phosphatase translocation from peripheral vesicles to the plasma membrane and MVBs inside PVs, with a probable role during parasite differentiation. We show that some PVs of vegetative trophozoites exhibit morphological characteristics of MVBs, harboring intraluminal vesicles (ILVs) with a mean diameter of 50 nm.

POSTER SESSION AFTERNOON

Amphi 100A

MoA-P5-14h00/14h05

First detection of zoonotic *Cryptosporidium parvum* in red-eared slider turtle of invasive alien species in water environment in poland

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Cryptosporidium infections occur in a wide range of vertebrates including mammals, birds and reptiles. However, cryptosporidiosis in reptiles is the least recognized parasitic disease with rarely manifested clinical symptoms. The aim of the study was an assessment of the prevalence of *Cryptosporidium* infections in turtles of invasive alien species (IAS) inhabiting different fresh water ecosystems (running and standing waters) in the south-eastern Poland. Faecal samples of 103 turtles and scrapings of intestinal mucosa (67 samples) were taken from IAS turtles belonging to the following species: red-eared slider turtle (Trachemys scripta elegans), yellow-bellied slider (Trachemys scripta scripta), false map turtle (Graptemys pseudogeographica) and cumberland slider (Trachemys scripta troostii). Animals were collected from river and lake habitats at Polesie National Park, Łęczna - Włodawa Lake District and Volhynian Polesie over a three-year period from 2015 to 2017. After a quarantine all trapped animals were subjected to euthanasia and necropsy. *Cryptosporidium* DNA was isolated using a modified alkali wash and heat lysis method followed by its detection using the 18SSU rRNA PCR-RFLPs. PCR amplicons were sequenced to confirm a correct identification of parasite species.

The presence of *Cryptosporidium* DNA was solely detected in one sample of intestinal scraping of red-eared slider found in Uściwierz Lake at Polesie National Park. Subsequent 18SSU rRNA sequence analysis revealed the presence of zoonotic *Cryptosporidium parvum*. A captured animal was healthy and did not show any signs of systematic infection. Likewise, there were no anatomopathological changes observed during necropsy. These results may signify that positive turtle served only as a passive carrier of the parasite. Meanwhile, the results provide further evidence on the role of IAS turtles in the environmental transmission of zoonotic *Cryptosporidium* species. Nevertheless, more comprehensive studies are needed to determine a real prevalence of *Cryptosporidium* infections in wild free-living IAS turtles in Polish water ecosystem.

This work was supported by the National Science Centre project “Invasive turtle species as a source and vector of animal and human pathogens” (Grant No. 2013/11/B/NZ7/01690) and conference participation by KNOW (Leading National Research Centre) Scientific Consortium „Healthy Animal - Safe Food" decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

MoA-P6 – 14h05/14h10

Cryptosporidium spp. (Apicomplexa: Cryptosporidiidae) in Passeriformes birds and biology of novel *Cryptosporidium* great-tit genotype and *Cryptosporidium* swallow genotype

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Faecal samples of wild passerines from Europe, New Zealand and South Africa were screened for the presence of *Cryptosporidium* by microscopy and sequencing of the small-subunit rRNA, actin and 70 kDa heat shock protein genes. *Cryptosporidium* oocysts and specific DNA were detected in 15 and 21 of 743 birds, respectively. Phylogenetic analysis revealed the presence of *Cryptosporidium* baileyi (n=9), *Cryptosporidium* galli (n=2), *Cryptosporidium* great-tit genotype (n=9) and *Cryptosporidium* swallow genotype (n=1). The biology of *Cryptosporidium* great-tit genotype was studied under experimental conditions. *Cryptosporidium* great-tit genotype was infectious for great tits (Parus major), Eurasian blue tits (Cyanistes caeruleus) and chickens (Gallus gallus f. domestica), with a prepatent period of 4-5 days post-infection (DPI) in tits and 20 DPI in chickens, but it was not infectious for SCID mice (Mus musculus) or budgerigars (Melopsittacus undulates). Experimentally infected tits remained infected for the duration of the study (50 DPI), with an infection intensity ranging from 2,000 to 3,000 oocysts per gram (OPG). Chickens inoculated with *Cryptosporidium* great-tit genotype failed to shed detectable oocysts at any time during the infection, although DNA was detected in faecal samples at least 50 DPI. Naturally infected great tits shed oocysts for more than 60 days, with an infection intensity ranging from 2,000 to 8,000 OPG. *Cryptosporidium* great-tit genotype infects the proventriculus and ventriculus, and oocysts measure 8.3×6.3 µm. None of the birds infected with *Cryptosporidium* great-tit genotype developed clinical signs. Genetic and biological data support the establishment of *Cryptosporidium* great-tit genotype as separate species of the genus *Cryptosporidium*. This study was funded by the Czech Science Foundation (18-12364S) and Grant Agency of University of South Bohemia (project No. 082/2017/Z). All of the experimental procedures were conducted in accordance with the law of the Czech Republic on the use of experimental animals, safety and use of pathogenic agents and were approved by the National Committee.

MoA-P7-14h10/14h15

Diversity of *Cryptosporidium* spp. in *Rattus norvegicus* in the Czech Republic

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Brown rats (Rattus norvegicus) from selected localities across the Czech Republic were screened for the presence of *Cryptosporidium* by microscopy and sequencing of the small-subunit rRNA and actin genes. *Cryptosporidium* oocysts were detected in five out of 390 faecal samples examined by microscopy following aniline-carbol-methyl violet staining. Fifty-four *Cryptosporidium* infections, including the five infections that were detected by microscopy, were detected using PCR/sequencing. Phylogenetic analysis revealed the presence of *Cryptosporidium* andersoni (3), C. muris (4), C. occultus (6), C. ryanae (1), *Cryptosporidium* rat genotype I (27) and *Cryptosporidium* rat genotype IV (13). *Cryptosporidium* occultus, with oocysts measuring 5.20 × 4.94 µm, inhabit the large intestine. *Cryptosporidium* rat genotypes I and IV were found in the small intestine, but oocysts of *Cryptosporidium* rat genotype I (4.89 × 4.63 µm) were significantly smaller than oocysts of *Cryptosporidium* rat genotype IV (5.77 × 5.47 µm). This study was funded by the Ministry of Education, Youth and Sports of the Czech Republic (LTAUSA17165) and Grant Agency of University of South Bohemia (project No. 082/2017/Z). All of the experimental procedures were conducted in accordance with the law of the Czech Republic on the use of experimental animals, safety and use of pathogenic agents and were approved by the National Committee.

MoA-P8-14h15h/14h20

Diversity of *Cryptosporidium* spp. in East- and West-European house mice

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A total 578 East-European house mice (Mus musculus musculus, MMM; n= 245) and West-European house mice (M. m. domesticus, MMD; n=333) from locations in the Czech Republic (n=54) and Germany (n=71) were screened for *Cryptosporidium* by microscopy and PCR/sequencing at the small subunit rRNA locus (SSU). *Cryptosporidium* oocysts were detected in 71 samples examined by microscopy following aniline-carbol-methyl violet staining and 123 samples examined by PCR. The PCR positive samples included the 71 samples that were positive by microscopy. Sequence analyses of SSU revealed the presence of *Cryptosporidium* muris (n=61), C. *parvum* (n=18), C. tyzzeri (n=63), C. *hominis* (n=3) and C. ubiquitum (n=2) in both house mouse subspecies. Co-infection with two or three *Cryptosporidium* spp. was detected in 15 animals. Statistical analyses revealed the different distribution of *Cryptosporidium* spp. between both studied mouse subspecies; MMD was parasitized (34.5%) twice as much than MMM (15.5%). There was no significant difference in morbidity between males and females. *Cryptosporidium parvum*, C. *hominis* and C. ubiquitum positive samples were additionally genotyped at 60 kDa glycoprotein gene (gp60). All MMM and MMD mice were solely infected with IXa and IXb gp60 subtypes of C. tyzzeri, respectively. All C. *parvum* and C. *hominis* positive mice were infected with IIa and Ib subtypes, respectively. Sequences of gp60 gene from C. ubiquitum formed a separate cluster that was closely related to Apodemus genotypes I and II. This study was funded by the Ministry of Education, Youth and Sports of the Czech Republic (LTAUSA17165) and Grant Agency of University of South Bohemia (project No. 082/2017/Z). All of the experimental procedures were conducted in accordance with the law of the Czech Republic on the use of experimental animals, safety and use of pathogenic agents and were approved by the National Committee.

MoA-P9-14h20/14h25

Novel *Cryptosporidium* genotypes in wild and cultured trout

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The available information about the prevalence, species and genotypes of *Cryptosporidium* in fish is very scarce. The presence of this waterborne protozoan parasite was investigated in 973 specimens of cultured and wild trout (Oncorhynchus mykiss; n = 360 and Salmo trutta; n = 613) from northwest Spain. The fish were dissected and nucleic acids were extracted from homogenized gastrointestinal tracts. By polymerase chain reaction (PCR) amplification and sequencing of fragments of the small subunit ribosomal (SSU-rRNA), 70 kDa heat shock protein (hsp70), 60 kDa glycoprotein (GP60) and actin loci of *Cryptosporidium*, a total of 57 fish (9 and 48 cultured and wild trout, respectively) were positive, giving an overall prevalence of 5.9%. Thus, *Cryptosporidium parvum* (IIaA15G2R1 and IIaA18G3R1 subtypes) was identified in 54 fish (7 and 47 cultured and wild trout, respectively) and sequences closer to *Cryptosporidium* molnari were obtained in the remaining 3 specimens (2 cultured and 1 wild trout). These sequences from cultured and wild trout represent novel piscine genotypes, named piscine genotype 9 and piscine genotype 10, that exhibited a 9.9% and 12.6% genetic distance from C. molnari, respectively. Future research involving molecular characterization at other loci, as well as histological and morphological studies, are required to confirm the species status of the novel genotypes. Moreover, the identification of the zoonotic species C. *parvum* in wild and cultured trout may indicate a risk to public health as trout may be a potential source of infection to humans. Thus, edible trout extend the range of foodstuffs involved in the transmission of cryptosporidiosis.

This study was funded by Xunta de Galicia (ED431C 2017/31).

MoA-P10-14h25/14h30

First report of *Cryptosporidium parvum* GP60 genotypes (IIaA15G2R1 and IIaA16G3R1) in wild ponies from northern Iberian Peninsula

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A total of 79 faecal samples from wild ponies of different breeds inhabiting several mountains in northern Iberian Peninsula were collected. Non-diarrhoeal samples were taken directly from the soil immediately after deposition and stored at 4ºC until their processing. The samples were homogenized, filtered through a set of two sieves (mesh size 150 and 45 µm) and concentrated in phosphate buffered saline 0.04 M pH 7.2/diethyl ether (2:1). By an immunofluorescence antibody test (IFAT), including 4',6-diamidino-2-phenylindole, *Cryptosporidium* spp. oocysts were observed in 3 samples. The subsequent polymerase chain reaction (PCR) amplification and sequencing of a fragment of the small subunit ribosomal (SSU-rRNA) gene, allowed to detected *Cryptosporidium* in other 8 samples, identifying *Cryptosporidium parvum* in all of them. Thus, a total of 11 samples (13.9%) were classified as positive by IFAT and PCR analysis. Subtyping of C. *parvum* isolates at the 60 kDa glycoprotein (GP60) locus showed the presence of subtypes IIaA15G2R1 and IIaA16G3R1 in this feral host. This work reports, for the first time, the occurrence of C. *parvum* subtypes IIaA15G2R1 and IIaA16G3R1 in wild ponies of Iberian Peninsula and suggests the potential role of these hosts in the sylvatic transmission of *Cryptosporidium*. Moreover, as several authors have suggested, it could also be possible that C. *parvum* is a parasite established in these wild ponies regardless of contact with humans or livestock.

This study was funded by Xunta de Galicia (ED431C 2017/31).

MoA-P11-14h30/14h35

First report of *Cryptosporidium parvum* subtype IIaA16G3R1 in cervids

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We characterized genetically the infections by *Cryptosporidium* in Mazama gouazoubira. By a non-invasive harvest methodology using trained sniffer dogs to locate fecal samples of cervids, 642 fecal samples were obtained from six Brazilian localities. The cervid species responsible for the excretion of each faecal sample were identified by the polymerase chain reaction (PCR) performed from the genomic DNA previously extracted. From this identification, 437 fecal samples of M. gouazoubira were selected for research of *Cryptosporidium* spp. performed through negative staining with malachite green and polymerase chain reaction (nPCR) followed by sequencing the amplified products. In the samples that were diagnosed the presence of parasite species with zoonotic potential, genotyping was also performed using nPCR with the subunit of GP60 gene. Statistical analysis consisted of the Fisher exact test to verify the association of the presence of the enteroparasite in relation to the presence of cattle in each locality, and the McNemar tests and Kappa correlation coefficient used to compare the results obtained between the two diagnostic techniques. In the fecal samples of M. gouazoubira the occurrences of *Cryptosporidium* were diagnosed in 1.6% (7/437) and 1.1% (5/437), respectively, through nPCR and microscopy. C. *parvum* was diagnosed in 100% (7/7) of the samples submitted to sequencing (18S gene). The IIaA16G3R1 subtype was diagnosed in five of the C. *parvum* samples submitted to genotyping (GP60 gene). This is the first world report of C. *parvum* in M. gouazoubira and subtype IIaA16G3R1 in cervids.
Keywords: deer, cryptosporidiosis, genotyping, oocysts.

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MoA-P12-14h35/14h40

First report of parasites of the *Cryptosporidium* genus in *Mazama americana*, *Mazama nana* and *Blastocerus dichotomus*

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We detected parasites of the Cryptosporium genus in fecal samples of free-living cervids from different regions of Brazil, an unpublished fact in the world literature. By a non-invasive harvest methodology, with the participation of sniffer dogs, 936 faecal samples were obtained from animals from 14 Brazilian locations. The cervid species responsible for the excretion of each faecal sample were identified by the polymerase chain reaction (PCR) performed from the genomic DNA previously extracted. From this identification, 563 fecal samples from *O.zotoceros bezoarticus*, B.*lastocerus dichotomus*, *Mazama nana*, *Mazama americana* and *Mazama bororo* were selected for research of *Cryptosporidium* spp. performed through negative staining with malachite green and polymerase chain reaction (nPCR) followed by sequencing the amplified products. In the samples that were diagnosed the presence of parasite species with *Cryptosporidium parvum*, genotyping was performed using nPCR with the GP60 gene subunit. In addition, sequencing using the actin gene was used for the molecular characterization of samples that were not identified by the 18S and GP60 genes. The association between the presence of *Cryptosporidium* and the occurrence of cattle in the locality where the samples were obtained was evaluated by Fisher's exact test. The comparison between the percentages of positivity obtained between the different diagnostic techniques was performed by the McNemar tests followed by the Kappa correlation coefficient. The presence of *Cryptosporidium* spp. in 1,42% (8/563) and 0.36% (2/563) of fecal samples of cervids, respectively analyzed by nested PCR and negative malachite green staining was detected. C. *parvum* isolated, IIaA16G3R1, was diagnosed in three fecal samples of *M. americana*, two of *M. nana* and one of B. dichotomus. *C. ryanae* was diagnosed in a sample of B*. dichotomus*. In a sample of *M. americana* the presence of the cervidae genotype was diagnosed, and the nucleotide sequence obtained through amplification of the actin gene did not show genetic similarity to any previously published sequence in GenBank. In unpublished way, C. ryanae was isolated in a faecal sample of B. dichotomus. The nucleotide sequence of the cervid genotype identified in this study did not show genetic similarity to any previously diagnosed published in GenBank, being this isolate titled genotype cervidae BR.
Keywords: deer, coccidia, *Cryptosporidium* ryanae, cervid genotype, oocysts.

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MoA-P13-14h40/14h45

Prevalence, molecular identification and risk factors for *Cryptosporidium* infection in edible marine fish: a survey across sea areas surrounding France

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Little information is available regarding *Cryptosporidium* prevalence in wild aquatic environments even it is well known that some parasites are both fish pathogens and recognized agents of zoonosis with a public health impact. To study the prevalence of *Cryptosporidium* spp. in edible marine fish in sea areas surrounding France, 1,853 specimens were collected as part of two campaigns. Nested PCR followed by sequence analysis at the 18S rRNA gene locus was performed in order to detect *Cryptosporidium* spp. The overall frequency of *Cryptosporidium* spp. in sampled fish reached 2.3% (35 out of 1,508) in a first survey and 3.2% (11 out of 345) in a second survey. Sequence and phylogenetic analysis of positive samples identified C. *parvum* (n=10) and seven genotypes which exhibited between 7.3% to 10.1% genetic distance from C. molnari, with the exception of one genotype which exhibited only 0.5% to 0.7% genetic distance from C. molnari. Among 31 analyzed fish species, 11 (35.5%) were identified as potential hosts for *Cryptosporidium*. A higher prevalence of *Cryptosporidium* spp. was observed in fish collected during the spring-summer period, in larger fish and in those caught in the Atlantic North East. Saithe (*Pollachius virens*) was the most frequently infected fish species. In fish infected by other parasites, the risk of *Cryptosporidium* infection increased 10-fold (OR: 9.95, CI: 2.32-40.01.04, P= 0.0002). Four gp60 subtypes, previously detected in terrestrial mammals were detected among the C. *parvum* positive samples: IIaA13G1R1, IIaA15G2R1, IIaA17G2R1 and IIaA18G3R1. These C. *parvum* subtypes may constitute an additional source of infection for other animals and in particular for humans. Histological analysis confirmed the presence of round bodies suggestive of the development of C. *parvum* within epithelial cells of digestive glands. This observation suggests that C. *parvum* is infecting fish rather than being passively carried. We report herein the first epidemiological and molecular data concerning the detection of *Cryptosporidium* in edible marine fish in different European marine areas broadening its host range and uncovering potential novel infection routes.

MoA-P14-14h45/14h50

Giardia spp. infection in a population of crested porcupine (*Hystrix cristata* L., 1758) from central Italy

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The crested porcupine (*Hystrix cristata L.*, 1758) is the largest rodent among the Italian fauna. It is a nocturnal and herbivorous mammal species that lives in burrows that could be self-made as well as made by other mammals, such as foxes and badgers. In Europe, the distribution of this species is mainly limited to Italy. Few studies deal with the parasite fauna of the crested porcupine. As part of a larger study on the health status of crested porcupine populations from Tuscany (central Italy), this investigation evaluated *Giardia* and *Cryptosporidium* infection in free-ranging crested porcupines living in woods of the province of Pisa (Tuscany, central Italy). During autumn-winter season 2018-2019, faecal samples were collected from juvenile and adult captured and/or road-killed individuals as well as from feeding areas and pathways. For parasitological analysis, faecal samples (n. 22) were examined by fresh and Lugol stained fecal smears and by a commercial rapid immunoassay for the search of *Giardia* and *Cryptosporidium* spp. fecal antigens (RIDA QUICK *Cryptosporidium*/*Giardia* Combi, R-Biopharm®, Darmstadt, Germany). Overall, 14/22 (63.6%) examined samples were found *Giardia* positive, while no positivity for *Cryptosporidium* was recorded. As observed in other wild rodent species, results here obtained revealed a high *Giardia* prevalence in examined crested porcupines. To date, different *Giardia* species, including various assemblages of *Giardia duodenalis*, *Giardia* microti and *Giardia* muris, have been identified among rodents. However, no data are available on *Giardia* species and genotypes infecting H. cristata. Further molecular studies aimed to evaluate the epidemiology and the potential zoonotic risk linked with *Giardia* infection in the crested porcupine population examined in this study, are in progress.

MoA-P15-14h50/14h55

Molecular identification of *Giardia microti* in captive vole *Microtus guentheri* (Mammalia: Rodentia) from Italy

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The genus *Giardia* includes several species distinguished by morphological, biological and molecular features. Currently, eight species within the genus are retained as valid: *Giardia* agilis in amphibians, G. ardeae and G. psittaci in birds, G. microti and G. muris in rodents, G. *duodenalis* (syns. G. intestinalis and G. lamblia) in most vertebrates including human, and two recently described species, G. peramelis, identified in Australian bandicoots, and G. cricetidarum detected in hamsters. In Italy, no identification of *Giardia* species other than G. *duodenalis* has been so far reported. The aim of the present study was the molecular characterization of *Giardia* isolates from Microtus guentheri (Günther's Vole) from Italy.

Fecal samples were collected from two *M. guentheri* individuals resulted positive to *Giardia* cysts by microscopic investigation and immunofluorescence. The voles were born in Milan (Northern Italy) from two gravid females imported from the Netherlands and kept for sale in a pet shop in Varese (Northern Italy). Positive feces were subjected to a nested PCR to amplify a 18S rRNA fragment for molecular characterization. Subsequently, a phylogenetic analysis was conducted to compare the obtained sequence with those of all other *Giardia* species available in GenBank for the 18S locus, using the Maximum Likelihood (ML) method by R software (ape and phangorn packages). Sequence analyses unambiguously identified the isolates as belonging to G. microti, showing 99% of identity with those of G. microti isolates available in GenBank. A well-defined cluster, supported by significant bootstrap values and corresponding to the G. microti cluster, including sequences obtained from M. guentheri, was evidenced in the ML tree, confirming species assignment. This study represents the first report of G. microti in Italy. The obtained results underline the role of national and international animal trade as a serious concern for domestic animals and native wildlife population health, and the need to control pet parasites to prevent the introduction of allochthonous parasitic species.

MoA-P16-14h55/15h00

Molecular epidemiology of giardiosis in Cuban paediatric population and its association with clinical data

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Giardia lamblia represents one of the most frequent intestinal parasites in our country, mainly associated with infections in children. To date, eight main assemblages of G. *duodenalis* have been described, but only A and B genetic groups are known to infect humans. The development of tools to dissect the molecular biology of different *Giardia* isolates, and the knowledge of the spectrum of symptoms associated with giardiasis, has led to the hunt for associations between particular assemblages and defined symptom patterns. Three cross-sectional studies were conducted in the period 2010-201, with the aim of describing the infection of G. lamblia, in children from Havana and the municipality of Fomento in Sancti Spiritus province, in addition to relate the results of the molecular characterization of this intestinal protozoon through two genetic markers with clinical variables collected in epidemiological questionnaires. The aim of this investigation was genetic characterization of G. *duodenalis* isolated from children with giardiasis diagnosed, and to compare the genetic results with clinical and epidemiological data. Our samples came from a mixture of clinical cases (Study 1) as well as asymptomatic children enrolled in a program of school (Study 2) and preschool (Study 3)-based surveillance. An infection rate of 22.8%, 11.9% and 10.8% was obtained in Studies 1, 2 and 3, respectively. In Studies 2 and 3, keeping dogs at home were significant risk factors for a *Giardia* infection. In addition, in Study 3 it was evidenced that the consumption of not boiled water increased significantly *Giardia* infection among children from Fomento municipality. A perfect concordance level (kappa index = 1) was obtained with the use of triosephosphate isomerase (tpi) and the intergenic sequences of the rDNA genes and a better percentage of typing (87.4%) of the stool samples studied. A total of 165 isolates of G. *duodenalis* were characterized and a predominance of assemblage B (49.1%) was observed over assemblage A (26.7%) and mixed infection by A + B assemblages (24.2%). The sub-assemblages AII (87%), BIII (85.7%), and the mixed infection AII-BIII (84.6%) were the most predominant ones. In children only infected with *Giardia*, the infection of assembly B was associated statistically with diarrhea or flatulence or abdominal pain, this association of assemblage B being significant statistically in preschool children in comparison with school children. In the future, novel molecular tools for a better discrimination of assemblages at the subassemblages level are needed to verify possible correlations between *Giardia* genotypes and symptomatology of giardiasis.

POSTER SESSION AFTERNOON

Amphi 100B

MoB-P5-14h00/14h05

Functional polymorphism of arginine deiminase, a putative *Giardia duodenalis* virulence factor

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Arginine depleting enzymes are considered virulence factors and the depletion of arginine is a well-known strategy of pathogens to evade immune effector mechanisms. The gut dwelling protozoan parasite *Giardia duodenalis* cause relevant morbidity in humans and animals. An arginine deiminase (ADI) secreted by the parasite has been implicated in virulence. We had reported before that sequence variation detected between G. *duodenalis* adi-alleles of different genotypes affects functional parameters of the enzyme. ADI sequences of different G. *duodenalis* genotypes had been cloned and purified in recombinant form and the Km value of the enzymes determined in vitro. Here, this data set is complemented with Km values of G. *duodenalis* ADI as determined in lysates of recent clinical parasite isolates. The results confirm that sequence variation causes changes in the activity of the respective enzyme variants and thus provide a further molecular argument to the concept that G. *duodenalis* ADI as a molecularly defined virulence factor is a quantitative trait of G. *duodenalis*.

MoB-P6-14h05/14h10

A new model based on human intestinal organoids to investigate *Giardia duodenalis* intestinal barrier interaction: Characterization of differentiation and cell type composition

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Giardia duodenalis describes a species complex of protozoan parasites causing acute and chronic intestinal disease with most of the developing countries considered as endemic regions. It is assumed that alterations of the intestinal barrier determine disease pattern and severity but current models to study this in vitro, such as CACO-2 cells, are limited and often show no or little direct impairment of barrier function. An improved in vitro model system for G. *duodenalis* infection is therefore highly desirable. For this purpose, we developed a human intestinal organoid derived, compartmentalized, two-dimensional infection model. Here, the state of differentiation and cell type composition as a function of different media compositions and interaction with G. *duodenalis* colonization is characterized via rt-qPCR of a panel of cell differentiation markers.

MoB-P7-14h10/14h15

Novel insights in the structural organization of the *Giardia intestinalis* cytoskeleton

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Giardia lamblia is a pathogenic protozoan that causes giardiasis; an intestinal illness characterized by symptoms as chronic diarrhea, nausea and cramps. The morphological stage of the life cycle responsible for establishing the parasitism is the trophozoite, a flagellated cell that colonizes the epithelial surface. The *Giardia* trophozoite has a characteristic pear-shaped body and presents two nuclei. It possesses a unique cytoskeleton composed basically of microtubular structures that form different arrays, including: four pairs of flagella, an adhesive disc composed of microtubules and microribbons, a median body and funis, which are two microtubular sheets associated the axonemes from the caudal flagella. While studies on molecular aspects of *Giardia* trophozoites have advanced, few papers addressed *Giardia* morphology, although the organizations of several cytoskeleton components are virtually unclear. For characterization of new cellular structures, the ultra-high-resolution scanning electron microscopy (UHRSEM) and helium ion microscopy (HIM) in combination with plasma membrane extraction protocols have a potential application. In this study we used UHRSEM and HIM to analyze the organization of the cytoskeleton in detergent membrane-extracted trophozoites. We also performed immunogold labeling using different antibodies to identify the post-translational modifications of tubulin, the major protein of the *Giardia* cytoskeleton. We observed that the microtubules from disc margin and ventral channel region appear more packaged when compared to microtubules to disc body. The crossbridges that connected adjacent microribbons are smaller in the ventral groove region of the disc and more resistant to breakage after detergent treatment. In the outer edge of the disc is possible to observe the lateral crest, a region composed of globular structures. This arrangement is present on dorsal and ventral side of the disc. The lateral crest is thinner in the region of disc overlap zone and thicker in the ventral groove region of the disc. Besides that, ring structures around the axonemes at the point of the emergence of the flagella were observed. Immunogold of membrane-extracted trophozoites allowed us to see that the several cytoskeleton structures of this parasite present distinct pattern of labeling for post-translational modifications of tubulin. For example, acetylation was observed in the nucleation zone of the disc, median body and funis, while glutamylation was observed in the all axonemes and flagella. Further ultrastructural analyses are being performed to a better characterization of these structures of G. intestinalis.

MoB-P8-14h15/14h20

The role of the Microribbon-Crossbridge Complex in the Structure and Function of *Giardia's* Ventral Disc

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Giardia lamblia is a single celled eukaryotic parasite that colonizes the small intestine and causes significant diarrheal disease worldwide. Motile trophozoites attach to intestinal villi with the ventral disc, a flexible, suction-cup shaped microtubule (MT) organelle, to resist peristalsis. Parallel, uniformly spaced MTs spiral to form a dome, with an overlap zone between the upper and lower portions. Novel protein complexes such as the microribbon-crossbridge (MR-CB) complex almost completely decorate the disc MTs. Microribbons are trilaminar sheets that extend into the cell from the MT spiral, and are connected by crossbridges. Aside from the obvious roles in infection, understanding how complex MT organelles are assembled and evolve can shed light on how morphological diversity evolved in different eukaryotes. Over 87 disc-associated proteins (DAPs) are known to comprise the disc, yet the functional and structural roles of the MR-CB remain unknown. The MRs that connect the spiral MT array of the disc may play a role in maintaining the curved disc structure. Crossbridges may be flexible, and could also mediate and/or maintain disc doming. Three SF-assemblin DAPs, (β-Giardin, δ-Giardin and SALP1) are known MR proteins. Prior work has identified one candidate crossbridge protein (DAP7268), morpholino knockdown of which causes an aberrant, flattened disc conformation. Additionally, we have developed CRISPR-Cas9 mediated knockdown (CRISPRi) in *Giardia*, which now allows us to rapidly create stable MR-CB mutants to evaluate their role in disc structure and function. Our findings indicate that the MR-CB complex stabilizes the domed disc spiral MT array that is required for parasite attachment. Using CRISPRi to stably knockdown several DAPs produces two notable phenotypes. Depletion of SALP1 results in a flattening of the microtubule spiral, with an enlargement of disc surface area. Targeting DAP5188, an Ankyrin repeat protein, with CRISPRi produces a loss of up to 90% of the disc's MR-CB structure. Furthermore, preliminary testing of the ability for SALP1 and DAP5188 knockdown cells to adhere to a glass surface has revealed an attachment defect. These results suggest that SALP1 is needed for the disc to form a three-dimensional dome, and that this is important in maintaining parasite adherence to a surface. Spacing between the microtubules of the disc spiral could be altered, and an examination by electron microscopy could provide further detail. Additionally, DAP5188 plays an essential role in either the assembly or maintenance of the disc, as evidenced by the gross morphological disturbance in 5188 knockdown cells.

MoB-P9-14h20/14h25

Defining *in vivo Giardia* physiology and commensal microbiota dysbiosis associated with parasite density during infection in mice

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Giardia lamblia is a microaerophilic protozoan parasite of humans and animals that causes significant diarrheal disease worldwide. While *Giardia* colonization of the small intestine occurs in a niche already inhabited by commensal microbiota, in vivo *Giardia*-microbiome interactions have been largely ignored in models of pathogenesis. We recently showed that *Giardia* infection in mice results in dysbiosis throughout the gastrointestinal tract characterized by blooms of aerobic bacteria and the depletion of obligate anaerobes. Altered redox chemistry at the site of infection and a shift to a more oxic microenvironment may benefit the parasite. *Giardia* is able to maximize its metabolic potential under in vitro microaerobic growth as compared to strictly anaerobic growth. Here we focus on *Giardia*-microbiome metabolic interactions at the primary site of infection and how parasite colonization density impacts the local ecological homeostasis of the gastrointestinal tract. We conducted a pilot metatranscriptomic study to interrogate microbiome diversity, abundance, and metabolism associated with both high-density and low-density areas of *Giardia* colonization. We infected cohorts of male and female C57/B6J mice with a constitutive bioluminescent bioreporter *Giardia* strain GDH_FLuc. We used bioluminescent imaging (BLI) to precisely sample gastrointestinal regions of the gut associated with high-density and low-density colonization. Total community genomic DNA and RNA was extracted and sequenced from regions with dense *Giardia* colonization and from control, uninfected animals. We are using total microbiome community metatranscriptomic profiling and metabolic analysis to estimate the diversity, abundance, and physiology of commensal bacteria in sites of *Giardia* colonization. In particular, we focus on microbiome carbon and nitrogen metabolism. This study allows us to develop a more comprehensive understanding of the ecological interactions between the host microbiome and *Giardia*, and lays the groundwork for development of mechanistic models to define and ultimately manipulate host-microbiome-parasite interactions.

MoB-P10-14h25/14h30

Comparison of mucin expression in non-mucinogenic cells infected with *Giardia lamblia*

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The adhesion of trophozoites to the intestinal epithelium is a key step in the establishment of the disease, and occurs after trophozoites have crossed the mucus layer. The mucus layer covering the gastrointestinal mucosa is considered the first line of defense against the infection by pathogens and it is mainly composed by highly O-glycosylated proteins called mucins. According to some studies, treatment of mammalian cells with mucinogenic compounds such as short chain fatty acid [1] (i.e. sodium butyrate) as well as infection with some enteropathogenic organisms, modulate the expression of mucins. Recent work [2] showed that human goblet-like cells (LS174T) infected with *Giardia* (GS/M) up to 3h had increased levels of MUC2 gene expression. Moreover, G. lamblia infection in mice was associated with increased Muc2 expression and degradation, and depletion of goblet cell mucin stores. The objective of this work was to evaluate whether infection of non-mucinogenic cells (HuTu-80 and Caco-2) with *Giardia* lamblia results in different levels of transcription and translation of mucins. For this, the expression of different mucins (MUC2 and MUC5AC) in control cells, cells co-incubated with trophozoites (up to 4 h) and cells treated with 1 mM sodium butyrate (butNa) was evaluated by RT-PCR, qPCR, Western blotting and immunofluorescence microscopy. Exposure of non-mucinogenic cells to trophozoites caused an increase in the transcript levels of MUC2 and MUC5AC as revealed by RT-PCR and qPCR. The levels of mRNAs for MUC2 and MUC5AC were respectively 4-fold and 0.3-fold higher in Caco-2 infected cells and 6.4-fold and 178-fold higher in Hutu-80 cells compared to controls. Immunofluorescence and western blot analysis using specific antibodies against MUC2 and MUC5AC showed that protein expression levels also were increased in infected cells however, expression levels between transcripts and proteins did not always correlate. The expression profile observed in non-mucinogenic cells (Hutu-80) infected with the parasite resembles that observed for the mucinogenic cells (LS174T). Such observation may suggest that this duodenal line also have the ability to produce and secrete mucins similar to LS174T cells. However, more studies are needed to confirm the mucinogenic character of Hutu-80 cells such as an analysis of the mucin profile expressed in this line and the determination of the percentage of goblet cells in the population.

[1] Hatayama, H. et. al, 2007, Biochemical and biophysical research communications, 356(3), 599-603.
[2] Amat, C.B. et al, 2017, The American jornal of pathology, 187(11), 2486- 2498.

MoB-P11-14h30/14h35

Metabolic stress in *Giardia*-infections: *in vivo* importance of arginine for parasite and host

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Nutrients in the intestinal lumen are essential resources for hosts, commensal bacteria, and pathogens of this niche. *Giardia duodenalis* infects the small intestine and is thought to scavenge the amino acid arginine. Its arginine metabolizing enzymes are differently regulated axenically or on host cells in vitro, and the metabolic pathway is predicted to be important for generating ATP in the parasite. Arginine deiminase (ADI) is a key parasite enzyme for arginine metabolism. ADI-mediated depletion of arginine e.g. modulates cytokine profiles of immune cells, demonstrating an arginine-dependent interplay between parasite and host. ADI also has the potential to deplete arginine in vivo during infection when parasites replicate. Considering the importance of arginine for both host and parasite we take a dual approach and use a mouse infection model to address effects of arginine depletion on host as well as on the parasite. First, G. *duodenalis* reproduction and cyst formation is analyzed in murine hosts fed zero-arginine diets, and compared to normal amino acid conditions. Secondly, host epithelial responses are analyzed in transcriptomes with a special focus on amino acid sensing signalling in the mechanistic Target of Rapamycin (mTOR) network. Epithelial-specific mTOR deletion mouse mutants are used under arginine-free food and control conditions. To our knowledge, this is the first in vivo study investigating the effect of arginine limitation on G. *duodenalis*, and analysis of host mechanisms which could sense G. *duodenalis*-induced metabolic stress.

MoB-P12-14h35/14h40

Phosphoinositide-binding proteins mark, shape and functionally modulate highly-diverged endocytic compartments in the parasitic protist *Giardia lamblia*

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The dorsal side of the *Giardia* lamblia (syn. intestinalis; *duodenalis*) trophozoite is enriched for organelles called peripheral vacuoles (PV). These organelles have been shown to mediate bulk fluid phase uptake in this parasite and are likely the only point of entry for nutrients. The PV protein interactome has been dissected by us and other groups over the past years and points to a notable level of complexity. PVs in their role as endolysosomal feeder organelles are associated to known endocytic protein interactomes which include clathrin (GlCHC), putative receptors, dynamin-like protein and receptor-associated adaptor protein 2. The absence of membrane-binding adaptor proteins such as epsin or AP180 known to be involved in endocytosis begs the question of how these protein complexes are able to bind and modulate events at specific membranes, specifically the plasma (PM) and PV membrane. In the same interactomes, we identified proteins that carry domains (PXD, FYVE and PH) for the specific binding of phosphorylated derivatives (PIPs) of the minor membrane phospholipid phosphatidylinositol. We therefore hypothesized that these would serve as PIP-binding adaptors to link and maintain GlCHC assemblies at the PM and PV membrane interface. We further hypothesized that a perturbation of PIP-binding protein levels and/or function would lead to impaired fluid-phase uptake by affecting PV functionality. To test these hypotheses, in this study we performed an in-depth functional characterization of all previously-identified PIP-binding proteins associated to clathrin at PVs. We defined their lipid-binding preferences and visualized their subcellular localizations using electron microscopy and both conventional and super resolution light microscopy. By manipulating protein levels and/or function we could elicit novel uptake and PV morphology-related phenotypes, thereby establishing a PIP-based connection between clathrin's unclear role in G. lamblia and PV-based endocytosis. Lastly, we propose an updated working model summarizing the complex networks between PIP-binding proteins and clathrin assemblies at PVs. The variety of PIP-binding modules in G. lamblia underscores their necessity for correct functioning of membrane traffic even in a protist so clearly marked by reduction in endomembrane complexity.

MoB-P13-14h40/14h45

Structural inheritance of microtubule organelles in *Giardia*

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The ventral disc, the eight flagella, and the median body are *Giardia*'s primary microtubule-based organelles. While the ventral disc and median body are assembled de novo during cell division, *Giardia*'s four pairs of flagella undergo a maturation cycle in which certain flagellar pairs are inherited and others are built de novo over several generations. This form of organellar inheritance has been termed "structural inheritance", as daughter cells inherit complete cellular structures (e.g., flagella) from the parental cells. The overall molecular mechanism or function of structural inheritance of flagella remains unclear. We are investigating the roles of tubulin post-translational modifications (PTMs) as a possible mechanism for structural inheritance and differentiation of *Giardia*'s microtubule arrays. In general the specialization of the microtubule cytoskeleton is regulated by the 'tubulin code', which includes the expression of diverse post-translational modifications of tubulin, or by the expression of different tubulin isotypes. To elucidate the pattern of structural inheritance of *Giardia*'s MT organelles in live cells over several generations, we designed pulse-chase imaging experiments using single and multi-color SNAP-tagged beta-tubulin strains and imaged for up to six generations. We confirm that the left caudal is the oldest flagellum, the right caudal flagellum is the third oldest, and the two anterior are the second oldest flagella. The ventral and posteriolateral flagella, as well as the ventral disc and median body, are built de novo each generation. To investigate role of PTMs in the structural inheritance of the flagella, we used CRISPR interference (CRISPRi) to transcriptionally repress each of the seven Tubulin-Tyrosine Ligase Like (TTLL) genes in *Giardia*. TTLLs are a widely conserved family of enzymes that modify alpha and beta tubulin post-translationally. Such PTM-tubulin is known to have differential interactions with MT motors or MT-binding proteins. We are quantifying changes in PTMs in TTLL knockdown mutants using antibodies against anti-polyglycylation, anti-tyrosination or anti-glutamylation. We expect a disruption in the modification patterns and/or in the overall inheritance of different flagellar pairs. Future pulse-chase experiments with TTLL knockdowns in the SNAP-tubulin background strain will enable us to determine the role of particular PTMs in flagellar inheritance.

MoB-P14-14h45/14h50

Functional polymorphism of arginine deiminase, a putative *Giardia duodenalis* virulence factor

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Arginine depleting enzymes are considered virulence factors and the depletion of arginine is a well-known strategy of pathogens to evade immune effector mechanisms. The gut dwelling protozoan parasite *Giardia duodenalis* cause relevant morbidity in humans and animals. An arginine deiminase (ADI) secreted by the parasite has been implicated in virulence. We had reported before that sequence variation detected between G. *duodenalis* adi-alleles of different genotypes affects functional parameters of the enzyme. ADI sequences of different G. *duodenalis* genotypes had been cloned and purified in recombinant form and the Km value of the enzymes determined in vitro. Here, this data set is complemented with Km values of G. *duodenalis* ADI as determined in lysates of recent clinical parasite isolates. The results confirm that sequence variation causes changes in the activity of the respective enzyme variants and thus provide a further molecular argument to the concept that G. *duodenalis* ADI as a molecularly defined virulence factor is a quantitative trait of G. *duodenalis*.

MoB-P15-14h50-14h55

High Cysteine Proteins play a major role during *Giardia intestinalis* interactions with host intestinal epithelial cells

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Giardia intestinalis colonizes the small intestine of humans and animals causing diarrheal disease known as giardiasis. This single celled eukaryotic parasite does not internalize into host cells but it rather attaches to the villi in the small intestine disrupting the proper functioning of the epithelial barrier. Here we have used an in vitro model of the parasite interaction with the host intestinal epithelial cells (IECs) to study by RNA-seq genome wide changes in gene expression from *Giardia* which might relate to persistence of infection and disease. Between hundred to 200 genes presented altered mRNA levels. Among the highly up-regulated genes we found several members of the High Cysteine Protein (HCP) family, genes involved in cellular redox balance and genes from the lipid and nucleic acids metabolic pathways. In contrast, kinases, cell cycle and structural proteins were down-regulated, inferring a reduced cell proliferation. Hypothetical proteins were the major group detected as well in all three time points, indicating the vast number of important genes during infection which are still completely unknown. QPCR validated part of the RNA-seq and detected that the interaction media per se induced some of the changes observed which were attenuated when IECs were present.

In our RNA-seq, 20 to 24 members of the HCP family were highly up-regulated in all time points analyzed and therefore we decided to further study this uncharacterized family and its involvement in parasite-host interaction. Immunofluorescence microscopy of HA-tagged HCPs localized them to peripheral vesicles (PVs), plasma membrane and internal compartments. During interactions they seemed to be secreted to the media.

MoB-P16 – 14h55/15h00

HuTu-80 cells as a model for *Giardia lamblia* infection

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Giardia lamblia is a microaerophilic protozoan that colonizes the small intestine of humans and other mammals, causing a disease known as giardiasis. The illness is characterized by a broad spectrum of symptoms that vary between asymptomatic to chronic infection. The parasite clings strongly to the epithelial cells leading to microvilli shortening, tight junction disturbance and apoptosis. [1] In most in vitro studies, infections with *Giardia* are performed in Caco-2 cells, due to its phenotypic, morphological and functional characteristics. These cells can polarize and differentiate into enterocytes, resembling small intestine cells, presenting features such as tight junctional complex, high transepithelial electrical resistance (TEER), microvilli, specific enzymes and uptake transporters. Recently, Caco-2 cells were suggested as a model for asymptomatic infection, since trophozoites do not affect Caco-2 barrier function significantly. [2] The main objective of this study was to evaluate the potential of HuTu-80 cells as a model for *Giardia* infection. One of the biggest challenges in these studies is to mimic the existents parameters in the intestinal environment, such as the low oxygen tension produced by the intestinal microbiota. Thinking about that, our studies were performed under hypoxia, aiming to better simulate the microenvironment in which trophozoites and cells are usually inserted. Infection of HuTu-80 cells with the parasite (up to 04 hours) resulted in a decrease in TEER by approximately 48 % (normoxia) and 80 % (hypoxia) but did not affect significantly the macromolecular flux (FITC-dextran). Infection also led to a decrease in ZO-2 expression and an increase in claudin-1 and claudin-7 expression, confirmed by western blotting and immunofluorescence. Since the expression and location of these tight junction proteins can be regulated by kinases proteins, we evaluated whether the infection changes the levels of PKC pan, PKC theta and pPKC theta (T538), observing a decrease in PKC theta levels and an increase in pPKC theta (T538). These results show that *Giardia* trophozoites induce changes in duodenum cells at a tight junction level, possibly interfering in signaling pathways, demonstrating that HuTu-80 cells can be used as a model for *Giardia* infectious.

[1] Ankarklev et al. Nat Rev Microbiol. 2010, Jun; 8(6): 413-22.
[2] Kraft et al. Front Cell Infect Microbiol. 2017, Sep; 7(421).

ORAL PRESENTATIONS

Mo-O18-15h00/15h20

Towards the development of nutritional interventions for cryptosporidiosis

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We are investigating the interaction between the intestinal microbiome and *Cryptosporidium* parasites. This research is centered on the hypothesis that the intestinal microbiome can impact the severity of cryptosporidiosis, a phenomenon observed with other enteric pathogens and frequently referred to as "colonization resistance". To investigate the response of *Cryptosporidium* parasites to changes in the intestinal ecosystem, we perturbed the native intestinal microbiota of mice infected with C. *parvum* or C. tyzzeri using various antibiotics and by feeding diets lacking fermentable fibers or diets supplemented with pre- and probiotics. The severity of the infection was measured by enumerating immuno-labelled oocysts in the feces using flow cytometry. We initially observed that a probiotic product intended for human consumption significantly aggravated the infection. In contrast, cryptosporidiosis in mice which ingested prebiotics was less severe. The mitigating effect of this treatment was also observed when comparing the course of the infection in mice consuming a diet containing 10% fermentable fiber and in mice deprived of dietary fiber. Using 16S amplicon sequencing, we found a significant association between the taxonomic profile of the gut bacterial microbiome and cumulative oocyst output over the course of the infection. Together, these results suggest that the effect of diet on cryptosporidiosis is mediated by the microbiome. Based on these observations, and the fact that *Cryptosporidium* parasites replicate intracellularly, our model predicts that the microbiome impacts parasite proliferation by modulating the metabolism of the host cell and/or by degrading the mucus layer secreted by the intestinal epithelium. Metabolites produced by bacterial fermentation of food ingredients, such as short-chain fatty acids, are known to impact the metabolism of enterocytes. Given the metabolic dependence of the parasite on the host cell, changes in the host cell metabolism resulting from the perturbation of the microbiome could limit or promote parasite proliferation. The intestinal microbiome is also known to modulate the secretion and turn-over of the epithelial mucus layer, and could thereby inhibit or facilitate host cell invasion by sporozoites and merozoites. Our observations on the impact of dietary interventions on cryptosporidiosis are significant because they could lead to the development of simple "nutraceuticals" to protect infants living in countries where cryptosporidiosis is common. In the absence of effective anti-cryptosporidial drugs, dietary interventions may play a role in limiting the severity of cryptosporidiosis. Such interventions may also enhance the effect of future anti-cryptosporidial treatments. Although dietary supplements are not expected to eradicate the infection, the approach may be sufficient to prevent severe symptoms, while enabling the host to develop immunity and break the cycle of recurrent infections, diarrhea and malnutrition.

Mo-O19-15h20/15h32

Genome evolution in *Cryptosporidium parvum*

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Cryptosporidium parvum is the most important zoonotic *Cryptosporidium* species, infecting humans, other primates, ruminants, and equine animals. Genetic characterizations of C. *parvum* isolates at the 60 kDa glycoprotein (gp60) locus have identified host-adapted subtype families. Among the common ones, the IIa subtype family is commonly found in cattle, IId subtype families in sheep and goats, and IIc subtype families in humans. The evolution of C. *parvum* at the genome level, however, is not clear. In recent years, we have sequenced the genome of 361 C. *parvum* isolates from various sources. Comparative genomics analyses of the data have identified significant differences in nucleotide sequences among these subtype families. For example, there are ~20,000 SNPs between IIa and IIc subtype families across the 9.1 Mb genome. Smaller genomic differences are present between IIa and IId subtype families, especially those from the same area, but there are geographically segregated populations within the IIa and IId subtype families. Other subtype families with more divergent genomes have been recently identified in China, such as the IIo subtype family in nonhuman primates and rodents. Most highly polymorphic genes among the three subtype families are subtelomeric ones encoding secretory proteins, especially the invasion-associated and immunodominant mucin proteins and members of the *Cryptosporidium*-specific SKSR gene family. These subtype families also differ in the copy numbers of subtelomeric genes encoding the MEDLE family of secretory proteins and insulinase-like proteases, with IIc having significantly fewer copies than IIa and IId. Thus, adaptation in C. *parvum* has led to the generation of geographically and host segregated populations that differ in reservoir hosts and public health significance. Molecular surveillance should be implemented to monitor their emergence in humans.

Mo-O20-15h32/15h44

Developing in vitro tools for investigating host-parasite interactions in *Cryptosporidium* spp

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The apicomplexan parasite *Cryptosporidium* is a worldwide distributed pathogen of the gastrointestinal tract causing potentially fatal infections in immunocompromised individuals, young children and juvenile livestock. Despite its prevalence and significance, the mechanisms of its pathogenicity and reproduction have remained elusive as a result of ineffective culturing techniques, for which it's complex life cycle maybe responsible. We present a new in-vitro cell culture for the propagation of *Cryptosporidium parvum* that significantly exceeds the production and longevity of the previous gold-standard cell type HCT-8 in all fields and new methods for investigating the biology of parasite and its host. We will show the new cell cultures infected with C. *parvum* produce more oocysts during their life span than HCT-8, displaying significantly longer life spans until total culture senescence and as a result of this are significantly easier to handle, more robust and cost-effective. By utilising a broad, multi-disciplined approach, including atomic force microscopy, fluorescence microscopy, proteomics and lipidomics, we conclusively show that the oocysts produced by these cell cultures are both morphologically and biochemically identical to those provided by animal models. In addition, using an 1H Nuclear Magnetic Resonance approach to metabolomics, we have explored the nature of the mouse gut metabolome as well as providing the first insight into the metabolome of an infected cell line. This practical and informative approach can spearhead our understanding of the *Cryptosporidium*-host biology and metabolic exchange and thus provide novel targets for tackling this parasite.

Mo-O21-15h44/15h56

Genetic basis for virulence differences of various *Cryptosporidium parvum* carcinogenic isolates

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Cryptosporidium parasites represent a major public health problem in humans and animals causing self-limited diarrhea in immunocompetent hosts and life-threatening disease in immunocompromised hosts. We formerly reported that C. *parvum* isolates of either animal or human origin were also able to induce digestive adenocarcinoma in a rodent model. Interestingly, three carcinogenic isolates of C. *parvum*, called DID, TUM1 and CHR, all subtyped as IIaA15G2R1 and obtained from fecal samples of naturally infected animals or humans, exhibited higher virulence than the commercially available C. *parvum* IOWA II isolate in our animal model in terms of clinical manifestations, mortality rate and time of onset of neoplastic lesions. Moreover, only mice inoculated by these three isolates developed extra gastro-intestinal lesions. In order to discover the potential genetic basis of the differential virulence observed between C. *parvum* isolates and to contribute to the understanding of *Cryptosporidium* virulence, whole genomes of the these three highly virulent carcinogenic C. *parvum* isolates were sequenced and compared to the C. *parvum* IOWA II reference genome. Following purification of parasites by Immunomagnetic separation (IMS), DNA extraction and whole genome amplification, sequencing of DNA amplified by Multiple Displacement Amplification (MDA) was performed using Ion Torrent sequencing technology for DID and TUM1, and Illumina technology (2x150bp) for CHR. Two different bio-informatic pipelines (i.e. MICRA and a custom pipeline) were then used in order to determine common genetic determinants in the three more virulent C. *parvum* isolates in comparison with the C. *parvum* IOWA II reference genome. We found 126 common single nucleotide variants (SNVs) corresponding to 91 coding DNA sequences (CDS) in the C. *parvum* genome that could explain this differential virulence. Of interest, we identified variants in several membrane and secreted proteins. Among the already described *Cryptosporidium* virulence factors, those identified in this work are all implicated in sporozoite invasion or intracellular multiplication/survival of the parasite in the host cell. Furthermore, this study has also identified new potential virulence factors whose functional characterization is now possible using CRISPR/Cas9 technology.

Mo-O22-16568/16h08

Comparative genomics of *C. hominis* IbA10G2 isolates from Europe

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Cryptosporidium hominis is a major cause of cryptosporidiosis in humans worldwide, and is the dominant species in low-income countries (Squire SA and Ryan U, Parasit Vectors, 2017, 10:195). Previous work, using both traditional and Next Generation Sequencing (NGS) molecular approaches, has revealed large genetic variability among C. *hominis* isolates in endemic areas. However, in industrialized countries, including Europe, the C. *hominis* IbA10G2 subtype is particularly prevalent, accounting for up to 80% of human cases, and has been associated to large waterborne outbreaks (Cacciò SM and Chalmers RM, Clin. Microbiol. Infect. 2016, 22, 471-480). The reasons for the high prevalence of this subtype are still speculative. A recent NGS-based study (Sikora et al., J. Clin. Microbiol, 2017, 55:844–858) showed an extremely low level of genetic variability among six IbA10G2 isolates from Sweden, pointing to a clonal expansion of this subtype.
In this study, additional genomes (n=19) were sequenced from archived human stool samples accounting for sporadic cases of cryptosporidiosis caused by the IbA10G2 subtype in the United Kingdom. Oocysts were purified from stool samples by immune-magnetic separation, followed by DNA extraction, qPCR to estimate target enrichment, a generic 16S rDNA PCR to verify the presence of bacterial contamination, and Whole Genome Amplification. An Illumina Hi-Seq platform was used to sequence libraries (2x150 bp, paired-ends), with production of 7.4-13.8 million reads. Trimmed reads were mapped against the C. *hominis* reference genome (UdeA01), and this showed that 89-98% of the reads were derived from *Cryptosporidium*, yielding an average genome coverage comprised between 112 and 214. To identify Single Nucleotide Polymorphisms (SNPs) in the IbA10G2 genomes, we applied stringent criteria (GATK with hard filters). The results showed that a very similar number of SNPs characterized the newly sequenced UK isolates (range, 2001-2227) and the previously reported ones (range, 860-2016). Of interest, the cumulative SNP distribution along the chromosomes was not random, with higher SNP counts observed close to telomeric regions of chromosomes 1, 2, and 6, and internal regions of chromosome 6. Both SNP-based phylogenetic and Principal Component analyses showed strong clustering of IbA10G2 isolates. We conclude that a low genetic variability is a shared feature of IbA10G2 genomes.

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Mo-O23-16h30/16h50

Comparative genomics of *Cryptosporidium* : the current picture

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Cryptosporidium is a protozoan parasite that infect humans and animals worldwide, causing a diarrheal disease that is particularly severe, and unfortunately poorly treatable, among the very young and the immunocompromised. Two species, *Cryptosporidium hominis* and C. *parvum*, are responsible for the vast majority of human cases of cryptosporidiosis. Not surprisingly, therefore, the first genome sequencing efforts focused on these two species and culminated about 15 years ago in the description of a small, compact genome (9.1 Mb) comprised of eight chromosomes, with a strong conservation in synteny between the C. *parvum* (IOWA) and C. *hominis* (TU502) isolates.

The introduction of high throughput sequencing techniques also referred to as Next Generation Sequencing (NGS), has had a big impact on the field of genomics allowing faster and cheaper data generation. However, intrinsic challenges in the in vitro and in vivo propagation of parasite isolates has limited the application of NGS to *Cryptosporidium*. Several groups have addressed this issue and improved methods for sample processing (including all steps from oocyst purification, robust DNA extraction and whole genome amplification) have been published. In turn, this has made possible to sequence *Cryptosporidium* genomes directly from fresh or archived fecal samples. Single cell whole genome sequencing techniques were also applied. In parallel to improved wet-lab procedures, efforts were also made to develop and test ad-hoc bio-informatics pipelines, as this should solve one of the perceived barrier in the use of NGS, that is to say, data analysis.

Research questions that have promoted genomic studies include understanding the extent of genetic variability of human and animal isolates, the role of recombination in the evolution of virulent strains and in the process of host adaptation, and the identification of species-specific genes.

These studies clearly demonstrated that large genetic variability is a common feature of *Cryptosporidium* isolates, and that the distribution of single nucleotide polymorphisms (SNPs) and indels is non-random, with more variability observed at telomeric and sub-telomeric regions of some chromosomes. Parasite isolates cluster differently based on genome-wide SNPs compared to what observed at the gp60 locus, indicating that better tools for molecular epidemiologic studies can be developed from genome data. The existence of C. *parvum* clusters related to different hosts (human, different ruminants) and/or different geographical origin is still unclear.

Other studies have shown that the essential role played by genetic recombination in the evolution of *Cryptosporidium*, and found that recombinant regions are enriched for positively selected genes and potential virulence factors. Furthermore, levels of genetic variability and population structures vary among C. *hominis* isolates from different geographical regions, likely reflecting the relative transmission rates and the likelihood of mixed infections.

The VIIth International *Giardia* and *Cryptosporidium* Conference (IGCC) represents the ideal venue to discuss recent results and major open questions in the field of *Cryptosporidium* genomics.

Mo-O24-16h50-17h02

Strand-specific RNA Sequencing in *Cryptosporidium parvum* Suggests Widespread and Developmentally Regulated Long Noncoding RNA Transcription and Intron encoded Small RNAs

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It is becoming increasingly clear that non-coding RNAs (ncRNAs) having critical and mechanistically diverse regulatory roles across a broad range of organisms. To date, the regulatory elements orchestrating gene expression and post-transcriptional modification during parasite development and host interactions in *Cryptosporidium* remain largely unknown. It has been shown that some specific parasitic ncRNAs from C. *parvum* are delivered into the host nucleus resulting in epigenetic transcriptional suppression of genes with effects on pathology. This finding demonstrates their importance and the need for a systematic analysis of ncRNA in *Cryptosporidium*. We are interested in constructing a more comprehensive transcriptome in C. *parvum*, emphasizing potential regulatory ncRNAs that play roles in parasitic development or the host-parasite interaction. In this study, libraries of strand-specific polyA-selected and non-polyA-selected RNA-seq libraries from different developmental stages were used to explore ncRNA expression in C. *parvum*.

The results revealed that ~20% of protein-coding genes are covered by transcription from the complementary strand, indicating a complex transcriptome and regulatory system. After removing potential transcriptional readthrough, the analysis of >200 high-quality long ncRNA (lncRNA) candidates (longer than 200 nucleotides) revealed that their abundance is associated with the *Cryptosporidium* lifecycle progression and their expressions shows a positive correlation with upstream gene expression. A subset of lncRNA expression peaks 48 hours post-infection when the parasite begins producing sexual stages. Evolutionary conservation of some lncRNA candidates was seen among *Cryptosporidium* species and even in other apicomplexans based on both primary sequence and expression data. Their potential function needs further study. Small RNA sequencing also enabled the prediction of intriguing small RNAs encoded in intronic regions.

In conclusion, ncRNA is a vital transcriptome component in *Cryptosporidium* with potential regulatory functions. PacBio Iso-seq is needed in the future to fully characterize the RNA repertoire to resolve the boundaries of overlapping and adjacent transcripts. This work has contributed to the initial characterization of the C. *parvum* non-coding transcriptome and may facilitate further insights into the roles of lncRNAs in parasitic development and parasite-host interactions.

Mo-O25-17h02/17h14

Development of SureSelect target enrichment for whole genome sequencing of *Cryptosporidium* directly from stool samples

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Cryptosporidium is the 2nd leading cause of severe diarrhea and mortality in young children and infants in Africa and southern Asia. So far, ~30 distinct species of *Cryptosporidium* are known to cause severe to moderate infections in humans, of which C. *hominis* and C. *parvum* are the major causative agents. Very few low-resolution typing and subtyping genetic markers exist to genotype and/or diagnose *Cryptosporidium*. Hence, there is an immediate need to a) develop high resolution genetic markers to genotype *Cryptosporidium* parasites at whole genome scale resolution directly from stool samples, b) to characterize subtypes associated with asymptomatic carriage in endemic regions, and c) to understand the potential for zoonotic transmission and the evolution of these protozoan parasites. We developed SureSelect target enrichment sequencing on an Illumina 2500 platform to understand the global population genetic structure of *Cryptosporidium*. Our platform has 75,000 probes that cover ~95% genome in order to hybridize *Cryptosporidium* DNA directly from stool samples. Initial spooling experiments show that SureSelect target enrichment can amplify 26.3nM of gDNA in the post capture library using only 3ng *Cryptosporidium* gDNA spiked into 200ng of gDNA from host stool. for DNA sequencing using the Illumina platform. Whole genome sequence data analyzed using PopNet, PCA, METAL, PLINK and POPSICLE software suites that measure genetic diversity and capture the population structure of *Cryptosporidium* spp. will be presented.

Mo-O26-17h14/17h26

Cryptosporidium parvum exports proteins into the cytoplasm of the epithelial host cell

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Infection with the protozoan parasite *Cryptosporidium* is a leading cause of diarrheal disease and child mortality worldwide. Cryptosporidiosis is typically self-limiting, but in the context of malnourishment or immunodeficiency the disease can be protracted and deadly. Upon infection, *Cryptosporidium* travels to the small intestine, where it drastically remodels the cytoskeleton of the epithelial cell to establish an intracellular but extracytoplasmic localization. We hypothesize that effector proteins exported into the host cell play critical roles in the initial establishment and maintenance of infection by modulating interaction with the host cell and host immunity. We assembled a prioritized list of candidate effectors based on a variety functional genomic and population genetic parameters. Using the CRISPR/Cas9 system to epitope tag the endogenous loci of candidate proteins, we have identified Medle 2 as the first example of a host targeted protein in *Cryptosporidium parvum*. Medle 2 is highly polymorphic and localizes to the cytoplasm of infected HCT-8 cells in tissue culture, and to the cytoplasm of intestinal epithelial cells in infected mice. The protein is not apparent in sporozoites, but is detectable in the host cell cytoplasm as early as 6 hours post infection, suggesting a rhoptry-independent delivery system that is assembled by the trophozoite only after invasion. Pharmacological and imaging experiments support this view. Medle 2 is expressed in cells infected with all life stages of the parasite and its expression increases over time with the number of intracellular parasites. Our current work aims to uncover the function of Medle 2 during C. *parvum* infection through cell biological, transcriptional and functional proteomic studies.

Mo-O27-17h30/17h50

Cryptosporidium and colon cancer: is there a causal link?

Gabriela Certad

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Cancers associated to infections are increasing at an alarming rate. The main recognized agents are the bacterium Helicobacter pylori (5.5 % of all cancer), the human papilloma viruses (5.2 %), the hepatitis B and C viruses (4.9 %), the Epstein-Barr virus (EBV) (1 %), the human immunodeficiency virus (HIV) together with the human herpes virus 8 (0.9 %) and the HTLV-I virus (0.03 %). Other pathogens such as parasites, in particular some trematodes, are also considered as carcinogenic agents. However, the contribution of intracellular eukaryotic parasites to cancer development is not well known. Based on clinical and epidemiological evidences, many reports highlight a potential association between parasitic protozoan infections and cancer. In particular, several evidences support an association between *Cryptosporidium* and the development of digestive neoplasia. In animals *Cryptosporidium* was found in intestinal polyps of naturally infected sheep and in aural-pharyngeal polyps of iguanas, even if signs of malignancy were not detected. Dysplastic changes in bile ducts have also been reported in an experimental model of *Cryptosporidium* infected IFN-δ knockout mice. Concerning humans, a survey including HIV-infected patients (known to be highly susceptible to *Cryptosporidium* infection) showed a higher incidence of colorectal cancer at earlier ages compared to immunocompetent controls. Other studies reported elevated colon squamous cell carcinoma risk in AIDS patients with cryptosporidiosis and bile duct carcinoma associated with *Cryptosporidium* infection in children with X-linked hyper-IgM syndrome. Finally, various epidemiological studies conducted in Poland in the last years strongly suggested a link between cryptosporidiosis and colorectal cancer.

To explore the dynamics of *Cryptosporidium* infection, an animal model of cryptosporidiosis using corticoid dexamethasone-treated adult SCID (severe combined immunodeficiency) mice, orally infected with C. *parvum* or C. muris oocysts, was implemented by our team. Intriguingly, only C. *parvum*-infected animals developed digestive adenocarcinoma even when they were infected with a single oocyst. The inoculation of animals was performed with the C. *parvum* IOWA strain. Additional strains were tested including “TUM1” (isolated from a calf in the USA) and “Did” (isolated from a patient in Lille, France), and these strains were found to be more virulent than IOWA. Indeed, both strains (TUM and Did) induced a higher mortality rate and the development of digestive neoplasia, with an earlier onset of neoplastic lesions and more rapid progression to invasive cancer. In addition, our team recently succeeded in the development of a three-dimensional (3D) in vivo like culture model from adult murine colon allowing biological investigations of *Cryptosporidium* infection. The resulting system allowed the reproduction of neoplastic lesions in vitro after only 27 days post-infection, providing new evidences on the role of the parasite in the induction of cancer. In parallel, the mechanisms involved in this neoplastic process have been explored and the pivotal role of the Wnt pathway together with the alteration of the cytoskeleton was emphasized. More recently, an epidemiological study conducted in Lebanon by our team reported a significant higher prevalence of *Cryptosporidium* among patients with recently diagnosed colon cancer before any treatment when compared to that of patients either with gastric cancer or without any cancer. These results suggest that *Cryptosporidium* is associated with human colon cancer being a potential etiological agent of this disease. More research in the field is required in order to identify mechanisms and molecular factors involved in this process.

Tuesday, 25th June, 2019

ORAL PRESENTATIONS

Tu-O1-8h00/8h20

A UK perspective on tools for identifying, investigating and preventing *Cryptosporidium* outbreaks

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Identification of *Cryptosporidium* cases is dependent on access to health-care, sample submission, and diagnostic and reporting practices. In the UK, *Cryptosporidium* is notifiable as a "causative agent" and so all diagnoses come to the attention of the public health agencies. However, what happens next is highly variable and this can impact the identification, classification and characterisation of outbreaks, which in turn affects their investigation, management and control. It is important to investigate the cause of outbreaks not only for immediate action for control but also for future prevention.

Cryptosporidium outbreaks in the UK in the 1990s were predominantly waterborne, with about one third overall attributed to mains drinking water. Since the introduction of specific legislation to control *Cryptosporidium* in drinking water, subsequently superseded by the water safety plan approach, drinking water quality has improved and *Cryptosporidium* outbreaks linked to mains water have become a rare event. Not so outbreaks linked to swimming pools and open farms, which in the last ten years accounted for 88% outbreaks in England and Wales. Foodborne outbreaks are an emerging issue.

Tools applied to outbreaks include microbiological (confirmation, genotyping), environmental (inspections, sampling), epidemiological / statistical (enhanced surveillance, exceedance monitoring, spatiotemporal analysis) methods. This paper will look at how current tools contribute to outbreak investigations and explore the tools needed for prevention of today's outbreaks.

Tu-O2-8h20/8h32

Epidemiology and distribution of *Giardia duodenalis* Genotypes in humans in Metropolitan Sydney, Australia

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giardiasis is an important widespread parasitic cause of human diarrhoea and knowledge of this disease in Sydney remains poorly understood. This study aimed to (1) describe the epidemiology of giardiasis and (2) detect and identify G. *duodenalis* genotypes affecting humans in Metropolitan Sydney.

A 1:2 matched case-control study of 190 confirmed giardiasis cases, notified to the South-Western Local Health District Public Health Unit from January to December 2016, was employed to explore the epidemiology of giardiasis in Sydney. Two groups of controls were selected to increase response rate; Pertussis cases and neighbourhood (NBH) controls. *Giardia* cysts in 105 human faecal samples collected from various tertiary hospitals and private (community) laboratories in Sydney, Australia, were genotyped by sequence analysis of the triosephosphate isomerase gene.

Overall, risk factors identified for giardiasis include being under 5 years of age, having a household member diagnosed with a gastrointestinal illness and having contact with farm animals, domestic animals or wildlife. Cases from overseas travellers were at increased risk of infection when compared with Pertussis cases. Genotyping results showed that the majority of samples belong to assemblage A, and only a small percentage (4%) belonged to assemblage B. Surprisingly, mixtures of genotypes A and B in individual cases were relatively common. Co-infections were observed in 39% of the samples, the most common co-infection being G. *duodenalis* with Blastocystis *hominis* (20%). This study provides new knowledge on the epidemiology of G. *duodenalis*, as well as new insights into the molecular diversity of this parasite in Sydney, Australia. This study provides information for enhanced surveillance and prevention strategies for metropolitan Sydney.

Tu-O3-8h32/8h44

Occurrence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in a large asymptomatic school children population in the Madrid area. Central Spain

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Background

Intestinal protozoan *Giardia duodenalis* and *Cryptosporidium* spp. are major contributors to the burden of childhood gastrointestinal illness globally. Giardiosis and cryptosporidiosis also represent an important public health threat in industrialized settings, where they are frequently associated with outbreaks of diarrhoea. In Spain, there is limited information regarding the frequency and molecular diversity of *G. duodenalis* and *Cryptosporidium* spp. in asymptomatic carriers.

Methods

A cross-sectional, molecular epidemiological study was conducted in children and their siblings (1-16 years) attending public (n = 10) and private (n = 3) schools in Leganés (n = 12) and Madrid (n = 1), Central Spain, during November 2017-June 2018. We collected stool samples and demographics from those accepting screening. Detection of *G. duodenalis* infections was achieved by qPCR; positive samples with qPCR Cq values <32 were subsequently assessed by multi-locus sequence genotyping (MLSG) of the gdh, bg, and tpi genes of the parasite. *Cryptosporidium* species and sub-genotypes were investigated at the ssu rDNA and the gp60 genes of the parasite.

Results

Stool samples were provided by 1,608 individuals (male/female ratio: 1.25; mean age: 6.9 years). Mean participation rate by school was 25% (range: 12–47%). G. *duodenalis* and *Cryptosporidium* spp. prevalences were 17% (95% CI: 15–19%) and 1.1% (95% CI: 0.6–1.7%), respectively. Two children were co-infected with both species. Prevalence rates varied largely among the schools surveyed (Table 1). A total of 24 *G. duodenalis*-positive samples were successfully typed revealing the presence of sub-assemblages AII (17%, 4/24), BIV (80%, 19/24) and BIII/BIV (4 %, 1/24). MLSG data at the three loci were available for seven samples. A total of 17 *Cryptosporidium*-positive samples were typed, allowing the identification of C. *hominis* (77%, 13/17) and C. *parvum* (18%, 3/17). A single sample was characterized at the genus level only. Two C. *hominis* and one C. *parvum* samples were assigned to sub-genotype IbA10G2 and family IId, respectively.

Table 1. Participation rates and prevalence of *G. duodenalis* and *Cryptosporidium* spp. among apparently healthy school children in the Madrid area, November 2017-June 2018.

School	No. students (total)	No. participating students	%	No. *Giardia*-positive	%	No. Crypto.-positive	%
1	850	238	28	60	25	0	0.0
2	990	137	14	35	26	0	0.0
3	440	125	28	25	20	1	0.8
4	234	70	30	11	16	3	4.3
5	475	56	12	4	7	1	1.8
6	446	67	15	1	1.5	0	0.0
7	561	146	26	13	9	0	0.0
8	459	214	47	24	11	8	3.7
9	400	74	19	8	11	1	1.4
10	353	146	41	14	10	0	0.0
11	450	141	31	44	31	2	1.4
12	485	128	26	25	20	0	0.0
13	180	66	37	12	18	1	1.5
Total	6,323	1,608	25	276	17	17	1.1

Conclusions

G. duodenalis, but not *Cryptosporidium hominis/parvum*, is a common finding in apparently healthy school children in Central Spain, mostly causing low-burden infections. Asymptomatic carriers might play an important role in the unnoticed dissemination of diarrhoea-causing pathogens at the community level, representing a public health concern for immunodebilitated individuals. The frequency and diversity of G. *duodenalis* assemblages/sub-assemblages and *Cryptosporidium* species/sub-genotypes identified here were essentially the same than those previously reported in symptomatic individuals from the same area.

Funding

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Tu-O4-8h44/8h56

Novel multi-locus genotypes of *Giardia duodenalis* isolates among children in rural communities in southern palawan, philippines

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Giardia duodenalis is a flagellated protist known to cause gastrointestinal disease called giardiasis. It is globally distributed and considered as the most common cause of water-borne diarrhea (WHO, 2007). It is a species complex having eight morphologically similar but genetically distinct genotypes (assemblage A-H) with inter- and intra-assemblage variation (sub-assemblages and subtypes). The detection of genetic variation and heterogeneous nucleotides when isolates were analyzed using multi-locus sequence typing (MLST) has been reported in various studies. This shows that genotyping using single locus only, may conceal the true picture of G. *duodenalis* genetic diversity. In the Philippines, there is limited information on the genetic distribution and diversity of G. *duodenalis*. The aim of the study was to identify the prevalence of G. *duodenalis*, its assemblages, sub-assemblages, and subtypes, including its genetic variation among the children in rural communities in southern Palawan, Philippines using multi-locus sequence analysis of the glutamate dehydrogenase (gdh) beta-giardin (bg), and triose phosphate isomerase (tpi).
G. *duodenalis* was detected in 18.7% (n=107) of stool samples using formalin ether concentration technique (FECT) and ssu-rRNA amplification. All of the positive samples were successfully sequenced, and 95% were typed as assemblage B. The remaining 5% were typed as assemblage A. “Assemblage swapping” was observed in one isolate (Ph13), typed as assemblage B at the gdh and bg loci and typed as assemblage A at the tpi specific locus. Moreover, 60% of the samples were amplified at the gdh locus, 55% at the tpi locus, and 45% at the bg locus.
In addition, single nucleotide polymorphisms (SNPs) that may indicate genetic variation and heterogeneous nucleotides were observed within assemblage B. The presence of heterogeneous nucleotide as observed in their chromatogram and phylogenetic tree indicates mixed infection at the sub-assemblage and subtype level. Some of the reference sequences used in the current study were isolated from animal origin based on their GenBank profiles, and may be considered as zoonotic genotypes, and thus of public health importance. Since, G. *duodenalis* is a pathogen transmitted by cyst-contaminated water, the presence of either zoonotic or anthroponotic assemblages, sub-assemblages, and subtypes of G. *duodenalis* may indicate that human and animal hosts were the possible source of cyst contamination in the water. The detection of two novel multi-locus genotypes (MLGs) despite the small sample size of the study may indicate that the MLGs existing in the country may be different from the previously described and identified MLGs in other countries. Also, there is a possibility that genetic diversity of G. *duodenalis* in the country may be underestimated. Future studies may analyze the association of G. *duodenalis* assemblages, sub-assemblages, subtypes, and MLGs with virulence, symptoms, and transmission patterns that can serve as a basis for more comprehensive and effective control of giardiasis.

Tu-O5-8h56/9h08

Recirculation of *Giardia duodenalis* genotype A in children after treatment with metronidazole: reinfection or parasitic resistance?

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Giardia duodenalis is an intestinal protozoan subdivided into assemblages A to H, according to its molecular characteristics and the host that infects. The assemblages A, B and E infect human and can circulate sympatrically. Here we aim to investigate the recirculation of these assemblages in individuals leaving in a high prevalence area of *Giardia* infection. Stool samples from 194 preschoolers attending a daycare center in a slum in Rio de Janeiro/Brazil were submitted to parasitological examination. Eighty-five G. *duodenalis*-infected subjects were included, treated with metronidazole, and followed-up up to 20 and 40 days after treatment. Those patients who still eliminate *Giardia* cysts were retreated and reevaluated for cure control. The DNA from *Giardia*-positive samples were extracted and submited to PCR, Nested-PCR, and sequencing for gdh and β-gia gene targets. *Giardia* assemblages A (n=43), B (n=21) and E (n=21) were identified. Only 36 subjects were reevaluated at the 1st cure control and 18 were negative. Curiously, those infected by assemblage B (n=2) and E (n=2) were still positive but now by the assemblage A. Then, at the 1st (18/36) and 2nd (14/17) control cure, all the G. *duodenalis* isolates were assemblage A. Though both persistence and reinfection could account to this picture, the level of homology of the *Giardia* nucleotide sequences obtained before and after treatment were compared. Fourteen isolates presented 100% of identity, at the 1st and 2nd cure control, indicating the subjects were infected by the same strain. Four *Giardia* sequences presented a distinct genotypic profile when isolates obtained before and after treatment were compared. To investigate whether metronidazole could induce a nucleotide alteration in the target genes evaluated herein (gdh and β-gia), *Giardia* trophozoite cell line was in vitro exposed continually during 5 weeks to metronidazole. No nucleotide alteration was observed even when high doses were employed, evidencing that differences of the genotypic profile were due to reinfection. We demonstrated that in high *Giardia* burden areas the reinfections are a common feature and, more seriously, it can occur soon after the end of therapy. As a consequence, constant and indiscriminate use of metronidazole can favor the selection of resistant strains. However, we were not able to provide molecular shreds of evidence for drug resistance. The present results raise the hypothesis that assemblage B and E can be more susceptible to treatment. Finally, assemblage A seems to be prone to recirculate in agglomerate areas.

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Tu-O6-9h08/9h20

C. *hominis* waterborne outbreak in a french military camp, 2017

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From 2015, several cases of acute gastroenteritis were reported every summer in a military camp in southwestern France. Symptomatology and epidemiology were consistent with foodborne origin, however investigations of pathogens in both stool and environmental samplings were constantly negative. In June 2017, two outbreaks of acute gastroenteritis were sequentially reported to the Center for Epidemiology and Public Health of Armed Forces (CESPA). and thanks to the recent availability of a new diagnostic tool (Filmarray gastrointestinal panel) in the CESPA, *Cryptosporidium* spp. was detected in stool samples in which *C. hominis* isolates with the *gp60* subtype IbA10G2 were also identified by the Centre National de Référence-Cryptosporidioses The same genotype was also exhibited by isolates from waters supplying both the military camp and the civilian population. Finally, with a 40% attack rate, 142 probable cases were identified in the military camp. Only military personels recently arrived and staying in the camp for a few months training presented gastroenteritis while the permanent military staff did not suffer from symptoms, which suggests a significant role of acquired immunity in controlling infection. Similarly, retrospective investigations on chronically exposed civilians did not reveal increased prevalence of gastroenteritis. Oocyst excretion in stools was followed up in patients until 3 months after diagnosis. One month post-diagnosis, microscopic oocysts and *Cryptosporidium* DNA were detected in 10/42 and 18/42 patients, respectively. Three months post-diagnosis, corresponding figures were 0/21 and 4/21. For several months, bottled water was supplied to the exposed populations, and further the network of water for human consumption was decontaminated by an ultrafiltration unit.

Tu-O7-9h30//9h42

Cryptosporidium spp. infection and carriage in rural Madagascar: cluster detection among humans and animals

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Introduction. Cryptosporidiosis is a main cause of childhood diarrhoea in sub-Saharan Africa. However, its transmission pathways and reservoirs are still under discussion. Humans and domesticated animals living in households within rural Madagascar were sampled to investigate geographical and microbiological transmission pattern.

Methods. Stool samples from humans, cows and sheep living in households in the Andina region were collected between May 2017 and March 2018. Households were randomly visited throughout the study period and samples were collected within a week. Specimens were screened for *Cryptosporidium* spp. by PCR-RFLP analysis of the small subunit rRNA gene and sequence analysis of the 60 kDa glycoprotein gene (gp60) for C. *hominis* and C. *parvum* was applied. Identical gp60 strains within households were identified to describe microbiological cluster and Average Nearest Neighbour Distances were simulated to detect geographical *Cryptosporidium* spp. cluster.

Results. Of the 332 households registered within the study region 252 (78%) could be included in the analysis. From 207 (82%) households human, from 223 (88%) cow and from 74 (29%) sheep samples were available. In total 3% (12/363) of the human, 3% (30/867) of the cow and 12% (41/332) of the sheep samples were tested positive for *Cryptosporidium* spp. The isolated subtypes differed between humans and animals. Infections in humans were due to C. *hominis* (8; 67%), C. viatorum/tyzzeri (2; 17%), and C. meleagridis (2; 17%), in cows due to C. xiaoi/bovis (22; 73%), C. ryanae (7; 23) and C. viatorum/tyzzeri (1; 3%), and infections in sheep were caused by C. xiaoi/bovis (41; 100%) solely. All of the C. *hominis* infections detected in humans could be allocated to the gp60 subtype IbA10G2. Two of these infections occurred simultaneously within one household. Geographical clustering was indicated in sheep ($p < 0.01$), however not in humans ($p = 0.23$) and cows ($p = 0.69$).

Conclusions. Even though humans and animals live in close proximity within the study area, no overlap in *Cryptosporidium* subtypes was detected. According to our results anthropological transmission seems unlikely. No local outbreaks in humans were detected but the overlap in gp60 subtypes suggest human-to-human transmission. Our results underline that improving hygiene and sanitation practices is the primary interventions to prevent infections among humans.

Tu-O8-9h42/9h54

The role of protozoan genotypic diversity in humans: Implications for the epidemiology of cryptosporidiosis and giardiasis in New Zealand

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Surveillance data from 2016 in New Zealand found that *Cryptosporidium* and *Giardia* were responsible for 5.4% and 7.4% respectively, of the total outbreaks in the country. However, there has been an inability to identify the same genotypes in epidemiologically linked cases using Sanger sequencing. Furthermore, the reportedly hyper-transmissible C. *parvum* genotype IIaA15G2R1 dominant in people and calves in most countries including the USA, UK, Spain and India is present but not dominant in New Zealand. Given the clinical effects of cryptosporidiosis and giardiasis are highly variable and the mechanisms underlying the epidemiological and phenotypic differences within these organisms are poorly understood, there are opportunities to use in vitro and next generation sequencing methods to address some of these questions. We hypothesise that 1) epidemiologically linked cases will share subtypes undetectable with consensus PCR and Sanger sequencing, and 2) that host-pathogen interactions are determined by genetic differences at the between species level and ecological differences at the between genotype level. To address our first hypothesis, we employed amplicon-based metabarcoding to characterise the within-host genetic diversity of *Cryptosporidium* and *Giardia* in humans from outbreaks that occurred in New Zealand between 2010 and 2018. To address our second hypothesis, we employ in vitro techniques to understand species and genotypic differences influencing the infectivity of these parasites. To do so we have adopted the newly characterised COLO-680N human cell line for the culture of *Cryptosporidium* and Caco-2 for *Giardia* and used flow cytometric analysis to assess levels of infectivity between isolates. Investigating the differences in human cell infectivity between isolates could provide insights into the molecular mechanisms of these diseases, through whole genome and transcriptomic sequencing, thereby informing future studies, guiding policy and leading to a better understanding of protozoa outbreak epidemiology.

Tu-O9-9h54/10h06

Transmission networks of *Cryptosporidium* spp. in rural sub-Saharan Africa: a multi-country study

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Introduction. High prevalence and mortality from Cryptosporidiosis among children in sub-Saharan Africa has been shown in recent years, however transmission dynamics and reservoirs are yet to be investigated. This multicentre study traces back *Cryptosporidium* positive children to their close human and animal contacts in order to identify transmission networks and reservoirs.

Methods. Stool samples from children below 5 years with diarrhoea were collected at hospitals in Gabon, Ghana, Madagascar and Tanzania. *Cryptosporidium* positive and negative initial children were followed to the community, where stool samples from all household members, neighbouring children (<5 years) and animal contacts (cows, sheep, goats and dogs) were obtained. Samples were screened for *Cryptosporidium* spp. by PCR-RFLP analysis of the small subunit rRNA gene and sequence analysis of the 60 kDa glycoprotein gene for C. *hominis* and C. *parvum*. Contact networks were identified and rate ratios (RR) calculated.

Results. Among 1,363 initial children 44 (20%), 47 (11%), 25 (11%) and 68 (14%) were diagnosed with *Cryptosporidium* spp. in Gabon, Ghana, Madagascar and Tanzania, respectively. The following species were diagnosed: 144 (79%) C. *hominis*, 26 (14%) C. *parvum*, 10 (5%) C. meleagridis, 2 (1%) C. felis and 1 (1%) C. xiaoi/bovis. Across the countries the proportion of infections ranged from 8% to 20% in household members (N=350), from 20% to 36% in neighbouring children (N=245) and from 11% to 15% in animals (N=338). Among 108 contact networks gp60 subtyping established 37 clusters, which contained 49% and 54% of *Cryptosporidium* positive household members and neighbours, respectively, but only 18% of *Cryptosporidium* positive animals. In comparison to *Cryptosporidium* negative initial children, positive initial children had an increased risk of having positive household members (RR = 2.5; 95%-Confidence Interval (CI): 1.5–5.2) or positive neighbouring children (RR = 2.7; 95%-CI: 1.6–4.8), but no risk of having positive animals (RR = 1.3; 95%-CI: 0.8–2.1) in their contact network.

Conclusions. Cryptosporidiosis in rural sub-Saharan Africa is characterized by clusters among human contacts, to which zoonotic transmission, despite close human-livestock contacts, seems to contribute only marginally. Shared sanitation facilities or water sources may be responsible for anthroponotic neighbourhood transmission. Public health programmes need to focus on improving hygiene and sanitation practices, particularly in the context of infant and childcare.

Tu-O10-10h06/10h18

Cryptosporidiosis outbreak within a middle school in western france, November 2017

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On Thursday 23 November 2017, a gastroenteritis outbreak was reported within a middle school in Loire-Atlantique western France, with more than 150 patients. Further epidemiological, microbiological and environmental investigations were performed. A retrospective cohort study of school students and adults was conducted using standardized questionnaires posted online the next day on the school local website. Faecal samples were analyzed successively for bacterial, viruses and parasites. Environmental specimens were analyzed. The attack rate was 61 % (180/293 respondents) and decreases significantly with the age from 75 % in ten-year-old students to 45 % in adults. Diarrhea were experimented by 98 % of the cohort; nausea and abdominal pain by 64 % and 62 % respectively (fever 33 %; vomiting 26 %). A peak of 75 cases occurred on Wednesday 22 November. While water consumptions weren't associated with the occurrence of the disease, eating canteen each lunch appeared as a significant risk factor [relative risk 1·6 (1·1-2·3)]. Bacteriological and virological investigations were found negative (15 samples), *Cryptosporidium* was finally detected in a national reference laboratory in stool samples using FilmArray® gastrointestinal panel (22 pathogens) and was then characterized at GP60 as C. *parvum* IIaA15G2R1. Among foods served at canteen 6 days before (incubation period), there was an organic unpasteurized cottage cheese. It was served from a single pot of 5 kg to all half-boarders. A survey of the producer showed the presence of calves infected with the same virulent zoonotic genotype close to the cheese production room; shedding was from 80 000 to 570 000 oocysts per gram of faeces. Phylogenetic tree inferred from MSC6-7 locus analysis showed the genetic closeness of illness students isolates with bovine isolates compared to human without any contact with bovine harboring the same GP60 subtype. Human cryptosporidiosis appears in France largely under-diagnosed. Routine prescriptions for the biological diagnosis of persistent diarrhea should be improved specifying parasitological research. This is to our knowledge the first cryptosporidiosis outbreak with a link to dairy product in France.

Tu-O11-10h40/11h00

Giardia and *Cryptosporidium* : should we always consider both?

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Giardia and *Cryptosporidium* both were included in 2004 in the WHO Neglected Diseases Initiative. Both pathogens are significant causes of diarrhea and nutritional disorders in institutional and community settings. They are also significant waterborne pathogens. However in the ranking of foodborne parasites *Cryptosporidium* is usually ranked higher than *Giardia* although giardiasis is more prevalent and giardiasis is costing more DALYs. Also in the scientific world *Giardia* seems to get less attention than Crypto.

Our knowledge of *Giardia* and *Cryptosporidium* has changed due to molecular techniques e.g. for diagnostics in humans with gastrointestinal symptoms. By implementing a multiplex PCR there is no need for laborious microscopic examinations or specific staining for Crypto and it is possible to test all samples for several pathogens in one test. But is it necessary to include both pathogens in routine diagnostic requests for all patients? An algorithm for diagnostic requests will be discussed: should we make a difference in age groups, are clinical symptoms indicative or are costs and lab logistics most important?

Tu-O12-11h00/11h12

Health Sequelae of Human Cryptosporidiosis – a 12 month prospective follow-up study

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Relatively little is known about the longer-term health effects of *Cryptosporidium* infection. There is growing evidence to suggest that, rather like some bacterial causes of gastroenteritis, *Cryptosporidium* infection may have longer-term consequences. Acute bacterial gastroenteritis leads to post-infectious irritable bowel syndrome (PI-IBS) in up to 26% of patients. C. *parvum* in animal models can also induce pathophysiological features consistent with PI-IBS. Anecdotally, *Cryptosporidium* infection has also been associated with the development of reactive arthritis, Reiter's syndrome, acute pancreatitis and haemolytic uraemic syndrome.

A small number of previous studies have investigated post-acute symptoms after cryptosporidiosis but most did not include both C. *hominis* and C. *parvum* infections, and some did not have a follow-up period of sufficient duration to be able to identify PI-IBS, a diagnosis which requires symptoms to have been present for at least 6 months. We carried out a cohort study of laboratory-confirmed cryptosporidiosis cases in Wales, UK, and sought to investigate the development of potential post-infection sequelae of both C. *parvum* and C. *hominis* over a 12-month time period, with particular attention to PI-IBS. All patients with laboratory-confirmed, genotyped cryptosporidiosis in Wales aged between 6 months -45 years of age over a two year period were contacted. They were asked to complete questionnaires (paper or online) at baseline, 3 and 12 months after diagnosis. Presence/absence of IBS was established using Rome III criteria. 205/515 cases participated (40% response rate). At 12 months, over a third of cases reported persistent abdominal pain and diarrhea, 28% reported joint pain and 26% reported fatigue. At both 3 and 12 months, the number reporting fatigue after C. *hominis* infection was statistically significantly greater than after C. *parvum* ($p<0.05$). 10% of cases met the full Rome III criteria for IBS. A further 27% met all criteria apart from 6 months' duration and another 23% had several features of IBS but did not fulfil strict Rome III criteria. There was no significant difference between C. *parvum* and C. *hominis* infection with regard to PI-IBS. In conclusion, a substantial number of patients are likely to experience continued gastrointestinal symptoms for a prolonged period after *Cryptosporidium* infection. It would be helpful in terms of patient management to advise patients of this following a diagnosis of cryptosporidiosis.

Tu-O13-11h12/11h24

Three year population based *Cryptosporidium* study in the Netherlands : risk factor and long term sequelae

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Background: Since 2012, cryptosporidiosis increased in the Netherlands, but there was no information available about risk factors and the long term sequelae of this infection. From April 2013 until April 2016, a 3-year population-based case-control study was carried out, coupled with genotyping, to identify risk factors for sporadic cryptosporidiosis and a study of long term sequela.
Methods: *Cryptosporidium* cases were laboratory confirmed and C. *hominis* or C. *parvum* species was determined. Cryptosporidiosis cases were invited to complete a follow-up questionnaire 4 months after diagnosis. Frequencies of symptoms in the pre- to post-infection phases were compared with those of a population control group. We analyzed data by study year, combined and by species. We performed single-variable analysis, and variables with a P value of ≤ 10 were included in a multivariable logistic regression model adjusting for age, sex, and season.
Results. The study included 609 cases and 1548 frequency-matched controls. C. *parvum* was the predominant species in the first 2 study years, shifting to C. *hominis* in the third year. Household person-to-person transmission and eating barbequed food were strongly associated with being a case. Eating tomatoes was negatively associated. When the analysis was stratified by study year, person-to-person transmission was an independent risk factor. Analysis by species identified different risk factors for cases infected with C. *parvum* and C. *hominis*. Of the 731 available cases for the follow up study, 443 (60%) responded and 308 (42%) could be included. Of these 58% were female; 30% were infected with C. *hominis* and 70% with C.*parvum*. Compared to before illness, cases were significantly more likely to report dizziness (OR = 2.25), headache (OR = 2.15), fatigue (OR = 2.04), weight loss (OR = 1.82), diarrhoea (OR = 1.50), abdominal pain (OR = 1.38) or joint pain (OR = 1.84).
However, symptoms of joint pain and headache occurred among cases after illness at a rate that was not significantly different from that observed in the general population. There were no significant differences in post-infection symptom occurrence between C. *hominis* and C. *parvum*.
Conclusion. Providing information about *Cryptosporidium* exposure during outdoor activities and improvements in hygiene within households could prevent future sporadic infections. The disease burden of cryptosporidiosis extends beyond the acute phase of the infection, with cases reporting both intestinal and extra-intestinal symptoms up to 4 months following infection.

Tu-O14-11h24/11h36

Intestinal epithelial cell damage and plasma cytokines levels can be related to giardiasis in Brazilian pre-school children

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giardiasis is caused by fecal-oral transmission of cysts *Giardia duodenalis*, a flagellated intestinal protozoan present in water, food or transmitted zoonotically through domestic animals stools. It is an infectious disease of global importance and its prevalence can reach up to 90% in low-income areas, although prevalence is not restricted to those areas. Children are most susceptible to the disease and here we found that in Brazilian preschoolers from 10 months to four years old levels of IL-5 and IL-8 were decreased and IL-10 and IL-17 were increased in plasma. Importantly, intestinal fatty acid binding protein (IFABP) levels were higher in *Giardia*-infected children comparing to children without any parasitic intestinal infection, suggesting damage in intestinal epithelial cells. Genotyping of *Giardia* identified in stool belonged to assemblages to A, B and E and no correlation was found between assemblages and IFAB or cytokines levels. These results can be important to better elucidate systemic immune response to *Giardia duodenalis* in children.

Financial support: CNPq, CAPES, IOC/FIOCRUZ

Tu-O15-11h46/11h58

Validation of a multilocus variable number tandem repeat scheme for *Cryptosporidium parvum* subtyping during outbreak investigations

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Cryptosporidium parvum is the most common zoonotic *Cryptosporidium* species infecting humans and a wide range of animals, posing a challenge for public health. Subtyping of isolates becomes crucial to investigate sources of contamination and routes of transmission and in doing so, identify appropriate interventions. In the event of a cryptosporidiosis outbreak, subtyping C. *parvum* commonly relies on the DNA sequence analysis of a highly genetically variable 60 KDa glycoprotein (gp60). Although this marker is useful for allele discrimination and inferring linkage to point source infections, the recombinant nature of this parasite demands a multilocus-based method for more reliable typeability. While whole genome sequencing would provide the most discriminatory approach, it is time consuming and too expensive to be implemented in clinical laboratories and for inter-laboratory surveillance. Furthermore, validated pipelines are lacking. Genetic loci containing a variable number of tandem repeats (VNTRs), when used in multilocus variable number tandem repeat analysis (MLVA), can enable rapid characterisation of outbreak isolates and infer epidemiologic linkage. However, there is currently no standardised genotyping scheme for *Cryptosporidium*. Thus, in the present study which extends the work presented previously, to establish a multi-locus genotyping fragment-sizing scheme we validated a panel of seven VNTR markers for typeability potential and their efficiency to discriminate between isolates that are epidemiologically linked and those that are not. We amplified fragments of the corresponding seven markers through two multiplex PCRs using combinations of four (four-plex) and three markers (three-plex). Using labelled-forward primers, the four-plex PCR was set to amplify markers cgd1_470_1429, cgd4_2350_796, cgd8_4440_NC_506, cgd8_4840_6355 and the three-plex PCR, markers cgd5_10_310, cgd5_4490_2941, cgd6_4290_9811. Markers were validated systematically for repeatability and reproducibility, typeability, discriminatory power and epidemiological concordance. A panel of 262 samples were investigated, including human, animal, spatio-temporal variation, sporadic cases and five different outbreaks. Our study showed that this MLFT method improved typeability and discriminatory capabilities over current gp60 sequence analysis by producing further discrimination of samples from outbreak investigations in a cost- and time-efficient manner.

Tu-O16-11h58/12h10

Global population structure and genetic diversity of *Cryptosporidium hominis*

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Cryptosporidium is the second leading cause of death due to diarrhoeal disease worldwide, particularly among infants, young children, and the immunocompromised. Despite its global importance, its prevalence and transmission remain high due to a lack of treatment options and limited tools to accurately track transmission and identify infection sources in a local context.
Human cryptosporidiosis is caused by two major species, C. *hominis* and C. *parvum*. Current studies of the molecular epidemiology, species sub-structuring and transmission dynamics of these species are largely limited to single locus genotyping or use of a small number of marker genes/loci. There is no standardized approach for the application of these markers for population genetic studies of *Cryptosporidium*. Further, they have not been shown to accurately resolving population structure or reflect the underlying genetic relationships within and among populations.
The draft C. *parvum* and C. *hominis* genome were published in 2004. However, resequencing of non-reference field isolates has only recently been undertaken. We have compiled and undertaken comprehensive genomic variant and population structure analysis of all currently available (n ~ 81) and 28 newly sequenced C. *hominis* isolates (from infected individuals in Ghana, Madagascar, Gabon and Tanzania). We assess global variation in the genome, population structure and 'hotspots'. Finally, we examined overall population structuring within and among these isolates using whole genome data and compared this to existing 'population' marker loci for the species.
Our analysis indicates there is substantial population structuring within C. *hominis* at continental and possibly sub-continental or national scales. This includes regional genetic clusters in southern Europe, South Asia, and eastern versus western Africa, as well as evidence of nation-scale subclades within each of these regions. We show that this complex structure is not reflected by major, currently used population markers, such as the 60 kilodalton glycoprotein gene. We identify highly polymorphic genes across the genome, explore them for evidence of a deviation from neutral selection, and discuss their potential as novel, robust population markers for multi-locus genotyping.

Tu-O17-12120/12h22

Human microsporidiosis in France in 2018 : Data from the French microsporidiosis network

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Introduction. Few data are available about epidemiology and clinical impact of microsporidiosis in humans. Clinical interest for microsporidiosis has emerged with the HIV pandemic and considerably dropped down in the 2000s following the introduction of antiretroviral therapies. New susceptible populations have emerged, especially solid organ transplant (SOT) recipients, hematological malignancies, and other chronic diseases requiring immunosuppressive or immunomodulatory treatments. Then, we have initiated in 2018 the creation of the French microsporidiosis network. Forty-seven French medical laboratories collaborate to this network, including 36 university hospitals, 5 hospitals, 2 medical institutes and 4 private medical laboratories.

Material and Methods. Microsporidiosis cases were reported from January to December 2018 on a website allowing anonymization of patient data. Molecular investigations were performed on microsporidia isolates with species identification, and, for *E. bieneusi,* a genotyping based on Internal Transcribed Spacer (ITS) sequencing and a Multi-Locus Genotyping (MLG) by sequencing of three microsatellite markers.

Results. Over the 12 months period, 67 cases were recorded. All cases were intestinal infections, mostly in immunocompromised patients (n=57, 85%) with a sex-ratio of 1.68 (M/F). Age of patients ranged from less than 4 years old (y/o) to >75 y/o (median 50-54 y/o). Among immunocompromised patients, 59% were SOT (kidney transplants only), 22% HIV, 11% hematological malignancies, 4% cancer, 2% auto-immune diseases and 2% other immunocompromised. Major symptoms were diarrhea (80%) and weight loss (40%). Microsporidiosis in immunocompromised patients required hospitalization in 25% of cases, 60% of cases being already hospitalized. Specific anti-microsporidia treatment was initiated in 55% of patients and a decrease in immunosuppressive therapy was performed in 48%.

Enterocytozoon bieneusi was involved in 86.4% of cases, followed by *Encephalitozoon intestinalis* (9.1%) and *E. hellem* (2.3%). Eleven ITS genotypes of *E. bieneusi* were identified, with 3 new genotypes. Genotypes C and Wilboar3 were the most frequent genotypes. MLG analyses revealed a high genetic diversity between French strains belonging to a same ITS genotype.

Discussion. Our results highlight changes in microsporidiosis epidemiology in France, with a shift from HIV patients to SOT recipients. The impact on SOT prognosis has to be investigated as half of patients have undergone a decrease in immunosuppressive therapy. *Enterocytozoon bieneusi* remains the most prevalent species, with a high genetic diversity. More data and isolates are required for accurate analysis of genotypes distribution and understanding of contamination sources. To conclude, the French microsporidiosis network is an original initiative that will provide update data about human microsporidiosis.

Ethical statement: All patients were informed and were free to oppose to the anonymous report of their medical data

Tu-O18-12h22/12h34

Using biofilms to monitor *Cryptosporidium* contamination in surface water

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Current sample methodology for *Cryptosporidium* in water (EPA Method 1623) relies on filtering a 10-L sample. A sample deemed negative by Method 1623 does not mean that oocysts are not (or have not recently been) present in the water supply, only that no oocysts were captured in that 10-L sample.

We have shown that biofilm sampling provides comparable oocyst detection to filtration-based methods. Waterborne oocysts attach to aquatic biofilms, and biofilm sampling provides an integrated look at oocyst contamination over the time that the biofilm has been growing in the water. We deployed glass slides (the substrate for biofilm growth) at an urban water treatment plant (WTP) intake and a sewage-contaminated creek for 2-week intervals over one year; we also filtered water at these locations every two weeks. Biofilms were scraped from slides and filters were eluted; oocysts were detected from the scraped biomaterial and the eluted filter material by immunomagnetic separation (IMS) and an immunofluorescent assay (IFA). At the WTP, oocysts were detected in 36% of slides and 32% of filters; at the creek, oocysts were detected in 56% of slides and 59% of filters. While detection by biofilms and filtration did not necessarily agree on a given sample day, similar detection frequencies and seasonal trends were observed from both sampling methods. These data suggest that analyzing environmental biofilms in lieu of filters may provide a comparable assessment of oocyst contamination in a water supply. Furthermore, biofilm collection is cheaper than filtration ($3/set of glass slides compared to $110/filter) and could thus be performed more frequently and at more locations to monitor oocyst contamination.

Benthic rock biofilm sampling was also tested. Rock scrapings collected upstream and downstream of a sewage outfall (single sample date) were processed by IMS and IFA: 5 oocysts were counted upstream compared to 19 downstream. At a second outfall, oocysts were detected in 16% and 50% of upstream and downstream rock scrapings, respectively.

Although biofilm composition and development varies across seasons and geographic locations, biofilm sampling at strategic locations throughout a watershed could be used to identify point sources and hot spots of oocyst contamination that warrant additional monitoring with the more expensive EPA filtration method. Biofilm sampling could also supplement filtration-based *Cryptosporidium* monitoring to permit oocyst monitoring at more locations than are economically feasible with the current EPA method.

Tu-O19-12h34/12h46

Evolution of anthroponosis in *Cryptosporidium*

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Human cryptosporidiosis is the leading protozoan cause of diarrhoeal mortality worldwide, and a preponderance of infections is caused by *Cryptosporidium hominis* and C. *parvum*. Both species consist of several subtypes with distinct geographic distributions and host preferences (i.e. generalist zoonotic and specialist anthroponotic subtypes). The evolutionary processes driving the adaptation to human host, and the population structure remain unknown. In this study, we analyse 21 whole genome sequences to elucidate the evolution of anthroponosis. We show that C. *parvum* splits into two subclades, and that the specialist anthroponotic subtype IIc-a shares a subset of loci with C. *hominis* that are undergoing rapid convergent evolution driven by positive selection. Subtype IIc-a also has an elevated level of insertion-deletion (indel) mutations in the peri-telomeric genes, which is characteristic also for other specialist subtypes. Genetic exchange between subtypes plays a prominent role throughout the evolution of *Cryptosporidium*. Interestingly, recombinant regions are enriched for positively selected genes and potential virulence factors, which indicates adaptive introgression. Analysis of 467 gp60 sequences collected across the world shows that the population genetic structure differs markedly between the main zoonotic subtype (isolation-by-distance) and the anthroponotic subtype (admixed population structure). Finally, we show that introgression between the four anthroponotic *Cryptosporidium* subtypes and species included in this study has occurred recently, probably within the past millennium.

Tu-O20-14h00/14h20

The detection and persistence of *Cyclospora cayetanensis*

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Cyclospora cayetanensis oocysts have been associated with foodborne outbreaks since the early 1990s, mostly attributed to the consumption of contaminated berries. Since 2015, imported salad greens and cilantro in the US were implicated in several Cyclospora foodborne outbreaks. In 2018, two important outbreaks affecting more than 760 individuals in the US were described implicating different food items and sources of these products. Detection of oocysts in food commodities during outbreak investigations have been very infrequent. These limitations can be attributed to the lack of an enrichment step for the detection of oocysts in fresh produce and environmental samples. Detection of oocysts can vary because of the food commodity type and denaturalization of oocysts under various environmental conditions.

To address these food types and environmental challenges, the use of hollow fiber filters have resulted in better recovery of oocysts and higher water filtration capacity. The use of various molecular methods targeting the 18S, ITS, and mitochondrial DNA in clinical and environmental samples have been evaluated. These detection methodologies were used in two endemic locations. Monthly samples were collected for one year. The prevalence of Cyclospora in the population with its marked seasonality observed in humans is similar to the ones noted in environmental samples. These results provide a better understanding of the prevalence of Cyclospora in endemic locations and potential control points to prevent transmission.

Tu-O21-14h20/14h32

Opportunistic intestinal protozoan and coccidian infection among various groups of immunocompromised patients in Nepal

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Introduction: Infection of intestinal parasites predominantly coccidian and protozoa are responsible for severe recurrent and persistent diarrhea among immunocompromised patents. Such infections of gastrointestinal tract are major cause of mortality and morbidity in developing countries. The present study aim was to find out the burden of different intestinal opportunistic parasites in various groups immunocompromised patient.
Methods: The study was conducted between April 2016 to September 2017 in tertiary hospital of Kathmandu in various groups of immunocompromised patients, who are HIV- seropositive, malignancy with or without immunosuppressive drugs, or with chronic renal failure, diabetes mellitus. After obtaining informed consent, a total of 678 soft, loose or watery samples were collected and examined microscopically for ova, cyst and oocysts using wet mount preparations and stained with modified Kinyoun acid-fast stain.

Results: A total of 678 stool samples examined, 126 (18.6%) samples were detected intestinal opportunistic parasites in which males were found higher 76 (60.3%) than females 50 (39.7%). The parasites identified were *Giardia* lamblia (43), *Cryptosporidium parvum* (36), Cyclospora cayentenensis (18), Entamoeba histolytica (12), Isospora belli (6), Trichuris trichura (5), Hymenolepis nana (4) and Strongyloides stercoralis (2). The highest group of infected with parasites was patients with HIV-seropositive 54(42.8%), followed by malignancy 28 (22.2%), Chronic renal failure 20 (15.8%), diabetic patients 13 (10.3) and the least 11 (8.7%) was found among post transplant with regular immunosuppressive drug. The infection rate of opportunistic parasitic infection was significantly higher in watery stool than in the formed stool samples (P< 0.0001).
Conclusion: Opportunistic parasitic infection was frequently associated with HIV-seropostive patients with diarrhea in Nepal. Stools of all HIV positive patients along with malignancy with or without immunosuppressive drugs, chronic renal failure and diabetic patients must thoroughly be investigated to identify causative agent for proper treatment and management.

Tu-O22-14H32/14h44

Profiling the diversity of *Cryptosporidium* species and genotypes in wastewater treatment plants in Australia using next generation sequencing

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Wastewater recycling is an increasingly popular option worldwide to reduce pressure on water supplies due to population growth and climate change. *Cryptosporidium* spp. are among the most common parasites found in wastewater and understanding the prevalence of human-infectious species is essential for accurate quantitative microbial risk assessment (QMRA) and cost-effective management of wastewater. The present study conducted next generation sequencing (NGS) to determine the prevalence and diversity of *Cryptosporidium* species in 730 raw influent samples from 25 Australian wastewater treatment plants (WWTPs) across three states: New South Wales (NSW), Queensland (QLD) and Western Australia (WA), between 2014 to 2015. All samples were initially screened for the presence of *Cryptosporidium* at the 18S rRNA (18S) locus using quantitative PCR (qPCR). Oocyst numbers were determined directly from the qPCR data using DNA standards calibrated by droplet digital PCR. Positives were characterized using NGS of 18S amplicons and were also screened using C. *parvum* and C. *hominis* specific qPCRs. The overall *Cryptosporidium* prevalence was 11.4% (83/730): 14.3% (3/21) in NSW; 10.8% (51/470) in QLD; and 12.1% (29/239) in WA. A total of 17 *Cryptosporidium* species and six genotypes were detected by NGS. In NSW, C. *hominis* and *Cryptosporidium* rat genotype III were the most prevalent species/genotype (9.5% each). In QLD, C. galli, C. muris and C. *parvum* were the three most prevalent species (7.7%, 5.7%, and 4.5%, respectively), while in WA, C. meleagridis was the most prevalent species (6.3%). The oocyst load/litre ranged from 70 to 18,055 oocysts/L (overall mean of 3,426 oocysts/L: 4,746 oocysts/L in NSW; 3,578 oocysts/L in QLD; and 3,292 oocysts/L in WA). NGS-based profiling demonstrated that *Cryptosporidium* is prevalent in the raw influent across Australia and revealed a large diversity of *Cryptosporidium* species and genotypes, which indicates the potential contribution of livestock, wildlife and birds to wastewater contamination.

Tu-O23-14h44/14h56

Development and Application of Bioinformatics Tools: Automation of Species/Genotype Identification and Genetic Subtyping of *Cryptosporidium*

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To date, 39 species and over 60 genotypes of *Cryptosporidium* have been described. Additionally, numerous subtypes of C. *parvum* and C. *hominis*, the most common species infectious to humans, have been characterized based on the polymorphic, glycoprotein 60 (gp60) gene. This molecular data plays a crucial role in classifying *Cryptosporidium* for understanding potential sources of infection and routes of transmission, whether anthroponotic or zoonotic. Both species identification and subtyping are primarily based on PCR amplification and traditional Sanger sequencing. However, the analysis of *Cryptosporidium* 18S and gp60 Sanger sequences is time consuming, reader biased, and frequently plagued with mixed sequences in the sample, resulting in undecipherable sequence chromatograms. To aid in resolving these issues, we developed two tools that can analyze Sanger sequence chromatograms for both 18S and gp60 gene targets. These Python-based programs are simple to use as they only require the user to upload an ABI sequence file. Once uploaded, the programs determine either the species/genotype name for the 18S reader or the family/subtype for the gp60 reader and outputs the results in FASTA format to enable BLAST database searches. The 18S gene analysis tool was designed to accurately analyze two difficult sequence analysis scenarios that result in overlapping peaks. These include polymerase slippages due to homopolymer regions found in C. *hominis* and the presence of mixed sequences that are frequently encountered in animal and water samples. The program is able to decipher these mixed chromatograms by calculating the log ratio of each overlapping peak and then using sequence comparisons with *Cryptosporidium* reference sequences to classify the sequence of each species in the sample. Use of the 18S tool increased the number of species/genotypes identified in samples in our analysis of *Cryptosporidium* in raw drinking water sites in Quebec, including 18S mixed sequences that could not be processed manually. We also identified the species in samples containing mixed *Cryptosporidium* in positive stool samples from humans in the UK and Ontario, Canada, and in adult dairy cattle samples from across Canada. In summary, we have developed two bioinformatics tools that provide reproducible sequence classifications and capabilities of identifying species/genotypes and gp60 subtypes, all of which amounts to large time saving benefits. These programs will be made publically available both via easy installable Conda packages and Galaxy servers.

Tu-O24-14h56/15h08

A New Protocol for Molecular Detection of *Cyclospora cayetanensis* as contaminants of Berry Fruits

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Cyclospora cayetanensis is a coccidian parasite that has been associated with foodborne outbreaks of gastrointestinal illnesses. Epidemiological investigations indicated that some of the outbreaks were linked to prepackaged vegetable trays (containing broccoli, cauliflower and carrots) sold at a convenience store chain and salads (containing carrots, romaine lettuce, spinach, kale, and varietal red-leaf lettuce) sold at a fast-food chain. Given that the sensitivity of molecular techniques, such as polymerase chain reaction (PCR), is known to be considerably better than that of microscopy techniques, there have been considerable efforts directed towards development and validation of new protocols for detection of C. cayetanensis. Molecular detection methods often target conserved regions of the genome, e.g., 18s rRNA, that are found in multiple copies. Though the multi-copy feature is beneficial for assay's sensitivity, it is also highly subject to cross-reactivity of primers and or probe with related species due to its conserved nature. In our lab, we have noted this to be a problem (cross-reactivity with Toxoplasma gondii) with the primers and probe set in the method currently used by FDA for detection of Cyclospora contaminating fresh produce. Therefore, the present study was aimed at developing a new protocol for detection of C. cayetanensis, with emphasis on specificity, targeting the ITS-1 region of the genome, between the 18S and 5.8S rRNA genes. This target was chosen due to the high variation, even in closely related species, due to its non-coding nature. The new assay was evaluated for various performance characteristics, including specificity, efficiency, linearity, repeatability, and robustness. It showed good efficiency (102 %), linearity (r2 = 0.999) and repeatability (standard deviation of Cq 0.2 (95 % CI: 0.2, 0.3). The robustness test identified factors that impacted the assay's performance significantly. There was no cross-reactivity with Toxoplasma gondii, *Cryptosporidium parvum*, Eimeria mitis, and Cystoisospora canis when tested both in silico and in vitro. The method was also improved by incorporating an internal control as a duplex to monitor inhibition and the efficiency of DNA extraction. The duplex assay also showed a good performance (efficiency of 100% and r2 of 0.99). The findings of the present study, including suitability of the ITS-1 as a PCR target and potential for standardizing the new assay for use in the food testing laboratories will be discussed.

Tu-O25-15h28/15h40

Prevalence of *Giardia* and *Cryptosporidium* in the Viennese urban water bodies: a first overview

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In Austria, giardiasis is not a reportable disease therefore, data on the epidemiology of the disease is scarce. However, in the past years, there has been a rise of chronic cases and cases refractory to treatment, the sources of infection remaining unknown. In urban settings, waterborne pathogens can be transmitted via wastewater, sewer overflows as well as via cross-contamination from animals to humans through direct or indirect transmission routes. The objective of this work was to study possible sources and pathways of fecally-transmitted pathogens in urban surface water bodies used for recreation and for the production of drinking water.
In a monitoring campaign over one year, the microbiological water quality of wastewater and urban rivers and surface waters of the city of Vienna was investigated at multiple levels including *Cryptosporidium* spp., *Giardia* and the prevalence of its different genotypes, as well as bacterial standard fecal indicators (SFI). Results obtained showed that the concentrations of SFI, including E. coli, Enterococci and C. perfringens spores were between 2-3 logs higher in Influent wastewater samples in comparison to Effluent wastewater samples and approximately 5 logs higher than in surface water samples. *Giardia* was more prevalent in Influent wastewater samples than *Cryptosporidium*, however the latter one was more prevalent in Effluent and surface water samples. The concentration of each parasite varied according to the water matrix analysed varying from 25-1846 (oo)cysts/100mL in Influent water samples, from 0-10 (oo)cysts/100mL in Effluent water samples and from 0-2 (oo)cysts/100mL in surface water samples. *Giardia* isolates detected belong mainly to the assemblage AI and AII, however we also detected the assemblages BIII,C and a new assemblage, yet to be described, in some of the water samples analyzed.
Studies like the present one, might help to elucidate the source of *Giardia* spp. and *Cryptosporidium* spp. as well as other fecally-transmitted pathogens within the urban environment. Moreover, it will help to implement protection measures of the Viennese water resources for a safer use and a better and more sustainable management of them.

This study was supported by the Vienna Science and Technology Fund (WWTF), Project [ESR17-070].

Tu-O26-15h40/15h52

The impact on water quality from improved land management decisions for *Cryptosporidium* control in a catchment with a history of public water supply contamination

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Cryptosporidium, in particular C. *parvum*, is an important zoonotic parasite which represents a threat to animal health, water quality and public health. The main reservoirs of C. *parvum* are known to be farm livestock, cattle in particular, but the contribution from wildlife in water catchments is unclear. The aim of this study was to establish *Cryptosporidium* prevalence, species and genotypes present in livestock, deer and water samples from a water catchment in the north of Scotland with a history of *Cryptosporidium* contamination in the public water supply. Study results were used to identify and implement improved land management strategies and record the impact of these on water quality over the two years following the catchment improvements, using data from Scottish Water from routine testing for *Cryptosporidium*. Faecal samples were collected from livestock from four farms located throughout the catchment, as well as from wild red deer herds and roe deer free-ranging across all four farms in 2014. Water samples were collected at three points within the catchment area in the same time period. Nested species specific multiplex PCR, targeting the 18S rRNA gene was used to detect and speciate *Cryptosporidium*, following which a multilocus fragment typing (MLFT) tool and GP60 sequencing was used to genotype C. *parvum* positive samples. Results indicated a very high prevalence of *Cryptosporidium* with a predominance of C. *parvum* in livestock, deer and water samples. Four GP60 subtypes were detected with the most prevalent subtype (IIaA15G2R1) being found in all host species and on all farms. Each animal species had a different predominant GP60 genotype and as each of these genotypes was isolated from water samples, it was established that all of these species were contributing to water contamination. MLFT further differentiated these GP60 genotypes into 6 highly related multilocus genotypes. The predominance of C. *parvum* in livestock and deer suggested that they represented a significant risk to water quality and public health, with genotyping results suggesting that all animal species had a role to play in contamination of the water sources. Discussions between land managers, water industry catchment officers and scientists, resulted in improved land management strategies in the catchment area. These improvements have impacted positively on the quality of the water supply with respect to *Cryptosporidium* contamination. Historical and current data will be presented to illustrate the high impact of the research and subsequent outputs to water quality and public health in this catchment.

Tu-O27-15h52/16h04

Cryptosporidium and *Giardia* in Dairy Calves--30 years of observation

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Sources of *Cryptosporidium* and *Giardia* are of continuing interest and importance to public health protection. The literature amply describes the distribution of these organisms among the humans and animals worldwide and provides details on many important characteristics of their presence, propagation inter and intra species, and of their distribution into the environment. Beginning in the mid 1980's with colleagues and students, initially seeking a source of the organism for use in laboratory projects, we began to accumulate data on *Cryptosporidium* and *Giardia* in dairy cattle, focussing calves based on early reports that shedding was most prominent in neonatal animals. As local dairies and their calves were screened to identify a convenient and productive source, data on characteristics of cryptosporidiosis in these animals began to accumulate. Continued research pertaining to development and refinement of monitoring procedure and on the characteristics of these organisms in water treatment processes, in light of the inability to cultivate *Cryptosporidium* in a practical laboratory system led to the regular collection of calf faecal samples for screening and isolation of oocysts. Continued demand for laboratory oocyst populations resulted in refinement of procedure and regular collection from a single local source...partly for convenience but with the initial idea that the resulting organisms would have reasonably consistent characteristics.

The object of this paper is to summarize data now accumulated from literally hundreds of roughly monthly collections from predominantly two dairies, one near Seattle Washington USA, the other near Sydney NSW Australia. Also, to present more detailed data from two projects: one in Washington that sampled from 10 consecutively born (female only) Holstein Friesian calves, following oocyst and coproatigen shedding and serum antibody levels daily for 30 days, then less frequently through 90 days; the other in NSW examining the genetic characteristics among *Cryptosporidium* from a large (2500 milking Holstein Friesians) having three geographically separate locations.

Major features of the accumulated data include: 1) substantial consistency with data on dairy cattle published elsewhere; 2) consistent evidence of *Cryptosporidium* infection in virtually every calf within the first 30 days post partum, peaking in the 7-15 day period; 3) consistent evidence of *Giardia* infection in the same populations, most evident in the 15-30 day period; 4) clear evidence of infection in each of 10 consecutively-born calves as early as 3 days post partum; 5) evidence of *Cryptosporidium* shedding in parent adults, but requiring more careful screening due to low shedding levels and large faecal volumes; 6) substantial genetic stability of *Cryptosporidium* among representatives of a single dairy herd (calves) across a 1-5 year sampling period, and across three geographically spaced locations.

Tu-O28-16h04/16h16

First detection of *Giardia lamblia* and *Cryptosporidium* spp. in surface waters in Serbia

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Giardia lamblia and *Cryptosporidium* spp. cysts/oocysts can stay viable for several weeks or months in the environment. Water is a major source of infection for humans and animals. In Serbia, sources of drinking water as well as surface waters are routinely surveyed for the presence of enteric bacteria, viruses and some chemical pollutants, but detection of protozoa is not routinely performed because it is not required by current legislation, but also because of lack of the necessary methodology and expertise. We have thus initiated a research study to assess the presence of and level of contamination with *Giardia* lamblia and *Cryptosporidium* spp. in surface waters, and to gain insight into the population genetics of these pathogenic protozoan species in Serbia. The locations for sample collection were selected along the rivers flowing from the major river catchments of the central, eastern and western parts of the country. These waters are used for recreation, irrigation and as drinking water sources. Water samples were processed according to the EPA1623 protocol while species determination was performed by PCR-RFLP. The study is still underway but the results obtained thus far showed a significant presence (40%) of both protozoan species. Most locations with a positive finding were in close proximity to urban centers. The *Giardia* lamblia isolates were identified as belonging to Assemblage A. As these are in fact associated with most human infections, this finding is worrisome. *Cryptosporidium* spp. was detected in the vicinity of a large dairy farm, suggesting the possibility of inadequate containment of effluent from the farm. The presented results indicate inadequate wastewater and effluent treatment, which is not surprising as only 10% of the country's wastewater is being processed. Biosafety measures need to be introduced to prevent further contamination of surface waters. Our research is the first step towards the prevention of waterborne giardiasis and cryptosporidiosis outbreaks in Serbia. This work was supported by a grant from the Ministry of Education, Science and Technological Development of the Republic of Serbia (no. III 41019), and by the COST Action FA1408 – European Network for Foodborne Parasites in Europe (EURO-FBP).

Tu-O29-16h16/16h28

Cryptosporidium in water: what makes a good method for genotyping?

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Genotyping *Cryptosporidium* oocysts detected in water is an important tool in incident and catchment management, providing information about the identity and often the likely sources of the parasite, and the potential risk to public health. However, material available for genotyping is often limited to that available on single microscope slides; a balance between the competing needs of sensitivity, specificity, cost effectiveness and speed must be struck in developing a good genotyping method. There is an increasing trend for water testing laboratories in England and Wales to send *Cryptosporidium*-positive microscope slides for genotyping, regardless of numbers of oocysts seen. A recent audit of 346 slides received between 2015 and 2018 showed a positive relationship between typability and oocyst numbers, with just 19/127 (15%) of those with one oocyst typable, 36/73 (49%) of slides with 2-4 oocysts typable, and 112/146 (77%) that had 5 or more oocysts typable. In 2018 more than half of slides submitted for genotyping had only one oocyst seen, and providing useful genotyping data from this material remains a challenge. In recognition of the declining frequency of detection in drinking water and numbers of oocysts seen, which is in itself a public health success, and the continuing importance of genotyping these small numbers, we have undertaken to review current methods and aim to develop a more effective protocol for slide genotyping. As part of the Aquavalens project (www.aquavalens.org) we began to address this challenge by developing a sensitive, species-specific real-time PCR for C. *hominis* and C. *parvum*, but developing a real-time PCR-based pan-genus assay detecting all species and genotypes was challenged by lack of specificity. Investigating the multi-copy SSU rRNA (18s) gene provides robust species identification, therefore in the current project we have revisited this, aiming for >50% typability of slides with <5 oocysts seen. Sequence analysis of PCR positives will be used to identify species and genotypes but the assay must have an acceptable level of specificity to avoid unnecessary sequencing therefore a probe-based real-time PCR assay will be explored. The molecular detection and differentiation of low copy numbers of a gene with conserved and polymorphic regions, which shares sequence similarities between unrelated organisms, from a biomic environmental sample containing unknown inhibitors is a mighty challenge that we will share in our presentation.

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POSTER SESSION

Amphi 100A

TuA-P1-16h30/16h35

Prevalence of *Cryptosporidium* spp, *Giardia duodenalis* and *Toxoplasma gondii* in three leafy green vegetables usually consumed in Marrakech region, Morocco.

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Introduction: The presence of protozoan parasites in fresh vegetables can be a source of human contamination. They are responsible for human diseases that represent an important public health problem in several countries, including Morocco. The presence of these parasites is mainly due to insufficient treatment of irrigation water, lack or non-application of hygiene practices and environmental pollution.

The aim this work is to determine the prevalence of *Cryptosporidium spp.*, *Giardia duodenalis* and *Toxoplasma gondii* in fresh leafy green vegetables that are usually consumed raw or slightly cooked in the region of Marrakech, Morocco.

Material and Methods: A total of 108 vegetable samples, including lettuce (36), parsley (36) and coriander(36), were obtained from three different markets in Marrakech (the wholesale vegetable market, a supermarket and rural retailers of Ghmate village(30 Km from Marrakech). Vegetable samples were randomly collected per month from each market, from April 2018 to April 2019. Samples were washed in stomacher bags using an elution buffer (PBS-Tween 80), with vigorous shaking. The eluates were centrifuged, and the supernatants discarded. The resulting pellets were examined for parasites, using modified Ziehl-Neelsen staining and Immunochromatography technique. As they were also subjected to DNA extraction using FastDNA™ Spin Kit For Soil, then analyzed by qPCR after a step of development for the detection of these 3 protozoan in the studied matrices.

Results: The investigation of *Cryptosporidium spp, Giardia duodenalis* and *Toxoplasma gondii*, in the different pellets obtained after samples pretreatment is currently in progress. The available results using Ziehl-Neelsen staining and immunochromatography test showed the absence of *Cryptosporidium spp* and *G. duodenalis*, whereas qPCR analysis revealed the presence of *T. gondii* oocysts and *G. duodenalis* cysts in fresh leafy green vegetables sold in Marrakech. The results will be presented at the conference.

Conclusion: This work allowed the detection of the targeted parasites, in addition to other parasitic forms such as *Cyclospora* that could represent a risk for human health. The results encourage us to continue the investigation of the three parasites in vegetables, and to expand the study area in order to identify supply sources that could represent a risk for consumers and so to provide local authorities and health services with a mapping of areas to be monitored.

TuA-P2-16h40/16h45

Monitoring of *Cryptosporidium parvum* in fruits, vegetables, herbs and sprout seeds

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Fresh vegetables, fruits, herbs and sprout seeds are usually prepared and consumed raw, without cooking or heating that leads to a risk of foodborne infection. Besides bacterial and viral agents these infections may also be caused by parasitic agents. These parasitic infections are often referred as neglected, although such infections may have had serious consequences for human health. Our goal was to assess the parasitic contamination of vegetables, fruits, herbs and sprout seeds, either raw or frozen. Specifically, the work focused on the detection of *Cryptosporidium parvum* (CP) in samples that come from the markets and farms predominantly from Europe. During five-year monitoring, we tested almost 1100 samples using qPCR assay targeting hsp70. Our results revealed that there were 2.3% positive samples for CP. Fresh vegetables (lettuce, cucumber, carrot) and fresh strawberries were the most contaminated samples with this agent. Even the level of positivity was low this contamination may pose risk of alimentary infection for human health especially for immunocompromised individuals.

This work was supported by the Ministry of Agriculture, Czech Republic RO0518.

TuA-P3-16h45/16h50

Presence of *Giardia intestinalis* in ready to eat foods of plant origin

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The protozoan parasites such as *Giardia intestinalis*, *Cryptosporidium parvum*, *Toxoplasma gondii* are pathogens that pose significant risks to public health worldwide. Water for drinking, washing or irrigation is one of the sources of parasites (or their life stages) for foodstuff, generally. Especially in ready to eat foods (RTE) which are not thermally treated, the presence of low level of parasites/life stages may pose risk of foodborne infection. The aim of the study was to monitor of RTE foods (herbs, friuts and vegetables) for the presence of *Giardia* intestinalis (GI). During five-year monitoring, 1088 samples of fresh or frozen fruits and vegetables, herbs and sprouts were collected and analyzed. Samples originated from fields, fruit farms or markets. Country of origin of samples was not only the Czech Republic but also other EU countries. By a newly developed DNA isolation and GI-specific real time PCR (targeting β – giardin gene) was processed one hundred grams per sample. None sample of sprouts (31 tested samples) was found to be positive for GI DNA presence. Presence of GI DNA in amounts 1-10 organism/gram was found in one sample of herbs (227 tested samples), in 15 samples of vegetable (574 tested samples) and in two samples of fruits (249 tested samples).

This work was supported by the Ministry of Agriculture, Czech Republic RO0518.

TuA-P4-16h50/16h55

Investigation On *Cyclospora Cayetanensis* In Fresh Produce In Italy

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In the USA, large outbreaks of cyclosporiasis have increasingly been linked to various types of fresh produce imported from South-American countries. Despite some outbreaks of cyclosporiasis have been recorded in Germany and Sweden, C. cayetanensis has received much less attention as a foodborne pathogen in Europe compared to the U.S. In Italy, the high prevalence of C. cayetanensis DNA detected in water, soil, vegetables, and in humans indicate the need to investigate more in depth the public health significance of Cyclospora in this country.
The aim of this study is to investigate the prevalence of C. cayetanensis as a contaminant of fresh produce -ready to eat (RTE) mixed salads and berries- sold on the Italian market, using validated methodologies.

The sampling regime has been based on testing samples in pools with an expected prevalence of 0.6%, 95 % confidence and 1.15 % of precision. To estimate the prevalence, we chose a pool size of 9 packages of fresh produce each month, for a total of 54 samples per month and 72 pools per one year. RTE mixed salads and berries packages were bought from Italian food stores. After collection, they were transferred to the laboratory and then washed before their expiry date using the FDA washing procedure (BAM 19b). After concentration, the pellets were subjected to molecular analyses for the detection of C. cayetanensis whereas an aliquot of each pool was examined by microscopy. Two real-time qPCR assays, one based on the 18S ribosomal RNA gene, according to the BAM 19b (Assay 1) and, a new, specific qPCR assay, targeting the ITS1 region (Assay 2), were set up. Both these methods showed correct amplification from the internal amplification control as well as the positive control, with consistent Ct values across the replicates. So far, a total of 27 RTE mixed salad packages, belonging to three industrial brands, and 27 berries packages (blueberries and blackberries imported from Perù and Mexico, respectively, and raspberries grown in Italy) have been collected and washed. Six pools and a total of 54 aliquots (9 per pool) were obtained. Four samples (two from RTE mixed salads belonging to two different brands and two from raspberries and blueberries) were tested in duplicate in qPCR using Assay 2 molecular analysis.

Preliminary microscopy and molecular results will be presented. Once the study is completed, we anticipate that the molecular tools that have been developed cooperatively in different partner labs will provide not only data on the prevalence of Cyclospora in fresh produce in Italy but also a shared methodological direction for wider monitoring of fresh produce at European level.

TuA-P5-16h55/17h00

Presence of *Giardia intestinalis* in ready to eat foodstuff

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The protozoan parasites such as *Giardia intestinalis*, *Cryptosporidium parvum*, *Toxoplasma gondii* are pathogens that pose significant risk to public health worldwide. Water for drinking, washing or irrigation is one of the sources of parasites (or their life stages) for foodstuff, generally. Especially in ready to eat foodstuff (RTE) which are not thermally treated, the presence of low level of parasitic life stages may pose risk of foodborne infection. The aim of the study was to monitor of RTE foods (herbs, fruits and vegetables) for the presence of *Giardia* intestinalis (GI). During five-year monitoring, 1081 samples of fresh or frozen fruits and vegetables, herbs and sprout seeds were collected and analyzed. Samples originated from fields, fruit farms and markets. The origin of samples was not only the Czech Republic but also other EU countries. For the detection of GI molecular analyses based on real time PCR targeting β – giardin gene applied. None sample of sprout seeds (31 tested samples) was found to be positive for GI DNA presence. Presence of GI DNA in amounts 1-10 organism/gram was found in one sample of herbs (227 tested samples), in 15 samples of vegetable (574 tested samples) and in two samples of fruits (249 tested samples). Altough the total positivity of the samples was only 1.66% the occurrence of the parasitic stages is still significant for human health.

This work was supported by the Ministry of Agriculture, Czech Republic RO0518.

TuA-P6-17h00/17h05

Cyclospora cayetanensis infections in Sweden – underdiagnosed or uncommon?

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In recent years an increase of infection with Cyclospora cayetanensis have been reported from several countries as well as outbreaks, often linked to imported fresh produce or traveling. Infection with Cyclospora is not notifiable in Sweden and thus there are no records of the number infected annually. One food-borne outbreak linked to imported sugar snap peas occurred in Sweden 2009, but since then there has been few reports of findings.

The aim of this study was to investigate the number of Cyclospora infections diagnosed in Sweden in 2016 and 2017 as well as to get an overview of diagnostic methods used.

In January 2018, all clinical parasitological laboratories in Sweden were invited to participate. Twenty laboratories responded and answered a questionnaire. Seventeen laboratories perform diagnostics for Cyclospora and the remaining three forward such samples to others.

All 17 laboratories perform microscopy after concentration and modifed Ziehl-Neelsen (mZN) staining of samples that has a specific request for Cyclospora diagnostics. One laboratory also has a multiplexed PCR where the parasite is included. Ten laboratories state they screen for Cyclospora in all samples with a general request for microscopy of parasites in wet smear. Six laboratories have different criteria for when they stain with mZN even without a specific request.

Several laboratories bring up the problem of this infection being unusual and the difficulties to maintain competence when positive samples are rare even though all laboratories participate in external quality assessment programs. Rarely samples have a specific Cyclospora request and it is possible that positive samples are being missed when not performing mZN microscopy. Another issue of concern is the implementation of multiplexed PCR systems for parasites where Cyclospora is seldom included. PCR analyses also results in fewer samples being analyzed by microscopy.

In 2017 seven cases of Cyclospora infection were identified by three laboratories and in 2016 ten cases were found by five laboratories. During the last ten years seven laboratories reported findings, approximately 40 to 75 cases in total. This imply that ten of the laboratories have not had a positive finding of the parasite in the last ten years.

It is difficult to conclude if the parasite is uncommon or underdiagnosed in Sweden. To ensure the capability for detection and good patient diagnostics it is important to maintain or improve the competence at the laboratories by for example participating in control programs and be aware of the difficulties.

TuA-P7-17h05/17h10

Multi-locus genotyping provides epidemiological insights about a potential common source of infection with *Enterocytozoon bieneusi* microsporidia in a hematological unit.

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Microsporidiosis is a rare water- or foodborne infection that mostly causes digestive disorders in immunocompromised hosts. Over a four day-period only, three patients with hematological malignancies housed in Hematology unit at a single French hospital were diagnosed with Enterocytozoon bieneusi microsporidiosis. This unusually high incidence was investigated using a molecular genotyping approach: stools obtained from the three patients with microsporidiosis were processed for sequence typing (ST) analysis at the internal transcribed spacer (ITS) rDNA locus. Moreover, 3 micro- and 1 minisatellite were sequenced and concatenated for Multi-Locus Genotyping (MLG). Twenty-four other E. bieneusi strains, from epidemiologically-unrelated patients of the same hospital, were studied in parallel using the same concatenating method. Eight isolates from two other centers were also included. Interestingly, Enterocytozoon bieneusi isolates from the three patients with hematological malignancies belonged to a new genotype closely related to genotype C. None of the other strains from the study belonged to that new genotype. MLG brought evidences of high similarity between strains from clustered-cases, while it showed substantial discrepancies to the various strains collected from all the epidemiologically-unrelated individuals. Overall, these data indicated a valuable discriminant power of MLG approach compared to the previously widely-used ITS genotyping. They also confirmed the link between the three patients housed in Hematology department, and suggested a potential common source of contamination, or even cross-transmission. This study also highlighted the importance of prophylactic means in such a population.

TuA-P8-17h10/17h15

Evaluation of a ceramic candle filter to remove *Cryptosporidium* oocysts in drinking water

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Ceramic filters represent a common and effective household water treatment technology, particularly in that regions of developing countries where there is not access to safe drinking water. The filtration process using ceramic filters not only removes the microbial contamination of water, but also reduce significantly the turbidity of water. In addition, some commercial ceramic filters contain silver salts that help to disinfect and prevent the growth of microorganisms, and activated carbon that adsorbs chemical pollutants, particles and microorganisms, improving the capacity of retention of the filter. This study evaluates the effectiveness of a ceramic candle filter to remove *Cryptosporidium* oocysts in drinking water at a household level. According to the guidelines of the World Health Organization, three types of water [well, general test water (GTW) and challenge test water (CTW)] were evaluated. Water volumes of 10 L were spiked with 1×106 oocysts of *Cryptosporidium parvum* per liter and passed through the ceramic filter. Oocyst quantification was performed in the effluents on days 1, 3, 6, 7, 8 and 10 of the assays. *Cryptosporidium* oocysts were detected only on day 8 in well water (10 oocysts/L; 4.9 logarithmic reduction units); on day 7 in GTW (30 oocysts/L; 4.4 logarithmic reduction units); and, on day 3 in CTW (10 oocysts/L; 4.9 logarithmic reduction units). In addition, a statistically significant decrease ($P<0.0001$) in the turbidity of the CTW (37.3±5.1 NTU vs 1.3±1.1 NTU) was observed. However, a statistically significant decrease ($P<0.001$) in the mean filtration flow rate with the increase of the turbidity of the water was also observed [1.6±0.3 L/h; 1.7±0.3 L/h; and, 1.1±0.5 L/h for the well water (≈1.0 NTU); GTW (<1.0 NTU); and, CTW (37.3±5.1 NTU), respectively. In conclusion, this ceramic filter provides a high level of protection against diarrheal diseases caused by waterborne parasitic protozoa. Moreover, it improves the appearance of water and, consequently, favors the acceptance of this technology by the communities to which it is destined.
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TuA-P9-17h15/17h20

Molecular characterization of *Cryptosporidium* in Algerian HIV/AIDS patients

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Background

Cryptosporidium is an important cause for chronic diarrhoea and death in HIV/AIDS patients. Since the lack of effective specific treatment for cryptosporidiosis in immunocompromised patients, it is estimated that diarrhoea occurs in 90% HIV/AIDS patients in developing countries. In industrialized nations, access to highly active antiretroviral therapy (HAART) has significantly reduced the morbidity and mortality by cryptosporidiosis. Standing out as one of the countries in the Middle East and North Africa (MENA) region with the most advanced health responses, Algeria provided HAART free of charge since 1998. Despite the fact that HIV-infected patients under HAART have currently reduced risk of suffering from an opportunistic infection; gastrointestinal opportunistic infection still occur. Currently, there are no data on *Cryptosporidium* species and subtype families in HIV/AIDS patients in Algeria. This study aims at identifying *Cryptosporidium* species and subtype families prevalent in Algeria and contributing to the epidemiology mapping of *Cryptosporidium* in the MENA region.

Materials and Methods

From 2016 to 2018, 350 faecal specimens were obtained from in-patients (hospitalized) and out-patient follow-up with HIV/AIDS positive status associated with or without diarrhoea attending El Hadi Flici Ex El- Kettar hospital, Alger city, Algeria and were screened for the presence of *Cryptosporidium* by using microscopy. Positive samples were submitted to the French National Reference Centre - Cryptosporidiosis for molecular analysis (species/genotype) by DNA sequencing of the 18S rRNA and gp60 gene respectively.

Results

Out of 350 samples, 33 (9.4%) were positive for *Cryptosporidium* by microscopy. 22 isolates were successfully amplified at the 18S rRNA and gp60 locus. Based on sequence analysis: 68,18% were identified as *C. parvum* with family subtypes IIa-7, and IId-8, whilst 5 cases (22,72%) was caused by *C. hominis* (family subtypes Ia-2 and Ib-3) and 9.09% by *C. felis*.

Conclusion

The predominance of the zoonotic subtype families of *C. parvum* IIa and IId in this study suggests that animal-to- human transmission may be a common transmission route of *Cryptosporidium* in HIV/AIDS patients in Algeria. Minimizing contact with animals and maintaining good hygiene practices should be advocated to reduce the transmission.

	Species			
Genotypes	***C. felis***	***C. hominis***	***C. parvum***	**Total**
C. felis	1			1
C. felis	1			1
IaA14		1		1
IaA22R2		1		1
IbA10G2		1		1
IbA13G3		2		2
IIaA14G2R1			1	1
IIaA15G2R1			3	3
IIaA16G2R1			1	1
IIaA20G1R1			1	1
IIaA21G1R1			1	1
IIdA16G1			4	4
IIdA19G1			4	4
Total	**2**	**5**	**15**	**22**

POSTER SESSION

Amphi 100B

TuB-P1-16h30/16h35

Investigations on co-infections with Bovine corona virus and *Cryptosporidium parvum* using *in vitro* methods: experimental setup and preliminary results

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Intestinal infections resulting in diarrhea are a major health problem in calves that may result in reduced weight gain, increased mortality rate and high risk of acquiring disease later in life. According to the Norwegian Dairy Herd Recording System (NDHRS), diarrhea accounts for almost 40% of calf diseases. A study in Norway showed that Rotavirus (RoV) and *Cryptosporidium parvum* were the most common enteric pathogens responsible for diarrhea, and seropositivity for Bovine Coronavirus (BCoV) increased the risk of diarrhea by >72%. Young calves are highly susceptible to acquiring diarrhea during their first month of life. RoV, BCoV, and C. *parvum* are the most significant (75-95%) enteropathogens among neonatal calves worldwide. Several studies have shown that a field study alone is insufficient to understand the severity of such co-infection, and requires an experimental study with large numbers of animals infected with different enteropathogens. Both BCoV and C. *parvum* infect the small intestinal enterocytes, and in vitro cultures in relevant cell lines have been established for both pathogens. Our overall objective is to study the interactions of BCoV and C. *parvum* during the course of co-infections in cell culture models. Established cell lines will be infected withBCoV alone C. *parvum* alone. Both pathogens simultaneously. Both pathogens sequentially (first one and then the other). Measured outcomes will be on the infected cells: (changes in morphology and mRNA expression) and dynamics in the pathogen establishment depending on the presence or otherwise of the other pathogen.

In the present study, HRT-18G cell lines were either be uninfected, infected with BCoV (fecal and respiratory isolates) alone, or infected with C. *parvum*, and expression of eukaryotic mRNA investigated as a reflection of the host cell response. Preliminary results and the experimental protocols will be described in this presentation.

TuB-P2-16h35/16h40

The challenge of inferring host shifting in *Cryptosporidium* parasites with complex life cycles and population strategies

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Parasites sometimes infect new hosts, expanding their host range and causing new disease aetiologies. The selection acting on genes may then change due to host-specific adaptive alterations, particularly when parasites cross between evolutionarily distant hosts. Genetic diversity inferred from sequence data can provide insights into the colonization and evolution of these new infectious diseases in humans and other hosts, but rely heavily on key sets of assumptions. We explore the effects of these standard analyses in a system of protozoan parasites of the genus *Cryptosporidium*, which comprises about 30 species that infect different vertebrate hosts. A few *Cryptosporidium* species predominantly or exclusively infect people. However, species previously restricted to other animals now sometimes infect humans, effectively expanding their ecological niche. Characterizing the genetic diversity of *Cryptosporidium* from humans and the animals we interact with has important implications for transmission, immunogenicity and pathogenesis. Still, few studies attempt to disentangle these factors, perhaps because the parasite has a complex life cycle, with both sexual diploid and asexual haploid life stages, environmentally resistant oocysts, and reports of a spectrum of populations from panmictic to clonal. Here, we analyse sequences for a global sample representing multiple *Cryptosporidium* species reported in humans and other animals. We estimate population genetic diversity parameters and show that, in isolation, these parameter estimates can sometimes lead to misleading conclusions regarding the evolutionary processes acting during the invasion of new hosts by parasites, such as *Cryptosporidium*, that have complex life cycles. However, the framework emphasized here, which combines theoretical and empirical expectations, has the potential to provide powerful new prediction opportunities regarding the emergence of pathogens and host shifting to humans.

TuB-P3-16h40/16h45

Role of Paneth cells during infection of neonatal mice by *Cryptosporidium parvum.*

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Cryptosporidium parvum is a zoonotic apicomplexan parasite responsible for a diarrheal disease named cryptosporidiosis. This protozoan parasite is found worldwide and is transmitted by contaminated water. The immature intestinal immune system in very young animals and children under 5 places them at high risk of developing severe cryptosporidiosis. Paneth cells (PC) are specialized intestinal epithelial cells located at the base of intestinal crypts producing antimicrobial peptides (AMPs) that develop and mature after birth. We and others have already described in vitro that antimicrobial peptides such as CRAMP and CCL20 can alter the viability of sporozoites of C. *parvum* (1). We therefore wondered whether PCs and the AMPs that they produce can participate in the protective innate immune response against the parasite. By using a mouse model of neonatal cryptosporidiosis, we investigated the role of Paneth cells in the innate immune response against C. *parvum*. We first compared the susceptibility to C. *parvum* of mice genetically modified to be depleted of PCs (Sox9flox/flox-vil-Cre mice) and observed an increased level of infection when PCs are absent, associated with a reduced expression of AMPs. We also determined the effect of *Cryptosporidium parvum* infection on PC development and activity. By immunofluorescence, we observed on intestinal sections that C. *parvum* infection decreases the number of granule-positive-PCs and lysozyme-positive-PCs in neonatal mice. Altogether, these first results clearly demonstrate that PCs are important contributors of the innate protective immune response in mice and that lyzozyme, already described to be efficient in vitro on C. *parvum* sporozoite viability, may be involved in this effect.

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TuB-P4-16h45/16h50

Cryptosporidium parvum can subvert the host immune response through manipulation of CRAMP expression during neonatal infection

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Due to the immaturity of their immune system, neonates are highly sensitive to intestinal infections. During the neonatal period, antimicrobial peptide (AMP) composition differs substantially from that of adults. This is the case in the small intestine for the cathelicidin-related antimicrobial peptide (CRAMP) expressed preferentially in the neonatal period while conversely other AMPs such as Reg3γ are expressed later in life. Among enteric neonatal diseases, Cryptosporidiosis is a zoonotic disease and is highly prevalent in children less than 5 years old in developing countries and in neonatal ruminants worldwide. *Cryptosporidium parvum* is the etiological agent of this diarrheal disease and infects exclusively epithelial cells. Innate immunity is important to control the acute phase of infection in neonates with dendritic cells and IFNγ playing a major role. Antimicrobial peptides are important contributors of innate immunity, but the role of CRAMP, which is elevated in the intestine of neonates has never been investigated during Cryptosporidiosis so far.
In this work, we observed in the neonatal murine model of cryptosporidiosis that unlike other antimicrobial molecules such as Reg3© and Lysozyme, CRAMP expression was significantly reduced in the intestine during infection. By using different genetically modified mouse models, we demonstrated that the reduced CRAMP expression was independent of IFN©, a pro-inflammatory cytokine strongly produced during infection, but also of Myd88, an adaptor molecule involved in innate immune signalling. We also excluded the role of gut flora in this response. When C. *parvum* infected neonatal mice orally received exogenous CRAMP to compensate the reduced expression of this AMP, the parasitic load of neonates was significantly decreased. In addition, when free parasites were in direct contact with CRAMP, this AMP affected the viability of sporozoites. All together, these data suggest that C. *parvum* induces the reduction of CRAMP expression to escape the anti-parasiticidal effect of CRAMP.

TuB-P5-16h50/16h55

Influence of prior excystation procedures on *Cryptosporidium parvum* proliferation in HCT-8 host cell culture

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Background: *Cryptosporidium* spp. have been responsible for a number of waterborne and foodborne outbreaks worldwide. The common detection of *Cryptosporidium* oocysts in diversified food matrices makes it imperative to develop strategies to assess their viability and infectivity for food safety and public health significance. In vitro excystation which has been widely used as a viability indicator, is usually performed to obtain sporozoite host cell invasion and further parasite proliferation which represents an inexpensive, time-saving and ethical alternative to the “gold standard” experimental rodent infections for infectivity evaluation. The aim of this study was to investigate the effect of excystation protocols on *Cryptosporidium parvum* proliferation in cell cultures.

Materials/methods: For excystation, fresh C. *parvum* oocysts from infected calves faeces (INRA, Nouzilly, France) were purified using immunomagnetic separation and subjected to either hypochlorite, trypsin, sodium taurocholate or combined hypochlorite with sodium taurocholate treatment. Excystation ratios were sequentially assessed and monitored by light microscopy. Treated oocysts (104) at optimum excystation time, untreated control oocysts or heat-inactivated oocysts were inoculated onto HCT-8 cell monolayers. Cells were harvested at 24, 48 and 72hrs post-inoculation, DNA was extracted from cell culture and quantitative PCR (CC-qPCR) was performed to evaluate the parasite infectivity. Oocysts inoculated into wells without HCT-8 cells were used as “background” controls.

Results: The highest excystation rate was obtained after oocysts incubation with both sodium taurocholate alone and combined hypochlorite with sodium taurocholate at 37°C for one hour. Resulting sporozoites yielded however low proliferation rates in HCT-8 cell cultures compared to untreated oocysts.

Conclusion: The results show that the CC-qPCR assay presents a valid alternative to the mouse infectivity assay provided that oocysts did not undergo any excystation treatment before inoculation

TuB-P6-16h55/17h00

Identification and localization of the *Cryptosporidium parvum* Gigantic Extracellular protein (CpGE)

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The search for antigenic and distinctive proteins of *Cryptosporidium* represents a key step to design an immune therapy against this parasite. Our project is focused on the identification of immunogenic proteins expressed at the oocysts/sporozoite stage and particularly those involved in or activated by the excystation process. In a previous study some sporozoite proteins have been identified by means of the immunological screening of an expression library that expresses peptides derived from C. *parvum* proteome (Mol Biochem Parasitol. 2007 Apr;152(2):159-69). Among these peptides, a short peptide of 36 aminoacids, referred to as IC4, has been identified as a small part of a big protein (3082 aminoacids) expressed at the sporozoite stage defined Gigantic Extracellular protein of C. *parvum* (CpGE). In this study CpGE was structurally characterised as a large modular protein with a unique assembly of domains and typical traits of a membrane protein which includes a signal peptide and a transmembrane domain. A singular feature of this protein is the presence of domains of different taxonomic origins that are an Ig-like 5 (Big 5) domain, a motif exclusively found in bacteria, and six Sushi (CCP) domains that are characteristic motifs of metazoan proteins. A search for homology revealed that related proteins, which conserve a similar architecture, are present in Apicomplexa exclusively in genera Gregarina, Eimeria, Neospora and Toxoplasma and in some distantly related organisms of the phylum Chromerida. The IC4 peptide was used to immunize mice producing CpGE-specific antibodies to identify and localize this protein in sporozoites. Therefore, CpGE was identified by immunoblot in oocyst/sporozoite extract as a protein of approximately 200 kDa, the quantity of which did not vary significantly in the passage from the quiescent oocyst to the mobile sporozoite. In immunofluorescence experiments CpGE was localized in the apical portion of sporozoites and the CpGE labelling appeared to be internal to cell membrane. This observation was confirmed at the ultrastructural level using immunogold labelling and electron microscopy that showed all the gold particles located internally in the apical complex region and prevalentely associated with dense granules. The structure and the cellular localization of CpGE indicate that this protein is associated with membrane of dense granules and will be exposed on the sporozoite surface after discharge of dense granules content into the extracellular space.

Giardia: A natural CERN Laboratory to Study Evolution of Complex Cellular Events

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Giardia, in addition to its role in developing diarrhoeal disease giardiasis, appeared to be an important eukaryotic organism where we can study the evolution of various cellular organelles, present in higher eukaryote. Though *Giardia* is a eukaryote but its organellar system is completely different than other eukaryotes e.g. yeast and human. The endo-lysosomal system in *Giardia* is quite different where distinct early endosome, late endosome and lysosome are absent, instead they have peripheral vesicles (PVs). PVs are considered as predecessor of endo-lysosomal system. In higher eukaryotes the early to late transition of endosome is characterized by formation of small intraluminal vesicles (ILV) and this vesicle formation is driven by a protein complex known as Endosomal Sorting Complex Required for Transport or ESCRT. In higher eukaryotes there are five ESCRTs, ESCRT-0, -I, -II, -III and -IV. Each of them consists of several small subunits called class E VPS proteins (vesicular protein sorting). ESCRT proteins act sequentially on the cytosolic face of a receptor which is ubiquitinated and marked for degradation in the lysosome. The ESCRT pathway components are divided into two parts cargo recognition and vesicle formation. In *Giardia* we could not find any homologues of ESCRT-0 and -I, the components mainly involved in cargo recognition. But components that are involved in ILV formation, subset of ESCRT-II, -III and -IV, are present exclusively. Surprisingly all the VPS proteins of ESCRT pathway that interact with ubiquitin residue of cargo are absent in *Giardia*. Although there are ubiquitin interacting proteins in *Giardia*, they are not part of ESCRT pathway. We took functional complementation approach to see whether *Giardia* putative ESCRT proteins can function in the context of yeast ESCRT pathway. Our results indicated that the test was positive for GlVps25, GlVps2, all the two orthologues of GlVps46 and all the three orthologues of GlVps4 but GlVps22, GlVps20 and GlVps24 failed to complement the corresponding ESCRT proteins in yeast. We have seen the localization of GlVps4a and GlVps46a orthologues in *Giardia* cells. They appear to localize in the cell periphery and the bare zone which are crowded by PVs. We have used lucifer yellow, a well-known chemical that specifically stains endosome and lysosome, to stain *Giardia* PVs. The localization of GlVps4a, GlVps46a and lucifer yellow staining pattern indicated that *Giardia* putative ESCRT proteins are associated with PVs.

TuB-P9-17h10/17h15

Epidemiology of human cryptosporidiosis cases in immunocompromised patients in France, 2015-2018

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Since January 2015, an online declaration was available for members of the French National Network on the surveillance of human cryptosporidiosis to report confirmed cases. From 2015 to 2018, a total of 737 cases were reported online by 50 tertiary care hospitals and 6 private laboratories, Among them, 345 (47%) concerned immunocompromised patients. of which solid organ transplantation (SOT) recipients and HIV infected patients represented 50% and 26%, respectivel.. In SOT recipients, cryptosporidiosis appeared 6 months, between 6 months and one year, and more than one year post-transplantation in 18%, 13% and 69% of cases, respectively. The commonly used MMF and tacrolimus anti-rejection treatments were administered in 93%) of SGOT patients. The occurence of cryptosporidiosis was associated with blood CD4+ lymphocyte counts lower than 50/mm^3 or 100/mm^3 in 16% and 84% of HIV-infected patients, respectively, In both immunocompetent and immunocompromised patients, *C. parvum* and *C. hominis* were detected in 72 and 25% of patients, respectively. Of 81 genotyped isolates, 21 and 11 exhibited different *C. parvum* and *C. hominis* genotypes, with a predominance of IIaA15G2R1 and IbA10G2 gp60 subtypes. Interestingly, 2 genotypes i.e. IA11G3T3 (n=3) and IIdA15G1 (n=4 were only present in immunocompromised patients. Worthy of note is a fatal course of disease in 5% of immunocompromised patients (*vs* none of immunocompetent patients) which underlines the importance of screening for cryptosporidiosis in immunocompromised patients suffering from diarrhea.

TuB-P10-16h15/16h20

***Giardia*: an under-reported foodborne parasite**

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Foodborne zoonotic pathogens are a serious public health issue and result in significant global economic losses. Despite their importance to public health, epidemiological data on foodborne diseases including giardiasis caused by the enteric parasite, *Giardia duodenalis*, are lacking. This parasite is estimated to cause 28.2 million cases of diarrhoea each year due to contamination of food, but very few foodborne outbreaks have been documented due to the limitations of current detection as well as surveillance methods. The current method for the recovery of *Giardia* cysts from food matrices using immunomagnetic separation requires further standardisation and cost reduction before it can be widely used. It also should incorporate downstream molecular procedures for genotyping, and traceback and viability analyses.

.Foodborne giardiasis can be potentially controlled through improvements in national disease surveillance systems and the establishment of Hazard Analysis and Critical Control Point interventions across the food chain. Studies are needed to assess the true prevalence and public health impact of foodborne giardiasis.

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Wednesday, 26th June, 2019

ORAL PRESENTATIONS

We-O1-8h/8h20

Giardiosis in domestic mammals: clinical importance and public health consequence

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Giardia duodenalis (syn. G. lamblia, G. intestinalis) is the protist responsible for giardiosis, the most common and widely spread intestinal parasitic disease worldwide, affecting humans and many other mammals' species with different genotype (assemblage). In rodents, there are also three other species of *Giardia*: G. muris, G. microti, and G. cricetidarum recently described in three hamsters' species. The infection occurs following cysts ingestion through either contaminated food, water, or environment. The cysts are infectious at very low dose; they can survive during several months in the environment and are relatively resistant to disinfectants. After cysts ingestion, *Giardia* excysts in the upper small intestine to release replicating trophozoites that are responsible for the production of symptoms. In the gut, *Giardia* cohabits with the host's microbiota.

The disease is characterized by an intestinal malabsorption, notability for lipids, diarrheas, weight loss, and abdominal pains. In humans, giardiosis is well recognized to have an important impact on public health, in particular nutritional deficiencies in the children. In domestic mammals, its role in diarrhoeic symptoms is more subject for debate mainly in livestock. As the common rule is the circulation of multiple pathogens in the same time in livestock herds but also in dogs or cats kennels, it is difficult to separate the precise role of each pathogen. Moreover some authors think that in many cases, *Giardia* acts more like a commensal than a pathogen, it depends of its relations with the microbiota community (including bacteria, archaea, viruses and eukaryotes). In this context, diagnosis for *Giardia* infection and for clinical or subclinical giardiosis is difficult and depend if it is for an individual or for a group of animals.

Treatment of giardiosis is difficult because there are only few drugs which can be used against *Giardia* and because recontamination is very often seen even in pets

Finally, the consequences of animal giardiosis for public health are variable and depend of different factors. First of all, it depends of the genotype of the *Giardia* because two assemblages are zoonotic (A and B) but the others are not, and mixed infections are possible. Nevertheless identification of assemblages isn't possible in routine examination. Secondly even with zoonotic assemblages, the frequency of human contamination isn't known.

We-O2-8h20/8h32

Long-term monitoring of *Cryptosporidium* in animals inhabiting drinking water catchments in three states across Australia

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As part of long-term monitoring of *Cryptosporidium* in water catchments serving Western Australia, New South Wales (Sydney) and Queensland, Australia, *Cryptosporidium* was characterised in a total of 5,774 faecal samples from 17 known host species and 7 unknown bird samples, in 11 water catchment areas over a period of 30 months (July 2013 to December 2015). All samples were initially screened for *Cryptosporidium* spp. at the 18S rRNA locus using a quantitative PCR (qPCR). *Cryptosporidium*-positive samples were then typed by sequence analysis of the 18S gene and subtyped at the glycoprotein 60 (gp60) locus. The overall prevalence of *Cryptosporidium* across the various hosts sampled was 18.3% (1,054/5,774; 95% CI, 17.3-19.3). Of these, 873 samples produced clean Sanger sequencing chromatograms, and the remaining 181 samples, which initially produced chromatograms suggesting the presence of multiple different sequences, were re-analysed by Next- Generation Sequencing (NGS) to resolve the presence of *Cryptosporidium* and the species composition. The overall prevalence of confirmed mixed infection was 1.7% (98/5,774), and in the remaining 83 samples, NGS only detected one species of *Cryptosporidium*. Of the 17 *Cryptosporidium* species and four genotypes detected (Sanger sequencing combined with NGS), 13 are capable of infecting humans; C. *parvum*, C. *hominis*, C. ubiquitum, C. cuniculus, C. meleagridis, C. canis, C. felis, C. muris, C. suis, C. scrofarum, C. bovis, C. erinacei and C. fayeri. Oocyst numbers per gram of faeces (g-1) were also determined using qPCR, with medians varying from 6,021 - 61,064 across the three states. The significant findings were the detection of C. *hominis* in cattle and kangaroo faeces and the high prevalence of C. *parvum* in cattle. In addition, two novel C. fayeri subtypes (IVaA11G3T1 and IVgA10G1T1R1) and one novel C. meleagridis subtype (IIIeA18G2R1) were identified. This is also the first report of C. erinacei in Australia. Future work to monitor the prevalence of *Cryptosporidium* species and subtypes in animals in these catchments is warranted.

We-O3-8h32/8h44

Genetic diversity of *Cryptosporidium* in fish

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Fish as a host for *Cryptosporidium* present a transmission route via (1) being a food source for humans and animals and (2) releasing oocysts into the surrounding water including drinking water. *Cryptosporidium* has been described in both fresh and marine water piscine species with parasitic stages located either on the stomach or intestinal surface, or at both sites. Currently, three species of piscine *Cryptosporidium* are recognised: (1) *Cryptosporidium* molnari, which was characterised genetically in 2010, (2) *Cryptosporidium* scophthalmi which was first described in 2004 and a C. scophthalmi-like isolate was characterised genetically in 2015 and (3) *Cryptosporidium* huwi (previously piscine genotype 1). Recent genetic typing has uncovered a large diversity of additional genotypes and species of *Cryptosporidium* infecting fish, many of which appear to be non-zoonotic as well as some zoonotic species. Understanding this diversity has important evolutionary, biological and public health implications and will be discussed.

We-O4-8h44/8h56

Propagation of *Cryptosporidium baileyi* and *C. parvum* in chickens following *in ovo* inoculation

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The genus *Cryptosporidium* comprises species of protist parasites that infect epithelial cells in the microvillus border of the gastrointestinal tract, lungs and the bursa of Fabricius of vertebrate hosts. Most *Cryptosporidium* species and genotypes have a narrow host specificity, and, species with a broad host range rarely infect different classes of vertebrates. Rodent models are most frequently used to propagate oocysts of *Cryptosporidium*, but these are expensive and only work for a limited number of *Cryptosporidium* species. In the present study, we examined whether chickens inoculated in ovo were more susceptible to infection by C. baileyi and C. *parvum* than chickens inoculated following hatching. Ten chicken embryos on the 9th day of incubation and ten one-day-old chickens were inoculated with a dose of 1,000,000 viable oocysts of C. baileyi or C. *parvum*. Faecal samples of all hatched chickens were individually screened daily from the first day of hatching for the presence of *Cryptosporidium* oocysts and specific DNA using microscopy and PCR/sequencing. Development of embryos was checked at the 9th and 18th day. *Cryptosporidium* baileyi and C. *parvum* were infectious for both one-day-old chickens and nine-day-old embryos. Following embryo inoculation, hatched chickens shed oocysts of C. baileyi from the first day after hatching, with an infection intensity up to 45,000,000 oocysts per gram of faeces (OPG), and died 8-16 days post-hatching. Chickens inoculated with C. baileyi at one day of age shed oocysts for at least 30 DPI with maximum infection intensity of 220,000 OPG. Chickens hatched from embryos inoculated with C. *parvum* shed oocysts ranging from 2,000 to 50,000 OPG for at least 30 days. One-day-old chickens inoculated with C. *parvum* failed to shed detectable oocysts at any time during the infection, although DNA was detected intermittently in faecal samples. In ovo inoculation appears to be an effective approach to propagate C. baileyi and C. *parvum* in chickens. This study was funded by the Czech Science Foundation (18-12364S) and by the Grant Agency of the University of South Bohemia (project No. 017/2017/Z). All of the experimental procedures were conducted in accordance with the law of the Czech Republic on the use of experimental animals, safety and use of pathogenic agents and were approved by the National Committee.

We-O5-9h18/9h30

Cryptosporidium spp. (Apicomplexa: Cryptosporidiidae) in Psittaciformes birds and biology of *Cryptosporidium* avian genotype III

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Faecal samples of pet birds from the order Psittaciformes were screened for the presence of *Cryptosporidium* by microscopy and sequencing of the small-subunit rRNA, actin and 70 kDa heat shock protein genes. *Cryptosporidium* infections were detected in 27 of 402 birds. Phylogenetic analysis revealed the presence of *Cryptosporidium* baileyi (n=4), C. avium (n=2) and *Cryptosporidium* avian genotype III (n=21). The biology of *Cryptosporidium* avian genotype III was characterized under experimental conditions. *Cryptosporidium* avian genotype III was infectious for cockatiels (Nymphicus hollandicus), with a prepatent period of six days post-infection (DPI), but not for budgerigars (Melopsittacus undulates), chickens (Gallus gallus f. domestica) or SCID mice (Mus musculus). Experimentally infected cockatiels remained infected for the duration of the study (30 DPI), with an infection intensity ranging from 4,000 to 60,000 oocysts per gram (OPG). Naturally infected cockatiels shed oocysts for more than 5 months, with an infection intensity ranging from 10,000 to 30,000 OPG. *Cryptosporidium* avian genotype III infects the proventriculus and ventriculus, and oocysts measure 7.4×5.8 µm. None of the birds infected with avian genotype III developed clinical signs. Genetic and biological data support the establishment of avian genotype III as separate species of the genus *Cryptosporidium*. This study was funded by the Czech Science Foundation (18-12364S) and Grant Agency of University of South Bohemia (017/2018/Z). All of the experimental procedures were conducted in accordance with the law of the Czech Republic on the use of experimental animals, safety and use of pathogenic agents and were approved by the National Committee.

We-O6-9h30/9h42

What is the source of *Cryptosporidium parvum* infection for beef and dairy calves?

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Cryptosporidium parvum infection has been a major restraint for the cattle industry and cryptosporidiosis is the most commonly diagnosed cause of neonatal enteritis in calves in the United Kingdom.
We conducted longitudinal epidemiology studies on three farms in Scotland; two dairy and one beef farm. These farms had ongoing cryptosporidiosis issues for many years. For all the farms faecal samples were collected from pregnant cows in the run up to calving and their calves for the first few weeks of life. We used a differential sedimentation method to concentrate oocysts from the adult faecal samples and the calf samples were processed directly for DNA extraction. The DNA samples were screened with a nested multiplex PCR to speciate any *Cryptosporidium* spp. present and then we used gp60 typing and satellite marker fragment size analysis to genotype C. *parvum* positive samples.

The calves on each farm had a single predominant C. *parvum* genotype and this genotype stayed stable for many years, for one farm the genotype in the calves has not changed for over 10 years. The satellite marker analysis has shown that the adult cattle had more C. *parvum* genotype diversity than the calves. In the dairy industry the genotypes in the adult cattle were mostly different to the genotypes found in the calves, while on the beef farm some adult cattle were shedding the same genotype as the calves. We conclude that adult dairy cows are not a main source of *Cryptosporidium* infection for the calves due genotype differences between the adults and the very young calves. This may also happen on the beef farms but due to co-housing of adult and young cattle is it more difficult to see. We predict that the neonatal calves pick up and amplify the parasite from the environment in which they are kept, which can explain the persistence of the same predominant genotypes on these farms over several years. It may also indicate that the predominant genotypes are better adapted for transmission in neonatal calves.

We-O7-9h42/9h54

Occurrence and zoonotic potential of *Cryptosporidium* in horses from the Netherlands

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BACKGROUND: Horses can shed zoonotic *Cryptosporidium* species and genotypes, such as C. *parvum*, C. *hominis* and *Cryptosporidium* horse genotype. These species/genotypes can be further divided into subtypes, which have more or less zoonotic potential. In the Netherlands, little is known about the occurrence and public health relevance of *Cryptosporidium* in horses. Previously, 18-34% of horse fecal samples were found positive with rapid immunoassays, but these percentages could not be confirmed.

AIM: To assess the occurrence and zoonotic potential of *Cryptosporidium* shed by Dutch horses.

METHODS: Individual fecal samples from 211 horses of different age categories and origin were collected and screened with a *Cryptosporidium* specific 18S qPCR. For confirmation, positive samples were tested with a nested GP60 PCR, and subsequently sequenced in case of a positive result.

RESULTS: Confirmation with GP60 PCR yielded positive results for 2 horse samples. DNA sequence analysis showed one belonged to C. *parvum* subtype IIaA15G2R1 and one to C. *parvum* subtype IIaA16G1R1. Both are commonly detected subtypes, with IIaA15G2R1 being one of the most prevalent subtype of C. *parvum* in livestock, horses and humans in industrialised countries.

CONCLUSION: According to our findings, the presence of zoonotic *Cryptosporidium* subtypes among horses in the Netherlands is low.

We-O8-9h54/10h06

Wildlife vectors of *Cryptosporidium parvum* as contributors to parasite transmission between farms and to water sources

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Cryptosporidium parvum is an environmentally ubiquitous, zoonotic parasite which is one of the commonest causes of neonatal calf enteritis in both the beef and dairy industries worldwide. It is also a public health issue and causes significant problems for the water industry. C. *parvum* oocysts have the ability to survive in the environment for long periods of time if climatic conditions are favourable and are particularly robust in the fresh water environment. Although the processes by which oocysts are transferred from host to water courses is unclear, transmission via wildlife vectors has been suggested by several studies. This study aimed to identify the role of wildlife in C. *parvum* transmission between farms and, where appropriate, in Scottish water sources, by investigating the prevalence and genotypes of C. *parvum* present in wildlife and any public water sources on farms where cryptosporidiosis has been an issue in calves and/or water contamination episodes have been reported.

Faecal samples were collected from livestock and co-grazing wildlife on farm and surrounding water bodies, if appropriate, for DNA extraction and nested PCR, targeting the 18S rRNA gene, for the detection of *Cryptosporidium* parasites. Positive samples were sequenced to determine *Cryptosporidium* species and GP60 sequencing was then used to genotype C. *parvum* positive samples. Water samples, where available and supplied by Scottish Water from routine sampling were analysed as above. Water, wildlife and livestock samples from farms in geographical areas where cryptosporidiosis had been reported as a calf health and welfare problem, showed a high prevalence of C. *parvum*. GP60 analysis of wildlife and livestock samples illustrated a predominance of identical genotypes of C. *parvum* between the animal species, but interestingly there were typically more strains present in wildlife compared with livestock. This suggests that C. *parvum* is being transmitted between wildlife and livestock and illustrates a potential transmission route for farm to farm transmission of C. *parvum*. The spread of C. *parvum* between farms has been thought traditionally to be mainly due to livestock movement. However, these studies have shown that mechanical transport of oocysts by water or movement of wildlife is possible and can result in calf health and welfare issues as well as contamination of water supplies with C. *parvum*. The transmission of the parasite in the environment should therefore not be ignored or underestimated.

We-O9-10h30/10h42

Identification of *Cryptosporidium parvum* subtype diversity using next generation sequencing in pre-weaned calves in Argentina

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Cryptosporidium spp. are ubiquitous enteric parasites that infect a broad range of hosts including humans and animals. The parasite is transmitted via the fecal-oral route through direct contact (human to human/animal or animal to animal/human) or indirectly via contaminated water and food. Molecular methods are now widely used in epidemiological studies of *Cryptosporidium* infections to identify species, genotypes, and subtypes; however, the extent of within-host genetic diversity remains largely unexplored. Precise identification of subtype diversity is essential to understand the epidemiology and sources of *Cryptosporidium* transmission to humans. Next Generation Sequencing (NGS) of amplicons offer the possibility to more effectively identify mixed infections and low abundance subtypes. To determine *Cryptosporidium parvum* subtype diversity circulating in dairy calves in Argentina, 147 C. *parvum*-positive specimens collected from pre-weaned calves (<6 weeks) from three provinces (Buenos Aires, Cordoba, and Santa Fe) were examined using a next-generation amplicon sequencing approach targeting the glycoprotein 60 (GP60) gene. A comparison of subtypes identified by Sanger sequencing and NGS was conducted. NGS identified the same subtypes initially identified by Sanger sequencing, but frequently identified additional subtypes in the same sample. These data support the capability of NGS to detect mixed infections and low abundance subtypes. Further studies in which subtype diversity is explored are needed to better understand within-host genetic diversity and the transmission dynamics of this parasite, and its public health implications.

We-O10-10h42/10h54

Molecular identification, genotyping, and subtyping of individual *Cryptosporidium* oocysts isolated from bovine faeces with special reference to zoonotic significance

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The ability of the SSU rRNA based nested PCR and Restriction Fragment Length Polymorphism (PCR-RFLP) to identify and genotype *Cryptosporidium* spp. from a single oocyst in bovine faecal samples was evaluated in this study. In addition, subtyping was carried out by sequencing of the 60 kDa glycoprotein (gp60) gene from the same individual oocysts. Faecal samples were collected from 40 pre-weaned calves (5-20 days old) from 7 dairy farms located in 3 different counties within the Finger Lakes region of Upstate New York. All the samples were microscopically positive for *Cryptosporidium* spp. A total of 400 individual *Cryptosporidium* oocysts (10 single oocysts from each calf sample) were purified and analyzed using a nested PCR targeting SSU rRNA gene. The SSU rRNA gene was amplified in 324 (81%) individual oocysts. All SSU rRNA amplified individual oocysts DNA was genotyped using PCR-RFLP. C. *parvum* was the only identified species; 107 single oocysts generated PCR products from the A gene, 18 generated PCR products from the B gene and 199 generated PCR products from both. Phylogenetic analysis of the gp60 gene sequences of 79 C. *parvum* individual oocysts revealed that all sequences were subtype IIaA15G2R1 and displayed 99.4-100% and 99.1-100% identity of nucleotides and amino acids, respectively. These sequences were identical (100%) in oocysts from 35 calves and exhibited mutations in the non-repeat region in those of 5 other calves. The examination of the genetic structure of DNA from individual oocysts with genotyping and subtyping provides methodology to more clearly define the genetic characteristics of *Cryptosporidium* present on farms and within individual animals.

We-O11-10h54/11h06

Cryptosporidium and *Giardia* in Dairy Calves-30 years of observation

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Sources of *Cryptosporidium* and *Giardia* are of continuing interest and importance to public health protection. The literature amply describes the distribution of these organisms among the humans and animals worldwide and provides details on many important characteristics of their presence, propagation inter and intra species, and of their distribution into the environment. Beginning in the mid 1980's with colleagues and students, initially seeking a source of the organism for use in laboratory projects, we began to accumulate data on *Cryptosporidium* and *Giardia* in dairy cattle, focussing calves based on early reports that shedding was most prominent in neonatal animals. As local dairies and their calves were screened to identify a convenient and productive source, data on characteristics of cryptosporidiosis in these animals began to accumulate. Continued research pertaining to development and refinement of monitoring procedure and on the characteristics of these organisms in water treatment processes, in light of the inability to cultivate *Cryptosporidium* in a practical laboratory system led to the regular collection of calf faecal samples for screening and isolation of oocysts. Continued demand for laboratory oocyst populations resulted in refinement of procedure and regular collection from a single local source...partly for convenience but with the initial idea that the resulting organisms would have reasonably consistent characteristics.

The object of this paper is to summarize data now accumulated from literally hundreds of roughly monthly collections from predominantly two dairies, one near Seattle Washington USA, the other near Sydney NSW Australia. Also, to present more detailed data from two projects: one in Washington that sampled from 10 consecutively born (female only) Holstein Friesian calves, following oocyst and coproantigen shedding and serum antibody levels daily for 30 days, then less frequently through 90 days; the other in NSW examining the genetic characteristics among *Cryptosporidium* from a large (2500 milking Holstein Friesians) having three geographically separate locations.

Major features of the accumulated data include: 1) substantial consistency with data on dairy cattle published elsewhere; 2) consistent evidence of *Cryptosporidium* infection in virtually every calf within the first 30 days post partum, peaking in the 7-15 day period; 3) consistent evidence of *Giardia* infection in the same populations, most evident in the 15-30 day period; 4) clear evidence of infection in each of 10 consecutively-born calves as early as 3 days post-partum; 5) evidence of *Cryptosporidium* shedding in parent adults, but requiring more careful screening due to low shedding levels and large faecal volumes; 6) substantial genetic stability of *Cryptosporidium* among representatives of a single dairy herd (calves) across a 1-5 year sampling period, and across three geographically spaced locations.

We-O12-11h06/11h18

MCS6-7 sequences as markers of the bovine origin of *Cryptosporidium parvum* isolates from infected bovines and humans

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Understanding and investigating the sources and routes of transmission of *Cryptosporidium spp*. isolates is particularly important for surveillance, outbreak investigations and risk management. While molecular characterization of *Cryptosporidium spp*. is widely based on the gp60 locus, more informative multi-locus genotyping has proved to have high epidemiological relevance. Yet for this purpose, there is presently no consensus multilocus procedure for isolate genotyping, and defining the optimal set of relevant loci is still an open problem.

In the present study, investigating the *Cryptosporidium* MCS6-7 microsatellite as an epidemiological marker was based on its polymorphism which was documented in previous multilocus investigations, and its function as a serine repeat antigen gene.

The aim of the study was to consider MCS6-7 sequences to distinguish isolates of bovine and non-bovine origin Isolates exhibiting the IIaA15G2R1 gp60 subtype (which is predominant in isolates obtained in France from both infected cattle and humans) were obtained from 1/ bovines, 2/ humans in contact with bovines, 3/ humans from bovine-free environments 4/ symptomatic patients experiencing a cryptosporidiosis outbreak likely due to contamination by unpasteurized cottage cheese and 5/ isolates sampled from infected calves from the cheese producer farm.

Based on MCS6-7 sequences, phylogenetic tree analysis determined that isolates from cattle and humans exhibiting the *Cryptosporidium parvum* IIa15G2R1 subtype were clustered in two groups, i.e. 1/ isolates form cattle and humans with prior contact with bovines, 2/ vs isolates from humans living in cattle-free environments).
Present results suggest that combined C. *parvum* MCS6-7 and gp60 sequencing provides a marker to distinguish isolates of bovine and non-bovine origin and prompt further investigation especially in the context of cryptosporidiosis outbreaks.

POSTER SESSION MORNING

Amphi 100A

WeA-P1-11h30/11h35

Molecular characterization of *Crypstosporidium* isolates from diarrheal dairy calves in France

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Cryptosporidium is an obligate intracellular protist parasite infecting a wide range of vertebrate hosts with significant intestinal diseases in both animals and humans, some species being zoonotic. Cattle and especially calves have been identified as the most common reservoir of this protist. However, little is known about the genetic of *Cryptosporidium* in calves in some regions of France. The aim of this study was to detect and isolate *Cryptosporidium* spp. in faecal samples from naturally infected pre-weaned calves (≤ 45 days-old) in France. A total of thirty-five diarrhoeic pre-weaned calves faecal samples were collected from twenty-six dairy cattle farms with or without diarrhoea in six departments (French administrative provinces). The screening was established microscopically by the detection of *Cryptosporidium* oocysts using an immunofluorescence (IF) staining method. IF-positive samples were then analysed to determine species by PCR-RFLP and sequencing targeting the 18S rRNA gene. C. *parvum* positive samples were subtyped through the analysis of the partial 60 kDa glycoprotein (gp60) gene. Data were then integrated into a phylogenetic tree analysis. IF revealed the presence of *Cryptosporidium* oocysts in 31 out of 35 (88%) samples. Combining results of 18S rRNA gene analysis, C. *parvum* was detected in 30 samples. Subtyping analysis in 27/30 samples (90%) of the C. *parvum* isolates revealed two zoonotic subtype families, IIa (24/27) and IId (3/27). Four sub-types were recognized within the subtype family IIa including IIaA15G2R1 (21/27) which is the hypertransmissible subtype the most frequently reported worldwide, IIaA17G3R1 (1/27), IIaA17G1R1 (1/27) and IIaA19G1R1 (1/27). Two subtypes were recognized within the IId subtype family including IIdA22G1 (2/27) and IIdA27G1 (1/27). These findings illustrate the high prevalence of *Cryptosporidium* in calves in dairy herds and increase the diversity of the molecular characteristics of C. *parvum* isolates with the first description of IIaA17G3R1, IIaA19G1R1 and IId subtypes in France. The presence of zoonotic subtype families of C. *parvum* species (IIa, IId) in this study suggests that pre-weaned calves are likely to be an important reservoir of zoonotic C. *parvum*, and highlights the importance of animal to human transmission risk of cryptosporidiosis.

WeA-P2-11h35/11h40

Molecular characterization of zoonotic *Cryptosporidium* and *Giardia duodenalis* pathogens in Algerian sheep

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Little is known about the presence of *Cryptosporidium* spp. and *Giardia duodenalis* in Algerian sheep, nor their potential role as zoonotic reservoirs. This study aimed to investigate the occurrence and the distribution of these two protists in lambs. A total of 83 fecal samples were collected from lambs (< 40 days old) on different 14 farms. Samples were screened for *Cryptosporidium* spp. and *Giardia duodenalis* presence with Immunofluorescence technique. Nested PCR of the small subunit ribosomal RNA (rRNA) gene, followed by restriction fragment length polymorphism (PCR-RFLP) and sequence analyses were used to identify *Cryptosporidium* species. Then, C. *parvum* was further subtyped by sequencing the highly polymorphic 60kDa glycoprotein (gp60) gene. For G. *duodenalis*, nested PCR of the glutamate dehydrogenase (gdh) and triose phosphate isomerase (tpi) genes were applied and then PCR-RFLP was used to determine G. *duodenalis* assemblages. *Cryptosporidium* oocysts and *Giardia* cysts were detected in 36/83 (43%) and 23/83 (28%) of fecal samples, respectively. Of the 21/36 (58%) *Cryptosporidium* samples that were microscopically-positive, 16/21 (76%) were identified as C. *parvum*, and 5/21 (24%) C. ubiquitum. From 15 C. *parvum* isolates, 2 subtypes were identified within the subtype family IIa including IIaA21G2R1 (3/15) and IIaA13G2R1 (1/15), while IIdA16G1 (11/15) was the only subtype within IId subtype family. Of the 16/23 (69%) G. *duodenalis* microscopically-positive samples, the most frequent assemblage was the ruminant-specific assemblage E (10/16), followed by assemblage D (4/16), and A + E mixed assemblages (2/16). This study reports for the first time the identification and genotyping of both *Cryptosporidium* spp. and *Giardia duodenalis* from lambs in Algeria. This is also the first description of assemblage D in small ruminants. The presence of zoonotic C. *parvum* subtype families (IIa, IId) and C. ubiquitum, as well as the zoonotic G. *duodenalis* assemblage A, indicates that sheep could play an important role as a potential reservoir for zoonotic protists.

WeA-P3-11h40/11h45

Prevalence of potentially zoonotic assemblages of *Giardia duodenalis* in domestic and wild animals in Scotland

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Giardia duodenalis is of interest to scientists in both the medical and veterinary fields, as two assemblages (A and B) are known to be zoonotic. The aims of this study were firstly to determine the prevalence of *Giardia* in faecal samples collected from domestic livestock and wildlife species from across Scotland and secondly to determine the potential for zoonotic transmission of parasites found. This study included samples from cattle (n=388), sheep (n=84), rabbits (n=296), wood mice (n=40), field voles (n=17), bank voles (n=132) as well as four species of wild deer (red (n=278), roe (n=131), sika (n=41) and fallow (n=7)). DNA was extracted from each sample and nested PCRs were performed in duplicate to detect *Giardia* β-giardin (bg) DNA. The results from this study showed that every species examined tested positive for the presence of *Giardia* DNA. Prevalence ranged from 69.0% in rabbits (233/296) to 2.4% (1/41) in sika deer. Interestingly the wood mice all tested negative using the bg primers, however 27.5% (11/40) tested positive using *Giardia* specific ssu rRNA gene primers. Potentially zoonotic assemblages (A or B) of G. *duodenalis* were found in all species tested, with the exception of wood mice where a 98.6% (209/212 bp) sequence identity to an unnamed *Giardia* sp. previously found in wood mice (Apodemus sylvaticus) was detected. In cattle the predominant assemblage identified was assemblage E (77%) followed by B (18%) and A (5%) whilst in the rabbits, the most common assemblages were A, B and D (29%, 27% and 26% respectively). In voles, assemblages A, B and E were identified (49%, 9% and 14%) as well as 100% (439/439 bp) sequence identity to an unnamed *Giardia* sp. parasite previously identified in Microtus sp. (voles). Finally, in the deer a majority 30 /40 (75%) of the positive samples were identified as assemblage A, though assemblages B, D and E were also found, but in much lower numbers (8%, 7% and 10% respectively). The sequences of the assemblage A and B parasites identified during this study (in all host species except wood mouse) demonstrated 100% sequence identity (NCBI BLAST) to the published sequences of human infectious assemblages of the parasite. This data indicates that all of the ruminant and wildlife species studied where G. *duodenalis* DNA was identified could be contributing to the zoonotic transmission of the parasite in Scotland.

WeA-P4-11h45/11h50

Zoonotic species *Cryptosporidium parvum* in cattle in Slovakia

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Domestic ruminants represent typical hosts for protozoan parasites of the *Cryptosporidium* genus. Farm animals may serve as important sources of zoonotic species or genotypes of *Cryptosporidium* spp. which may have negative effects on human health. To date, the IIaA17G1R1 subtype has been confirmed in cattle and in oncological patients in Slovakia (Danišová et al., 2016; Hatalová et al., 2018). Our objective was to identify the subtypes of *Cryptosporidium* spp. in calves younger than 35 days because to date there are only a few records of the prevalence of this species in calves in Slovakia. Danišová et al. (2016) confirmed as much as 70 % prevalence of C. *parvum*, the IIaA17G2R1 subtype, in calves younger than 1 month. In our study, we diagnosed the oocysts of *Cryptosporidium* spp., applying the Ziehl-Neelsen acid resistant staining technique, in 31.5 % (17/54) of calves bred on a farm in Zemplínska Teplica. Applying the nest PCR molecular method, we confirmed the zoonotic species of C. *parvum*. Subsequently, we performed subtyping of five microscopically positive specimens at the GP60 locus. The obtained sequences were compared to the sequences from the GenBank and the isolates were classified as subtypes IId and IIa. The IIdA17G1 subtype (GenBank KY499053.1) was identified in four isolates and the IIaA17G1R1 subtype (GenBank JX258865.1) in one isolate (Mravcová et al., 2019).

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WeA-P5-11h50/11h55

Occurrence of *Cryptosporidium* suis in Italian pig farms

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Among *Cryptosporidium* spp. infecting pigs, *Cryptosporidium* suis and *Cryptosporidium* scrofarum are the most common species, while infections caused by the highly zoonotic species *Cryptosporidium parvum* are less frequently observed. In pigs, *Cryptosporidium* prevalence and species may depend on production systems and age, with C. suis infecting all age categories and C. scrofarum infecting older pigs. In Italy, no recent data are available on *Cryptosporidium* spp. infections in pigs. The present study evaluated the occurrence and species of *Cryptosporidium* in different pig managements systems in Italy.
Individual faecal samples collected from 51 suckling piglets, including 11 diarrhoeic and 40 asymptomatic animals, and 93 asymptomatic sows (total 144 animals) from four pig farms, were examined. More specifically, in three farms adult pigs were reared outdoor, while in the fourth farm animals were reared indoor (intensive farm). Faecal samples were examined by a commercial rapid immunoassay (RIDA QUICK *Cryptosporidium*, R-Biopharm®) to detect *Cryptosporidium* faecal antigens. Samples found positive at the immunoassay were stored at -20°C and subsequently tested by a nested PCR assay targeting the 18S ribosomal DNA gene. Positive PCR samples were sequenced and *Cryptosporidium* species determined by BLAST comparison with sequences available in the GenBank database.

At the immunoassay, an overall *Cryptosporidium* spp. prevalence of 6.25% (9/144) was found. More specifically, five sows (5/93, 5.4%) and four suckling piglets (4/51, 7.8%) were found positive. PCR confirmed the infection in all suckling piglets (7.8%), but sows were found all negative. Molecular analysis revealed C. suis as the cause of the infection in all PCR positive animals. Moreover, all C. suis positive suckling piglets were from the intensive farm and all of them were diarrhoeic animals. Results from this study confirm previous observations about the occurrence of C. suis in young piglets. Moreover, this is the first report of C. suis infection in pigs from Italy.

Table 1. Prevalence of *Cryptosporidium* found by immunoenzymatic and PCR assays from four Italian pig farms.

Animals	(n)	Immunoassay (%)	PCR (%)
Suckling piglets	51	7.8%	7.8%
Sows	93	5.4%	0.0%
Diarrhoeic piglets	11	45.5%	45.5%
Asymptomatic piglets	40	0.0%	0.0%
Outdoor	59	0.0%	0.0%
Indoor	85	10.6%	4.7%
Total	144	6.25%	2.8%

WeA-P6-11h55/12h00

A snapshot of *Cryptosporidium* spp infection in livestock in France: Public health risk concern

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Cryptosporidiosis becomes a major public health and veterinary concern. Domestic animal, livestock, wildlife, and human can be potential reservoirs that contribute this parasite genera to food and surface waters and transmitted to other hosts through the fecal-oral route. On a One Health approach and to promptly and adequately respond to a possible source during an outbreak investigation, genotype data from animals and the environment is needed.
The aim of this study collects knowledge about the occurrence and genotype of this parasite among livestock in France.
-In the French Basque Region, during a study conducted on ewes (n=80) and lambs (78) showing no signs of diarrhea at the time of sampling. Polymerase Chain Reaction (PCR) targeting the 18S ribosomal RNA gene, confirmed by sequencing, was conducted on the fecal samples to detect *Cryptosporidium* genomic DNA and determine *Cryptosporidium* identity. The prevalence of *Cryptosporidium*-positive fecal samples was 8,86% (14/158). *Cryptosporidium parvum* (n=9); C. xiaoi (n=3) and C. ubiquitum (n=2) were identified. GP60 subtyping analysis identified C *parvum* isolates belonged to family subtype IIa and IId. Subtype IIdA24G1 was more prevalent (n=6). This subtype was already identified and considered as the probable aetiologic agent for Cryptosporidiosis outbreak in Sweden (2010).
- Molecular analyses targeting small subunit ribosomal RNA as well as glycoprotein 60 (GP60) genes from fecal specimens from 630 from calves in different French regions revealed that the *Cryptosporidium* were detected in 227 fecal samples (36,03%) and C. *parvum* IIaA15G2R1 was the most common and widely distributed (144/227) followed by IIaA16G3R1(n=42). While C. *parvum* IIaA15G2R1 is the most reported subtype involved in cryptosporidiosis outbreaks, C. *parvum* IIaA16G3R1 were also reported in an outbreak of cryptosporidiosis associated with raw milk consumption (USA). 15 cases of C. *hominis* infection was also found in calves. The predominant C. *hominis* IbA9G3 (n=9) genotype was present in all regions and more frequent in the western region. C. *hominis* IbA9G3 was already reported in an outbreak of cryptosporidiosis in children attending daycare center in the USA.
Presence of *Cryptosporidium* predominant subtypes (involved in human cryptosporidiosis outbreaks elsewhere), in livestock and by implication in the environment in France, reported in this study highlight the increasing risk of transmission to humans.

WeAP7-12h00/12h05

Evaluation for associations amongst *Giardia duodenalis* Assemblages and fecal score of dogs

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Giardia duodenalis is a species complex comprising at least eight Assemblages. Most infected dogs harbor the host-adapted Assemblages C and D (~70%) and 30% harbor the zoonotic genotypes. Humans and dogs with giardiasis can experience a variety of clinical manifestations ranging from the absence of clinical signs to acute or chronic diarrhea. In human studies, the association between clinical signs and Assemblage type have reported conflicting results. One study reported that dogs with diarrhea were most likely to harbor Assemblages C and D, however only a small number of dogs were typed.

The aim of this study was to evaluate for associations between G. *duodenalis* Assemblages and different fecal scores using feces of client-owned dogs from the United States.

Fecal samples with a confirmed *Giardia* positive result by microscopic examination were collected from a commercial diagnostic laboratory (IDEXX) and classified on arrival into our laboratory using a standardized fecal scoring system. PCR assays and the sequencing of the amplified genes (beta giardin, glutamate dehydrogenase, triose phosphate isomerase) were performed following published protocols. A multiple alignment with other sequences from the Genbank was performed first and then phylogenetic analysis was assessed by Neighbor-Joining analysis using MEGA X with bootstrap support in 1000 replicates.

Three hundred samples were tested by the three PCR assays; 100 samples were from dogs with normal stool consistency and 100 samples from dogs with diarrhea were PCR positive for one or more genes Most of the samples in both the diarrhea and normal groups typed as dog specific Assemblages (D or C) by at least one gene (Table 1). Phylogenetic analysis of the three genes place the isolates from Assemblages A, B, C and D, separated from each other with strong bootstrap support. The clustering was similar among the bg and gdh genes. Subgrouping with low bootstrap values was observed among Assemblages C and D in the tpi analysis.

Table 1: PCR results from the diarrhea and non-diarrhea samples from G. *duodenalis* positive dogs.

No differences between Assemblages and clinical signs were detected in this sample set. The lack of association between clinical signs and Assemblages in this particular population might be related to the population sampled. The role of gut microbiota and the presence of other co-infections should also be considered when analyzing associations amongst Assemblages and clinical signs.

WeA-P8-12h05/12h10

The assemblages of *Giardia duodenalis* circulating among humans and animals in Slovakia

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At present, there is a lack of information on epidemiology and epizootiology of giardiasis in Slovakia and on the spread of this infection in populations living in various social and economic conditions or in animals. Within our research, the faecal specimens were examined applying the Faust flotation and concentration technique for the presence of cysts of *Giardia duodenalis*. Microscopically positive specimens were used for genotyping of G. *duodenalis* assemblages. DNA was extracted using the ZR Fecal DNA MiniPrep™ kit and then subjected to a nested PCR. The nested PCR protocol was used to amplify a partial sequence of the triosephosphate isomerase (tpi) gene, β-giardin (bg) gene and glutamate dehydrogenase (gdh). Applying the molecular biology methods, we were the first in Europe to prove the presence of canine genotype C of *Giardia duodenalis* in a female patient from Košice, hence confirming the possible role of dogs in the zoonotic transmission of this pathogen (Štrkolcová et al., 2015). Our results also represent the initial evidence of the occurrence of zoonotic assemblages A and B of *Giardia duodenalis* in children aged below 14 years who attended nursery and elementary schools in two towns in Slovakia (Štrkolcová et al., 2016), as well as the initial evidence of assemblages C, D and F of *Giardia duodenalis* in dogs and cats in Slovakia. Also, we confirmed the zoonotic assemblage B isolated from Lemur catta taken from a zoological garden in Košice in Slovakia. In 2018, the assemblage F was confirmed for the first time in Slovakia in children living in poor environmental conditions (Pipíková et al., 2018).

Acknowledgements:
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WeA-P9-12h10/12h15

Investigations on zoonotic cryptosporidiosis in Northern Ethiopia

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Livestock farming is an important source of livelihoods in rural communities of Ethiopia and children play a major role in livestock activities. In these communities, livestock fulfil multiple functionalities including being a source of human nutrition, transport, and a tool for agriculture. Parasitic diseases, including cryptosporidiosis, can have a significant negative impact on these communities, both directly on livestock mortality, morbidity and reduced production, and also due to the potential for transmission to humans.

The role of livestock in the transmission of cryptosporidiosis to humans is poorly understood in Ethiopia, largely because most previous studies have been based on Modified Ziehl-Neelsen staining that does not enable species identification. However, some molecular studies have identified C. *parvum* subtype IIa as the major cause of human cryptosporidiosis in Ethiopia, which strongly suggests that zoonotic transmission of this parasite is occurring. This contrasts with studies from other areas of Africa where C. *hominis* infections tends to predominate in the human population, and zoonotic transmission appears to occur less often.

Tigray, in Northern Ethiopia, is primarily an area of subsistence farming, and therefore any impacts on animal health can have a substantial impact on livelihoods. Furthermore, due to close contact between people and their animals, the risk for transmission of zoonotic pathogens between livestock and humans is considered to be high.

The MEZCRYPT project will investigate the prevalence and species/genotypes of *Cryptosporidium* and *Giardia* infection in dairy calves, lambs, goat kids, and farmers, including children involved in livestock activities, assess the relative importance of zoonotic cycles in these infections, and determine whether water and fresh produce are a potential source of transmission in rural communities of the districts. In the first stage of the project, between October 2018 and January 2019 faecal samples were collected from 209 calves, 269 lambs, 258 goat kids, and 221 children and adults from Enderta, Kileteawlalo, Hintalowajerat and Raya Azebo districts of Tigray. In addition, questionnaires were completed from 221 farmers regarding livestock management and risk factors for infection.

Analysis is in progress, and we would like to present preliminary results regarding the occurrence of infection with *Cryptosporidium* in both animals and people, and potential risk factors for animal and human infection in this region based on the questionnaire data.

WeA-P10-12h15/12h20

Frequency and molecular diversity of *Giardia duodenalis* and *Cryptosporidium* spp. in captive non-human primates and their keepers in Spain and rescued wild chimpanzees (Pan troglodytes) in Sierra Leone

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Background

Giardia duodenalis and *Cryptosporidium* spp. are common diarrhoea-causing enteric pathogens in human (HP) and non-human (NHP) primates. Given the remarkable genetic similarity among them, we hypothesized that HP and NHP can harbour infections by similar, or even identical, species/genotypes of *Giardia* and *Cryptosporidium*, and that zoonotic/anthroponotic transmission may occur under favourable epidemiological conditions.

Methods. A molecular-based epidemiological survey was conducted in captive NHP and their keepers in four Spanish zoological gardens (Madrid Zoo Aquarium, Faunia, Córdoba Zoo, Santillana del Mar Zoo) and in rescued chimpanzees at the Tacugama Chimpanzee Sanctuary (TCS) in Sierra Leone. Faecal samples were collected during October 2018-January 2019. Detection of G. *duodenalis* infections was achieved by qPCR, and positive samples were subsequently assessed by multi-locus sequence genotyping of the gdh, bg, and tpi genes of the parasite. *Cryptosporidium* species and sub-genotypes were investigated at the ssu rDNA and the gp60 genes of the parasite.

Results:

Faecal specimens from HP (n=42) and 25 different genera of NHP (n=226) were collected in the Madrid Zoo Aquarium (17+62), Faunia (6+38), the Córdoba Zoo (15+28), the Santillana del Mar Zoo (3+31), and the TCS (1+67). G. *duodenalis* was found in 2.4% of HP and in 23.0% of 15 different genera of NHP. *Cryptosporidium* was identified in 4.8% of HP and 1.8% of four different genera of NHP (Table 1). All HP infected by G. *duodenalis* (n=1) or *Cryptosporidium* spp. (n=2) belonged to the Madrid Zoo Aquarium. Molecular analyses revealed the presence of G. *duodenalis* sub-assemblage AI in a single sample of the genus Cebus, and BIV in members of the genera Callimico (n=1), Callithrix (n=1), Lemur (n=7) and Pan (n=1). *Cryptosporidium hominis* was identified in two HP and a member of the genus Pan from the Madrid Zoo Aquarium. At the Santillana del Mar Zoo C. *hominis* was found in a sample of the genus Callithrix, and C. *parvum* in samples of the genera Macaca (n=1) and Seguinus (n=1).

Table 1. Prevalence rates of G. *duodenalis* and *Cryptosporidium* spp. found in the different genera of human and non-human primate populations investigated in the present study.

Genus	Total	No.	No. *Giardia*-positive	%	No.	Crypto.-positive	%
Aotus	3	0	0.0	0	0.0		
Callimico	4	2	50.0	0	0.0		
Callithrix	4	3	75.0	1	25.0		
Cebuella	2	0	0.0	0	0.0		
Cebus	15	3	20.0	0	0.0		
Cercocebus	3	3	100	0	0.0		
Cercopithecus	4	1	25.0	0	0.0		
Colobus	6	0	0.0	0	0.0		
Eulemur	5	0	0.0	0	0.0		
Galago	2	1	50.0	0	0.0		
Gorilla	8	2	25.0	0	0.0		
Homo	42	1	2.4	2	4.8		
Hylobates	7	1	14.3	0	0.0		
Lemur	17	8	47.1	0	0.0		
Leontopithecus	5	0	0.0	0	0.0		
Macaca	7	0	0.0	1	14.3		
Mandrillus	12	1	8.3	0	0.0		
Mico	1	1	100	0	0.0		
Pan	78	22	28.2	1	1.3		
Papio	9	1	11.1	0	0.0		
Perodicticus	1	0	0.0	0	0.0		
Pithecia	3	0	0.0	0	0.0		
Pongo	8	0	0.0	0	0.0		
Seguinus	7	2	28.6	1	14.3		
Sarmiri	7	0	0.0	0	0.0		
Varecia	8	1	12.5	0	0.0		
Total	268	53	19.8	6	2.2		

Conclusions

G. *duodenalis* BIV (the most common sub-assemblage circulating in the European human population) was frequently detected in captive NHP. Similarly, C. *hominis* was identified in a captive chimpanzee and two of his keepers at the Madrid Zoo Aquarium. Although preliminary, these data seem to suggest that zoonotic/anthroponotic transmission of G. *duodenalis* and C. *hominis* in zoological gardens may be a more frequent event than previously anticipated.

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WeA-P11-12h20/12h25

Transmission of *Cryptosporidium parvum* from cattle to a veterinary student in Slovakia

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In Slovakia, human cryptosporidiosis has been observed very rarely. The first records date back to 1987 when there were seven cases of the disease caused by *Cryptosporidium* spp. diagnosed in patients with HIV/AIDS (Čatár and Sobota, 1987). The folowing occurrence was observed in 2013 in two children; one of them was a seven years old boy who was present with clinical symptoms of this disease. DNA typing was applied to identify the IbA10G2 subtype of C. *hominis* (Ondriska et al., 2013) in these children. At present, there are records of infections in dogs and children living in Roma settlements or children's homes in the Eastern Slovakia caused by the C. muris species (Počátková et al., 2013, Hasajová et al., 2014). In our study, we describe a case of human cryptosporidiosis caused by the *Cryptosporidium parvum* species. The patient, a 23-year old man, was present with typical clinical symptoms of cryptosporidiosis (abdominal pain, profuse diarrhoea and dehydration). The oocysts were probably transmitted through a direct contact with farm animals. The infection had a severe clinical development accompanied with a very large number of watery diarrhoea episodes per day. Applying the Ziehl-Neelsen staining method and the ELISA immunological assay (faecal), we identified the presence of cryptosporidia oocysts in faecal specimens. Applying the nested PCR, we amplified the SSU rRNA and confirmed the presence of the C. *parvum* species; subsequently, we performed sequencing of GP60 gene and identified the zoonotic subtype IIdA15G1 (Mravcová et al., in press). Acknowledgements: This study was funded by the projects of the Scientific Grant Agency of the Ministry of Education of the SR and the Slovak Academy of Sciences, VEGA 1/0536/18

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WeA-P12-12h25/12h30

Multilocus sequence typing strategies for the characterization of *Giardia duodenalis* Assemblage A isolates from human and animals in Italy

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Giardia duodenalis (syn. *Giardia* lamblia, *Giardia* intestinalis) is a food- and waterborne protozoa affecting humans and a wide range of domestic and wild animals. Several molecular assays have been developed to unravel the complex epidemiology of this infection. G. *duodenalis* is characterized by the existence of eight genetic groups (Assemblages), two of which (A and B) are found in both humans and animals and are therefore potentially zoonotic, whereas the remaining six (C-H) are relatively host-specific. Despite of the rapid accumulation of sequence data and the refinement of the assays, many biological and/or epidemiological features specifically peculiar of each major human-pathogenic Assemblages A and B remain still unclear. Older studies have traditionally assigned *Giardia* isolates to one or other of these Assemblages, based only on the sequences derived from a single gene locus, or part thereof, representing a very small fragment of that isolate’s genome. The majority of recent surveys attempting to determine the occurrence of different G. *duodenalis* Assemblages A and B in particular host populations are now based on a multilocus genotyping approach (MLST). The aim of the present study was to apply MLST strategies for the characterization of G. *duodenalis* Assemblage A isolates of diverse origin. Fecal samples were collected from humans and a large number of animal species. Feces resulted positive to *Giardia* cysts by microscopic investigation and by immunofluorescence were subjected to PCR and sequenced by using the standard small subunit (18S), β-giardin (BG), glutamate dehydrogenase (GDH) and triose phosphate isomerase (TPI) typing methods. All isolates assigned to the Assemblage A were furtherly analysed by the recently described MLST approach based on a set of three genetic loci [NEK Kinase 15411, High cysteine membrane protein group 2 (HCMP22547) and the Caffeine-induced death protein 1-like protein (CID1)] showing high genetic variability. Phylogenetic analysis was performed by comparison of the obtained sequence with those retrieved from NCBI GenBank by MEGA7 for all the loci analysed. *Giardia* 18S, BG, GDH and TPI sequences from a total of 32 samples (human = 18, animals = 14) were obtained. Eighteen isolates identified as Assemblage A were analysed by the new multi-locus typing scheme. The increased resolution of the genetic variability within the Assemblage A allowed valuable indication on the epidemiology and zoonotic transmission of the parasite at different geographical scale.

WeA-P13-12h30/12h35

Cryptosporidiosis in non-human primate in Ethiopia a Public health problem

Ambachew Wortea Hailu; Damien Costa; Beyene Petros; Haileeyesus Adamu; Loic Favennec; Romy Razakandrainibe

Background
Chlorocebus pygerythrus (vervet monkey) and *Colobus guereza* (colobus monkey) are among the most widely distributed non-human primate (NHP) species in Ethiopia. These monkeys are extremely adaptable and are frequently found in suburban areas which overlap with their home ranges, where they come in frequent contact with humans. Acting as a reservoir for a variety of pathogens, less is known about occurrence of cryptosporidiosis in these monkeys and their role as source of transmission. Our goal was to investigate *Cryptosporidium* infection and to assess cross-species transmission using molecular methods.

Methodology/Principal Findings
Paired sample (n=185) was taken from monkeys and human living in same area. The molecular characterization of *Cryptosporidium* spp. was conducted by nested PCR analyses of the small subunit rRNA (SSU rRNA) and GP60. Analysis revealed a prevalence of *Cryptosporidium* of 21,62 % (40/185) in humans and 17,83% (33/185) in non-human primates. *Cryptosporidium parvum* and *C. hominis* were the only species identified in humans; *Cryptosporidium cuniculus*, *C. hominis* , *C. baileyi* and C. *parvum* in monkeys. In monkeys 29/33 isolates were successfully subtyped and revealed 14/29 C *hominis* and 15/29 C. *parvum*. The identification of C. *hominis* subtype IaA20 and C. *parvum* IIaA17G1R1 in both human and monkey suggests potential cross-species transmission. In this study, *C. hominis* subtype richness and diversity was higher in monkey compared to human: IeA11G3T3, IaA26, IbA10G2 and IdA24 were also identified (*C. hominis* subtypes found in human samples: IaA20 and IdA21). Within the *C. hominis* subtype family Ie, human infections in developing countries are caused mostly by IeA11G3T3. IbA10G2 was also reported, a subtype which is distributed on all inhabited continents and has been implicated in some waterborne and foodborne outbreaks of human cryptosporidiosis.

Conclusions
The study revealed very important public health problem caused by probably an extensive transmission of cryptosporidiosis between NHP and humans. NHP have been shown to harbor same subtypes found in human living in their close proximity hence may serve as a reservoir for *Cryptosporidium*. Further studies are needed to identify the exact route(s) of *Cryptosporidium* transmission in the studied areas, which will aid in controlling of the spread of the infectious agent.

WeA-P14-12h35/12h40

Interreg 2 seas Project: Health For Dairy Cows, H4DC.

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Pathogens are not bound by national borders. Trade between farms and breeding centres can be a risk factor for pathogens, including *Cryptosporidium*, to spread far and wide. In cattle farms, cryptosporidiosis is a common disease difficult to control without good breeding practices, efficient detection tools and effective pharmaceuticals. Juvenile infected animals are typically infected with *Cryptosporidium* species, which results in stunted growth. Thus, cryptosporidiosis leads to a higher mortality rate that further threatens the economic viability of a sector. To address this concern, the Interreg-2-seas European Union programme approved the Health-for-Dairy-Cows (H4DC) Project. To tackle these issues, we will adopt a threefold approach

(1) Disseminated pilot farms across France, Belgium, The Netherlands and England will serve as operational demonstrators to proof and transfer new husbandry practices. Application of such developed practices will aim to prevent economic damage due to stunting growth and/or mortality of calves. That approach will also be held to improve cattle health and wellbeing. Pilot farms will subsequently be used to test a low-cost and easy-to-use in-situ parasite detection system developed by the H4DC consortium. (2) Accelerate the discovery of new anti-*Cryptosporidium* pharmaceuticals will be managed by the development of an innovative electronic device for faster analysis of the effects of various compounds tested. (3) A marketing study linked to a business model and strategies to transfer technologies developed in H4DC project to the end users (biotech/pharmaceutical companies) will finally be undertaken. One of the most original features of this project is its methodological approach. We aim to involve farmers throughout the process in order to co-develop pilot farms. This inclusion of farmers in the initial planning combined with the expertise of various partners (Belgian, Dutch, English and French) will engender proposals for infectious risk management procedures, which will work in the context of their regional and individual constraints. Moreover, development of a *Cryptosporidium* detection system will provide a technological breakthrough. Furthermore, this collaborative project will allow us to adapt our sensitive and fast-acting pilot electronic microsystem for the R&D laboratories in pharmaceutical companies' needs. This system, which uses living cells to detect the effect of pathogens, doesn't exist yet in the analytical tool market and could be adapted in the near future to a broad range of pathogens. Importantly, under the "one health" approach, by reducing *Cryptosporidium* infection and excretion in farms, the H4DC project will also aim to decrease the risk of cryptosporidiosis in humans.

WeA-P15-12h40/12h45

Cryptosporidium horse genotype infection in immunocompromised child with Crohn's disease.

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Inflammatory Bowel Diseases (IBDs), including both Crohn's disease (CD) and ulcerative colitis (UC), are characterized by chronic, relapsing inflammation of the gastrointestinal tract of unknown aetiology. Among factors generally considered as a cause of the inflammation arising both genetic and immunological predispositions as well as exposition to infective factors are taken into an account. Since there is no causative treatment of these diseases immunosuppressive drugs that reduce inflammation are generally used. Recently, increasing incidence of IBD, especially in newly industrialized countries, has been observed, and one challenging aspect is the development of the diseases in younger children.

Here we present for a first time a case of *Cryptosporidium* horse genotype infection in a 13-year-old immunosuppressed female patient suffering from CD. Patient has been receiving biological treatment (infliximab) for 9,5 years at the time of examination due to CD and rheumatoid arthritis. Due to recurrent diarrhoea regardless of CD activity, stool patient was referred for tests for intestinal infection. Screening of stool samples by microscopy using an aniline-carbol-methyl violet stating showed a presence of *Cryptosporidium* sp. oocysts. Following phylogenetic analysis of small-subunit rRNA and 60 kDa glycoprotein genes of *Cryptosporidium* revealed the presence of *Cryptosporidium* horse genotype, which is the third case of this *Cryptosporidium* species in humans. Whereas the previous two human cases of *Cryptosporidium* horse genotype were caused by subtype family VIb, which never have been found in horses before, the patient in this study was infected with subtype family VIa. The patient has had documented every-week horse riding for 4 years. One of the seven horses from the paddock, where the patient was riding, had an infection with the same *Cryptosporidium* subtype. According to our finding, cryptosporidial infections should be considered in IBD persons, especially when immunosuppressed, as one of the agents causing diarrhoea. Moreover, due to high risk of zoonotic transmission, phylogenetic analysis of *Cryptosporidium* in IBD children should be performed in detail.

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WeA-P16-12H45/12H50

Tools for understanding the public health risks of *Cryptosporidium* in swimming pools

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Swimming pools are the most common setting for outbreaks of cryptosporidiosis in the UK. To improve risk assessment, direct appropriate interventions, and encourage good management, we developed new tools and gathered information to supplement guidance first issued in 2011 (www.publichealthwales.org/cryptopoolguidance).

We have depicted a *Cryptosporidium* transmission pathway in swimming pool settings for training and communication purposes, especially when annotated with control measures and potential failures. This highlights that bather hygiene is a critical control point. It also underpins the development of quantitative risk assessment models by identifying the data points required, including oocyst concentration.

To investigate the background occurrence and concentration of oocysts in pool water and filter backwash, and any relationships with pool parameters, we undertook a sample survey over 10 weeks in summer of 2017. High-volume, in-line sample modules (IDEXX Filta-Max xpress®) were installed at six volunteer leisure pools. Up to 1000L water was tested weekly by standard methods. At three pools, 1L filter backwash water was also sampled weekly. Pool water was tested for bacterial indicators, pH, residual free and combined chlorine, temperature, turbidity and total dissolved solids. Oocysts were detected in 12/59 (20%) pool water samples (60 to 999L) and at least once in each pool; 8/12 (66%) detections were in August when bather loads were highest. Counts ranged from 0 to 1.16 oocysts per 10L, mean 0.04. Oocysts were detected in 2/29 (7%) backwash samples, and coincided with detections in pool water. Colony counts of routine pool indicators were all zero apart from aerobic colony counts (ACC); these were satisfactory (≤10 cfu/ml) in 41/57 (72%) samples. There was no relationship between ACC failures (>100 cfu/ml) and *Cryptosporidium* detections.

To validate standard methods for oocyst detection in drinking water for pool water, we investigated oocyst recovery rates in samples spiked with 99 pre-stained *Cryptosporidium* oocysts (ColorSeedTM, TCS Biosciences). The mean recovery was 54.8% (range 46.5-61.6%), and well within the acceptable range for drinking water. The adjusted sample survey counts ranged from 0 to 2.11 oocysts per 10L, mean 0.08. Oocyst recoveries from backwash samples prepared by centrifugation, IMS and IFM and seeded with 99 pre-stained oocysts were more variable; mean 50.7% (range 0-82.9%).

Cryptosporidium risk was greatest when bather loads were highest. Acceptable oocyst recovery rates for pool water allow adjusted counts to be generated and could contribute to geographically-valid infection risk estimates, if UK swimmer behaviour data are obtained.

Funding: Pool Water Treatment Advisory Group

POSTER SESSION MORNING

Amphi 100B

WeB-P1-11h40/11h45

The enteric syndrome in calves, the consequence of infections with *Cryptosporidium parvum*, *Giardia duodenalis* and *Eimeria* spp. under production conditions, and the therapeutic efficacy of Azithromycin

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The investigations were carried out between July 2017 and August 2018 in the Parasitic Diseases Clinic of The Faculty of Veterinary Medicine Iași, and in a dairy cow breeding farm in the sub-mountain area, in order to determine the causes of mortality losses in calves with trabecular enteric syndrome, rebel to anti-infectious treatment. 144 calves were clinically and coproscopically examined; the necropsy exam was done in three cases. The fecal samples were collected by rectal stimulation, in sterile stool specimen containers. The parasitic elements were highlighted by smear method, flotation methods and immunodiagnostic. The smears were stained by the Romanovksi rapid method for *Giardia*, the modified Ziehl-Nielsen method for *Cryptosporidium* and the rapid Giadia Ag (VetExpert) test, for coproantigens.

The qualitative (Willis, Wisconsin) and quantitative (Flotac) flotation methods revealed the invasive elements consisting of protozoa and helminths. From the dead calves' intestine fragments were collected which were preserved in 10% formaldehyde, cut to 5 µm and stained by the Masson trichromic method. Examination and microphotography were performed on the Leica 750 DM photonic microscope.
Clinically, the diseased calves showed diarrhea with aqueous feces and blood strips, hemorrhagic feces with mucous membranes or gullible feces, rapid dehydration, refusal of food, prolonged decubitus, hypothermia and lethal end. In some cases, enteral disorders have been associated with neurological and bronchopulmonary syndromes. Coproscopically, *Giardia duodenalis* trophozoites and cysts were identified with a prevalence of 10.41% (15/144), *Cryptosporidium parvum* oocysts, with a prevalence of 37.5% (53/144) and Eimeria spp. oocysts with a prevalence of 23.61% (34/144). From the Eimeria genus, the species E. bovis, E. zuernii, E. subsferica, E. cilindrica, were morphologically identified Histopathologically, the intestinal mucosa revealed tissue destructions, severe atrophy with the disappearance of intestinal villi, blocking of glandular crypts and enterocytes with schizogonic and gametogonic stages of eimeries. The enteric syndrome that evolved in the calves had polyfactorial etiology in which the parasite protozoa *Cryptosporidium parvum*, *Giardia duodenalis* and Eimeria spp. had a significant contribution. The therapy with Azithromycin, a single dose of 500 mg/day, orally, for 3-5 days, has limited until stopping, the mortality in calves.

Key words: calves, enteric syndrom, polyfactorial etiology, *Cryptosporidium*, *Giardia*, Eimeria, therapy, Azithromycin.

WeB-P2-11h45/11h50

Drug Susceptibility Testing in *Giardia lamblia*: Cysteine strongly affects the effectivities of Metronidazole and Auranofin, a novel and promising antimicrobial

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The microaerophilic parasite *Giardia* lamblia annually causes hundreds of millions of human infections which are treated with antiparasitic drugs. Metronidazole is the most often prescribed drug but novel drugs with improved characteristics are constantly being developed. One of these novel drugs is auranofin, an antirheumatic relabelled for the treatment of parasitic infections. Drug effectivity is commonly assessed in susceptibility assays using in vitro cultures of a given pathogen. However, drug susceptibility assays can be strongly affected by certain compounds in the growth media such as cysteine. Cysteine is added in large amounts as an antioxidant but is generally highly reactive and known to modulate the toxicity of metronidazole in several microaerophilic parasites. When growing G. lamblia in an anaerobic cabinet, cysteine concentrations could be reduced to 20% of normal levels without affecting viability of the parasite. The IC50 for metronidazole was found to be clearly elevated in the presence of high cysteine concentrations. In the case of auranofin, the protective effect of cysteine was even more extreme, providing protection to concentrations up to 12-fold higher as observed in the presence of low-level cysteine. With three other drugs tested, the effect of cysteine was less pronounced. Oxygen was found to have a less marked impact on metronidazole and auranofin than cysteine but bovine bile which is standardly used in growth media for G. lamblia, displayed a marked synergistic effect with metronidazole

WeB-P3-11h50/11h55

Anti-*Giardia duodenalis* activity of nicotinamide

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The SIR2 family of NAD+ -dependent deacetylases, collectively called sirtuins, are proteins highly conserved from archaea to higher eukaryotes. Notably, sirtuins found in various human parasites (especially the Plasmodium, Trypanosoma, and Leishmania species) have shown to play relevant roles in key processes such as growth, proliferation, differentiation and survival under various conditions. Due to the fact that parasitic sirtuins have emerged as promising anti-parasitic therapeutic targets, in the present work we carried out a comparative analysis in silico of the five sirtuins identified in *Giardia duodenalis* together with a virtual screening focused in their interaction with NAD+ and nicotinamide, a non-competitive inhibitor of deacetylation activity of sirtuins. Structures obtained from homology modelling present a bilobed structure with the larger domain consisting predominantly of a modified Rossmann fold, found in several diverse NAD(H)/NADP(H) binding enzymes, and the minor domain that contains a structural zinc atom motif. Though some residues from the NAD+ binding site and the Zn-binding domain were highly conserved, overall the rest of the proteins structure is poorly conserved. Docking simulations of these proteins predicts nicotinamide binding, which suggests that *Giardia* sirtuins can also be inhibited by this drug. To evaluate this, *Giardia* intestinalis trophozoites (Assemblage A1, strain WB, ATCC 30957) viability was tested in presence of nicotinamide through MTT assays. These results revealed an anti-parasitic activity with nicotinamide alone or in combination with metronidazole. Furthermore, cell cycle analysis using propidium iodide showed that nicotinamide treatment decreased cell population in G1 phase and increased it in G2/M phase in a dose-dependent manner, which could suggest that DNA damage could be involved with arrest in G2/M phase and triggering cell death as has been shown for SIRT1, SIRT6 and SIRT7. Our data support a potential use of *Giardia duodenalis* sirtuins as targets for new drug discovery and treatments

CONFLICT OF INTEREST

The authors declare no conflict of interest.

WeB-P4-11h55/12h00

Cell-penetrating peptide dramatically improves the efficacy of Nitazoxanide in the inhibition of *Cryptosporidium parvum* growth

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Cryptosporidium parvum is one of the most common causes of diarrhea worldwide in neonatal calves and this pathogen is also life-threatening for humans; especially in malnourished children and immunodeficient patients such as HIV patients, transplanted-organs patients (Ventura et al., 1997, Kotloff et al., 2013). Currently, there is no vaccine and only one drug, Nitazoxanide (NTZ) of moderate efficacy (Schnyder et al., 2009), approved by FDA for *Cryptosporidium* treatment in human. In this study, we have investigated the potential of the cell-penetrating peptid (CPP) oligoarginine to increase the uptake of NTZ by cells and thereby to improve its efficacy. For this purpose, oligoarginine was synthetically attached to NTZ (NTZ-CPP) and tested for the inhibition of *Cryptosporidium* growth in comparison to the unmodified drug NTZ 24 hours post infection. Parasite growth was monitored by quantitative reverse transcription PCR after RNA extraction from C. *parvum* infected HTC-8 cells. Quantitative PCR was performed on the target gene 18S rRNA of C. *parvum* by primers Cp18S-1011F (5′-TTG TTC CTT ACT CCT TCA GCA C-3′) and Cp18S-1185R (5′-TCC TTC CTA TGT CTG GAC CTG-3′). Data were normalized to the expression of the housekeeping gene 18S rRNA of host cells with primers Hs18S-1F (5′-GGC GCC CCC TCG ATG CTC TTA-3′) and Hs18S-1R (5′-CCC CCG GCC GTC CCT CTT A-3′) (Zhang and Zhu, 2015). The percentage of inhibition of parasite reproduction obtained by NTZ and NTZ-CPP were calculated according to the ΔΔCt method. We observed a drastic improvement of drug activity by NTZ-CPP as compared to NTZ. These results are highly attractive and represent an encouraging step toward developing an alternative therapy for this relevant parasitic infection.

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WeB-P5-12h00/12h05

Histone deacetylase inhibitors affect growth and cell organization of *Giardia intestinalis* trophozoites

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Giardia intestinalis is a parasitic protozoan and the causative agent of giardiasis, a waterborne infectious disease. It presents two morphological stages during its life cycle the cyst and the trophozoits that are adapted to different environments conditions. *Giardia* trophozoits inhabit the upper portion of small intestine of mammalian host and attach to surface of epithelial cells, leading to parasitism. For over five decades, giardiasis treatment is based on the use of nitro compounds of which metronidazole is the most commonly used drug in many countries. The efficacy of the therapy varies according to the age and immunological conditions of the patient, period and dose, and may still cause several side effects. Parasite resistance to metronidazole has emerged over the decades, contributing to failure rates on the treatment. In this context, the need to develop new approaches to the chemotherapy of giardiasis is widely recognized. Epigenetic mechanisms as acetylation and deacetylation of histone proteins have become target of several studies because they are associated with different cellular processes. Histone acetylation is controlled by the activity of histone acetylases (HATs), which contribute to chromatin decondensation and activation of transcription, and histone deacetylases (HDACs), which promote chromatin condensation and gene silencing. Class I and III HDACs have been found in various parasitic protozoa (including G. intestinalis) where they were associated with antigenic variation regulation, proliferation, cytoskeleton dynamic and cell differentiation. In view of the biological role, inhibitors of histone deacetylases may become interesting alternatives for the treatment of parasitic diseases. In this study, effects of class I and III HDACs inhibitors KV-30, KV-46 and KH-TFMDI, (synthesized by Prof. Franz Bracher group) were evaluated on cell proliferation, viability, cell cycle and ultrastructure, as well as on histone acetylation and tubulin expression of the G. intestinalis trophozoites. These inhibitors decreased the trophozoites proliferation and viability at 24, 48 and 72 h of incubation. Scanning electron microscopy analysis revealed membrane surface alterations and flagella internalization. Results obtained by light microscopy methodologies and western blot indicated that KH-TFMDI, inhibitor of sirtuin I and II, did not affect microtubule cytoskeleton dynamics. These cells eventually died by a mechanism which lead to nuclear damage and the formation of multi-lamellar bodies as observed by transmission electron microscopy analysis and biochemical approaches. Taken together, these data show that class I and III HDACs inhibitors have significant effects against G. intestinalis trophozoites growth and structural organization and suggest that histone deacetylation pathway should be explored on this protozoon as target for chemotherapy.

WeB-P6-12h05/12h10

Efficacy of anti-diarrheal traditional plant used in Côte d'Ivoire against *Cryptosporidium parvum* in both in vitro and in vivo model

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Cryptosporidiosis are an important cause of diarrhoea. However in absence of an effective treatment, it is mainly treated symptomatically. This study aims to explore the traditional Ivorian pharmacopoeia to identify new effective compounds against *Cryptosporidium parvum*.
An ethnobotanical survey was conducted in different parts for the country, using a questionnaire focussing on anti-diarrheal therapeutic habits. Collection of the plants was done in collaboration with traditional practitioners and identification of the specimens was done at the National Floristic Center. For each plant decoction and hydroalcoholic extraction method were used on leaves and root bark. Extracts were dehydrated at 37°C for 48 hours. Cytotoxicity of products was first evaluated by Trypan blue method after 48h of contact with cell cultures. Anticryptosporidial effect of extracts was evaluated in vitro on HCT-8 cell lines and in vivo on CD-1 neonatal mice according to Mammeri et al., 2018. HCT-8 cells were infected with 104 oocysts of C. *parvum* (Iowa strain, Atlanta, USA), and incubated with plant extracts at a final concentration of 500 µg/mL during 48h. For in vivo test, five-day-old suckled mice (CD-1) were infected orally (by gavage) with 105 oocysts and treated by the same way at D-1, D+1, and D+4. To evaluate the infection load Counts of oocysts and endogenous *Cryptosporidium* were determined using immunofluorescence techniques. Sulfate of paromomycin, a drug used in veterinary medicine, has been used as a reference molecule. 18 plants were collected and identified, mostly from the North and West parts of the country. 40 extracts were obtained, out of which 28 were used for further investigations. In vitro, six (6/28) extracts showed greater efficacy (60-80% effectiveness compared to the positive control) than paromomycin (50-60% effectiveness compared to the positive control) at equal concentration (500 µg/mL). Cytotoxic of these products doesn't appear before 1000 µg/mL. At 100 µg/mL concentration BT12 and AC19 extracts still harboured equivalent efficacy with paromomycin (at 500 µg/mL). In vivo tests are in process to confirm the anticryptosporidial activity reported in vitro. This study confirms that traditional medicine can be an important source of new effective treatment against cryptosporidiosis.

Mammeri, M., Chevillot, A., Thomas, M., Polack, B., Julien, C., Marden, J.-P., Auclair, E., Vallée, I., Adjou, K.T., 2018. Efficacy of chitosan, a natural polysaccharide, against *Cryptosporidium parvum* in vitro and in vivo in neonatal mice. Exp. Parasitol. 194, 1–8. https://doi.org/10.1016/j.exppara.2018.09.003

Keywords: Anticryptosporidial, Pharmacopoeia, Immunofluorescence, Cytotoxicity, Cryptosporidiosis

WeB-P7-12h10/12h15

Screening of 61 plant extracts endemic in Madagascar for anti-cryptosporidial activity:

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Background: Diarrhea is one of the most common diseases in children, especially in developing countries with poor sanitation and/or poor personal hygiene standards. The protozoan *Cryptosporidium* is a leading cause of diarrhea morbidity and mortality in children younger than 5 years. Infection leads to self-limited diarrheal disease in immunocompetent individuals and can be life threatening in immunocompromised patients. Currently, nitazoxanide is the only proven anti-parasitic treatment for *Cryptosporidium* infections. However, it is not effective in severely immunocompromised patients. Medicinal plants are globally valuable sources of new drugs. In developing countries, large sections of the population who cannot afford or access formal health care systems, still rely on traditional practitioners and herbal medicines which are financially affordable and generally effective for their primary care. The aim of this study is to investigate the anticryptosporidial properties of 56 endemic plants in Madagascar.

Materials and Methods: Fresh C. *parvum* oocysts (10^4) purified from infected calves faeces (INRA, Nouzilly, France) using immunomagnetic separation were inoculated onto HCT-8 cell monolayers. 61 Crude methanol extracts obtained from 56 endemic plants in Madagascar were tested for potential anti-cryptosporidial activity by inhibiting the ability of *Cryptosporidium* oocysts to infect HCT-8 cells. Each plant extract was tested in duplicate at different concentration ranging from 0.031mg/ml to 1mg/ml. Treated wells were harvested at 48hrs post-inoculation and DNA was extracted. CC-qPCR assay was performed for evaluation of infectivity. Oocysts inoculation onto wells with and without HCT-8 cells were used as *Cryptosporidium* proliferation and inhibition of growth controls respectively.

Results: Out of 61 plant extracts, 30 were completely processed, the remaining extracts are currently being analyzed. 8/30 seem to have anti-cryptosporidial activity as Ct value from wells corresponding to these extracts are similar from wells without HCT-8 cells but inoculated with oocysts.

Conclusion: This study contributes new data to the existing knowledge of anti-diarrheal especially the anti-cryptosporidial activity of endemic flora of Madagascar. Additional tests should be performed to confirm this activity. Other common parasitic agents of diarrhea will be included in a new screening test.

WeB-P8-12h15/12h20

Standardization of single-tube nested real-time PCR and genetic sequencing for detection and species characterization of avian *Cryptosporidium* spp

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Cryptosporidium spp. causes gastrointestinal and respiratory diseases in domestic and wild birds. For detection and identification of *Cryptosporidium* spp. in fecal samples, conventional two-step nested PCR assays followed by agarose gel electrophoresis and genetic sequencing are usually performed. The aim of this study was to standardize a single-tube nested real-time PCR assay followed by genetic sequencing to detect and characterize avian *Cryptosporidium* species and genotypes. The assay was performed to amplify a fragment of ~ 430 bp of 18S rRNA gene in the CFX96 Real-Time PCR Detection System (Bio-Rad) using SsoFast EvaGreen Supermix (Bio-Rad), two sets of primers with different annealing temperatures, and genomic DNA samples originated from avian *Cryptosporidium* species and genotypes. Conditions of the first and second cycling, such as primer concentrations and time and temperature of annealing and extension steps, were optimized. The conditions of the assay were: 98 ºC for 2 min was first followed by 20 cycles of denaturation at 98 ºC for 5 s and annealing/extension at 70 ºC for 30s. This was followed by 35 cycles of denaturation at 98ºC for 5 s, annealing at 63 ºC for 5 s, and extension at 72º C for 30 s. Fluorescence signal acquisition occurred at the second cycling step. Melting curve analysis was performed from 70 °C to 95 °C. Amplified fragments were purified using ExoSAP-IT (Thermo Fisher Scientific) and submitted to bidirectional sequencing for confirmation of *Cryptosporidium* species/genotype. PCR products were also analyzed by agarose gel electrophoresis to check the size of DNA band and absence of non-specific amplification. Assay sensitivity was determined by the analysis of DNA extracted from diluted suspensions of C. *parvum* oocysts counted in a Neubauer chamber. The detection limit was approximately 0.5 oocyst (2 sporozoites) per reaction. No nonspecific amplification was detected by agar gel electrophoresis, melting curve analysis, and genetic sequencing. Although melting temperature analysis was useful for checking assay specificity, genetic sequencing must be performed for identification of *Cryptosporidium* species or genotype. Our results show that single-tube nested real-time PCR could be used as an alternative to conventional nested PCR with the advantages of lower turnaround time and lower risk of carry-over contamination. However, studies with larger number of samples from different locations and from many species of birds are needed to further validate the assay for avian samples.

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WeB-P9-12h20/12h25

A four-plex qpcr-hrm assay for the detection and quantification of human diarrhea parasites

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Intestinal parasitic diseases occur worldwide and their diagnosis poses considerable challenges, especially in low-endemicity countries. *Cryptosporidium* spp., Entamoeba histolytica, *Giardia* intestinalis, (and, arguably, Blastocystis spp.) are among the most important and common parasitic protozoans causing diarrhoea. Microscopic diagnosis of these parasites is limited by its sensitivity and specificity as compared to molecular methods. Several multiplex real time PCR assays have been developed to diagnose these important parasites however most assays included the use of hydrolysis probes, increasing the cost of stool examination. In this study, a real time PCR protocol followed by a high-resolution melting (HRM) curve analysis of the PCR products has been designed to diagnose these parasites in a 4-plex reaction. Clinical samples (143) with laboratory diagnostic data were blindly used to test the method. The melting temperatures were 75°C, 78°C, 80°C, 81.5°C and 83.5°C for E. histolitica, *Cryptosporidium* spp., Blastocystis spp., G. Intestinalis assemblage A and G. Intestinalis assemblage B. Comparing the 4-plex results to those of the laboratory diagnostic, the data were concordant at 95%.

Good sensitivity and specificity were achieved using clinical specimens and, if needed, each of the respective infections can be detected in a single-plex format using the relevant primer-pair of the four-plex panel. This convenient and cost-effective method could contribute to a quicker, yet accurate, diagnosis and more precisely targeted therapies of parasite derived diarrhoea.

WeB-P10-12h25/12h30

Validation and application of a salivary antibody assay for measuring exposure to *Cryptosporidium*

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Oral fluid (saliva) is a useful potential alternative specimen to blood serum for the evaluation of antibody responses to infection. Antibodies to gastro-intestinal pathogens from saliva have been reported to be good indicators of recent infection, including *Cryptosporidium* spp. (Egorov et al, 2010). We have previously presented the adaptation of an assay reported by Griffin et al (2011) that used recombinant *Cryptosporidium* proteins-coupled to magnetic microspheres to detect anti-*Cryptosporidium* antibodies in cryptosporidiosis cases (at IGCCV, Uppsala, 2014). That work demonstrated that the assay was reproducible and had an acceptable dynamic range. We have now investigated the assay by comparing IgG responses to recombinant *Cryptosporidium* 15 kDa sporozoite antigens in two longitudinal studies.

In the first phase of the investigation, serum and saliva samples from 11 laboratory confirmed cases of cryptosporidiosis were tested at up to six time points over an 18-month period post-diagnosis. Antibody responses measured in both serum and saliva peaked <60 days post diagnosis. There was no significant difference between sera and saliva (Kruskal-Wallis H (equivalent to Chi square) P=0.10, respectively). The assay provides a means of testing saliva as a good alternative to serum for detecting recent exposure to *Cryptosporidium*.

In the second phase, we used the salivary antibody assay to compare the intensity of IgG responses from a known exposed population (a class of Veterinary Science students who experienced an outbreak of C. *parvum* following calf handling classes, n=56 recruits) and a presumed unexposed, comparison group (Business Studies students who did not undertake animal handling classes, n=30 recruits). Samples were collected at four time points after the outbreak: 2, 6, 12 and 15 months. At +2 months IgG responses were significantly higher in the exposed group than the comparison group. Although retention among the comparison group in particular was poor, sufficient recruits remained for analysis. At +6 and +12 months, IgG responses remained higher in the exposed group than the comparison group. At +15 months, no samples were available for analysis from the comparison group; the IgG response in the exposed group had declined at each time point from that observed at +2 months.

References

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WeB-P11-12h30/12h35

Evaluation of LAMP detection of *Giardia* DNA for microfluidic environment

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Giardia duodenalis is one among the most important human protozoan parasites causing gastrointestinal illness worldwide. The clinical characteristics of acute human giardiasis include abdominal cramps, diarrhoea, nausea and weight loss. These symptoms may persist for a few weeks or become a chronic reoccurring disease G. *duodenalis* infection in cattles, goats, sheeps and humans can cause nutrient malabsorption which can result in a reduction of weight gain. There is no doubt that *Giardia* cysts are distributed in wastewater treatment facilities and surface waters globally. Among food-borne diseases, giardiasis causes a considerable burden at the global level. Thus, a reliable, cheap and fast parasite identification in different matrices (faecal material, water or food samples) is a key in risk management. Microscopic examination of stool specimens and water pellets remains the cornerstone of diagnostic testing for *Giardia* species, although molecular methods in all matrices and immunological assays in faecal samples can effectively replace microscopic approaches. DNA amplification methods are reliable ways to identify the DNA of *Giardia* and isothermal amplification methods overcome the problem of thermocycling. Microfluidic devices are the optimal solutions to miniaturize and automate standard DNA amplification methods including significantly reduced volume of expensive reagents, manual steps and reaction time. For waterborne protozoan pathogens a high-throughput concentration and separation microfluidic system has been presented by Jimenez et al. in 2016. The maximum recovery rate from drinking or distilled water was 86% for *Giardia duodenalis* cysts. In this work we tested and compared all published *Giardia* LAMP (loop-mediated isothermal amplification) protocols, furthermore we optimized them for microfluidic environment. Optimization included the careful selection of temperature range and adjusting reaction components concentration in the reaction mixture in order to obtain the lowest detection limit and shortest reaction time. In case of positive LAMP reaction, the turbidity change can be detected by the naked eye or adding fluorescent dye to produce a color change in the solution enabling endpoint detection. The optimized and evaluated LAMP protocol is rapid, reliable and cost-effective for Lab-on-a-chip platforms to identify *Giardia* species in different origin of samples.

WeB-P12-12h35/12h40

Validation of a semi-quantitative real-time PCR assay for the diagnosis and the monitoring of *Giardia intestinalis* infection in canine faeces samples according to the French standard NFU47-600-2

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Infection of young dogs with *Giardia* intestinalis is very common in France (prevalence of around 30% in puppies under 6 months of age) and is a major clinical problem, especially in breeding facilities. The diagnosis is currently performed using ELISA (pen side test) which do not give quantitative information, or by visualization and numeration of the cysts by microscopy after concentration of faeces. But this method remains manipulator dependent with a lack of detectability notably for chronic infection.

In order to obtain a reliable, sensitive and semi-quantitative diagnosis that can easily be carried out in a routine laboratory, we have developed a complete tool combining firstly a ready-to-use quantitative duplex real-time PCR kit (qPCR Premium® *Giardia* intestinalis ; BioDev) to detect all *Giardia* intestinalis assemblies A to F (beta-giardin target) by FAM labelling and an exogenous internal positive control (IPC) by Cy5 labelling, and secondly a method of preparation samples and extraction-purification of nucleic acids to obtain an optimized yield (BioPrep® *Giardia* and BioExtract® column, BioSellal). This tool has been validated according to the recommendations of the French standard NFU47-600-2. Thus, for the PCR part, the following characteristics were determined: LODPCR (5 copies / PCR), Efficiency (99%) and Linearity Domain (between 10 copies corresponding to the LOQPCR and 1.106 copies / PCR). Various modes of faecal preparation were compared in terms of the extraction yield of known faeces positives for *Giardia*: either mechanical lysis, or thermal and chemical lysis, or mechanical lysis followed by thermal and chemical lysis; or no pretreatment at all before nucleic acids extraction-purification. It is the association of mechanical and thermal-chemical lysis that reproducibly gives the best extraction yield.

Finally, the complete method part was characterized by the determination of the diagnostic sensitivity and specificity. We analyzed a hundred dog faeces with or without an evocative clinic in order to determine an infection threshold of clinical interest making it possible to distinguish between a chronic carriage and a clinical infection.

WeB-P13-12h40/12h45

Effect of potassium dichromate medium on preservation of *Giardia duodenalis* cysts in faecal specimens of three different livestock species

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Background
High quality DNA is required for producing reliable and reproducible results in PCR-based diagnosis of enteropathogens. When DNA extraction and purification from fresh faecal specimens is not technically or logistically feasible, storage media become particularly handy. Among them, potassium dichromate is particularly suited for the medium to long-term preservation of stool samples. Here we assess the efficacy of this medium for preserving *Giardia* cysts in faecal material of three different livestock species for up to three years.

Methods
A total of 45 faecal samples from Algerian cattle (n = 9), sheep (n = 27) and goats (n = 9) with a positive result for G. *duodenalis* by conventional microscopy after the zinc sulfate flotation concentration technique were stored in 2.5% potassium dichromate at 4 ˚C for 1, 2 or 3 years. DNA extraction and purification were carried out after extensive washing of samples to remove the medium. The presence of the parasite was then re-assessed by a real-time PCR method.

Results
Overall, *Giardia*-positive results by qPCR were obtained in 100%, 93.3%, and 75.0% of the samples after 1, 2, and 3 years storage in 2.5% potassium dichromate (Table 1). All faecal samples from cattle origin tested positive by qPCR after 2 years storage. All faecal samples from sheep origin tested positive by qPCR after 1- or 2-years storage, but this percentage decreased to 75% after 3 years storage. All faecal samples from goat origin tested positive by qPCR after 1-year storage, but only 68% after 2 years storage. G. *duodenalis* cysts in goat´s stool samples may be more sensitive to long-storage periods (>2 years) in 2.5% potassium dichromate medium than those in cattle´s and sheep´s samples.

Table 1. Number of faecal samples from livestock species with a *Giardia*-positive result by conventional microscopy (CM) or real-time PCR (qPCR) after 1, 2- or 3-years storage in 2.5% potassium dichromate.

Host		1 year			2 years			3 years		
	Total	CM	qPCR	%	CM	qPCR	%	CM	qPCR	%
Cattle	9	0	0		9	9	100	0	0	
sheep	27	16	16	100	3	3	100	8	6	75
Goat	9	6	6	100	3	2	66.7	0	0	
Total	45	22	22	100	15	14	93.3	8	6	75

Conclusions
Our data indicate that 2.5% potassium dichromate is an effective media to preserve faecal samples from livestock origin for downstream molecular procedures for at least 1 year. The host origin and composition of the faecal sample may influence the preservation of *Giardia duodenalis* cysts.

WeB-P14-12h45/12h50

Evaluation of the FTD stool parasites Fast track diagnostic kit

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Background: Molecular diagnostic kits in parasitology are rising from recent years. Today, many kits are available for both *Giardia lamblia*, *Cryptosporidium* species and/or *Entamoeba histolytica* DNA investigations. Accordingly, to the specific expert assessment of the French National reference center expert laboratory, we evaluated performances of the FTD stool parasites fast track diagnostic kit.

Method: 24 DNA extracts obtained from clinical fecal samples were tested according to manufacturer's instructions of the FTD stool parasites Fast track diagnostic kit. Extracts were perfomed using QiaAMP DNA power fecal kit (Qiagen). The real time PCR was performed on CFX 96 (biorad). Finally, 4 extracts of *Cryptosporidium hominis* (*C. hominis*), 6 of *C. parvum*, 1 of *C. felis*, 1 of *C. canis*, 1 of *C. xiaoi*, 1 of *C. ubiquitum*, 5 of *Giardia lamblia* and 5 mix (*Giardia lamblia* +/- *Cryptosporidium* sp.) were evaluated.

Results: obtained results were satisfying. No cross-reaction was detected. All *Giardia Lamblia* positive extracts were detected; only the *C. xiaoi* DNA extract was not detected. The evaluated *C. parvum* DNA extracts corresponded to a range of concentration from 10^5 to 1 oocyst(s)/mL and were all detected underlying an optimal limit of detection.

Conclusion: results suggests that the FTD stool parasites fast track diagnostic kit harbored very satisfying performances and could be used in routine for *Giardia* and *Cryptosporidium* species DNA detection.

WeB-P15-12h50/12h55

Multicentric evaluation of the real-time PCR assay Amplidiag Stool Parasites for *Cryptosporidium* spp. detection in human stools *Cryptosporidium* National Network

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Cryptosporidium spp. are protozoan parasites that cause widespread gastrointestinal illness. Parasitic gastroenteritis laboratory diagnosis based on microscopy in combination with various staining methods are time consuming. Rapid and accurate high-quality laboratory detection methods for the identification of etiologic agents associated with the disease are urgently needed for proper surveillance and appropriate treatment.

This study was to analyse performance of the qPCR Amplidiag Stool Parasite Kit (Mobidiag, Finland) for molecular detection of *Cryptosporidium* spp. oocysts on selected samples. The sensitivity and specificity of this new PCR assay was assessed on a multicentric basis (3 geographically distinct clinical sites in France), using well-characterized positive and-negative human stool samples, and the efficiencies of extraction methods were assessed.

Ten DNA extracts from 200µL PBS or 200mg negative stools seeded with 100, 200, 500, 1000 or 10000 C. parvum oocysts respectively, 40 DNA extracts from *Cryptosporidium* positive stool samples (*C. hominis, C. parvum*, *C. felis*, *C. cuniculus, C. canis, C. meleagridis*, C. genotype tamia, and new undescribed C. genotype) provided by the Crypto-Anofel network swere analyzed. In order to study the specificity of the kit, extracts from 3 *Enterocytozoon bieneusi* positive stool samples, 10 Giardia duodenalis positive stool samples and 10 Candida albicans positive stool samples were analysed; 100 negatives samples, extracted following the manufacturer's instructions and 20 raw positive samples with *Cryptosporidium*, were sent blindly to the participating laboratories

Regardless of the number of oocysts in the seeded samples, they have all been found positive for both *Cryptosporidium*. Of the 40 DNA from Cryptosporidium positive samples, 3 samples with *Cryptosporidium* was found negative in one of the 3 participating laboratories and positive by two laboratories. For each laboratory, the specificity of the kit is 100%, and the sensitivity varies from 94.12% to 100%. A consensus on Cryptosporidium qPCR results gives a sensibility of 98.04% and a specificity of 100%. The interlaboratory variability was examined, ANOVA showed no laboratory effect (p=0.238) attesting to the good reproducibility between laboratories. *Cryptosporidium* DNA extraction methods applied by each of the participating laboratories with the 20 raw positive samples does not affect the results, they are all found positive

Conclusion

To conclude, this one-step quantitative PCR is well suited to the routine diagnosis of cryptosporidiosis since practical conditions, including DNA extraction, have been positively assessed. With a positive and negative predictive value of 100% and 99.01% respectively, the kit can be used for the detection of Cryptosporidium.

POSTER SESSION AFTERNOON

Amphi 100A

WeA-P17-14h00/14h05

Cohort study of associations between intestinal protozoa infection and intestinal barrier function, nutritional status, and neurodevelopment in infants in São Tomé

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Giardia lamblia and *Cryptosporidium* are prevalent etiologic agents of enteric infections in infants from LMIC. Host-parasite interactions lead to mucosal inflammatory response and increased intestinal permeability. Clinically this result in a negative impact on growth and neurodevelopment. This study aimed to analyze the associations between enteric parasitic infections and intestinal barrier function, nutritional status and neurodevelopment in asymptomatic infants in Sao Tome. A birth cohort study with a follow-up until 24 months of age was implemented. Anthropometry was assessed monthly including attained growth (weight-for-length z- score, length-for-age z-score – LAZ, and length-for-age difference – LAD), growth velocity (weight velocity z-score – WAVZ, and length velocity z-score – LAVZ), and undernutrition (wasting and stunting, using <-1SD cut-off). Neurodevelopment was screened using Bayley Infant Neurodevelopmental Screening score. Fecal biomarkers for intestinal inflammation (S100A12) and permeability (A1AT) were measured at 24 months of age. Enteric parasites were examined quarterly using microscopic techniques. Different statistical models were used to explore associations between enteric parasitic infections and intestinal barrier function, nutritional status, and neurodevelopment. A total of 475 neonates were enrolled and 280 infants completed 24 months follow-up. *Giardia* lamblia and helminths were the most prevalent parasites. The multivariable analysis showed 1) infants with *Giardia* lamblia and helminths infections had a tendency toward an increase of 23.6 % and of 24.1 % in the inflammatory biomarker, respectively; those infected by any enteric parasite had a tendency toward an increase of 33.6% in the permeability biomarker; additionally, this biomarker was 100% higher in wasted infants and 50% higher in those stunted; 2) infants with *Giardia* lamblia and helminths infections showed a significant association with a decrease in linear growth (by -0.10 and -0.16 of LAZ and by -0.32 and -0,48 of LAD, respectively); those with *Cryptosporidium* spp. infection displayed a significant association with a decrease in weight and length velocities (-0.43 WAVZ and -0.55 LAVZ); 3) *Giardia* lamblia infection and stunting were independently and significantly associated with a 1.69 and 2.37 increased risk of poor development, respectively. The underestimated role of enteric parasites as etiologic agents of subclinical infections was confirmed. These parasitic infections showed a tendency of association with intestinal barrier dysfunction and significant associations with decreased linear growth and risk of poor neurodevelopment.

WeA-P18-14h05/14h10

Prevalence of intestinal parasites and associated risk factors in primary school children in lahore, pakistan

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Intestinal parasites are the major reason for morbidity and deaths all over the world especially in the third world. Poverty, lack of education, poor sanitation, unclean drinking water, hot and moist environment are the conditions responsible for these parasitic ailments. Poor personal hygiene among children is considered an effective cause of parasitic invasion. In present study the prevalence of intestinal parasites and their associated risk factors were determined among the primary school children of Lahore, Pakistan. A total of 150 faecal samples were collected from the children of 3-15 years of age belonging to Private and Government Schools. Different techniques like Direct smear method, Formalin ether concentration technique, Sedimentation technique and McMaster were used to identify different stages of intestinal parasites. Different stages of parasites were identified by these techniques. The parasites found were *Giardia* lamblia (4.66%), Entamoeba histolytica (3.3%), Ascaris lumbricoides (4.66%), Taenia saginata (4%), Hymenolepis nana (2%), Trichuris trichura (2.66%) and Enterobius vermicularis (4%). A. lumbricoides was found to the most frequent of all parasites. The prevalence was found to be more among the individuals, with poor hygiene, having lack of education especially of mothers because they play a big role in child's upbringing and maintaining his good health. Educating cleanness alertness on parasitic diseases and application of helpful strategies for parents to raise socioeconomic circumstances may decrease the load of infection.

Key Words: Prevalence, intestinal parasites, associated risks, personal hygiene

WeA-P19-14h10/14h15

Retrospective analysis of *Cryptosporidium* species in Western Australian human patients (2015-2018), and emergence of the C. *hominis* IfA12G1R5 subtype

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Cryptosporidium species are a major cause of diarrhoea worldwide. In the present study, a retrospective analysis of 109 microscopically *Cryptosporidium*-positive faecal specimens from Western Australian patients with gastrointestinal symptoms, collected between 2015 and 2018 was conducted. Sequence analysis of the 18S rRNA and the 60 kDa glycoprotein (*gp60*) gene loci identified four *Cryptosporidium* species; *C. hominis* (86.2%, 94/109), *C. parvum* (11.0%, 12/109), *C. meleagridis* (1.8%, 2/109) and *C. viatorum* (0.9%, 1/109). Subtyping at the *gp60* locus identified the emergence of the previously rare *C. hominis* IfA12G1R5 subtype in 2017 as the dominant subtype (51.2%, 21/41). This subtype has also recently emerged as the dominant subtype in the United States but the reasons for its emergence are unknown. This is also the first report of *C. viatorum* in humans in Australia and a novel subtype variant (XVaA3g) was identified in the one positive patient.

WeA-P20-14h15/14h20

Cryptosporidium chipmunk genotype I – an emerging cause of human cryptosporidiosis in Sweden

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Cryptosporidium infection has been a notifiable disease in Sweden since 2004 and molecular typing of cases has been conducted within different studies and occasionally during outbreaks and source tracking. Between 2006 and 2017, we identified eight sporadic cases of human infection with *Cryptosporidium* chipmunk genotype I whose natural hosts are mainly chipmunks, squirrels and deer mice. However, this species is known to infect humans as well and is considered an emerging human pathogen in the USA.

In 2018, the Public Health Agency of Sweden initiated a microbiological surveillance program for *Cryptosporidium*. The aim is to determine species and subtypes of all domestic cryptosporidiosis cases in order to better understand the national epidemiology as well as to detect outbreaks.

Between August and November 2018 eight cases of *Cryptosporidium* chipmunk genotype I infected in different parts of the country were identified. It was the third most common species (3.5 %) of all investigated cases infected in Sweden during 2018, only C. *parvum* (84.5 %) and C. *hominis* (8.8 %) occurred more frequently.

All samples were investigated at the small subunit rRNA and the polymorphic 60 kDa glycoprotein (gp60) genes. The same gp60 subtype, XIVaA20G2T1, which has only been reported from Sweden, was identified in all successfully subtyped isolates. This includes a case of zoonotic transmission between squirrels and a woman working at a small animal rehabilitation center, as confirmed by analysis of both human and squirrel fecal samples.

In Europe only one human case of *Cryptosporidium* chipmunk genotype I, from France, has been reported outside Sweden, but the organism has been identified in red and grey squirrels as well as Pallas squirrels from Italy. There are only red squirrels in Sweden and, as all the isolates (humans and squirrels) harbored the same subtype, it is likely that the human cases were infected by this host. However, the prevalence of *Cryptosporidium* chipmunk genotype I among squirrels in Sweden is unknown and needs to be investigated.

Cryptosporidium chipmunk genotype I was the third most common species causing human cryptosporidiosis in Sweden both during the newly established surveillance program as well as in earlier studies. It is therefore important to follow its public health significance by continuous monitoring through the national microbiological surveillance program.

WeA-P21-14h20/14h25

Presence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in asymptomatic schoolchildren and symptomatic subjects seeking medical attention in the province of Zambezia, Mozambique

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Background
Giardia duodenalis and *Cryptosporidium* spp. are among the most important diarrhoea-causing agents in Sub-Saharan countries. Infections by these pathogens disproportionally affect children aged 30 suggestive of light parasite burdens. Molecular analyses revealed the presence of G. *duodenalis* sub-assemblages BIII (20%, 1/5) and BIV (80%, 4/5), and three different species of *Cryptosporidium* including C. *hominis* (40%, 4/10), C. *parvum* (40%, 4/10) and C. felis (20%, 2/10).

Conclusions
Our data indicate that G. *duodenalis* was a common finding both in symptomatic and asymptomatic children in the province of Zambézia, mostly causing light infections. This fact explain, at least partially, the failure to genotype most of these samples. *Cryptosporidium* infections were far less common. The finding of two infections by C. felis strongly suggest a zoonotic transmission event from dogs.

Funding
This study was funded by the ISCIII under project PI16CIII/00024.

WeA-P22-14h25/14h30

Genetic diversity of *Giardia duodenalis* and *Cryptosporidium* spp. in symptomatic individuals attending public hospitals in Spain. A multicentre study

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Background
Giardia duodenalis and *Cryptosporidium* spp. are important contributors to the global burden of gastrointestinal illness. Infections by these pathogens affect more frequently individuals living in poor-resource settings, but also represent a serious public health concern in industrialized countries. Multicentre, molecular-based studies targeting individuals with gastrointestinal symptoms are important to ascertain the molecular diversity of G. *duodenalis* and *Cryptosporidium* in clinical populations.

Methods
Stool samples from clinical patients with a positive result for G. *duodenalis* and *Cryptosporidium* spp. by microscopy, immunochromatography, and/or PCR in public hospitals of the Autonomous Regions (AR) of Madrid (6), La Rioja (1) and Comunidad Valenciana (1) were collected during January 2017-December 2018. G. *duodenalis*-positive samples were confirmed by qPCR and subsequently assessed by multi-locus sequence genotyping of the gdh and bg genes. *Cryptosporidium* species and sub-genotypes were investigated at the gp60 and ssu rDNA loci.

Results
Stool samples positive for G. *duodenalis* (n=642) and *Cryptosporidium* spp. (n=266) from the hospitals 12 de Octubre (128+32), Fundación Jiménez Díaz (27+13), La Paz (66+10), Principe de Asturias (16+2), Puerta de Hierro (177+37), and Severo Ochoa (98+42) in the AR of Madrid, La Fe (92+18) in the AR of Comunidad Valenciana and San Pedro (38+112) in the AR of La Rioja were collected. Overall, 41.7% (268/642) and 62.8% (167/266) of the *Giardia*- and *Cryptosporidium*-positive samples were successfully genotyped. *Giardia* infections were caused by assemblages B (71%), A (28%), or mixed infections of both (1%). Sub-assemblages BIV (49%, 131/268) and AII (19%, 52/268) accounted for most of infections within assemblages B and A, respectively. Three *Cryptosporidium* species were detected including C. *hominis* (75%, 125/167), C. *parvum* (24%, 40/167), and C. meleagridis (1%, 2/167). Sub-genotyping data at the gp60 marker were available for 77% (127/167) of the samples, revealing the presence of families Ia (1%), Ib (53%), Id (2%) and Ie (1%) within C. *hominis*, IIa (16%) and IId (3%) within C. *parvum*, and IIIb (1%) within C. meleagridis. IbA10G2 (47%, 78/167) and IIaA15G2R1 (11%, 18/167) were the most prevalent sub-genotypes circulating in the surveyed population.

Conclusions
In line with previous European molecular studies, G. *duodenalis* sub-assemblage BIV and C. *hominis* sub-genotype IbA10G2 are responsible for most of the symptomatic cases of giardiosis and cryptosporidiosis identified in Spain. Absence of animal-specific species/genotypes in the surveyed clinical population seem to indicate that giardiosis/cryptosporidiosis must be primarily of anthroponotic origin.

Funding
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WeA-P23-14h30/14h35

The epiCrypt study protocol: investigating household transmission of *Cryptosporidium* in England and Wales

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Cryptosporidium causes 3-4,000 cases of diagnosed illness in England and Wales every year. Outbreaks may only represent a small proportion of cases, and the amount of sporadic disease has not been sufficiently established. Contact with infected people is a risk factor and secondary cases may represent an underestimated and unreported burden of sporadic disease which could be prevented.

There are no published household-level studies in the UK which ascertain the frequency of, risk factors for, or pathways to, on-going spread of infection.

The epiCrypt study is a year-long observational study to identify secondary spread in 400 households with a case of *Cryptosporidium*. This project will estimate the amount of secondary spread that occurs in the home and potentially identify asymptomatic infections which might have a role in transmission.

The study team identify cryptosporidiosis cases in North West England and Wales and invite them and their household to take part. Each household completes a questionnaire and each household member is asked to provide a stool sample. Clinical, demographic, and household variables are collected and analysed to investigate associations with secondary spread.

Stool samples of participating household members are tested for *Cryptosporidium* at the *Cryptosporidium* Reference Unit; samples are scored against the Bristol stool scale, and screened and quantified using immunofluorescence (IF) microscopy and real-time PCR. Positive samples are speciated using validated real-time PCR for C. *parvum* and C. *hominis* or n18s PCR for all *Cryptosporidium* spp. and genotypes. To characterise patterns of transmission, *Cryptosporidium* DNA will be retained for subtyping and possibly whole genome sequencing (WGS).

The study began in Winter 2018 with a pilot phase to evaluate study design. Recruitment processes are working, viable samples are being collected, and laboratory protocols are feasible. We have successfully located original index case samples from the reference laboratory which allows us to establish *Cryptosporidium* species of the index case, observe dates of symptomatic illness in the home, and comment on likely direction of infection between people in individual households.

The study has been approved by the North West – Liverpool East NHS Research Ethics Committee (Reference: 18/NW/0300) and the Confidentiality and Advisory Group (Reference 18/CAG/0084).

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WeA-P24-14h35/14h40

Cryptosporidiosis in French nationals traveling abroad (2015-2018)

Costa D[1,2], French national network on surveillance of human cryptosporidiosis, Gargala G[1,2], Dalle F[3], Razakandrainibe R[1,2], Favennec L[1,2].

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Since January 2015, an online procedure was available to correspondent members of the French Centre National de Référence -Cryptosporidioses (CNR-C) to report confirmed cases of human cryptosporidiosis. From 2015 to 2018, a total of 6 different *Cryptosporidium* species were identified, *i.e.* *C. parvum*, *C. hominis*, *C. felis*, *C. meleagridis*, *C. ubiquitum* and *C. erinacei* representing respectively 70, 28, 0.8, 0.4, 0.4 and 0.4% of all species (n=235). *C. hominis* were observed in 51% in French travellers and *C. parvum* in 44% (N=39). The representations of *Cryptosporidium gp60* subtypes of isolates from patients differed from those of non-travelers. As in the entire population, IbA10G2 and IaA22R2 *C. hominis* gp60 subtypes were dominant in travelers but not the IIaA15G2R1 *C. parvum* genotype. In travellers 24 different *Cryptosporidium* subtypes were reported (vs 52 in non-travelers) and 10 subtypes were exclusively present in travelers. Present results reflect the large diversity of anthroponotic *Cryptosporidium* species and the predominance of *C. hominis* worldwide, which contrast with the predominance of *C. parvum*, (especially with the IIaA15G2R1 *gp60* subtype) in human cryptosporidiosis cases due to infectious contacts in France.

WeAP25-14H40/14H45

Epidemiology of human giardiasis**. A 14 years survey**

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The purpose of the research was to look for novel epidemiological data on the prevalence and clinical presentation in a long-time interval in a specific area. All patients admitted to a tertiary center for infectious diseases serving an entire county were tested for *Giardia* infection over a period of 14 years. The positive cases were recorded and analyzed regarding demographic data, month of incidence, clinical complaints. Longitudinal trends were assessed. In the total number of 54,623 patients studied, the incidence of giardiasis was 4.47%. The annual incidence presented a descending trend. The most frequent cases of giardiasis were recorded in patients aged 15-44 years, in females (F:M ratio 1.5), and in urban areas. The most frequent symptoms were the loss of appetite (71.24%) and abdominal pain (69.07%). Monthly incidence was higher in June (10.20%), July (10.65%) and August (10.49%). This epidemiological study carried out on a long interval in a specific area shows changes in the yearly prevalence, monthly incidence and demographic characteristics, allowing a better knowledge of *Giardia* spp. Infection

Keywords : *Giardia*; giardiasis; Prevalence; Digestive symptoms; Parasitic intestinal diseases; Zoonoses

WeA-P26-14H45/14H50

Genetic Diversity of *C. hominis* in an Urban Slum Population in Southern India

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Introduction: *Cryptosporidium* spp. is one of the commonest causes of diarrhea in children in low to middle income countries. In India alone, cryptosporidiosis has been estimated to contribute to 3.9-7.1 million diarrheal episodes each year in children under 2. Community based cohort studies on cryptosporidiosis in early childhood have been carried out in a population of ~45,000 people in a 2.6 sq km area of semi-urban slum communities in Vellore, Southern India for over a decade. Methods: Between 2008 to 2011, 176 children residing in this area were enrolled in a study on the effect of bottled drinking water on cryptosporidiosis and were followed up from weaning till the age of 2 years. Subsequently, between 2009 to 2013, 410 children were enrolled in a birth cohort study on immune response to cryptosporidiosis and were followed up till the age of 3 years. In both studies, diarrheal and monthly surveillance stools were collected and species and subtypes were determined by PCR and sequencing of the SSU rRNA and gp60 loci. Results: ~70% of children had at least one episode of cryptosporidiosis detected by PCR with a predominance of asymptomatic infections. Multiple infections were documented in 40-60% of children. *Cryptosporidium* spp. was associated with 8-9% of all diarrheal episodes and was more severe and of longer duration than non-cryptosporidial diarrhea. C. *hominis* was the predominant species (70-80%) followed by C. *parvum* (12-17%). Other zoonotic isolates identified were C. felis, C. meleagridis and C. muris. C. *hominis* gp60 subtyping with 239 samples typed showed that nearly half were IE (47.2%) followed by IB (20.5%), IA (13.4%) and ID (10%). When tandem repeat sequences were analyzed, among C. *hominis* IE, A11G3T3 was the most common variant followed by A6G2 and A7G3. Among the IB samples, a majority were A9G3R2 and all 5 C. *hominis* IF were A13G1R2. Other subtypes C. *hominis* IA, ID and IG were more diverse with C. *hominis* IA A23 and A19 and C. *hominis* ID with A14G1, A21 and A9. Among the C. *parvum*, the anthroponotic subgenotype, C. *parvum* IICA5G3R2 was identified most often followed by C. *parvum* IIM A7G1. Our studies indicate a significant diversity of C. *hominis* subtypes and mostly anthroponotic subtypes of C. *parvum* in this community. A more detailed analysis in the context of global diversity of subtypes is needed to identify potential pathways of introduction of these genetic variants into these densely populated areas.

WeA-P27-14H50/14H00

Epidemiological characteristics of cryptosporidiosis cases in France, 2015-2018

Costa D[1,2], French national network on surveillance of human cryptosporidiosis, Gargala G[1,2], Dalle F[3], Razakandrainibe R[1,2], Favennec L[1,2].

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Since January 2015, an online procedure was available to correspondent members of the French Centre National de Référence -Cryptosporidioses (CNR-C) to report confirmed cases of human cryptosporidiosis and contribute to surveillance. From 2015 to 2018, a total of 737 cases were reported online by 50 tertiary care hospitals and 6 private laboratories. Two age subgroups were predominant, *i.e.* children (< 9 years old) and young adults (20-34 years old) who represented respectively 28 and 22% of all reported cases. In particular, young children appeared strongly concerned by cryptosporidiosis: 11% of all cases were reported in 7 to 27 months children. Livestock contact, tap water/well water consumption, recreational swimming and close contact with patients with diarrhea were reported in 37%, 22%, 18% and 14% of cases, respectively. The majority of cases occurred in summer (57% of cases occurred between July and October). Ratios of individuals with a documented immunocompetence or immunocompromised status were 48% and 47% respectively. Immunocompromised subjects consisted predominantly of solid organ transplanted (SOT) (50%) and HIV infected patients (26%). Among anti-graft rejection treatment, numerous cases were reported in patients treated with tacrolimus (49%) and/or MMF (41%). Diarrhoea (80% of all cases), abdominal pain (29%), weight loss (23%), vomiting (20%), nausea (19%) and fever (19%), were the most reported symptoms and lasted less than 2 weeks in 77%. Hospitalization was required in 53% of all cases (54% for cryptosporidiosis symptoms and 41% for symptoms associated with underlying diseases). Six different *Cryptosporidium* species (*C. parvum, C. hominis, C. felis, C. ubiquitum, C. meleagridis* and *C. erinacei*) and 62 different genotypes were identified. *C. parvum* was predominant (70% of cases), frequently exhibiting the IIaA15G2R1 *gp60* subtype. *C. hominis* was found in 25% of cases with frequently the IaA22R2 and IbA10G2 *gp60* subtype*s.* Nitazoxanide therapy was used in 21% of cases. A fatal course of disease was reported in 5% of cases, all of them with an immunodeficient condition. Present results suggest that in France, cryptosporidiosis is common in children and young adults, especially during summer. Around half of the cases occurred in immunocompetent subjects whereas all lethal cases occurred in immunodeficient patients. Data document the dominant prevalence in France of *C. parvum*, cryptosporidiosis, likely due to contacts with livestock animals.

WeA-P28-14H55/15H00

Evaluation of an immunochromatographic test CORIS Crypto-Strip® for the detection of *Cryptosporidium parvum* in stool samples.

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Introduction

Cryptosporidiosis is a major cause of persistent diarrhea in developing countries, due to the presence of *Cryptosporidium* sp oocysts in the gastrointestinal tract. This pathology is particularly important to diagnose in immunocompromised persons, especially among HIV-positive ones. Optical microscopy using Kinyoun stain remains the reference method. However, in view of the limitations of microscopic examination, alternative techniques (IF, ELISA, PCR) have been developed. All of these methods are sensitive but costly and require qualified staff. That's why we have evaluated the performance of a rapid immuno-chromatographic test, Crypto-strip® (Coris Bioconcept), that detects *Cryptosporidium parvum* oocyst antigens in unconcentrated stool in 15 minutes.

Material and methods

A total of 46 human faeces (17 positive and 29 negative for *Cryptosporidium* sp oocysts), from enteritis patients addressed to our laboratory, were tested by standard methods and Kinyoun staining were included to evaluate the performance of Crypto-strip® comparing to optical microscopy. Stools containing other parasites (Entamoeba histolytica/E. dispar, Cyclospora sp…) were included to evaluate the specificity of the test. The sensitivity and specificity parameters as defined by Bouyer, were determined by the immunochromatographic test in relation to optical microscopy. The Crypto-strip® was used according to the manufacturer's instructions.

Results

In the study, 8 of the 17 positive known samples were positive and 28 of the 29 negative *Cryptosporidium* sp samples were negative (47.1% sensitivity, 96.6% specificity, 78.3% reliability).

Conclusion

In this study Crypto-strip® has a poor sensitivity but an excellent specificity for the detection of *Cryptosporidium parvum* in stool. This loss of sensitivity, could be explained in part by the use of monoclonal antibodies specifically directed against *Cryptosporidium parvum*, so, the other nine "false" negative samples could be caused by other species, like C. *hominis*. However, this test shows an excellent specificity, and is fast, simple to perform and easy to interpret. The real evaluation of its sensitivity requires a prospective study to compare its performance to either microscopy and genotyping methods.

Evaluation of the immunochromatographic CORIS *Giardia*-Strip® test for rapid diagnosis of *Giardia lamblia* in stool.

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Introduction

Giardia lamblia is one of the most common intestinal parasites causing severe diarrhea among children and immunocompromised people. In Morocco, its prevalence is 20% (24% in rural areas). Routine diagnosis is based on microscopic identification of trophozoites and/or cysts in faeces, using direct mounts and concentration methods. This diagnosis is labour-intensive and requires a great deal of expertise, including qualified staff, for these reasons Rapid antigen tests have been developed as an alternative.

We have evaluated the performance of an immunochromatographic one-step test, *Giardia*-strip® (Coris Bioconcept), using monoclonal antibodies against the cyst membrane antigens of G. lamblia.

Material and methods

The performance of this test was evaluated with known positive (n=12) and negative (n=36) stool specimens for G. lamblia, identified by optical microscopy as gold standard. Faeces with other parasites (Entamoeba histolytica/E. dispar, Blastocystis sp, E. coli, etc.) were included to evaluate the specificity of the test. The *Giardia*-strip® was used according to the manufacturer's instructions.

Results

By the *Giardia*-strip®, 10 of the 13 known positive specimens were positive and 34 of the 35 *Giardia*-negative samples were negative, compared to optical microscopy which is considered as the reference method (90.9% sensitivity, 91.9% specificity, 91.7% reliability).

Conclusion

Giardia-strip® has an excellent sensitivity and specificity for the detection of G. lamblia antigens in stool samples. The test is easy to perform, inexpensive, suitable for the single sample analysis and has a short turn-around-time (15 minutes). This test could be an important aid in some cases (especially of low parasite emission), however it cannot replace optical microscopy for recognition of other parasites.

POSTER SESSION AFTERNOON

Amphi 100B

WeB-P16-14h00/14h05

Understanding metronidazole resistance in *Giardia duodenalis*: Identifying patterns by transcriptomics combined with biochemical analysis of two oxygen-insensitive nitroreductases

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Giardia duodenalis is an intestinal protozoan parasite causing diarrhoea and abdominal cramping in approximately 300 million patients every year. The standard treatment is oral medication with metronidazole, an nitroimidazole drug selectively targeting anaerobic cells. Metronidazole is taken up as prodrug and has to be activated by redox enzymes to its active form. Under aerobic conditions this activation reaction is quickly and non-enzymatically reversed explaining the selectivity. Even though metronidazole has been in use since the early 1960s resistance against this drug only recently became a problem in the clinical setting. In contrast to that, it is easy to generate resistant G. *duodenalis* mutants in vitro. However, in-vitro mutants do often have severe growth and attachment defects and are therefore seldom able to establish infections in immunocompetent hosts possibly explaining the slow resistance development. Vice versa resistant lines isolated from patients cannot be grown and analysed in vitro. Hence, changes in genome, transcriptome and proteome of resistant lines created in vitro were explored and searched for common features as possible common resistance mechanisms. In this project we continue this search by analysing the transcriptome of two additional resistant lines (WB-M1 and WB-M2) and one revertant line (WB-M1NR) and comparing our results with the literature.

One important group of enzymes that was repeatedly reported to be changed in transcription and expression are diverse nitroreductases, a group of proteins known to play a key role in metronidazole resistance in other organisms. Nitroreductases can often either activate the drug or change it to an inert form. Both functions are very important in the context of drug resistance development. In G. *duodenalis* nitroreductase 1 and nitroreductase 2 are strongly associated with resistance towards metronidazole as activating respectively deactivating reagents in all resistant lines analysed to date and have been characterised previously.

Here we report the heterogeneous expression and purification of the two remaining nitroreductases, oxygen insensitive nitroreductase 1 (GL50803_15307) and hypothetical nitroreductase (GL50803_8377) respectively. Both enzymes were reported as differently expressed in several resistant lines prompting us to investigate their function closer. With our new transcriptomics data we will verify whether all four nitroreductases play a role in conferring metronidazole resistance in G. intestinalis in the lines named above while at the same analysing the two less characterised nitroreductases biochemically. Furthermore, we will look for additional common gene expression patterns in resistant vs non-resistant cell lines.

WeBP17-14h05/14h10

Giardia 14-3-3 protein post-translational modifications: Assemblage specificity and correlation with cell growth

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The dimeric 14-3-3s are a family of highly conserved 30 kDa acidic proteins with multiple isoforms eventually expressed in a tissue- and/or stage-dependent fashion. 14-3-3s regulate a plethora of cellular processes interacting with hundreds of client proteins via the recognition of conserved Ser/Thr phosphorylated binding motifs. At the same time, post-translational modifications (PTMs) of 14-3-3 itself, particularly phosphorylation, can add a further regulatory level for finely tune the interactions with targets and/or hamper 14-3-3 dimerization. The flagellated protozoan *Giardia duodenalis* encodes for a single 14-3-3 isoform constitutively phosphorylated at Thr214. In addition, the C-terminal Glu246 is subject to polyglycylation (polyGly), a common tubulin polymodification consisting in the addition of a stretch of glycine residues to the γ-carboxyl group of the glutamate. Mutational analysis have demonstrated that polyGly length affects the nuclear localization of g14-3-3 and encystations timing. Here we further explore how 14-3-3 and its PTMs levels change during *Giardia* trophozoite growth and between Assemblages. Using specific pAbs, we observed a distinct alteration of the polyglycylation level for 14-3-3 and tubulin in *Giardia* trophozoites throughout 72h growth. The variations of the polyglycine chain length were further confirmed by 14-3-3 affinity chromatography and MALDI-TOF analysis. Neverthless, PTMs alteration did not directly correlate with the expression level of the polyglycylase and the two deglycylases enzymes. Cell density and medium depletion also affected 14-3-3 expression and PTMs level. When trophozoites of the reference Assemblage A, B and E were compared after 48h of growth, the 14-3-3 intracellular distribution and PTMs extent were markedly different. Intriguingly, difference in 14-3-3 PTMs were also observed among different isolates of the same Assemblage opening up to the possible involvement of 14-3-3 in processes related to pathogenicity. In summary, the obtained preliminary data shed new light on the mechanisms that regulate *Giardia*l 14-3-3 activity and point out on assemblage specific behaviour of this multifunctional adaptor protein.

WeB-P18-14h10/14h15

Epigenetic control of gene expression during differentiation of *Giardia intestinalis*

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Giardia intestinalis presents a simple life cycle: the environment-resistant cysts and the disease-causing vegetative trophozoites. Between these developmental stages, *Giardia* needs to rapidly adapt to establish an infection in a new host. The cyst wall of *Giardia* protects the parasite from the harsh environmental conditions it has to endure outside the host's intestine. On the other hand, when a putative host ingests water or food contaminated with *Giardia* cysts, a signal transduction cascade triggered by the low pH of the stomach allows the release of trophozoites in the upper small intestine. Once in the small intestine, trophozoites produce asymptomatic infection or remain unnoticed in asymptomatic individuals. Differentiation of *Giardia* parasites from trophozoites to cysts is essential for transmission of the parasite We have recently characterized the gene expression changes related to this process using RNA Seq. We used our newly developed in vitro encystation protocol to generate differentiating cells from each 1.5 h of the 30 h encystation process. Transcriptional changes during the entire differentiation from trophozoites to cysts were studied using RNA sequencing (RNA-Seq). A high level of periodicity was observed for up- and down-regulated genes, both at the level of the entire transcriptome and putative regulators. The transcriptomic analyses were complemented by analyses of the methylation and histone modification status of selected encystation-regulated genes and these analyses suggest an important role of epigenetic regulation of gene expression during G. intestinalis encystation. We also characterize the effect of the DNA methylase inhibitor 5'-aza-2' deoxycytidine (5-aza-dC) on *Giardia* trophozoites and differentiation, observing that de-methylation effects encystation.

WeB-P19-14h15/14h20

High-resolution, quantitative proteome of *Giardia duodenalis* during encystation

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Encystation in *Giardia duodenalis* is a seemingly simple, yet critically important and highly complex developmental process that is essential for the parasite's transmission. Large-scale studies of the major changes induced in *Giardia* during the encystation process have greatly improved our understanding. However, although RNA-seq methods have achieved high-coverage characterization of the encystation process at a transcriptional level, significant limitations in the sensitivity of proteomic methods have limited a similar depth of understanding of the protein expressional changes. Currently, proteomic data are available for just ~16% of predicted protein coding genes during encystation. By contrast, 97% of these genes are identifiable as transcripts in this stage. Many of these transcripts may indeed be under translational repression in the cyst stage. Understanding this provides important insights into the mechanisms and regulation of encystation and excystation, but requires a more comprehensive characterization of the cyst proteome.

Using cutting-edge, mass spectrometry methods, we have generated the first high-resolution proteome for *Giardia duodenalis* across encystation, identifying over 4,000 proteins and reproducibly quantifying ~3,600 proteins across biological replicates, effectively tripling the currently published proteome coverage. We use this approach to comprehensively explore proteomic changes during trophozoite growth, low-bile encystation priming, mid-encystation and in mature cysts. Our analysis is the first quantifying changes in trophozoites during low bile priming prior to in vitro induction of encystation, documenting events that may provide crucial insights into early signalling in trophozoites pre-empting encystation. Our dataset also identifies 43 of the 45 lipid metabolic enzymes at protein level and demonstrates a concerted upregulation of a set of lipid metabolic enzymes during the progression of encystation. These include ~10 lipid metabolic enzymes, including an unannotated phospholipase B like enzyme which we have verified using HMM and structural modelling, and which is upregulated almost 4-fold in the cyst, and may indicate new roles of lipid metabolism in mediating encystation.

This new high-resolution proteome dataset will be of value for large scale transcriptomic-proteomic correlation studies, verifying protein expression, as well as providing insights into parasite transmission for developing strategies to block transmission blocking drugs.

WeB-P20-14h20/14h25

Characterization of the secreted cysteine protease CP17516 as a potential virulence factor

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Giardia intestinalis is an intestinal protozoan parasite causing diarrhoea infections in humans. Worldwide there are an estimated 280 million symptomatic *Giardia* infections (giardiasis) annually, affecting mainly young children in developing countries. Despite this, *Giardia* virulence factors and disease mechanisms and are poorly characterized. However, secreted proteins are known to contribute to the parasite's pathogenicity. Of these proteins many cysteine proteases (CPs) were identified to be highly upregulated and secreted during co-infections with intestinal epithelial cells (IECs) in vitro. CPs have a clear link to *Giardia* virulence and have been shown to be responsible for the degradation of several immune factors as well as contribute to the disruption of the intestinal epithelial barrier. The uncharacterized CP, CP17516, was shown to be secreted during *Giardia*'s interaction with IECs by two independent secretome studies. This secreted CP is glycosylated and contains a secretion signal peptide (SSP). Interestingly, CP17516 has an unusual active site lacking the common cysteine and histidine residues (C101S and H245L) found in most other CPs. Therefore, we started to characterize the potential function of CP17516 during infection. First, we localized the protein in trophozoites to the endoplasmic reticulum and cytoplasmic vesicle-like structures (a localization pattern similar to other characterized CPs) by epitope tagging. After this we went on to characterize its activity. Recombinant CP17516 expressed by Pichia pastoris showed no activity towards any tested substrate. Further, we attempted to perform substrate phage display but no substrate specificity could be identified. Therefore, it appears that this CP might have a different function than previously known and characterized CPs. Currently we are investigating if and what host or parasite proteins CP17516 binds to during an infection. Our goal is to co-immunoprecipitate CP17516 with its potential host receptor. Further we are using live-imaging to track the protein during a co-infection with IECs in vitro.

WeB-P21-14h25/14h30

How does ISC system work in the mitosomes of *Giardia intestinalis*?

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Mitosomes of *Giardia* intestinalis are one of the most reduced mitochondria found to date. They do not have any DNA and their proteome is extensively reduced - just a few tens of proteins are identified as mitosomal. The only known metabolic pathway is the synthesis of iron-sulphur clusters (ISC pathway)

The ISC pathway consists of two phases. During the early ISC pathway the [2Fe-2S] cluster is formed by IscS, Fdx, IscU and Grx5. The late ISC pathway serves for the formation of [4Fe-4S] cluster and its transfer to target apoproteins. IscA, Nfu1 and BolA are among the proteins employed in the late phase. However, the actual role of the mitosomal ISC pathway and the exchange of metabolites between the mitosome and the cytosol remain unknown. In this project, we tackle these unknowns by two biochemical approaches: (i) we attempt to establish affinity purification of the whole mitosomes from the cell lysate. The technique is based on specific biotinylation of the outer mitosomal membrane proteins. We have already chosen the most suitable protein candidate and now we are testing its inducible expression to minimize the experimental artifacts. (ii) we also isolate individual component of ISC pathway (Grx5, Nfu1) to co-purify missing functional components. We have identified G. intestinalis BolA homologue, which is normally present in ISC pathway of only aerobic organisms. GiBolA is localized in the mitosomal matrix and interacts with several other ISC components. Interestingly, despite the presence of three mitosomal components of the late ISC pathway (BolA, IscA and Nfu1) no mitosomal [4Fe-4S] cluster-containing substrate has been identified yet. Our aim is to identify such substrate or to demonstrate that [4Fe-4S] cluster is directly transported across two mitosomal membranes to the cytosolic apoprotein.

WeB-P22-14h30/14h35

Characterization of the proteome and dynamics of encystation specific vesicles of *Giardia intestinalis*

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The process of encystation is an important part of life cycle of many pathogenic organisms. *Giardia* intestinalis is an intestinal parasite of various vertebrates including humans from the supergroup of Excavata. The parasite leaves the body of the host in the form of cysts, which are infectious to other hosts. The encystation is triggered by increased pH and higher concentration of bile in the lower parts of the gastrointestinal tract. During the encystation process specific endomembrane organelles are formed, called the encystation-specific vesicles (ESVs) that serve for the accumulation of material for the future cyst wall (CWM). This material is consecutively transported from the lumen of the ESVs to the surface of the cell. CWM is composed of a fibrillar matrix, containing three paralogous cyst wall proteins (CWP 1-3) and a *Giardia*-specific β-1,3-GalNAc homopolymer. Because all three CWPs are rich in leucine and cysteine, it´s assumed that these aminoacids are involved in protein-protein interactions between the CWPs and other probable proteins. ESVs are also the sites of processing of CWM proteins, including several posttranslational modifications. During the maturation of ESVs, a condensed core is formed with a fluid phase at the edge, where CWP1 and the N-terminal fragment of CWP2 are present. This fragment was cleaved from pro-CWP2 when the core condensated. Compared to the fluid phase, the core of ESVs contains the C-terminal part of CWP2 and the entire CWP3. In this project, we strive to characterize the progressive conversion from the metabolically active cell to the resting stage of the cyst. To this aim we employ two methods, which enable high affinity purification of CWP1 and its interaction partners in the ESVs: (i) we have established in vivo biotinylation of CWP1, which is then purified upon chemical crosslinking to its interaction partners (ii) we are introducing the proximity labelling biotinylation using promiscuous mutant of biotin ligase – TurboID fused to known ESV proteins. In the combination with the live-cell microscopy we would like to improve our knowledge on the overall transformation of the parasite into the infectious cyst.

WeB-P23-14h35/14h40

Novel protein of *Giardia intestinalis* links the function of the mitosomes and the encystation specific vesicles

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Majority of *Giardia* intestinalis genes encode proteins of unknown function. However, it is mainly these unknowns, which represent unique biology of this important intestinal parasite. The highly reduced mitochondria known as mitosomes belong among such *Giardia* distinctive features and multiple mitosomal proteins of unknown function have been described to date.
One such protein, referred to as GiMlf1IP (GL50803_16424), has drawn our attention as the interacting partner of known mitosomal proteins from all tested mitosomal subcompartments - the inner and the outer membrane and the mitosomal matrix. GiMlf1IP has atypical phylogenetic distribution, as outside of the Hexamitidae family it can be found only in Viridiplantae and Metazoa, where it localizes to the nucleus as putative transcription factor of Mlf1IP (Myelodysplasia-myeloid leukemia factor 1-interacting protein) family. Using specific polyclonal antibody, we show that, in contrast to previous reports, the protein is not in the mitosomes but predominantly at discrete foci right next to the organelles. In addition, the protein was found at the rim of the adhesive disc and, interestingly, it colocalizes with the encystation specific vesicles (ESVs) in the later stages of encystation. GiMlf1IP possesses C-terminal intrinsically disordered region, which have been shown to mediate dynamic protein-protein interactions and enable creation of “membraneless compartments” and allow quick response to the current state of the cell. We are characterising these “membraneless compartments” by affinity purification of chemically crosslinked complexes and by CRISPRi technique, which has been recently introduced to *Giardia*. In addition, we are studying the dynamics of GiMlf1IP localization upon the environmental stimuli and during the life cycle of the parasite.

WeB-P07-12h10/12h15

Is *Cryptosporidium parvum* able to induce intestinal neoplasia in vitro?

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The apicomplexan parasite, *Cryptosporidium*, is a major public health problem in humans and animals. The Global Burden of Disease 2015 Study outlines that *Cryptosporidium* is the second leading cause of death due to diarrhea in children under 5 years old. Additionally, our team has reported that *Cryptosporidium parvum* is able to induce the development of invasive gastrointestinal neoplasia in an immunocompromised rodent model. We also found that *Cryptosporidium* spp were detected significantly more often in colonic biopsies of patients with colon adenocarcinoma before any treatment than in the control group. However, the study of the pathophysiology of this parasite, which is of crucial interest for the understanding of its link with cancerogenesis is hampered by the lack of a continuous in vitro culture system. Recently, we developed a three-dimensional (3D) culture model from adult SCID mice colon that allowed the completion of the parasite life cycle with production of newly formed oocysts. Then, having this system available, the following two questions were investigated: (i) is C. *parvum* able to induce neoplasia in a *in vitro* system in which there is no influence of the host immune response? (ii) is C. *parvum* able to develop and induce neoplasia in a 3D culture system based on colon explants from immunocompetent mice? For answer these questions, we performed a 3D culture using colon explants from either adult SCID or wild type C57BL/6JRj mice that were co-cultured with 25 excysted C. *parvum* oocysts. Culture explants were stopped periodically and subsequently analyzed histologically. Strikingly, we noted the development of low-grade intraepithelial neoplasia as soon as 27 days post-infection in both systems conceived with colon explants from either immunosuppressed or immunocompetent mice. These results are very interesting as they provide new evidences of the carcinogenic power of C. *parvum*, confirming observations obtained previously in the animal model and in epidemiological studies. In addition, it seems that the host immune response is not involved in the mechanisms of C. *parvum*-induced carcinogenesis. This topic should be further investigated as we know, for now on, that around 20% of human cancers have infectious origin. Consequently, if the causal link between *Cryptosporidium* and cancer is clearly established in human tissues, a significant number of digestive cancers could be prevented.

WeB-P25-14h45/14h50

Is oxygen a crucial factor for the survival of *Cryptosporidium* during solar water disinfection?

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Solar water disinfection (SODIS) is a simple, effective, sustainable and low-cost treatment that uses solar radiation as a biocide to improve the microbiological quality of drinking water. It has been shown that several factors influence the efficacy of SODIS method such as temperature, radiation intensity, turbidity, dissolved oxygen and salts, organic matter, the material and shape of the container, and the time of exposure. This work evaluates the influence of oxygen in the efficacy of SODIS procedures against the waterborne enteropathogen *Cryptosporidium*. Polyethylene terephthalate (PET) bottles of 330 mL were filled with well water containing 3 oxygen concentrations (approximately, 100%, 85% and 15% of saturation), spiked with *Cryptosporidium parvum* oocysts (23x106 oocysts/L) and exposed to simulated solar radiation at an intensity of 30 W/m2 of UV radiation (290-390 nm). After 2, 4 and 6 hours of exposure, oocyst survival was assessed by an induced excystation technique and the inclusion/exclusion of the fluorogenic vital dye propidium iodide (PI) technique. A statistically significant decrease in the oocyst viability was observed as the exposure time increases. After 6 hours of exposure, statistically significant differences in the percentages of induced excysted oocysts were detected in the samples with the higher oxygen concentrations with respect to that ones with lowest concentration (52.93±10.87%; 53.80±8.35%; 72.89±4.14% for dissolved oxygen saturations of 100%, 85% and 15% respectively). However, these differences were not appreciated by the PI inclusion/exclusion technique (54.20±6.95%; 57.89±7.80%; 54.66±6.08% for dissolved oxygen saturations of 100%, 85% and 15%, respectively). In conclusion, high levels of dissolved oxygen slightly increase the efficacy of solar disinfection of drinking water against *Cryptosporidium*. This study was funded from the European Union´s Horizon 2020 Research and Innovation Programme under grant agreement number 688928.

WeB-P26-14h50/14h55

Study of DNA repair protein recruitment and chromatin remodeling during DNA Double-Strand Break repair process in *Giardia duodenalis*

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Giardia duodenalis is a flagellated protozoan parasite that causes giardiasis. During its life cycle, several rounds of DNA replication, cytokinesis and karyokinesis occur accompanied by inevitable DNA damage inherent to cell metabolism. In those processes DNA repair mechanisms must be functional to maintain genomic stability. The most severe lesion is the DNA double-stranded break (DSB) since if one single DSB is not repaired this may cause apoptosis.

The DSB´s are also generated by nucleases like Cas9 nuclease from CRISPR/Cas bacterial system, which is a programmable nuclease that can be targeted by one guide-RNA (gRNA) molecule designed to the cleavage site of interest.

The aim of this work was to determine GdMre11 and GdDMC1B proteins recruitment and chromatin remodeling during the DNA repair process of a directed DSB (dDSB) generated by Cas9.

dDSB was generated by transfecting transgenic G. *duodenalis* trophozoites, either GdMre11-3HA or GdDMC1B-HA, with an inducible expression plasmid for both Cas9 (with 2X SV40 NLS on each side and a MYC tag) and gRNA (directed against cwp1 gene locus in *Giardia* genome) driven from P1 promoter. Protein recruitment to dDSB and chromatin remodeling process were evaluated through chromatin immunoprecipitation (ChrIP) technique using anti HA antibody and anti phospho H2A.X antibody, respectively.

Cas9, GdMre11-3HA and GdDMC1B-HA proteins expression was verified by Western blot after Cas9 expression induction with doxycycline (10 µg/mL) at different times (0 and 12 hrs). ChrIP assay was performed at different distances from dDBS up to 5 Kb on either side of the dDSB and by PCR analysis. Results showed localization of GdMre11-3HA, GdDMC1B-HA and phosphorylated GdH2A proteins nearby dDBS, which strongly suggest these proteins are the repair proteins involve in the DBS repair process via homologous recombination in *Giardia*.

Further analysis with ChrIP and qPCR will allow to obtain detailed quantification in the recruitment of repair proteins as well as in changes on the chromatin distribution around a DSB during the DNA repair process.

WeB-P27-14h55/15h00

Characterization of the proteome and dynamics of encystation specific vesicles of *Giardia intestinalis.*

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The process of encystation is an important part of life cycle of many pathogenic organisms. *Giardia* intestinalis is an intestinal parasite of various vertebrates including humans from the supergroup of Excavata. The parasite leaves the body of the host in the form of cysts, which are infectious to other hosts. The encystation is triggered by increased pH and higher concentration of bile in the lower parts of the gastrointestinal tract. During the encystation process specific endomembrane organelles are formed, called the encystation-specific vesicles (ESVs) that serve for the accumulation of material for the future cyst wall (CWM). This material is consecutively transported from the lumen of the ESVs to the surface of the cell. CWM is composed of a fibrillar matrix, containing three paralogous cyst wall proteins (CWP 1-3) and a *Giardia*-specific β-1,3-GalNAc homopolymer. Because all three CWPs are rich in leucine and cysteine, it´s assumed that these aminoacids are involved in protein-protein interactions between the CWPs and other probable proteins. ESVs are also the sites of processing of CWM proteins, including several posttranslational modifications. During the maturation of ESVs, a condensed core is formed with a fluid phase at the edge, where CWP1 and the N-terminal fragment of CWP2 are present. This fragment was cleaved from pro-CWP2 when the core condensated. Compared to the fluid phase, the core of ESVs contains the C-terminal part of CWP2 and the entire CWP3.

In this project, we strive to characterize the progressive conversion from the metabolically active cell to the resting stage of the cyst. To this aim we employ two methods, which enable high affinity purification of CWP1 and its interaction partners in the ESVs: (i) we have established in vivo biotinylation of CWP1, which is then purified upon chemical crosslinking to its interaction partners (ii) we are introducing the proximity labelling biotinylation using promiscuous mutant of biotin ligase – TurboID fused to known ESV proteins. In the combination with the live-cell microscopy we would like to improve our knowledge on the overall transformation of the parasite into the infectious cyst.

ORAL PRESENTATIONS

We-O13-15h00/15h20

Detection of *Giardia duodenalis* and *Cryptosporidium* spp. in stools : Is microcopy still accurate ?

Favennec L.

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Since Antoni van Leeuwenhoek discovered the flagellate *Giardia duodenalis*, microscopy has been widely used for the diagnosis of giardiasis. Although labour-intensive, time-consuming, lacking sensitivity and requiring repeated stol examination due to sporadic *Giardia* shedding, microscopy exhibited improved sensitivity and specificity by using fluorescent antibody staining. ELISA provide rather sensitive *Giardia* antigen detection while the sensitivity and specificity of immunochromatographic tests appear more variable. As a result of the limitations of microscopic and immunological assays, DNA-based detection methods have been developed and exhibit numerous advantages such as increased sensitivity and specificity, ability to combine multiple targets in one multiplex assay, and a rapid turnaround time, particularly when PCR is coupled to automated DNA extraction.

In contrast with *Giardia*, *Cryptosporidium* spp. has been for a long time considered pathogenic only in immunodeficient patients. Due to the size of oocysts and the lack of efficacy of common staining methods, widely used techniques have been developped using more specific stains mainly fuschine, which are time consuming, exhibit low sensitivity, and require a high level of skill for optimal interpretation, thus hampering diagnosis. Other microscopic techniques such as auramine staining or phase contrast microscopy (Heine technique) are more sensitive and for the latter much less time-consuming which eases routine diagnosis. They also allow for a diagnosis of others Coccidia including Cystoisospora and Cyclospora. Immunological assays for *Cryptosporidium* have been widely developped, however a number of immunochromatographic reagents do not detect other species than *parvum* and *hominis* thus limiting their use for the diagnosis of immunocompromized patients

Highly specific PCR-based molecular genetic methods which may detect asymptomatic carriers have been developped for the detection of *Cryptosporidium*. They are however particularly dependent on the quality and purity of the starting DNA material, so the choice of an efficient DNA extraction method is a critical step. Notable differences in performance have been observed between commercially available DNA-extraction kits, depending on parasite species and infection burden.

In addition, multiplex PCR assays have become available for the simultaneous detection and identification of common enteric protozoan parasites including *Cryptosporidium* and *Giardia* (as well as bacteria and/or viruses). They allow rapid investigation of undiagnosed infections, to save time in detecting specific organism and providing early specific therapy. However, they are presently expensive and not in use for routine diagnosis. Emerging technologies including NGS and the use of biosensors are under development.

We-O14-15h20/15h32

The CerTest VIASURETM PCR simplex and multiplex assays for the detection of *Giardia intestinalis*, *Entamoeba* sp and *Cryptosporidium* sp.: comparative evaluation with two commercial multiplex PCR kits and routine in-house simplex PCR assay

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Diagnosis of intestinal protozoal infections still relies mainly on microscopic examination. However, this approach is time-consuming, especially in non-endemic countries where the prevalence of gastrointestinal parasites is low, and its performances is dependent on the operator's expertise. Recently, many commercial PCR kits have been developed in order to improve the diagnostic sensitivity of these parasitic infections. The aim of this study was to evaluate the performances of the commercial CerTest VIASURETM multiplex and simplex qPCR assays (San Mateo de Gállego Zaragoza, Spain), for the detection of G. intestinalis, E. histolytica and *Cryptosporidium* sp. in stool samples, and to compare them with (i) two commercial multiplex qPCR assays (i.e. FAST-TRACK Diagnostics FTD Stool ParasiteTM (Esch-sur-Alzette, Luxembourg) and DIAGENODE Gastroenteritis/Parasite panel ITM (Liège, Belgique)) and (ii) an in-house simplex qPCR assays for the detection of G. intestinalis, E. histolytica, E. dispar and *Cryptosporidium* sp. (Dijon University Hospital, France) A panel of 174 stool samples collected between 2007 and 2017 was retrospectively included in this study. Overall, 86 samples were positives at least for one of the three protozoan parasites detected by the commercial qPCRs assays, 58 samples were negative for G. intestinalis, Entamoeba sp. and *Cryptosporidium* sp. but positive for other parasites and 30 samples were totally negative. We first compared all the PCR assays with microscopic examination as the gold standard and evidenced that the simplex assays did better results than the multiplex assays. In all, our in-house simplex PCR assays displayed the highest performances for the detection of G. intestinalis, E. histolytica, E. dispar and *Cryptosporidium* spp. (96.9/96.5%; 100/100%; 100/96.7% and 100/100% sensitivity (Se) / specificity (Sp), respectively). As a consequence, we finally compared the performances of each commercial qPCR assays with our in-house simplex qPCR assays as the reference. In all, the CerTest VIASURETM simplex qPCR assays showed the highest Se/Sp for the detection of G. intestinalis, E. histolytica, E. dispar and *Cryptosporidium* spp. (i.e. 94.4/93.5%; 100/100%; 96.1/100%; 100/99.3% respectively). All three multiplex qPCRs assays showed equivalent performances for the detection of Entamoeba histolytica. (i.e. 80% Se/100% Sp) but the multiplex CerTest VIASURETM kit did better for *Cryptosporidium* sp., when compared with the others (i.e. 100% Se/99.3% Sp). Finally, all three commercial multiplex qPCRs assays reported here showed limited performances for G. lamblia detection (i.e. 77.1/97.8%; 86.1/91.3% and 69.4/97.1% Se/Sp for the CerTest VIASURETM, FAST-TRACK Diagnostics FTD Stool ParasiteTM and the DIAGENODE Gastroenteritis/Parasite panel ITM multiplex qPCRs assays respectively).

We-O15-15h32/15h44

Auramine-phenol staining and light-emitting diode fluorescence microscopy and a rapid dipstick test for detection of *Cryptosporidium* infection: a diagnostic accuracy study in a university hospital and a rural health centre in Ethiopia

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Introduction: *Cryptosporidium* is a leading cause of diarrhoeal disease and mortality among children in low and middle-income countries, especially in Sub-Saharan Africa and South Asia. Clinical trials of novel treatment options depend on rapid, accurate, low-cost, point of-care diagnostic tools. A promising candidate is auramine-phenol staining visualised with light-emitting diode fluorescence microscopy (LED-AP), which is currently being rolled out for tuberculosis diagnosis. Aim: This study evaluated the diagnostic accuracy of LED-AP for detecting *Cryptosporidium* in stool samples from children under 5 years with diarrhoea. We also evaluated a new lateral flow dipstick test that can be stored at ambient temperature. Methods: The study was a prospective diagnostic accuracy study (phase III study) that included enrolment of non-diarrhoeal controls in two sites in in Southwest-Ethiopia: Jimma University Specialised Teaching Hospital and a rural Health Centre 20 km away. We aimed for at least 75 *Cryptosporidium*-positive diarrhoea cases. After clinical examination and interviews, samples were tested at the point-of-care by LED-AP and twice weekly by dipstick. The testing was done fully blinded and results was later compared against a battery of reference tests including quantitative PCR. Odds ratios and quantitative cut-offs for diarrhoea-associated infection will be used to determine whether an episode of diarrhoea is likely to have been due to *Cryptosporidium* infection. Results: Enrolment lasted from February 2017 until July 2018. Preliminary analysis shows a *Cryptosporidium* prevalence of about 8% in cases with diarrhoea by LED-AP. Data validation and reference testing will be finished by spring 2018 followed by the final data analysis. Diagnostic accuracy results for LED-AP and dipstick testing - sensitivity, specificity, NPV, PPV - will be presented at the conference.

Conclusion: This is the first diagnostic study evaluating auramine-phenol staining and LED-AP as a point-of-care diagnostic test for cryptosporidiosis in a low-income country, with a prospective study design and attempting to integrate testing with routine care. LED-AP is promising as a “dual-use” technology and can be easily established on top of existing hospital or health centre lab infrastructures, requiring no new equipment or supplies, and minimal additional training.

We-O16-15h44/15h56

Integrated selective *Cryptosporidium* EWOD concentrator

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Cryptosporidium is an Apicomplexan genus that is pathogenic to humans and animals. The linked disease (cryptosporidiosis) is a severe diarrheal disease, which can be deadly for immunodeficient people or toddler in Africa and Asia. This growing challenge requires the development of new automated tools to facilitate detection and analysis of contaminated samples.

In this perspective, development of Micro Electro Mechanical Systems applied to biology (BioMEMS) would be a valuable asset. Indeed, BioMEMS technologies have enabled the design of integrated platforms able to perform many biochemical operations. However, these devices often struggle to concentrate oocysts from volumes relevant to ecological or biological sampling strategies. In particular, it is difficult to go from a few tens of µL to a few tens of nL.

In IGCC-5, our team presented a novel *Cryptosporidium* oocyst electrical concentrator device. We obtained state-of-the-art concentration factors of 12 ± 0.4 times in the sub-region of a 10 µL drop. Furthermore, this device had the ability to selectively concentrate parasites of different genus out of a mix. We demonstrated this by segregating C. *parvum* oocysts from either *Giardia* lamblia cysts or C. Muris oocysts.

Since these observations, two major results were obtained. We have firstly fully modelled the physics behind this effect and identified the parameters controlling the electro-hydrodynamics forces used in this label free concentrator effect. We have furthermore integrated this concentrator in an electrowetting-on-dielectric (EWOD) platform. We show that using EWOD, it is possible to extract the oocysts which were concentrated at the edge of the drop and transfer them to a droplet of a few hundred nanoliters. The complete process enables us to obtain up to x4 concentration factor with an efficiency of 70 ± 6%. This work represents the first device able to concentrate parasites and manipulate them in such a small volume.

We-O17-16h10/16h30

Therapeutic aspects of cryptosporidiosis

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The unique localisation of the parasite in a parasitophorous vacuole and distinct structural and biochemical composition may contribute to the lack of effectiveness of most anti-protozoal drugs. During the last 20 years, data have been accumulated on safety and efficacy of nitazoxanide (NTZ) in infected immunocompetent individuals. Efforts to find new treatments must address the clinical needs, mainly those of immunocompromised patients for whom the efficacy of NTZ is limited and who need appropriate supportive measures until the cellular immunity is restored. There is no evidence of endogenous synthesis of isoprenoid precursors by *Cryptosporidium* sp. which have an access to these precursors from the host. Drug repurposing screen has revealed that statin drugs, inhibitors of human HMG-CoA reductase and isoprenoid synthesis block C. *parvum* growth. Combinations of drugs might be effective and would be a means of preventing the development of resistance and for example, atorvastatin and NTZ showed a synergistic effect. Triacsin C and other drugs inhibiting fatty acyl-CoA-binding protein, located in the parasitophorous vacuole (PV) membrane, and fatty acid-CoA synthetase, have been shown effective against C. *parvum* growth, both in cell culture and in mice. A novel piperazine-based lead compound, MMV665917, from the open access malaria box was shown to be a potent inhibitor in acute and chronic mouse models suggesting a parasiticidal activity whose compound effectiveness could depend to eliminate parasites in the absence of a competent immune system. Several bumped kinase inhibitors (BKI) compounds which inhibit calcium-dependent protein kinase 1 improved diarrhea in calves. Moreover, BKI 1369 reduced the parasite burden and disease severity in the C. *hominis* gnotobiotic pig model and offers positive prospects of future therapy. Nevertheless, the cardiotoxicity risk of MMV665917 and these BKI compounds will require careful investigation. The pyrazolopyridine KDU731, inhibitor of Plasmodium lipid kinase PI(4)K8 of *Cryptosporidium* and imidazopyridine compounds targeting the *Cryptosporidium* methionyl-tRNA synthetase (MetRS) have potent in vitro activity against C. *parvum* and C. *hominis*. Imidazopyridine compounds are most probably parasiticidal and have controlled C. *parvum* infection in 2 different murine models. Clofazimine, a drug for the treatment of leprosy, has been established as a potential repurposing candidate for the treatment of cryptosporidiosis and has entered human trials. Oxidoreductase inosine 5'-monophosphate dehydrogenase (IMPDH) is required for the conversion of adenosine into guanine nucleotides and CpIMPDH inhibitors have been shown effective in vitro and in vivo. A specific antibody against a parasitic protein CP2 conjugated with nanoparticles to deliver an inhibitor of C. *hominis* bifunctional thymidylate synthase-dihydrofolate reductase to the PV showed significant in vitro efficacy compared to either the inhibitor or nanoparticles alone, highlighting the importance of drug delivery to intestinal cells. The routes of drug uptake are still poorly known and it is unclear whether, for intestinal cryptosporidiosis, the optimal anticryptosporidial agent should be absorbed systemically and/or retained in the gastrointestinal tract. Studies with BPI indicate that intestinal levels of compound better correlate with activity than do plasma levels. In case of extra-digestive cryptosporidiosis, therapeutics acting at all sites of infection are desirable. Ideally, the treatment of extra-digestive or severe cryptosporidiosis calls for an injectable compound and aminoxanide, a new amino-ester thiazolide derivative and the first soluble form of NTZ could be a candidate.

ORAL PRESENTATION

We-O19-16h42/16h54

New potent and selective anti-*Giardia* compound series

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Giardia duodenalis infects a wide array of hosts and is the most frequently reported human intestinal parasite. On an annual basis this parasite is responsible for ~1 billion human infections of which >200 million result in symptomatic disease. While infections are more prevalent in the developing world, this parasite is ubiquitous. In developed countries the prevalence of giardiasis is 2-7%. However, rates are higher in sub-populations where the burden is primarily borne by children (>60% in Australian Aboriginal children). *Giardia* infection can result in severe and chronic disease, causing malabsorption, weight loss and failure to thrive in children. There is also evidence that infection is linked to post-infection disorders including irritable bowel syndrome. Despite growing evidence of *Giardia* associated morbidity, current treatment options are sub-optimal. As an example, the commonly used 5-nitroimidazole, metronidazole (MTZ), is associated with side-effects and drug resistance. It is also distasteful and must be taken in multiple doses over 5-7 days, factors which results in poor compliance, treatment failure, rapid re-infection and parasite resistance. In addition, there is increasing evidence that MTZ has a collateral effect on host microbiome. To improve giardiasis treatment options in the long-term we recently screened compounds from the Compounds Australia Open Access Scaffold Library for anti-*Giardia* activity (2451 compound subset; 2/scaffold; Z-factor 0.75). Rational selection based on activity, novelty, and chemical liabilities identified three compound series with potent (IC50≤1µM) and selective activity for G. *duodenalis*. These compound series have been chosen as starting points for anti-*Giardia* drug development. The most promising compound identified to date is active against assemblage A, B and metronidazole resistant parasites (290-fold more potent than metronidazole) and has a *Giardia* vs human selectivity index of >9000. Preliminary in vivo studies with this compound suggest no toxicity at up to 100X the calculated therapeutic dose and ~75% efficacy at a dose of 0.7mg/kg daily for 3 days. Further in vivo and activity studies with this compound and other lead series candidate are now under way and will be presented.

We-O20-16h54/17h06

Metronidazole drug-resistance in *Giardia*: emerging roles of epigenetic and post-translational modifications and sub-species variation

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Metronidazole (Mtz) is the frontline drug for multiple anaerobic bacteria and microaerophilic protists, including *Giardia duodenalis*. However, Mtz treatment failure is common, and *in vivo* drug resistance is increasing globally. Mtz resistance phenotypes in *Giardia* is complex: *in vivo* isolates often lose resistance *in vitro*, and resistance *in vitro* is rarely genetically fixed, with reversion to sensitivity after drug selection ceases, or via passage through the life cycle.

Our lab has contributed to extensive systems biology exploring Mtz resistance in *Giardia*, including isolate and subspecies variation, as well as phenotype plasticity via post-translation protein modifications (PTMs). Recently, we applied quantitative proteomics to identify differentially expressed proteins (DEPs) in three seminal Mtz resistant (MtzR) lines compared to their isogenic, Mtz-susceptible, parental line. We also undertook the first post- translational analysis of Mtz resistance, immunoblotting changes in protein acetylation, methylation, ubiquitination and phosphorylation between isogenic lines, and explored phenotype plasticity in MtzR lines across 12 weeks after removing drug selection. Our data confirms DEPs in the antioxidant network, glycolysis and electron transport, as well as substantial changes in PTM networks, particularly protein acetylation (KAc) and Mtz resistance. This includes substantial cross-resistance to deacetylase inhibitor Trichostatin A (TSA) in Mtz resistant lines.

To complement this work, we have recently undertaken the first high-resolution, site- specific investigations of KAc in *Giardia*. Thus far we have identified over 2000 KAc sites in over 12% of non-deprecated genes, demonstrating links to genetic regulation, antioxidant and glycolysis systems with implications for regulation of Mtz resistance. Furthermore, we have generated new Mtz resistant lines in both Assemblage A and B genome isolates, and interrogated these with high-resolution, multiplexed mass spectrometry. We have quantifying over 3000 and 2000 DEPs in these, respectively, which reveal Mtz resistance may manifest differently between sub-species, with significant implications for parasite treatment and control.

We-O21-17h06/17h18

Developing Therapeutics to Reduce *Cryptosporidium* Morbidity and Mortality Among Children in Low-Resource Settings

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Cryptosporidium is a leading cause of diarrheal disease among impoverished children in low resource settings. There are no vaccines and only one drug (nitazoxanide) is approved for treatment of cryptosporidiosis. Nitazoxanide has poor efficacy in malnourished children and is ineffective in severely immunocompromised patients. Therefore, there is an urgent need for new treatments to reduce the burden of diarrheal disease and for their appropriate integration with other interventions, such as oral rehydration, vaccines, and improvements in water, sanitation, and hygiene. In addition to the substantial burden of acute diarrhea, there is an emerging understanding of the contribution of *Cryptosporidium* to the chronic condition of environmental enteric dysfunction and its devastating sequelae of malnutrition, growth stunting, reduced oral vaccine effectiveness, and cognitive dysfunction. In many endemic countries, the burden due to chronic, asymptomatic infection may exceed that of acute diarrheal disease. This further underscores the need for new interventions to reduce this burden.

We-O22-17h18/17h30

Parenteral aminoxanide as a novel route to treat cryptosporidiosis

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Cryptosporidium spp. normally causes self-limiting diarrhea in immunocompetent individuals but infection can be life-threatening in young children and immunocompromised individuals. Nitazoxanide (NTZ) is the only approved treatment for cryptosporidiosis in immunocompetent but has modest efficacy in highly immunosuppressed people for whom disseminated disease might occur. Due to its poor intestinal absorption, its bio-avaibility profile is not ideal to treat a disseminated cryptosporidiosis. Aminoxanide, a new amino-acid ester thiazolide, has been shown effective against *C. parvum in vitro* development. The present study was undertaken to investigate the *in vivo* anticryptosporidial activity of aminoxanide, which is a water-soluble prodrug of tizoxanide, the active circulating metabolite of nitazoxanide.

For *in vivo*, six weeks old gerbils (*Meriones unguiculatus*) were immunosuppressed by low-protein diet and intraperitoneal injection of dexamethasone. Gerbils were infected with 2 x 10^5 *C. parvum* oocysts by oral gavage and allowed to develop infection for 24 hours before the onset of treatment. NTZ was given orally in pure DMSO suspension. Aminoxanide was suspended in 0.9% NaCl for oral gavage or for intramuscular or intraperitoneal injection. Doses were based on results obtained in previous NTZ efficacy studies on *C. parvum* infection.

In an immunosuppressed model of *C. parvum* infection, a 5-day treatment with a daily intramuscular dose of 100 mg/kg aminoxanide, compared with a daily 400 mg/kg dose of nitazoxanide, resulted in a 72.6% and 67.9% oocyst shedding inhibition, respectively. Aminoxanide improved *C. parvum*-induced intestinal pathology (shortening of villi, goblet cell depletion) and inflammation (lymphocyte infiltration of lamina propria).

These data suggest that severe cryptosporidiosis could benefit from parenteral administration of aminoxanide. This proof of concept study demonstrates efficacy of injection-mediated treatment of cryptosporidiosis with aminoxanide, leading to reduction of parasite burden and histological improvement in *C. parvum* infected gerbils. Aminoxanide provides an injectable form of tizoxanide that NTZ was unable to do and represents a good candidate for the first injectable treatment of cryptosporidiosis.

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François	Alexandre	
Favennec	Loic	*We-O22- We-O14- We-O13- WeA-P27- WeA-P24- WeB-P14- WeB-P7- WeA-P13- WeA-P6- We-O12- TuB-P9- TuB-P5- TuA-P1- Tu-O17- Tu-O10- Tu-O6*
Follet	Jerome	*We-O16- WeA-P14- WeA-P1- WeA-P2- MoA-P13*
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Gagnaire	Aurélie	*Mo-O4*
Gantois	Nausicaa	*WeB-P23- Mo-O21- MoA-P13-*
Gargala	Gilles	*We-O22- We-O17- WeA-P27- WeA-P24- WeB-P14- WeA-P6- We-O12- TuB-P9- Tu-O6-*
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Giangaspero	Annunziata	*TuA-P4*
Globokar	Madja	
Gomez-Couso	Hipolito	*TuA-P8- MoA-P9- MoA-P10-*
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Gruttner	Jana	
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Hagen	Karl	*Mo-O15*
Hanevik	Kurt	*We-O15- Mo-O12- Su-O8*
Hartdegen	Marton	*WeB- P11*

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Hilton	Nicholas	*Mo- O15 MoB-P 8*
Holthaus	David	*MoB-P6*
Holubova	Nikola	*MoA-P6 MoA-P7 We-O4 We-05*
Horackova	Vendula	*WeB-P20*
Iacob	Olimpia	*WeB-P1*
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Jellison	Kristen	*Tu-O18-*
Jex	Aaron	*We-O20- WeB-P18- TuB-P7- Tu-O16- Su-O17- Su-O16- Su-O5- Su-O4*
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kapel	Nathalie	*Tu-O17*
Karadjian	Grégory	
Katzer	Frank	*WeA-P3- We-O8- We-O6- Tu-O26-*
Kaupke	Agnieska	*MoA-P5-*
Khan	Asis	*Mo-O25*
Kicia	Marta	*WeA-P15*
Kifleyohannes	Tsegabirhan	
Kissinger	Jessica	*TuB-P7- Mo-O24- Mo-O9- Su-O12-*
Klotz	Christian	*MoB-P14- MoB-P11- MoB-P6- MoB-P5- Mo-O6- Su-O8-*
Kooyman	Frans	*Su-O7*
Kopacz	Zaneta	*WeA-P15*
Kortbeek	Titia	*Tu-O13- Tu-O11*
Kraft	Martin	*MoB-P16- MoB-P6- Mo-O6-*
Krakovka	Sascha	*WeB-P15*
Krumkamp	Ralf	
Krumrie	Sarah	
Kvac	Martin	*WeA-P15- We-O4- We-O5- Mo-O20- MoA-P7- MoA-P8- MoA-P6-*
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Lalle	Marco	*WeBP16- Su-O5- Su-O6- Su-O3-*
Lamande	Sonia	*TuB-P4- TuB-P3- Su-O19– Su-O18*
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Laurent	Fabrice	*TuB-P4- TuB-P3- Mo-O1- Su-O19- Su-O18*
Lefebvre	Marion	
Lemeteil	Denis	*Tu-O17*
Liotta	Janice	*We-O10*
Lucio-Forster	Araceli	
Loury	Pascaline	
Lugonja	Bozo	*MoA-P4- Mo-O10*
Ma'ayeh	Showgy	*Mo-O5*
Maertens	Brecht	*Mo-O13- Mo-O4-*
Maloney	Jenny	*We-O9- Mo-O3- Su-O10-*
Mammeri	Mohamed	*WeB-P6- WeA-P1*
Mank	Theo	
Markova	Lenka	*WeB-P26- WeA-P6*
Mattei	Sara	

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Melan	Xavier	
Meireles	Marcelo	*WeB-P8- MoA-P11- MoA-P12- MoA-P2-*
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Motychova	Alzbeta	*WeB- P21*
Mravcova	Kristina	*WeA –P4 WeA –P8 WeA –P11*
Nguyen	Tran	*WeB-P4*
Nourrisson	Céline	*TuA-P7- Tu-O17-*
Ogbuigwe	Paul	*Tu-O8*
Oliveira	Bruno	*Mo- O8 MoO18 MoA-P8 MoA-P11*
Oliveira	Luiz	*MoA-P8 MoA-P11*
Ongerth	Jerry	
Ortega	Ynes	*Tu-O20*
Ortega-Pierres	Guadalupe	*Mo-O16*
Pantchev	Nikola	
Paziewska-Harris	Anna	*MoA-P4- Mo-O10-*
Peirasmaki	Dimitra	*MoB-P15- Su-O2*
Perrucci	Stefania	*WeA-P5- MoA-P14-*
Petersen	Heidi Huus	
Pezier	Tiffany	*TuB-P4- TuB-P3- Su-O19- Su-O18*
Poirier	Philippe	*TuA-P7- Tu-O17-*
Polack	Bruno	*WeB-P12- WeB-P6- WeA-P1- We-O1- Tu-O10*
Punter	Ken	
Rafferty	Steven	*MoB-P3- Su-O11- Su-O8-*
Razakandrainibe	Romy	*We-O22- WeA-P27- WeA-P24- WeB-P14- WeB-P7- WeA-P13- WeA-P6- We-O12- TuB-P9- TuB-P5- TuA-P9- TuA-P1- Tu-O10- Tu-O6-*
Riba	Ambre	
Robert-Gangneux	Florence	
Robertson	Lucy	*We-O15- WeA-P9- We-O2- TuB-P1- TuA-P4- Tu-O28- Tu-O24- Tu-O22-*
Roelfsema	Jeroen	*We-O7- Tu-O13*
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Rojas	Laura	*WeB-P17- MoA-P16-*
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Rossignol	Céline	
Ryan	Una	*We-O19- WeA-P19- We-O3- We-O2- TuB-P10- Tu-O22- MoA-P13*
Saghaug	Christina	*Su-O8*
Santin-Duran	Monica	*We-O9- Su-O10-*
Santos	Rui	*Tu-O14- Mo-O14*
Sateriale	Adam	*TuB-P7- Mo-O26- Mo-O24- Mo-O9-*
Sawant	Manasi	*WeB-P23*
Schalling	Henk	
Scorza	Andréa	
Shakya	Ruchika	*TuB-P1*

Sherchand	Jeevan	*Tu-O21*
Silva	Maria	*Mo- O3*
Singer	Steven	*Tu-O14- Mo- O3*
Slana	Iva	*TuA-P5- TuA-P3- TuA-P2*
Souza	Juliana-Bizarri	*MoB-P16-*
Souza	Maria	
Stairs	Courtney	*WeB-P20- Mo-O7-*
Starcevich	Hannah	
Striepen	Boris	*TuB-P7- Mo-O26- Mo-O24- MO-O17- Mo-O9*
Strkolcova	Gabriela	
Svard	Staffan	
Szelig	Adam	
Tesmegen	Tamirat	
Thomas	Myriam	*WeB-P12- WeB-P6- WeA-P2 Tu-O19- Mo-O10*
Tichkule	Swapnil	*Tu-O16- Su-O16- Su-O5-*
Tonelli	Renata	*MoB-P16- MoB-P10*
Tonkin	Christopher	
Tosini	Fabio	*TuB-P6*
Tottey	Julie	*TuB-P4*
Troell	Karin	*WeA-P20*
Tsantarlis	Katherine	*MoB-P10*
Tsaousis	Anastasius	*WeA-P14- Mo-O20-*
Tumova	Pavla	
Tyler	Kevin	*WeA-P14- Tu-O19*
Uiterwijk	Mathilde	*We-O7*
Vallée	Isabelle	*WeB-P6- WeA-P2*
Valot	Stéphane	*We-O14- Tu-O17- Tu-O10-*
Villena	Isabelle	*TuB-P5- TuA-P1- Tu-O17*
Vinopola	Martina	
Vlandas	Alexis	*We-O16- WeA-P14*
Warrenfeltz	Susanne	*Su-O12*
Weir	William	
Wells	Beth	*WeA-P3- We-O8- Tu-O26*
Whatley	Rangimarie	
Widmer	Giovanni	*Mo-O18- Mo-O8*
Williams	Gareth	*MoB-P13- MoA-P4- Mo-O15-*
Williams	Joseph	*Mo- O15 MoB-P13*
Xiao	Lihua	*WeA-P19- TuB-P10-We-O10- Mo-O19*
Xu	Feifei	*Mo-O7- Su-O5- Su-O2-*
Ydring	Elsie	*WeA-P20*
Yee	Janeth	*MoB-P3- Su-O11*
Yin	Jigang	*Su-O20*
Yordanova	Yvet	*Mo-O2*
Zahedi Abdi	Alireza	*WeA-P19- We-O2- We-O3- Tu-O22*