INTERNATIONAL WORKSHOPS ON OPPORTUNISTIC PROTISTS
IWOP12
TARRYTOWN, NEW YORK

August 5th to 9th 2012
International Workshops on Opportunistic Protists
IWOP-12, August 5th through 9th 2012

Tarrytown House Estate and Conference Center, Tarrytown, NY

The objectives of the IWOP meetings are to: (1) Serve as a forum for exchange of new information among active researchers concerning the basic biology, molecular genetics, immunology, biochemistry, pathogenesis, drug development, therapy, and epidemiology of the immunodeficiency-dependent diseases (e.g., as in AIDS patients) caused by eukaryotic protists; and (2) Foster the entry of new and young investigators into these underserved research areas. The IWOP meeting focuses on opportunistic protists; e.g. the free-living amoebae, Pneumocystis, Cryptosporidium, Toxoplasma, the Microsporidia, and kinetoplastid flagellates.

ORGANIZING COMMITTEE

Louis M. Weiss, Albert Einstein College of Medicine.
Melanie T. Cushion, University of Cincinnati College of Medicine, and VA Medical Center.
Enrique J. Calderon, Seville University
Edna S. Kaneshiro, University of Cincinnati
Elizabeth S. Didier, Tulane Primate Center
Francine Marciano-Cabral, Medical College of Virginia
Olga Matos, New University of Lisbon
Anthony P. Sinai, University of Kentucky
Lihua Xiao Center for Disease Control

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The National Institutes of Health
Burroughs Welcome Fund
American Type Culture Collection
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Phthisis Diagnostics
Waterborne, Inc.

The next International Workshops will be in Spain in 2014.

IWOP-13 (http://iwop2014.atlantacongress.org/)
Sunday August 5th
3:00 PM REGISTRATION
6:00 PM DINNER (Winter Palace)
7:45 PM PLENARY SESSION (Mary Duke Ballroom)

Monday August 6th
7:30 AM BREAKFAST
8:30 AM DIAGNOSTICS
10:10 AM COFFEE BREAK
10:40 AM DRUG DEVELOPMENT AND BIOCHEMISTRY
12:20 PM LUNCH
2:00 PM MOLECULAR BIOLOGY
3:30 PM COFFEE BREAK
6:00 PM DINNER
7:30 PM COMMUNITY INITIATIVES AND ROUND TABLE DISCUSSIONS
8:00 PM to 10:00 PM POSTER SESSION A

Tuesday August 7th
7:30 AM BREAKFAST
8:30 AM IMMUNOLOGY
10:10 AM COFFEE BREAK
12:10 PM LUNCH
2:00 PM CLINICAL EFFECTS OF INFECTION
4:10 AM COFFEE BREAK
4:30 PM COMMUNITY INITIATIVES AND ROUND TABLE DISCUSSIONS
6:00 PM DINNER
8:00 PM POSTER SESSION B
Wednesday August 8th

7:30 AM       BREAKFAST
9:00 AM       CELLULAR BIOLOGY
10:10 AM      COFFEE BREAK
12:00 PM      LUNCH
1:30 PM       EPIDEMIOLOGY and TAXONOMY
3:30 PM       COFFEE BREAK
6:10 PM       DINNER
8:00 PM       RECEPTION AND CLOSING EVENT

Thursday August 9th

8:00 AM       BREAKFAST AND INFORMAL MEETINGS
               SPECIAL INTEREST GROUPS, COLLABORATION GROUP MEETINGS

DEPARTURE

All of the meeting sessions will be held in the Mary Duke BallRoom (Biddle Mansion).
Meals will be served in the Winter Palace (Biddle Mansion).
Sunday August 5th

3:00 PM      REGISTRATION

6:00 PM     DINNER

7:45 PM    PLENARY SESSION

7:45    Introduction and welcome.  LOUIS M. WEISS, Albert Einstein College of Medicine, Bronx, NY

8:00    T1. Pneumocystis: Perspectives on the Past and the Future.  PETER D. WALZER, University of Cincinnati, VA Medical Center, OH, USA.

9:00    T2. EuPathDB: an integrated eukaryotic pathogen genome resource.  BRIAN BRUNK for the EuPathDB Project.  University of Pennsylvania, Philadelphia PA and University of Georgia, Athens GA

9:30    T3. The ATCC Protistology Collection: A Biological Resource for Parasitologists.  ROBERT E. MOLESTINA.  American Type Culture Collection, Manassas, VA 20110.

Monday August 6th

BREAKFAST

8:30AM   DIAGNOSTICS

Session Chairs: CRYSTAL R. ICENHOUR and ENRIQUE J. CALDERÓN

8:30    T4. Development of Commercially-Viable Diagnostics.  CRYSTAL R. ICENHOUR; Phthisis Diagnostics, Charlottesville, VA.

8:50    T5. Serum markers (1-3)-Beta-glucan and lactate dehydrogenase levels for the diagnosis of Pneumocystis pneumonia (PcP) in HIV+ patients.  FRANCISCO ESTEVES1*, MARGARIDA SERINGA1, ROBERT BADURA2, BRUNO DE SOUSA1, CAMILA FERNANDES1, FRANCISCO ANTUNES2, JORGE GASPAR3, CHAO-HUNG LEE4, OLGA MATOS1; 1Instituto de Higiene e Medicina Tropical, CMDT/UNL, Lisboa, Portugal.  2Hospital de Santa Maria, FM/UL, Lisboa, Portugal.  3Faculdade de Ciências Médicas, CIGMH/UNL, Lisboa, Portugal.  4Indiana University School of Medicine, Indianapolis, Indiana, USA.

9:10    T6. Modeling Waterborne Pathogen Limit of Detection by Most Probable Number PCR.  SCOTT P. KEELY*, SARAH E. STAGGS, MICHAEL W. WARE, ERIC N. VILLEGAS; National Exposure Research Laboratory ,United States Environmental Protection Agency, Cincinnati, OH, USA.

9:30    T7. Identification of Nosema bombycis by Loop-mediated Isothermal Amplification.  LIU JI-PING YANG JI-LONG WEI JIAN-YING YAN YU-WEI LI XIANG-LIN ZOU ZHEN-HUA College of Animal Science, South China Agriculture University, Guangdong Provincial Key Lab of Agro-Animal Genomics and Molecular Breeding, Guangzhou 510642, China
9:50  T8. Clinical utility of β-D-glucan as a serological marker for *Pneumocystis jiroveci* colonization in lung transplant recipients with cystic fibrosis. ENRIQUE J. CALDERÓN1*, VICENTE FRIAZA1, ESTHER QUINTANA2, NIEVES RESPALDIZA1, MARIA LUZ CALERO1, RUBEN MORILLA1, RAFAEL TERAN1, ELENA CAMPAÑO1, FRANCISCO J. MEDRANO1, JOSE M. VARELA1, FRANCISCO J. DAPENA2, CARMEN DE LA HORDA1.

1Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocio/ CSIC/Universidad de Sevilla, and CIBER de Epidemiología y Salud Pública, Seville, Spain. 2Cystic Fibrosis Unit, Virgen del Rocio University Hospital, Seville, Spain.

COFFEE BREAK

10:40AM  DRUG DEVELOPMENT AND BIOCHEMISTRY

Session Chairs: EDNA KANESHIRO and GUAN ZHU

10:40  T9. Development of endochin-like quinolones for the treatment and prevention of toxoplasmosis. LORRAINE JONES-BRANDO1*, CLAUDIA BORDON1, ROLF WINTER2, AARON NILSEN2, J. STONE DOGGETT2, MIKE RISCOE2, ROBERT YOLKEN1; 1Johns Hopkins University, MD, USA, 2Oregon Health & Science University, OR, USA.

11:00  T10 Accumulation of Myeloid-Derived Suppressor Cells in the Lungs during *Pneumocystis* Pneumonia. CHEN ZHANG, GUANG-SHENG LEI, SHOUJIN SHAO, PAMELA J. DURANT, AND CHAO-HUNG LEE; Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN 46202

11:20  T11. All-trans Retinoic Acid in Combination with Primaquine Clears *Pneumocystis* Infection and Eliminates Myeloid Derived Suppressor Cells that Accumulate in the Lung during *Pneumocystis* Pneumonia. GUANG-SHENG LEI, CHEN ZHANG, SHOUJIN SHAO, PAMELA J. DURANT, AND CHAO-HUNG LEE. Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN 46202

11:40  T12. Complementation of *Saccharomyces cervisiae* Sterols Expressing the *Pneumocystis carinii* S-Adenosylmethionine:Sterol C-24 Methyltransferase. EDNA S. KANESHIRO1, LAURA Q. JOHNSTON1, EDWARD A. WRIGHT1, JOSE-LUIS GINER2. 1Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, USA, 2Department of Chemistry, State University of New York, ESF, Syracuse, New York, USA.

12:00  T13. Acyl-CoA Synthetases (ACSs) in the zoonotic pathogen *Cryptosporidium parvum*. FENGGUANG GUO*, HAILI ZHANG, GUAN ZHU; Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX, USA

LUNCH

2:00 PM  MOLECULAR BIOLOGY

Session Chairs: NICOLAS CORRADI and SCOTT P. KEELY

2:00  T14. Microsporidian tiniest genomes: more malleable than previously anticipated? NICOLAS CORRADI, University of Ottawa, Department of Biology, Ottawa, ON, Canada
2:20 T15. Determining the Genome Sequence of Cryptosporidium meleagridis.
SCOTT P. KEELY*, SARAH E. STAGGS, MICHAEL W. WARE, ERIC N. VILLEGAS; National Exposure Research Laboratory, United States Environmental Protection Agency, Cincinnati, OH, USA.

JANET YEE1,2*, CHASE REAUME1, CHRISTINE OUELLET2*, AMBER OLSON1, KIN CHAN3, CHI-YIP HO3; 1Environmental and Life Sciences Graduate Program, 2Biochemistry Program, Trent University, Peterborough, ON, Canada; 3Microarray Laboratory, Samuel Lunenfeld Research Institute, University of Toronto, Toronto, ON, Canada.

3:00 T17. Microsporidian genome analysis reveals evolutionary strategies for growth of obligate intracellular pathogens.
CHRISTINA A. CUOMO1†, CHRISTOPHER A. DESJARDINS1†, MALINA A. BAKOWSKI2†, JONATHAN GOLDBERG1, AMY T. MA2, JAMES J. BECNEL3, ELIZABETH S. DIDIER4, LIN FAN1, DAVID I. HEIMAN1, JOSHUA Z. LEVIN1, SARAH YOUNG1, QIANDONG ZENG1, EMILY R. TROEMEL2; 1The Broad Institute of MIT and Harvard, Cambridge, MA, USA. 2University of California San Diego, La Jolla, CA, USA. 3USDA, Gainesville, FL, USA. 4Tulane National Primate Research Center, Covington, LA, USA.

3:10 T18. De novo Assembly of the Pneumocystis jirovecii Genome from a Single Bronchoalveolar Lavage Fluid of a Patient with Pneumocystis Pneumonia.
O.H. CISSÉ1,2 *, M. PAGNI2, P.M. HAUSER1; 1Institute of Microbiology, Centre Hospitalier Universitaire Vaudois and University of Lausanne. 2Swiss Institute of Bioinformatics, Lausanne, Switzerland.

COFFEE BREAK

4:00 T19. Identification and functional characterization of stage-specific cis-regulatory elements in Toxoplasma gondii.
MIN LIU, YUNYING YAO, SHANLIANG SHEN, KUN WU, PEILLIANG YANG, HONGJUAN PENG, XIAOGUANG CHEN*. Department of Parasitology, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, Guangdong 510515, P.R.China.

ZOI TAMPAKI, SHEILA C. NARDELLI, EDUARDO FAJARDO, KAMI KIM. Departments of Microbiology & Immunology and Medicine, Albert Einstein College of Medicine, Bronx, NY, 10461. USA.

SHEILA C NARDELLI1,2; FA-YUN CHE2; NATALIE SILMON DE MONERRI3; HUI XIAO2; EDWARD NIEVES4,5; CARLOS MADRID-ALISTE*; SERGIO ANGEL6; WILLIAM J. SULLIVAN7;; RUTH H. ANGELETTI4,5; KAMI KIM1,3 AND LOUIS M. WEISS2,3. Departments of 1Microbiology & Immunology, 2Pathology, 3Medicine, 4Developmental and Molecular Biology, and 5Biochemistry, Albert Einstein College of Medicine, Bronx, NY, USA; 6Laboratorio de Parasitologia Molecular, IIB-Intech, Conicet-UNSAM, Buenos Aires, Argentina; Departments of 7Pharmacology & Toxicology and 1Microbiology & Immunology, Indiana University School of Medicine, Indianapolis, IN, USA.

5:00 T22. In silico analysis identifies Toxoplasma gondii proteins with capacity to target to the mammalian cell nucleus and influence epigenetic regulation of host genes.
GENEVIEVE SYN**, JENEFER M. BLACKWELL, SARRA E. JAMIESON AND RICHARD W. FRANCIS; Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, Subiaco, Western Australia, Australia.
5:20 T23. CpArray15K: The first oligonucleotide microarray for Cryptosporidium parvum and its application to study the transcriptome in the parasite oocysts. HAILI ZHANG,1 FENGGUANG GUO,1 HUAIJUN ZHOU,2 GUAN ZHU1: 1Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX, USA, 2Department of Animal Science, University of California, Davis, CA, USA.

5:40 T24 Genes and gene pathways involved in bradyzoite-tachyzoite interconversion in Toxoplasma gondii. MATTHEW MCKNIGHT CROKEN3, YAN-FEN MA1, RONALD C. TAYLOR4, MENG MARKILLIE4, GALYA ORR4, STEVEN WILEY4, LOUIS M. WEISS1,2, KAMI KIM1,3 Departments of Pathology1, Medicine2 and Microbiology & Immunology3, Albert Einstein College of Medicine, Bronx, NY 10461, USA. EMSL4, Pacific Northwest National Laboratory, Redmond, Richland, WA 99352

DINNER

7:30 PM COMMUNITY INITIATIVES AND ROUND TABLE DISCUSSIONS

8:00 PM to 10:00 PM POSTER SESSION A

PA1. Characterization of a putative C-24(28) sterol reductase (erg4) in Pneumocystis carinii. THOMAS M. SESTERHENN1,*, ALEKSEY POROLLO3, MELANIE T. CUSHION1,2; 1University of Cincinnati College of Medicine, Cincinnati, OH, USA, 2Cincinnati Veteran's Affairs Medical Center, Cincinnati, OH, USA, 3Department of Environmental Health, University of Cincinnati, Cincinnati, OH, USA.

PA2. Identification of Pentamidine Analogs as Candidate Anti-Pneumocystis Compounds by In Vitro Efficacy and Toxicity Screenings. MARGARET COLLINS1, DOROTA MACIEJEWSKA2, MICHAEL LINKE3, PETER D. WALZER1,3, ALAN ASHBAUGH1, KEELEY LYNCH1, MELANIE T. CUSHION1,3 1University of Cincinnati, College of Medicine, Department of Internal Medicine2Medical University of Warsaw, Faculty of Pharmacy, 3Department of Organic Chemistry 3Cincinnati Department of Veterans Affairs Medical Center

PA3. S-adenosyl Methionine and KL-6 as serological markers for diagnosis of Pneumocystis Pneumonia (PcP) – Preliminary results. SÓNIA S. CALÉ1, OLGA MATOS2, BRUNO DE SOUSA2, JORGE GASPAR3, ROBERT BADURA4, FRANCISCO ANTUNES4, FRANCISCO ESTEVES2; 1Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal. 2Faculdade de Ciências Médicas, CIGMH/UNL, Lisboa, Portugal. 3Faculdade de Ciências Médicas, CIGMH/UNL, Lisboa, Portugal. 4Hospital de Santa Maria, FM/UL, Lisboa, Portugal.

PA4. Interleukin-8 and β-D-glucan serum levels in Chronic obstructive pulmonary disease patients colonized by Pneumocystis jirovecii. ELENA CAMPANO*, EDUARDO MARQUEZ, VICENTE FRIAŻA, LAURA RIVERO, RUBÉN MORILLA, JUAN M. PRAENA-FERNÁNDEZ, NIEVES RESPALDIZA, JOSE M. VARELA, FRANCISCO J. MEDRANO, FRANCISCO ORTEGA, ENRIQUE J. CALDERÓN, CARMEN DE LA HORRA. Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocio/ CSIC/Universidad de Sevilla, and CIBER de Epidemiologia y Salud Pública, Seville, Spain.

PA5. Pneumocystis colonization in patients receiving anti-tumoral necrosis factor (anti-TNF) drugs. SABEL MARTÍN-GARRIDO, RUBÉN MORILLA, JUAN M. PRAENA-FERNÁNDEZ, GUSTAVO WISSMANN, ELENA CAMPANO, VICENTE FRIAŻA, BERTA

PA7. *Pneumocystis pneumonia in patients receiving Rituximab.* SABEL MARTIN-GARRIDO1,2, EVA M. CARMONA1, ULRICH SPECKS1, ANDREW H. LIMPER1; 1Division of Pulmonary and Critical Care Medicine, Mayo Clinic College of Medicine, Rochester, MN, USA, 2Servicio de Medicina Interna, Hospital Universitario Virgen del Rocío, Seville, Spain.

PA8. Evaluation of Caspofungin and Caspofungin in association with Trimethoprim-sulfamethoxazole (TMP-SMZ) in the rodent model of *Pneumocystis* – Preliminary study. MARIA LUIZA LOBO1*, FRANCISCO ESTEVES1, FERNANDO CARDOSO1, MELANIE CUSHION2, FRANCISCO ANTUNES3, OLGA MATOS1; 1Instituto de Higiene e Medicina Tropical, CMDT/UNL, Lisboa, Portugal, 2University of Cincinnati College of Medicine, Cincinnati, Ohio, USA, 3Faculdade de Medicina, HSM/UL, Lisboa, Portugal.


PA10. Endochin-like quinolones have remarkable efficacy against infection by *Toxoplasma gondii*. CLAUDIA BORDÓN1*, J.STONE DOGGETT2, ROLF WINTER2, AARON NILSEN2, MICHAEL RISCOE2, ROBERT YOLKEN1, LORRAINE JONES-BRANDO1; 1Johns Hopkins University, MD, USA, 2Oregon Health & Science University, OR, USA.

PA11. A novel *Cryptosporidium* protein with a C-type lectin domain. SEEMA R. BHALCHANDRA1*, JACOB LUDINGTON1,2, HONORINE D. WARD1,2; 1Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center, 2Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, MA.

PA12. Spontaneous cystogenesis *in vitro* of a Brazilian strain of *Toxoplasma gondii*. TATIANA C. PAREDES-SANTOS1, ERICA S. MARTINS-DUARTE1, RICARDO W.A. VITOR2, WANDERLEY DE SOUZA3, MARCIA ATTIA1, ROSSIANE C. VOMMARO1; 1Universidade Federal do Rio de Janeiro, Brazil, 2Universidade Federal de Minas Gerais, Brazil, 3Instituto Nacional de Metrologia, Qualidade e Tecnologia-Inmetro, Brazil.

PA13. Absence of *Pneumocystis* Dihydropteroate Synthase Mutants in Brittany, France. SOLENE LE GAL1*, FLORENCE ROBERT-GANGNEUX2, CELINE DAMIANI3, MICHELE VIRMAUX1, ANNE TOTET3, JEAN-PIERRE GANGNEUX2, GILLES NEVEZ1; 1University of Brest, LUBEM EA 3882, SFR 148, Brest, 2University of Rennes, INSERM U1085, Rennes, 3University of Picardy-Jules Verne, EA 4285 UMI INERIS 01, Amiens, France.

PA14. Matches of *Pneumocystis jirovecii* Genotypes between Pulmonary and Exhaled Air Samples from Patients with *Pneumocystis Pneumonia*. CELINE DAMIANI1, SOLENE LE GAL2, FIRAS CHOUKRI3, JEAN MENOTTI3, CLAUDINE SARFATI3, FRANCIS DEROUVIN3, GILLES NEVEZ2*, ANNE TOTET1; 1University of Picardy-Jules Verne, EA 4285 UMI INERIS.
PA15. Selection and characterization of scfv antibodies against *Toxoplasma gondii* tachyzoites from phage display libraries. FERNANDO CARDOSO*, OLGA MATOS, UEI Parasitologia Médica, IHMT- Universidade Nova de Lisboa, Rua da Junqueira, 100, 1349-008 Lisboa, Portugal.

PA16. The Role of Mouse Dendritic Cells in *Cryptosporidium parvum* Infection. BRAMCHETNA BEDI1, NINA MCNAIR2, JAN R. MEAD1,2*. 1Atlanta VA Medical Center, Decatur, GA 30033 2Emory University, Department of Pediatrics, Atlanta, GA 30322


PA18. A role for IFN-g in *Pneumocystis*-induced bone marrow failure in IFNAR-deficient mice. KATHERINE GAUSS, MICHELLE MOZER, STEVEN SEARLES, NICOLE MEISSNER. Montana State University, MT USA.

PA19. Effect of ciprofloxacin derivatives against *Toxoplasma gondii in vitro*. ERICA S. MARTINS-DUARTE1, FRANCINE DUBAR2, CRISTOPHE BIOT3, WANDERLEY DE SOUZA1, ROSSIANE C. VOMMARO 1; 1Instituto Federal do Rio de Janeiro - Instituto de Biofísica Carlos Chagas Filho. 2Université Lille Nord de France - Unité de Catalyse et Chimie du Solide. 3Université Lille Nord de France, Université de Lille1 - Unité de Glycobiologie Structuraleet Fonctionnelle.

PA20. Profiles of cytokines and immunoglobulins in *Cryptosporidium parvum* infection: usefulness of Luminex® xMAP technology VERA CODICES1*, CATARINA MARTINS2, CARLOS NOVO2,3, BRUNO DE SOUSA1, MIGUEL BORREGO2, OLGA MATOS1; 1Instituto de Higiene e Medicina Tropical, CMDT/UNL, Lisboa, Portugal; 2Centro de Estudos de Doenças Crônicas, CEDOC, Faculdade de Ciências Médicas, UNL, Lisboa, Portugal; 3Instituto de Higiene e Medicina Tropical, UNL, Lisboa, Portugal.

PA21. Progress in the *Pneumocystis carinii* Genome Assembly and Annotation ALEKSEY POROLLO1, JAROSLAW MELLER1,2, A. GEORGE SMULIAN1,3, MELANIE T. CUSHION1,2,3 1University of Cincinnati College of Medicine, 3223 Eden Avenue, Cincinnati, OH 45267, USA 2Cincinnati Children’s Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA 3Cincinnati Veterans Administration Medical Center, 3200 Vine Street, Cincinnati, OH 45220, USA

PA22. Studies of an Investigational New Anti-Pneumocystis Compound (IKT061) from in vitro Screening to in vivo Therapeutics in Mice. MICHAEL LINKE2,3, BILAL S. ABUASAL1, PETER D. WALZER2,3, ALAN ASHBAUGH2, MARGARET COLLINS2, KEELEY LYNCH2, LARRY SALLANS3, MILTON H. WERNER3, PANKAJ DESAI1, MELANIE T. CUSHION2,3; 1University of Cincinnati, College of Pharmacy, Department of Basic Pharmaceutical Sciences 2University of Cincinnati, College of Medicine, Department of Internal Medicine, 3 Cincinnati Department of Veterans Affairs Medical Center, 4University of Cincinnati, R. Marshall Wilson Mass Spectrometry Facility, 5Inhibikase Therapeutics, Inc, Atlanta, GA 30339
Tuesday August 7th

BREAKFAST

8:30 AM  IMMUNOLOGY

Session Chairs: BOHUMIL SAK and MAGALI MORETTO

8:30 AM  T25  Nitric oxide resistance mechanisms of Toxoplasma gondii. SINI SKARIAH, ROBERT BEDNARCZYK, NATHANIEL KIM, BRIAN LYNCH, EMMA IACONETTI, NICHOLAS GERMANO, DANA MORDUE*; New York Medical College, Valhalla, NY, USA.

8:50 AM  T26  The Pulmonary Host Response to Pneumocystis murina Asci (Cysts) and Trophic Forms during Clearance of Infection. ALAN D ASHBAUGH 1, MICHAEL J LINKE 2, MELANIE T CUSHION 1.2*, 1 University of Cincinnati, Cincinnati, OH, 2 Veterans Affairs Medical Center, Cincinnati, OH.

9:10 AM  T27  Early intestinal mucosal immune response against per oral microsporidial infection. MAGALI MORETTO, JASON GIGLEY, TAYLOR GOULD, CHRISTINA DEMERVILLE AND IMTIAZ KHAN. Department of Microbiology, Immunology and tropical medicine, George Washington University, Washington 20037, DC.

9:30 AM  T28  Activation of Alveolar Macrophages in Response to Pneumocystis in Neonatal Mice is Defined by Enhanced Expression of Alternative Markers CATHRYN KURKJIAN1, BRIAN S. MURPHY1,2, DAVE J. FEOLA1,3, BETH A. GARVY1,2,4; 1Department of Microbiology, Immunology, and Molecular Genetics, 2Division of Infectious Diseases, Department of Internal Medicine, and 3Department of Pharmacy Practice and Science, College of Pharmacy, University of Kentucky, Lexington, KY, USA, 4Veteran’s Affairs Medical Center, Lexington, KY, USA

9:50AM  T29  Alveolar macrophages in neonatal mice are inherently unresponsive to Pneumocystis infection CATHRYN KURKJIAN1, MELISSA HOLLIFIELD1,4, J. LOUISE LINES1, AMY ROGOSKY1, KERRY M. EMPEY2, MAHBOOB QURESHI1,3, STEPHEN A. BROWN4, BETH A. GARVY1,3,4; 1Departments of Microbiology, Immunology, and Molecular Genetics, 2Clinical Pharmaceutical Sciences, and 3Internal Medicine, University of Kentucky, Lexington, KY, USA and 4Veteran’s Affairs Medical Center, Lexington, KY, USA

COFFEE BREAK

10:40AM  T30  Macrophage Apoptosis Inhibition by Microsporidia Infection. YULIYA Y. SOKOLOVA1,2, LISA C. BOWERS1, XAVIER ALVAREZ1, AND ELIZABETH S. DIDIER*; 1 Tulane National Primate Research Center, Covington, LA USA, 2 Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia.

11:00AM  T31  Modulation of Macrophage Phenotype for Enhanced Pneumocystis Clearance. JING WANG, SAMIR BHAGWAT, FRANCIS GIGLIOTTI, TERRY WRIGHT. Department of Pediatrics, University of Rochester School of Medicine, Rochester, NY, USA

11:20AM  T32  Latent Encephalitozoon cuniculi infection is reactivated after factitious immunodeficiency in mice. BOHUMIL SAK1, MICHAELA KOTKOVÁ1, MARTIN KVÁČ1,2; 1 Institute of Parasitology, BC ASCR, v.v.i., Czech Republic, 2 Faculty of Agriculture, University of South Bohemia in Ceské Budejovice, Czech Republic.
11:50 AM  T33  Deviated systemic immune responses to Pneumocystis lung infection due to a defective type-I-IFN-system can trigger osteoporosis
MICHELLE MOZER, STEVE SEARLES, KATHERINE GAUSS, NICOLE MEISSNER,
Department of Immunology and Infectious diseases, Montana State University,

LUNCH

2:00 PM  CLINICAL EFFECTS OF INFECTION
Session Chairs: MONICA SASSI and PHILIPPE HAUSER

2:00 PM  T34  Outbreaks of Pneumocystis Pneumonia in Two Renal Transplant Centers Linked to a Single Strain of Pneumocystis: Implications for Transmission and Virulence
MONICA SASSI1, CHIARA RIPAMONTI1, NICOLAS J. MUELLER2, HIROHISA YAZAKI3, GEETHA KUTTY1, LIANG MA1, CHARLES HUBER5, EMILE GOGINENI1, SHINICHI OKA3, NORTHIKO GOTO6, THOMAS FEHR7, SARA GIANELLA8, REGINA KONRAD9, ANDREAS SINS9, and JOSEPH A. KOVACS1
Critical Care Medicine 1 and Department of Laboratory Medicine 5, NIH Clinical Center, National Institutes of Health, Bethesda, MD, USA; Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland 2; AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan 3; Division of Viral Immunology, Center for AIDS Research, Kumamoto University, Kumamoto, Japan 4; Department of Transplant and Endocrine Surgery, Nagoya Daini Red Cross Hospital, Nagoya, Japan 6; Division of Nephrology, University Hospital Zurich, Zurich, Switzerland 7; University of California, San Diego, La Jolla, CA, USA 8; Bavarian Health and Food Safety Authority (Bavarian LGL), Veterinaerstrasse 2, 85764 Oberschleissheim, Germany 9.

2:20 PM  T35  A specific Pneumocystis jirovecii genotype may worsen outcome of pneumonia in HIV–infected patients.
M. RABODONIRINA1, L. VAILLANT2, P. TAFFE3, A. NAHIMANA4, R.-P. GILLIBERT5, P. VANHEMS2,5, P.M. HAUSER6,7; 1Laboratoire de Parasitologie, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Universitée Claude-Bernard Lyon I, France. 2Hospices Civils de Lyon, Hôpital Edouard Herriot, Service d’Hypogène, Epidémiologie et Prévention. 3Data Center, Swiss HIV Cohort Study, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Switzerland. 4Hospital Preventive Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne. 5Université de Lyon 1; CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive. 6Institute of Microbiology, Centre Hospitalier Universitaire Vaudois and University of Lausanne.

2:40 PM  T36  The chronic Toxoplasma infection can alter mice’s behavior, intelligence and emotion.
MIN LIU, YUN-YING YAO, SHANLIANG SHEN, KUN WU, HONG-JUAN PENG, XIAO-GUANG CHEN*. Department of Parasitology, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, Guangdong 510515, P.R.China.

3:10PM  T37  High prevalence of Pneumocystis Jirovecii infections among Mozambican children <5 years of age admitted to hospital with suspected pneumonia
QUIQUE BASSAT, CRISTINA O’CALLAGHAN-GORDO, LUIS MORAIS, SÓNIA MACHEVO, NÚRIA DIEZ-PADRISA, BETUEL SIGAUQUE, PEDRO L. ALONSO, VICENTE FRIAZA, ELENA CAMPANO, ENRIQUE J. CALDERÓN*, ANNA ROCA. 1 Barcelona Centre for International Health Research, (CRESIB, Hospital Clínica-Universitat de Barcelona), Barcelona, Spain; 2 Centro de Investigación-emSaúde de Manhiça (CISM), Maputo, Mozambique 3Instituto de Biomedicina de Sevilla and CIBER de Epidemiología y Salud Pública. Virgen del Rocio University Hospital, Seville, Spain.
3:30 PM T38 Microsporidia and Cryptosporidia in HIV infected patients in St.
Petersburg, Russia. Olga I. Sokolova1, Anton V. Demyanov2, Vasily F. Dedov2, Elizabeth S.
Didier4, and Yuliya Y. Sokolova3,5 1S.P. Botkin Memorial Hospital, Medical Faculty, St.
Petersburg State University; 2State Research Institute of Highly Pure Biopreparations; 3Institute
of Cytology RAS, St. Petersburg, Russia; 4Tulane National Primate Research Center,
Covington, LA; 5School of Veterinary Medicine, LSU, Baton Rouge, LA, USA

3:50 PM T39 Predictors of Clinical Stability and Relapse in HIV-associated
Pneumocystis Pneumonia. EUNICE J. KIM1, MATTHEW W. FEI2, LEAH G. JARLSBERG3,
CATHERINE A. SANT1, ALEXANDRA SWARTZMAN1 AND LAURENCE HUANG1*. 1Department of Medicine, San Francisco General Hospital, University of California San Francisco, San Francisco, CA, USA.

COFFEE BREAK

4:30 PM COMMUNITY INITIATIVES AND ROUND TABLE DISCUSSIONS

4:30 PM T40 Ibero-American Network about Pneumocystosis, a project supported
by the Ibero-American Programme for Science, Technology and Development (CYTED)
ENRIQUE CALDERÓN. Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del
Rocío/ CSIC/Universidad de Sevilla, and CIBER de Epidemiología y Salud Pública, Seville,
Spain.


DINNER

8:00 PM POSTER SESSION B

PB1. Identification and genetic analysis of Pneumocystis from thoroughbred foals.
THOMAS M. SESTERHENN1,2, ROBIN W. ALLISON3, MELANIE T. CUSHION4; 5University of
Cincinnati College of Medicine, Cincinnati, OH, USA, 2Cincinnati Veteran’s Affairs Medical
Center, Cincinnati, OH, USA, 3Oklahoma State University, Department of Veterinary
Pathobiology, Stillwater, OK, USA.

PB2. One-year follow-up study of Giardia spp and Cryptosporidium species in
recreational waters from Spain. ANA L. GALVÁN1,2, ANGELA MAGNET1, FERNANDO
IZQUIERDO1, CARMEN FERNANDEZ-VADILLO4, REGINA H.S. PERALTA3, SOLEDAD
FENOY1, CARMEN DEL AGUILA1; 1Laboratorio de Parasitología, Facultad de Farmacia,
Universidad San Pablo CEU, Urbanización Montepríncipe, 28668, Boadilla del Monte, Madrid,
España, 2Escuela de Microbiología, Universidad de Antioquia, Medellín, Colombia, Becaria
Colciencias, 3Universidad Federal Fluminense, Departamento de Patologia.

PB3. Molecular characterization of human pathogen microsporidia and Cyclospora
cayetanensis during a one-year follow-up study from several drinking water treatment
plants and wastewater treatment plants. ANA L. GALVÁN1,2, ANGELA MAGNET1, FERNANDO
IZQUIERDO1, SOLEDAD FENOY1, CRISTINA RUEDA1, CARMEN FERNANDEZ-
VADILLO1, NUNO HENRIQUES-GIL1, CARMEN DEL AGUILA1; 1Parasitology laboratory, San
Pablo CEU University, Urbanización Montepríncipe, Boadilla del Monte, Madrid, Spain,
2Escuela de Microbiología, Universidad de Antioquia, Medellín, Colombia, Becaria
Colciencias.

PB 4. Low incidence of Acanthamoeba spp. in contact lens wearers from Madrid, Spain.
THIAGO S. GOMES1, ANGELA MAGNET1, FERNANDO IZQUIERDO1, SARA BUENO2,
MARIA LUISA SANCHEZ RODRIGUEZ², SOLEDAD FENOY¹, CARMEN DEL ÁGUILA¹; ¹Parasitology laboratory, San Pablo CEU University, Urbanización Montepríncipe, Boadilla del Monte, Madrid, Spain, ²Optical laboratory, San Pablo CEU University, Urbanización Montepríncipe, Boadilla del Monte, Madrid, Spain

PB5. Molecular characterization of Cryptosporidium in children and cattle populations from Romania. PATRÍCIA VIEIRA¹, NARCISA MEDERLE², MARIA LÚISA LOBO³, OVIDIU MEDERLE³, LIHUA XIAO⁴, GHEORGE DARABUS², OLGA MATOS¹; ¹Instituto de Higiene e Medicina Tropical, CMU/UNL, Lisboa, Portugal, ²Parasitology Department, Faculty of Veterinary Medicine, Timisoara, Romania, ³Faculty of Medicine, Timisoara, Romania, ⁴Division of Foodborne, Waterborne and Environmental Diseases, CDC, Atlanta, GA, USA.

PB6. Use of Molecular Standards as High Quality PCR Controls for Parasites. LINH N.K. NGUYEN*, LAURA A. SAHNOW, CRYSTAL R. ICENHOUR; Phthisis Diagnostics, Charlottesville, VA.

PB7. Molecular and morphological characterization of Agmasoma penaei parasitizing Litopenaeus setiferus from the Gulf of Mexico, and analysis of microsporidian diversity in marine decapods inferred from SSUrDNA phylogenies. YULIYA Y. SOKOLOVA¹, JOHN P. HAWKE²; ¹Dept. Comparative Biomedical Studies, ²Dept. Pathobiological Sciences, School of Veterinary Medicine LSU, Baton Rouge LA, USA.

PB8. Molecular characterizations of Cryptosporidium, Giardia, and Enterocytozoon in humans in Kaduna State, Nigeria. BEATTY V. MAIKAI¹,², JARLATH U. UMHO², IDRIS A. LAWAL³, AYUBA C. KUDI⁴, CLARA L. EJEMBI⁵, VICTOR A. MAIKAI⁶, LIHUA XIAO¹; ¹Division of Foodborne, Waterborne and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA, ²Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, ³Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, ⁴Department of Veterinary Medicine and Surgery, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, ⁵Department of Community Medicine, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria, College of Agriculture and Animal Science, Ahmadu Bello University, Mando Road, Kaduna, Nigeria.

PB9. Epidemiology of Giardia and Cryptosporidium in Kolkata, India. SANDIPAN GANGULY* Dept. of Parasitology, National Institute of Cholera & Enteric Diseases, Indian Council of Medical Research, Kolkata, West Bengal, India.

PB10. Experimental cryptosporidiosis in pigs: host and age specificity of Cryptosporidium Muris, C. Tyzzeri, C. Suis and Cryptosporidium Pig Genotype II. MARTIN KVÁC¹,², MICHAELA KESTRÁNOVÁ², DANA KVETONOVA¹, MICHAELA KOTKOVÁ¹, JANA KALINOVA¹, YNÉS ORTEGA³, JOHN MCEVOY⁴, BRIANNA STERLING⁴, BOHUMIL SAK¹; ¹Institute of Parasitology, BC ASCR, v.v.i., Czech Republic, ²Faculty of Agriculture, University of South Bohemia in Ceské Budejovice, Czech Republic, ³Center for Food Safety, Department of Food Science & Technology, University of Georgia, Griffin, USA, ⁴Veterinary and Microbiological Sciences Department, North Dakota State University, Fargo, USA.

PB11. Enterocytozoon bieneusi and Encephalitozoon cuniculi in horses kept under different management systems in the Czech Republic. PAVLA WAGNEROVÁ¹,², BOHUMIL SAK, DANA KVETONOVA², ZITA BUNATOVÁ¹, HANA CIVIŠOVÁ¹, MIROSLAV MARŠÁLEK¹,
PB12. Diversity of *Cryptosporidium* spp., *Encephalitozoon* spp. and *Enterocytozoon bieneusi* in great apes in different level of habituation. BOHUMIL SAK1,*, ANNA MYNÁROVÁ2, KLÁRA PETRZELKOVÁ3,4,5, DANA KVETONOVÁ1, KATERINA POMAJBÍKOVÁ3, DAVID MODRÝ1,3, BÁRBORA KALOUSOVÁ6, MARTIN KVÁC1,7; 1Institute of Parasitology, BC ASCR, v.v.i., Czech Republic, 2Faculty of Science, University of South Bohemia in Ceské Budejovice, Czech Republic, 3Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, 4Institute of Vertebrate Biology, ASCR, Czech Republic, 5Liberec Zoo, Czech Republic, 6Faculty of Science, Masaryk University, Czech Republic, 7Faculty of Agriculture, University of South Bohemia in Ceské Budejovice, Czech Republic

PB13. The variability of *Cryptosporidium* spp. infecting rodents of family Muridae in the Czech Republic and the Slovak Republic. VERONIKA RAŠKOVÁ1,2, MICHAL STANKO3, JAN VÁVRA4, BOHUMIL SAK2, DANA KVETONOVÁ2, MARTIN KVÁC1,2,*; 1Faculty of Agriculture, University of South Bohemia in Ceské Budejovice, Czech Republic, 2Institute of Parasitology, BC ASCR, v.v.i., Czech Republic, 3Institute of Zoology, SAS, Slovak Republic, 4Bishop gymnasium of J. N. Neumann, Ceské Budejovice, Czech Republic

PB14. *Encephalitozoon* and *Enterocytozoon* (microsporidia) spores in stool from pigeons and exotic birds. MARIA ANETE LALLO1,2*, PATRÍCIA CALÁBRIA2, LILIANE MILANELO3; 1Universidade Paulista (UNIP); 2Universidade Cruzeiro do Sul (Unicsul); 3Parque Ecológico do Tietê

PB15. Free-living South American coatis (*Nasua nasua*) can be source of infection of *Encephalitozoon* and *Enterocytozoon* (microsporidia) spores for humans. MARIA ANETE LALLO1,2*, PATRÍCIA CALÁBRIA2, LILIANE MILANELO3; 1Universidade Paulista (UNIP); 2Universidade Cruzeiro do Sul (Unicsul); 3Parque Ecológico do Tietê

PB16. Study of the prevalence of *Cryptosporidium* in lambs for estimate the zoonotic risk in the region of Algiers. KHELEF D.1, XIAO L.2, BAROUDI D.1; 1Laboratory of Parasitology-Mycology, Department of Cattle and Avian Pathology, High National Veterinary , School of Algeirs , Algeria, 2Centers for Disease Control and Prevention, Atlanta ,Georgia, USA

PB17. Characterization of multiple polymorphisms in *Pneumocystis jirovecii* by multiplex PCR/Single-Base Extension (MPCR/SBE) - Preliminary results CAMILA B. FERNANDES1, FRANCISCO ESTEVES3”, OLGA MATOS 1 1Instituto de Higiene e Medicina Tropical, CMDT/UNL, Lisboa, Portugal.

Wednesday August 8th

BREAKFAST

9:00 AM CELLULAR BIOLOGY

Session Chairs: ANN CALI and ANDREW LIMPER

9:00 AM T41 Escapology taught by *Toxoplasma gondii*: tachyzoite evasion from vacuole loaded with Irga6 in mouse embryonic fibroblasts. MARIALICE HEIDER1, YASUHIRO TAKASHIMA2, JONATHAN C. HOWARD1; 1Department of Cell Genetics, Institute
9:20AM T42 All Glucans are Not Created Equal: Evidence for β-1,6 Glucans in the *Pneumocystis carinii* Cell Wall. ANDREW H. LIMPER, DEANNE M. HEBRINK, SEHER IQBAL, THEODORE J KOTTOM. Thoracic Diseases Research Unit, Mayo Clinic, Rochester, MN USA 55905.

9:40AM T43 *Anncalia algerae* sporoplasms contain a Multilayered Interlaced Network (MIN) which has Golgi-like structural and enzymatic properties. PETER M. TAKVORIAN¹, KAROLYN F. BUTTLE², DAVID MANKUS², CARMEN A. MANNELLA³, LOUIS M. WEISS²,³ ANN CALI¹; ¹Department of Biological Sciences, Rutgers University, Newark, New Jersey, USA, ²Department of Pathology, Division of Tropical Medicine and Parasitology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY, USA, ³Resource for Visualization of Biological Complexity, Wadsworth Center, New York State Dept of Health, Empire State Plaza, Albany NY, USA, ⁴Department of Medicine, Division of Infectious Diseases, Albert Einstein College of Medicine,1300 Morris Park Avenue, Bronx, NY, USA.

10:00AM T44 Thigmotropism in *Pneumocystis carinii*: Contact Mediated Cell-Signaling Regulates Cell Wall Assembly Control Pathways. ANDREW H. LIMPER¹, THEODORE J. KOTTOM¹. ¹Thoracic Diseases Research Unit, Mayo Clinic, Rochester, MN USA 55905.

COFFEE BREAK

10:50AM T45 Identification and Characterization of Toxoplasma gondii Cyst Wall Proteins. TADAKIMI TOMITA¹, YAN FEN MA¹, YANSEN XAN¹, BARBARA FOX², DAVID J. BZIK³ AND LOUIS M. WEISS¹,² * Departments of ¹Pathology and ²Medicine, Albert Einstein College of Medicine, Bronx, NY, USA. Department of ³Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH, USA.

11:10AM T46 A case for host involvement in the glycosylation of *Toxoplasma* tissue cysts. BECCA ELLER¹, ARUN DATTA², BERT C. LYNN³ and ANTHONY P. SINAI¹; *¹Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky College of Medicine, 800 Rose Street, Lexington KY 40536. ²National University, 11255 N. Torrey Pines Road, La Jolla, CA 92037. ³Department of Chemistry, University of Kentucky, A053 ASTeCC Building, Lexington, KY 40506

11:30AM T47 Enterocytozoonidae Revisited. ANN CALI**, PETER MICHAEL TAKVORIAN¹, OSWALDO PALENZUELA², MARIA JOSÉ REDONDO², and ARIADNA SITJÀ-BOBADILLA²; ¹Department of Biological Sciences, Rutgers University, Newark NJ, USA, ²Instituto de Acuicultura Torre de la Sal (IATS-CSIC), Castellón, Spain.

LUNCH

1:30 PM EPIDEMIOLOGY and TAXONOMY

Session Chairs: LIHUA XIAO and MARTIN KVÁC

1:30 PM T48 *Cryptosporidium ubiquitum*, an Emerging Zoonotic Pathogen in Humans. NA LI,¹,² YAOYU FENG,¹ LIN WANG,¹ MONICA SANTIN-DURAN,² RONALD FAYER,³ LONGXIAN ZHANG,⁴ JINZHONG CAI,⁵ LIHUA XIAO**; ¹East China University of Science and Technology, Shanghai, China, ²Centers for Disease Control and Prevention, Atlanta, Georgia, USA, ³Agricultural Research Service, U. S. Department of Agriculture,
1:50 PM T49 Direct evidence of airborne excretion of Pneumocystis carinii during infection in immunocompetent rats. Lung involvement and antibody response. J. MENOTTI1*, A. EMMANUEL1, C. BOUCHEKOUK1, M. CHABE2, F. CHOUKRI1, M. POTTIER2, C. SARFATI1, E. M. ALIOUAT2, F. DEROUIN1. 1Department of Parasitology-Mycology, E.A.3520, Paris-Diderot University and Saint Louis Hospital APHP, Paris, France; 2Biography and Diversity of Emerging Eukaryotic Pathogens (BDEEP), Center for Infection and Immunity of Lille (CIIL); Inserm U1019, CNRS UMR 8204, Université Lille-Nord de France, Lille, France.

2:10PM T50 Transmission characteristics of Pseudoloma neurophilia in the zebrafish, Danio rerio. JUSTIN L. SANDERS*, VIRGINIA WATRAL, MICHAEL L. KENT; Oregon State University, Corvallis, OR, USA.

2:30PM T51 First identification of the avian zoonotic specie Cryptosporidium meleagridis in broiler chickens and turkeys in Algeria using molecular and conventional methods and about a new GP60 subtype family detected. DJAMEL BAROUDI1*, JAMEL KHELEF1, RACHID GOUCEM1, KARIM ADJOU3, LIHUA XIAO3; 1Laboratory of Parasitology-Mycology, Department of Cattle and Avian Pathology, High National Veterinary School of Algiers, El-Harrach, Algeria, 2UMR-BIPAR, ANSES-ENVAlfort, Paris, France, 3Centers for Disease Control and Prevention, 4770 Buford Highway Chamblee, Atlanta, GA, USA.

2:50PM T52 Potentially pathogenic free-living amoebae isolated from recreational areas of rivers in Tehran, Iran. MARYAM NIYYATI1*, ZOHERH LASJERDI1, MAHDIIEH NAZAR1, ALI HAGHIGHI1, EHSAN NAZEMALHOSSEINI MOJARAD2; 1Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran, 2Research Center for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

3:10PM T53 Identification and Differentiation of two Nosema spp. Isolated from Pieris rapae and Hemerophila atrilineata LIU JI-PING1,2*, HAO JUAN1,2 AND LIAO SEN-TAI1,2; 1College of Animal Science, South China Agriculture University, Guangdong Provincial Key Lab of Agro-Animal Genomics and Molecular Breeding, Guangzhou 510642, China; 2Guangdong Provincial Agricultural Academy Institute, Guangzhou 510640, China

COFFEE BREAK

4:00 PM T54 Cryptosporidium suis and Cryptosporidium pig genotype II: its associations with age and husbandry practices. MARTIN KVÁC1,2, KAREL NEMEJC1, MIACHELA KESTRÁNOVÁ1, BOHUMIL SAK2, DANA KVETONOVÁ2, NADEZDA KERNEROVÁ1, MICHAEL ROST3, VITALIANO A. CAMA4; 1Faculty of Agriculture, University of South Bohemia in Ceské Budejovice, Czech Republic, 2Institute of Parasitology, BC ASCR, v.v.i., Czech Republic, 3Faculty of Science, University of South Bohemia in Ceské Budejovice, Czech Republic, 4Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

4:20PM T55 Detection of Multiple Zoonotic Infectious Agents in Cattle with Blastocystis, Cryptosporidium, Giardia, and Enterocytozoon by Molecular and Microscopic Methods. R. FAYER*, M. SANTIN, D. MACARISIN; Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland, USA

4:50PM T56 Anthroponotic Enteric Parasites in Free-range Rhesus Monkeys in a Public Park, China JIANBIN YE1,2, LIHUA XIAO2, JINGBO MA1, AND YAOYU FENG1*; 1East
5:10 PM T57 Molecular characterizations of Cryptosporidium, Giardia, and Enterocytozoon in humans in Kaduna State, Nigeria BEATTY V. MAIKAI A, B, JARLATH U. UMOH B, IDRIS A. LAWAL C, AYUBA C. KUDI D, CLARA L. EJEMBI E, VICTOR C. MAIKAI F, LIHUA XIAO A,* a Division of Foodborne, Waterborne and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA; bDepartment of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria; cDepartment of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, d Department of Veterinary Medicine and Surgery, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria; e Department of Community Medicine, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria; f College of Agriculture and Animal Science, Ahmadu Bello University, Mando Road, Kaduna, Nigeria.

5:30PM T58. First identification of the avian zoonotic specie Cryptosporidium meleagridis in broiler chickens and turkeys in Algeria using molecular and conventional methods and about a new GP60 subtype family detected. DJAMEL BAROUDI 1*, DJAMEL KHELEF 1, RACHID GOUCEM 1, KARIM ADJOU 3, and LIHUA XIAO 3; 1Laboratory of Parasitology-Mycology, Department of Cattle and Avian Pathology, High National Veterinary School of Algiers, El-Harrach, Algeria, 2UMR-BIPAR, ANSES-ENVALfort, Paris, France, 3Centers for Disease Control and Prevention, 4770 Buford Highway Chamblee, Atlanta, GA, USA.

5:50PM Summary and closing comments. LOUIS M. WEISS, MELANIE T. CUSHION 1
TALK SESSIONS
ABSTRACTS

T1 to T58
T1. Pneumocystosis: Perspectives on the Past and the Future. PETER D. WALZER, University of Cincinnati, VA Medical Center, OH, USA.

*Pneumocystis* (Pc) is an opportunistic fungal pathogen found in the lungs of humans and other animals in nature. Research on Pc has been hampered by the lack of a reliable *in vitro* culture system. Pc is an important cause of pneumonia (PcP) in immunocompromised hosts; Pc respiratory tract colonization has been associated with a decline in lung function. One of the least studied areas of Pc research is its ecology. This presentation focuses on the past and future perspectives of the interaction of Pc with the environment.

Pc can be transmitted by close contact and the airborne route, and the cyst is the likely transmissible form. It is also possible that an additional, undetected stage may exist in the air. The effects of environmental factors on cyst viability are poorly understood. Outbreaks of PcP are occurring more frequently in renal transplant recipients than in the past, and geographic clusters of PcP have occurred in HIV+ patients. Studies of HIV+ patient populations have identified temperature and outdoor activities (hiking, gardening) as independent risk factors for PcP.

One way to advance Pc ecological research is to learn from investigations of other organisms. Studies of invasive mycoses caused by inhalation of spores have identified specific climatological factors that affect the occurrence of coccidiomycosis in the normal host and hospital outbreaks of aspergillosis in immunosuppressed patients. Respiratory viruses (influenza) have added information about contagion, crowding, air pollutants, and the value of animal models.

More basic and clinical research is needed to better understand the interaction of Pc and the environment. The use of tools that are already available or adapted from studies of other organisms is a good first step to achieving this goal.

[Supported by NIH Grant HL-090335 and Department of Veterans Affairs]
The Eukaryotic Pathogen Genome Database (http://www.EuPathDB.org) is an NIH/NIAID funded Bioinformatics Resource Center, providing an online portal to the complete genome sequence, annotations and integrated functional genomics data from multiple eukaryotic pathogens. These include *Entamoeba spp.* (AmoebaDB.org), *Cryptosporidium spp.* (CryptoDB.org), *Babesia spp.* and *Theileria spp.* (PiroplasmaDB.org), *Plasmodium spp.* (PlasmoDB.org), *Toxoplasma gondii*, *Neospora caninum* and *Eimeria tenella* (ToxoDB.org), *Giardia* (GiardiaDB.org), *Trichomonas vaginalis* (TrichDB.org) and kinetoplastids (TriTrypDB.org).

The EuPathDB component databases enable scientists to ask questions about complex genomic-scale data using an intuitive graphical interface. Data types include the underlying genomic sequences and annotations, transcript level data (SAGE-tag, EST, microarray and RNA sequence data), protein expression data, epigenomic data, population-level and isolate data. In addition, automated genomic analyses provide the ability to search for gene features, subcellular localization, motifs (InterPro and user defined), function (Enzyme commission annotation and GO terms) and evolutionary relationships.

EuPathDB feature highlights include:

- Graphical search method allows building complex searches in a step-wise manner. Strategies can be saved, modified and shared. An example strategy can be viewed by following this link: [http://plasmodb.org/plasmo/im.do?s=19eaaf6ea54f7244](http://plasmodb.org/plasmo/im.do?s=19eaaf6ea54f7244)
- Gene basket, allows cherry picking genes of interest for later integration into a strategy.
- A favorites page provides a location to store and organize genes of interest for future rapid access.
- User comments can be added to records in EuPathDB and can be associated with images, files, PubMed and Genbank records, etc.
- Column analysis tools are available to generate word cloud graphics and histograms of results.
- A genomic colocation tool enables searches based on the relative genomic locations. For example, identifying genes based on their location relative to a DNA motif.
Various species of pathogenic protozoan parasites have evolved a series of strategies to evade the immune system and establish chronic infections in the host. Elucidating the underlying mechanisms used by highly adapted parasites to block or subvert host cellular processes offers new targets for therapeutic intervention or vaccine development. In this context, the accessibility of reference strains and reagents is critical to the generation of studies aimed at deciphering the intricate aspects of the host-parasite relationship. Protozoan strains deposited in culture collections are in fact considered ‘biological standards’ as they are critical components of comparative studies. The Protistology Collection at the American Type Culture Collection (ATCC®) houses the largest and most diverse repository of parasitic protozoa in the world. The primary role of the collection over the years has been the acquisition, authentication, preservation, and distribution of reference parasite strains to the wider scientific community. Characterization of deposited cultures includes a variety of tests such as viability, purity, phenotypic properties, and genotypic analysis. Establishing seed and distribution stocks for every strain is a common practice to make certain that cultures distributed to researchers are closely similar to the original material provided by the depositor. Over the last decade resources within the collection have expanded to include a variety of biomaterials such as genomic DNAs, polyclonal antisera, monoclonal antibodies, and expression clones. This presentation will provide an overview of biological resources available to the researcher, a discussion on the benefits of depositing, current research projects, and future perspectives.
The translation of technologies from academic settings to commercially-viable products is desirable for many biotechnology companies. There are two critical pieces of the translational puzzle: knowledge and pain. The academic side is best suited for the pursuit of knowledge. And while knowledge is powerful, the company must identify a customer pain which can be resolved by the translated knowledge. This session will explore differences between academic and industry research and how the two can efficiently work together for the improvement of human health.
In order to develop less invasive methodologies for laboratorial/clinical analysis of *Pneumocystis jirovecii* (Pj) pneumonia (PcP), the aim of the present study was to evaluate the usefulness of (1-3)-β-D-Glucan (BG) and lactate dehydrogenase (LDH) as serological markers in the diagnosis of PcP in HIV+ patients.

Pulmonary specimens and sera were obtained from 100 HIV+ Portuguese patients for diagnostic purpose (1994-2011). Sera were collected from 20 blood donors (negative control group). PcP cases were confirmed by using indirect immunofluorescence with monoclonal antibodies (IF) and nested-PCR. Data on clinical diagnosis of PcP (symptoms, PaO₂, chest radiographs), T CD4+ counts, and anti-Pj prophylaxis and therapy were available. BG and LDH levels in serum were measured using specific quantitative microplate-based assays according to standard procedures (Fungitell Assay, Associates of Cape Cod, MA, USA; LDH Quantification Assay Kit (BioVision, Inc.).

Of the 100 HIV+ patients studied, 69 were PcP diagnosed (66 proven PcP cases and three patients not fulfilling the clinical criteria for PcP) (median: BG 270 pg/ml, LDH 707 U/L) and 31 were PcP negative (12 were colonized by Pj presenting subclinical infection and 19 were not infected by Pj) (median: BG 45 pg/ml, LDH 420 U/L). The median serologic markers levels detected for the colonization cases and for the patients without Pj infection were 67 pg/ml and 36 pg/ml for BG, and 474 U/L and 419 U/L for LDH, respectively. In the blood donors the median BG level was 31.0 pg/ml (lower limit), while the median LDH level was 141 U/L. The BG and the LDH positive sera were statistically associated with PcP cases (90%, \(P < 0.001\) for BG; 93%, \(P = 0.01\) for LDH) and with cases fulfilling clinical diagnostic criteria for PcP (83%, \(P < 0.001\) for BG; 91%, \(P = 0.04\) for LDH).

We thank to Associates of Cape Cod, Inc. for providing free BG detection kit. Supported by “FCT” Ref. PTDC/SAU-MII/104231/2008 and “AIDFM de Lisboa”.

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T5. Serum markers (1-3)-Beta-glucan and lactate dehydrogenase levels for the diagnosis of *Pneumocystis* pneumonia (PcP) in HIV+ patients. FRANCISCO ESTEVES1, MARGARIDA SERINGA1, ROBERT BADURA2, BRUNO DE SOUSA1, CAMILA FERNANDES1, FRANCISCO ANTUNES2, JORGE GASPAR3, CHAO-HUNG LEE4, OLGA MATOS1; 1Instituto de Higiene e Medicina Tropical, CMDT/UNL, Lisboa, Portugal. 2Hospital de Santa Maria, FM/UL, Lisboa, Portugal. 3Faculdade de Ciências Médicas, CIGMH/UNL, Lisboa, Portugal. 4Indiana University School of Medicine, Indianapolis, Indiana, USA.
Quantitative Microbial Risk Assessment (QMRA) is a statistical technique for assessing pathogen risks associated with pathogen exposure from drinking water. QMRA requires detection assays that are both quantitative and sensitive enough for low-abundance pathogens typical in environmental samples. USEPA Method 1623 is the approved method for estimating levels of *Cryptosporidium* and *Giardia* (oo)cysts present in water. This method of filtration, immunomagnetic separation, and enumeration of the target organisms by epifluorescent microscopy can detect a single (oo)cyst. Although this approach is effective for monitoring various water matrices, it can be technically challenging and labor intensive. More importantly, it does not differentiate between pathogenic or nonpathogenic species, and it cannot determine the human infectious potential of the (oo)cysts detected. Moreover, this approach does not detect other pathogenic protozoa, like *Toxoplasma gondii*. Nevertheless, Method 1623 has provided critical values for establishing QMRA estimates of risks associated with *Cryptosporidium* and *Giardia* in drinking water. One strategy to address these limitations is PCR. PCR has been successfully used for the detection and genotyping of *Cryptosporidium* and *Giardia* in both clinical and environmental samples. However, little is known about the robustness of PCR for quantification of low-level concentrations of these parasites. Because environmental (oo)cyst densities are below the normal quantitation range of PCR and little is known about the analytic sensitivity of PCR for both pathogens, alternative approaches are desirable. One such alternative is using Most Probable Number (MPN) PCR. The MPN-PCR is a statistical and molecular approach based on the Poisson distribution of estimating the density of low-abundance targets without an external standard. Research is underway to determine the detection limits and uncertainties due to clumping or PCR inhibitors by MPN-PCR for several waterborne protozoan pathogens. Ultimately, this approach will provide additional data for QMRA analyses of these pathogens in drinking water.
The microsporidian *Nosema bombycis*, a well-known protistan pathogen of silkworms pebrine disease, now transferred to fungi, infects all tissues and developmental stages from the embryo to the adult, and is high pathogenic to pest insects in mulberry trees. In order to develop a loop-mediated isothermal amplification (LAMP) assay for the rapid, and specific detection of *Nosema bombycis*. On the basis of separation, purification of *N. bombycis* spores from artificial propagating silkworms and collection of microscopic samples of female moths from the silkworm eggs' production in the field. Selecting the pseudogene of 16SSrRNA of *N.bombycis* as the identification target gene, LAMP primers for detecting species-specific for *N. bombycis* were designed with the online software program of Primer Explorer V3, and screened basing on the specificity, sensitivity and practicability to the field samples. The results as follows, firstly, over six groups of LAMP primers were designed. Then, all of their effectiveness, specificity and reaction time were investigated respectively; one group of primers FI2/BI2 and F2/B2 were screened. Secondly, the primers were used to detect the species specificity to *Nosema bombycis*. LAMP have the positive reaction in the samples of *N.bombycis*, and the mixture of *N.bombycis* and BmNPV polyhedron, and the microsporidian spores isolated from *Prodenis litura* after incubation for 60 minutes at 63°C, respectively. the results were also confirmed by PCR. Finally, we found that the sensitivity of LAMP were related with the extracting template DNA from spores. DNA extracted by boiling precipitation method was 10^4 times higher than that of DNA extracted by CTAB method, and was 100 fold than that of identification with PCR, the lower limit concentration was 3.6 ×10^3 spores/mL of the *N.bombycis*. Furthermore, 40 microscopic samples of female moths were compared by LAMP and PCR, of which 67.5% and 47.5% samples were confirmed by LAMP and PCR, respectively. Pre-denaturizing could be helpful of reducing the false positive. The LAMP assay is a rapid, specific, sensitive and convenient method for *N.bombycis*. This assay is suitable for rapid diagnosis in *N.bombycis* infection.

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Cystic fibrosis (CF) is one of the most common indications for lung transplantation worldwide. P. jirovecii is a major cause of fatal pneumonia in immunosuppressed lung transplant patients. However, there is no data about P. jirovecii colonization in lung transplant recipients with CF. The aim of this study was to analyze the frequency and dynamic evolution of P. jirovecii colonization among patients with CF after lung transplantation and the utility of β-D-glucan as serological marker. The study included 11 lung transplant recipients (LTR) and 7 CF patients who attended a specialized unit. All them were followed for a 1-year period and underwent a clinical and biological examination every 6 months in conjunction with use of a standardized questionnaire. Also, six individuals without CF were included as control. An individual colonized with P. jirovecii was defined as a subject, with no symptoms or signs of PcP, whose respiratory specimen contained P. jirovecii DNA detectable by nested PCR. At baseline, P. jirovecii colonization was detected in five (45.5%) LTR, but everyone except for one were colonized sometime during the follow-up period. β-D-glucan levels were measured in all cases by using the Fungitell test kit as recommended by the manufacturer. Median β-D-glucan was 87.6 ± 37.7 pg/mL in P. jirovecii colonized LTR, compared with 80 ± 29.3 pg/mL in LTR without colonization (P = 0.59). Other possible fungal infections were explored using a ITS PCR-based approach. Our results demonstrate that P. jirovecii colonization is common and may appear at any time after lung transplantation in patients with Cystic fibrosis despite use of chemoprophylaxis. Serum level of β-D-glucan is not useful for detecting P. jirovecii colonization in Cystic fibrosis patients. Therefore, frequent monitoring of this patients using molecular techniques is thus necessary for detecting P. jirovecii colonization.

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T9. Development of endochin-like quinolones for the treatment and prevention of toxoplasmosis. LORRAINE JONES-BRANDO,1*, CLAUDIA Bordón,1 ROLF Winter,2 AARON NILSEN,2 J. STONE DOGGETT,2 MIKE RISCOE,2 ROBERT Yolken,1; 1 Johns Hopkins University, MD, USA, 2 Oregon Health & Science University, OR, USA.

Toxoplasmosis causes significant morbidity worldwide. Disease symptoms can range from mild to life-threatening. In addition, infection with *Toxoplasma gondii*, as determined serologically, is associated with an increased risk of developing schizophrenia or other serious psychiatric diseases. Therefore it could be beneficial to anti-*T. gondii*-positive schizophrenia patients to treat and eliminate the Toxoplasma infection and thereby alleviate the psychiatric symptoms. A major obligation of the Stanley Laboratory at Johns Hopkins is to develop new, specific and highly effective drugs to treat and prevent schizophrenia. Consequently our lab is actively seeking and developing safe and highly potent drugs to treat and ultimately eradicate toxoplasmosis. A library of more than 70 novel endochin-like quinolones (ELQs) was synthesized and examined for in vitro activity against *T. gondii*. Both the parent compound endochin and the ELQs proved to be both highly efficacious (IC50 range = 1.4 x 10^-8 pM – 17 µM) against *T. gondii* infection and relatively non-cytotoxic (75% of ELQs TD50 ≥ 320 µM). Standard replication and invasion assays showed that a majority of the ELQs are active on both replicating intracellular as well as purified extracellular tachyzoites. Further, we found that the extent and longevity of the effect on extracellular parasites is time and dose dependent. Selected compounds are parasiticidal against tachyzoites while others are parasitistatic. The mode of action of these compounds has not been fully elucidated but preliminary experiments show that these quinolones inhibit *T. gondii* oxygen consumption and thus could be acting on the parasite electron transport chain. Experiments investigating the mode of action are ongoing. Collectively our results provide hope that an optimized ELQ can be developed for use in the treatment and prevention of toxoplasmosis.

[Supported by The Stanley Medical Research Institute and NIH Grant RO1 AI079182]
Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of hematopoietic precursors with the ability to adversely affect the host immunity. They have been shown to accumulate in pathological conditions such as cancer and some microbial diseases. In the mouse and rat models of Pneumocystis pneumonia (PcP), we found a distinct population of cells with MDSC-like morphology in the bronchoalveolar lavage (BAL) fluid, constituting up to 50% of the total BAL cells. These cells were not seen in the BAL fluid from normal animals or from Pneumocystis-infected animals that had been successfully treated for PcP with the combination of trimethoprim and sulfamethoxazole. With flow cytometry, these cells were found to express the characteristic MDSC surface markers Gr-1 and CD11b in mice, or CD11bc and His48 in rats. Using RT-PCR, we demonstrated that these cells produced high levels of arginase-1 and iNOS mRNA. These cells were shown to suppress CD4+ T-cell proliferation in response to stimulation by anti-CD3 and anti-CD28 antibodies. Adoptive transfer of these cells to normal mice caused lung damage as indicated by elevated levels of albumin and lactate dehydrogenase in the BAL fluid. These experiments provide evidence of the presence of MDSCs in the lungs during PcP. [Supported by NIH Grants R01 AI062259 and R03 AI091418]
All-trans Retinoic Acid in Combination with Primaquine Clears *Pneumocystis* Infection and Eliminates Myeloid Derived Suppressor Cells that Accumulate in the Lung during *Pneumocystis* Pneumonia. GUANG-SHENG LEI, CHEN ZHANG, SHOUJIN SHAO, PAMELA J. DURANT, AND CHAO-HUNG LEE. Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN 46202

Myeloid-derived suppressor cells (MDSCs) have been found to accumulate in the lungs of mice and rats with *Pneumocystis* pneumonia (PcP). We hypothesized that treatment with all-trans retinoic acid (ATRA) may effectively control *Pneumocystis* (Pc) infection by inducing the differentiation of MDSCs into functional macrophages. In rodent models of PcP, we found that 5 weeks of ATRA treatment reduced the number of MDSCs in the lungs and cleared Pc infection. We also found that ATRA in combination with primaquine (PMQ) was as effective as the combination of trimethoprim and sulfamethaxazole for treatment of PcP and completely eliminated MDSCs and Pc organisms in the lungs in 2 weeks. No relapse of PcP was seen after 3 weeks of the ATRA-PMQ combination treatment. Prolonged survival of Pc-infected animals was also achieved by this regimen. Results of our study establish the foundation for development of an alternative treatment for PcP and may also serve as a model for development of novel therapies for other chronic infections with MDSC accumulation. [Supported by NIH Grants R01 AI062259 and R03 AI091418]
The S-adenosylmethionine:sterol C-24 methyltransferase (SAM:SMT), coded by the erg6 gene, adds methyl groups to the C-24 position of the sterol side chain. Most fungi can add a single methyl group and produces C-28 ergosterol by action on the major precursor zymosterol. In contrast the Pneumocystis carinii SAM:SMT can add one or two methyl groups at this position producing C-28 and C-29 sterols and prefers lanosterol and 24-methylenelanosterol over zymosterol as substrates. The erg6 gene is not an essential gene for cell viability. A haploid Saccharomyces cerevisiae erg6 knockout mutant was transformed with the P. carinii erg6 gene and the sterol composition was analyzed to determine the nature of complementation. The sterol compositions of S. cerevisiae wild type, S. cerevisiae Δerg6, S. cerevisiae Δerg6 transformed with the S. cerevisiae erg6, and S. cerevisiae Derg6 transformed with the P. carinii erg6 were analyzed. C-28 and C-29 sterols were present indicating the P. carinii SAM:SMT was functional in the yeast cell. Ergosterol was the major sterol in all strains. Previous studies on the S. cerevisiae erg11 knockout mutant expressing the P. carinii erg11 gene (codes the sterol C-14 demethylase enzyme) demonstrated excellent complementation by producing a sterol composition similar that of wild-type yeast. Unlike the erg11 transformants, the S. cerevisiae erg6 knockout transformed with the P. carinii erg6 gene had a sterol profile distinct from that of wild-type yeast. These data are consistent with earlier studies indicating that the function of the P. carinii SAM:SMT is unique from that of S. cerevisiae and other fungi.

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T13. Acyl-CoA Synthetases (ACSs) in the zoonotic pathogen Cryptosporidium parvum.

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Cryptosporidium parvum may cause severe infections in both humans and animals, for which no fully effective treatments are available. The C. parvum genome encodes three fatty acyl-CoA synthetase (ACS) genes, namely CpACS1, CpACS2 and CpACS3, which may serve as a novel drug target as this group of enzymes play an essential role in all organisms by catalyzing the first step of reaction required in all fatty acid metabolisms. We have cloned all 3 CpACS genes, and successfully expressed CpACS1 and CpACS2 as recombinant proteins, which allowed us to elucidate their biochemical features and develop an assay for identifying inhibitors for potential drug development. Our functional analyses using recombinant proteins have shown that both CpACS1 and CpACS2 were able to hydrolyze C10 to C20 fatty acids with the highest activity towards C16 palmitic acid and C18 stearic acid. More detailed analysis revealed that both enzymes displayed Michaelis-Menten kinetics towards both fatty acid and cofactor ATP. More importantly, their activity could be inhibited by the inhibitor triacsin C (IC₅₀ = 4.1 μM and 1.2 μM on ACS1 and ACS2, respectively). We have also shown that CpACS1 and CpACS2 genes were expressed in all life cycle stages, implying their importance in the parasite development. Intriguingly, CpACS2 protein was localized to the unique parasitophorous vacuolar membrane (PVM) structure that separates enclosed parasite from the environment, suggesting that this protein might be involved in fatty acid transport. Our data not only validate the function of CpACS1 and CpACS2, but also build the foundation to further explore them as drug targets in this virtually untreatable parasite.

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Microsporidia are obligate intracellular pathogens of medical and ecological importance. The genomes of many members of the group have been studied extensively, revealing their remarkably reduced gene content and, in some cases, the mechanisms involved in their extreme compaction. Indeed, microsporidia often recognized for harboring very small genomes for eukaryotes, and to encode for a reductive number of biochemical pathways that perfectly mirror their ultra-adaptation for an obligate intracellular lifestyle. To date, most studies of microsporidian genomics have focused on the causes and consequences of reduction, and only a few have revealed the capacity of some of the most reduced members of the group to acquire an arsenal of genes by horizontal gene transfer (HGTs). The current presentation will review our current knowledge about the genomics of microsporidia, and highlight how horizontal gene transfers have played a major role in the origin and evolution of these parasites. Finally, the utility of next generation sequencing to explore the presence of polymorphism and ploidy levels within and across species that are relevant to the health of many humans will be shown and discussed.
Cryptosporidium species are important waterborne pathogens that cause diarrheal disease outbreaks worldwide. Although the genomes of *C. parvum* and *C. hominis* have been described, little is known about the genes of *C. meleagridis*. The goal of this study was to determine the sequence of the *C. meleagridis* genome (CMG). Using an initial stock of $10^8$ oocysts approximately 1 million 400 bp reads were obtained and assembled by Roche 454 Genome Sequencer and GS De Novo Assembler software, respectively. The CMG contigs contained a total of $8.9 \times 10^6$ bp, with a 37X coverage, and an N50 of $2 \times 10^5$ bp. The twelve largest CMG contigs ranged from 2 to $6.1 \times 10^5$ bp for a total of $4.8 \times 10^6$ bp. Preliminary genomic comparisons revealed that the CMG contigs overlapped with approximately $8.9 \times 10^6$ bp of the 9.1MB (97.8%) genome of *C. parvum* isolate IOWA and large stretches of conserved synteny were observed. Overall, the average identity was $89.2 \pm 5.5$ %, with the longest CMG contig ($6.1 \times 10^5$ bp) aligning with nucleotides 225,740 to 845,098 of *C. parvum* chromosome 1 ($88.8 \pm 3.8$ % identical). To determine if equivalent results could be achieved using an oocyst amplified via whole genome amplification, a single oocyst was isolated using a fluorescence activated cell sorter and subjected to multiple displacement amplification (MDA) prior to pyrosequencing. Results from this approach produced similar amounts and read lengths, although the contigs were shorter, primarily because of the presence of bacterial DNA sequences. Nevertheless, the contigs were evenly distributed throughout the genome, with an average coverage of $87 \pm 30$ Kb per *C. parvum* chromosome.
The focus in our laboratory is to study the biology of the protozoan parasite, *Giardia intestinalis*, by using molecular and biochemical approaches. I will present recent data on two of our current projects:

i) Cell cycle-dependent gene expression. As the cell cycle controls the proliferation of Giardia during infection, and the differentiation of trophozoites into infectious cysts, the study of gene expression during the cell cycle would provide insights on how to inhibit this process in the treatment of giardiasis. We use counterflow centrifugal elutriation (CCE), a drug-free and physical separation procedure, to obtain fractions of Giardia trophozoites for cell cycle studies. Quantitative PCR (qPCR) analysis of CCE fractions enriched in S-phase cells showed an increase in Giardia histone mRNAs, and fractions enriched in G2/M cells showed an increase in mRNAs from putative cyclin-B and cyclin-B-like genes. While such observations are consistent with the expression pattern of these genes during the cell cycle in other eukaryotic cells, the transcript levels of the giardial orthologs of other cell cycle genes have expression patterns that suggest possible differences in the control of the Giardia cell cycle compared to other eukaryotes.

ii) Comparative analysis of gene expression among different Giardia assemblages. Molecular genotyping classified Giardia isolates into seven different assemblages (A to G). In clinical studies, the severity and length of giardiasis are correlated with infection by Giardia from specific assemblages. Moreover, some Giardia assemblages can infect only a single species while other assemblages can infect many different species. We employ microarray analysis to compare the expression profiles of Giardia isolates representing assemblages A and B, as well as two isolates of assemblage B from two different mammalian hosts. We identified differentially-expressed genes among these isolates that have potential roles in virulence and host-adaptation.

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Obligate intracellular pathogens are ubiquitous, but many questions remain about how they evolved. Microsporidia comprise a large phylum of obligate intracellular eukaryotes that are fungal-related parasites responsible for widespread disease. We identified a new genus in microsporidia called **Nematocida**, which contains natural pathogens of *Caenorhabditis* nematodes and provide model systems for studying microsporidian pathogenesis. We sequenced the genomes of two *Nematocida parisii* strains isolated from wild-caught *C. elegans* in France and the genome of a *Nematocida* sp1 strain isolated from wild-caught *C. briggsae* in India. We also performed deep-sequencing of transcripts from a timecourse of *N. parisii* infection. Examination of pathogen gene expression revealed compact transcripts and a dramatic takeover of host cells by *Nematocida*.

We used these *Nematocida* genomes, together with other microsporidian genomes, to perform phylogenomic analysis. With a set of 53 orthologous genes we refined phylogeny of the Microsporidia and identified evolutionary events of gene loss, acquisition, and modification. In particular, we found that all microsporidia lost the tumor suppressor gene Retinoblastoma, which could accelerate the parasite cell cycle. We found that microsporidia acquired transporters that could import nucleosides to fuel rapid growth. Also, microsporidian hexokinases gained secretion signal sequences, and in a functional assay these were sufficient to export proteins out of the cell; thus hexokinase may be targeted into the host cell to reprogram it toward biosynthesis.

Similar molecular changes appear during formation of cancer cells and may be evolutionary strategies adopted independently by microsporidia to proliferate rapidly within host cells. In addition, analysis of genome polymorphisms revealed evidence for a sexual cycle that may provide genetic diversity to alleviate problems caused by clonal growth. Altogether these events may explain the emergence and success of these diverse intracellular parasites.

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De novo Assembly of the *Pneumocystis jirovecii* Genome from a Single Bronchoalveolar Lavage Fluid of a Patient with *Pneumocystis* Pneumonia. O.H. CISSÉ\textsuperscript{1,2} *, M. PAGNI\textsuperscript{2}, P.M. HAUSER\textsuperscript{1}; \textsuperscript{1}Institute of Microbiology, Centre Hospitalier Universitaire Vaudois and University of Lausanne. \textsuperscript{2}Vital-IT group, Swiss Institute of Bioinformatics, Lausanne, Switzerland

*Pneumocystis jirovecii* can only be recovered out of the microbiome present in the lungs of patients. Hence, *P. jirovecii* DNA is available only in limited amount and extensively contaminated by that from human host and other microorganisms. *P. jirovecii* genome was de novo assembled starting from a single bronchoalveolar lavage fluid of a patient with a heavy load of *P. jirovecii*. The sample was enriched in *P. jirovecii* cells using immuno-precipitation. The whole DNA was randomly amplified and sequenced using Roche 454 shotgun and Illumina paired end technologies. A Bioinformatics strategy of sequence reads filtration and genome assembly was developed to overcome the technical difficulty to assemble a eukaryote genome out of a mixture of DNAs. The genome was progressively reconstructed using an iterated process that separated the *P. jirovecii* reads from those of other organisms. The process ended up in a genome scale assembly of 358 contigs totaling 8.1 Mb. Gene prediction yielded 3878 peptides, which covered a large repertoire of important metabolic pathways. However, most of the genes specifically dedicated to the biosynthesis of amino acids were absent. The requirement for amino acids, the reduced size and low GC content of the genome, and the strict host specificity strongly suggest obligate parasitism of *P. jirovecii*. 

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Identification and functional characterization of stage-specific cis-regulatory elements in Toxoplasma gondii. MIN LIU, YUNYING YAO, SHANLIANG SHEN, KUN WU, PEILIANG YANG, HONGJUAN PENG, XIAOGUANG CHEN*. Department of Parasitology, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, Guangdong 510515, P.R.China.

Toxoplasma gondii is a member of the phylum Apicomplexa, which exhibit complicated life cycles, involving transmission and differentiation within and between different hosts. The infection of Toxoplasma gondii includes acute stage and chronic stage, their biological basis is the tachyzoite and bradyzoite. Thus, finding the cis-regulatory elements in tachyzoite and bradyzoite promoters will be important to understand the underlying mechanism for toxoplasmosis caused by the interconversion between tachyzoite and bradyzoite. In the present study, the putative cis-regulatory elements upstream of functionally related groups of tachyzoite- and bradyzoite-specific genes were identified by using a dual luciferase model adapted in Toxoplasma gondii. Mapping of cis-regulatory elements in several tachyzoite and bradyzoite promoters led to the identification of core sequences that are involved in the control of stage-specific gene expression in RH and PRU strains. Sequential deletion of the surface antigens SAG2A promoter from -2092bp to -192bp upstream of start codon resulted in no significant change from -2092bp to -500bp and a nearly two-fold increase in reporter luciferase expression when deletion from -500bp to -192bp, which implied that the sequences between -500bp and -192bp might have the function in repressing gene-expression. The result of reporter assays show that sequential deletion of the SAG2C promoter from -2055bp to -500bp are able to induce but no significant change in luciferase expression, indicating that the core promoter would locate in -500bp upstream of gene. The surface related antigen SRS1 promoter from -2043bp to -500bp are able to induce relatively high luciferase expression compared to the positive control, and there is 40% decrease in luciferase expression when deletion from -1500bp to -1000bp, indicating that the range from -1500bp to -1000bp and -500bp to start codon might have cis-acting elements. The results in the present study shows that the core regulatory elements of SAG2A, SAG2C and SRS1 located in no more than 500 bp upstream of start codon and some enhancing and repressing elements might existed in the upstream region of the promoter. We will further confirm these results by using site mutagenesis analysis and electrophoresis mobility shift assay (EMSA). These works will provide an insight into the regulatory mechanisms of tachyzoite- and bradyzoite-specific genes expression. [Supported by the NSFC project (No.31030066) and GDUPS (2009) to XG CHEN].
Epigenetic regulation of gene expression has emerged as an important aspect of parasite biology. *Toxoplasma gondii* has a rich repertoire of chromatin remodeling molecules. Amongst them there are JmjC domain histone demethylases (JmjCs) and histone lysine-specific demethylases (LSDs). The JmjC class consists of several subfamilies that remove methyl groups from the modified H3K4, H3K9, H3K27, or H3K36 residue, whereas LSD1 specifically targets mono- and dimethylated H3K4 or H3K9. The enzymes that regulate histone methylation have proven to be very important for several biological processes but there little known about these molecules in *T. gondii*. *T. gondii* JmjC1 protein is the only JmjC domain protein in the parasite that has also a JmjN domain, and phylogenetic analysis shows that TgJmjC1 is highly homologous with the human histone demethylases JARID, JMJD2 and JMJD3 that have been described to demethylate H3K4, H3K36/K9 and H3K27 respectively. Genetic studies suggest that TgJmjC1 is essential for parasite's survival. Genome wide chromatin immunoprecipitation analysis coupled to microarrays (ChIP-chip) of TgJmjC1-HA shows that JmjC1 is excluded the promoter regions of active genes and co-localizes with the H3K4me1 histone mark on top of active open reading frames. Furthermore, comparative ChIP-chip analysis of JmjC1 and Sir2A deacetylase, a repressor of transcription and heterochromatin marker, shows that these molecules are enriched on the promoter regions of inactive bradyzoite genes. Additionally, JmjC1 and Sir2A are highly enriched on characteristic heterochromatin areas of the chromosome at the centromere as well as on regions at the very end of the chromosomes. Mass-spectrometry analysis of protein-complexes that are associated with JmjC1-HA revealed a number of candidate proteins including HDAC3. Based on these results we speculate that JmjC1 has a repressive role on inactive genes as well as a role in maintaining the heterochromatic regions of the chromosome. Future *in vitro* and *in vivo* studies will help to elucidate the role of JmjC1 in *Toxoplasma* as well as to investigate possible cross talk mechanism of JmjC1 with Sir2A and HDAC3 deacetylases.
Histones are small, basic proteins that constitute the major protein component of chromatin. As in other eukaryotes, *Toxoplasma* DNA is wrapped around an octamer formed by two copies of each histone (H2A, H2B, H3 and H4) forming the nucleosome. All histones are formed by a globular domain that contains the histone-fold domain as well as flexible domains that are exposed and are the site of post translational modifications (PTMs), such as acetylation, methylation and phosphorylation. The PTMs in *T. gondii* histones have been studied mainly using antibody-based techniques. In order to obtain a full repertoire of PTMs, histones were enriched using standard acid-extraction protocols and analyzed by combined proteomic approaches. Histones were Asp-N or Trypsin-digested in solution or after SDS-PAGE separation followed by MS. Alternatively, samples were fractioned by HPLC followed by top-down MS or in gel digestion followed by MS. The spectra were obtained from LC-MS/MS using ETD or HCD on a NanoAcquity UPLC coupled to an LTQ-Orbitrap Velos. To facilitate more efficient peptide identification, we used the MASCOT algorithm to identify histone PTMs using a small database containing only *T. gondii* histones. Identified PTMs were further validated using the MS/MS spectra against a complete database consisting of a complete *T. gondii* and human protein sequence database, including non-histone proteins. Using this approach we were able to identify over 100 PTMs. *T. gondii* histones especially H3 and H4, are very similar to human histones, and as expected, several modifications are conserved between both species. On the other hand, we were able to identify unique histone modifications, including some within the globular domain. In addition, we identified histone succinylation, a recently described histone mark whose role is under investigation. *T. gondii* histone PTM mapping is a first step of analysis required to understand how epigenetic regulation can affect different processes in this pathogenic parasite. Supported by NIH grants RC4AI092801 (KK, WJS), R01AI087625 (KK), R01AI39454 (LMW), 5T32AI070117-04 (SCN) and AI083162 (SOA, WJS).
In silico analysis identifies *Toxoplasma gondii* proteins with capacity to target to the mammalian cell nucleus and influence epigenetic regulation of host genes. 

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The ubiquitous eukaryotic protozoan parasite *Toxoplasma gondii* can impair chromatin remodeling in mammalian host cells. Susceptibility to congenital toxoplasmosis is associated with variants in host genes that are under epigenetic regulation. We questioned whether *T. gondii* secretes proteins that localize to the host cell nucleus and modulate DNA methylation and chromatin remodeling pathways. We used *in silico* tools to identify *T. gondii* secreted proteins as candidates for translocation to the host cell nucleus and infer function from their domain architecture. Using ToxoDB, we derived 334 secreted proteins for *T. gondii* from published secretome proteomics analysis, together with known dense granule, microneme and rhoptry proteins. We compared two pipelines to identify candidates with properties that would facilitate translocation to the host cell nucleus. Pipeline 1 (PredictNLS-ACCpro) used PredictNLS to identify classical nuclear localization signals (NLS), ACCpro to determine whether the NLS was surface exposed, InterproScan for protein sequence classification and identification of domains associated with nuclear functions, and TMHMM to determine whether the protein has a transmembrane domain (TMD; unlikely to be nuclear localised). This identified 82 proteins with an exposed NLS; 18 predicted (InterproScan) to have a nuclear functional domain. Pipeline 2 (NucPred) replaced PredictNLS-ACCpro with NucPred, which uses an ensemble of 100 sequence-based predictors to make a Boolean decision about whether a protein spends time in the nucleus. NucPred identified 122 proteins predicted to go to the nucleus, 70 overlapping with PredictNLS-ACCpro. Of the 52 additional NucPred proteins, 8 were predicted (InterproScan) to have a nuclear functional domain. None of the 26 proteins identified across both pipelines to have a nuclear function were predicted to have a TMD. These 26 proteins were primary candidates for assessment of putative epigenetic function, top amongst which are histone modifying enzymes, a DNA methyltransferase, 2 SWI2/SNF2 DNA-dependent ATPases and 4 DEAD Box helicases.
Cryptosporidium parvum is a globally recognized zoonotic parasite and an important opportunistic pathogen in immunocompromised patients. However, little is known on the metabolic dynamics in the parasite, and the study is hampered by the lack of molecular and genetic tools. Here we report the development of the first Agilent microarray for C. parvum (CpArray15K) that covers all predicted ORFs in the parasite genome. Global transcriptome analysis using CpArray15K coupled with real-time qRT-PCR uncovered a number of unique metabolic features in oocysts, the infectious and environmental stage of the parasite. The oocysts were found to be highly active in the protein synthesis based on the high levels of expressions of genes associated with ribosome biogenesis, transcription, and translation. The proteasome and ubiquitin associated components were also highly active, implying that this stage of parasite may employ the protein degradation pathways to recycle amino acids to overcome its incapability to synthesize amino acids de novo. The energy metabolism was featured by the surprisingly highest level of expression of lactate dehydrogenase (LDH) gene in oocysts. The observation was confirmed by qRT-PCR, immunofluorescence microscopy and western blot analysis. We also studied the parasite responses to the UV-irradiation, and observed complex and dynamic regulations in gene expressions, although the fold changes were less dramatic than expected. Notable changes included the increased activations in DNA repair and intracellular trafficking. Among the stress-related genes, TCP-1 family members and some thioredoxin-associated genes appear to play more important roles in the recovery of UV-induced damages in the oocysts. Our observations also implied increased activities in cytoskeletal rearrangement and intracellular membrane trafficking in the parasite oocysts upon UV irradiation. These observations shed new light on how the parasite oocysts adapt and respond to the generally hostile environment and common stresses such as UV irradiation.

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T24 Genes and gene pathways involved in bradyzoite-tachyzoite interconversion in *Toxoplasma gondii*. MATTHEW MCKNIGHT CROKEN3, YAN-FEN MA1, RONALD C. TAYLOR4, MENG MARKILLIE4, GALYA ORR4, STEVEN WILEY4, LOUIS M. WEISS1,2, KAMI KIM1,3 Departments of Pathology1, Medicine2 and Microbiology & Immunology3, Albert Einstein College of Medicine, Bronx, NY 10461, USA. EMSL4, Pacific Northwest National Laboratory, Redmond, Richland, WA 99352

*Toxoplasma gondii* is an opportunistic parasitic pathogen infecting around 30% of the world's population according to the CDC. During the asexual stage of its lifecycle, the parasite is capable of differentiating between a fast replicating, invasive form, called a tachyzoite, and a slow growing, cyst forming stage, called a bradyzoite. While tachyzoites can cause acute disease in animals, they are treatable. On the other hand, bradyzoite cysts are asymptomatic, but resistant to pharmaceuticals, capable of persisting for the lifetime of the host, and reemerging to establish a new acute infection. Using high-throughput sequencing of mRNA, we have characterized the transcriptomes of both tachyzoites and in vitro bradyzoites (alkaline/low CO₂ induced) for three different strains representing the three canonical lineages of the parasite: RH (type I), P (type II), and CTG (type III). Here, we identify genes differentially expressed between tachyzoite and bradyzoite in each strain as well as genes that are expressed differently between strains under the same conditions. Notably, we have identified several genes which have proposed functions as chromatin modifiers as well as many genes containing an AP2 DNA binding domain, which are regulated either up or down in response to bradyzoite stress conditions. Genes of the AP2 family are thought to function as transcription factors in Apicomplexa. The genes we identify may prove to be key transcriptional regulators of differentiation. To validate our gene lists, we have integrated data from expression microarrays, chromatin immunoprecipitation (ChIP-chip/seq) and expressed sequence tag (EST) experiments. Using Gene Set Enrichment Analysis (GSEA), we further identify whole gene pathways that may be differentially regulated during parasite conversion.
Nitric oxide resistance mechanisms of Toxoplasma gondii. SINI SKARIAH, ROBERT BEDNARCZYK, NATHANIEL KIM, BRIAN LYNCH, EMMA IACONETTI, NICHOLAS GERMANO, DANA MORDUE*; New York Medical College, Valhalla, NY, USA.

Like all successful intracellular pathogens Toxoplasma modifies its host cell to create a permissive environment for growth. However, it is not clear if reprogramming the infected host cell is sufficient or if the parasite has evolved additional mechanisms to protect itself against host cell defenses whose presence cannot be completely prevented/eliminated or to repair damage from cell autonomous immunity. For example, Toxoplasma can suppress host cell production of inducible nitric oxide synthase but sufficient amounts of nitric oxide (NO) are often produced to impair parasite replication. Our overall goal is to determine mechanisms used by Toxoplasma to protect itself against cell autonomous immunity. Mechanisms Toxoplasma has evolved to protect itself within its host cell likely underlie its ability to use phagocytes to disseminate to the brain and CNS, to survive to establish chronic infection, and to persist within cysts long term to enable reactivation of disease. In our studies we used an unbiased approach to let Toxoplasma identify for us the defenses it has evolved to protect itself from cell autonomous immunity. These studies led to the isolation of 12 independent Toxoplasma mutants impaired for survival/replication following activation of infected macrophages (MO) but with normal replication in naïve MO. Interestingly, all of the mutants share a similar phenotype, an inability to withstand nitrosative stress; a phenotype that can be reproduced by NO donors. NO sensitivity correlates with NO-dependent fragmentation of the parasites’ single mitochondrion. Ongoing studies are using these mutants to identify genes important for Tg resistance to NO to enable functional characterization of their proteins and determine mechanistically how they protect Toxoplasma against NO. The availability of 12 Toxoplasma mutants that share such a similar phenotype provides a unique set of tools to dissect Toxoplasma genes and pathways important for resistance to nitrosative stress.

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The cell wall of Pneumocystis has been reported to contain an abundance of $\beta$-glucans and the glucan synthesis genes (FKS1, FKS2, and Rho1) have been identified in the Pneumocystis carinii genome. We have shown that treatment of Pneumocystis infected mice with inhibitors of $\beta$-1, 3-D-glucan synthesis, the echinocandins, dramatically shift the ascus and trophic populations to one made up almost entirely of trophic forms with a significant reduction in mortality. We designed these experiments to use the echinocandins as a molecular tool to assess the host response to each life cycle phase.

Mice were exposed to Pneumocystis murina infected mice and immune suppressed for 5 weeks. Mice were then treated with either anidulafungin or vehicle for 3 weeks and then all mice were taken off immune suppression and sacrificed at days 1, 7 and 14. Microscopic analysis was performed to quantitate organism burdens and differential leukocyte counts. Flow cytometry was also performed to attain T cell counts.

No asci were found in the anidulafungin treated mice and similar levels of trophic forms were seen in all mice at each timepoint. At days 7 and 14, a significantly higher percentage of neutrophils was found in the untreated mice. At day 1, there was a significantly higher percentage of CD4+ T cells in the untreated mice and at day 7, there was a significantly higher percentage and total number of CD8+ T cells in the untreated mice. Measurement of $\beta$-1,3-D-glucan was associated with the presence of asci suggesting this pro-inflammatory compound was responsible for the more robust immune response during reconstitution. These results indicate a unique host immune response to each life cycle phase. Future studies will be performed to further define the ascus and trophic forms role in host pathogenesis.

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Microsporidia is an opportunistic pathogen, which, in addition to HIV infected individuals and organ transplant patients, also affects aging population. The infection is primarily acquired through ingestion of contaminated food or water. Microsporidia is an intracellular parasite recently reclassified as a fungus, which triggers a strong Th1 response against per oral challenge and CD8+ T cells are a critical component of the protective immunity against this pathogen. Residing in the lining of the epithelium of the gut, intraepithelial lymphocytes (IEL) have been shown to be one of the very first lines of defense against several pathogens. Moreover, IEL population is mainly comprised of CD8+ T cells. Our laboratory has reported that adoptive transfer of immune intestinal IEL to SCID mice can partially protect them against a lethal infection and protection was mediated by cytolytic activity and IFNg production. However, early steps leading to the IEL immune response generation against microsporidial or any other oral infection remains unknown. In the current report, we demonstrate that microsporidia infect the gut rapidly and by day 2 post-infection, disseminate to the mesenteric lymph nodes (MLN). This trafficking was cell dependent as FTY720 treatment (an S1P receptor agonist) significantly reduced the number of parasites detected in the MLN. Also, dendritic cell (DC) subsets in the lamina propria were activated early after infection and trafficked to MLN to activate the IEL response. These findings were further strengthened by the observation that IEL response was substantially reduced following early and transient FTY720 treatment or using CCR7−/− animals (which have been shown to have defective trafficking of intestinal DC). Our observations provide strong correlative evidences that IEL priming occurs in the MLN after trafficking of activated DC to this site. (NIH AI043693. AI071778)
Activation of Alveolar Macrophages in Response to *Pneumocystis* in Neonatal Mice is Defined by Enhanced Expression of Alternative Markers

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Alveolar macrophages (AMs) are the first line of defense against *Pneumocystis* (PC). We have previously described that neonatal AMs, due to both intrinsic defects and the external lung environment, do not respond to PC infection with the same efficiency as adults. This results in a delayed immune response and clearance of infection. To further examine neonatal macrophage activation, macrophage phenotype was examined following PC infection. Neonatal AMs developed an alternative phenotype defined by mannose receptor (MR), Ym1 and arginase expression, which was delayed but prolonged, and in some instances enhanced, as compared to that observed in adults. Infiltrating CD4 T cells and neutrophils expressed IL-4 at the time of immune response to PC in neonates, presumably driving this phenotype. Additionally, macrophages within the airways expressed IL-4 during resolution of infection possibly to sustain an alternative phenotype. Deficiencies in IL-4 or IL-4R\(\alpha\) result in faster clearance of PC infection, though a marked decrease in arginase was observed in these models, suggesting defects in lung repair and remodeling following resolution of PC infection. We hypothesize that, upon developing an immune response to PC infection, neonatal AMs develop a more robust alternative phenotype compared to that observed in adults to help maintain lung integrity within the developing lung environment. [Supported by NIH grant NIH-HL62053-12]
Alveolar macrophages in neonatal mice are inherently unresponsive to Pneumocystis infection

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Pneumocystis pneumonia was first diagnosed in malnourished children and has more recently been found in children with upper respiratory symptoms. We previously reported that there is a significant delay in the immune response in newborn mice infected with Pneumocystis compared to adults. This delay is characterized by failure of neonatal lungs to up-regulate proinflammatory cytokines and attract T cells into the alveoli. Here we report that regardless of the age at which we infected the mice, they failed to mount an inflammatory response in the alveolar spaces until they were 21 days of age or older. Anti-inflammatory cytokines had some role in dampening inflammation since IL-10-deficient pups cleared Pneumocystis faster than wild type pups and neutralization of TGFβ with specific antibody enhanced T cell migration into the lungs at later time points. However, the clearance kinetics was similar to control pups suggesting that there is an intrinsic deficiency in the ability of innate immunity to control Pneumocystis. However, we found using an adoptive transfer strategy that the lung environment contributes to association of Pneumocystis organisms with alveolar macrophages implying no intrinsic deficiency in the binding of Pneumocystis by neonatal macrophages. Using both in vivo and in vitro assays, we found that Pneumocystis organisms were less able to stimulate translocation of NFκB to the nucleus of alveolar macrophages from neonatal mice. These data indicate that there is an early unresponsiveness of neonatal alveolar macrophages to Pneumocystis infection that is both intrinsic and related to the immunosuppressive environment found in neonatal lungs. [Supported by NIH grant NIH-HL62053-12]
Microsporidia continue to cause opportunistic enteric and systemic infections in immune-compromised individuals worldwide and also cause persistent infections in otherwise healthy mammalian hosts. Macrophages can be activated during innate and adaptive immune responses to kill intracellular microsporidia yet some organisms escape to continue infection. Earlier studies by others demonstrated that *Encephalitozoon spp.* inhibited apoptosis in non-phagocytic host cells, and the purpose of this study was to determine if microsporidia can also inhibit apoptosis in phagocytic cells such as macrophages. THP1-differentiated macrophages infected with live *Encephalitozoon cuniculi* or *Vittaforma corneae* inhibited staurosporine-induced apoptosis three days later as determined by lower levels of TUNEL staining and caspase 3 activity compared with uninfected control macrophages induced with staurosporine. Conversely, THP1 macrophages incubated with dead microsporidia and treated with staurosporine three days later exhibited significantly higher levels of apoptosis than THP1 macrophages treated only with staurosporine. PCR apoptosis pathway micro-array analysis corroborated these bioassay findings. Anti-apoptosis genes including BLC2 and TP53 were significantly up-regulated in macrophages infected with microsporidia for three days while pro-apoptosis genes such as FADD, CASP3, CD40LG, LTA, and several TNF-family genes were up-regulated in the macrophages incubated with dead organisms. Interestingly, the inhibition of apoptosis was more pronounced with *E. cuniculi*, which replicates within parasitophorous vacuoles, than *V. corneae*, which replicates in close association with endoplasmic reticulum in the cytoplasm. These results open the door to consider targeting apoptosis pathways for controlling microsporidia infections. [Supported by a Tulane Research Enhancement Fund grant and national primate research center support from NIH via Grant OD011104.]
Despite general advances in patient care, Pneumocystis pneumonia (PcP)-related mortality remains unacceptably high. Therefore, we need better understanding of the biology of the host-pathogen interaction to identify alternative treatment strategies for PcP. T cell-derived signals are critical for host defense against PcP. However, the host’s T helper (TH)-mediated immune response also contributes to PcP-related immunopathogenesis. The precise downstream effector mechanisms by which TH cells mediate these processes are undefined, impairing our ability to design optimal therapeutic strategies. Macrophage phenotype and effector functions are regulated by TH cells. TH1 signals induce a classical macrophage activation phenotype (CAM), while TH2 signals produce an alternative activation phenotype (AAM). However, the relative contributions of these distinct macrophage activation programs to host defense and/or immunopathogenesis have not been determined. Our studies demonstrate that it is possible to control macrophage phenotype during PcP. Importantly, shifting the macrophage activation program towards AAM resulted in attenuated PcP-related immunopathogenesis while at the same time enhancing macrophage-mediated Pc clearance. These results provide further evidence that immune modulation of macrophage function could be a novel therapeutic strategy for PcP.
Latent *Encephalitozoon cuniculi* infection is reactivated after factitious immunodeficiency in mice. BOHUMIL SAK1,*, MICHAELA KOTKOVÁ1, MARTIN KVÁC1,2; 1Institute of Parasitology, BC ASCR, v.v.i., Czech Republic, 2Faculty of Agriculture, University of South Bohemia in Ceské Budejovice, Czech Republic

Microsporidia are obligate intracellular parasites that cause severe infections with lethal outcome in highly immunocompromised hosts. However, with the increasing use of molecular methods in routine diagnostics, these pathogens are more frequently reported in immunocompetent individuals with latent infection opening the question about the potential risk of reactivation of latent microsporidiosis in case of immunosuppression. The different course of infection depending on the immunological status of the host has been successfully imitated also in murine models. We used immunocompetent BALB/c mice and immunodeficient SCID mice as an experimental model for the study of the course of microsporidiosis caused by *Encephalitozoon cuniculi* using molecular detection of the parasite in numerous tissues of the host. Whereas *E. cuniculi* spread all over the host body and caused a lethal microsporidiosis in SCID mice, the infection in BABL/c mice remained asymptomatic despite the parasite expansion into many organs in acute phase of infection. While treatment with albendazole led to disappearance of microsporidia from all examined organs in BALB/c mice, only temporary reduction of number of affected organs with infection re-dissemination after treatment termination was observed in SCID mice. Application of immunosuppressive (dexamethazone) caused the microsporidiosis reactivation connected with spreading into most of host’s organs in BALB/c mice with chronic infection. Although the number of affected organs was not so high in BALB/c mice treated with albendazole, the fact, that the infection is able to be reactivated virtually from zero, is groundbreaking. All these findings clearly showed that latent microsporidiosis pose a life-threatening risk factor especially for individuals under chemotherapy and transplant recipients of organs originating from infected donors.

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Deviated systemic immune responses to Pneumocystis lung infection due to a defective type-I-IFN-system can trigger osteoporosis

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Pneumocystis (PC) is an opportunistic fungal pathogen causing fatal pneumonia in patients with severe immune-deficiencies, such as those with AIDS or on immunosuppressive therapy. However, low-grade PC colonization without progression to pneumonia is common in two patient groups: those who are HIV+ and receiving HAART and those who are HIV- but with underlying chronic pulmonary diseases, such as COPD, that require steroid treatment. Interestingly, in both groups osteoporosis is a common complication causing significant co-morbidity and mortality. The type-I-IFN system is bone protective, and can be suppressed in both groups, either due to incomplete recovery of type-I-IFN-producing pDCs under HAART or by steroid-mediated repression.

We recently demonstrated that type-I-IFNs are induced in lungs and serum of PC-infected WT and RAG-/- mice. This response is abrogated in mice lacking the IFNAR-receptor (IFNAR). As a consequence, lymphocyte-competent IFNAR+/+ mice develop local and systemic immune deviations with chronic lung pathology following delayed PC clearance and transient bone marrow (BM) depression with extramedullary hematopoiesis. Mice lacking lymphocytes and IFNAR (IFrag-/ mice) can not clear the pulmonary infection and develop rapidly progressing BM failure due to apoptotic cell death. Here we demonstrate that PC-infected IFNAR+/+ and IFRag-/ mice also show inflammation-induced accelerated osteoclastogenesis and bone loss. These changes are associated with a pro-osteoclastogenic BM-cytokine profile uniquely in mice lacking IFNAR. Chronic pulmonary diseases in humans are often treated with steroids, which promote both PC lung infection and osteoporosis. Steroid-treatment of IFNAR-competent WT and RAG-/- mice strongly suppressed systemic type-I-IFN-response to PC lung infection and appeared to accelerate osteoclastogenesis in the BM when compared to treated but uninfected mice. This implicates and defines the pathophysiological connection between disease manifestations of two seemingly independent organ systems, lung and bone, via PC lung infection and an impaired type-I-IFN-system as it may occur during HIV infection and steroid treatment.

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Outbreaks of Pneumocystis Pneumonia in Two Renal Transplant Centers Linked to a Single Strain of Pneumocystis: Implications for Transmission and Virulence

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Background: Better understanding of the epidemiology and transmission of human Pneumocystis should lead to improved strategies for preventing Pneumocystis pneumonia (PCP). There have been numerous reports of clustered outbreaks of PCP at renal transplant centers over the past two decades. It has been unclear whether these outbreaks were linked epidemiologically to one or several unique strains, which could have implications for transmission patterns or strain virulence.

Methods: Restriction fragment length polymorphism (RFLP) analysis was used to compare Pneumocystis isolates from 3 outbreaks of PCP in renal transplant patients in Germany, Switzerland, and Japan, as well as non-transplant isolates from both HIV infected and uninfected patients.

Results: RFLP analysis with its high discriminatory power demonstrated that a single Pneumocystis strain caused pneumonia in transplant patients in Switzerland (7 patients) and Germany (14 patients). This strain was different from the strain that caused an outbreak in transplant patients in Japan, as well as strains causing sporadic cases of PCP in non-transplant patients with or without HIV infection from diverse locations world-wide.

Conclusions: Two geographically distinct clusters of PCP in Europe were due to a single strain of Pneumocystis. This suggests either enhanced virulence of this strain in transplant patients, or a common, but unidentified, source of transmission. Additional studies comparing the strains responsible for outbreaks in renal transplant patients and in other susceptible populations, are needed to better define the transmission of human Pneumocystis and emphasize the need to develop better parameters for determining susceptibility to PCP so that prophylaxis can be continued during periods of enhanced susceptibility.

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A specific *Pneumocystis jirovecii* genotype may worsen outcome of pneumonia in HIV–infected patients. M. RABODONIRINA¹, L. VAILLANT², P. TAFFE ³, A. NAHIMANA⁴, R.-P. GILLIBERT², P. VANHEMS²,⁵, P.M. HAUSER⁶; ¹Laboratoire de Parasitologie, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Université Claude-Bernard Lyon I, France. ²Hospices Civils de Lyon, Hôpital Edouard Herriot, Service d’Hygiène, Epidémiologie et Prévention. ³Data Center, Swiss HIV Cohort Study, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Switzerland. ⁴Hospital Preventive Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne. ⁵Université de Lyon 1; CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive. ⁶Institute of Microbiology, Centre Hospitalier Universitaire Vaudois and University of Lausanne.

*Pneumocystis jirovecii* dihydropteroate synthase (DHPS) mutations have been suggested to be associated with failure of sulfa prophylaxis. Their impact on outcome of *P. jirovecii* pneumonia (PCP) remains controversial. *P. jirovecii* DHPS polymorphisms and genotypes were determined in 112 PCP episodes of 110 HIV-infected patients using PCR-single-strand conformation polymorphism. Death occurred in 18.8% (N=21) of the episodes and was attributed to PCP in 18 cases. Thirty-three percent (N=37) of the PCP episodes involved a *P. jirovecii* strain carrying one or both DHPS mutations. DHPS mutations had no impact on PCP outcome within one month (*P*=0.35), whereas *P. jirovecii* type 7 and the need mechanical ventilation at PCP diagnosis were associated with an increased risk of death due to PCP (relative hazard=4.2; *P*=0.05; 95%CI=1.0-17.9; relative hazard=5.0; *P*=0.002; 95%CI=1.8-13.5, respectively). Mechanical ventilation was also associated with an increased risk of sulfa treatment failure at five days (odds ratio=34.2; *P*=0.002; 95%CI=3.6-321.3).
The chronic Toxoplasma infection can alter mice’s behavior, intelligence and emotion. MIN LIU, YUN-YING YAO, SHANLIANG SHEN, KUN WU, HONG-JUAN PENG, XIAO-GUANG CHEN*. Department of Parasitology, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, Guangdong 510515, P.R.China.

Toxoplasma gondii is a ubiquitous intracellular parasite which chronically infects approximately two billion people worldwide. Previous studies have demonstrated that T. gondii has the ability to alter the behavior of infected rodent and humans. Furthermore, a number of studies have suggested T. gondii infection is associated with an increased risk of factor for the development of schizophrenia and depression in humans. However, the mechanism of T. gondii manipulates host behavior remain unclear, and there is lack of data about the changes of depression behavior, learning and spatial memory capacity of chronic T.gondii infectedanimal for a better understanding the effect of the infection on the change of host behavior. The objective of this study was to investigate and evaluate the effects of chronic T.gondii infection on emotion and cognitive function in mice. Kunming mice (outbred) were peroral inoculated with avirulent T. gondii strain Prugniaud (a Type II strain) cysts and tested in the Open filed test (OFT), Forced swimming test (FST), Morris water maze (MWM) and Step-through passive avoidance task (STPAT) in the sixth month post-inoculation. Compared to uninfected mice, chronic infected mice exhibited behavioral abnormalities, less activity and exploration during the first exposure to the open field; longer immobility time during the FST; worse spatial and recognition memory in the MWM test; a greater number of errors in Step-through passive avoidance task. Although the observed changes in behavior can not be directly used to explain the effect of chronic T. gondii infection on the human diseases such as schizophrenia and depression, this mouse model of chronic T. gondii infection could provide evidence that the chronic T. gondii infection has a positive effect on depression and learning processes of mice. [Supported by the NSFC project (No.31030066) and GDUPS (2009) to XG CHEN]
High prevalence of Pneumocystis Jirovecii infections among Mozambican children <5 years of age admitted to hospital with suspected pneumonia

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Background: We aimed to assess the specific prevalence of Pneumocystis jirovecii (PJ) infections among children <5 years of age admitted to hospital with clinically severe pneumonia, in an area with very high HIV prevalence.

Methods: Within a study assessing the etiology of pediatric pneumonia, we recruited during 12 months 835 pediatric patients. Collection of standardized clinical data, chest X-rays and screening for 12 respiratory viruses using polymerase chain reactions (PCR) were routinely performed, together with tests for invasive bacterial infection (IBI), malaria and HIV. For a random sample of 100 patients, investigations on PJ infection in nasopharyngeal aspirates were performed using a tri-sequential PCR strategy, assessing two multicopy mitochondrial genes (mtLSU y mtSSU) and a third unicopy one, linked to sulfa drug resistance (DHPS).

Results: Fourteen (14%) of the patients tested positive for at least one of the PJ genes. Five patients showed triple (mtLSU, mtSSU and DHPS) gene positivity, while further 4 showed double (mtLSU and mtSSU) positivity. Five further cases tested solely positive for mtLSU. Of the 14 positive patients, three died, and all but one had a clinical picture of probable or possible Pneumocystis jiroveci pneumonia (PCP). HIV co-infection was present in 54,5% of the patients (6/11, for three patients no HIV results were available), and surprisingly, 50% (7/14) had a concomitant IBI (3 cases of Strept. pneumoniae, 1 Haemophilus influenzae b, 1 Pseudomonas aeruginosa, 1 E.coli and 1 Strept. group B). Three patients showed also positive P. falciparum parasitemias. 30% (4/13) of the patients were also co-infected with respiratory viruses (all rhinoviruses, some with double infection including HMPv, Flu A, ADV).

Conclusion: PCP is a severe and highly prevalent infection among Mozambican children admitted with severe pneumonia and coexists with other common pediatric infections. Evaluation of all study samples is underway.

Funding source: This study was supported by a grant from the World Health Organization (WHO-C6-181-489).
Microsporidia were identified in stool specimens by light microscopy and PCR in 30 (18.9%) of 159 HIV-infected patients presenting to the S.P. Botkin Memorial Clinical Hospital of Infectious Diseases, St. Petersburg, Russia. Unexpected was the higher prevalence of *Encephalitozoon intestinalis* in 21 (12.8%) patients compared to *Enterocytozoon bieneusi* in two patients (1.2%). *Encephalitozoon cuniculi* was detected in three patients; one with strain I and two with strain II. *Encephalitozoon hellem* was detected in one patient, and two patients were identified with *Microsporidium* species. One patient was infected with both *E. intestinalis* and *E. cuniculi*. In two patients, the microsporidia species were not identified. No statistically significant differences in gender, age, and stage of AIDS were observed between the microsporidia-positive and -negative HIV-infected patients. Patients diagnosed with microsporidia were significantly more likely to exhibit < 100 CD4+ T cells/mL blood (P = 0.01) and weight loss > 10% (P = 0.04) than patients not diagnosed with microsporidia. Cryptosporidia were detected in 17 of total 99 (17.2%) patients by PCR and light microscopy. *Cryptosporidium parvum* was diagnosed in eleven (64.7%), *C. hominis* – in six patients (35.3%) by RFLP, verified by direct sequencing. Patients with cryptosporidiosis demonstrated lower average CD4+ lymphocytes numbers (P = 0.05) and increased frequency of long lasted (>10 days) diarrhea (P = 0.06).

This is the first report describing the diagnosis of microsporidia in HIV-infected patients in Russia, and the first detection of *E. cuniculi* strain II in a human. Prevailing *Encephalitozoon intestinalis* and *Cryptosporidium parvum* over *Enterocytozoon bieneusi* and *C. hominis* might suggest zoonotic rout of infection in the studied patient group.

Supported by CRDF (RUB2-002707-SP-05), RFBR (10-04-00943), NIH (RR 00164), and Tulane Research Enhancement Fund.
Background: *Pneumocystis* pneumonia (PCP) is a common opportunistic infection affecting HIV-infected patients. Despite decades of experience in treating this disease, little has been reported on the expected clinical course of HIV-associated PCP. Gaining a better understanding of when clinical response occurs and factors associated with clinical stability and relapse are important in caring for this patient population.

Methods: We performed a retrospective study of 475 hospitalized cases of HIV-associated PCP seen at San Francisco General Hospital from January 1, 1999 through December 31, 2006. Medical chart review was performed using standardized forms and pre-determined definitions. Fever, tachycardia, tachypnea, and supplemental oxygen requirement were evaluated at baseline and daily during the hospitalization.

Results: At least one vital sign abnormality was seen in 93% of the study population. Nearly 26% had abnormal values for all 4 vital signs at baseline. Median time to improvement for fever, tachycardia, and tachypnea was 2 days. Resolution of oxygen supplementation took a median of 4 days (p<0.001 compared to fever, tachycardia, and tachypnea). Factors associated with a slower time to clinical stability included a lack of pre-hospital PCP prophylaxis and more severe PCP. Relapse of vital sign abnormalities occurred in a proportion of patients; 23% had recurrence in fever, 36% in tachycardia, 42% in tachypnea, and 28% in supplemental oxygen requirement. Relapse in any vital sign was associated with longer hospitalization. A relapse in fever, tachycardia, or tachypnea was associated with death.

Conclusions: Vital sign abnormalities are common in HIV-associated PCP. The clinician can expect most patients to improve in 2-4 days, with improvement in supplemental oxygen requirement lagging behind resolution of fever, tachycardia and tachypnea. A relapse in any vital sign is associated with a worse outcome.

Funding Source: NIH K24 HL087713 and R01 HL090335.
The Ibero-American Programme for Science, Technology and Development (CYTED) was created in 1984 through an International Framework Agreement signed by 19 Latin American countries, Spain and Portugal. The CYTED Programme is an intergovernmental multilateral Science and Technology cooperation programme, which aims to combine different perspectives and visions to promote cooperation in Research and Innovation for the development of the Latin America region. The principal objective of the CYTED Programme is to contribute to harmonious development in the Latin America region by setting up mechanisms for cooperation between research groups of universities, R+D centers and innovative companies in Latin American countries, targeting scientific and technological breakthroughs that are transferable to systems of production and social policies. Since 1995, the CYTED Programme has been officially included among the Cooperation Programmes at the Summits of Latin American Heads of State and Government. The “Red Iberoamericana sobre Pneumocystosis” (Pneumocystosis Ibero-American Network) is a Thematic Network supported by CYTED Programme that was created in 2012. This Network involved 11 research group from eight countries (Chile, Venezuela, Cuba, Mexico, Brazil, France, Portugal and Spain). The main objective of this Network is the promotion of joint scientific research, the transfer of knowledge and practices, and the exchange of scientists and technicians mainly between member groups but also with other groups in the field of Pneumocystis infection. [Supported by Red Iberoamericana sobre Pneumocystosis, CYTED 212RT0450].
Recent work has shed light on an intracellular resistance mechanism against *Toxoplasma gondii* mediated by the IFN inducible immunity-related GTPases (IRG) in mice. In IFN-γ-stimulated cells, several IRG proteins load onto the parasitophorous vacuole (PV) membrane leading to disruption of the PV followed by the death of the parasite and shortly thereafter necrotic death of the host cell. Toxoplasma is a very successful parasite and developed a number of strategies to escape from the host cell defence. Those strategies include phosphorylation of IRG resistance proteins by virulent Toxoplasma, sequestration of cellular functions of the immune system to assure propagation, including the hijacking of migratory leucocytes and modulation of migratory properties of infected cells among other several mechanisms. Given the important role of stage conversion of Toxoplasma tachyzoites into slower growing bradyzoites for the establishment of a chronic infection, it is of interest whether IRG proteins are capable of mediating the destruction of bradyzoite-containing PVs or if stage conversion is a strategy used by the parasite to “evade” the IRG resistance system allowing persistence. To address this question, we are using a transgenic *T. gondii* stably transformed with genes encoding red fluorescent (DsREd-Express) and green fluorescent protein (GFP) under the control of the tachyzoite-specific SAG1 and bradyzoite-specific BAG1 promoters, respectively. During our studies we faced, unexpectedly, with one more lesson from escapology by the apicomplexa parasite *Toxoplasma gondii*. [Supported by SFB670]
T42  All Glucans are Not Created Equal: Evidence for β-1,6 Glucans in the Pneumocystis carinii Cell Wall. ANDREW H. LIMPER, DEANNE M. HEBRINK, SEHER IQBAL, THEODORE J KOTTOM. Thoracic Diseases Research Unit, Mayo Clinic, Rochester, MN USA 55905.

Pneumocystis organisms continue to cause severe pneumonia in immune compromised hosts. Inflammation is a predominant cause of respiratory impairment during Pneumocystis pneumonia. Our prior studies have shown a significant role for cell wall β-glucans in stimulating lung inflammation from both macrophages and lung epithelial cells. Cell wall β-glucans are comprised of particulate homo-polymers of D-glucose, linked through β-1,3 backbones and variable β-1,6 side chain structures. Wide ranging host responses have been observed to various fungal derived glucan preparations, presumably on the basis of the tertiary structures of these complex carbohydrates. Initial work in Pc has focused largely on β-1,3 glucan components. However, more recent investigations in other yeasts have suggested important roles for β-1,6 glucans in mediating inflammation. On this basis, we sought to document evidence of β-1,6 glucans in the cell wall of P. carinii. Immune staining using β-1,6 specific antibodies revealed specific staining of P. carinii cysts walls. Next, homology cloning efforts allowed us to characterize a functional β-1,6 glucan synthase, namely Kre6 from P. carinii, which, when expressed in kre6 (deficient) Saccharomyces cerevisiae, complemented resistance to calcofluor. We subsequently isolated β-1,6 glucan enriched fractions from P. carinii cell walls. These soluble carbohydrate fractions showed low level inflammatory activation when cultured with macrophages. However, when the soluble β-1,6 glucan enriched fractions were rendered into a particulate format on beads, as they would be present in an intact Pc organism, the macrophages responded with a vigorous TNF-α inflammatory response. These inflammatory responses could be significantly dampened by inhibition of host cell plasma membrane micro-domain function with PDMP, in a fashion parallel to unfractionated P. carinii cell wall carbohydrate. Taken together, these studies indicate that β-1,6 glucans are present in the Pc cell wall and that these components contribute to lung inflammation during infection

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Annallia algerae sporoplasts contain a Multilayered Interlaced Network (MIN) which has Golgi-like structural and enzymatic properties. PETER M. TAKVORIAN1,2*, KAROLYN F. BUTTLE3, DAVID MANKUS3, CARMEN A. MANNELLA3, LOUIS M. WEISS2,4 ANN CALI1; 1 Department of Biological Sciences, Rutgers University, Newark, New Jersey, USA, 2Department of Pathology, Division of Tropical Medicine and Parasitology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY, USA, 3 Resource for Visualization of Biological Complexity, Wadsworth Center, New York State Dept of Health, Empire State Plaza, Albany NY, USA, 4Department of Medicine, Division of Infectious Diseases, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY, USA.

The multilayered interlaced network (MIN) is an organelle that has been identified in the sporoplasm during extrusion from germinating Annallia algerae spores. In previous studies we demonstrated that the MIN serves a dual function. The first is structural, surrounding the sporoplasm immediately below its plasma membrane and attached to the end of the polar filament where it maintains the integrity of the sporoplasm as it is pulled through the everting polar tube. The second is secretory, depositing its dense contents on the surface of the sporoplasm shortly after discharge, providing the material for the precocious plasma membrane thickening, characteristic of the genus, Annallia. In the current study two enzyme histochemical procedures have been applied, demonstrating that the MIN has cis and trans Golgi-related enzymatic functions. The presence of Golgi in the Microsporidia has been previously documented in other microsporidial genera, including Glugea and Paranosema. Microsporidial Golgi has been shown to be developmentally regulated, varying in its appearance and presence. The current study has enabled us to extend our understanding of the microsporidial Golgi presence in the sporoplasm and demonstrates the MIN-Golgi enzymatic function for extracellular secretion in Annallia algerae. This report further illustrates the three dimensional morphology of the MIN as obtained by high voltage TEM and tomographic reconstructions. These reveal that the MIN is composed of a complex series of interconnected flattened sacs, varicose tubules and fenestrated flattened sheet-like structures, similar to the appearance of typical Golgi cisternae but lacking transport vesicles and buds. [Supported by NIH grant 5R01AI031788-19 and The National Center for Research Resources, NIH, grant RR01219]
Thigmotropism in *Pneumocystis carinii*: Contact Mediated Cell-Signaling Regulates Cell Wall Assembly Control Pathways. ANDREW H. LIMPER¹, THEODORE J. KOTTOM¹.

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*Pneumocystis jirovecii* shows a predilection for the lung microenvironment and continues to inflict serious pneumonia in immune compromised patients. To learn details of the molecular regulation of the life cycle in these genetically intractable fungi, studies were performed using the *Pneumocystis carinii* (*Pc*) model in rats. Thigmotropism is a process, often discussed in plant biology, where an organism grows or moves in response to contact or touch stimuli. In this light, we previously documented stimulated proliferation of *Pc* organisms following binding to lung epithelial cells. We have further shown that the binding of the organism is facilitated by the mammalian matrix proteins fibronectin and vitronectin, and that *Pc* utilizes an integrin-like molecule termed PcInt1 to bind lung proteins and cells. In recent investigations, we utilized studies of differential gene expression and cloning and characterization of downstream regulatory proteins to define the consequences to the organisms of *Pc* binding to lung alveolar epithelial cells and matrix proteins. These studies revealed that following binding to lung cells, *Pc* induce the activation of a central regulatory PcSte20 MAP kinase which subsequently phosphorylates the cell wall biosynthesis regulatory kinase termed PcCbk1. Now, we further have observed that the PcCbk1 kinase further stimulates the PcAce2 transcription factor that regulates gene expression for cell wall biosynthetic and remodeling genes. Our functional analyses demonstrate that this cell-signaling cascade induced by interactions of *Pc* with the host lung environment (epithelial cells and matrix proteins) can stimulate fungal proliferation, cell wall remodeling and virulence factors. Based on these data, we posit that the interactions of *Pc* with the microenvironment of the lung provide key signals that promote life cycle progression and cell wall remodeling in this organism. Better understanding of these life cycle regulatory pathway promise new insights to control this important infection.

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Identification and Characterization of Toxoplasma gondii Cyst Wall Proteins

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Toxoplasma gondii forms tissue cysts during initial infection which persist in the host and are involved in disease relapses. A prominent characteristic of these cysts is the cyst wall which protects the parasites from environmental stress including the immune system. Identifying cyst wall components should help elucidate mechanism(s) of parasite persistence. To identify cyst wall proteins two approaches were utilized: (1) a proteomic analysis of cyst wall preparations and (2) the development of cyst wall specific monoclonal antibodies (mABs). Using various cyst wall specific mABs the corresponding antigens were purified by immunoprecipitation from parasite lysates. The purified antigens were analyzed by mass spectrometry and corresponding genes identified using EPICdB and ToxoDB (EuPathdB). mAB SalmonE stained the cyst wall uniformly, but not the tachyzoite parasitophorous vacuole and the identified protein was designated TgCST250. The localization of TgCST250 was verified by a polyclonal antibody raised against expressed recombinant protein. TgCST250 is highly glycosylated and reacts with DBA. A knockout (KO) of TgCST250 was performed in T. gondii PruΔKU80. Although the TgCST250 KO did not lose its ability to differentiate into bradyzoites, the number of brain cysts in mice was greatly reduced and KO cysts were much more fragile than wild type cysts suggesting a defect in cyst wall formation. A second cyst wall protein reacting with mAb PufferM has also been characterized. Using our proteomic approach the gene corresponding to mAB PufferM, TgCST88 has been identified and we are in the process of evaluating the phenotype of KO parasites.

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A case for host involvement in the glycosylation of *Toxoplasma* tissue cysts.

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Despite their central role in the pathogenesis of *Toxoplasma gondii* little is known about the composition and functions of proteins associated with the cyst and cyst wall. The tissue cyst is defined by the presence of a heavily glycosylated cyst wall with additional glycoproteins present in the tissue cyst matrix. Their identity remains largely unknown. Lectins and monoclonal antibodies against glycan moieties indicate the presence of both sialic acid and complex N-linked glycans in the tissue cyst wall and/or matrix. The detection of these moieties in the tissue cyst is surprising in light of the fact that the *Toxoplasma* genome does not encode any of the enzymes needed for the synthesis, activation or transfer of sialic acid or the enzymatic machinery to synthesize complex N-glycans. This presents the possibility that *Toxoplasma* may acquire these modifications from the host cell. We now have data that strongly implicate not only host sugars but more critically host enzymes in the modification of the tissue cyst wall and matrix. Using host cell mutants with specific defects in the sialylation pathway we are now dissecting the mechanistic basis underlying the sialic acid modification of *Toxoplasma* proteins particularly those associated with the tissue cysts. These findings suggest a mechanisms whereby *Toxoplasma* may redirect host ER and Golgi vesicles followed by fusion with PVM to deliver the requisite modifying enzymes to the vacuole. We believe that the subversion of host activities resulting in the decoration of the tissue cyst with host glycans may have a role in the relative immunological invisibility of tissue cysts that appear to be disguised as immunological self. Confirmation of this pathway may also contribute to the vast host range of the parasite whereby host specific modifications can be promoted.

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Enterocytozoonidae Revisited. ANN CALI¹*, PETER MICHAEL TAKVORIAN¹, OSWALDO PALENZUELA², MARIA JOSÉ REDONDO², and ARIADNA SITJÀ-BOBADILLA² ;¹ Department of Biological Sciences, Rutgers University, Newark NJ, USA, ² Instituto de Acuicultura Torre de la Sal (IATS-CSIC), Castellón, Spain.

The family Enterocytozoonidae was established in 1990 for the human-infecting microsporidium, Enterocytozoon bieneusi. It was the first microsporidium described with the formation of multiple polar filaments within each sporogonial plasmodium. The polar filament formation was accomplished by structures/organelles not previously observed in microsporidia and yet the resultant spores were diagnostic for the microsporidia. When polar filament (pf) formation has been studied, its development has been observed after the last sporogonic cell division, in the stages identified as sporoblasts. The E. bieneusi organism presented a new pf developmental process which was sufficiently significant to warrant formation of a new family. Since E. bieneusi was the only microsporidium with these features, all its developmental features became synonymous with those of the family. In the 20+ years since the establishment of this family, four new genera and several species have been added. They include the genera Nucleospora, Paranucleospora/Desmozoon, Hepatospora, and Enterospora. The host range includes humans, mammals, fish, and marine invertebrates. Our investigations into the development of a new microsporidium in farmed gilthead sea bream (Sparus aurata) fish have brought the need to revisit the family definition to the forefront. The features unifying the family are the precocious polar filament formation and its related structures. However, many other microsporidial features are variable, including their occurrence in the host cell nucleus or cytoplasm, direct contact with the host cytoplasm or in an isolating parasitophorous vesicle, and independent or diplokaryotic nuclei, features that we normally use to separate the microsporidial parasite groups. The molecular phylogeny inference using the SSU rDNA from these genera has also demonstrated their relatedness and groups them as a monophyletic clade. With the ongoing debate concerning the value of morphology vs molecular characteristics, this family may present the advantage of using both. The unifying structural and developmental processes found in these organisms with a revision of the family definition will be presented.
Cryptosporidium ubiquitum is emerging as an important pathogen for zoonotic cryptosporidiosis. We report here the development of a subtyping technique for C. ubiquitum based on sequence analysis of the 60 kDa glycoprotein gene and the characterization of the subtype distribution in 53 isolates from humans and animals in various geographic areas. Four subtype families, XIIa, XIIb, XIIc, and XIIId, were found, with one major subtype family (XIIa) in domestic and wild ruminants in the Old and New World, and three subtype families in wild rodents in the United States. All four subtype families were detected in humans in the same areas. Domestication of sheep was postulated to be a major factor in the spread of the XIIa subtype families around the world. The finding of potentially host-adapted C. ubiquitum populations and sheep farming as a key factor in their spread improve our understanding of the transmission of C. ubiquitum in humans [Supported by NSFC Grant 31110103901].
Direct evidence of airborne excretion of Pneumocystis carinii during infection in immunocompetent rats. Lung involvement and antibody response.

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Background: Pneumocystis pneumonia (PCP) remains a serious opportunistic infection among immunocompromised patients. Both animal and human studies are in favor of an airborne transmission of Pneumocystis and support the role of PCP patients and colonized hosts as potential sources of Pneumocystis. As we previously showed that Pneumocystis was detectable and quantifiable in the close environment of individuals with PCP, our aim was to study the airborne shedding of Pneumocystis by immunocompetent hosts.

Methods: In two experiments, Sprague Dawley rats were inoculated with 106 cryopreserved P. carinii organisms and followed for 60 days. P. carinii was quantified by qPCR in the surrounding air and in the lungs of infected rats, and anti-P. carinii antibodies were titrated in serum.

Results: A transient air excretion was observed between d14 and d22 in the first experiment and between d9 and d19 in the second experiment, and was related to the peak of infection in lungs. IgM and IgG anti-P. carinii antibody increase preceded clearance of P. carinii in the lungs and cessation of airborne excretion. In rats receiving a second challenge 3 months after the first inoculation, Pneumocystis was only detected in the lungs of 2 of 3 rats at d2 post challenge and was never detected thereafter; anti-Pneumocystis antibody determinations showed a typical secondary antibody response and all air samples were PCR negative.

Conclusions: This study provides the first direct evidence that an immunocompetent host can excrete Pneumocystis following a primary acquired infection. Lung infection was apparently controlled by the immune response since fungal burdens decreased to become undetectable as specific antibodies reached high titers in the serum. This immune response was apparently protective against reinfection 3 months later. The evidence that P. carinii can be excreted by healthy individuals also warrants refining preventive measures for naive immunocompetent hosts and immunocompromised patients.

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Vertical transmission of Microsporidia occurs in several species infecting invertebrates, and there is strong, but indirect, evidence of this for a few species infecting fishes. The zebrafish, Danio rerio, is a very important model in biomedical research, and the microsporidium, Pseudoloma neurophilia, is prevalent in these fish in laboratory facilities. This host-parasite system provides an excellent vertebrate model for investigating transmission and development of Microsporidia as pathogen-free fish are available from our SPF facility and larval fish are especially susceptible to experimental infection. Pseudoloma neurophilia is frequently observed in developing oocytes and ovaries of adult zebrafish and we consistently detect parasite DNA by PCR in eggs and water collected at spawning. Recently, we detected large numbers of spores in developing embryos within eggs spawned by infected adults. We also observed infections in post-hatch fry and juveniles from infected parents that were reared under controlled laboratory conditions. While some early infections are clearly the result of vertical transmission, it is possible that many highly infected embryos will not develop into viable larval fish but instead provide large numbers of spores to infect other tank mates by horizontal transmission. By feeding spores to larval fish and examining whole-body sagittal sections stained with either H&E, the Luna stain, or our in situ probe based on the small subunit rRNA gene of the parasites, we were able to visualize the early stages of infection in numerous tissues. We have also observed evidence of infection by P. neurophilia by direct contact (i.e. via the skin) and are currently working to determine the tissue distribution of the parasite by exposing pre-feeding embryonic fish.[Supported by NIH NCRR 5R24RR017386-02 and P40 RR12546-03S1]
Several studies have been conducted worldwide on avian cryptosporidiosis, including the zoonotic species Cryptosporidium meleagridis, third important species of Cryptosporidium which affecting human. In Algeria, no study have been conducted on this subject. In the present study an investigation is conducted, on the research of Cryptosporidium spp. in chickens and turkeys, mainly C. meleagridis. During the investigation, a total of 92 subjects, dead or sick, were collected, including 48 chickens and 44 turkeys, from 13 and 11 farms respectively, in classical buildings or in greenhouses. Upon autopsy, a section of the ileum is levied, on which three sections were made. Two of them are used for the identification of Cryptosporidium spp. by conventional methods (staining and histology), and the third for molecular methods: PCR-RFLP, PCR and sequencing of 18S rRNA and GP60. Molecular methods show forty-three (46.73%) samples positive to Cryptosporidium, from which 39.58% in chickens and 54.54% in turkeys. Molecular analysis show that positive samples contain predominantly C. meleagridis (88.33%), followed by C. baileyi (9.33%), and one mixed infections by the two species (2.32%) from chickens. All positive detected turkeys were infected by C. meleagridis, while chickens frequently host this species, with 29.16% against 8.33% to C. baileyi. In parallel, the GP60 analysis allows the detection of a new subtype family of C. meleagridis. Also, the young age, during the start-up and growth, seems to favor this zoonotic species, in the two types of birds. The identification of C. meleagridis with great frequency in chickens and turkeys shows the emerging zoonotic potential of these two animals species, strongly farmed in Algeria.

[Supported by HNVS of Algeirs]
A survey was conducted to determine the presence of free-living amoebae (FLA), especially *Acanthamoeba* and *Naegleria*, in river recreation areas in Tehran Province, Iran. All rivers surveyed were associated with human activity, and two were also a source of municipal tap water. Fifty-five water samples from 10 major rivers were screened for FLA and identified by morphological characters, PCR amplification targeting specific genes for *Acanthamoeba* (DF3 region of Rns gene) and other FLA (ITS PCR), and homology analysis. The percent of positive FLA isolates was 27.3%, of which 80% were *Acanthamoeba*, assigned to the T4 and T15 genotype, and 20% were *Naegleria*. Isolation of *Acanthamoeba* T4 genotype (91.7%) from recreation areas could be a health threat and a sanitary risk associated with human activity where young people and tourists congregate in summer. Posting of warning signs and education of high risk individuals are important for disease prevention. To the best of our knowledge this is the first report of genotype T15 (clustered with *A. jacobsi*) identified in Iran and the first report of the distribution of FLA such as *Naegleria* (*N. pagei, N. clarki, and N. fultoni*) in recreation areas in rivers of Tehran Province using molecular methods. Supported by Shahid Beheshti University of Medical Sciences, Tehran, Iran
Identification and Differentiation of two Nosema spp. Isolated from Pieris rapae and Hemerophila atrilineta

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Microsporidia are a group of obligate intracellular parasites, and widely existing, which have caused more difficulty in identifying different species and controlling the silkworm Pebrine disease. This paper was about studying on the identification and the genetic variation of two microsporidian, isolated from two insects, Pieris rapae (CFD) and Hemerophila atrilineta (SCH), from its life cycle, ultrastructure, SSU-rRNA and ITS. The results were listed as follows:

(1) The life cycle of these two insects' microsporidian, CFD and SCH, respectively, are very similar comparing its parasitizing in silkworm. But the duration of life cycle of these two parasites are obviously shorter. CFD's was 72h and SCH's was 96h, respectively. (2) The original spores collected from pests are disunity; its shape is tending to uniform after propagating in silkworms repeatedly. They are mostly oval and smooth. CFD and SCH were showed that the spore wall has three layers, polar filament is a five-layer structure, and isofilar, and two parts of polaroplast, and all spores are binucleate. But the numbers of polar coils and posterior vacuole have bigger difference within different generations under TEM. (3) The phylogenetic analysis based on the partial SSU-rDNA gene fragment shows that original spores of CFD and SCH fell in the clade of Nosema/Vairimorpha complex group and the other four generations fell in the clade of true N. bombycis group. (4) Two spores ITS were showed higher variation. The different clones from the same PCR amplication were also showed bigger difference. The change trends in different generations were still unclear. Multiple alignment show that ITS of this two insects' microsporidia have three high variation sections in different length, in other conservative section there exists three high variation point. Based on the above-mentioned results, two microsporidia, isolated from CFD and SCH respectively, are all belong to Nosema. In gene level, especially in the phylogenetetic analysis with SSU-rDNA data showed the microsporidia have a select adaptability and differentiations in alternative host.

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T54 Cryptosporidium suis and Cryptosporidium pig genotype II: its associations with age and husbandry practices. MARTIN KVÁC,1,2* KAREL NEMEJC,1 MIACHELA KESTRÁNOVÁ,1 BOHUMIL SAK,2 DANA KVETONOVÁ,2 NADEZDA KERNEROVÁ,1 MICHAEL ROST,3 VITALIANO A. CAMA4; 1Faculty of Agriculture, University of South Bohemia in České Budejovice, Czech Republic, 2 Institute of Parasitology, BC ASCR, v.v.i., Czech Republic, 3Faculty of Science, University of South Bohemia in České Budejovice, Czech Republic, 4Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA

We examined 1620 fecal samples of pigs of all age categories on 22 farms in the Czech Republic for identification and genotyping of Cryptosporidium. Cryptosporidium spp. were found on 16 of 22 farms with a mean prevalence of 23.1%. Overall, 217 (13.4%) specimens were positive by microscopy and 355 (21.9%) by PCR. Genotyping results obtained using swine Cryptosporidium species-specific primers identified 39.7% of pigs infected only with C. suis, 35.8% only with Cryptosporidium pig genotype II, while 23.3% of the pigs surveyed had concurrent infections. One sample had C. parvum and three C. muris. The analysis by age showed that Cryptosporidium suis was primarily detected among piglets (pre-weaned), while Cryptosporidium pig genotype II was only detected among starters, specially those weaned at 4-weeks of age. Pigs raised on straw bedding were more likely to have Cryptosporidium than pigs raised on slats/slurry systems. Experimental infection clearly revealed susceptibility of both 4 and 8 week old pigs to C. suis. While parasitological, molecular and histology examination confirmed susceptibility of 8 week old pigs to Cryptosporidium pig genotype II, 4 week old pigs were not being infected with this genotype. [Supported by MSM LH11061, MSM 6007665806 and GAJU 022/2010/Z].
Feces from 47 dairy cattle ranging in age from neonates to multiparous cows were examined and 9, 10, 24, and 17 were found positive for Blastocystis spp., Cryptosporidium spp., Giardia duodenalis, and Enterocytozoon bieneusi, respectively, by PCR. Eight 3- to 5-month-old cattle were concurrently infected with 3 or 4 of these parasites. This is the first report to identify multiple concurrent infections with these four potentially zoonotic protist pathogens in cattle. None of the cattle exhibited signs of illness or effects of infection on growth and are regarded as healthy carriers, potentially a source of infection for humans and other animals. A new commercially available immunofluorescence (IFA) microscopic test confirmed 6 of 7 available PCR-positive Blastocystis specimens and identified one IFA positive cow that was PCR negative.
Although non-human primates are genetically related to humans, and they have frequent contact with humans in some areas, their role in the transmission of enteric parasitic diseases remains unclear. In this study, we characterized enteric protists from rhesus monkeys which were in close contact with humans in a popular public park in Guizhou, China. A total of 411 fecal specimens were analyzed by PCR for Cryptosporidium spp., Giardia duodenalis and Enterocytozoon bieneusi, which were found in 45 (10.9%), 35 (8.5%) and 116 (28.2%) specimens, respectively. Within Cryptosporidium, the anthroponotic C. hominis was the dominant species, being found in 39 specimens, and belonging to six subtypes. Cryptosporidium parvum was found in five specimens, all belonging to the anthroponotic IIc subtype family. Only the human-pathogenic Assemblages A and B were found within G. duodenalis, with the former all belonging to the anthroponotic A-II subtype. The E. bieneusi detected were mostly (107/116) belonging to group I, which most human-pathogenic E. bieneusi genotypes belong to. Thus, rhesus monkeys in close contact with humans are mostly infected with anthroponotic Cryptosporidium spp., Giardia duodenalis, and E. bieneusi, and therefore represent potential reservoirs of human-pathogenic protists.

[Supported by NSFC Grant 31110103901]
The use of molecular diagnostic tools in epidemiological investigations of Cryptosporidium, Giardia, and Enterocytozoon has provided new insights into their diversity and transmission pathway. In this study of 157 stool specimens from 0-70-year-old patients, a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis of the small subunit (SSU) rRNA gene was used to detect and differentiate Cryptosporidium spp., and DNA sequence analysis of the 60 kDa glycoprotein (gp60) gene was used to subtype C. hominis and C. parvum. Giardia duodenalis and Enterocytozoon bieneusi in the specimens were detected using PCR and sequence analysis of the triosephosphate isomerase (TPI) gene and internal transcribed spacer (ITS), respectively. Cryptosporidium hominis and C. parvum were found in two (1.3%) and one (0.6%) specimen respectively, comprising of Ia and Ile (with 8 nucleotide substitutions) subtype families. The G. duodenalis A2 subtype was detected in five (3.2%), while four genotypes of E. bieneusi, namely A, type IV, D and WL7 were found in 10 (6.4%) specimens. Children two years or younger had the highest occurrence of Cryptosporidium (4.4%) and Enterocytozoon (13.0%) while children of 6 to 17 years had the highest Giardia infection rate (40.0%). No Cryptosporidium, Giardia and Enterocytozoon were detected in patients older than 60 years. Enterocytozoon had high infection rates in both HIV positive (3.3%) and HIV negative (8.3%) patients. Results of the study suggest that anthroponotic transmission may be important in the transmission of Cryptosporidium spp. and G. duodenalis while zoonotic transmissions may also play a role in the transmission of E. bieneusi in humans in Kaduna State, Nigeria.
Several studies have been conducted worldwide on avian cryptosporidiosis, including the zoonotic species Cryptosporidium meleagridis, third important species of Cryptosporidium which affecting human. In Algeria, no study have been conducted on this subject. In the present study an investigation is conducted, on the research of Cryptosporidium spp. in chickens and turkeys, mainly C. meleagridis. During the investigation, a total of 92 subjects, dead or sick, were collected, including 48 chickens and 44 turkeys, from 13 and 11 farms respectively, in classical buildings or in greenhouses. Upon autopsy, a section of the ileum is levied, on which three sections were made. Two of them are used for the identification of Cryptosporidium spp. by conventional methods (staining and histology), and the third for molecular methods: PCR-RFLP, PCR and sequencing of 18S rRNA and GP60. Molecular methods show forty-three (46.73%) samples positive to Cryptosporidium, from which 39.58% in chickens and 54.54% in turkeys. Molecular analysis show that positive samples contain predominantly C. meleagridis (88.33%), followed by C. baileyi (9.33%), and one mixed infections by the two species (2.32%) from chickens. All positive detected turkeys were infected by C. meleagridis, while chickens frequently host this species, with 29.16% against 8.33% to C. baileyi. In parallel, the GP60 analysis allows the detection of a new subtype family of C. meleagridis. Also, the young age, during the start-up and growth, seems to favor this zoonotic species, in the two types of birds. The identification of C. meleagridis with great frequency in chickens and turkeys shows the emerging zoonotic potential of these two animals species, strongly farmed in Algeria.

[Supported by HNVS of Algeirs]
POSTER SESSION A
ABSTRACTS

PA1 to PA22
PA1. Characterization of a putative C-24(28) sterol reductase (erg4) in *Pneumocystis carinii*. THOMAS M. SESTERHENN¹,*, ALEKSEY POROLLO³, MELANIE T. CUSHION¹,², ¹University of Cincinnati College of Medicine, Cincinnati, OH, USA, ²Cincinnati Veteran’s Affairs Medical Center, Cincinnati, OH, USA, ³Department of Environmental Health, University of Cincinnati, Cincinnati, OH, USA.

Previous work from other laboratories has shown that *Pneumocystis carinii* lacks detectable levels of ergosterol, the major fungal sterol, and instead utilizes cholesterol scavenged from the host as its major sterol. However, our laboratory and others have provided evidence of the functionality of enzymes in the early and intermediate steps of the sterol biosynthetic pathway including the production of lanosterol. In addition, in vitro screening assays showed that these enzymes were susceptible to inhibitors targeting these reactions. Sequence data from the *Pneumocystis* Genome Project (PGP) identified several putative genes encoding enzymes in the early part of the biosynthetic pathway but genes for later steps were never identified. Additional sequence data has now allowed identification of a putative C-24(28) sterol reductase (Pcerg4), the enzyme that catalyzes the final step of ergosterol biosynthesis pathway in fungi. To elucidate if this is a functioning gene and catalyzes the production of ergosterol, the putative Pcerg4 was expressed in an erg4 knockout strain of *Saccharomyces cerevisiae*. Deletion of erg4 is not a lethal mutation in *S. cerevisiae*, but results in a lack of ergosterol, a build up of its precursor ergosta-5,7,22,24(28) tetraenol, and an altered sensitivity to a number of compounds. The putative Pcerg4 was expressed in the Scerg4 knockout strain which resulted in no perceptible growth changes, giving evidence of a functional enzyme. Studies are ongoing to determine ergosterol levels.
Pentamidine isethionate, designated an orphan drug by the US FDA, is used for both prophylaxis and therapy in the treatment of *Pneumocystis jiroveci (Pj)* pneumonia, although severe toxic and allergic side effects can result. Modifications of the pentamidine molecule to increase efficacy and decrease toxicity are a target of previous and new drug development in our laboratory.

A series of pentamidine analogs were synthesized (in the laboratory of DM) to evaluate heteroatoms, sulfobenzene groups, and alkanediamide groups when targeted against *Pneumocystis carinii (Pc)* in vitro, and to assess toxicity in mammalian lung tissue cultures. A bioluminescent ATP detection assay was used to measure the viability of Pc isolates in culture with and without test compounds. Compounds were screened at multiple concentrations for three consecutive days to yield 50% Inhibitory Concentration (IC$_{50}$) values.

Of the 20 compounds tested, 5 were found to have marked activity against Pc (IC$_{50}$ of 0.10 to 0.99µg/ml), 13 compounds had moderate activity (IC$_{50}$ of 1.0 to 9.99µg/ml), 1 compound had slight activity (IC$_{50}$ of 10.0 to 49.9µg/ml), and 1 was ineffective with no reduction in Pc ATP (IC$_{50}$ ≥50µg/ml). In general, higher activities were achieved with the inclusion of a heteroatom in the aliphatic linker. Compounds demonstrating marked, moderate, and slight activity against Pc were subsequently tested for toxicity on A549 human lung epithelial carcinoma cell and L2 rat lung epithelial carcinoma cell monolayers. The ATP content of monolayers was not reduced (IC$_{50}$ >100µg/ml) in A549 cells treated with all19 analogs and L2 cells treated with 16 of 19 analogs. The pentamidine analogues were ranked for efficacy against Pc and low toxicity to mammalian cells. A proof of principle compound, PEN10 is now undergoing assessment in the in vivo model of infection.

Supported by the Veterans Affairs Department.
PA3. S-adenosyl Methionine and KL-6 as serological markers for diagnosis of *Pneumocystis* Pneumonia (PcP) – Preliminary results. SONIA S. CALÉ, OLGA MATOS, BRUNO DE SOUSA, JORGE GASPAR, ROBERT BADURA, FRANCISCO ANTUNES, FRANCISCO ESTEVES; 1Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal. 2Instituto de Higiene e Medicina Tropical, CMDT/UNL, Lisboa, Portugal. 3Faculdade de Ciências Médicas, CIGMH/UNL, Lisboa, Portugal. 4Hospital de Santa Maria, FM/UL, Lisboa, Portugal.

*Pneumocystis jirovecii* (Pj) is an opportunistic pathogen responsible for one of the most severe infections in immunocompromised patients, *Pneumocystis* pneumonia (PcP). Currently, the laboratory diagnosis of PcP is based on cytochemical staining, indirect immunofluorescence with monoclonal antibodies and PCR on induced sputum and bronchoalveolar lavage samples. Studies showed that *Pneumocystis* species have a need for exogenous S-adenosyl Methionine (SAM) since they are unable to synthetize this compound. Other studies suggest that *Krebs von den Lungen*-6 (KL-6), a mucin-like glycoprotein expressed on type II alveolar pneumocytes and bronchiolar epithelial cells, may be used as a sensitive indicator of various types of interstitial pneumonitis. The aim of the present work is to evaluate these two potential serological markers (SAM and KL-6) for PcP diagnosis. Sera and pulmonary specimens were obtained from 65 HIV+ and seven HIV- Portuguese patients for diagnostic purpose (1997-2012). Sera were collected from 25 blood donors (negative control group). PcP cases were confirmed by using indirect immunofluorescence with monoclonal antibodies (IF) and nested-PCR.

Of the 72 patients studied, 47 were PcP positive (median: SAM 38 nmol/L; KL-6 1416 U/mL) and 25 were PcP negative (10 were colonized by Pj presenting subclinical infection and 15 were not infected by Pj) (median: SAM 36 nmol/L; KL-6 556 U/mL). The median serologic markers levels detected for the colonized carriers were 21 nmol/L (SAM) and 542 U/mL (KL-6), while in the patients without Pj infection were 60 nmol/L (SAM) and 670 U/mL (KL-6). In the blood donors the median SAM serological level was 75 nmol/L, while the median KL-6 level was 459 U/mL. Sera presenting high levels of KL-6 (>570 U/mL) were statistically associated with diagnosed PcP cases (77%, *P*<0.001). These preliminary results suggest that KL-6 might be a candidate to use in PcP diagnosis, mostly when used in association with other serologic markers.

Supported by “Centro de Malária e Outras Doenças Tropicais (CMDT)"

Interleukin-8 (IL-8) is a potent chemoattractant and activator of neutrophils and other immune cells that play an important role in pathophysiology of COPD. Patients with COPD colonized by *Pneumocystis jirovecii* show a high levels of circulating IL-8. Several previous studies indicate that airway epithelial cells release IL-8, in response to *Pneumocystis* and purified *Pneumocystis* cell wall beta-glucans. So, *P. jirovecii* cell wall beta-glucans could be responsible for the increased production of IL-8 in COPD patients. We addressed whether there is a relation between IL-8 and beta-D-glucan serum levels in COPD patients. Serum samples from 35 COPD patients were analyzed to determine IL-8 level using Multicitokin Kit and Beta-D-glucan using Fungitell assay. Identification of *P. jirovecii* colonization was done analyzing respiratory samples by nested-PCR assay that amplifies the mt LSU rRNA gene. *P. jirovecii* colonization was identified in 13 cases (37%). *Pneumocystis*-colonized COPD patients showed higher mean levels of IL-8 (20.7±19.7 vs 8.9 ± 10.8 pg/ml, p < 0.001) and beta-D-glucan (44.5 ± 52.1 vs 24.6 ± 27 pg/ml, p= 0.565) than did non-colonized patients. However, we were not able to establish any relation between both variables. This work confirms results of previous study showing that *Pneumocystis*-colonization in COPD patients induces increase in IL-8 but a relation between IL-8 and β-D-glucan serum levels cannot be demonstrate. However, further studies are necessary to clarify this question.

[Supported by Red Iberoamericana sobre Pneumocystosis, CYTED 212RT0450]
Biologic agents targeting TNF-α mediated immunomodulatory effects that include decreasing expression of pattern-recognition receptors and IFN-γ production, increasing monocytes apoptosis and failure of maintain of granulomas. Their use is increasing in systemic diseases because is well demonstrated that these drugs reduce disease severity. But the appearance of opportunistic infections, including Pneumocystis pneumonia (PCP), is increasing. Pneumocystis colonization could be a risk factor for PCP in patients undergo anti-TNF drugs, but prevalence of this situation is unknown. Our objectives were to determine the prevalence of Pneumocystis jirovecii colonization in patients receiving treatment with anti-TNF (infliximab, adalimumab or etanercept) and if there is difference in prevalence by type of drug or underlying diseases, and potential risk factors associated. we examined 195 oropharyngeal wash samples collected from 63 controls with systemic diseases not receiving biological treatments, 62 patients receiving infliximab, 40 receiving etanercept and 30 receiving adalimumab with systemic diseases. We define colonization when we detected Pneumocystis DNA using a specific RT-PCR for the mitochondrial large-subunit rRNA gene of P. jirovecii in the samples of individuals without sings or symptoms of PCP. We also collected demographic data, underlying conditions, use of other drugs and habits in order to know potential risk factors. Pneumocystis colonization was detected in 41 patients (21%) without statistically significant differences between groups. In a multivariate regression model only the use of steroids and metotrexato were significantly and independently associated with risk of Pneumocystis colonization. The use of steroids and metotrexato are risk factor for P. jirovecii colonization in patients receiving anti-TNF drugs. In this group of patients anti-Pneumocystis treatment could be necessary to prevent PcP.

[Supported by Red Iberoamericana sobre Pneumocystosis, CYTED 212RT0450]
Acute interstitial pneumonia (AIP) is a rare and rapidly fatal form of diffuse lung injury. It is characterized by the presence of diffuse alveolar damage and rapid progression to severe respiratory failure. AIP etiology is unknown, although it has been proposed that infectious agents could be the trigger of the process. Recently it has been shown the high prevalence of *P. jirovecii* colonization among patients with idiopathic interstitial lung diseases. However there is any data in AIP patients. Therefore, this study aims to investigate the presence of *P. jirovecii* in AIP patients. We identified all cases of biopsy-confirmed AIP treated in our hospital during the last 10 years with available samples of bronchoalveolar lavage (BAL). The presence of *P. jirovecii* was studied by PCR, using primers for the large mitochondrial subunit gene of this organism. Positive and negative controls in each case were used together with a direct sequencing for establishing the genotype. Five cases of AIP (only one female) aged between 48 and 75 years were available. In all cases mechanical ventilation was required in an intensive care unit. There were no cases of HIV infection and microbiological culture of BAL samples were negative in all cases. The presence of *P. jirovecii* DNA was identified in all 5 cases. Only two of the patients received empirical treatment with cotrimoxazole, one of them was the only survivor to the episode of AIP. Our data suggest a possible role of *P. jirovecii* in the development of AIP. However, additional studies are necessary in order to unravel the role of *P. jirovecii* infection in the pathophysiology of AIP that allow us to design rational strategies for prevention and treatment.

[Supported by Red Iberoamericana sobre Pneumocystosis, CYTED 212RT0450]
Background: *Pneumocystis* Pneumonia (PcP) is an opportunistic fungal infection. Although T-cell immunity is classically related to *Pneumocystis* defense, recent data supports roles for B-lymphocytes in the development of PcP in animals, and we have observed several cases of PcP in patients receiving rituximab. These observations prompted a systematic review of our experience to define the spectrum of clinical presentations in which PcP has occurred in the setting of rituximab therapy.

Methods: Using a computer-based search, we reviewed patients who received rituximab and developed PcP at Mayo Clinic Rochester over the years of 1998-2011 in order to establish the underlying conditions, clinical course, possible risk factors and potential association between this drug and the development of PcP.

Results: Over this period, 30 patients developed PcP during treatment with rituximab. The underlying diseases included hematologic malignancies in 90% of cases. Glucocorticoids were used in 73% of these patients under different chemotherapeutic regimens. Three patients (10%) developed PcP in the setting of rituximab without concomitant chemotherapy or significant glucocorticoid exposure. Of these 30 patients, 88% developed acute hypoxemic respiratory failure and 53% required ICU admission. The clinical course was fatal in 30%.

Conclusion: PcP can occur in association with rituximab with the majority of cases having also received cytotoxic chemotherapy or significant doses of glucocorticoids. The clinical course of cases of rituximab associated PcP can be quite fulminant with significant mortality. Primary prophylaxis should be considered in patients at risk, and secondary prophylaxis provided unless immune reconstitution is well assured.
PA8. Evaluation of Caspofungin and Caspofungin in association with Trimethoprim-sulfamethoxazole (TMP-SMZ) in the rodent model of Pneumocystis – Preliminary study. MARIA LUÍSA LOBO1*, FRANCISCO ESTEVES1, FERNANDO CARDOSO1, MELANIE CUSHION2, FRANCISCO ANTUNES3, OLGA MATOS1;
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This study intends to evaluate Pneumocystis susceptibility to low doses of caspofungin and caspofungin in association with TMP-SMZ, in Balb/c immunosuppressed mice, infected intranasally with P. murina. The caspofungin was administered intravenously, once daily at 0.1mg/kg, 0.05mg/kg, and 0.001mg/kg and TMP-SMZ was administered by oral gavage (12.25mg/62.5mg/day), for 21 days. Efficacy was based on a reduction in organism burden in treated vs untreated mice determined by microscopic quantification (Gomori methenamine silver-stain) and RT-qPCR targeting the P. murina large subunit ribosomal RNA gene (mtLSU). The β-1,3-D-glucan levels in treated and untreated mice were measured as an additional marker of infection. The RT-qPCR data obtained suggest that, even at the lowest concentrations tested, the efficacy of caspofungin in association with TMP-SMZ is higher than the efficacy of the drugs used alone. For instance, at the 14th day of treatment with caspofungin (0.1mg/kg)+TMP-SMZ was observed a 127-fold reduction in organism burden. While TMP-SMZ and caspofungin (0.1mg/kg) administered alone gave respectively a 64-fold and 2-fold reduction in organism burden. P. murina was not detected in the lungs of mice after 21 days treatment with either the caspofungin+TMP-SMZ or with TMP-SMZ administered alone. P. murina was still detectable in the lungs of mice treated with caspofungin alone, on the 21st day of treatment, adding evidence that this drug alone will not eradicate Pneumocystis infection. The results of this preliminary study suggest the potential benefit of the combined treatment with caspofungin in low doses and TMP-SMZ for PcP. Further studies involving a larger number of mice and time points over the treatment period are required, to confirm the efficacy of the drug combination and to determine the shortest period of drugs administration. However, this study provides a significant contribution to the development of better approaches to applying the selected drugs for treatment of PcP patients with minimal toxicity to the host.

[Supported by Merck Sharp & Dohme Corp.]

Background: *Encephalitozoon intestinalis* and *Enterocytozoon bieneusi* are responsible for the majority of enteric microsporidial infections in humans. Currently, microsporidiosis is under-reported due to the lack of adequate clinical diagnostic testing. The development of a molecular diagnostic test for Microsporidia has been limited by a lack of available organisms and genomic DNA for these pathogens. This study demonstrates a novel, multiplex real-time PCR assay for *E. intestinalis* and *E. bieneusi* using synthetic molecular standards as surrogates for genomic DNA.

Methods: Primers and hybridization probes for *E. intestinalis* and *E. bieneusi* were designed for multiplex PCR on the LightCycler® 2.0 (LC). A molecular standard of each organism was synthesized and used as the target DNA. Internal control DNA was designed and detected by hybridization probes in a different channel. The functionality of the primers, synthetic molecular standards, and internal control DNA was verified by end-point PCR followed by gel electrophoresis. This assay was further multiplexed and optimized on the LC.

Results: Gel electrophoresis results of end-point PCR yielded amplification products of 143bp for *E. bieneusi* target DNA, 142bp for *E. intestinalis* target DNA, and 233bp for the internal control target DNA. All bands were well-defined and bright. Cycle threshold (Ct) values (mean ± standard deviation) of single-plex real-time PCR were 22.34 ± 0.08 for *E. intestinalis* and 20.96 ± 0.15 for *E. bieneusi*. Ct values of multiplex real-time PCR were 22.01 ± 0.65 for *E. intestinalis* and 21.91 ± 0.16 for *E. bieneusi*.

Conclusions: A multiplex, real-time PCR assay for the detection and differentiation of *E. intestinalis* and *E. bieneusi* was demonstrated to be functional. The data presented provided consistent results between single and multiple DNA targets indicating an efficient PCR assay. This multiplex assay affords tremendous promise to clinical laboratories for the accurate diagnosis of *E. intestinalis* and *E. bieneusi* infections.

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Endochin-like quinolones have remarkable efficacy against infection by *Toxoplasma gondii*. CLAUDIA BORDÓN1*, J.STONE DOGGETT2, ROLF WINTER2, AARON NILSEN2, MICHAEL RISCOE2, ROBERT YOLKEN1, LORRAINE JONES-BRANDELORO1; 1Johns Hopkins University, MD, USA, 2Oregon Health & Science University, OR, USA.

The development of new, specific drugs for the treatment and prevention of toxoplasmosis is one of the major goals of The Stanley Laboratory. Endochin and endochin-like quinolones (ELQs) exhibit potent antiplasmodial activity. In addition, endochin reportedly showed inhibitory activity against experimental toxoplasmosis (*Am J Trop Med Hyg.* 1951 Jan; 31(1):12-7) and thus represents a potentially effective scaffold upon which to synthesize a library of derivatives for testing in vitro and subsequently in vivo. Accordingly, we have synthesized and examined more than 70 novel ELQs for in vitro activity against *Toxoplasma gondii* and found that the majority are remarkably efficacious (IC50 range = 1.4 x 10^-8 pM – 17 µM) and mostly parasite specific (75% of ELQs TD50 ≥ 320 µM). In addition, these quinolones are effective against both extracellular and intracellular parasites as shown by standard invasion and replication assays, respectively. The majority of these ELQs significantly inhibit both tachyzoite invasion and attachment to host cell; ≥50% completely inhibit intracellular replication or limit replication to just one doubling. Furthermore, a selected number of ELQs are parasiticidal in vitro. The site of action of these compounds has not been conclusively elucidated as yet although initial experiments suggest that these quinolones act on the parasite cytochrome bc1 complex. We conclude that the ELQs are exceptionally effective against *T. gondii* and thus we propose that they are excellent candidates for drug development for the prevention and treatment of toxoplasmosis.

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A novel Cryptosporidium protein with a C-type lectin domain

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Cryptosporidium is a water-borne pathogen that is a significant cause of gastrointestinal disease worldwide, particularly in the immunocompromised. However, the molecular mechanisms underlying Cryptosporidium-host cells interactions and the proteins that mediate them are not well understood. In higher eukaryotes, proteins containing C-type lectin domains (CTLDs) are known to mediate diverse cell-cell interactions. However, proteins with CTLDs have not been reported in apicomplexan parasites. We identified and cloned a C. parvum protein containing a CTLD. Analysis of the deduced amino acid sequence revealed a signal peptide, a transmembrane domain, a CTLD and a mucin domain that is predicted to be heavily O-glycosylated. It has homologues in both C. hominis and C. muris, in which the relative positions and sequences of these domains are highly conserved. The protein homology/analogy recognition engine Phyre2 was utilized to model the tertiary structure of the C. parvum CTLD-containing protein and the 3DLigandSite server was used to identify four predicted ligand binding clusters which included Ca2+ ions, galactose, N-acetylgalactosamine, fucose and mannose, suggesting that the protein is a calcium-dependent lectin. In order to study its function, the full length coding sequence was PCR-amplified from cDNA of C. parvum infected Caco-2A cells using specific primers that include a hemagglutinin (HA) tag. The amplified sequence was cloned into the Toxoplasma gondii expression vector pHLEM under the control of the GRA1 promoter and upstream of the GRA2 3'UTR. Transfected parasites were cloned by limiting dilution and positive clones were identified by IFA using an anti-HA antibody. Therecombinant protein was purified by HA-immunoaffinity chromatography and migrated with a Mr of 120 kDa by SDS PAGE and localized to dense granules of tachyzoites by IFA. Transfected tachyzoites and the purified recombinant protein will be used for further investigations into the role of this protein in Cryptosporidium-host cell interactions.

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PA12. Spontaneous cystogenesis in vitro of a Brazilian strain of Toxoplasma gondii

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The conversion of T.gondii tachyzoites into bradyzoites and the formation of tissue cysts have a crucial role in the establishment of chronic toxoplasmosis. In this work we investigated the cystogenesis of the EGS strain isolated from human amniotic fluid in the epithelial cells LLC-MK2 using specific markers against bradyzoite (anti-BAG-1), tachyzoite (anti-SAG-1) and cyst wall components (lectin Dolichus bifloruns (DBA)-FITC) by fluorescence microscopy. We observed which parasites of the EGS strain had converted to intracellular cysts spontaneously 4 days after infection, up to 30% of infected cells were DBA positive. Tissue cysts were BAG-1 positive parasites (mature cysts), but the majority of infected cells harbored parasitophorous vacuoles (PV) containing BAG-1 or SAG-1 positive parasites (immature cysts). The capacity of spontaneous cystogenesis was also observed in the muscle cell line (L6), the cell glial line (C6), human fibroblast (HFSF) and embryonic cell (HEK-293). Transmission and scanning electron microscopy showed that the thickness and electron density of the cyst wall increased with the maturation of the cysts. Also, the cyst matrix tubules were shorter than those from the intravacuolar network of the PV and were immersed in granular electron dense material. With this work, we demonstrated that the EGS strain spontaneously converts to bradyzoites in culture cells without artificial stress conditions, resulting in a high burden of cysts in vitro constituting a useful tool to study this stage of the Toxoplasma gondii life cycle.

Supported by CNPq, FAPERJ and CAPES
PA13. Absence of *Pneumocystis* Dihydropteroate Synthase Mutants in Brittany, France. SOLÈNE LE GAL¹, FLORENCE ROBERT-GANGNEUX², CELINE DAMIANI³, MICHELE VIRMAUX¹, ANNE TOTET³, JEAN-PIERRE GANGNEUX², GILLES NEVEZ¹. ¹University of Brest, LUBEM EA 3882, SFR 148, Brest, ²University of Rennes, INSERM U1085, Rennes, ³University of Picardy-Jules Verne, EA 4285 UMI INERIS 01, Amiens, France.

Dihydropteroate synthase (DHPS) is the target of sulfonamides, which are widely used for *Pneumocystis jirovecii* infection prophylaxis and treatment. Two major mutations have been described at nucleotide positions 165 and 171 of the DHPS locus of *P. jirovecii*. Prior exposure to sulfonamides and the city of patient residence have been identified as predictors of mutants in the USA. Because data on mutant frequency in France are still limited, in this study we assessed this frequency in Brittany, France.

Archival *P. jirovecii* specimens from 84 patients monitored at Rennes university hospital, and from 85 patients monitored at Brest university hospital, were genotyped at the DHPS locus using a RFLP assay. Rennes and Brest are the 2 main urban centers in Brittany and are located 250 km apart.

*Pneumocystis* typing was successful in 63/84 patients from Rennes. No mutant was identified. *Pneumocystis* typing was successful in 64/85 patients from Brest. Wild types were detected in 62/64 patients, whereas mutants were detected in 2 patients (2/64, 3.1%). Medical chart analysis pointed out that these 2 patients were vacationers and usually lived in Paris. Taking into account the usual city of patient residence, the corrected frequency of mutants in Brest is 0%. Thus, the study carried out in 2 hospital centers suggests that mutant frequency in Brittany is 0%. This frequency is lower than that reported in other French cities, Lyon (33-36%) Paris (18.5-40%), Amiens (8%), and other European cities, Copenhagen (20%), London (17-36%), Seville (22-35.5%), Milan (9%), Barcelona (5.5-33%), Lisbon (7-27%), and Zurich (10 %), and similar to that reported in Stockholm (0%).

The results show that *P. jirovecii* infections involving mutants are infrequent in Brittany and that the city of patient residence is a predictor of mutants in France and Europe as previously postulated in the USA.

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PA14. Matches of *Pneumocystis jirovecii* Genotypes between Pulmonary and Exhaled Air Samples from Patients with *Pneumocystis* Pneumonia. CELINE DAMIANI¹, SOLENE LE GAL², FIRAS CHOUKRI³, JEAN MENOTTI³, CLAUDINE SARFATI³, FRANCIS DEROUIN², GILLES NEVEZ²*, ANNE TOTET¹.¹University of Picardy-Jules Verne, EA 4285 UMI INERIS 01, Amiens, ²University of Brest, LUBEM EA 3882, SFR 148, Brest, France, ³University Paris Diderot, EA 3520, Paris.

Dihydropteroate synthase (DHPS) is the enzymatic target of sulfonamides, the main drugs used for *Pneumocystis* pneumonia (PCP) prophylaxis and treatment. Studies of the DHPS locus have revealed two major non-synonymous mutations at positions 165 and 171, which confer potentially lower sensitivity to sulfonamides to mutant *Pneumocystis jirovecii* (*P.jirovecii*) organisms. Inter-individual transmission of *Pneumocystis* by the airborne route has been demonstrated in animal models and is probable in humans. We recently quantified *P.jirovecii* in the air surrounding patients with PCP, suggesting that the fungus is exhaled from infected patients and then spreads into their surrounding air. Because matches of *P.jirovecii* genotypes between pulmonary and air samples would strengthen this hypothesis, we conducted DHPS genotyping of *P.jirovecii* isolates from PCP patients and from the air in their close environment. DHPS genotyping was based on a PCR restriction fragment length polymorphism assay that enables detection of mutations at positions 165 and 171.

DHPS genotyping was successful for all 15 pulmonary samples and 6/15 air samples. A wild genotype was identified in 12/15 pulmonary samples and in 4/6 air samples. Mutant genotypes were identified in 6/15 pulmonary samples and in 2/6 air samples. A full match of DHPS genotypes was found for 6 pairs of samples.

Genotype matches in pairs of pulmonary and air samples pointed out that *P.jirovecii* organisms detected in the air were in fact exhaled by infected patients into their surrounding environment. Moreover, the first data of the airborne spread of DHPS mutants that we obtained emphasize the risk of nosocomial transmission of potentially sulfonamide-resistant organisms. This study provides additional arguments for applying measures to prevent the airborne transmission of *P.jirovecii* in healthcare settings.

[Supported by the “ANSES”, contract number 2011/1/053]
PA15. Selection and characterization of scfv antibodies against *Toxoplasma gondii* tachizoites from phage display libraries. FERNANDO CARDOSO*, OLGA MATOS, UEI Parasitologia Médica, IHMT- Universidade Nova de Lisboa, Rua da Junqueira, 100, 1349-008 Lisboa, Portugal.

*Toxoplasma gondii* is an obligate intracellular protozoan parasite that infects a variety of mammals and birds and causes toxoplasmosis. Diagnosis of toxoplasmosis in humans is made by biological, serological, histological, or molecular methods or by some combination of these. In order to obtain a recombinant antibody against *T. gondii* and use it in the diagnosis we can use the phage display technology in order to rapidly obtain a new diagnostic tool. In this work we used three phage display libraries: the human synthetic scfv Tomlinson I+J libraries, (MCR, UK) and Griffin scfv library (Griffin.1 library) to select *T. gondii* tachozoites RH (EP) strain scfv binding phages. The antigen was obtained by *in vitro* culture of tachozoites in Vero or Hells cells. The tachozoites were maintained *in vitro* until attached to the cell culture flask (1 month). In the panning protocol the washing steps include a high salt (500 mM NaCl) buffer to remove the low binding and unspecific phages. The phage elution and infection were performed in one step by mixing with *E. coli* TG1 for 1 hour. From the 3th panning, 90 clones were selected from each library and scfv-phage were then tested by Indirect-ELISA. The specificity and the usefulness of these scfv-phage were investigated by ELISA and immunofluorescent assays. We are in the process of testing the cross reactivity to other *T. gondii* strains. Acknowledgments: The *T.gondii* strains were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, USA and the scfv libraries from MCR, UK.
Dendritic cells (DCs) play an important role in eliciting an immune response and are also the first line of defense against pathogens by activating an innate immune response. In order to better understand the role these cells play in host immune responses to \textit{C. parvum}, we investigated the effects of sporozoites and soluble antigens on the function and phenotype of mouse DCs derived from bone marrow cells. We found that DCs could be activated by \textit{C. parvum} antigen as indicated by an increase in maturation markers, as well as the production of the cytokines interleukin-12 p40, IL-2, IL-1beta, and IL-6. In particular, significant increases in expression of IL-12p70 were observed from mouse DCs in response to solubilized sporozoite antigen and the recombinant antigens, Cp40 and Cp23. Additionally, a small but significant increase in IL-18 expression was observed following treatment with Cp40. These responses were MyD88 dependent as parasite antigens were not able to activate bone-marrow dendritic cells isolated from MyD88 knockout mice as measured by cytokine expression. We also looked at the effects of depletion of dendritic cells using a transgenic mouse model CD11c-DTR/EGFP that expresses the simian diphtheria toxin receptor under the control of the CD11c promoter. Mice depleted of DCs cells with the injection of diphtheria toxin (>90% of CD11c DCs as determined through flow cytometry) were highly susceptible to \textit{C. parvum} infections compared to controls. Infection levels were 10 fold higher in mice depleted of DCs than in controls at 3 days post infection (P< 0.05). By understanding how DCs are activated and the types of antigen needed to generate these responses, more efficacious therapies can be developed.
Toxoplasmosis is the disease caused by *Toxoplasma gondii*, an obligate intracellular parasite and the infection produces a wide range of clinical syndromes in humans and other animals. The infection is mainly acquired by ingestion of undercooked or raw meat containing viable cysts, or acquired by ingestion of food and/or water contaminated with oocysts shed by felids. The congenital infection is also possible when women get infected during pregnancy. In humans, this parasite has a simple life cycle consisting of two stages: tachyzoite and bradyzoite. The former is a rapidly growing, obligate intracellular form of *T. gondii* present in acute infections. The surface of *T. gondii* tachyzoites and bradyzoites is covered with surface antigen SAG1 or SAG2 families. These molecules play an important role in host cell invasion, immune modulation and/or virulence attenuation, although they may also provide protection, needed by the parasite, to survive in the environment. The objective of this work was to produce monoclonal antibodies to the surface antigens and use them to characterize the surface of *T. gondii* tachyzoite by immunological assays. *T. gondii* strain RH was grown in vivo in Balb/c for 3 days and the parasite was collected and purified by percoll gradient. The antigen (tachyzoite) was heat inactivated before the immunization. The antigen was mixed with incomplete Freud’s adjuvant plus MPL-TDM adjuvant and the Balb/c mice were immunized 4 times at 2-3 weeks intervals. To obtain the MAbs, one cell fusion was done between splenocytes and Sp2/AG-14 cell partner, using PEG. The hybridoma screening was by ELISA against tachyzoites and we obtained 45 positive wells in a total of 220 wells. After cell expansion the selected hybridoma cell lines were cloned by the limiting dilution method. Immunological assays based on ELISA, western-blot and immunofluorescent are in progress, to confirm the ability to detect surface proteins in *T. gondii* tachyzoite and to determine if there are cross reactions with other Apicomplexan parasites.
The continuous loss of CD4 T cells and type-I-interferon (IFN)-producing plasmacytoid dendritic cells (pDC) in AIDS patients results in increased susceptibility to AIDS-defining illnesses such as *Pneumocystis* (PC) pneumonia. Regenerative bone marrow (BM) failure resulting in peripheral pancytopenia is an additional problem in these patients, although the mechanisms responsible are poorly understood. Whether there is a direct connection between BM failure, progressive immune deficiency, and increased demand on hematopoiesis due to recurrent opportunistic infections is not known.

We recently reported a connection between deviated systemic immune responses to low grade pulmonary PC infection, BM failure, and a defective type-I-IFN system using IFNAR-deficient mouse models (IFNAR−/−, IFNa-receptor-deficient; IFrag−/−, IFNa-receptor- and lymphocyte-deficient). We demonstrated increased oxidative stress and global caspase activity, progressive loss of neutrophils, increased levels of the pro-apoptotic cytokine TRAIL, and apoptosis of hematopoietic cells in the BM of affected mice in response to *Pneumocystis* lung infection. The stimulus triggered by PC lung infection that induces systemic effects and BM failure in our model is not understood.

Towards this goal, cytokine profiles of bronchoalveolar lavages (BAL) from PC-infected IFNAR−/− and IFrag−/− mice showed significantly high levels of IFNg already by day 4 post-infection, followed by high levels in the serum at day 7, which proceeded induction of BM failure in these mice. IFNg is known to regulate the pro-apoptotic cytokine TRAIL and also to play a role in stem cell destruction and inhibition of hematopoietic activity in aplastic anemia in humans. FACs analysis of lung homogenates revealed innate NK cells as the early producers of IFNg in the lungs of PC-infected IFrag−/− mice. These data suggest that, in the absence of type-I-IFNs, exuberant production of IFNg by NK cells in PC-infected lungs may be the initiating signal that sets off a series of events resulting in BM failure in our model.
Toxoplasma gondii is one of the most important pathogens affecting immunocompromised patients. However, there are few options of treatment against toxoplasmosis, which are often associated to side effects. Thus, the discovery of new compounds against Toxoplasma gondii is extremely important. Fluoroquinolone is a known class of topoisomerases II and IV inhibitors in prokaryotes, but has demonstrated a broad spectrum of activity against many other eucaryotic pathogens, including T. gondii. Herein we evaluated the antiproliferative effect of four new ethyl-ester derivatives of ciprofloxacin against the tachyzoites of T. gondii in vitro. The treatment of infected cultures with concentrations of ciprofloxacin up to 20μM for 24 and 48h just reduced modestly the parasite proliferation. On the other hand, the four novel derivatives were very active and inhibited parasite proliferation with IC_{50} at concentrations lower than 3μM after 24 and 48h (Reference: MedChem Comm 2011, 2, 43). In addition, two derivatives were able to inhibit parasite proliferation with IC_{50} at nanomolar range. The study of the cytotoxic effect of the compounds against LLC-MK2, epithelial cell line, showed a wide therapeutic index. The cellular effects evaluated by transmission electron microscopy of parasites treated with the most two active compounds showed the main effect observed for cipro derivatives was cell division arrestment leading to the formation of gathered daughter cells. Observations of treated parasites by fluorescence microscopy utilizing an antibody for the inner membrane complex (anti-IMC1 antibody) and the probe sytox green for nucleus demonstrated that parasites can divide their nucleus entirely, but can not complete cytokinesis. Indeed, experiments labeling the apicoplast (anti-Cpn60 antibody) showed that treatment caused apicoplast enlargement. Accordingly to the results obtained, chemical modifications on ciprofloxacin structure were efficient to improve the anti-T. gondii activity of the new derivatives. [Supported by: CNPq, CAPES and FAPERJ]
Profiles of cytokines and immunoglobulins in Cryptosporidium parvum infection: usefulness of Luminex® xMAP technology

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Cryptosporidium parvum is the enteric protozoa most frequently associated with diarrhea outbreaks. Infection with this organism triggers a complex innate and cell mediated adaptive immune response. To date, there are no studies applying the Luminex xMAP® technology to determine profiles of cytokines and immunoglobulins in the context of an infection by C. parvum. In order to contribute to a better knowledge of the immune response to this infection, analysis of immune mediators in serum of immunocompetent mice inoculated with C. parvum oocysts was done, using Luminex. ELISA assays were used to determine Ig isotype and ascertain if the profile of immune mediators fits the Luminex one. Specific-pathogen-free BALB/C mice were inoculated with 1x10^6 oocysts of C. parvum at days 0 and 22. Peripheral blood was aseptically collected from mice euthanized on specific days. As for the xMAP technology, two kits were used to evaluate mouse cytokines and immunoglobulins in serum samples, respectively: the Bio-Plex Pro™ Mouse Cytokine Standard Group I 23-Plex and the Milliplex® MAP Mouse Immunoglobulin Isotyping kits. ELISA assays were performed with the Mouse Mono Ab-ID Kit. Infection was confirmed by the presence of C. parvum DNA in feces by a nested-PCR assay of the 60-kDa glycoprotein. Luminex results show predominance in the secretion of IgG1 and IgG2a. These data were confirmed by ELISA. Analysis of the produced cytokines suggested a preferential Th1 over the Th2 response, with emphasis on the increase of TNF-α and IFN-γ and also the hematopoiesis related cytokine (GM-CSF). In this work, we report and discuss preliminary experimental results which suggest the advantage of using Luminex® xMAP technology to study these immune mediators, from a single small volume sample.

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PA21. Progress in the Pneumocystis carinii Genome Assembly and Annotation
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Pneumocystis pneumonia (PCP) is an opportunistic infection in patients with compromised immune systems. The infection is caused by the fungus that belongs to the genus, Pneumocystis, and its members are found in a variety of mammals. While adaptation to the lung environment of a host is essential to the pathogen, the metabolic strategies used by these fungi to grow and survive in this context are largely unknown. Considerable impediments to standard approaches for investigation of this unique pathogen include the lack of a long term in vitro culture system and the absence of an ex vivo cultivation method. Therefore, many fundamental scientific questions about the basic biology, metabolism, and life cycle of Pneumocystis remain unanswered. In this work, we present the progress in the sequencing of the Pneumocystis carinii genome, a species infecting rats. Furthermore, the first genome wide annotation of Pneumocystis enzymes is presented. Annotation is based on sequence homology using two bioinformatics tools (Blast2GO and DETECT). First insights in metabolic strategies used by the fungus are discussed. All sequences are available via Pneumocystis Genome Project at http://pgp.cchmc.org/.
PA22. Studies of an Investigational New Anti-Pneumocystis Compound (IKT061) from \textit{in vitro} Screening to \textit{in vivo} Therapeutics in Mice. MICHAEL LINKE$^{2,3}$, BILAL S. ABUASAL$^1$, PETER D. WALZER$^{2,3}$, ALAN ASHBAUGH$^2$, MARGARET COLLINS$^2$, KEELEY LYNCH$^2$, LARRY SALLANS$^4$, MILTON H. WERNER$^5$, PANKAJ DESAI$^1$, MELANIE T. CUSHION$^{2,3}$; $^1$University of Cincinnati, College of Pharmacy, Department of Basic Pharmaceutical Sciences $^2$University of Cincinnati, College of Medicine, Department of Internal Medicine, $^3$ Cincinnati Department of Veterans Affairs Medical Center, $^4$University of Cincinnati, R. Marshall Wilson Mass Spectrometry Facility, $^5$Inhibikase Therapeutics, Inc, Atlanta, GA 30339

\textit{Pneumocystis jirovecii} pneumonia (PCP) remains a significant cause of morbidity and mortality and new treatment options are needed. We identified IKT061 as a promising new PCP therapeutic. In an \textit{in vitro} system, IKT 061 resulted in a dose-dependent reduction in \textit{P. carinii} viability with an IC50 of 1.22µg/ml. Pharmacokinetics/pharmacodynamics studies were conducted to support development of IKT 061 for the treatment of PCP. These studies showed that the oral bioavailability of IKT061 was low ranging from 3-10%; however, intraperitoneal (i.p.) dosing provided over 70% availability. The oral AUC and Cmax of the IKT061 increased proportionally relative to the dose demonstrating that IKT061 exhibits dose-proportional PK. A therapeutic study was conducted with IKT061 in the \textit{P. murina} (Pm) corticosteroid immunosuppressed mouse model. Mice were treated with IKT061 with 20 and 50 mgs/kg/d i.p. twice daily for 7, 14, and 21 days. Negative controls were treated with vehicle alone. Infection levels were evaluated by microscopic enumeration of Pm organisms in the lungs. At day 7, significantly fewer organisms were detected in mice treated with IKT061 than in the negative controls. At days 14 and 21, similar numbers were detected in the IKT061 treated mice and the negative controls. Interestingly, IKT061 demonstrated a dose-dependent improvement in survival. At d14, only 50% of the negative control mice were still alive; however, in mice treated with 20 and 50 mgs/kg/d of IKT061, 66% and 100% of the mice, respectively, were still alive. At d21 there was no difference in survival. Long term effects of the steroids and cumulative damage from the Pm infection to the lung may diminish the activity of IKT061 and explain the lack of efficacy of IKT061 at the later time points. In conclusion, IKT061 demonstrated anti Pc activities in \textit{in vitro} and \textit{in vivo} and potentially represent an innovative new therapy for PCP.

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POSTER SESSION B
ABSTRACTS

PB1 to PB17
Identification and genetic analysis of *Pneumocystis* from thoroughbred foals. THOMAS M. SESTERHENN\(^1\), ROBIN W. ALLISON\(^3\), MELANIE T. CUSHION\(^{1,2}\); \(^1\)University of Cincinnati College of Medicine, Cincinnati, OH, USA, \(^2\)Cincinnati Veteran’s Affairs Medical Center, Cincinnati, OH, USA, \(^3\)Oklahoma State University, Department of Veterinary Pathobiology, Stillwater, OK, USA.

*Pneumocystis* is a ubiquitous organism that has been found in the lungs of nearly every mammalian species. There have been several reports of *Pneumocystis* being identified in thoroughbred foals, but to date only a single sequence of the mitochondrial large ribosomal subunit (mtLSU) has been obtained from these organisms and submitted to the public databases. Tracheal washes taken from two foals that had been diagnosed with *Pneumocystis* by microscopic examination were obtained. DNA was extracted and amplified with PCR primers targeted to *Pneumocystis* genes: mtLSU, 18s ribosomal subunit, dihydrofolate reductase, and thymidylate synthase. The products were sequenced and aligned against other known *Pneumocystis* sequences for comparison and generation of phylogenetic trees. Although there was not always 100% identity between the sequences from the two foals, they consistently claded together and usually had a higher percentage of identity to each other than to the other *Pneumocystis* sequences. The mtLSU sequences from the foals also differed significantly from the previously published horse *Pneumocystis* mtLSU sequence. This is of interest because the previous sequence came from an English foal while the foals in this study were from the United States. Based on this information, we plan to begin the process of formally naming *Pneumocystis* isolated from horses.
PB2. One-year follow-up study of *Giardia* spp and *Cryptosporidium* species in recreational waters from Spain. ANA L. GALVÁN¹,², ANGELA MAGNET¹, FERNANDO IZQUIERDO¹*, CARMEN FERNANDEZ-VADILLO¹, REGINA H.S. PERALTA³, SOLEDAD FENOY¹, CARMEN DEL AGUILA¹; ¹Laboratorio de Parasitología, Facultad de Farmacia, Universidad San Pablo CEU, Urbanización Montepríncipe, 28668, Boadilla del Monte, Madrid, España; ²Escuela de Microbiología, Universidad de Antioquia, Medellín, Colombia, Becaria Colciencias; ³Universidade Federal Fluminense, Departamento de Patologia.

Waterborne cryptosporidiosis and giardiasis represent a serious threat to human health due to their ubiquitous distribution and the resistance of the cysts to harsh environmental conditions, various disinfectants and some treatment practices. This study was undertaken to monitor the presence of *Giardia* spp and *Cryptosporidium* in 5 natural pools from Spain during one year. Water samples were collected according to US-EPA Method 1623, and concentrated by the IDEXX Filta-Max® system. To evaluate the presence of *Cryptosporidium* spp and *Giardia* spp, 1 ml of the concentrate samples was processed by immunomagnetic separation (IMS) followed by immunofluorescence (IFA) microscopy. *Cryptosporidium* species were detected based on PCR-restriction fragment length polymorphism and sequence analyses of the ssuRNA gene. Among 40 samples processed by IFA, 14 were positive for *Giardia* spp (35%), 5 positive for *Cryptosporidium* spp (12.5%) and 3 positive for both parasites (7.5%). Parasite concentration ranged from 0.1 to 1.2 *Cryptosporidium* spp. oocysts per liter and from 0.6 to 209 *Giardia* spp cysts per liter. Using PCR, 17.5% of samples (7/40) were *Cryptosporidium* positive and only *C. andersoni* (4/7) and *C. muris* (2/7) were identified, with one sample of undetermined species. *Giardia* spp. was present throughout the year with an elevated presence in spring (43%) while *Cryptosporidium* was more frequent in summer (57%) and winter (29%). Our results reveal the presence of *Giardia* spp and *Cryptosporidium* in recreational waters from Spain which supports the view that they are a potential source of infection.

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PB3. Molecular characterization of human pathogen microsporidia and *Cyclospora cayetanensis* during a one-year follow-up study from several drinking water treatment plants and wastewater treatment plants. ANA L. GALVÁN¹,², ANGELA MAGNET¹, FERNANDO IZQUIERDO¹, SOLEDAD FENOY¹, CRISTINA RUEDA¹, CARMEN FERNANDEZ-VADILLO¹, NUNO HENRIQUES-GIL¹, CARMEN DEL AGUILA¹; ¹Parasitology laboratory, San Pablo CEU University, Urbanización Montepríncipe, Boadilla del Monte, Madrid, Spain, ²Escuela de Microbiología, Universidad de Antioquia, Becaria Colciencias.

Recent studies suggest the involvement of water in the epidemiology of *Cyclospora cayetanensis* and several species of microsporidia. A total of 223 samples from 4 drinking water treatment plants (DWTP), 7 wastewater treatment plants (WWTP), and 6 locations of influence (LI) on 4 river basins from Madrid (Spain) were analyzed during one year. Microsporidia and/or *Cyclospora spp.* were detected by PCR in 52% (117 out of 223) of samples, with 49% (109 out of 223) positive for microsporidia, 9% (20 out of 223) positive for *Cyclospora spp.* and 5.4% (12 out of 223) positive for both parasites. Human pathogen microsporidia species were detected including *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon cuniculi* and *Annclalia (Brachiola) algerae*. *E. bieneusi* C, D and D-like genotype and *E. cuniculi* I and III genotype were also identified. *C. cayetanensis* was identified in 17 of 20 samples. To our knowledge, this is the first study that shows a one-year follow-up of *C. cayetanensis* in drinking water treatment plants and wastewater treatment plants worldwide. Additionally, we present for the first time a one-year study on the presence and molecular characterization of human pathogen microsporidia in drinking water, wastewater and locations of influence in Spain. These results provide information on water as a potential infection source for *Cyclospora* and microsporidia in Spain.

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PB 4. Low incidence of *Acanthamoeba* spp. in contact lens wearers from Madrid, Spain. THIAGO S. GOMES1*, ANGELA MAGNET1, FERNANDO IZQUIERDO1, SARA BUENO2, MARIA LUISA SANCHEZ RODRIGUEZ2, SOLEDAD FENOY1, CARMEN DEL ÁGUILA1, 1Parasitology laboratory, San Pablo CEU University, Urbanización Monteperpríncipe, Boadilla del Monte, Madrid, Spain, 2Optical laboratory, San Pablo CEU University, Urbanización Monteperpríncipe, Boadilla del Monte, Madrid, Spain.

*Acanthamoeba* keratitis (AK) is a painful and potentially blinding corneal infection caused by *Acanthamoeba* spp., an ubiquitous free-living amoebae which can be found in diverse habitats. Although several outbreaks seem to be associated with contaminated CL solutions, the great majority of AK cases occur in CL wearers with inadequate CL practices. Environmental studies in Madrid have demonstrated a high presence of *Acanthamoeba* in tap water; this fact induced us to study the influence of its high presence in the risk of AK acquisition by CL wearers. A campaign was designed to collect CL from healthy individuals in Madrid (Spain). One hundred-sixty one individuals participated by contributing their discarded CL and answering a hygiene habits questionnaire. These CL were cultured on non-nutrient agar plates seeded with inactivated *E. coli* and the samples were considered negative if after 20 days of culture no cyst or trophozoites were observed. Among the 161 samples investigated, the presence and multiplication of *Acanthamoeba* trophozoites was detected in only one sample, showing an incidence value of 0.62%. The hygiene habits reported by the CL owner included some recognized risk factors, such as rinsing with tap water and showering while wearing CL. The CL owner also reported feeling some eye discomfort, as did 46.6% of the other individuals in the study. Thus, the results obtained suggest a low prevalence of *Acanthamoeba* spp. in healthy CL wearers from Madrid. This information is important in understanding *Acanthamoeba*’s incidence in healthy carriers from continental Europe, since a higher prevalence has only been seen in studies in the Canary Islands and the United Kingdom. To understand if the differences observed may be explained by good hygiene habits of the citizens from Madrid, by a lower pathogenicity of the strains present in this city environment or to other factors, more studies are necessary.

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PB5. Molecular characterization of *Cryptosporidium* in children and cattle populations from Romania. PATRÍCIA VIEIRA\(^1\), NARCISA MEDERLE\(^2\), MARIA LUÍSA LOBO\(^1\), OVIDIU MEDERLE\(^2\), LIHUA XIAO\(^4\), GHEORGE DARABUS \(^2\), OLGA MATOS\(^1\);
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*Cryptosporidium* species are ubiquitous apicomplexan parasites that infect both gastrointestinal epithelium of humans and vertebrate hosts. *Cryptosporidium* infection has several transmission routes, being diarrhea the most frequent sign of disease, and especially immunocompromised patients can develop severe infection, often fatal. In recent years, molecular tools have improved the knowledge about the epidemiology of these parasites. Some loci have revealed high degree of polymorphisms allowing characterize genetic diversity at subtype level, leading to a better understanding of sources of infection and transmission dynamics in humans and animals. With this work we intend to genetically characterize *Cryptosporidium* spp. organisms from human and animal origin, from Romania, using nested-PCR techniques applied to two loci, small subunit ribosomal RNA (SSU rRNA) and 60 kDa glycoprotein (GP60), with the purpose to understand the main reservoirs present in this country. In the present study, 41,4% (12/29) of bovine samples were positive by SSU rRNA and 51,7% (15/29) by GP60, while 18,2% (2/11) and 27,3% (3/11) of human samples showed a positive result to SSU rRNA and GP60, respectively. Sequence analyses revealed that all isolates were *Cryptosporidium parvum*. Two subtype families were identified, IIA and IId. Unlike several reports, the most widely distributed subtype family IIA, was identified in only one isolate with IIA16G1R1 subtype. The other positive samples had the following distribution: four subtypes in family IId, IIdA27G1 (n=7), IIdA25G1 (n=4), IIdA21G1a (n=1) and IIdA22G1 (n=5). All were detected in bovines with the exception of the subtype IIdA22G1 that was observed in bovine and human fecal samples. These results show a remarkable different epidemiologic perspective from previous Romanian reports, since the four subtypes of family IId identified in this study have never been identified previously in this country, showing that transmission dynamics in Romania is more complex than previous conclusions. Furthermore, the importance for public health of the subtypes identified only in bovines remains unclear.
Background: PCR-based pathogen detection typically relies on the use of positive control DNA extracted from clinical samples, which is limited in amount, varied in quality, and easily degrades. Molecular Standards are easier to produce, store, maintain, and distribute than clinical samples. Added benefits are the fact that these Molecular Standards are not infectious and provide more consistent control material than use of clinical samples. This study evaluated the use of G-Sphere® Molecular Standards as positive controls for various parasites.

Methods: G-Sphere® Molecular Standards for Cryptosporidium, Giardia, and Microsporidia were designed, manufactured, and compared to genomic DNA for each parasite by PCR amplification. Limit of detection (LOD), linearity, reproducibility, and efficiency for PCR was determined for each parasite. Positive and negative controls were included and each PCR was replicated 10 times.

Results: G-Sphere® Molecular Standards consistently demonstrated analytical LOD values tenfold lower than that of the corresponding genomic DNA for each parasite. Standard curves were constructed by plotting threshold cycle number versus log_{10} (gene copy number per reaction) for standard and genomic DNAs. The R^2 values of the linear regression were above 0.9 for both DNA types over a quantification range of five orders of magnitude. PCR efficiencies for standard and genomic DNA amplification ranged from 90-110% for each parasite.

Conclusions: Although Molecular Standards had a lower LOD, the efficiency, quantification, and reproducibility of standard and genomic DNA was comparable. Thus, Molecular Standards for parasites are suitable for use as both positive and quantification controls.

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**PB7. Molecular and morphological characterization of *Agmasoma penaei* parasitizing *Litopenaeus setiferus* from the Gulf of Mexico, and analysis of microsporidian diversity in marine decapods inferred from SSUrDNA phylogenies.**

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*Agmasoma penaei* described initially within the genus *Thelohania* by Sprague (1950) causes epizootics of “cotton disease” among the white Atlantic shrimp *Litopenaeus setiferus*. Microsporidium prevalence can range from 1 to 90% with high levels having devastating effects on populations. Normally the infection rate does not exceed 1%, however within the last two years there have been reports of increased levels of infection in the areas of the Gulf of Mexico adjacent to the BP oil spill. In June 2012 a few shrimp from Plaquemines parish LA with the symptoms of microsporidiosis were delivered to the Louisiana Aquatic Diagnostic Laboratory for identification. Light and EM examination indicated the infection was caused by a microsporidium producing subpersistent roundish pansporoblasts with 8 spores (3.6x2.1um,median) and possessing anisofilar (2+6) polar filaments. These features allowed us to identify the microsporidium as *Agmasoma penaei* (=*Thelohania penaei* Sprague 1950, n.comb Hazrad and Oldacre, 1973) within the family Thelohaniidae sensu Hazard and Oldacre 1973. Two ultrastructural descriptions of “*Agmasoma penaei*” exist: one of the organism parasitizing *Penaeus notialis* from Senegal coast of Atlantic, another from the farm-reared Pacific white shrimp *Litopenaeus vannamei* from Thailand. Unlike previous authors we obtained the microsporidium from the type host species and type locality for sequencing. Comparison of the type SSU rDNA sequence (c.1300bp) to *A.penaei* from Thailand revealed 95% similarity, which suggests two different species, a conclusion supported by several ultrastructural dissimilarities. No sequence is available for *A.penaei* from Senegal. Alignment of *A.penaei* sequences with 19 microsporidia from decapods (GenBank), and consequent phylogenetic analysis did not reveal any meaningful homology. Neither phylogenies supported existence of the “Thelohaniidae” clade. Moreover, most taxa belong to unrelated clades, reflecting poor sampling of decapod microsporida. We presume, that the genus *Agmasoma* includes parasites of the superfamily Penaeoidea of the ancient suborder Dendrobranchiata, a sister group to suborder Pleocyemata, which comprises lobsters, crayfish, crabs and shrimps. Supported by Louisiana Department of Wildlife and Fisheries.
PB8. Molecular characterizations of Cryptosporidium, Giardia, and Enterocytozoon in humans in Kaduna State, Nigeria. *BEATTY V. MAIKAI1,2, JARLATH U. UMOH2, IDRIS A. LAWAL3, AYUBA C. KUDI4, CLARA L. EJEMBI5, VICTOR A. MAIKAI6, LIHUA XIAO1; 1Division of Foodborne, Waterborne and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA, 2Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, 3Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, 4Department of Veterinary Medicine and Surgery, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria, 5Department of Community Medicine, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria, College of Agriculture and Animal Science, Ahmadu Bello University, Mando Road, Kaduna, Nigeria.

The use of molecular diagnostic tools in epidemiological investigations of Cryptosporidium, Giardia, and Enterocytozoon has provided new insights into their diversity and transmission pathways. In this study of 157 stool specimens from 0 to 70-year-old patients, a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis of the small subunit (SSU) rRNA gene was used to detect and differentiate Cryptosporidium species, and DNA sequence analysis of the 60 kDa glycoprotein (gp60) gene was used to subtype C. hominis and C. parvum. Giardia duodenalis and Enterocytozoon bieneusi in the specimens were detected using PCR and sequence analysis of the triosephosphate isomerase (tpi) gene and internal transcribed spacer (ITS), respectively. Cryptosporidium hominis and C. parvum were found in two (1.3%) and one (0.6%) specimen respectively, comprising of Ia and Ile (with 8 nucleotide substitutions) subtype families. The G. duodenalis A2 subtype was detected in five (3.2%), while four genotypes of E. bieneusi, namely A, type IV, D and WL7 were found in 10 (6.4%) specimens. Children two years or younger had the highest occurrence of Cryptosporidium (4.4%) and Enterocytozoon (13.0%) while children of 6 to 17 years had the highest Giardia infection rate (40.0%). No Cryptosporidium, Giardia and Enterocytozoon were detected in patients older than 60 years. Enterocytozoon had high infection rates in both HIV-positive (3.3%) and HIV-negative (8.3%) patients. Results of the study suggest that anthroponotic transmission may be important in the transmission of Cryptosporidium spp. and G. duodenalis while zoonotic transmissions may also play a role in the transmission of E. bieneusi in humans in Kaduna State, Nigeria.

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PB9. Epidemiology of Giardia and Cryptosporidium in Kolkata, India.
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Few studies in the past have examined the genetic diversity and zoonotic potential of Cryptosporidium and Giardia duodenalis among dairy cattle in India. To assess the contribution of these animals as a source of generating human Cryptosporidiosis and Giardiasis, fecal samples from 180 calves, heifers and adults and 51 dairy farm workers of two dairy farms in West Bengal, India were genotyped by PCR-RFLP analyses and direct sequencing of the 18S rRNA and β-giardin genes of Cryptosporidium sp. and Giardia sp. respectively. Phylogenetic analysis was carried out on the DNA sequences obtained in the study and those available in GenBank. The overall prevalence of Cryptosporidium sp. and Giardia sp. in cattle were found to be 11.66% & 12.22% respectively, though it was observed that infections by both these parasites were more prevalent in young calves than in adult cattle. The occurrence of C. parvum, C. bovis, C. ryanae and C. andersoni in cattle followed an age-related pattern. One C. suis-like genotype was also detected in a calf. Dairy farm workers were infected with C. hominis, C. parvum and a novel C. Bovis genotype. Furthermore, zoonotic G. duodenalis Assemblage A1 was identified in both calves and workers, although the most prevalent genotype detected in cattle was a novel Assemblage E subgenotype. These observations clearly suggest that there is a potential risk of zoonotic transmission of Cryptosporidium sp. and G. duodenalis infections between cattle and humans in dairy farms of India.

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Pigs are probably susceptible to infection of at least 7 different Cryptosporidium species or genotypes. Despite intensive recent field research the susceptibility of pigs to rodent-adapted as well as age specificity of pig-adapted cryptosporidia remains unknown. Four and 8 week old pigs (three animals per isolate) were inoculated with Cryptosporidium muris, C. tyzzeri, C. suis and Cryptosporidium pig genotype II at a dose of 1 × 10^7 oocysts per animal. Both categories of pigs inoculated with C. muris or C. tyzzeri showed no detectable infection and no clinical symptoms of cryptosporidiosis during 30 days post infection (DPI), and no macroscopic changes were detected in the digestive tract after necropsy. Any developmental stages were detected in gastrointestinal tract tissues neither by histology nor PCR throughout the duration of the experiment. The infectivity of these isolates was verified on SCID mice, which started to shed oocysts from 4 to 8 DPI. Experimental infection revealed susceptibility of both 4 and 8 week old pigs to C. suis. While parasitological, molecular and histology examination confirmed susceptibility of 8 week old pigs to Cryptosporidium pig genotype II, 4 week old pigs were not being infected with this genotype. Both C. suis and Cryptosporidium pig genotype II were detected in small and large intestine. Based on our findings, it can be concluded that pigs are not susceptible to C. muris or C. tyzzeri infection, C. suis does not have age specificity and Cryptosporidium pig genotype II is not infectious for pre-weaned pigs.

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PB11. *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* in horses kept under different management systems in the Czech Republic. PAVLA WAGNEROVÁ1,2, BOHUMIL SAK,2,† DANA KVETONOVÁ2, ZITA BUNATOVÁ1, HANA CIVIŠOVÁ1, MIROSLAV MARŠÁLEK1, MARTIN KVÁC1,2; 1Faculty of Agriculture, University of South Bohemia in České Budejovice, Czech Republic, 2Institute of Parasitology, BC ASCR, v.v.i., Czech Republic.

Faecal samples were collected from 377 horses from 23 farms with various management systems in the Czech Republic. Microsporidia were found on 16 farms in total. Of these, 66 (17.3%) and 26 horses (6.9%) were found to be positive for *E. bieneusi* and *E. cuniculi*, respectively. The prevalence of *E. cuniculi* in horses over 3 years of age was significantly higher (10.0%) compared to younger horses (4.0%). No significant differences in prevalence were observed between males and females for both microsporidia. The significantly higher infection rates of *E. bieneusi* and *E. cuniculi* were recorded in horses kept in stables than those on pasture. Two genotypes of *E. cuniculi* (I and II) and 15 genotypes of *E. bieneusi* including six previously described and nine novel genotypes were detected. The most common genotype detected was *E. bieneusi* genotype D identified in 34 (51.5%) horses out of the 66 positive ones. The identification of *E. bieneusi* genotype D, EpbA, G and WL15, which was previously reported in pigs, humans, racoons or horses, indicates that horses could be a potential source of zoonotic infection in humans.

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PB12. Diversity of Cryptosporidium spp., Encephalitozoon spp. and Enterocytozoon bieneusi in great apes in different level of habituation. BOHUMIL SAK1,*, ANNA MYNÁROVÁ2, KLÁRA PETRZELKOVÁ3,4, 5, DANA KVETONOVÁ1, KATERINA POMAJBÍKOVÁ3,5, DAVID MODRÝ1,3, BARBORA KALOUSOVÁ6, MARTIN KVÁC1,7; 1Institute of Parasitology, BC ASCR, v.v.i., Czech Republic, 2Faculty of Science, University of South Bohemia in Ceské Budejovice, Czech Republic, 3Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, 4Institute of Vertebrate Biology, ASCR, Czech Republic, 5Liberec Zoo, Czech Republic, 6Faculty of Science, Masaryk University, Czech Republic, 7Faculty of Agriculture, University of South Bohemia in Ceské Budejovice, Czech Republic

Due to growing ecotourism and research purposes several groups of free-ranging great apes have been habituated to human presence. Humans can act as reservoir of pathogens for great apes and thus close contact with them can pose a risk to their health and vice versa. Two hundred and fifty chimpanzees, western and eastern gorillas in different level of habituation, 175 wild and domestic animals and 47 humans from several research sites across Africa were sampled for the occurrence of Cryptosporidium, Encephalitozoon and Enterocytozoon bieneusi. Sequence analyses determined 59 individuals positive for above mentioned infections. This is the first report of Cryptosporidium bovis, C. muris and C. meleagridis infection in great apes. The most prevalent microsporidium species was Encephalitozoon cuniculi (E. cuniculi genotype I: 20 cases, E. cuniculi genotype II: 4 cases). Out of 11 detected genotypes of Enterocytozoon bieneusi, eight novel genotypes were described. E. bieneusi genotypes D, C and EpbA previously recorded in human and domestic animals were the most prevalent. A comparison of the prevalence between unhabituated and habituated animals showed a higher prevalence of these parasites in habituated individuals, suggesting positive impact of human-ape contact on microsporidia and cryptosporidia infections.

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PB13. The variability of Cryptosporidium spp. infecting rodents of family Muridae in the Czech Republic and the Slovak Republic. VERONIKA RAŠKOVÁ1,2, MICHAL STANKO3, JAN VÁVRA4, BOHUMIL SAK2, DANA KVETONOVÁ2, MARTIN KVÁC1,2,*; 1Faculty of Agriculture, University of South Bohemia in Ceské Budejovice, Czech Republic, 2Institute of Parasitology, BC ASCR, v.v.i., Czech Republic, 3Institute of Zoology, SAS, Slovak Republic, 4Bishop gymnasium of J. N. Neumann, Ceské Budejovice, Czech Republic

A total of 364 fecal samples from rodents of family Muridae were collected from 2009 to 2012, namely Apodemus agrarius (104), A. flavicollis (114), A. sylvaticus (2), Clethrionomys glareolus (71), Microtus arvalis (2), M. subterraneus (2), Mus musculus (62) and Rattus norvegicus (7). DNA was extracted from all samples. Nested PCR was performed to amplify the partial sequence of SSU rRNA, GP60 and actin gene of Cryptosporidium. Total of 55 samples (15.1 %) were positive for Cryptosporidium spp. The sequence analyses of PCR-positive specimens identified Cryptosporidium muris (14), C. andersoni (1), C. parvum (2), C. tyzzeri (1), C. ubiquitum (12), C. canis (2), Cryptosporidium vole genotype (1), Cryptosporidium muscrat genotype II (2) and Cryptosporidium SW1 genotype (1). In addition we detected 13 novel Cryptosporidium genotypes (in 19 samples). We detected C. muris and C. ubiquitum in most rodent species. Rodent specific C. tyzzeri infection was detected in one sample from Mus musculus only. Environmental genotype Cryptosporidium SW1 was found in Microtus subterraneus, so it could be considered as natural host of this Cryptosporidium genotype. We detected 8 new genotypes in Apodemus spp. Out of them 3 genotypes were closely related to Cryptosporidium deer mouse genotype IV and 5 to C. canis. In C. glareolus we found 1 genotype closely related to Cryptosporidium marsupial genotype II, 1 to C. squirrel genotype and 2 to C. muscrat genotype I. No sample containing mixed infection of Cryptosporidium spp. has been detected.

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PB14. *Encephalitozoon* and *Enterocytozoon* (microsporidia) spores in stool from pigeons and exotic birds. MARIA ANETE LALLO¹,²*, PATRÍCIA CALÁBRIA², LILIANE MILANÉLO³; ¹Universidade Paulista (UNIP); ²Universidade Cruzeiro do Sul (Unicsul); ³Parque Ecológico do Tietê

Microsporidia are considered to be a cause of emerging and opportunistic infections in human beings, and the species that infect humans can also infect a wide range of animals, which raises concerns of zoonotic transmission. Disease associated with microsporidial parasites has been described in birds. *E. hellem* has been found most frequently in avian hosts and based on these reports it has commonly been assumed that there is a zoonotic threat. In this investigation, we performed a survey of microsporidia occurrence in fecal samples from 196 birds, including 106 free-ranging and 90 pigeons. The families of the free-ranging birds included Psittacidae, Emberizidae and Icteridae. Pigeons used for fecal sample collection were placed in individual cages after capture in order to obtain fecal samples. We found 48 (24.5%) positive results among 196 fecal samples analyzed by PCR-based analysis and a Gram-Chromotrope staining techniques, and the prevalence was higher in pigeons (31.1%) than the free-ranging birds (18.8%). Of 90 fecal samples of pigeons, 28 (31.1%) contained variable number of microsporidian spores, these 16 (17.8%) were positive for *E. hellem*, 7 (7.8%) for *E. bieneusi*, 3 (3.3%) for *E. intestinalis*, and 2 (2.2%) for *E. cuniculi* (Table 1). A total of 20 of 106 (18.8%) free-ranging birds had microsporidian spores, in which 16 (15.1%) tested positive for *E. hellem* and 4 (3.7%) animals were positive for *E. bieneusi* (Table 1). The most popular parrots (blue-fronted parrot) breeders were those who had higher prevalence among the infection (22.7%). Other species were also popular high occurrence rates, such as mealy parrot (20%), peach-fronted parakeet (20%), scaly-headed parrot (20%), budgerigars (20%), yellow-finch grassland (20%) and double-collared seedeater (20%). This study emphasizes the importance of exotic birds and pigeons as a potential source of microsporidia infection for humans living in large cities.
Free-living South American coatis (*Nasua nasua*) can be source of infection of *Encephalitozoon* and *Enterocytozoon* (microsporidia) spores for humans.

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Microsporidia are considered to be a cause of emerging and opportunistic infections in human beings, and the species that infect humans can also infect a wide range of animals, which raises concerns of zoonotic transmission. Epidemiology of human and animal microsporidiosis has been the focus of many studies as prevalence of the disease has increased greatly in recent years. Both infected people and animals are sources of infection and contaminate the environment by releasing spores in feces, urine and other secretions. In this investigation, we performed a survey of *Encephalitozoon* microsporidia occurrence in fecal and urine samples of 60 South American coatis (*Nasua nasua*). Fecal and urine specimens were examined using PCR-based analysis and a Gram-Chromotrope staining technique. Results showed that 24 (40%) coatis were positive for these parasites. We found 19 (31.7%) positive results among 60 fecal samples analyzed by both techniques, and of these positive samples, 7 (11.7%) were positive for *E. cuniculi*, 4 (6.7%) were positive for *E. intestinalis*, 4 (6.7%) were positive for *E. hellem*, and 4 (6.7%) coatis tested positive for *E. bieneusi*. Only 5 (8.4%) urine samples tested positive for *E. cuniculi* as assessed by the two techniques. No fecal or urine sample displayed more than one type of microsporidian species. We have demonstrated that free-living South American coatis can be a source of infection of the genus *Encephalitozoon* and *Enterocytozoon* affecting the human population in large cities as 40% of coatis investigated in this study tested positive for these parasites.
In Algeria, several studies have been led on Cryptosporidium in calves, but very few on lambs, in spite of the important livestock. For this, a study is undertaken, to research this protozoan in these animals, in the region of Algiers. During which 147 fecal samples were collected, stored at 4°, and then analyzed by using the enrichment Ritchie's simplified method, followed by the Ziehl-Neelsen modified at the laboratory of Parasitology-Mycology of ENSV of Algiers. Statistical calculations were made by the Chi-square test. The result shows that from the 147 samples analyzed, the parasite was isolated in 51 cases (34.69%). The study of the influence of some parameters demonstrated that the parasite is more found in diarrheal stools than in the stool without diarrhea (56.06%), (17.28%) respectively. Moreover, the age seems to play a role in the emergence of this infection. Indeed, the infection begins early; the protozoan was isolated at 02 days-old and is more frequently isolated in the age group between 1-7 days-old (50.94%). On the other hand, the study showed the influence of the type of farming, intensive farming is the most affected than the extensive one (55.93%) and (20.45%) respectively. After the found of this high frequency of Cryptosporidium, other studies by using molecular methods are necessary to identify the specie of Cryptosporidium in lambs because of the novel zoonotic species of Cryptosporidium identified from these animals which are strongly farmed in Algeria.
PB17. Characterization of multiple polymorphisms in Pneumocystis jirovecii by multiplex PCR/Single-Base Extension (MPCR/SBE) - Preliminary results

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Pneumocystis jirovecii (Pj) is known for causing specific infections in the respiratory tract of their hosts, mostly in immunocompromised patients, leading to a severe, and often fatal, pneumonia known as Pneumocystis pneumonia (PcP). The characterization of the genetic diversity of Pj has shown that some specific single-nucleotide polymorphisms (SNPs) have been recognized as the markers of choice to study the population genetics, geographical distribution, modes of transmission, drug susceptibility/resistance and virulence of specific genetic subtypes. The aim of the present work was to identify Pj SNPs and potential multilocus genotypes (MLG) associated with clinical data of PcP cases, using Multiplex PCR in association with Single-Base Extension (MPCR/SBE).

Forty one pulmonary specimens tested previously and found to be positive for Pj, collected between 2001 and 2012, from Portuguese HIV+ were randomly selected and included in this multilocus study. MPCR was used for simultaneous amplification of four genomic regions: dihydrofolate reductase (DHFR), mitochondrial large-subunit rRNA (mtLSU rRNA), superoxide dismutase (SOD) and dihydropteroate synthase (DHPS). Six SNPs correlated previously with parameters of disease were genotyped by SBE: mt85, SOD110, SOD215, DHFR312, DHPS165 and DHPS171.

Through the analysis of the results, 23 different Pj MLG were identified. Statistical correlations between mt85C, mt85T and SOD110T, and the clinical data were observed: mt85C was associated with low to moderate parasite load, while mt85T was associated with high parasite load; SOD110T was more frequent in cases of PcP with favorable follow-up. However, no statistically significant association between the SNPs in DHFR and DHPS genes and the clinical data was observed.

The present work confirmed the importance of some specific Pj SNPs and their potential association with clinical parameters of disease, suggesting that multilocus SNP/MLG characterization may be used to provide useful information in what concerns to genetic markers in Pj infection, enhancing the research of this important pathogen.

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