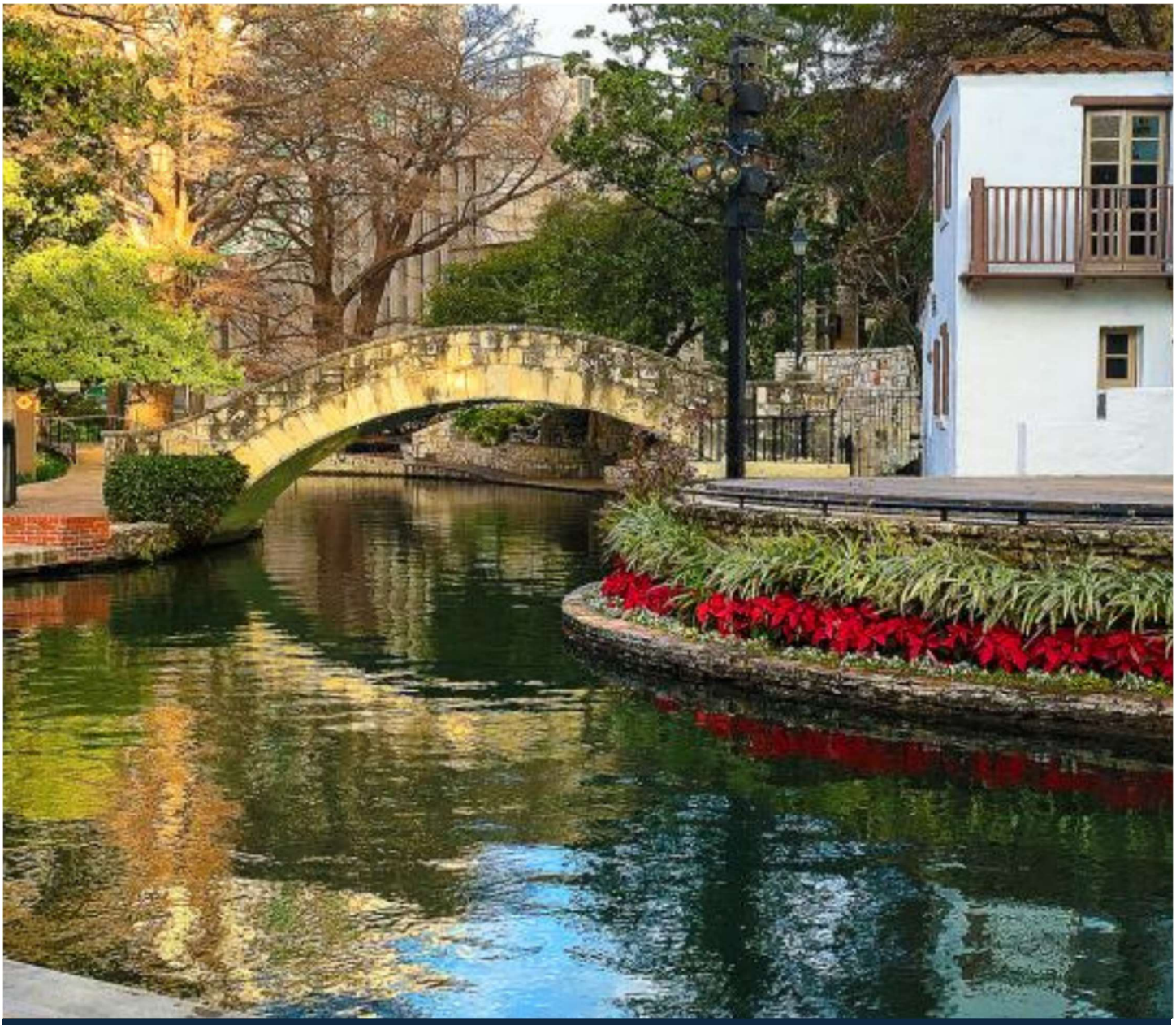

68th Annual Coccidioidomycosis Study Group Meeting Abstracts

April 4-5, 2024 | The Menger Hotel | San Antonio, TX

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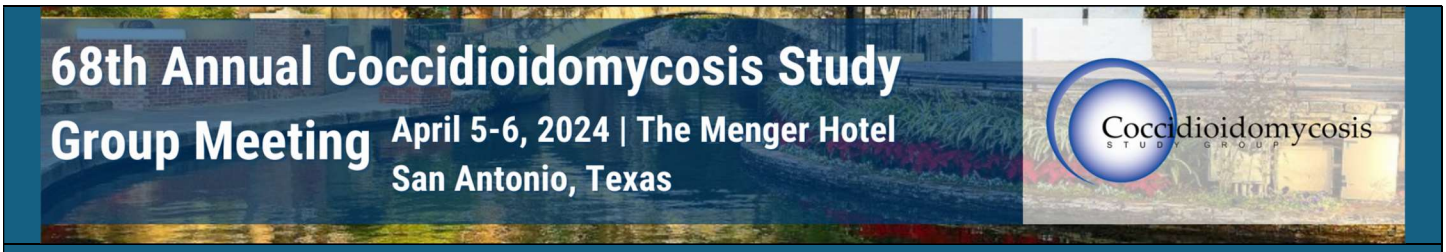
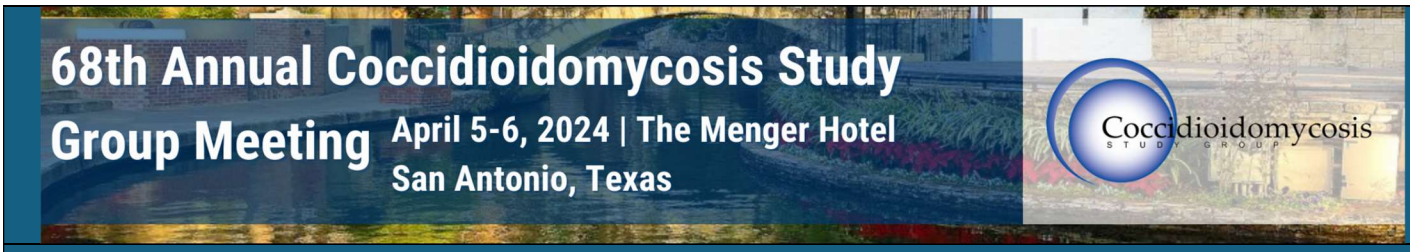
Coccidioidomycosis STUDY GROUP



Meeting Agenda
Day 1: Friday, April 5, 2024

7:00 am – 4:00 pm	Registration Open
7:00 am – 7:50 am	Continental Breakfast
8:00 am – 8:15 am	Opening Remarks: *President:* Neil Ampel, MD, *Local Host:* Chiung-Yu Hung, PhD, UTSA College of Sciences
8:15 am – 10:15 am	Scientific Section I | Epidemiology | *Moderator: Neil Ampel, MD*
8:15 am – 8:35 am	**Understanding Valley Fever: California's First Statewide Enhanced Surveillance for Coccidioidomycosis, June 2022–July 2023** Gail Sondermeyer Cooksey, Natalie Dassian, Shambhavi Mishra, Alyssa Nguyen, Seema Jain, Duc Vugia, Akiko Kimura California Department of Public Health, Richmond, CA, USA
8:35 am – 8:55 am	**Estimated Burden of Coccidioidomycosis in the United States—2019** Samantha Williams, Kaitlin Benedict, Malavika Rajeev, Tom Chiller, Brendan Jackson, Mitsuru Toda Centers for Disease Control and Prevention, Atlanta, USA
8:55 am – 9:15 am	**Coccidioidomycosis in New Mexico: Current Knowledge, Future Research Questions, and the Question Of What's in a Geopolitical Border** Morgan Gorris[1], Paris Salazar-Hamm[2], Kimberly Kaufeld[1], Sarah Shrum Davis[2], Donald Natvig[2] [1]Los Alamos National Laboratory, Los Alamos, USA. [2]University of New Mexico, Albuquerque, USA
9:15 am – 9:35 am	**Retrospective Observational Cohort Analysis of Disseminated Coccidioidomycosis Patients in a US Nationwide Claims Database: A Descriptive Analysis** Mark Bresnik[1], Fariba Donovan[2,3], Lia Pizzicato[4], Vamshi Ruthwik Anupindi[4], Anna Ratiu[4], Mitchell Dekoven[4], Belinda Lovelace[3] [1]F2G, Ltd., Manchester, United Kingdom. [2]The Valley Fever Center for Excellence, University of Arizona College of Medicine-Tucson, Tucson, Arizona, USA. [3]The Division of Infectious Diseases, Department of Medicine, University of Arizona College of Medicine-Tucson, Tucson, Arizona, USA. [4]IQVIA, Falls Church, Virginia, USA
9:35 am – 9:55 am	**Analysis of Coccidioidal Test Data from a Single Commercial Laboratory in Arizona, 2019–2022** Thomas Williamson, Irene Ruberto, Guillermo Adame Arizona Department of Health Services, Phoenix, USA
9:55 am – 10:15 am	**Epidemiology of Coccidioidomycosis in the Veterans Health Administration, 2013-2022** Cynthia Lucero-Obusan[1], Rishi Deka[1], Patricia Schirmer[1], Gina Oda[1], Mark Holodniy[1,2] [1]US Department of Veterans Affairs, Palo Alto, USA. [2]Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, USA [1]University of Texas at San Antonio, San Antonio, USA. [2]South Texas Center of Emerging Infectious Diseases, San Antonio, USA



Day 1: Friday, April 5, 2024

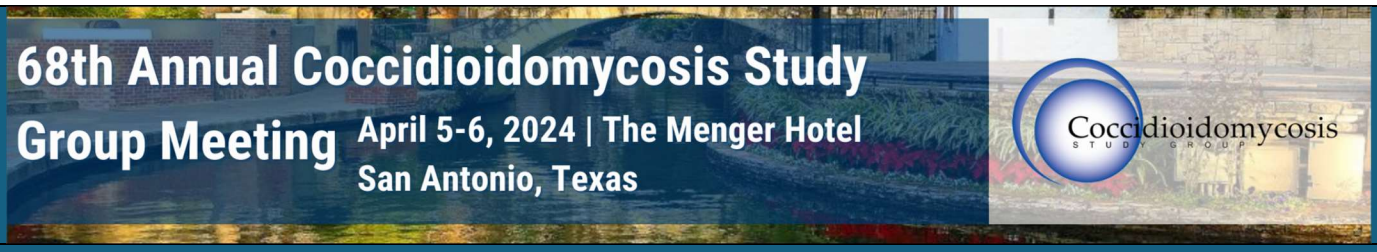
10:15 am – 10:45 am	**Refreshment Break/Visit Our Sponsors**
10:45 am – 12:25 pm	**Scientific Section II | Ecology | *Moderator: Chiung-Yu Hung, PhD***
10:45 am – 11:05 am	**Detection Of Airborne *Coccidioides* Spores Using Unmanned Aircraft Systems in the Carrizo Plain, California: A Pilot Study** Sarah Dobson[1], Amanda K. Weaver[2], Molly Radosevich[2], Phinehas Lampman[3], Tim Wallace[4], Lisa I Couper[2], John Taylor[2], Leda Kobziar[3], Justin Remais[2], James Markwiese[5], Jennifer Head[1] [1]University of Michigan, Ann Arbor, USA. [2]University of California at Berkeley, Berkeley, USA. [3]University of Idaho, Coeur d'Alene, USA. [4]Black Mountain UAS, LLC, Creston, USA. [5]U.S. Environmental Protection Agency, Corvallis, USA
11:05 am – 11:25 am	**Exploring the Link Between Local Construction and Coccidioides Aerosolization in the Phoenix Metropolitan Area** W. Tanner Porter[1], Allison Glazer[1], Jennifer Collins[2], Rebecca Sunenshine[2], Neil Ampel[3], David Engelthaler[1] [1]Translational Genomics Research Institute, Flagstaff, USA. [2]Maricopa County Department of Public Health, Phoenix, USA. [3]The University of Arizona, Tucson, USA
11:25 am – 11:45 am	**A Comparative Analysis of Soil And Air Surveillance of Coccidiodes Posadasii in Arizona** Marieke Ramsey[1], Savannah Marriot[1], Megan Ruby[1], Amelia Stout[2], Emily Luberto[1], Daniel Kollath[1], Matthew Fraser[2], Pierre Herckes[2], Bridget Barker[1] [1]Pathogen and Microbiome Institute at NAU, Flagstaff, USA. [2]Arizona State University, Tempe, USA
11:45 am – 12:05 pm	**Distribution of *Coccidioides Immitis* in Relation to Soil Microbial Community Composition in a Minimally Disturbed Native Grassland Plain in the Southern San Joaquin Valley of California** Molly Radosevich[1], Lisa Couper[1], Jennifer Head[2], Amanda Gomez-Weaver[1], Simon Camponuri[1], Grace Campbell[1], Liliam Montoya[1], John Taylor[1], Justin Remais[1] [1]UC Berkeley, Berkeley, USA. [2]University of Michigan, Ann Arbor, USA
12:05 am – 12:25 pm	**Culture-free Genomic Analysis of *Coccidioides Posadasii* DNA Present in Complex Environmental Samples** Jason Sahl[1], Nathan Stone[1], Marieke Ramsey[1], Daniel Kollath[1], Matthew Fraser[2], Amelia Stout[2], Pierre Herckes[2], Bridget Barker[1], Paul Keim[1], David Wagner[1] [1]Northern Arizona University, Flagstaff, USA. [2]Arizona State University, Tempe, USA
12:25 pm – 2:00 pm	**Lunch & Clinical Symposium Panel Discussion: Cavitary Coccidioidomycosis** **Moderators:** *Neil Ampel, MD, Fariba Donovan, MD, PhD* **Panelists:** *Janice Blair, MD, John Galgiani, MD, George Thompson III, MD, Royce Johnson, MD, Joshua Malo, MD, Stephanie Worrell, MD, FACS, Kayitha Yaddanapudi, MD*

68th Annual Coccidioidomycosis Study Group Meeting

April 5-6, 2024 | The Menger Hotel
San Antonio, Texas

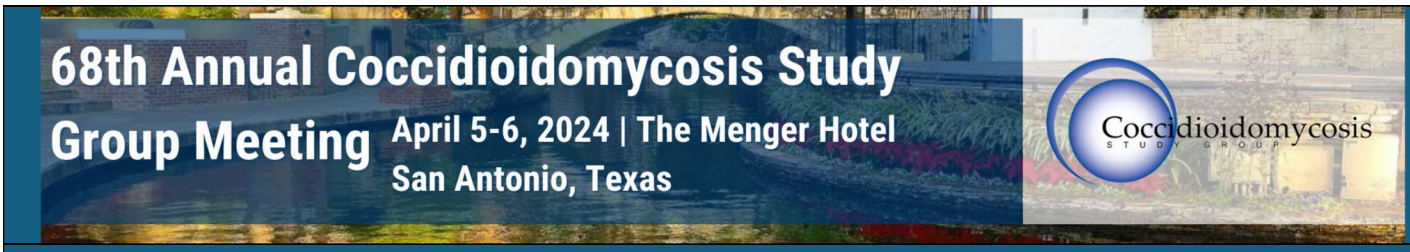
Day 1: Friday, April 5, 2024

2:00 pm – 3:40 pm	**Scientific Section III | Modelling | *Moderator: Fariba Donovan, MD, PhD***
2:00 pm - 2:20 pm	**Coccidioidomycosis Seasonality In California: Climate Determinants and Spatiotemporal Variability of Seasonal Dynamics, 2000-2021** Alexandra K. Heaney[1], Simon K. Camponuri[2], Jennifer R. Head[3], Phil Collender[2], Amanda Weaver[2], Gail Sondermeyer-Cooksey[4], Alexander Yu[4], Duc Vugia[4], Seema Jain[4], Abinash Bhattachan[5], John Taylor[2], Justin Remais[2] [1]University of California San Diego, San Diego, USA. [2]University of California Berkeley, Berkeley, USA. [3]University of Michigan, Ann Arbor, USA. [4]California Department of Public Health, Richmond, USA. [5]Texas Tech University, Lubbock, USA
2:20 pm – 2:40 pm	**Prolonged Dry Seasons Lengthen Coccidioidomycosis Transmission Seasons: Implications for a Changing California** Simon Camponuri[1], Jennifer Head[2], Phillip Collender[1], Amanda Weaver[1], Alexandra Heaney[3], Kate Colvin[1], Abinash Bhattachan[4], Gail Sondermeyer-Cooksey[5], Duc Vugia[5], Seema Jain[5], Justin Remais[1] [1]University of California, Berkeley, Berkeley, USA. [2]University of Michigan, Ann Arbor, USA. [3]University of California, San Diego, San Diego, USA. [4]Texas Tech University, Lubbock, USA. [5]California Department of Public Health, Richmond, USA
2:40 pm – 3:00 pm	**Projecting the Effects of Rainfall on Coccidioidomycosis Case Dynamics in the San Joaquin Valley of California in Response to Climate Change** Morgan E. Gorris[1], Grace Leito[2,3], Staci A. Hepler[4], David M. Kline[5], Andrew W. Bartlow[1], Kimberly A. Kaufeld[1] [1]Los Alamos National Laboratory, Los Alamos, USA. [2]Oak Ridge Institute for Science and Education, Los Alamos/Washington DC, USA. [3]University of Arizona, Tucson, USA. [4]Wake Forest University, Winston-Salem, USA. [5]Wake Forest University School of Medicine, Winston-Salem, USA
3:00 pm – 3:20 pm	**Optimizing a Bioinformatic Pipeline for Coccidioides Variant Identification and Species Assignment** Marco Marchetti, Katharine Walter University of Utah, Salt Lake City, USA
3:20 pm – 3:40 pm	**Genomics of Coccidiodes Posadasii in Arizona Derived from Patient Isolates Obtained from 2022-2023** Bridget Barker[1,2,3], Daniel Kollath[1], Marieke Ramsey[1], Dawn Birdsell[1], Ashley Itogawa[1], Amber Jones[1], Nicole Dulin[1], Tao Peng[2], Lourdes Lewis[2], Dave Wagner[1,3], Paul Keim[1,3], John Galgiani[2] [1]Northern Arizona University, Flagstaff, USA. [2]Valley Fever Center for Excellence, Tucson, USA. [3]Department of Biological Sciences, Flagstaff, USA
3:40 pm – 4:40 pm	**Keynote Address:** ***The Changing Coat of Cryptococcus and Implications for Vaccine Strategies*****, Jennifer Lodge, PhD,** Vice President for Research and Innovation, John Strohbehn University Distinguished Professor, Department of Molecular Genetics and Microbiology, Duke University
4:40 pm – 7:00 pm	**Poster Session**



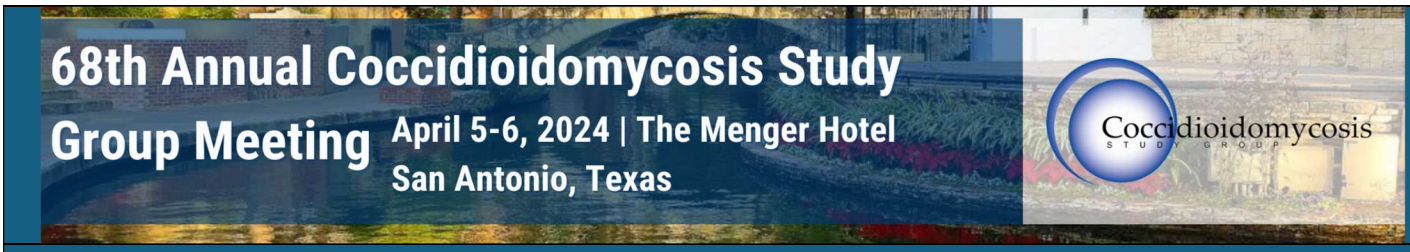
Day 2: Saturday, April 6, 2024

7:00 am – 5:00 pm	Registration Open
7:00 am – 8:00 am	Continental Breakfast
8:00 am – 10:00 am	Scientific Section IV | Basic Research | *Moderator: GR Thompson III, MD*
8:00 am – 8:20 am	**Assessing Myeloid-Derived Suppressor Cell (MDSC) Function in The Context of Coccidioidomycosis** Nawal Abdul-Baki, Austin Negron, Althea Campuzano, Matthew Mendoza Barker, Reimi Navarro, Kathryn West, Sarah Saeger, Chiung-Yu Hung South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, San Antonio, USA
8:20 am – 8:40 am	**Defining Roles of Eosinophils in Coccidioidomycosis** Althea Campuzano, Austin Negron, Nawal Abul-Baki, Chiung-Yu Hung UTSA, San Antonio, USA
8:40 am – 9:00 am	**Coccidioides Small RNA Atlas Reveals Stage-Specific Expression of Novel RNAs During Phase Transition** Jonathan Howard[1], Aidan Manning[1], Tahirah Williams[2], M. Lourdes Lewis[3], Clarissa Nobile[2], Lisa Shubitz[3], Sergei Kazakov[1], Sergio Barberan-Soler[1] [1]RealSeq Biosciences, Santa Cruz, USA. [2]University of California-Merced, Merced, USA. [3]University of Arizona, Tuscon, USA
9:00 am – 9:20 am	**Specialized Responses from Human Airway Epithelial Cells Shape Innate Immunity to *Coccidioides Posadasii*** Alfred Harding[1], Olivia Hepworth[2], Jennifer Reedy[2], Manalee Surve[2], Pritha Sen[3], Adam Haber[4], Rebecca Ward[2], Jayaraj Rajagopal[2], Bruce Klein[5], Jatin Vyas[2,6] [1]Massachusetts Institute of Technology, Cambridge, USA. [2]Massachusetts General Hospital, Boston, USA. [3]Brigham and Women's Hospital, Boston, USA. [4]Harvard School of Public Health, Boston, USA. [5]University of Wisconsin School of Medicine, Madison, USA. [6]Harvard Medical School, Boston, USA
9:20 am – 9:40 am	**Analysis Of CLEC7A Variation and Expression in Coccidioidomycosis Patients Reveals Mixed Association with Disease Severity** Samantha L. Niles-Jensen[1,2,3], Sarah J. Spendlove[4], Alexis V. Stephens[5], George R. Thompson[6,7,8], Royce H. Johnson[9,10,11], Arash Heidari[9,10,11], Rasha Kuran[9,10,11], Aaron Carlin[12], Harold Pimentel[1,3], Manish J. Butte[1,2,5], Valerie A. Arboleda[1,2,3] [1]Department of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA. [2]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA. [3]Department of Computational Medicine, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA. [4]Kaiser Permanente Molecular Genetic Pathology Laboratory, Los Angeles, CA, USA. [5]Division of Immunology, Allergy, and Rheumatology, Department of Pediatrics, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA. [6]UC Davis Center for Valley Fever, UC Davis, Davis, CA, USA. [7]Department of Medical Microbiology and Immunology, UC Davis, Davis, CA, USA.



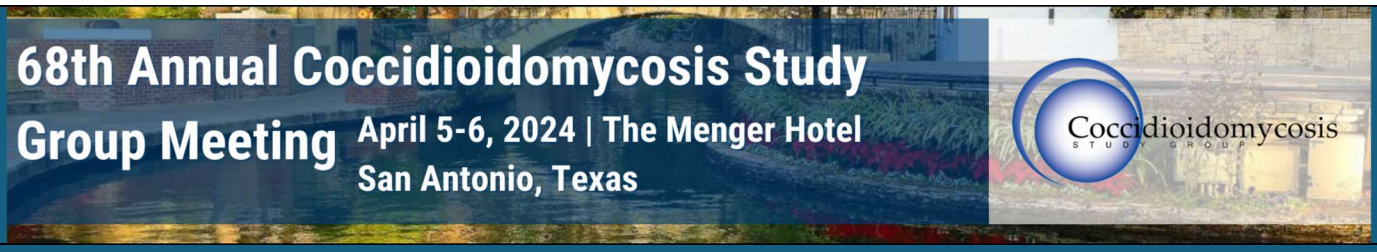
Day 2: Saturday, April 6, 2024

8:00 am – 10:00 am	**Scientific Section IV | Basic Research | *Moderator: GR Thompson III, MD (continued)***
9:20 am – 9:40 am	[8]Department of Medicine and Division of Infectious Diseases, UC Davis, Davis, CA, USA. [9]Department of Medicine, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA. [10]Valley Fever Institute, Kern Medical, Bakersfield, CA, USA. [11]Division of Infectious Diseases, Kern Medical, Bakersfield, CA, USA. [12]Department of Pathology, UCSD, San Diego, CA, USA
9:40 am – 10:00 am	**A Multi-Antigen Valley Fever DNA Vaccine Delivered by Gene Gun Induces Robust Antibody and Mucosal T Cell Responses And Protects Mice from High Dose Challenge with C. Posadasii** Deborah Fuller[1,2], James Fuller[1], Justin Ulrich-Lewis[1], Miles Corley[1], Daniel Kollath[3], Jesse Erasmus[4], Bridget Barker[3] [1]University of Washington, Seattle, USA. [2]Washington National Primate Research Center, Seattle, USA. [3]Northern Arizona University, Flagstaff, USA. [4]HDT Bio, Seattle, USA
10:00 am – 10:30 am	**Refreshment Break/Visit Our Sponsors**
10:30 am – 12:30 pm	**Scientific Section V | Clinical Research & One Health | *Moderator: John Galgiani, MD***
10:30 am – 10:50 am	**Spatiotemporal Analysis of Dog Serologic Data Unveils Critical New Perspectives on the Epidemiology of Coccidioidomycosis, an Emerging Fungal Disease** Jane Sykes[1], Amanda Weaver[2], Simon Camponuri[2], Kelly Crucillo[1], Helene Avocat[3], George Thompson[1], William Mills[4], Ellyn Mulcahy[3] [1]University of California-Davis, Davis, USA. [2]University of California-Berkeley, Berkeley, USA. [3]Kansas State University, Manhattan, USA. [4]United States Army Reserve, Fort Meade, USA
10:50 am – 11:10 am	**Evaluation of Macaque Serum using NAPPA to Screen Against the Coccidioides Expression Proteome** Megan Koehler[1], Lusheng Song[2], Francisca Grill[3], Bridget Barker[4,5], Erik Settles[4], Richard Grant[6], Deborah Fuller[6,7], Megan Fredericks[6,7], Lisa Shubitz[5], Daniel Powell[5,8], Marc Orbach[5,9], Edward Robb[10], John Galgiani[5,11], D. Mitchell Magee[2], Douglas Lake[1,3] [1]School of Life Sciences, Arizona State University, Tempe, USA. [2]Biodesign Institute, Arizona State University, Tempe, USA. [3]Cactus Bio, LLC, Phoenix, USA. [4]Pathogen and Microbiome Institute, Northern Arizona University,, Flagstaff, USA. [5]Valley Fever Center for Excellence, University of Arizona, Tucson, USA. [6]Washington National Primate Research Center, Seattle, USA. [7]Department of Microbiology, University of Washington, Seattle, USA. [8]Department of Immunobiology, The University of Arizona, Tucson, USA. [9]School of Plant Sciences, The University of Arizona, Tucson, USA. [10]Anivive Life Sciences, LLC, Long Beach, USA. [11]Department of Medicine, The University of Arizona, Tucson, USA
11:10 am – 11:30 am	**Identification of *Coccidioides* spp. Specific T Cell Clones and Antigens in Naturally Exposed Pig-Tailed Macaques (*Macaca Nemestrina*)** Allison Harmon[1], Paul Phillips[1], Megan Fredericks[2], Sandra Dross[2], Bridget Barker[1], Deborah Fuller[2], Paul Keim[1], Erik Settles[1] [1]Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, USA. [2]Washington National Primate Research Center, Seattle, USA



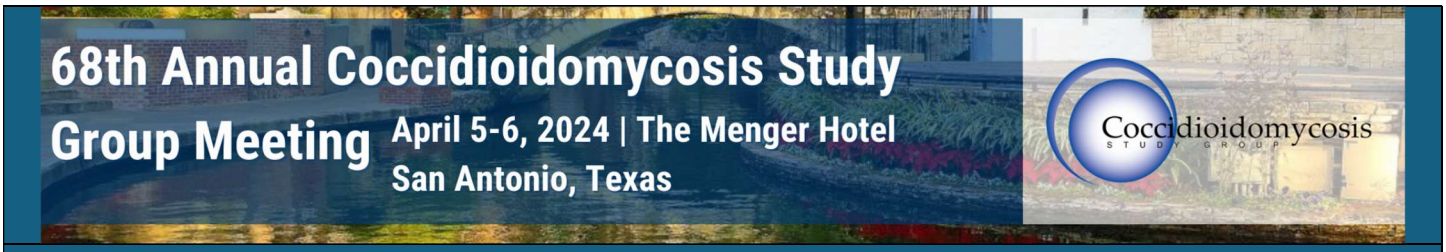
Day 2: Saturday, April 6, 2024

10:30 am – 12:30 pm	**Scientific Section V | Clinical Research & One Health | *Moderator: John Galgiani, MD (ctd)***
11:30 am – 11:50 am	**Characterization of Immune Responses in Pigtail Macaques Naturally Exposed to *Coccidioides* in Mesa, Arizona** Megan Fredericks[1,2], Sandra Dross[1,2], Oliver Mauer[1], Erik Settles[3], Richard Grant[2], Charlotte Hotchkiss[2], Deborah Fuller[1,2] [1]University of Washington, Department of Microbiology, Seattle, USA. [2]Washington National Primate Research Center, Seattle, USA. [3]Northern Arizona University, Pathogen and Microbiome Institute, Flagstaff, USA
11:50 am – 12:10 pm	**The Effect of DECTIN-1 Stalk Length in Coccidioides Mouse Infection** Daniel Powell[1,2], Lisa Shubitz[1], Christine Butkiewicz[1], Hien Trinh[1], Amy Hsu[3], Gary Ostroff[4], Jeffrey Frelinger[1], John Galgiani[1,2,5,6] [1]Valley Fever Center for Excellence, University of Arizona, Tucson, USA. [2]BIO5 Institute, University of Arizona, Tucson, USA. [3]NIAID, NIH, Bethesda, USA. [4]UMass Chan Medical School, Worcester, USA. [5]Department of Medicine, The University of Arizona College of Medicine-Tucson, Tucson, USA. [6]Department of Immunobiology, University of Arizona College of Medicine-Tucson, Tucson, USA
12:10 pm – 12:30 pm	**Suppressing Coccidioidomycosis in Naturally Infected Dogs by Frequent Dosing of Nikkomycin Z** David J Larwood[1,2], Lisa F. Shubitz[3] [1]Valley Fever Solutions, Tucson, USA. [2]UC San Francisco, San Francisco, USA. [3]Valley Fever Center for Excellence, University of Arizona, Tucson, USA
12:30 pm – 2:00 pm	**Business Meeting & Lunch**
2:00 pm – 4:00 pm	**Scientific Section VI | Clinical Research & Applied Science | *Moderator: Bridget Barker, PhD***
2:00 pm – 2:20 pm	**Addressing Diagnostic Inertia: A Quality Improvement Project to Increase Testing for Coccidioidomycosis in Community Acquired Pneumonia** Michael O'Shea, Ashlyn Brown, Amany Elshaer, Cody Cunningham, Jeremiah Bearss, Nneoma Alozie, Matt Biondi, Sandra Elmasry, Amogh Havanur, Avanika Mahajan, Juliana Savic, Bobak Seddighzadeh, Douglas Rappaport, Helene Labonte, Andrej Urumov, Janis Blair Mayo Clinic, Phoenix, USA
2:20 pm – 2:40 pm	**Olorofim for Treatment of Disseminated Coccidioidomycosis in Patients with Limited or No Therapeutic Options** Fariba M. Donovan[1,2,3], George R. Thompson III[4], Royce H. Johnson[5,6], Rasha A. Kuran[5,6], Thomas F. Patterson[7], Martin Hoenig[8], Joanna M. Schaenman[6], Shmuel Shoham[9], Steven M. Holland[10], Monica K. Sikka[11], Andrej Spec[12], Mark Bresnik Bresnik[13], John N. Galgiani[1,2,3,14], John H. Rex[13] [1]The Valley Fever Center for Excellence, University of Arizona College of Medicine-Tucson, Tucson, Arizona, USA. [2]The Division of Infectious Diseases, Department of Medicine, University of Arizona College of Medicine-Tucson, Tucson, Arizona, USA. [3]BIO5 Institute, University of Arizona, Tucson, Arizona, USA. [4]Division of Infectious Diseases, UC Davis School of Medicine, Sacramento, California,, USA. [5]Division of Infectious Disease, Valley Fever Institute,



Day 2: Saturday, April 6, 2024

2:00 pm – 4:00 pm	**Scientific Section VI | Clinical Research & Applied Science | *Moderator: Bridget Barker, PhD***
2:20 pm – 2:40 pm	Kern Medical,, Bakersfield, California, USA. [6]Division of Infectious Disease, Department of Medicine, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, USA. [7]University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA. [8]Division of Infectious Diseases, Medical University of Graz,, Graz, Austria. [9]Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. [10]Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, MD, USA. [11]Division of Infectious Diseases, Department of Medicine, Oregon Health & Science University, Portland, OR, USA. [12]Division of Infectious Disease, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA. [13]F2G, Ltd., Manchester, United Kingdom. [14]Department of Immunobiology, University of Arizona College of Medicine-Tucson, Tucson, Arizona, USA
2:40 pm – 3:00 pm	**Using a Dashboard to Improve Tracking of Coccidioidomycosis (CM) in Urgent Care Patients, Maricopa County Arizona.** John Galgiani[1,2], Anqi Lang[3], Jie Pu[3], Irene Ruberto[4], Jennifer Collins[5], Lia Koski[5], Thomas Williamson[4], Brandon Howard[5] [1]Valley Fever Center for Excellence, Department of Medicine, and Department of Immunobiology, College of Medicine-Tucson, University of Arizona, Tucson, USA. [2]BIO5 Institute, University of Arizona, Tucson, USA. [3]Data Analytics, Banner Health Systems, Phoenix, USA. [4]Arizona Department of Health Services, Phoenix, USA. [5]Maricopa County Department of Public Health, Phoenix, USA
3:00 pm – 3:20 pm	**Comparison of a Semi-Quantitative Anti-Coccidioidal Antibody Lateral Flow Assay to Immunodiffusion and Complement Fixation Antibody Titers** Francisca Grill[1,2], Erin Kaleta[2], Janis Blair[3], Richard Grant[4], Deborah Fuller[4,5], Megan Fredericks[4,5], Jared Jaffey[6], Lisa Shubitz[7], Kenta Reilly[2], Thomas Grys[2,1], Douglas Lake[8,1] [1]Cactus Bio, LLC, Phoenix, USA. [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Phoenix, USA. [3]Division of Infectious Diseases, Mayo Clinic, Phoenix, USA. [4]Washington National Primate Research Center, Seattle, USA. [5]Department of Microbiology, University of Washington, Seattle, USA. [6]Department of Specialty Medicine, College of Veterinary Medicine, Midwestern University, Glendale, USA. [7]University of Arizona, Tucson, USA. [8]School of Life Sciences, Arizona State University, Tempe, USA
3:20 pm – 3:40 pm	**Proteome-wide Discovery of Diagnostic Targets for Coccidioidomycosis** Evan Elko[1], Bridget Barker[1], Erik Settles[1], Jason Ladner[1], Kenneth Knox[2], John Altin[3], Heather Mead[3] [1]Northern Arizona University, Flagstaff, USA. [2]University of Arizona, Tucson, USA. [3]Translational Genomics Research Institute, Flagstaff, USA
3:40 pm – 4:00 pm	**Identification of Immune and Metabolic Biosignatures of Recovered or Disseminated Disease Spectrum of *Coccidioides* Infection and Associated Cellular Functional Pathways** Ramona Abbattista[1,2], Clarissa Santos Rocha[1], Sumathi Sankaran-Walters[1], Elise Buser[1], Kelly Crucillo[1], Marie Nearing[1], Ikaika Locque[1], George Thompson[1,3], Satya Dandekar[1] [1]Department of Medical Microbiology & Immunology, School of Medicine, University of California Davis, Davis, USA. [2]Department of Plant Sciences, University of California, Davis, Davis, USA. [3]Department of Internal Medicine: Infectious Diseases, University of California, Davis, Davis, USA



Day 2: Saturday, April 6, 2024

4:00 pm – 4:30 pm	Break
4:30 pm – 6:10 pm	Scientific Section VII | Case Reports | *Moderator: Janis Blair, MD*
4:30 pm – 4:50 pm	**Correlation of A1C levels with Outcomes in Patients With Diabetes Mellitus Who Contract Coccidioidomycosis** Rawan Elkurdi[1], Curtiss Cook[2], Janis Blair[1] [1]Division of Infectious Diseases, Mayo Clinic, Phoenix, USA. [2]Division of Endocrinology, Mayo Clinic, Phoenix, USA
4:50 pm – 5:10 pm	**Hypoxemic Respiratory Failure and Coccidioidomycosis-Associated Acute Respiratory Distress Syndrome** Arash Heidari[1], Simmer Kaur[1], Skyler Pearson[2], Augustine Munoz[1], Harleen Sandhu[3], Gursimran Mann[2], Michael Schivoe[2], Amir Zeki[2], Derek Bays[2], Machelle Wilson[2], Timothy Albertson[2], Royce Johnson[3], George Thompson[2] [1]Kern County, Bakersfield, USA. [2]UC-Davis, Sacramento, USA. [3]Kern Medical, Bakersfield, USA
5:10 pm – 5:30 pm	**Placental Abruption Caused by Reactivation of Coccidioidomycosis During Pregnancy** Katherine Arn[1], Adrienne Carey[1], Souha Haydoura[2], Allan Seibert[2], TW Jones[1] [1]University of Utah, Salt Lake City, USA. [2]Intermountain Health, Salt Lake City, USA
3:20 pm – 3:40 pm	**Immunomodulation in the Treatment of Disseminated Coccidioidomycosis** Alexis V Stephens[1], Timothy J Thauland[2], Rina Nagarajan[2], Maria I Garcia-Lloret[2], Manish J Butte[2 3] [1]Institute of Precision Health, University of California, Los Angeles, CA, USA. [2]Division of Immunology, Allergy, and Rheumatology, Department of Pediatrics, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA. [3]Department of Microbiology, Immunology, and Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, California, USA.
3:40 pm – 4:00 pm	**Role of Glycemic Control in Severity and Outcomes of Coccidioidomycosis in Patients with Diabetes Mellitus in Central California** Geetha Sivasubramanian[1], Yueqi Yan[2], Pavel Diaz[3], Seema Policepatil[4] [1]University of California, San Francisco, Fresno, USA. [2]University of California, Merced, Merced, USA. [3]University of California, Merced, USA. [4]Veteran's Affairs Medical Center, Fresno, USA
6:30 pm – 8:30 pm	Closing Banquet/Poster Awards/Travel Awards Recognition

Podium Presentations

Scientific Section I | Epidemiology

Understanding Valley Fever: California's First Statewide Enhanced Surveillance for Coccidioidomycosis, June 2022–July 2023

Gail Sondermeyer Cooksey, Natalie Dassian, Shambhavi Mishra, Alyssa Nguyen, Seema Jain, Duc Vugia, Akiko Kimura

California Department of Public Health, Richmond, CA, USA

Estimated Burden of Coccidioidomycosis in the United States—2019

Samantha Williams, Kaitlin Benedict, Malavika Rajeev, Tom Chiller, Brendan Jackson, Mitsuru Toda

Centers for Disease Control and Prevention, Atlanta, USA

Coccidioidomycosis in New Mexico: Current Knowledge, Future Research Questions, and the Question Of What's in a Geopolitical Border

Morgan Gorris[1], Paris Salazar-Hamm[2], Kimberly Kaufeld[1], Sarah Shrum Davis[2], Donald Natvig[2]

[1]Los Alamos National Laboratory, Los Alamos, USA. [2]University of New Mexico, Albuquerque, USA

Retrospective Observational Cohort Analysis of Disseminated Coccidioidomycosis Patients in a US Nationwide Claims Database: A Descriptive Analysis

Mark Bresnik[1], Fariba Donovan[2,3], Lia Pizzicato[4], Vamshi Ruthwik Anupindi[4], Anna Ratiu[4], Mitchell Dekoven[4], Belinda Lovelace[3]

[1]F2G, Ltd., Manchester, United Kingdom. [2]The Valley Fever Center for Excellence, University of Arizona College of Medicine-Tucson, Tucson, Arizona, USA. [3]The Division of Infectious Diseases, Department of Medicine, University of Arizona College of Medicine-Tucson, Tucson, Arizona, USA. [4]IQVIA, Falls Church, Virginia, USA

Analysis of Coccidioidal Test Data from a Single Commercial Laboratory in Arizona, 2019–2022.

Thomas Williamson, Irene Ruberto, Guillermo Adame

Arizona Department of Health Services, Phoenix, USA

Epidemiology of Coccidioidomycosis in the Veterans Health Administration, 2013-2022

Cynthia Lucero-Obusan[1], Rishi Deka[1], Patricia Schirmer[1], Gina Oda[1], Mark Holodniy[1,2]

[1]US Department of Veterans Affairs, Palo Alto, USA. [2]Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, USA
[1]University of Texas at San Antonio, San Antonio, USA. [2]South Texas Center of Emerging Infectious Diseases, San Antonio, USA

Understanding Valley Fever: California's First Statewide Enhanced Surveillance for Coccidioidomycosis, June 2022–July 2023

Gail Sondermeyer Cooksey, Natalie Dassian, Shambhavi Mishra, Alyssa Nguyen, Seema Jain, Duc Vugia, Akiko Kimura

California Department of Public Health, Richmond, CA, USA

Abstract

Introduction: Reported annual cases of coccidioidomycosis, commonly known as Valley fever (VF) have steadily increased in California, with 7000-9000 annual cases in recent years. However, reported CA VF epidemiologic data are limited to demographics and laboratory results. In June 2022, the CA Department of Public Health (CDPH) launched an enhanced surveillance pilot to interview a subset of reported VF patients to better describe CA VF epidemiology and target interventions.

Methods: CDPH conducted standardized phone interviews with non-incarcerated, adult residents of CA who had at least one incident *Coccidioides*-positive laboratory result reported to CDPH from June 1, 2022– July 19, 2023. Demographic, clinical, and possible exposure data were collected. Reported patients were randomly selected for interview and sampled to provide adequate representation of residents in three different regions of CA. Descriptive analyses were weighted by region of residence and age group to represent non-incarcerated, adult VF patients reported during the survey period.

Results: A total of 2721 (32.1%) of 8469 patients reported during the survey period were eligible for interview; 427 (16%) were interviewed. Majority of patients were <60 years of age (60%, median age 53 years), of Hispanic/Latino (39%) or White, Non-Hispanic (28%) race-ethnicity, with 42% reporting no major comorbidities. While approximately 86% reported symptoms consistent with VF, only 65% knew of their *Coccidioides*-positive laboratory result at time of interview. Median days from symptom onset to VF diagnostic testing was 28 days (interquartile range 12-172). Of 245 employed patients, 60% reported exposure to outdoor dust at work. About 15% of patients reported travel to or work in an area of higher VF incidence than their region of residence. More than 39% reported exposure to dust from nearby construction. About 51% of patients received antibiotics prior to VF testing; 53% reported taking antifungal medication following VF diagnosis. Over 33% of patients were hospitalized (median 6 days), and 73% missed work or school for a median of 13 days. Only 25% reported being fully recovered at the time of interview, which occurred a median of 36 days from illness onset.

Conclusion: In a population of primarily healthy adult CA residents, VF led to severe outcomes including hospitalization, missed work and school, and long symptom duration. Some patients were not aware of their positive VF results at time of interview, and there were delays in testing. These results highlight the need for increased public and provider awareness, improved diagnostics, and better prevention to reduce the burden of VF in CA.

Estimated Burden of Coccidioidomycosis in the United States—2019

Samantha Williams, Kaitlin Benedict, Malavika Rajeev, Tom Chiller, Brendan Jackson, Mitsuru Toda

Centers for Disease Control and Prevention, Atlanta, USA

Abstract

Introduction: Roughly 10,000–20,000 coccidioidomycosis cases are reported annually in the United States. The true disease burden is likely substantially higher than the reported total, since public health surveillance does not capture patients who do not seek medical care, get misdiagnosed by medical professionals, and are not reported to public health authorities. A more accurate estimate of coccidioidomycosis cases is essential for better clinician and patient awareness, and to inform public health and policy decision-making.

Methods: To estimate incident symptomatic coccidioidomycosis cases, hospitalizations, and deaths nationwide, we developed multi-stage multiplicative models using the Centers for Disease Control and Prevention's National Notifiable Diseases Surveillance System 2019 case reports and multipliers accounting for healthcare-seeking behavior, underdiagnosis, underreporting, and in-hospital mortality. Multipliers were obtained from literature review and expert opinion. Regional estimates were generated using endemicity levels categorized as high (Arizona and California), low (Nevada, New Mexico, Texas, Utah, Washington), or unknown endemicity (all other states and the District of Columbia). Model outputs are represented by mean point estimates with 95% credible intervals (CrI).

Results: We estimated approximately 299,000 (95% CrI: 225,000–394,000) incident symptomatic coccidioidomycosis cases in 2019. High-endemic states accounted for the highest burden (136,000; 95% CrI: 102,000–181,000), followed by states of unknown endemicity (109,000; 95% CrI: 70,000–165,000) and low-endemic states (53,000; 95% CrI: 36,000–76,000). Nationally, we estimated approximately 25,000 hospitalizations (95% CrI: 20,000–31,000) and 1,000 deaths (95% CrI: 700–1,300) associated with coccidioidomycosis.

Conclusion: The estimated national burden of symptomatic coccidioidomycosis in 2019 is 11–20 times higher than the number of cases reported through national surveillance. Improved awareness, diagnostic testing practices, and reporting are needed to enhance our understanding of coccidioidomycosis epidemiology and better patient outcomes.

Coccidioidomycosis in New Mexico: Current Knowledge, Future Research Questions, and the Question Of What's in a Geopolitical Border

Morgan Gorris[1], Paris Salazar-Hamm[2], Kimberly Kaufeld[1], Sarah Shrum Davis[2], Donald Natvig[2]

[1]Los Alamos National Laboratory, Los Alamos, USA. [2]University of New Mexico, Albuquerque, USA

Abstract

Introduction: From 2006 to 2019, the New Mexico Department of Health reported 705 cases of coccidioidomycosis, representing an average yearly rate of 2.5 cases per 100,000 population. Sequencing studies demonstrate that, as expected, most infections are caused by *Coccidioides posadasii*, although a few cases from northern New Mexico resulted from *C. immitis*. Except for the predominance of *C. posadasii*, there are striking and puzzling differences in the rates of coccidioidomycosis between New Mexico and its immediate neighbor to the west, Arizona. From 2006 to 2019, Arizona reported 116,568 cases, representing a yearly rate of >100 cases per 100,000 population, 40 times the incidence rate in New Mexico. The two states have substantial overlap in terms of geography and climate, characterized by arid lands to the south and mountainous, less arid regions to the north. Both receive rain in the winter months and monsoon rain in summer months. We are exploring the potential roles of efficiency of diagnosis, human population density, land use, and climate that may account for the dramatic difference in case numbers between the two states.

Methods: The results presented here are based on a combination of molecular surveys, New Mexico and Arizona case reports, published models regarding the roles of climate and environmental factors in shaping the distributions of *Coccidioides*, and new analyses of public health data available for human cases of coccidioidomycosis in the two states.

Results: Isolates of *Coccidioides* from New Mexico patients are underrepresented in genetic studies of the genus. We have now characterized three dozen New Mexico isolates, most of which are *C. posadasii*. Targeting gene sequences known to differ between the two species, we previously identified three *C. immitis* isolates from northwestern New Mexico among a total of 18 (Hamm et al. 2019, J. Fungi 5:74). We recently characterized an additional 17 isolates from New Mexico residents that were all *C. posadasii*. Reported human infections and a survey of *Coccidioides* in small mammals (Salazar-Hamm et al. 2022, Front. Fungal Biol. 3:996574) show skewing toward southern and northwestern New Mexico. Models that link the distribution of *Coccidioides* to rainfall, temperature and other environmental factors suggest substantial overlap between New Mexico and Arizona in the types of habitats predicted to be favorable for presence (Gorris et al. 2018, GeoHealth 2:6-24; Gorris et al. 2019, GeoHealth 3:308–327). We modeled the likelihood that a coccidioidomycosis case will be detected throughout the southwestern United States, which shows much lower values in New Mexico compared to Arizona (Hepler et al., Am. J. Epidemiology, in press), indicating there may be substantial under diagnosing. It is likely that other factors are also involved, for example differences in the scale and types of agriculture near metropolitan areas. We are attempting to identify additional factors that account for the differences in case counts between the two states.

Conclusion: The distribution of *Coccidioides* in New Mexico is becoming clearer, based on reports of human infections and targeted sampling, but there are still holes in our understanding. The possibility that cases are critically under reported in New Mexico is a viable and concerning hypothesis.

Retrospective Observational Cohort Analysis of Disseminated Coccidioidomycosis Patients in a US Nationwide Claims Database: A Descriptive Analysis

Fariba Donovan[1,2], Mark Bresnik[3], Lia Pizzicato[4], Vamshi Ruthwik Anupindi[4], Anna Ratiu[4], Mitchell Dekoven[4], Belinda Lovelace[3]

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Abstract

Introduction: Central nervous system (CNS) coccidioidomycosis (cocci) treatment is lifelong. Despite the availability of several antifungal agents, treatment for CNS cocci is challenging for experts, even in endemic regions. To date, few studies have examined real-world patient demographics and clinical characteristics in CNS cocci patients, and, to our knowledge, all have been conducted as single-center studies spanning decades of follow-up time with limited sample sizes. The objective of this study was to describe baseline demographic, clinical characteristics, and healthcare costs for patients with both CNS and non-CNS disseminated cocci (DISCO) in a large US nationwide claims database.

Methods: A retrospective, observational cohort analysis was conducted using a linked patient population from IQVIA's US hospital charge data master, professional fee claims, and prescription claims sources. The hospital database is used to identify hospitalizations and associated resource use from 450 hospitals, the professional fee claims database includes professional medical claims representing 70% of physician activity, and the prescription database contains information regarding dispensed prescriptions sourced from retail, mail, long-term and specialty pharmacies. Given the data is de-identified, IRB review was not required. Newly diagnosed patients with CNS or non-CNS DISCO between October 1, 2015 to November 30, 2022 were identified and placed into two cohorts. Patients ≥18 years of age with ≥1 medical claim with an International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM) diagnosis code were selected (CNS DISCO: B38.4; Non-CNS DISCO: B38.7, B38.3, or B38.81). The first such claim was defined as the index date (i.e., Time 0). Patients with evidence of multiple invasive fungal infections (other than cocci) on the index date, missing gender, or no days of post-index follow up were excluded. Patient demographics, clinical characteristics, and costs were reported during the baseline period, defined as 6-months prior to, not including the index date. Descriptive statistics including mean, SD, and median for continuous data and absolute/relative frequencies for categorical data were presented for all baseline demographic, clinical characteristics, and billable healthcare costs.

Results: In total, 2,218 patients were identified with DISCO, of which 626 were CNS and 1,592 were non-CNS DISCO patients. Baseline demographics in both cohorts were similar with mean age of ~55 years old, ~57% male, 83% in the Western US region and ~70% had commercial insurance. Eighty-two percent of CNS and 85% of non-CNS DISCO patients were identified with the first ICD-10-CM in the outpatient setting (see Table). Thirty-two percent of patients in both cohorts had a Charlson Comorbidity index of 3+, indicating moderate and severe baseline comorbid status. The most common comorbidities included diabetes and pneumonia. Common baseline utilization of medications included azoles and diabetes medications. Nineteen percent of CNS and 17% of non-CNS DISCO patients had a prior inpatient stay. Among those with an inpatient stay, more than 50% in both cohorts required an intensive care unit (ICU) admission.

Conclusion: Baseline data from this retrospective US claims database analysis suggest patients with CNS and non-CNS DISCO had notable baseline comorbidities and healthcare resource utilization prior to diagnosis. Future analyses will examine antifungal treatment patterns, healthcare resource utilization, all-cause mortality, and costs in the post-diagnostic period.

Table: Baseline (6-months prior to, not including the index date) Clinical Characteristics, Healthcare Resource Utilization, and Cost	**Cohorts**	
	CNS DISCO (n=626)	Non-CNS DISCO (n=1,592)
Diagnosis setting on the index date (%)		
Inpatient	18%	15%
Outpatient	82%	85%
Cocci characteristics (%)		
Pulmonary cocci	27%	31%
CCI Score Categories (%)		
0	38%	38%
1-2	30%	29%
3+	32%	32%
Comorbidities (%)		
Diabetes	28%	26%
Pneumonia	28%	23%
Obesity	16%	12%
Sepsis	13%	13%
COPD	11%	10%
Asthma	7%	8%
Autoimmune conditions*	6%	6%
Cancer	6%	6%
HIV	6%	6%
Immunodeficiency*	6%	5%
Medications (%)		
Azoles	46%	45%
Diabetes medications	24%	21%
Corticosteroids in 30 days prior to index date	13%	13%
Antineoplastics/chemotherapies	5%	5%
Immunosuppressants	4%	4%
Polyenes	1%	1%
Hospitalizations (%)		
Inpatient stay (%)	19%	17%
ICU stay (%) among hospitalized patients	55%	53%
Baseline Costs (mean, SD)		
Total Healthcare Costs	$50,456 ($133,278)	$43,281 ($105,976)

Analysis of Coccidioidal Test Data from a Single Commercial Laboratory in Arizona, 2019–2022

Thomas Williamson, Irene Ruberto, Guillermo Adame
Arizona Department of Health Services, Phoenix, USA

Abstract

Introduction: The Arizona Department of Health Services obtained coccidioidal test data, both positive and negative results, from a single commercial laboratory to evaluate trends in testing practices and compare them to recommendations set by the Infectious Disease Society of America (IDSA). The commercial laboratory reported 67.8% of all laboratory observations submitted to ADHS by Electronic Laboratory Report between 2019–2022.

Methods: Data was obtained for coccidioidal tests: enzyme immunoassay (EIA), immunodiffusion (IMDF), and complement fixation (CF), performed by a single commercial laboratory between 01/01/2019 and 12/31/2022. The commercial laboratory shared 967,641 test results, however, after exclusions, only 925,390 observations (95.6%) from 337,219 patients were evaluated. Entries were excluded if no laboratory results were reported or if the patient lived outside of Arizona as determined by zip code. Tests were classified as either 'Positive' or 'Negative,' with negative tests distinguished by titers of <1:2 if performed on serum and <1:1 for cerebrospinal fluid specimens. Results of 'Equivocal' or 'Indeterminant' were also considered negative. For the analysis, percent positivity was calculated by dividing the number of positive tests by the total number of tests performed and multiplying by 100.

Results: The number of annual tests performed increased from 211,935 in 2019 to its peak of 255,867 in 2021, but then declined to 242,292 in 2022. A similar trend was observed among the number of patients tested as rates increased from 83,618 in 2019 to its peak of 99,767 in 2021 (annual range: 83,618–99,767 patients). The mean number of annual tests performed per patient remained consistent at 2.5 to 2.6. Patients tested were predominantly female (annual range: 54.9–57.2%) and resided in Maricopa County (annual range: 70.9–71.4%), with a median testing age of 60 years.
From 2019 to 2022, the commercial laboratory performed 634,736 (68.6%) EIA, 176,998 (19.1%) IMDF, and 113,656 (12.3%) CF tests. Patient-level analysis showed that 39,183 (11.6%) reported at least one positive coccidioidal test, of which, 10,443 (26.7%) reported EIA IgM positive; this patient subset is missing or reporting a negative EIA IgG test alongside the positive EIA IgM. Specific to those patients reporting positive EIA IgM, 10,044 (96.2%) received subsequent IMDF or CF testing, with 3,626 (36.1%) reporting IMDF positive and 155 (1.5%) CF positive. Of those patients who did not receive or reported negative EIA testing, 5,830 (14.9%) tested positive by either IMDF or CF. Annual percent positivity increased from 9.4% in 2019 to its peak at 13.2% in 2020, however, it later fell to 8.2% in 2022.

Conclusion: Commercial testing data offers insight into clinician diagnostic practices. The data showed that a majority of patients (96.2%) received subsequent testing after a positive EIA IgM as recommended by 2016 IDSA guidelines. About half of these patients with positive EIA were confirmed by a positive IMDF, suggesting a high false-positivity rate, which is expected since most EIA tests have a low specificity (McHardy I. et al, 2023). Thereby highlighting the importance of confirmatory testing as recommended by the IDSA. The limited use and percent positivity of CF tests is not surprising, as this test is less sensitive than the others and recommended for disease staging and prognosis rather than diagnosis.

Epidemiology of Coccidioidomycosis in the Veterans Health Administration, 2013-2022

Cynthia Lucero-Obusan[1], Rishi Deka[1], Patricia Schirmer[1], Gina Oda[1], Mark Holodniy[1,2]

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Abstract

Introduction: The objective of this study was to characterize the epidemiology of laboratory-confirmed coccidioidomycosis infections, including risks for severe infection, hospitalization, and mortality, among a national cohort of US Veterans during 2013-2022. US Veterans represent a potentially high-risk population for severe coccidioidomycosis infections given most enrollees in the Veterans Health Administration (VHA) are males with higher rates of underlying comorbidities and a greater potential for environmental dust exposure during military service and desert training exercises. Prior studies among US Veterans during the 1950–1960s provide historical context for the natural history of coccidioidomycosis, including disseminated infections, but more recent patient data are lacking. Strengthening the evidence base for the association of coccidioidomycosis infections with severe morbidity outcomes will help guide prevention, testing, treatment, and control efforts, including potential future vaccines for coccidioidomycosis. Improving our understanding of coccidioidomycosis risk factors is important for targeted prevention strategies and to reduce delays in diagnosis and ineffective treatment.

Methods: Using electronic health record data from adults tested for coccidioidomycosis between January 1, 2013 and December 31, 2022, we analyzed differences in baseline demographics (age, sex, race/ethnicity, birth country, comorbidities, residence, and Charlson Comorbidity Index score) between 4,204 coccidioidomycosis-test-positive and 63,322 test-negative Veterans. The distributions of demographic characteristics as well as comorbidities at the time of coccidioidomycosis testing were compared between test-positive and test-negative patients using Pearson's chi-square test for categorical variables and the Mann–Whitney–Wilcoxon (MWW) test for continuous variables. Log-binomial regression models with adjusted risk ratios (aRRs) were used to evaluate risk factors associated with coccidioidomycosis including dissemination, hospitalization, and mortality. Annual state and county rates for coccidioidomycosis positivity were calculated per 100,000 Veterans in care, based on the VHA Support Service Center Capital Assets Unique Patients data cube by the estimated date of onset, utilizing the earliest positive coccidioidomycosis test result. Statistical analysis was performed using R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria). The data utilized in this study were obtained for the purpose of public health operations in the VHA and the study was deemed to meet the requirements of public health surveillance as defined in 45 CFR 46.102(l)(2). This project was approved by the Stanford University Institutional Review Board (Protocol ID 47191, "Public Health Surveillance in the Department of Veterans Affairs") and written informed consent was waived.

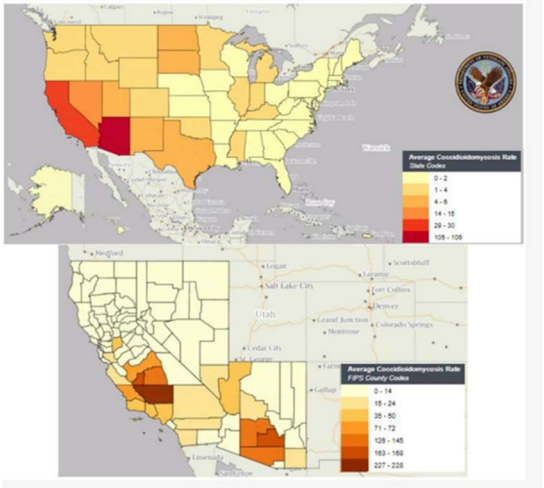
Results: The number of tests performed increased over time during the study period, from 17,061 in 2013 to 36,445 in 2022. Case counts and incidence rates were highest in select counties in Arizona and California where *Coccidioides* is endemic (Figure). Coccidioidomycosis-positive Veterans were younger, more likely to be male,

Epidemiology of Coccidioidomycosis in the Veterans Health Administration, 2013-2022 (continued)

and Philippine-born than those testing negative. The risk factors most highly associated with being coccidioidomycosis-positive included Native Hawaiian/Pacific Islander (aRR 1.068 [95%CI: 1.039–1.098]), Asian (aRR 1.060 [95%CI: 1.037–1.083]), Black (aRR 1.029 [95%CI: 1.022–1.036]), American Indian/Alaska Native (aRR 1.026 [95%CI: 1.004–1.048]) race, and Hispanic/Latino ethnicity (aRR 1.021 [95%CI: 1.013–1.028]). Black race (aRR: 1.058 [95%CI: 1.037–1.081]) and Hispanic/Latino ethnicity (aRR 1.018 [95%CI: 1.0003–1.036]) were also associated with disseminated coccidioidomycosis, strengthening the evidence for the association of coccidioidomycosis, including severe infections, with specific racial and ethnic groups. There were no statistically significant differences in hospitalization within 45 days of testing or 30-day all-cause mortality. The annual number of coccidioidomycosis-coded hospitalizations and outpatient visits among test-positive individuals increased over the study period, from 113 hospitalizations and 1,800 outpatient visits in 2013 to 196 hospitalizations and 4,137 outpatient visits in 2022.

Conclusion: During 2013–2022, testing, identification and healthcare utilization of US Veterans with coccidioidomycosis increased in VHA. Demographic features of patients testing positive for coccidiomycosis were consistent with previous reports, particularly among certain racial and ethnic groups. Rates of coccidioidomycosis for counties in Arizona and California followed similar trends compared with reported surveillance data over the period analyzed. As a national healthcare system, this analysis provides a supplemental source of data on coccidioidomycosis in jurisdictions where coccidioidomycosis is not reportable and surveillance data are lacking or inconsistent. Additional emphasis on targeted public health prevention strategies and education are needed to ensure at-risk patients are receiving timely testing, appropriate treatment, and follow-up.

Figure 1. Average coccidioidomycosis-positive rate per 100,000 Veterans in care, by state (upper panel) and select counties (lower panel), Veterans Health Administration, 2014–2022. Crude rates were calculated per 100,000 Veterans in care by state and county (based on Core Facility Uniques in VSSC Unique Patients Data Cube, Core Facility Uniques) by estimated date of onset using the earliest positive coccidioidomycosis test date. AZ, CA, and NV counties with at least 1 VHA coccidioidomycosis-positive case per calendar year or 4 or more positive cases in a single calendar year are displayed. All other counties are shown in the lightest color as rates are potentially unreliable due to low case counts. Veterans in care are tabulated by VHA fiscal year (1 October to 30 September each year).



Scientific Section II | Ecology

Detection Of Airborne *Coccidioides* Spores Using Unmanned Aircraft Systems in the Carrizo Plain, California: A Pilot Study

Sarah Dobson[1], Amanda K. Weaver[2], Molly Radosevich[2], Phinehas Lampman[3], Tim Wallace[4], Lisa I Couper[2], John Taylor[2], Leda Kobziar[3], Justin Remais[2], James Markwiese[5], Jennifer Head[1]

[1]University of Michigan, Ann Arbor, USA. [2]University of California at Berkeley, Berkeley, USA. [3]University of Idaho, Coeur d'Alene, USA. [4]Black Mountain UAS, LLC, Creston, USA. [5]U.S. Environmental Protection Agency, Corvallis, USA

Exploring the Link Between Local Construction and Coccidioides Aerosolization in the Phoenix Metropolitan Area

W. Tanner Porter[1], Allison Glazer[1], Jennifer Collins[2], Rebecca Sunenshine[2], Neil Ampel[3], David Engelthaler[1]

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A Comparative Analysis of Soil And Air Surveillance of Coccidiodes Posadasii in Arizona

Marieke Ramsey[1], Savannah Marriot[1], Megan Ruby[1], Amelia Stout[2], Emily Luberto[1], Daniel Kollath[1], Matthew Fraser[2], Pierre Herckes[2], Bridget Barker[1]

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Distribution of *Coccidioides Immitis* in Relation to Soil Microbial Community Composition in a Minimally Disturbed Native Grassland Plain in the Southern San Joaquin Valley of California

Molly Radosevich[1], Lisa Couper[1], Jennifer Head[2], Amanda Gomez-Weaver[1], Simon Camponuri[1], Grace Campbell[1], Liliam Montoya[1], John Taylor[1], Justin Remais[1]

[1]UC Berkeley, Berkeley, USA. [2]University of Michigan, Ann Arbor, USA

Culture-free Genomic Analysis of *Coccidioides Posadasii* DNA Present in Complex Environmental Samples

Jason Sahl[1], Nathan Stone[1], Marieke Ramsey[1], Daniel Kollath[1], Matthew Fraser[2], Amelia Stout[2], Pierre Herckes[2], Bridget Barker[1], Paul Keim[1], David Wagner[1]

[1]Northern Arizona University, Flagstaff, USA. [2]Arizona State University, Tempe, USA

Detection Of Airborne *Coccidioides* Spores Using Unmanned Aircraft Systems in the Carrizo Plain, California: A Pilot Study

Sarah Dobson[1], Amanda K. Weaver[2], Molly Radosevich[2], Phinehas Lampman[3], Tim Wallace[4], Lisa I Couper[2], John Taylor[2], Leda Kobziar[3], Justin Remais[2], James Markwiese[5], Jennifer Head[1]

[1]University of Michigan, Ann Arbor, USA. [2]University of California at Berkeley, Berkeley, USA. [3]University of Idaho, Coeur d'Alene, USA. [4]Black Mountain UAS, LLC, Creston, USA. [5]U.S. Environmental Protection Agency, Corvallis, USA

Abstract

Introduction: Coccidioidomycosis is an emerging fungal infection caused by inhalation of airborne spores of the *Coccidioides* genus. While *Coccidioides immitis* has been detected in soils across California, successful recovery of *C. immitis* from air samples has so far been limited in California, hindering understanding of the environmental factors that give rise to dispersal of arthroconidia. Here, we examine a novel sampling strategy – air filtration with unmanned aircraft systems (UAS) – as a means of detecting *C. immitis* in aerosolized soil dust.

Methods: We conducted a study in September 2023 at 14 locations across the Carrizo Plain National Monument, an area with confirmed *C. immitis* soil presence. We completed 41 20-minute flights using two UAS equipped with an 8 L/min aerosol sampler and Purple Air particulate matter (PM) Monitor. At each site, we sampled air under ambient conditions using a single flight at 10-20 m altitude and under a simulated high-wind event using paired flights at <5 m altitude (to mobilize dust) and 10-20 m altitude (to capture downwind spores that may be present in dust). We concurrently collected soils (n=168) using an interrupted radial transect design. We used qPCR to determine presence of *C. immitis* DNA on filters and soil samples. We used logistic regression models to identify associations between *C. immitis* detection and flight altitude, PM concentration, wind speed, temperature, and humidity.

Results: *Coccidioides* was detected in 32% of precinct soil samples and 21% of surface soil samples. We will present this evidence of *Coccidioides* detection on filters, including estimated associations between *Coccidioides* detection, wind, and PM.

Conclusion: Our findings will inform a larger-scale sampling campaign being conducted over the coming year, which is aimed at understanding the aerosolization of *C. immitis* and potential transport in dust-generating events such as high winds and wildfires. Our study will also influence efforts to control and prevent coccidioidomycosis in endemic regions.

Exploring the Link Between Local Construction and Coccidioides Aerosolization in the Phoenix Metropolitan Area

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Abstract

Introduction: *Coccidioides*, the causative agent of Valley fever, is an endemic fungal pathogen in arid regions of the western hemisphere. Although *Coccidioides* has been an established pathogen for more than 120 years, little is known about the drivers of *Coccidioides* arthroconidia aerosolization and how this impacts public health. Previously, we conducted air surveillance in the Phoenix, AZ metropolitan area to understand the distribution, and prevalence of aerosolized *Coccidioides* across an 18-month period. Airborne arthroconidia prevalence was highly variable across space and time, with no obvious spatial or temporal characteristics, suggesting that local events may drive the prevalence. Here, we explore the effect of hypothetical localized drivers of increased arthroconidia prevalence, including construction and development.

Methods: We have leveraged our previous surveillance campaign to understand if the prevalence of aerosolized *Coccidioides* relates to the amount of local construction and undeveloped land around filter locations. Construction sites and undeveloped land around filter locations were quantified using high-resolution satellite data from Google Earth. The relationship between air filter prevalence and construction and/or land development was quantified using statistical models.

Results: Overall, the relationship between prevalence and construction was limited across all sites; however, after removing a likely outlier site, a positive relationship was identified between construction, undeveloped lands, and *Coccidioides* filter prevalence, with Pearson's correlations between 0.51 and 0.73. The reasons for the discordance within the outlier site are still under investigation.

Conclusions: Air surveillance provides critical insights into *Coccidioides* aerosolization, with high variations in prevalence across space and time, and are not uniformly driven by geographic location. High-resolution site-level land use data such as local construction and area development may be able to begin to explain these variations in a highly metropolitan region. We plan to improve land use and case data resolution to further elucidate these linkages.

A Comparative Analysis of Soil And Air Surveillance of Coccidiodes Posadasii in Arizona

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Abstract

Introduction: The distribution and ecology of *Coccidioides* is not well understood, despite being an environmentally acquired pathogen with a high disease burden. To date, there are no large-scale temporal studies monitoring *Coccidioides posadasii* presence in the soil and air at one location simultaneously. In this study, we sampled soils from one location in the endemic region of Arizona monthly for one year to detect the presence of *C. posadasii* as well as sampling the air every 6 days for one year. Chemical analyses were conducted on air filters to uncover relationships between *C. posadasii* detection and PM10, organic carbon, key elements, and ions. We found both spatial and temporal variability in *Coccidioides* presence in the soil, a disconnect between *Coccidiodes* in air and soil, and relationships between air variables and *Coccidioides*.

Methods: Soil samples were collected monthly from September 2022 to September 2023 from fourteen burrow systems. Each burrow system ranged from one to four soil collections per month depending on the number of burrow entrances. In total, fifty samples were collected per month. Soils were collected using a garden trowel or kitchen spoon depending on soil depth and put directly into sterile 50-milliliter collection containers; trowels and spoons were sterilized with 10% bleach between soil samples. DNA extractions were completed on 250mg samples in duplicate using the QIAGEN DNeasy Power Soil Pro kit and then quantified in triplicate with presence/absence real-time qPCR using CocciDX assay (Bowers JR *et al.* 2019; Saubolle MA *et al.* 2018; Litvintseva AP *et al.* 2014).

Quartz air filters are collected every 6 days and analyzed for PM10, key ions, key elements, and organic carbon. Gravimetric mass determination was used to determine the total mass of PM10 in the air. Ion Chromatography was used to determine the mass of key ions in the atmosphere such as sulfate, nitrate, potassium, calcium, and ammonium. Carbon analysis was determined by thermal optical measurements to determine the organic carbon and the elemental carbon on the air filters. Key elemental detection was determined by HF digestion followed by ICPMS to get the total amounts of key elements. In addition, phenol/chloroform DNA extractions were conducted on quartz filters and quantified in triplicate with presence/absence real-time qPCR using CocciDX assay (Bowers JR *et al.* 2019; Saubolle MA *et al.* 2018; Litvintseva AP *et al.* 2014).

A Comparative Analysis of Soil And Air Surveillance of Coccidiodes Posadasii in Arizona *(continued)*

A multiscale hierarchical occupancy model was used to analyze the probability of *Coccidioides* occurrence at a location, the conditional probability of species occurrence in a sample of a location given that the species is present at that location, and the conditional probability of species detection in a subsample of a sample given that the species is present in the sample. Logistic regression models were used to compare monthly occurrences to positive air filters. All statistical analyses were conducted through RStudio.

Results: In the soil, there was lower detection of *C. posadasii* in October 2022 and March of 2023. Conversely, in air filters, high detection of *C. posadasii* was found in October 2022 and March 2023. Of the fourteen targeted burrow systems, systems 11,12, 13, and 14 showed nearly 100% positivity from each burrow entrance and experienced a decrease in presence of 40% in October 2022 and March 2023. This trend was also seen in the sites 1-10 during October 2022 and March 2023. Throughout the sampling year, PM10 remained relatively low indicating little dust pollution for the site location. However, months of increased recorded PM10 had higher detection of *C. posadasii* in the air filters. Higher detection of elemental carbon was found compared to anthropogenic carbon indicating that the particulate matter at this specific location is due to local soil dispersion and not anthropogenic pollution. There was no significant correlation between organic carbon, key elements, or key ions.

Conclusion: These findings provide a preliminary understanding of the temporal presence of *C. posadasii* at one site annually. The contrast between *Coccidioides* soil detection and presence in the air provides researchers fundamental understanding of concerning time points of the year when infection rate may increase due to high dispersed arthroconidia. Our study is a major step in estimating disease transmission of *Coccidioides* spp.

Distribution of *Coccidioides Immitis* in Relation to Soil Microbial Community Composition in a Minimally Disturbed Native Grassland Plain in the Southern San Joaquin Valley of California

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Abstract

Introduction: Understanding the distribution and spread of *Coccidioides* in the soil is important for minimizing the risk of human exposure but remains challenging due to the pathogen's highly variable presence across a given region. Its distribution is linked, in part, to climatic conditions, soil characteristics, and the presence of mammalian host reservoirs. The soil microbial community in endemic regions may also be an important driver, though this remains poorly understood. In this study, we explored the role of the soil microbial community on *Coccidioides* presence within the Carrizo Plain National Monument - a large, minimally disturbed grassland ecosystem near Taft, California, and the site of an ongoing, longitudinal study to examine the effects of rodents and their burrows on *Coccidioides* presence in soils.

Methods: Soil samples (n=318) were collected in April 2021 at the Carrizo Plain National Monument from 20 experimental plots (established in 2007) across two pastures with differential cattle grazing history. Soil was collected using a factorial design from rodent burrows and nearby topsoil in areas with and without an exclosure that blocked rodent occupancy of burrows for as many as 14 years. DNA was extracted from soil samples and analyzed via a qPCR assay for the presence of *Coccidioides*. Then, ITS2 (fungal) and 16S (bacterial) amplicon sequencing was conducted on the soil isolates using the Illumina platform, and sequences were processed and mapped to fungal and bacterial taxa. Associations between the presence of *Coccidioides* and microbial composition, structure, and diversity in soils were examined, as were relationships between the soil microbial community and pasture status, sample type (burrow or surface) and rodent exclosure status of sampled sites.

Results: For both fungi (species-level analysis) and bacteria (family-level analysis), pasture status (grazed or never grazed), sample type (burrow or surface), rodent exclosure status (exclosed or not exclosed), and *Coccidioides* status (present or not present) were all significantly associated with beta diversity ($p < 0.01$) based on PERMANOVA testing, though the variables explained a small proportion of the variance (R^2 = 3.0, 7.4, 3.0, and 1.2%, respectively, for fungi; 1.8, 8.8, 1.3 and 1.1%, respectively, for bacteria). *Coccidioides* was detected via qPCR but not represented among the ITS2 sequences, likely due to its low abundance relative to other fungi. However, the *Onygenaceae* family (within which *Coccidioides* resides) was represented in 23% (73/318) of all samples at a relative abundance of 0.1% or greater. Within this subset of samples that were positive for members of the *Onygenaceae* family, 27.4% (20/73) were ground surface samples while 72.6% (53/73) were collected from rodent burrows.

Conclusion: The sample type (rodent burrow or surface soil) was the most significant factor associated with bacterial and fungal community composition, suggesting that rodents and their burrows may create unique environmental niches for microbes due to temperature and humidity regulation and differential nutrient availability. Similarly to *Coccidioides*, other members of the fungal family *Onygenaceae* are observed at a greater frequency inside burrows than in surface soils, suggesting they may occupy similar environmental niches.

Culture-free Genomic Analysis of *Coccidioides Posadasii* DNA Present in Complex Environmental Samples

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Abstract

Introduction: *Coccidioides posadasii,* a fungal species primarily found in Arizona, causes Valley Fever following inhalation of blowing dust and soil carrying the pathogen. Culturing *C. posadasii* from the environment can be difficult to impossible, which limits our understanding of the risk posed to humans by environmental *C. posadasii* strains.

Methods: We developed a targeted DNA capture and enrichment system designed to selectively capture, amplify, sequence, and genotype *C. posadasii* DNA present in DNA extracts obtained from environmental samples. The system was initially designed to target all coding region sequences identified in Silveria, a laboratory strain with a completed genome. The candidate probes were then screened *in silico* against a diverse collection of *C. posadasii* genomes generated from clinical isolates, as well as genomes from non-target but closely related fungal species, to ensure specificity and sensitivity. A final set of 292,408 RNA probes was ordered from Agilent Technologies.

Results: We tested this *C. posadasii* DNA capture and enrichment system on a diverse set of DNA extracts obtained from complex samples, including air filters, soil, and infected mouse tissue. An optimized bioinformatics workflow was used to align enriched reads against Silveria, identify mutations (*e.g.*, SNPs), and use the resulting data to place enriched samples within a global phylogenetic framework of all publicly available *C. posadasii* genomes. Paired enrichments/isolates from soil from the same field site yielded highly similar genotypes, demonstrating the utility of the enrichment approach to provide high resolution genotyping information without culturing or whole genome sequencing. Soil, air filter, and mouse enrichments from a single Arizona field site over a similar time frame identified multiple circulating genotypes, suggesting that our understanding of local *C. posadasii* distribution in the environment is limited by current methodological approaches.

Conclusions: The *C. posadasii* DNA capture and enrichment system that we developed can be used to characterize the diversity of *C. posadasii* in complex samples using ~50% of the core genome of this species. Use of this system on additional environmental samples will provide researchers with a more comprehensive understanding of *C. posadasii* distribution and diversity in the environment and inform risk assessment activities that can improve targeted strategies to reduce human exposure.

Scientific Section III | Modelling

Coccidioidomycosis Seasonality In California: Climate Determinants and Spatiotemporal Variability of Seasonal Dynamics, 2000-2021

Alexandra K. Heaney[1], Simon K. Camponuri[2], Jennifer R. Head[3], Phil Collender[2], Amanda Weaver[2], Gail Sondermeyer-Cooksey[4], Alexander Yu[4], Duc Vugia[4], Seema Jain[4], Abinash Bhattachan[5], John Taylor[2], Justin Remais[2]

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Prolonged Dry Seasons Lengthen Coccidioidomycosis Transmission Seasons: Implications for a Changing California

Simon Camponuri[1], Jennifer Head[2], Phillip Collender[1], Amanda Weaver[1], Alexandra Heaney[3], Kate Colvin[1], Abinash Bhattachan[4], Gail Sondermeyer-Cooksey[5], Duc Vugia[5], Seema Jain[5], Justin Remais[1]

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Projecting the Effects of Rainfall on Coccidioidomycosis Case Dynamics in the San Joaquin Valley of California in Response to Climate Change

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Optimizing a Bioinformatic Pipeline for Coccidioides Variant Identification and Species Assignment

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Genomics of Coccidiodes Posadasii in Arizona Derived from Patient Isolates Obtained from 2022-2023

Bridget Barker[1,2,3], Daniel Kollath[1], Marieke Ramsey[1], Dawn Birdsell[1], Ashley Itogawa[1], Amber Jones[1], Nicole Dulin[1], Tao Peng[2], Lourdes Lewis[2], Dave Wagner[1,3], Paul Keim[1,3], John Galgiani[2]

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Coccidioidomycosis Seasonality In California: Climate Determinants and Spatiotemporal Variability of Seasonal Dynamics, 2000-2021

Alexandra K. Heaney[1], Simon K. Camponuri[2], Jennifer R. Head[3], Phil Collender[2], Amanda Weaver[2], Gail Sondermeyer-Cooksey[4], Alexander Yu[4], Duc Vugia[4], Seema Jain[4], Abinash Bhattachan[5], John Taylor[2], Justin Remais[2]

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Abstract

Introduction: Coccidioidomycosis, an emerging fungal disease in the western U.S., exhibits seasonal patterns that are poorly understood, including periods of strong cyclicity, aseasonal intervals, and variation in seasonal timing that have been minimally characterized, and unexplained as to their causal factors. Coccidioidomycosis incidence has increased markedly in recent years, and our limited understanding of intra- and inter-annual seasonality has hindered the identification of important drivers of disease transmission, including climate conditions.

Methods: We analyzed data on all reported incident cases of coccidioidomycosis in California from 2000-2021 to characterize seasonal patterns in incidence, and conducted wavelet analyses to assess the dominant periodicity, power, and timing of incidence for 17 counties with consistently high incidence rates. We assessed associations between seasonality parameters and measures of drought in California using a distributed lag nonlinear modeling framework.

Results: All counties exhibited annual cyclicity in incidence (i.e., a dominant wavelet periodicity of 12 months), but there was considerable heterogeneity in seasonal strength and timing across regions and years. On average, 12-month periodicity was most pronounced in the Southern San Joaquin Valley and Central Coast. Further, the annual seasonal cycles in the Southern San Joaquin Valley and the Southern Inland regions occurred earlier than those in coastal and northern counties, yet the timing of annual cycles became more aligned among counties by the end of the study period. Drought conditions were associated with a strong attenuation of the annual seasonal cycle, and seasonal peaks became more pronounced in the 1-2 years after a drought ends.

Conclusions: We conclude that drought conditions do not increase risk of coccidioidomycosis onset uniformly across the year, but instead promote increased risk concentrated within a specific calendar period (September to December). The findings have important implications for public health preparedness, and for how future shifts in seasonal climate patterns and extreme events may impact spatial and temporal coccidioidomycosis risk.

Disclaimer: The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of opinion of the California Department of Public Health or the California Health and Human Services Agency.

Prolonged Dry Seasons Lengthen Coccidioidomycosis Transmission Seasons: Implications for a Changing California

Simon Camponuri[1], Jennifer Head[2], Phillip Collender[1], Amanda Weaver[1], Alexandra Heaney[3], Kate Colvin[1], Abinash Bhattachan[4], Gail Sondermeyer-Cooksey[5], Duc Vugia[5], Seema Jain[5], Justin Remais[1]

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Abstract

Introduction: Seasonal transmission of infectious diseases is often influenced by climatic conditions. Coccidioidomycosis, a fungal disease caused by exposure to soil-borne *Coccidioides* spp., exhibits pronounced seasonal transmission, with incidence typically peaking in the fall months. Yet the role of climate in determining the timing and duration of transmission seasons remains poorly understood, limiting our ability to predict and prepare for seasonal changes in disease risk.

Methods: Using weekly data based on the estimated date of disease onset of all reported incident coccidioidomycosis cases in California census tracts from 2000-2020, we developed a distributed-lag Markov state-transition model to estimate the effects of temperature, precipitation, and soil moisture on the timing of transmission season onset and end, as determined by the breakpoint in the slope of the pre- and post-peak seasonal incidence time series.

Results: We found that transitions from cooler, wetter conditions to hotter, drier conditions accelerated season onsets. Dry conditions (i.e., 10^{th} percentile of precipitation) in the spring shifted season onset an average of 2.9 weeks (95% CI: 1.0-4.0 weeks) earlier compared to wet conditions (i.e., 90^{th} percentile of precipitation). Transitions from hotter, drier conditions to wetter, cooler conditions accelerated the end of the season, with dry conditions in the fall extending the transmission season by an average of 1.1 weeks (95% CI: 0-2.0 weeks) compared to wet conditions. Together, when dry conditions occurred in both the spring and fall, the transmission season was extended by an average of 4.0 weeks (95% CI: 2.0–5.0 weeks).

Conclusion: As California is expected to experience prolonged dry seasons with climate change, our findings suggest this shift may lengthen the time at which populations are at elevated infection risk.

Projecting the Effects of Rainfall on Coccidioidomycosis Case Dynamics in the San Joaquin Valley of California in Response to Climate Change

Morgan E. Gorris[1], Grace Leito[2,3], Staci A. Hepler[4], David M. Kline[5], Andrew W. Bartlow[1], Kimberly A. Kaufeld[1]
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Abstract

Introduction: Climate change is expected to shift the timing (i.e., seasonality) and amount of precipitation (here, rainfall) in California. Generally, it's expected that rainfall will become more infrequent, but with increased intensity. Because of these opposing signals, there is relatively large uncertainty across different climate models in how precipitation in this region will change. These changes, in turn, could shift the seasonality and amplitude of coccidioidomycosis cases, as it would affect the growth and dispersion of *Coccidioides*. Our goal was to create a predictive model of coccidioidomycosis incidence in the San Joaquin Valley of California, then use this model and projections of climate to assess how coccidioidomycosis case dynamics may change in response to varying future climate scenarios throughout the 21st century.

Methods: We used county-level, monthly case counts of coccidioidomycosis from the US Centers for Disease control and Prevention National Notifiable Diseases Surveillance System database through a data use agreement. We used 2000-2015 data to create our predictive model of case incidence and tested our model performance on years 2016-2019. We used lagged measures of average rainfall as the predictor of coccidioidomycosis incidence. For baseline climate conditions, we used precipitation from the PRISM dataset through Oregon State University. For climate projections, we analyzed 19 different climate models from the LOCA2 downscaled CMIP6 dataset. We analyzed three future climate change scenarios, ranging from moderate to large greenhouse gas emissions and resultant global warming. We converted monthly coccidioidomycosis cases to incidence, then detrended incidence to account for changes that are less likely related to climate and more likely related to disease awareness and reporting. We define the San Joaquin Valley of California as Fresno, Kern, Kings, San Luis Obispo, and Tulare Counties.

Results: Initial results show that by year 2080, a large greenhouse gas emissions scenario is likely to increase overall case burden in the San Joaquin Valley of California, result in more intense coccidioidomycosis case seasonality, and favor an earlier seasonal peak of cases. A moderate greenhouse gas emissions scenario may favor an even greater increase in case burden and seasonality, but the seasonal peak of cases may remain similar. There is a larger amount of precipitation variability and resultant uncertainty in the change of coccidioidomycosis dynamics for the large greenhouse gas emissions scenario than the moderate greenhouse gas emissions scenario.

Conclusion: The range of projections in rainfall for the San Joaquin Valley of California makes it challenging to say for certain how climate change may impact coccidioidomycosis case dynamics. The potential for case burden to increase, even in response to a moderate greenhouse gas emissions scenario, suggests the development and stockpiling of coccidioidomycosis therapeutics and a human vaccine may reduce the health and economic impacts of coccidioidomycosis in response to climate change.

Optimizing a Bioinformatic Pipeline for Coccidioides Variant Identification and Species Assignment

Marco Marchetti, Katharine Walter

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Abstract

Introduction: Genomic methods have been powerfully applied to characterize the evolutionary history of *Coccidioides* and distinguish between local and travel-associated infections. However, the information within *Coccidioides* genomic variation has not been fully leveraged to inform our understanding of Coccidioides transmission nor to inform clinical decision making. One limitation is that *Coccidioides* variant identification tools have not been standardized or benchmarked, and existing tools do not assign species from *Coccidioides* sequence data.

Methods: We developed and benchmarked a variant identification pipeline, *cocci-call,* to (a) assign *Coccidioides* species and (b) identify genome-wide variants. *cocci-call* is based on open-source bioinformatic tools, and importantly assigns *Coccidioides* species and includes a taxonomic filtering step, *Kraken,* to exclude contaminating reads, which distinguishes it from the generic fungal pathogen variant identification pipeline, *mycosnp*, developed by the Center for Disease Control & Prevention. To measure performance of *cocci-call,* we generated a genomic "truth" set of variants by aligning reference genomes with *nucmer*. We measured pipeline performance on 10 synthetic Illumina sequence data generated *in silico* from each species reference genome. We additionally measured the accuracy of *cocci-call* in assigning species for the synthetic sequence data and all previously published *Coccidioides* sequencing data.

Results: Before calibration, *cocci-call* identified SNP variants with 0.74 recall (sensitivity) and 0.33 precision for *C. immitis* and 0.82 recall and 0.30 precision for *C. posadasii*. *mycosnp* identified SNP variants with 0.49 recall and 0.91 precision for *C. immitis* and 0.67 recall and 0.90 precision for *C. posadasii*. The majority of false positive SNP variants identified by *cocci-call* fell within repetitive genomic regions. We are currently defining a genomic BED file of repetitive, error-prone regions to exclude from variant files, to improve precision of *cocci-call.* While *mycosnp* does not assign species, *cocci-call* assigns *Coccidioides* species in both *in silico* read sets and previously published isolates with 100% accuracy.

Conclusion: We developed and benchmarked an open-source tool to assign *Coccidioides* species and identify genome-wide variants. This tool could enable further studies of *Coccidioides*, such as genome-wide association studies to identify genomic determinants of antifungal resistance and other virulence phenotypes.

Genomics of Coccidiodes Posadasii in Arizona Derived from Patient Isolates Obtained from 2022-2023

Bridget Barker[1,2,3], Daniel Kollath[1], Marieke Ramsey[1], Dawn Birdsell[1], Ashley Itogawa[1], Amber Jones[1], Nicole Dulin[1], Tao Peng[2], Lourdes Lewis[2], Dave Wagner[1,3], Paul Keim[1,3], John Galgiani[2]

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Abstract

Introduction: The human fungal pathogen *Coccidioides posadasii* is comprised of 2 main populations: Arizona and Texas/Mexico/South America. Within the Arizona population, two main subpopulations have been suggested, representing isolates from patients living primarily in either Pima County or Maricopa County at the time of isolation. There was no clonal structure observed within the subpopulations, indicating that the isolates are recombining and reproducing sexually. However, most of these isolates did not have extensive metadata associated with them and were collected over a period of 30 years, which makes genome-wide association studies (GWAS) difficult. We proposed to collect isolates prospectively over a short period of time to determine if clusters of patient isolates are discovered, if any clonality could be observed, and complete GWAS with clinical phenotypes.

Methods: Coccidioides isolates were collected by Sonora Quest diagnostics during routine clinical microbiology diagnostics beginning in 2022. Isolated were grown in a BSL3 research laboratory at the University of Arizona to ensure purity and create glycerol stocks. Stocks were divided and half sent to Northern Arizona University for DNA extraction and preliminary phenotypic analysis (e.g. slow, normal, fast growth, pigmentation, aerial hyphae, etc.). DNA was extracted with organic acids or Promega Wizard kit. DNA was QC'd with Qubit (Invitrogen) and 0.7% gel electrophoresis. 1 ug of DNA of each isolate was submitted to the PMI sequencing core, sequencing library processed, and genomes sequenced with Illumina NextSeq instrument. Genomes were processed for single nucleotide polymorphisms (SNPs) and a phylogenetic tree was created from the SNP matrix.

Results: Preliminary analysis indicates that isolates from Arizona patients in our sample are primarily placed in the Maricopa clade of the Arizona subpopulation. No clustering or clonality has been observed, reflecting high diversity. Genomes are admixed and there is an even distribution of isolates having either a MAT1-1 or MAT1-2 idiomorph in the haploid genome. We anticipate approximately 800 isolates in total to be processed by July 2024 and GWAS to be completed in early 2025.

Conclusion: A clear understanding of the origin of infection for coccidioidomycosis and genomic landscape of the fungus is critical for determining localized outbreaks and potential for emerging pathogenic strains. The high diversity of the Arizona population of *C. posadasii* has made this difficult. Furthermore, detection of antifungal resistance, genetic basis of virulence, and other clinically relevant phenotypes can be determined using GWAS methods. To date this has not been completed for either species of *Coccidioides*, even though differences in clinical outcome and patterns of dissemination could be attributed to pathogen genotype.

Scientific Section IV | Basic Research

Assessing Myeloid-Derived Suppressor Cell (MDSC) Function in The Context of Coccidioidomycosis

Nawal Abdul-Baki, Austin Negron, Althea Campuzano, Matthew Mendoza Barker, Reimi Navarro, Kathryn West, Sarah Saeger, Chiung-Yu Hung

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Defining Roles of Eosinophils in Coccidioidomycosis

Althea Campuzano, Austin Negron, Nawal Abul-Baki, Chiung-Yu Hung

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Coccidioides Small RNA Atlas Reveals Stage-Specific Expression of Novel RNAs During Phase Transition

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Specialized Responses from Human Airway Epithelial Cells Shape Innate Immunity to *Coccidioides Posadasii*

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Analysis Of CLEC7A Variation and Expression in Coccidioidomycosis Patients Reveals Mixed Association with Disease Severity

Samantha L. Niles-Jensen[1,2,3], Sarah J. Spendlove[4], Alexis V. Stephens[5], George R. Thompson[6,7,8], Royce H. Johnson[9,10,11], Arash Heidari[9,10,11], Rasha Kuran[9,10,11], Aaron Carlin[12], Harold Pimentel[1,3], Manish J. Butte[1,2,5], Valerie A. Arboleda[1,2,3]

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Scientific Section IV | Basic Research *(continued)*

A Multi-Antigen Valley Fever DNA Vaccine Delivered by Gene Gun Induces Robust Antibody and Mucosal T Cell Responses And Protects Mice from High Dose Challenge with C. Posadasii

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Assessing Myeloid-Derived Suppressor Cell (MDSC) Function in The Context of Coccidioidomycosis

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Abstract

Introduction: Studies of failure of immune responses leading to disseminated coccidioidomycosis is of utmost importance for understanding pathogenesis of *Coccidioides* species. Both laboratory animal studies and clinical patients' data show that cases of disseminated coccidioidomycosis cause reduced Th1 and Th17 $CD4^+$ T cells, and increased infiltration of neutrophils in the lungs. It has been demonstrated that *Coccidioides* components such as spherule outer wall (SOW) fraction and associated lipids can reduce fungicidal activity of $Ly6G^+$ neutrophils in the lungs, while their roles in immunomodulation is still elusive. Moreover, it was shown that as spherules mature H_2O_2 produced by human neutrophils becomes less effective. We hypothesize that those recruited $Ly6G^+$ immune cells may be myeloid-derived suppressor cells (MDSCs), which can reduce T cell immunity. In this study, we apply immunophenotyping and immunological functional assays to characterize pulmonary $Ly6G^+$ myeloid cells. We further evaluate the immunosuppressive function of both *in vitro* bone marrow-derived MDSCs (BM-MDSCs) and the $Ly6G^+$ cells isolated from the lungs of *C. posadasii*-challenged mice upon exposure to arthroconidia and spherules.

Materials and Methods: We vaccinated C57BL/6 mice subcutaneously with a live-attenuated strain of *C. posadasii* (ΔT) twice at a 14-day interval. Four weeks following the final vaccination, mice (n=10 mice per group) were challenged intratracheally with 53 spores isolated from the C735 strain. On days 7 and 11 post challenge, single-cell suspensions from the lungs and spleen were prepared and flow cytometry analysis was performed (n=5 mice per group). MDSCs were enriched from the lungs or spleen of either vaccine-protected (n=2), non-protected (n=3), and naïve mice (n=3) and cultured *in vitro* with violet proliferation dye (VPD) and anti-CD3- and anti-CD28-coated beads to activate T cells for 72 hr before subjected to flow cytometry analysis. BM-MDSCs were treated with *Cp.* for 24 hours, and the expression of iNOS, Arginase-1, FasL, and PD-L1 was assessed by flow cytometry (n=3).

Results*:* Here we demonstrate that the percentage of $CD11b^+$ $Ly6G^+$ but not $CD11b^+$ $Ly6C^+$ cells were significantly increased in the lungs of unvaccinated *Coccidioides*-infected mice (average of 65.8%), compared with vaccine-protected mice (average of 8.2%) on day 11 post-challenge. MDSCs isolated from the lungs of non-protected mice had a 22-fold increase of $PD\text{-}L1^+$ cells as compared with vaccine-protected mice. Additionally, $Ly6G^+$ cells from the lungs or the spleen of non-protected mice suppressed the proliferation of $CD4^+$ T cells to a greater extent at 1:1, 1:2, 1:4, and 1:8, as compared to those isolated from vaccine-protected or non-challenged mice. BM-MDSCs treated with *Cp.* significantly increased the

Assessing Myeloid-Derived Suppressor Cell (MDSC) Function in The Context of Coccidioidomycosis *(continued)*

expression of PD-L1 (average of 72.3%) and iNOS (average of 18.8%) , as compared with media (average of 42.7%), (average of 0.2%) respectively.

Conclusions*:* Our findings suggest that the $CD11b^+$ $Ly6G^+$ cells function as MDSCs due to their suppressive function in *Cp.* infected mice. These data suggest that MDSCs play a potential role in pathogenesis of *Coccidioides* by inhibiting the proliferation and activation of $CD4^+$ T cells. Further research will focus on Coccidioides molecules that can induce development, differentiation and recruitment of MDSCs into the infected lungs. These data will uncover how fungal virulence regulates MDSC function, ultimately leading to detrimental immune responses.

Defining Roles of Eosinophils in Coccidioidomycosis

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Abstract

Introduction: Eosinophils, granulocytes with diverse immune functions, display elevated levels (>500 cells/ µL) in both pulmonary and disseminated Coccidioidomycosis (CM) meningitis. Although eosinophils are known for their involvement in parasitic infections and allergic reactions, their contribution to fungal clearance or disease exacerbation remains unclear. Previous studies evaluating vaccine correlates in a live-attenuated vaccine model (DT) noted that decreased fungal burden was associated with a moderate influx of various types of phagocytes, including a significant increase of eosinophils in the vaccinated mice compared to control mice at two weeks post-challenge. Therefore, we hypothesize that primed eosinophils from vaccinated mice aid in fungal clearance during CM via IL-5-mediated signaling. In contrast, unprimed eosinophils may contribute to immune dysregulation and exacerbate disease.

Materials and Methods: Flow cytometry analysis evaluated infiltrating phagocytes following a *Coccidioides* challenge in immunized and non-immunized mice. C57BL/6 mice were immunized and boosted twice at two-week intervals with either the recombinant subunit vaccine (GCP-rCpa1) or adjuvant alone (GCP-MSA). Challenged naïve mice serve as a control for primary infection. We depleted mice one day before challenge and then every other day using anti-IL-5 or IgG isotype control. Next, eosinophils were enriched by positive selection of $F4/80^+$ cells, and their phagocytic and anti-fungal capabilities were evaluated in the absence of IL-5 using an *ex-vivo* killing assay and phagocytosis assay by image flow cytometry. Furthermore, bone marrow-derived eosinophils (BMDEos) were used to evaluate their interaction with *Coccidioides in vitro*.

Results: Increased pulmonary eosinophil infiltration was noted from GCP-rCpa1 immunized mice compared to non-immunized cohorts. Next, we evaluated the uptake capabilities of pulmonary phagocytes exposed to Calcofluor white-stained *Coccidioides* using image flow cytometry. At 12 DPC, neutrophils made up the majority of infiltrating cells. Cell numbers of DCs, macrophages, and eosinophils were increased, making up over 30% of $CD45^+$ leukocytes in the immunized mice compared to GCP-MSA group (<12%), or unvaccinated control (<5%). Moreover, the recruited phagocytes in the vaccinated mice effectively engulfed arthroconidia. Anti-IL-5 depletions did not exacerbate disease progression in both immunized and control mice. However, anti-IL-5 depletion resulted in hindered anti-coccidioidal killing. We showed that BMDEos could detect arthroconidia and early endospores. Furthermore, BMDEos were capable of killing *Coccidioides* following incubation for 24 hours *in vitro*, likely by phagocytosis and other mechanisms under investigation.

Conclusions: Altogether, our data suggests diverse mechanisms of eosinophil recruitment to the lungs infected with *Coccidioides* among vaccinated and non-vaccinated mice. Eosinophils are capable of killing *Coccidioides* arthroconidia, and young spherules. Primed pulmonary eosinophils can take up and kill *Coccidioides*, while naïve eosinophils cannot. However, eosinophils are dispensable during a pulmonary *Coccidioides* infection, presumably due to immune cell function redundancy. Future studies will evaluate the genotypic and phenotypic differences between immunized and non-immunized eosinophils (Type 1 vs 2) and contribute to the knowledge gap in innate immune responses to *Coccidioides* infection.

Coccidioides Small RNA Atlas Reveals Stage-Specific Expression of Novel RNAs During Phase Transition

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Abstract

Introduction: Over the last quarter century, incidences of the mammalian fungal disease coccidioidomycosis (colloquially known as “Valley Fever” or “Cocci”) have been slowly on the rise. While the current impact on human health is proportionally minor to other pathogenic organisms, a brewing “fungal apocalypse”2 deems it necessary to begin designing and implementing non-culture-based diagnostics (e.g., biomarkers) for rapid detection of Valley Fever and other fungal infections. Small non-coding RNAs (sRNAs; e.g. miRNAs, snoRNAs, piRNAs, snRNAs, tRNAs, etc.) and RNA fragments derived from small and larger (e.g., mRNA, lncRNA, rRNA) RNAs have arisen as a promising subclass of biomarkers due to their abundance in human biofluids, (relative to cell-free DNA and larger RNAs), and their potential in providing broad, dynamic insights into pathological processes. Their use in the detection and monitoring of human disease are revolutionizing the world of clinical diagnostics, and fungal infections are no exception. However, many standard approaches to sRNA sequencing library preparation exclude incompatible fragmented RNA sequences, which are likely to hold significant biological and diagnostic power compared to their full-length, parental RNAs.

Methods: As a “proof of principle, RealSeq Biosciences has accurately identified both full-length and fragmented small RNAs (sRNAs) using RealSeq® -Biofluids (RealSeq® -BF) library preparation kit in conjunction with the RiboMarker® platform, establishing a “first of its kind” small RNA atlas from total RNA extracted from Coccidioides across mycelia, arthroconidia, and spherule morphologies. Additionally, small RNAs were profiled from mycelia- and arthroconidia-conditioned media to ascertain small RNAs that might be found in the extracellular space of these saprobic stages.

Results: We characterized the small RNA transcriptome of three Coccidioides life stages at unprecedented high resolution. Leveraging both the standard RealSeq-Biofluid and RiboMarker® methods, we reveal canonical small RNA transcripts as well as an expanded set of RNA fragments, respectively, facilitating the discovery of stage-specific expression and processing of RNAs, and informing on the biological roles these RNAs may play in saprobic and parasitic life cycles. A subset of these intracellular RNAs are also observed to exist in the extracellular space, either in cell-free and exosomal fractions of conditioned media, suggesting their possible association with extracellular vesicles.

Coccidioides Small RNA Atlas Reveals Stage-Specific Expression of Novel RNAs During Phase Transition *(continued)*

Furthermore, we exploited our data to better annotate the small RNA genes of Coccidioides, adding 100s of loci corresponding to known RNA classes as well as novel small RNA producing loci with yet-described functions. A comparison of our small RNA sequencing to previous data highlighting transcribed start regions (TSRs) of RNA polymerase II (Pol II) in Coccidioides revealed numerous overlaps in Pol II transcription and our described small RNA loci, further validating their expression.

When comparing the expression levels of a variety of known and novel small RNAs, we observe large transcriptional changes between saprobic (mycelia, arthroconidia) and parasitic stages (spherule), consistent with previous mRNA sequencing results across the same morphologies. Moreover, characterization of the small RNA transcriptome using the RiboMarker® platform revealed distinct profiles of RNA fragmentation, specifically in transfer RNAs. Additionally, these fragmentation profiles modulate between saprobic and parasitic stages, suggesting the importance of tRNA processing during the spherule stage compared to mycelia and arthroconidia, suggesting the dynamic regulation of downstream processes including protein translation and gene expression.

Conclusions: Our findings provide insights into the small RNA transcriptional programs underlying the phase transition of Coccidioides and highlight differential expression and RNA fragmentation being central to Valley Fever switch from saprobic to parasitic cycles. These findings and the data generated will provide a resource of putative RNA biomarkers for Coccidioidomycosis diagnostics and a stepping-stone to combat the increasing disease incidence and expanding geographic range of Valley Fever. On a broader scope, this study also provides a proof of principle for the utility of the RealSeq RiboMarker® platform, in combination with RealSeq-Biofluids library preparation kit, for detecting otherwise hidden RNAs and RNA fragments that may prove useful as diagnostic tools, and further our understanding of Coccidioides biology and Valley Fever dissemination.

Specialized Responses from Human Airway Epithelial Cells Shape Innate Immunity to *Coccidioides Posadasii*

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Abstract

Introduction: As the first point of contact and initial site of infection, the lung epithelium mounts early host defense against pathogens through PRR recognition, secreting antimicrobial proteins, and chemokines that recruit immune cells. Unfortunately, most prior work on the epithelial response to fungi has been limited by the use of monomorphic and oncogenic cell lines that lack the cellular diversity of airway epithelium. The use of cell lines precludes an assessment of cell type-specific antifungal mechanisms and an understanding of distinct epithelial responses to clinically relevant fungal respiratory pathogens. The human airway epithelium is composed of eight distinct cell subtypes. *We posit that distinct cell types in human airway epithelia elicit fungal pathogen-specific responses to coordinate protective immune responses.*

Methods: To circumvent limitations of immortalized cell lines, we used a novel method for extended culturing of human subject-derived pulmonary hAECs. These cells are isolated from the sputum, bronchoalveolar lavage (BAL), or surgical explants. Basal cells isolated from these samples are differentiated at an air-liquid interface (ALI) culture system into complete airway epithelium. These cells recapitulate the diversity of conducting airway epithelium with common cell types (ciliated cells, goblet cells, club cells, basal cells) and rare cell types (tuft cells, neuroendocrine cells, and ionocytes). We infected hAECs with *Coccidioides posadasii* (*Cp*) for 18h and performed scRNA-seq. We compared data of infected vs. uninfected hAECs with a yield of 15,000 cells suitable for sequencing.

Results: Interestingly, *Cp* induced the greatest amount of DEGs within secretory cells. Indeed, other cell types showed little DEGs. In sharp contrast, *Aspergillus fumigatus* (*Af*) triggered the greatest amount of DEGs in ciliated cells. These data indicate that specific cell sub-types are triggered differentially based on the specific fungi used as a stimulant. Having determined that secretory cells have the highest number of DEGs when infected by *Cp*, we next sought to understand whether these changes were specific to *Cp* (as compared to *Af*) or a general feature of infection by a pathogen. We next conducted further analysis of the scRNA-seq data generated to determine gene expression changes across all cell types. Across the four epithelial sub-types, we prioritized 41 genes differentially expressed in response to *Cp* as compared to *Af.* Within secretory cells, the largest cluster of DEGs was found in the cellular response to hypoxia and cytokine-mediated signaling. These pathways were not highly upregulated in other cell types or when infected by *Af.*

Conclusions: Together, these data pinpoint secretory cells as the major responders to *Cp* infection in human airway epithelium. These data support the hypothesis that recognition of Cp by human airway epithelial cells generates a specific signature that shapes the innate immune response.

Analysis Of CLEC7A Variation and Expression in Coccidioidomycosis Patients Reveals Mixed Association with Disease Severity

Samantha L. Niles-Jensen[1,2,3], Sarah J. Spendlove[4], Alexis V. Stephens[5], George R. Thompson[6,7,8], Royce H. Johnson[9,10,11], Arash Heidari[9,10,11], Rasha Kuran[9,10,11], Aaron Carlin[12], Harold Pimentel[1,3], Manish J. Butte[1,2,5], Valerie A. Arboleda[1,2,3]

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Abstract

Introduction: Coccidioidomycosis is a fungal infection with Coccidioides immitis or posadasii that usually has no symptoms or mild pulmonary symptoms. In a small subset of those infected, the disease spreads outside of the lungs, a potentially fatal form of the disease known as disseminated coccidioidomycosis (DCM). Previous research into the genetic causes of DCM identified membrane receptor protein Dectin1, encoded by the gene CLEC7A, as being necessary for fungal recognition. Mouse studies have established that a stalkless version of Clec7a (C57BL/6) or complete knockout of the gene (Dectin-1−/−) results in greater susceptibility to coccidioidomycosis infection. Previously identified putatively pathogenic variants in the gene include a stop gained mutation (rs16910526, Y238*) and missense variant (rs16910527, I223S) in the final exon. While some studies found homozygous and heterozygous carriers of rs16910526 were more susceptible to fungal infections, others found no association between genotype and susceptibility to disease.

Methods: To assess the association of CLEC7A variant genotypes with coccidioidomycosis symptoms, we carried out whole genome sequencing (WGS) and RNA-sequencing (RNAseq) for for 172 and 161 coccidioidomycosis patients, respectively. We combined the WGS from 167 samples that passed QC with whole exome sequencing (WES) from 468 coccidioidomycosis patients previously described in Hsu et al. 2022. All variants in CLEC7A were identified and annotated using Variant Effect Predictor (VEP). With the new WGS data as a validation cohort, we determined if patients with DCM had significantly higher rates of

Analysis Of CLEC7A Variation and Expression in Coccidioidomycosis Patients Reveals Mixed Association with Disease Severity *(continued)*

potentially pathogenic CLEC7A variants when compared to the gnomAD database (version 2.1.1) using a Fisher's Exact Test, as previously reported. However, membership in gnomAD doesn't guarantee that samples haven't had severe coccidioidomycosis or wouldn't develop it if exposed. For this reason, we also tested whether potentially pathogenic CLEC7A variants had significantly higher allele frequencies in DCM patients than in pulmonary-only patients (UVF and CPC). Since these patients have been exposed to Coccidioides but did not develop severe disease, variants that predispose carriers to more severe outcomes should be less common in this group. Finally, we assessed whether expression of CLEC7A and downstream cytokines was significantly altered in those with DCM or in carriers of potentially pathogenic variants.

Results: Some potentially pathogenic variants had significantly higher allele frequencies in the combined cohort than in gnomAD, including rs16910527, which is more common in individuals with African genetic ancestry. Variant rs16910526, which has a higher allele frequency in individuals with European ancestry, had a lower allele frequency in our sample than in gnomAD (OR=0.67, p=0.005) and did not have a significantly different allele frequency in DCM patients (N=182) than those with uncomplicated disease (N=359, p=0.08). However, both of the two homozygous carriers of the rs16910526 variant had severe disease (CPC or DCM). Allele frequency in severe disease patients (N=269) was double that of those with UVF (OR=2.07, p=0.007). In the transcriptomic data, CLEC7A was not significantly differentially expressed in DCM patients versus UVF, although the paralogous gene CLEC4D was upregulated (p=0.016).

Conclusions: Utilizing WGS and RNAseq from patients with coccidioidomycosis, we were able to replicate the association of some CLEC7A variants with dissemination risk and identify potential novel risk loci. We were unable to confirm that heterozygosity for rs16910526 is statistically associated with increased risk of DCM, but did observe a trend towards more severe disease in carriers.

A Multi-Antigen Valley Fever DNA Vaccine Delivered by Gene Gun Induces Robust Antibody and Mucosal T Cell Responses And Protects Mice from High Dose Challenge with C. Posadasii

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Abstract

Introduction: IFN-γ and Th17 T cell responses are associated with control of Coccidioides infections indicating an effective vaccine will likely need to induce these responses. In addition, localization of these responses to the lung mucosa may provide better protection at the site of exposure. Nucleic acid vaccines, including both DNA and RNA vaccines, induce robust CD4+ and CD8+ T cell responses including IFN-γ and Th-17 responses. We previously showed that gene gun delivery of DNA vaccines formulated with potent genetic adjuvants into the skin can substantially increase these responses in the spleen and lung mucosa. Here, we investigated the immunogenicity and protective efficacy of gene gun delivered adjuvanted DNA vaccines encoding known *Coccidioides* immunogens.

Methods: DNA vaccines expressing 3 known *Coccidioides* immunogens previously shown to afford some degree of protection when administered separately as recombinant protein vaccines were delivered by gene gun into the skin separately and in combination as a trivalent vaccine. Each DNA vaccine was delivered at 1mg doses directly into epidermal cells in the skin. To increase mucosal and Th1 T cell responses, the DNA vaccines were co-formulated with two plasmids expressing genetic adjuvants that were previously showed increase mucosal and Th1 T cell responses. Mice were primed and boosted 4 weeks apart. Three weeks after the boost, 5 mice per group were euthanized to analyze immune responses. Serum was analyzed for IgG antibodies against each antigen by ELISA. In addition, splenocytes and lung lymphocytes were stimulated with peptide pools representing each vaccine immunogen and then analyzed for IL-17 and IFN-γ T cell responses by a dual IFN-γ/IL-17 ELISPOT. Eight weeks following boost, another set of 15 mice per group were intranasally challenged with a high dose (500 conidia) of *C. Posadasii* Silveira. Five days post-challenge, a subset of 5 mice per group were sacrificed to measure fungal burden in the lung, spleen and brain and the remaining 10 mice per group were monitored for 21 days to analyze protection from disease. Mice reaching 15% weight loss were euthanized.

A Multi-Antigen Valley Fever DNA Vaccine Delivered by Gene Gun Induces Robust Antibody and Mucosal T Cell Responses And Protects Mice from High Dose Challenge with C. Posadasii *(continued)*

Results: The gene gun delivered adjuvanted DNA vaccines induced robust antibody responses and systemic and mucosal IFN-γ and Th17 responses in the spleen against each immunogen, and notably, Th-17 responses were higher in the lung than in the spleen. Following challenge, the trivalent DNA vaccines afforded 100% protection, from weight loss and mortality. One of the separate immunogens also afforded 90% protection when administered as a DNA vaccine but the other two separate DNA vaccines exhibited significant weight loss and mortality that was not significantly different from the unvaccinated controls. The DNA vaccine also afforded superior protection from lethality and weight loss when compared to a live attenuated (TKO) vaccine. Analysis of fungal burden showed the trivalent DNA vaccine reduced fungal burden by a substantial 2.5 logs, and CFU were undetectable in the spleen and brain only in this group, a result that suggests a synergistic effect in protection when all 3 immunogens are combined. A 2nd challenge study evaluating protective efficacy of the 3 immunogens as bivalent combinations showed that each combination afforded significant protection providing further evidence that all 3 immunogens likely contributed synergistically to the robust protective efficacy observed with the trivalent DNA vaccine.

Conclusion: These results show that a novel trivalent DNA vaccine delivered by gene gun induces robust antibody and mucosal and systemic IFN-γ and Th17 T cell responses and can afford complete protection from fungal dissemination and disease in mice. Our studies also provide strong evidence that all 3 immunogens contributed synergistically to protection when used in combination. Additional studies in progress aim to determine the precise immune mechanisms of protection by this vaccine.

Scientific Section V | Clinical Research & One Health

Spatiotemporal Analysis of Dog Serologic Data Unveils Critical New Perspectives on the Epidemiology of Coccidioidomycosis, an Emerging Fungal Disease

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Evaluation of Macaque Serum using NAPPA to Screen Against the Coccidioides Expression Proteome

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Identification of *Coccidioides* spp. Specific T Cell Clones and Antigens in Naturally Exposed Pig-Tailed Macaques (*Macaca Nemestrina*)

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Characterization of Immune Responses in Pigtail Macaques Naturally Exposed to *Coccidioides* in Mesa, Arizona

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Scientific Section V | Clinical Research & One Health *(continued)*

The Effect of DECTIN-1 Stalk Length in Coccidioides Mouse Infection

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Suppressing Coccidioidomycosis in Naturally Infected Dogs by Frequent Dosing of Nikkomycin Z

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Spatiotemporal Analysis of Dog Serologic Data Unveils Critical New Perspectives on the Epidemiology of Coccidioidomycosis, an Emerging Fungal Disease

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Abstract

Introduction: Coccidioidomycosis is an emerging infectious disease caused by *Coccidioides* spp., soil-borne fungi found in semi-arid regions of the southwestern U.S. as well as Central and South America. Coccidioidomycosis incidence in humans has increased dramatically in the southwestern U.S. over the past 20 years, yet limited disease surveillance and reporting has hindered our understanding of changes in distribution.

Methods: Here, we leverage ten years (2013 – 2022) of U.S.-wide serologic test results from domestic dogs, a species highly susceptible to coccidioidomycosis, to examine spatiotemporal trends and assess the utility of dogs as sentinels. Data on month, year, county, and state were gathered and mapped using GIS analysis. Monthly incidence/10,000 households was compared to human data for California and Arizona.

Results: Of 887,764 dogs, 37.3% were seropositive. The spatial distribution of cases progressively increased from 17743 cases in 3.3% of U.S. counties (2013) to 33503 cases in 13.4% of counties (2022). Expansion was particularly evident in Oregon, Utah, Colorado, New Mexico, Wyoming, Idaho, Montana, and Texas. Statewide dog and human incidence were significantly correlated in time in California (r = 0.58, 95% CI: 0.52–0.63) and Arizona (r = 0.61, 95% CI: 0.50–0.70). States with highest dog incidence were Arizona (87.1 infections/10,000 households), Nevada (0.78), California (0.67), New Mexico (0.53), Montana (0.35), Texas (0.34), Oregon (0.29), Colorado (0.26), Washington (0.20), Wyoming (0.19), and Idaho (0.17) (remaining states, 0.007-0.09).

Conclusion: This study identified potential novel endemic regions for coccidioidomycosis and should prompt investigation for unrecognized disease in these regions.

Evaluation of Macaque Serum using NAPPA to Screen Against the Coccidioides Expression Proteome

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Abstract

Introduction: Macaques are commonly used as models to evaluate vaccines and drugs prior to human clinical trials. The health of macaques in these colonies is important to ensure reliable research results. We analyzed serum antibodies from a breeding colony of pigtail macaques (PTM) in an endemic area for coccidioidomycosis who had a *Coccidioides* infection using a nucleic acid programmable protein array (NAPPA) to identify sero-reactive coccidioidal proteins.

Methods: NAPPA is a protein micro-array that allows for high-throughput serum screening against hundreds of proteins. DNA is printed onto a slide with micro-array followed by protein expression using an in-vitro transcription and translation (IVTT) mixture. Serum is then incubated on the slide with expressed proteins. Bound antibodies are detected with a fluorescent secondary anti-IgG. Sero-reactive proteins are scored using cutoff values and odds ratios.

Results: Serum from PTMs who were previously sero-positive for coccidioidomycosis by either Luminex or a rapid Ab test contained antibodies that bound to 47 coccidioidal proteins expressed on NAPPA–above a predetermined cutoff. In sero-positive specimens, the top 5 sero-reactive proteins were chitinase 1 (CTS1) (97%), endo-1,3-beta-glucanase (83%), peroxisomal matrix protein (64%), polyubiquitin (61%), and catalase (54%). Of the total 47 proteins, 9 proteins were also highly reactive in *Coccidioides* infected dogs. In the dogs, infection time course data was available, and we found 7 proteins that remained highly reactive throughout the time course, 3 of which included the top sero-reactive proteins in PTMs.

Conclusion: In addition to established sero-reactive antigens such as CTS1, highly reactive proteins identified by NAPPA screening may have the potential to be used for further analysis and monitoring of infection. Future infection studies will indicate if some of these proteins elicit antibodies earlier than CTS1, as demonstrated in dogs. The three proteins identified as highly reactive in dogs and PTM are especially interesting as cross-species markers of infection may lead to increased sensitivity and specificity in diagnostic monitoring assays with multiple antigens. To further validate the highly sero-reactive proteins seen in dogs and macaques, a human sera screening by NAPPA is underway.

Identification of *Coccidioides* spp. Specific T Cell Clones and Antigens in Naturally Exposed Pig-Tailed Macaques (*Macaca Nemestrina*)

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Abstract

Introduction: Coccidioidomycosis (Valley Fever (VF)) is frequently misdiagnosed and mistreated as bacterial or viral pneumonia due to similar clinical presentations. While infection in endemic areas is common, severe VF manifests in <5% of symptomatic cases and can be life-threatening. T cell responses play a vital role in vaccine-induced protection in mouse models and appear to be critical for resolution of infection in humans, so we set out to identify T cell clones and their associated antigens that are generated in pig-tailed macaques, a potential model for VF disease and vaccination. Our focus on the pig-tailed macaque (PTM) model is due to their established VF susceptibility and the availability of naturally infected colony animals. Thus, we set out to compare the T cell reactivity in PTM to VF patients.

Methods: Peripheral blood mononuclear cells (PBMCs) were collected from both humans and pig-tailed macaques. Macaque PBMCs were collected from two separate facilities, one of which contains naturally infected PTMs in an endemic area (Arizona), and a second facility in a non-endemic area with non-exposed PTMs (IACUC 4202-03, Washington National Primate Research Center). Additionally, human Valley Fever patient samples were collected from Arizona and Southern California (University of Arizona IRB#: STUDY00002062). The PBMCs were non-specifically expanded then stimulated with overlapping pools of *Coccidioides*-specific peptides following an antigen multiplexing scheme. The peptides were tiled and synthesized from a list of 27 *Coccidioides* antigens that have been identified to be upregulated early during infection in a mouse model. Cells were then sorted based on cell surface marker (CD3 and CD4), as well as a T cell activation marker (CD137). The activated T cells were sequenced, and the data were demultiplexed to associate T cell receptor (TCR) clonotypes with their stimulating *Coccidioides* antigens.

Results: A tiling scheme across the antigens was used for stimulating peptides since prediction of potential PTM T cell epitopes through MHC binding prediction is not possible. However, Human MHC binding prediction determined that many of the potential human epitopes were contained in the titling scheme. Using these VF specific peptides in antigen pools, we have identified 16 reactive antigens in our samples collected from PTM with a history or VF. Our results have confirmed reactivity of previously known *Coccidioides* antigens while also identifying 12 new antigens not previously associated with T cell immunogenicity. T cell responses were detected in primates that had an exposure history ranging from 130 to 3,111 days post symptom onset or VF antibody reactivity.

Conclusion: T cell reactive antigens will be prioritized for nucleic acid vaccinations. We are also expanding the study to evaluate whether these T cell antigens are immunogenic in human VF patients. This comparison will further support the PTM model for VF research. Finally, these antigens and their epitopes can be further investigated for potential diagnostic tools.

Characterization of Immune Responses in Pigtail Macaques Naturally Exposed to *Coccidioides* in Mesa, Arizona

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Abstract

Introduction: The development of a *Coccidioides* vaccine is imperative as the endemic regions of the pathogen spread due to climate change. Animal models of Valley Fever infection in humans are needed to gain a better understanding of the types of immune responses a vaccine will need to induce, and to evaluate candidate vaccines for immunogenicity and efficacy. Nonhuman primates are the closest model to humans in their anatomy, physiology, immune responses, and susceptibility to infections. Additionally, pigtail macaques (PTMs) bred at the Washington National Primate Research Center (WaNPRC) in Mesa, AZ are susceptible to natural exposure to *Coccidioides,* and their range of clinical manifestations closely align with human symptoms. Here, we characterized immune responses in PTMs naturally exposed to *Coccidioides*, with the goal of gaining a better understanding of the immune responses responding to the infection.

Methods: Forty-three PTMs (2.64-19.25 years, 3.66-18.29 kg) at the WaNPRC were sampled for blood and/or bronchoalveolar lavage (BAL). Animals had either a history testing positive for IgG against *Coccidiodes* F antigen (Prototek Reference Laboratory) without symptoms of VF infection (asymptomatic VF+), a history testing positive for IgG against VF and had VF symptoms (symptomatic VF+), or no exposure to *Coccidioides* (VF-). The frequencies of innate and adaptive immune cell subsets in whole blood and cells isolated from BAL were characterized by flow cytometry (Table 1). All samples were collected on a Symphony A3 (BD Biosciences) and analyzed using FlowJo™ 10.9.0.

Immunophenotyping Panel			
Antibody	**Fluorophore**	**Cell type marker**	**Cell activation marker**
CD3 (SP34-2)	BV650	x	
CD4 (OKT4)	BV605	x	
CD8 (RPA-T8)	BB700	x	
CD11b (ICRF44)	APC-Cy7	x	
CD11c (SHCL-3)	BV421	x	
CD14 (M5E2)	BV786	x	
CD16 (3G8)	Alexa700	x	
CD20 (2H7)	BV570	x	
CD45 (D058-1283)	PE-CF594	x	
CD66 (TET2)	FITC	x	
CD123 (7G3)	BUV661	x	
CD369 (15E2)	BUV737	x	
NKG2a (Z199)	PE-Cy7	x	
TCRγΔ (B1)	BUV395	x	
Live/Dead Stain (405nm)	-	x	
HLA-DR (L243)	BV711	x	x
PD-1 (EH12.2H7)	PE	x	x
CD69 (FN50)	BUV496		x
CD86 (IT2.2)	PE-Cy5		x

Table 1.

Characterization of Immune Responses in Pigtail Macaques Naturally Exposed to *Coccidioides* in Mesa, Arizona *(continued)*

Results: Symptomatic VF+ PTMs displayed lower levels plasmacytoid dendritic cells in the blood and BAL than asymptomatic VF+ PTMs, and asymptomatic VF+ PTMs displayed lower levels than VF- PTMs. In addition, in general, VF+ PTMs, both symptomatic and asymptomatic, exhibited lower frequencies of monocytes than VF- PTMs. VF- PTMs. In the BAL, symptomatic PTMs had higher frequencies of granulocytes but lower frequencies of plasmacytoid dendritic cells than asymptomatic VF+ and VF- PTMs. In general, VF+ animals, both asymptomatic and symptomatic, also had higher frequencies of both CD4+ and CD8+ T cells in the lung than VF- macaques. Notably, VF+ PTMs had higher levels of monocyte activation in the blood than VF- PTMs. Also, VF+ PTMs had higher levels of CD8+ T cell activation in the lung than VF- PTMs.

Conclusion: Our results show distinct differences in innate and adaptive immune cell subsets in PTMs naturally infected with *Coccidioides* and exhibiting symptoms of disease, when compared to VF+ PTMs that are asymptomatic or VF naive. Our results also show that symptomatic VF infection in PTMs is associated with T cell activation in the lung, a finding that is consistent with similar studies in humans. Limitations of this work include highly variable immune responses in VF+ animals that are likely due to variable sampling timepoints after the first positive test for VF. These findings support further study of naturally exposed PTMs as models for human infection and the development of an experimental infection PTM model to support preclinical evaluation of new vaccines and therapies for the prevention and treatment of Valley Fever.

The Effect of DECTIN-1 Stalk Length in Coccidioides Mouse Infection

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Abstract

Introduction: Previous work using Recombinant Inbred (RI) mice suggested a role for the length of the DECTIN-1 receptor in *Coccidioides* infection. Our interest in the role of DECTIN-1 was further strengthened by our recent evidence that human polymorphisms in the *Clec7A* gene and the associated signaling pathways were associated with more severe clinical coccidioidomycosis (CM). To critically test the role of DECTIN-1 stalk length on susceptibility to CM we generated an isogenic B6 mouse that expresses a long stalk variant of DECTIN-1.

Methods: The C57BL/6NJ-*Clec7a*$^{em1Pow/+}$ mouse was generated using CRISPR guide RNAs designed using the CRISPOR web tool. ssODNs with 30-70bp homology to sequences on each side of each gRNA-mediated double-stranded break were designed and ordered from IDT. Proper insertion was confirmed by sequence and surface expression of DECTIN-1 was confirmed by flow cytometry. Lung single cellular suspensions were made by digestion with collagenase and DNAse-I. Lung cell stimulations were performed using glucan-chitin particles (GCP) or live Δ*cps1*. Mouse infection studies were carried out using *Coccidioides* strain Cp1038 via intranasal inoculation. Organ burdens were determined by plating of serial dilutions of tissue homogenates on 2x GYE agar plates.

Results: Lung suspensions from B6 mice expressing a long stalk DECTIN-1 (B6-LSD) produced slightly more TNF-aafter stimulation with live Δ*cps1* compared to WT B6 mice. This increased production of TNF-a was even more pronounced when cells were stimulated with GCP. Given that the B6-LSD mice showed increased TNF-α production, we then challenged them with Cp1038 via intranasal inoculation. Cp1038 is lethal in WT B6 mice but develops a long-lasting controlled infection in B6xDBA2 F1 mice, who express both a short and long stalk DECTIN-1, similar to our B6-LSD mice. When challenged with Cp1038 B6-LSD mice survived for the entire length of the challenge, 104 days, with little outward signs of disease, whereas all B6 mice succumbed to infection by day 95. Organ burdens show that B6-LSD mice had lower levels of Cp1038 in the lung, spleen, liver, kidney and brain **(Figure 1)**.

Discussion: This work further highlights the role of DECTIN-1 stalk length in control of *Coccidioides* infection. While B6xDBA2 F1 mice are resistant to Cp1038, the heterogenous genetic background does not allow us to isolate specific genes which contribute to the control of Cp1038. By generating the B6-LSD mouse we are able to specifically test the hypothesis of long stalk DECTIN-1 contributing to control of *Coccidioides* infection. As cells from the B6-LSD mice control Cp1038 infection and respond to CPG better than their B6 litter mates it highlights the role of early immune events in the development of *Coccidioides* control. As DECTIN-1 is a receptor for b-glucan there is a possibility that the B6-LSD mouse could be more resistant to other fungal infections.

Suppressing Coccidioidomycosis in Naturally Infected Dogs by Frequent Dosing of Nikkomycin Z

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Abstract

Introduction: Coccidioidomycosis in dogs can be complicated with poor response to conventional drugs. In addition, intolerance of commercial drugs can preclude treatment. Nikkomycin Z (NikZ), a chitin synthase inhibitor, has previously shown efficacy and excellent tolerance in dogs with primary pulmonary coccidioidomycosis that were treated for 60 days. The short half-life of NikZ calls for highly divided dosing, shown to increase NikZ efficacy in mouse studies. Here, dosing was q. 4, 2 or 1 h during waking hours with bridge dosing at bedtime and optionally 2 am.

Methods: Three dogs were identified through compassionate use requests for administration of NikZ due to intolerance or failure to respond to oral azole antifungals. Histories, routine blood tests, coccidioidal serology, and imaging were reviewed prior to enrollment of dogs with probable or proven coccidioidomycosis. Dogs were treated solely with NikZ (excluding medications administered for unrelated health problems) at doses of 12 to 75 mg/kg for at least 60 days and up to 7 months.

Results: A 4 YO 32 kg SF Doberman pinscher with copper storage disease had pulmonary coccidioidomycosis and exhibited hepatotoxicity on fluconazole. On NikZ (31 mg/kg/day), liver enzymes stabilized, coughing nearly resolved, titer reduced from 1:8 to 1:2, and lung radiographs were near normal at the end of 17 weeks, and there was no recurrence in 6 months of follow up. A 6 YO 12 kg CM cavalier King Charles spaniel with pulmonary coccidioidomycosis had modest clinical and radiographic improvement after 4.5 months of treatment with fluconazole (12.5 mg/kg/day BID) but still had a severe cough. NikZ at 12.5 mg/kg per day given q 2 hrs gave noticeable improvement by day 4 and near normal lung radiographs at week 9. For a moderate, persistent cough the dose was doubled at week 10 to hourly dosing. At week 14 a CT scan and titer of 1:16 showed disease was still active. At week 18 the dose was doubled again to 50 mg/kg/day. The cough was finally negligible and therapy concluded at week 33 (7.5 months from start) with a titer of 1:4. The dog is without recurrence 24 months later. A 14 YO 4.6 kg SF shi tzu presented with recurrent CNS signs that were previously diagnosed as coccidioidomycosis by MRI, positive serology, and resolution with fluconazole therapy, which was subsequently lowered to 4 mg/kg SID because of severe hepatotoxicity. NikZ at 75 mg/kg/day for 30 days, then 50 mg/kg/day for 30 days gave 80-90% resolution of stumbling, ataxia, and mental depression, plus normalization of liver enzymes. After 4 additional months at the lower dose, dog was discontinued from all therapy. There was a very mild subjective worsening of stumbling, which became static without further treatment. The dog remained well with no CNS progression >1 year after stopping all treatment. Titer remained 1:4 throughout.

Conclusion: NikZ oral therapy given at 2-4 h intervals during waking hours resulted in improvement or resolution of clinical disease in three dogs with persistent natural infection and inadequate response or intolerance to azole therapy. Two dogs with liver issues contraindicating fluconazole experienced no liver toxicity. One dog with known CNS disease that recurred remains in remission without antifungal medication >1 year following 6 months of NikZ therapy. Two dogs with pulmonary coccidioidomycosis had resolution following 2[DL5] and 7 months of therapy. The results suggest a long-planned slow-release formulation will be efficacious, and convenient with QD dosing.

Scientific Section VI | Clinical Research & Applied Science

Addressing Diagnostic Inertia: A Quality Improvement Project to Increase Testing for Coccidioidomycosis in Community Acquired Pneumonia

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Olorofim for Treatment of Disseminated Coccidioidomycosis in Patients with Limited or No Therapeutic Options

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Using a Dashboard to Improve Tracking of Coccidioidomycosis (CM) in Urgent Care Patients, Maricopa County Arizona.

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Scientific Section VI | Clinical Research & Applied Science (continued)

Comparison of a Semi-Quantitative Anti-Coccidioidal Antibody Lateral Flow Assay to Immunodiffusion and Complement Fixation Antibody Titers

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Proteome-wide Discovery of Diagnostic Targets for Coccidioidomycosis

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Identification of Immune and Metabolic Biosignatures of Recovered or Disseminated Disease Spectrum of *Coccidioides* Infection and Associated Cellular Functional Pathways

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Addressing Diagnostic Inertia: A Quality Improvement Project to Increase Testing for Coccidioidomycosis in Community Acquired Pneumonia

Michael O'Shea, Ashlyn Brown, Amany Elshaer, Cody Cunningham, Jeremiah Bearss, Nneoma Alozie, Matt Biondi, Sandra Elmasry, Amogh Havanur, Avanika Mahajan, Juliana Savic, Bobak Seddighzadeh, Douglas Rappaport, Helene Labonte, Andrej Urumov, Janis Blair

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Abstract

Introduction: Coccidioidomycosis (CM) is common in Arizona, with an incidence of between 101-261 cases per 100,000 person years[1]. CM accounts for approximately 15-29% of cases of Community Acquired Pneumonia (CAP) in the state [2-4]. Despite high prevalence, CM testing is ordered in 6-15% of CAP in endemic areas, resulting in delayed diagnoses, use of antibiotics and increased emergency department usage[2, 5, 6]. Provider knowledge is a presumed barrier to CM testing[7].

Methods: We designed a mixed methods multi-stage quality improvement project in a single adult tertiary referral health system in the Phoenix Metropolitan Area to improve CM testing rates in people with CAP. Proportion of patients who were diagnosed in the Emergency Department (ED) with CAP who were tested for CM at baseline was measured between 8/6/23 and 10/31/23 using an electronic medical record search tool. A knowledge, attitudes, beliefs and practice (KABP) survey was distributed to Emergency Medicine (EM) and Primary Care Physicians (PCP) between 9/12/23 and 9/22/23. Descriptive statistics were used for Likert-style questions, and thematic analysis was used for open ended questions. On 11/1/23, we were invited to the EM Department meeting to present an educational module and to list concerns and barriers of valley fever testing. A link to an online CDC module with CME credit was provided. Articulated concerns were addressed within 2 weeks. The proportion of CAP patients who were tested for CM was re-evaluated from 11/1/23 to 12/5/23. Results were compared using a 2-tailed independent sample z-test for difference between two proportions.

Results: 36 of 80 people responded to the survey (45% response rate), of whom 14 (38.89%) were EM physicians (32% response rate). Difficulty arranging follow-up, delayed test results, test result interpretation and cost and benefits to patients were frequently cited as barrier to CM testing. Less than half of physicians (40%) agreed that universal testing for CM in all CAP patients would be beneficial, while 26% thought it could be potentially harmful. A majority of physicians (53%) estimated that the proportion of CAP cases tested for CM in their department was greater than 50%. When asked which interventions they would find most useful to improve the diagnosis and management of CM, physicians indicated that creation of an order set in the electronic medical record (12/33), a diagnostic algorithm in hospital guidelines (9/33), or support staff to relay CM results (6/33) would be more helpful than an online education module (0/33).

In the initial assessment, 9% (18 of 199) of people presenting to the ED with CAP were tested for CM. Following the EM Department meeting with an educational session and addressing initial concerns, the proportion of patients tested for CM doubled to 20.7% (19/92, p=0.04).

Conclusion: This study demonstrates that raised awareness, addressing concerns, and providing educational interventions can increase the rate of CM testing among patients with CAP. Despite a significant increase in CM testing following the intervention, 4 of 5 CAP patients presenting to the ED were not tested for CM.

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Olorofim for Treatment of Disseminated Coccidioidomycosis in Patients with Limited or No Therapeutic Options

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Abstract

Introduction: The mainstay of coccidioidomycosis (CM) treatment is the triazole class. Severe disseminated CM (DCM) disease requires lifelong treatment, as azole therapy suppresses rather than cures the disease. The side effects of triazoles and drug-drug interactions often limit the use of these agents. These limitations are prompting the development of novel antifungal treatments for DCM with shorter treatment duration and an improved quality of life. One such medication, olorofim, is a de novo pyrimidine biosynthesis inhibitor which selectively inhibits the catalytic activity of fungal dihydroorotate dehydrogenase (DHODH). In this multicenter study, we report the results of olorofim use in refractory DCM patients with limited or no therapeutic options.

Methods: We included adults ≥18 years of age who were able to take oral medications and had limited or no DCM treatment options with available therapies in a phase 2b open-label study of F901318/olorofim. Patients received olorofim (loading dose of 150 mg twice daily on Day 1, followed by 90 mg twice daily) for 84 days +/- 6 days (main phase), with extended therapy beyond Day 90 allowed (NCT03583164, Study 32). DRC-adjudicated overall response rates using the EORTC-MSG response criteria were assessed on Day 42

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(primary endpoint) and Day 84 (secondary endpoint) based on a composite of clinical, radiological, and mycological responses.

Results: A total of 41 patients enrolled in 10 US sites from 05/2019 to 08/2022. The age of patients ranged from 21 to 72 years (median 47 years), 33 were male and 8 were female. 30 patients had CNS disease (73.2%), 13 of these (43%) had hardware including ventriculoperitoneal (VP) shunt or Ommaya reservoir. The time from DCM diagnosis to olorofim initiation ranged from 47 days to 8 years (median 936 days, 2.6 years). This timeframe included therapy with azoles +/- amphotericin. The average treatment with olorofim was 382 days (median 353). The DRC-Adjudicated Clinical Response was Partial resolution in 28 (68.3%) of patients at Day 42 and 24 (58.5%) at Day 84 (see table). Complete resolution was observed in 3 (7.3%) patients at Day 42 and 3 (7.3%) patients at Day 84. Overall Response was 0% at both Day 42 and Day 84 as the slow pace of serologic improvement in coccidioidomycosis did not allow a conclusion of mycological clearance in this timeframe. In turn, this limited the composite Overall Response to Stable at best, even in patients showing a Complete Clinical Response. Hepatic injury considered at least possibly related to olorofim by the independent Hepatic Advisory Committee (HAC) was seen in 9 patients (22.0%) in the main phase and managed by dose reduction/pause, other than 1 patient (2.4%) requiring olorofim discontinuation, no patients had severe liver disease/death attributable to hepatic toxicity. A total of 16 patients (39%) experienced gastrointestinal (GI) Treatment-Emergent Adverse Events (TEAEs), of which 1 (2.4%) was related to olorofim.

Conclusion: Based on the Clinical Response component of the EORTC-MSG scoring system, olorofim displays a positive benefit-risk profile in a well-defined and limited population of patients with serious and/or life-threatening DCM infection with no or limited treatment options. Favorable Clinical Responses (Complete + Partial) were seen in the majority (65.9%) at Day 84. The composite Overall Response of the scoring system is less informative due to the slow pace of serological change in this infection. The hepatic injury signal is manageable with liver enzyme monitoring and dose modification. GI discomfort, mainly non-serious and self-limiting, includes nausea, vomiting, and diarrhea. These findings should prompt new studies to assess the earlier use of olorofim in serious DCM.

DRC-Adjudicated Clinical Response		
	Day 42 N (%)	Day 84 N (%)
Resolution-Complete	3 (7.3)	3 (7.3)
Resolution-Partial	28 (68.3)	24 (58.5)
Resolution-Complete or Partial	31 (75.6)	27 (65.9)
Failure-Stable	5 (12.2)	4 (9.8)
Failure-Progression	2 (4.9)	2 (4.9)
Failure-Not Evaluable	3 (7.3)	1 (2.4)
Failure-Missing	0	7 (17.1)

Using a Dashboard to Improve Tracking of Coccidioidomycosis (CM) in Urgent Care Patients, Maricopa County Arizona

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Abstract

Introduction: Since 2020, early diagnosis of CM has partially improved at Banner Urgent Care Services (BUCS) due to quarterly in-service clinician reminders that patients with community acquired pneumonia (CAP) should be tested serologically for CM. Erythema nodosum (EN) was also identified as a highly sensitive sign of CM, independent from CAP. To further the appropriate testing for CM in BUCS, a dashboard was developed in 2023 which displays past and current statistics about CAP, EN, and testing for CM. Providing clinicians with this daily-updated data may reenforce the testing of these patients for CM in standard practice. In this report, we use data from the dashboard to understand CM testing practices and how CM ebbs and flows within the community from year-to-year.

Methods: The newly created dashboard (Power BI, Microsoft Corporation, Redmond WA) is repopulated daily from the Banner enterprise data warehouse. Data for this analysis were retrieved in January 2024 for 2018-2023 from 45 BUCS clinics in Maricopa County, Arizona, staffed by approximately 150 clinicians: nurse practitioners (50%), physician assistants (35%), and physicians (15%). Data for all BUCS patients included visit dates, clinic location of visits, whether coded for CAP (ICD10 = J18.*) or EN (IDC10 = L52), whether a coccidioidal serology test was ordered, whether the test result was returned, and whether the serologic test result was positive or negative. Dashboard data were downloaded as daily results for this analysis. A single positive serologic test was considered diagnostic of CM. Patients were only counted once per year. Maricopa County Department of Public Health provided monthly confirmed CM case counts for 2020-2023 (2023 counts are provisional) by collection date of the first positive diagnostic specimen. Significance of differences between groups were determined by Fisher's exact chi-squared test, and correlations determined by Spearman rank correlation.

Results: During the study period after periodic reminders began, testing frequencies for CM increased in patients either with or without CAP (9.1-fold and 2.6-fold, respectively, $p<0.0001$). During 2020-2023, Maricopa County had 31,909 reported cases of CM, and BUCS contributed 724 (2.27%). The month-to-month correlation of non-BUCS case counts with BUCS diagnoses was strong ($r=0.86$, $p<0.0001$). In BUCS, CAP was diagnosed in 12,239 patients, of which 1,996 (16.3%) were tested for CM, and in 296 (14.8%) were positive. Of 163 EN patients, 56 (34%) were tested and 32 (57%) were positive. Only 7 patients had ICD10

Using a Dashboard to Improve Tracking of Coccidioidomycosis (CM) in Urgent Care Patients, Maricopa County Arizona *(continued)*

codes for both CAP and EN. The true number of patients with CM presenting as CAP can be estimated by multiplying the total CAP by the percent of tested CAP patients who were positive. Doing this and using 30-day moving average calculations to smooth out the day-to-day variability demonstrates a large variability in the ratio between CAP due to and not due to CM (Figure). At different times, the proportion of CAP due to CM was <5% and at others >40%. Apparent also is that the times of greatest CM prevalence varies from year to year. Testing frequency for CM in the study period was significantly correlated with total numbers of CAP ($r=0.56$, $p<0.0001$), consistent with national guidelines.

Conclusions: With sustained and repeated in-service reminders, testing for CM in BUCS has substantially increased since 2020. The BUCS CM dashboard provides a window into CM activity in patients suffering from CAP. Comparisons with MCDPH case numbers suggest that it may reflect CM activity county-wide. The percentage of CAP due to CM varies widely at different times and in different years. For this reason, the dashboard providing contemporaneous statistics may prove to be a useful resource to both BUCS clinicians and the Maricopa Community at large. EN complements CAP as a presentation of endemic CM and should also be routinely tested. This work represents a successful collaboration between private and municipal health efforts.

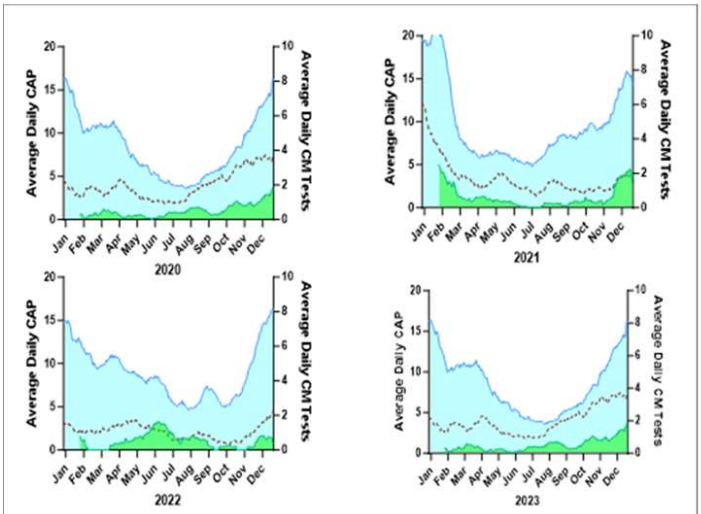

Figure. 30-day moving average of numbers of patients with all CAP (blue graphs), with CAP due to CM (green graphs), and with tests for CM (dotted lines) for the years 2020-2023.

Comparison of a Semi-Quantitative Anti-Coccidioidal Antibody Lateral Flow Assay to Immunodiffusion and Complement Fixation Antibody Titers

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Abstract

Introduction: Although the gold standard for diagnosing coccidioidomycosis requires microscopic or culture identification of *Coccidioides* organisms, a diagnosis is often made based on the collective interpretation of clinical and radiologic findings as well as laboratory antibody-based serologic testing. Traditional serologic assays for quantitative detection of anti-coccidioidal antibodies include immunodiffusion (ID) and complement fixation (CF), which may take several days for a result to be communicated back to a healthcare provider. Recently, a semi-quantitative lateral flow assay (LFA) that detects antibodies against coccidioidal chitinase 1 (CTS1) in 10-15 minutes was developed by our group. To further evaluate this test, we compared the quantifiable output of the LFA to antibody titers determined by CF or ID.

Methods: Human (n=58), macaque (n=24) and dog (n=76) serum or plasma specimens with previously positive serology by ID and/or CF were tested using the CTS1 LFA. Antibody titers of human specimens were previously determined by CF at a reference laboratory (Mayo Clinic Laboratories, Rochester, MN). Dog and macaque specimens had antibody titers determined by quantitative ID in our laboratory. Human sera were collected under an Arizona State University institutional review board (IRB)-approved protocol no. 0601000548 and Mayo Clinic IRB protocol no. 12-000965.

Results: When compared with CF (human) and ID IgG (macaque and dog) results, the CTS1 LFA had positive percent agreements of 91.4% and 100%, respectively. Since the CTS1 LFA is semi-quantitative, test line densities were correlated with CF and ID antibody titers using Spearman's nonparametric rank correlation analysis. A positive correlation was observed for all species tested (human: Spearman's ρ=0.69, 95% CI 0.51-0.80, p<0.0001; macaque: Spearman's ρ=0.72, 95% CI 0.55-0.84, p<0.0001; dog: Spearman's ρ=0.84, 95% CI 0.76-0.90, p<0.0001).

Conclusion: Using a LFA reader, the CTS1 LFA provides a semi-quantitative result that positively correlates to CF titers in humans and ID titers in animals which could supersede current testing that takes several days to weeks to obtain result. Our rapid test has the potential to help clinicians make treatment decisions and to longitudinally monitor patients in real time without diagnostic delay.

Proteome-wide Discovery of Diagnostic Targets for Coccidioidomycosis

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Abstract

There is great opportunity to discover immunogenic targets with improved diagnostic potential for the disease Coccidioidomycosis. However, the enormous diversity of possible Coccidioides specific antigens poses technical challenges in the identification of new targets recognized by patients' antibodies. PepSeq is a rapid, highly-multiplexed approach which can cost-effectively measure reactivity across large numbers of antibody targets simultaneously. Using this platform, we have designed the first proteome-wide peptide library, which covers both species of Coccidioides. Serum from known Coccidioidomycosis patients and healthy controls from outside the endemic region were assayed against the 244,000 plex 30mer library; then, antibody reactivity was mapped at the epitope and protein levels. We observed proteins and epitopes that were uniquely recognized by the disease cohort, and others that demonstrate reactivity in both sample sets. These results significantly expand our understanding of the breadth of patient antibody recognition of linear epitopes in the context of this important fungal disease.

Identification of Immune and Metabolic Biosignatures of Recovered or Disseminated Disease Spectrum of *Coccidioides* Infection and Associated Cellular Functional Pathways

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Abstract

Introduction: *Coccidioides* infection leads to acute or chronic pulmonary disease (Valley Fever) with a wide spectrum of disease severity. However, the mechanisms of coccidioidomycosis severity and therapeutic failures are not fully elucidated and correlates of disseminated or unresolved infection are not well established. We sought determine metabolic correlates of the disease spectrum and infection outcomes and identify pathways for diagnostic and therapeutic targeting.

Methods: We obtained serum and CSF samples that were divided in the four Groups as below:

Group 1: Clinical serum samples from 30 patients with *Coccidioides* infection were collected. Serum samples from 20 uninfected healthy individuals served as negative controls.

Group 2: Clinical serum and CSF samples were obtained from *Coccidioides* infected patients with (n=15) and without diagnosed meningitis (n=15).

Group 3: Longitudinal study of individuals with *Coccidioides* infection (n=18)

Group 4: *Coccidioides* infected patients with anti-fungal therapy (n=13) serum

Serum and CSF samples were evaluated for the immune parameters (*Coccidioides* antibody titers) and metabolomic changes (by untargeted metabolomic profiling) and clinical data. Raw data were processed, and statistical analysis was inferred. Significant metabolites were used for over representation analysis (ORA) and enriched metabolisms were plotted in network map. Machine learning predictive modeling for diagnostic based on serum-associated features was built and a metabolic biosignature consisting of 10 top predictive metabolites was developed that could serve as a predictive correlate of *Coccidioides* infection outcomes.

The metabolic biosignature was validated by evaluating the datasets obtained from the clinical samples of individuals recovered from *Coccidioides* infection and from longitudinal samples of individuals newly diagnosed with *Coccidioides* infection. The metabolic signature of *Coccidioides* infection was also

Identification of Immune and Metabolic Biosignatures of Recovered or Disseminated Disease Spectrum of *Coccidioides* Infection and Associated Cellular Functional Pathways *(continued)*

evaluated in the datasets from patients receiving anti-fungal therapy to identify the impact of the therapy on the metabolic changes. All these samples were correlated of metadata regarding patients' information (i.e. sex, age, ethnicity, IgG antibody complement fixation titers, CF titers, dates of blood collection and titer estimation, antifungal treatments).

Results: *Coccidioides* infection induced a substantial change in the serum metabolic profile compared to uninfected healthy controls. The most altered functional pathways included lipid metabolism, energy metabolism and inflammation. *Coccidioides* infection led to mitochondrial dysfunction during acute and disseminated stages of infection. Alteration of acylcarnitine profile and ω-oxidation of fatty acids constitute an early signature of *Coccidioides* infection along with dysregulation of host iron and heme metabolism. *Coccidioides* may increase iron uptake from the host for its own replication and survival therefore inducing dysregulation of iron and heme metabolism which causes impairing of mitochondrial function. Pro-inflammatory products produced due to mitochondrial dysfunction may promote inflammation and fungal dissemination. We identified host responses generated to counteract the fungal pathogens that were independent from antifungal treatment effects and were targeted to repair mitochondrial dysfunction. Based on these data, we developed a metabolic biosignature and examined its diagnostic potential. Our preliminary data analysis of longitudinal assessments validated the metabolic biosignature and identified cellular pathways contributing ton disease pathogenesis.

Conclusion: We have developed a metabolic biosignature of *Coccidioides* infection that has predictive and diagnostic potential for clinical outcomes. Our study identified most dysregulated cellular pathways during *Coccidioides* infection and provided insights into pathogenic mechanisms and clinical manifestations during *Coccidioides* infection and reveal new opportunities for the therapeutic approaches.

Scientific Section VII | Case Reports

Correlation of A1C levels with Outcomes in Patients With Diabetes Mellitus Who Contract Coccidioidomycosis

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Hypoxemic Respiratory Failure and Coccidioidomycosis-Associated Acute Respiratory Distress Syndrome

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Placental Abruption Caused by Reactivation of Coccidioidomycosis During Pregnancy

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Immunomodulation in the Treatment of Disseminated Coccidioidomycosis

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Role of Glycemic Control in Severity and Outcomes of Coccidioidomycosis in Patients with Diabetes Mellitus in Central California

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Correlation of A1C levels with Outcomes in Patients With Diabetes Mellitus Who Contract Coccidioidomycosis

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Abstract

Introduction: Prior studies of patients with diabetes mellitus (DM) and coccidioidomycosis (cocci) have suggested an association of poorer coccidioidal outcomes (such as relapse, lung cavities, or disseminated infection) in patients with poorer diabetes control; however, such associations were based on random blood glucose cutoff levels, prior to the widespread use of glycated hemoglobin (A1C). In the current study, we aimed to analyze the association between DM control (using A1C) and the clinical course of coccidioidomycosis, exploring potential modifiable risk factors in DM that could affect disease outcomes.

Methods: We performed an electronic search for patients with DM prior to symptomatic probable or proven coccidioidomycosis 1/1/17 – 10/13/22 and excluded patients who were immunosuppressed or pregnant at the time of initial coccidioidal infection or in the follow up period. From the electronic medical record, we collected patient demographics, details of comorbidities and morbidity score, diabetes control (A1C levels at time of coccidioidomycosis onset, and the subsequent 2, 6, 12, 18, and 24 months), and coccidioidal illness and follow up. The following endpoints were examined: cavitary illness and complications, episodes of coccidioidal relapse, antifungal administration, surgery, hospitalization, and extrapulmonary dissemination.

Results: 181 patients met criteria for inclusion. 127/181 (70.2%) were male, 136 (75.1%) white, 14 (7.7%) each Asian and African American, 6 (3.3%) native Hawaiian/pacific islander, 4 (2.2%) American indian/Alaskan native; median age 63 years (18-96), with an initial median A1C of 7.0% (range 4.8-16.0) at cocci diagnosis. 163/181 (90%) patients had at least 1 clinical follow up and were included in the group that we analyzed for outcomes. 142 (87.1%) were administered antifungals; 72 (44.3%) developed cavities during the follow up period; 14/72 (19.4%) had cavity complications (hemoptysis, hydropneumothorax or secondary infection). 127 (77.9%) improved and/or resolved their cocci infection, and 32 (19.6%) experienced relapsed infection; 17 (10.4%) had surgery for coccidioidomycosis, 12 (7.3%) experienced extrapulmonary dissemination. 75 (46%) were hospitalized for their coccidioidomycosis, and 1 (0.6%) died due to coccidioidomycosis. A1C levels correlated with presence of cavitary coccidioidal infection ($p<0.01$ up to 12 months), early hospitalization ($p<0.01$), hospitalization for cavitary disease ($p=0.049$ for A1C at 6 months), relapse of cavitary disease ($p=0.03$ for A1C at 24 months) and cavitary complications ($p= 0.049$ at 24 months). Initial analysis did not identify an A1C cutoff that correlated with cavity size or overall outcomes.

Conclusions: DM control, as manifested by A1C, correlates with presence of cavities, cavitary complications, and hospitalizations at the time of coccidioidal diagnosis. Higher A1Cs later in coccidioidal illness correlate with late cavitary complications, hospitalization, and clinical relapse. Whether interventions to improve DM control will result in better coccidioidal infections is not known but remains for future study.

Hypoxemic Respiratory Failure and Coccidioidomycosis-Associated Acute Respiratory Distress Syndrome

Arash Heidari[1], Simmer Kaur[1], Skyler Pearson[2], Augustine Munoz[1], Harleen Sandhu[3], Gursimran Mann[2], Michael Schivoe[2], Amir Zeki[2], Derek Bays[2], Machelle Wilson[2], Timothy Albertson[2], Royce Johnson[3], George Thompson[2]

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Abstract

Background: Severe coccidioidomycosis presenting with respiratory failure is an uncommon manifestation of disease. Current knowledge of this condition is limited to case reports and small case series.

Methods: A retrospective multicenter review of patients with coccidioidomycosis-associated acute respiratory distress syndrome (CA-ARDS) was conducted. It assessed clinical and laboratory variables at the time of presentation, reviewed the treatment course, and compared this cohort with a national database of patients with noncoccidioidomycosis ARDS. Survivors and nonsurvivors of coccidioidomycosis were also compared to determine prognostic factors.

Results: In this study, CA-ARDS (n = 54) was most common in males, those of Hispanic ethnicity, and those with concurrent diabetes mellitus. As compared with the PETAL network database (Prevention and Early Treatment of Acute Lung Injury; n = 1006), patients with coccidioidomycosis were younger, had fewer comorbid conditions, and were less acidemic. The 90-day mortality was 15.4% for patients with coccidioidomycosis, as opposed to 42.6% ($P < .0001$) for patients with noncoccidioidomycosis ARDS. Patients with coccidioidomycosis who died, as compared with those who survived, were older, had higher APACHE II scores (Acute Physiology and Chronic Health Evaluation), and did not receive corticosteroid therapy.

Conclusions: CA-ARDS is an uncommon but morbid manifestation of infection. When compared with a national database, the overall mortality appears favorable vs other causes of ARDS. Patients with CA-ARDS had a low overall mortality but required prolonged antifungal therapy. The utility of corticosteroids in this condition remains unconfirmed.

Placental Abruption Caused by Reactivation of Coccidioidomycosis During Pregnancy

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Abstract

Introduction: Pregnancy and immunosuppressive medications are well known risk factors for severe coccidioidomycosis (CM) [1,2]. Placental involvement from disseminated CM has been reported in the literature and is associated with a vast array of clinical outcomes for both the mother and fetus [2,3,4]. Presented here is a pregnant female with a history of rheumatoid arthritis (RA) who was diagnosed with placental abruption due to reactivation of coccidioidomycosis.

Methods: A retrospective review of the patient's record was performed. Literature search was conducted with PubMed.

Results: A 33-year-old G1P0 female with RA and a prior history of disseminated CM several years prior presented in her 26th week of pregnancy with mild/moderate vaginal bleeding. She had previously been treated with fluconazole for 2 years (2016-2018) for pulmonary and peritoneal CM. At the time of her diagnosis of disseminated CM, her immunosuppression was adalimumab. This was discontinued. Once pregnant, her RA treatment was resumed and her treatment regimen consisted of hydroxychloroquine and sulfasalazine. Routine Coccidioides serologies were obtained during her first trimester (04/2022) and did not show evidence of active disease: Coccidioides Ab by complement fixation (CF) was <1:2, IgM by immunodiffusion (ID) was not detected, IgG by ELISA 2.9, and IgM by ELISA 1.4 .

During the 26th week of pregnancy, she presented with rust colored/bloody vaginal discharge for 3 weeks. She was afebrile and did not endorse additional symptoms. A speculum exam revealed a normal cervical os; routine vaginal bacterial and fungal cultures were obtained (07/2022). She was diagnosed with chronic placental abruption. Beclomethasone was advised, however there were safety concerns in the setting of her prior disseminated CM. Repeat serologies (07/2022) revealed a slight increase in CF to 1:4, ID was now detected, and IgG by ELISA 5.7, and IgM by ELISA 2.4. Fluconazole 600 mg daily was initiated. One month later she was hospitalized for ongoing vaginal bleeding. Speculum exam revealed a friable, bloody cervical os, scattered with small, raised white plaques (Image 1). A microscopic exam of the vaginal fluid showed encapsulated organisms with budding yeast (Image 2 and 3). The fungal vaginal culture obtained from her initial presentation (07/2022) grew Coccidioides immitis. Repeat serologies revealed CF 1:16, ID detected, IgG by ELISA 7.2 and IgM by ELISA 2.6. Liposomal amphotericin B 3mg/kg IV every 24 hours was initiated and fluconazole was stopped. Due to ongoing bleeding, she required a C-section. Intraoperative findings revealed multiple 1-2 cm irregular nodules throughout the bowel and pelvic organs (Images 4, 5 and 6). The placenta was sent for pathology which showed fungal organisms, consistent with Coccidioides, identified within the chorion, adherent decidual tissue, and an adherent blood clot. A post-partum CT of the chest,

Placental Abruption Caused by Reactivation of Coccidioidomycosis During Pregnancy *(continued)*

abdomen, and pelvis did not reveal further evidence of disease. Her CF improved to 1:8 while on treatment with liposomal amphotericin B for three more doses post-partum, and she was discharged on fluconazole. She continued treatment for over 12 months; labs subsequently revealed a CF of <1:2 and ID not detected. As of December 2023, CF <1:2, ID was not detected, and her IgG/IgM by ELISA continue to downtrend while on fluconazole and her dose has been reduced to 600 mg daily.

Immediately following the delivery, the infant was started on positive pressure ventilation and transitioned to continuous positive airway pressure (CPAP). The pediatric infectious disease on-call team was contacted and recommendations were provided for full evaluation for congenital CM, including fungal blood cultures, cerebrospinal fluid (CSF) cell counts, protein, fungal cultures, and complement fixation antibody testing, as well as CSF, serum, and urine Coccidioides antigen testing, and serum CF antibody testing. Empiric treatment with fluconazole was initiated. Serial head ultrasounds and long bone radiography were performed as well. Ultimately, the infant's evaluation was normal/negative for all markers of disseminated infection or imaging abnormalities. Coccidioides CF antibodies were initially elevated at birth to 1:4 and fell to normal (<1:2) by 4 months of life, which was felt to be consistent with maternal transplacental serology rather than congenital CM . Fluconazole therapy was ultimately discontinued at five months of life, and serial CF antibodies remained negative thereafter. Over the ensuing year, the infant continued to do well clinically and develop normally with no concerns for CM or need for antifungal therapy.

Conclusion: This case demonstrates pelvic reactivation of CM during pregnancy, causing chronic placental abruption. Immunologic changes of pregnancy along with changes in the hormonal milieu in the setting of her immune impairments conferred by her underlying rheumatologic disease could have allowed for reactivation, and the need for beclomethasone probably worsened infection severity despite concurrent antifungal therapy. An increase in Coccidioides Ab by CF and ID correlated with an increase in severity of disease in our patient. Early detection and treatment likely contributed to favorable outcomes for both the mother and infant.

Immunomodulation in the Treatment of Disseminated Coccidioidomycosis

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Abstract

Introduction: The current approach relying on antifungal therapy is not sufficient for many patients with disseminated coccidioidomycosis. There are 5-10 cases known where interferon-gamma has been employed in severe, disseminated disease. We previously published a single case employing the IL4/IL13 blocker dupilumab in a child with disseminated disease and excessive Type 2 immune skewing. Here we present six additional cases where these immunomodulatory therapies were used in addition to antifungal treatment to facilitate disease resolution.

Methods: Cases were identified at UCLA by Infectious Disease experts in conjunction with Immunology . Informed consent was obtained. We performed intracellular cytokine staining on CD4+ cells and measured proportions of IFN-γ, IL-4, and IL-17-producing T cells prior to initiation of treatment. Patients with severe disease were given IFNγ. Patients who showed elevated Th2 responses were initiated on Dupilumab.

Results:

Case ID	Cocci Scoring	Highest Cocci Titer	Duration of Immunomodulation to Date	Post Immunomodulation Cocci Titer
1	4B	1:256	Interferon Gamma: 1.5 Years	Undetectable
2	5C	1:128	Dupilumab: 1 Year	1:2
3	4B	1:256	Dupilumab: 2 years	<1:2
4	3C	1:256	Dupilumab: 1 Year	<1:2
5	4B	1:392	Dupilumab: 6 months*	1:16
6	5C	1:256	Dupilumab: 4 months*	1:16
7	5B	1:512	Dupilumab: 3 Months*	1:16

*Ongoing

Conclusion: The results show that patients with disseminated coccidioimycosis may benefit from added immunomodulation. These observations provide evidence to support clinical trials of interferon gamma for severe disease and dupilumab for disseminated disease when type 2 immune skewing is present. Further research is needed to better characterize which patients will benefit from the addition of immunomodulatory medications.

Role of Glycemic Control in Severity and Outcomes of Coccidioidomycosis in Patients with Diabetes Mellitus in Central California

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Abstract

Introduction: Diabetes mellitus (DM) is a known risk factor for severe coccidioidomycosis. According to the Centers for Disease Control (CDC) data, Central California has counties with some of the highest burden of Diabetes mellitus in the entire country. We aim to study the role of glycemic control in the severity and outcomes of coccidioidomycosis in the Central California region given the high burden of DM in the area.

Methods: We did a retrospective analysis of patients with coccidioidomycosis and diabetes mellitus between the years 2014-2024 seen in a large referral center in Fresno, California. Cases were identified using International Classification of Diseases, Ninth or Tenth Revision codes. Data including demographics, predisposing factors, hemoglobin A1C, insulin usage, management, and outcomes of coccidioidomycosis were collected. Descriptive statistics were used to analyze the sample characteristics based on outcome measures. Measures of association were tested using the $\chi 2$ or Fisher exact test for categorical measures and the Wilcoxon-Rank sum test for continuous measures. The study was approved by the institutional review board.

Results: We identified 450 patients with DM and coccidioidomycosis during the study period. We included 80 patients with DM and Coccidioidomycosis for preliminary analysis. Five of these were excluded due to concurrent immunocompromising conditions and use of prolonged corticosteroids. Of the 75 patients included in the final analysis, 73% were male and 78% were Hispanic (Table 1). All of them were type 2 diabetics and 35% were insulin dependent. Patients who were insulin-dependent had statistically significant higher rates of complicated and cavitary lung disease (p-value .001 for both). They also required longer duration of anti-fungal therapy and higher rates of non-resolution of disease (p-value 0.05 and 0.023 respectively). The average hemoglobin A1C level was 8.7 gm/dl (=+/- 2.7 SD). Using hemoglobin A1C as a continuous variable, a higher A1C level was associated with higher rates of complicated and cavitary lung disease (p value 0.0004 and 0.0036 respectively) and lower rates of resolution of disease (p value 0.0005).

Role of Glycemic Control in Severity and Outcomes of Coccidioidomycosis in Patients with Diabetes Mellitus in Central California *(continued)*

Conclusion: Central California, with one of the highest burdens of Coccidioidomycosis, also has a heavy burden of uncontrolled DM. Our study shows that the level of glycemic control in patients with diabetes mellitus directly impacts the severity of pulmonary coccidioidomycosis, duration of anti-fungal therapy and rates of resolution. A multi-disciplinary approach to management, targeting tight glycemic control along with anti-fungal therapy is needed in patients with Coccidioidomycosis and DM.

Table 1: Sample characteristics

N=75	Total(n=75)	
	N	%
Sex		
Male	55	73.3
Female	20	26.7
Race		
Caucasian	14	18.7
Hispanic	59	78.7
African American	2	2.7
Ethnicity		
Hispanic	63	78.75
Non-Hispanic	17	21.25
Type 1 DM		
Yes	0	0
No	75	100
Type 2 DM		
Yes	75	100
No	0	0
Insulin Dependent		
Yes	26	34.7
No	49	65.3
	Total	
	Mean	SD
Age	57.7	12.0
HbA1C	8.7	2.6

Poster Presentations

Poster Presentations

1. Comparison of Abnormalities Identified at Chest Radiography Versus Computed Tomography in Coccidioidomycosis.

Sandhya Nagarakanti, Nikita Ashcherkin, Michael Gotway, Janis Blair
Mayo clinic, Phoenix, USA

2. Antifungal Activity of Brilacidin, a Nonpeptide Host Defense Molecule

David J. Larwood[1], David A. Stevens[2]
[1]Department of Pharmaceutical Chemistry, University of California-San Francisco, San Francisco, USA. [2]Division of Infectious Diseases and Geographic Medicine, Stanford University Medical School, Stanford, USA

3. Olorofim Demonstrates Potent In Vitro Activity Against Coccidioides Species Including Against Isolates With Reduced Fluconazole Susceptibility

Nathan Wiederhold[1], Hoja Patterson[1], D Ferrer[1], V Garcia[1], George Thompson[2], Tom Patterson[1]
[1]UT-San Antonio, San Antonio, USA. [2]UC-Davis, Sacramento, USA

4. Temporal Trends in Fluconazole and Itraconazole Susceptibility Against *Coccidioides*

Nathan Wiederhold[1], Hoja Patterson[1], Dora Ferrer[1], Victor Garcia[1], Laura Najvar[1], ChiungYu Hung[2], Jose Jopez-Ribot[2], George Thompson[3], Thomas Patterson[1]
[1]UT Health San Antonio, San Antonio, USA. [2]University of Texas at San Antonio, San Antonio, USA. [3]University of California at Davis, Davis, USA

5. *In vivo* Effectiveness of Fluconazole and Posaconazole Against *Coccidioides posadasii* Meningitis Caused by Fluconazole Resistant Isolates

Laura Najvar[1], Nathan Wiederhold[1], Rosie Jaramillo[1], Marcos Olivo[1], Jose Lopez-Ribot[2], ChiungYu Hung[2], George Thompson[3], Thomas Patterson[1]
[1]UT Health San Antonio, San Antonio, USA. [2]University of Texas at San Antonio, San Antonio, USA. [3]University of California at Davis, Davis, USA

6. Toward Better Treatments: Investigating Fungal Secreted Proteases in Coccidioidomycosis

Christina Homer, Elena Ochoa, Mark Voorhies, Anita Sil
University of California, San Francisco, San Francisco, USA

7. Creating a Precision Disease Risk Model For Coccidioidomycosis Using Historic and Current Genomics and Environmental Data

Cally Erickson[1], Tenley Housler[1], Kimberly Kaufeld[1], Morgan Gorris[2], Andrew Bartlow[1]
[1]Los Alamos National Laboratory, Los Alamos, USA. [2]Los Alamos National Laboratory, L, USA

8. Siloed Research Units: A Promising Strategy for Boosting Enrollment and Research Quality in Coccidioidomycosis Studies

Elizabeth Robison
UC Davis Medical Center, Sacramento, USA

9. Combining Unconventional Drugs with Current Treatments to Combat *Coccidioides*

Sarah Saeger[1], Kathryn West-Jeppson[1], Jieh-Juen Yu[1,2], Jose Lopez-Ribot[1,2], Chiung-Yu Hung[1,2]
[1]University of Texas at San Antonio, San Antonio, USA. [2]South Texas Center of Emerging Infectious Diseases, San Antonio, USA

10. Prevalence of Atopic Disorders Among Pediatric Patients With Pulmonary and Disseminated Coccidioidomycosis

Sarah Zhang[1], Brenna LaBere[2,1], James Woodward[2,1], Wassim Ballan[2,1], Katherine Perry[2,1], Keith Sacco[2,1]
[1]University of Arizona College of Medicine Phoenix, Phoenix, USA. [2]Phoenix Children's Hospital, Phoenix, USA

11. Real-World Diagnostic Performance in the Diagnosis of Coccidioidal Meningitis

Alexander Lietz, Rawan Elkurdi, Pool Jefferson Tobar Vega, Janis Blair
Mayo Clinic - Arizona, Phoenix, USA

12. Screening the ChemDiv Antifungal Drug Library Against Parasitic Spherules of Coccidioides posadasii

Pradeep Singh, Sarah Saeger, Jesus Rodriguez, Jieh-Jeun Yu, Jose Lopez-Ribot, Chiung-Yu Hung
Department of Molecular Microbiology and Immunology and South Texas Center of Emerging Infectious Diseases, The University of Texas at San Antonio, San Antonio, USA

13. Launching a Breath Analysis Study to Identify Volatile Biomarkers for Valley Fever

Jenna Diefenderfer[1,2], Emily A. Higgins Keppler[1,2], Janis E. Blair[3], Heather D. Bean[1,2]
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14. The Unknown "Knowns" of *Coccidioides* Susceptibility

Amy Hsu
NIAID/NIH, Bethesda, USA

15. Pediatric Coccidioidomycosis – Experience at an Academic Pediatric Infectious Disease Clinic in Southern Arizona

Jordan Gotwalt[1], Marian Lacy[1,2], Neil Ampel[1], Kareem Shehab[1,2]
[1]University of Arizona College of Medicine, Tucson, USA. [2]Banner Health University Medical Center, Tucson, USA

16. A Climate-driven, Seasonal Forecast of Relative Coccidioidomycosis Risk in Four Arizona Regions

Grace Leito[1,2], Kimberly A. Kaufeld[3], Staci A. Hepler[4], David M. Kline[5], Morgan E. Gorris[3]
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17. Evaluating Immunogenicity of an Eisosome Component Identified in *Coccidioides posadasii*

Reimi Navarro[1,2], Althea Campuzano[1,2], Austin Negron[1,2], Nawal Abdul-Baki[1,2], Matthew Mendoza Barker[1,2], Bryan Lam[1,2], Christina Barnes[1,2], Jieh-Juen Yu[1,2], Gary Ostroff[3], Chiung-Yu Hung[1,4]

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18. Evaluating the Efficacy of an mRNA Vaccine Against Coccidioidomycosis

Austin Negron[1], Althea Campuzano[1], Matthew Mendoza Barker[1], Nawal Abdul-Baki[1], Reimi Navarro[1], Sarah Saeger[1], Kathryn West[1], Venus Stanton[1], Florentina Rus[2], Kathrika Nagalekshmi[2], Jeremy Luban[2], Ernesto Soto[2], Gary Ostroff[2], Chiung-Yu Hung[1]

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19. Identification of Coccidioidomycosis Specific Immunoreactive Peptides and the Associated Immune Response

Mrinalini Kala[1], Mame Diarra Bousso Ndiaye[2], Jinhee Yi[2], John Altin[3], Paul Keim[2], Kenneth S Knox[1], Erik W Settles[2]

[1]University of Arizona, Phoenix, USA. [2]The Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, USA. [3]The Translational Genomics Research Institute (TGen), Flagstaff, USA

20. Detection of *Coccidioides* Galactomannan With a Monoclonal Antibody-based Lateral Flow Immunoassay

Amanda Burnham-Marusich[1], Jacqueline Kelly[1], Althea Mayberry[1], Kathleen Zayac[2], John Galgiani[3,4,5,6], Lourdes Lewis[3], Thomas Kozel[2]

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21. *Coccidioides* Species Hot Spots in the Arizona Landscape: Characteristics of Soils Harboring Valley Fever

Nastasia Baudin[1], Brooke Byars[1], Robert Root[1], Craig Rasmussen[1], Marieke Ramsey[2], Dan Kollath[2], Bridget Barker[2], Jon Chorover[1]

[1]University of Arizona, Tucson, USA. [2]Northern Arizona University, Flagstaff, USA

22. Urban Versus Rural Hospital Outcomes for Coccidioidomycosis 2016-2020

Laura Budvytyte[1], Sufyan Ibrahim, M.B.B.S[2], Janis E Blair, M.D.[3]

[1]Mayo Clinic Alix School of Medicine Arizona, Phoenix, USA. [2]Neuro-Informatics Laboratory Department of Neurological Surgery, Mayo Clinic, Rochester, USA. [3]Division of Infectious Diseases, Mayo Clinic, Phoenix, USA

23. Coccidioidal Tenosynovitis- A Case Series

Shikha Mishra, Jane Park, Michelle Fang, Michael Valdez, Carlos D'Assumpcao, Bianca Torres, Valerie Espinoza, Leila Moosavi, Royce Johnson, Rasha Kuran

Kern Medical, Bakersfield, USA

24. Acute Pulmonary Coccidioidomycosis with Bronchial Casts in Sputum

Shikha Mishra, Michelle Fang, Bianca Torres, Michael Valdez, Carlos D'Assumpcao, Royce Johnson, Rasha Kuran

Kern Medical, Bakersfield, USA

25. Disseminated Cocci Iridocyclitis: A Rare Presentation of Anterior Chamber Coccidioidomycosis in the Eye

Shikha Mishra[1], Kishan Ghadiya[2], Bianca Torres[1], Michelle Fang[1], Michael Valdez[1], Royce Johnson[1], Rasha Kuran[1]
[1]Kern Medical, Bakersfield, USA. [2]Ross University, Bakersfield, USA

26. Coccidioidal Empyema in the Making

Bianca Torres[1], Shikha Mishra[2,1], Michelle Fang[3,1], Michael Valdez[2,1], Carlos D'Assumpcao[2,1], Royce Johnson[2,1,4], Rasha Kuran[2,1,4]
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27. Case Series of Coccidioidomycosis and Malignancies in an Endemic Area

Rupam Sharma[1,2,3], Mia Yasonova[1,2,3], Akriti Chaudhry[1,3], Leila Moosavi[1,3], Arash Heidari[1,2,3], Royce Johnson[1,2,3], Rasha Kuran[1,2,3], Rahul Polineni[1,3], Everardo Cobos[1,3]
[1]Kern Medical, Bakersfield, USA. [2]Valley Fever Institute Kern Medical, Bakersfield, USA. [3]David Geffen School of Medicine UCLA, Los Angeles, USA

28. OPAT Liposomal Amphotericin B in an Immunocompetent Patient with Disseminated Coccidioidomycosis and Bone Involvement

Cynthia Ortiz-Lopez, Cristina Hernandez-Balboa, Emilio Castillo-Gonzalez, Laura Nuzzolo-Shihadeh, Eduardo Perez-Alba, Maria Teresa Ramirez-Elizondo, Reynaldo Lara-Medrano, Adrian Camacho-Ortiz
Servicio de Infectologia, Hospital Universitario "Dr Jose Eleuterio Gonzalez", Universidad Autonoma de Nuevo Leon, Monterrey, Mexico

29. Immuno-phenotyping Reveals Dysregulation of the CD8 T Cell Compartment in Coccidioidomycosis

Timothy Thauland, Alexis Stephens, Rina Nagarajan, Anviksha Srivastava, Manish Butte
UCLA, Los Angeles, USA

30. Meta-Analysis of Laboratory Diagnostics for Coccidioidomycosis

Donald Catanzaro[1], Mary Cowen[2]
[1]University of Arkansas, Fayetteville, Fayetteville, USA. [2]University of Arkansas, Fayetteville, USA

31. Proteome Wide Antibody Profiling of Pig-tailed Macaques with Naturally Acquired Valley Fever Using PepSeq

Evan Elko[1], Heather Mead[2], Luke Ryle[1], Georgia Nelson[2], Megan Fredericks[3], Deborah Fuller[3], John Altin[2], Jason Ladner[1]
[1]Northern Arizona University, Flagstaff, USA. [2]TGen, Flagstaff, USA. [3]University of Washington, Seattle, USA

32. Improvement of a Colorimetric Cell Viability Test for Testing Novel Antifungals on *Coccidiodes* spp.

Jesus Vazquez Rodriguez, Jieh-Jeun Yu, Chiung-Yu Hung, Jose L. Lopez-Ribot
Department of Molecular Microbiology & Immunology, and South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, San Antonio, USA

33. Drug Discovery and Drug Target Identification in *Coccidioides posadasii*

Kathryn West-Jeppson, Sarah Saeger, Pradeep Kumar Singh, Jieh-Juen Yu, Chiung-Yu Hung
University of Texas at San Antonio, San Antonio, USA

34. Impact of Dust on Climate, Health, and Disease Transmission in the California San Joaquin Valley

Adeola Fagbayibo, Estrella Herrera, Asa Bradman, Katrina Hoyer
University of California Merced, Merced, USA

35. Profiling Early Immune Responses in the Lung to *Coccidioides* Infection

Nadia Miranda[1,2], Oscar Davalos[2], Aimy Sebastian[2], Nicholas Hum[2], Margarita Rangel[2], Deepa Murugesh[2], Ashlee Phillips[2], Katrina Hoyer[1,2], Dina Weilhammer[2]
[1]University of California Merced, Merced, USA. [2]Lawrence Livermore National Laboratory, Livermore, USA

36. Whole Genome and RNA Sequencing of Large Coccidioidomycosis Patient Cohort Enables Genetic Characterization of Uncomplicated and Severe Disease

Samantha L. Niles-Jensen[1,2,3], Sarah J. Spendlove[4], Alexis V. Stephens[5], George R. Thompson[6,7,8], Royce H. Johnson[9,10,11], Arash Heidari[9,10,11], Rasha Kuran[9,10,11], Harold Pimentel[1,3], Manish J. Butte[1,2,5], Valerie A. Arboleda[1,2,3]
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37. Single-cell Transcriptomic Analysis Reveals the Dynamics of Immune Infiltration and Differentiation in the Lungs During C. Posadasii Infection

Oscar Davalos[1], Aimy Sebastian[1], Nicholas Hum[1], Nadia Miranda[2,1], Deepa Murugesh[1], Margarita Rangel[1], Ashlee Phillips[1], Gabriela Loots[3,1], Katrina Hoyer[2], Dina Weilhammer[1]
[1]Lawrence Livermore National Lab, Livermore, USA. [2]University of California, Merced, Merced, USA. [3]University of California, Davis, Davis, USA

38. Outcomes of Fluconazole Discontinuation in Solid Organ Transplant Recipients with at Least One *Coccidioides* Seropositivity

Matt Biondi[1], Simran Gupta[2,1], Priyal Shah[1], Matthew Buras[1], Holenarasipur Vikram[1], Janis Blair[1]
[1]Mayo Clinic AZ, Phoenix, USA. [2]Massachusetts General Hospital, Boston, USA

39. Spinal Cord Involvement with Coccidioidal Meningitis: A Case Series of 45 Adult Patients

Bianca Torres[1], Danish Khalid[1], Chelsea Dunn[1], Rupam Sharma[1], Lovedip Kooner[2], Michael Valdez[1,3], Shikha Mishra[1,3], Carlos D'Assumpcao[1,3,4], Royce Johnson[1,3,4], Rasha Kuran[1,3,4]
[1]Valley Fever Institute at Kern Medical, Bakersfield, USA. [2]Rio Bravo Family Medicine, Bakersfield, USA. [3]Department of Medicine, Division of Infectious Disease at Kern Medical, Bakersfield, USA. [4]Department of Medicine, David Geffen School of Medicine UCLA, Los Angeles, USA

40. Elucidating Key Interactions Between Macrophages and the Fungal Pathogen Coccidioides

Jane Symington, Bevin English, Mark Voorhies, Anita Sil
UCSF, San Francisco, USA

1. Comparison of Abnormalities Identified at Chest Radiography Versus Computed Tomography in Coccidioidomycosis.

Sandhya Nagarakanti, Nikita Ashcherkin, Michael Gotway, Janis Blair

Mayo clinic, Phoenix, USA

Abstract

Background: Chest radiographs (CXR) are often used as the initial imaging method for diagnosing pulmonary coccidioidomycosis. Chest computed tomography (CT) is superior to chest radiography for the detection of thoracic pathology, but the additional yield of CT over CXR for the detection of thoracic coccidioidal infection is not known. We aimed to determine whether CT is superior to CXR for supporting a diagnosis of acute pulmonary coccidioidomycosis.

Methods: We conducted a retrospective review of data from adult patients aged 18 years and older who were diagnosed with proven or probable pulmonary coccidioidomycosis between March 1, 2019, and December 31, 2020, at Mayo Clinic, Arizona. We reviewed patient information using a standardized data collection form that included demographic characteristics, clinical presentation, laboratory results, chest imaging findings, treatment modalities, and clinical outcomes.

Results: A total of 196 patients with acute pulmonary coccidioidomycosis were identified, with an average age of 56 years (range 17 to 95 years). 110 (56.1%) were male, 171 patients (87.2%) were White non-Hispanic/American Indian, 13 patients (6.6%) Hispanic/American Indian, and 5 (2.6%) were Black. 180 patients (92%) underwent a chest X-ray (CXR), and 131 patients (67%) had a chest CT scan. Both CXR and chest CT were performed in 116 patients (59%). Among them, coccidioidomycosis was proven in 22 patients (19%) and probable in 94 patients (81%).

Among 180 patients who underwent CXR, 26 (14.4%) appeared normal. Subsequently, 16 of these 26 patients (61.5%) underwent CT scans. All 16 CT scans (100%) revealed abnormalities: 10 showed pulmonary airspace abnormalities (62.5%), 12 lung nodules or masses (75%), 5 pathologically enlarged hilar lymph nodes (31.25%), 4 pathologically enlarged mediastinal lymph nodes (25%), 1 cavity (6.25%), and 1 pleural effusion (6.25%). The sensitivity of CXR and CT was 82.7% and 100%, respectively.

Conclusions: The diagnostic yield for pulmonary coccidioidomycosis increased by 22% using chest CT compared with CXR. Pulmonary air space abnormalities, nodules, and pathologically enlarged hilar and mediastinal lymph nodes seen in coccidioidomycosis patients were all more readily detected at CT chest than CXR. CT imaging may be advised when coccidioidomycosis infection is suspected despite normal CXR findings.

2. Antifungal Activity of Brilacidin, a Nonpeptide Host Defense Molecule

David J. Larwood[1], David A. Stevens[2]

[1]Department of Pharmaceutical Chemistry, University of California-San Francisco, San Francisco, USA. [2]Division of Infectious Diseases and Geographic Medicine, Stanford University Medical School, Stanford, USA

Abstract

Introduction: Natural host defensins, also sometimes termed antimicrobial peptides, are evolutionarily conserved. They have been studied as antimicrobials, but some pharmaceutical properties, undesirable for clinical use, led to the development of synthetic molecules with constructed peptide arrangements and/or peptides not found in nature. The exciting leading development currently is synthetic nonpeptide mimetics, whose physicochemical properties recapture the characteristics of the natural molecules, and share their biological attributes.

Methods: We studied brilacidin, an arylamide of this type, for its activity *in vitro* by broth dilution, against fungi (40 clinical isolates, 20 species) that the World Health Organization have highlighted as problem human pathogens.

Results: We find antifungal activity at low concentrations for many pathogens. The mode of MICs for coccidioidal isolates was 2 mcg/ml. Synergy with conventional, currently available, antifungals against other fungal pathogens has been shown. This presentation will review the physical chemistry of nonpeptide mimetics, their known mechanisms of action on fungi, and their actions on host defenses.

Conclusion: Our results indicate further screening for activity, particularly *in vivo*, is justified to evaluate this compound, and others of this new class of molecules, for possible clinical antifungal activity. That defensins have broadly immunostimulatory properties suggests direct antifungal activity as shown, coupled with boosting host immunity, could have benefits for patients with coccidioidomycosis and other invasive mycoses.

3. Olorofim Demonstrates Potent In Vitro Activity Against Coccidioides Species Including Against Isolates With Reduced Fluconazole Susceptibility

Nathan Wiederhold[1], Hoja Patterson[1], D Ferrer[1], V Garcia[1], George Thompson[2], Tom Patterson[1]

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Abstract

Introduction: *Coccidioides* are soil-dwelling fungi that are endemic in the western United States, northern Mexico, and parts of Central and South America. Disseminated coccidioidomycosis is difficult to treat and is associated with significant morbidity. Treatment of disseminated infections usually involves long-term administration of azole antifungals, such as fluconazole or itraconazole. Olorofim is a novel antifungal that targets the synthesis of fungal pyrimidine by inhibiting the dihydroorotate dehydrogenase enzyme, and it has been shown to have activity against a limited number of *Coccidioides* isolates. We evaluated the *in vitro* activity of olorofim against a large number of *Coccidioides* clinical strains, including those with reduced fluconazole susceptibility (MIC ³16 mg/L) and fluconazole resistance (MIC ³32 mg/L).

Methods: 202 clinical *Coccidioides* isolates from institutions across the U.S. over a 19-month period were included, representing both *C. immitis* and *C. posadasii* strains. Antifungal susceptibility testing was performed with olorofim, amphotericin B, fluconazole, and the extended spectrum azoles posaconazole, voriconazole, itraconazole, and isavuconazole by CLSI broth dilution methods. Geometric mean (GM) MICs, MIC50, MIC90, and modal MICs were determined.

Results: Olorofim demonstrated the most potent *in vitro* activity with a geometric mean (GM) MIC value of 0.010 mg/L, followed by amphotericin B (0.051 mg/L), posaconaozle (0.055 mg/L), itraconazole (0.097 mg/L), voriconazole (0.128 mg/L), isavuconazole (0.261 mg/L), and fluconazole (9.30 mg/L) (Table). Olorofim maintained *in vitro* potency against 43 isolates with reduced fluconazole susceptibility (GM MIC 0.010 mg/L, MIC90 0.03 mg/L, Mode 0.008 mg/L), and against 13 isolates that were fluconazole resistant (GM MIC 0.017 mg/L, MIC90 0.03 mg/L, Mode 0.008 mg/L). Although the MICs of the extended spectrum azoles remained relatively low against fluconazole resistant strains, there were larger increases in the GM MIC values of each compared to that of olorofim (GM MIC range 0.130-0.648 mg/L; fold-change 2.37-3.90).

Conclusions: Olorofim demonstrated potent *in vitro* activity against a large number of *Coccidioides* clinical isolates, and its activity was maintained against strains with reduced susceptibility or resistance to fluconazole. Further studies are warranted to determine the clinical efficacy of olorofim in the treatment of coccidioidomycosis caused by fluconazole resistant isolates.

4. Temporal Trends in Fluconazole and Itraconazole Susceptibility Against *Coccidioides*

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Abstract

Introduction: Fluconazole and itraconazole are often used in the treatment of coccidioidomycosis. Our group has previously reported reduced fluconazole susceptibility for a high percentage of *Coccidioides* clinical isolates between 2001-2015 (minimum inhibitory concentrations [MIC] ≥16 mg/ml 37.3%; Thompson et al. *Antimicrob Agents Chemother* 2016). We reviewed more recent fluconazole and itraconazole MICs against *Coccidioides* clinical isolates to assess trends changes in susceptibility patterns over time.

Methods: The laboratory database in the Fungus Testing Laboratory, UT Health San Antonio, was queried for antifungal MIC data against *Coccidioides* species between 2001-2023. All susceptibility testing had been performed by broth dilution according to CLSI M38 methods. The results were divided into 3 time periods: 2001-2015 (as previously reported), 2016-2019, and 2020-2023. MIC parameters (i.e., MIC value at which 90% of isolates inhibited [MIC90], geometric mean [GM] MIC) and the percentage of isolates with elevated fluconazole MICs (i.e., ≥16, 32, and 64 mg/ml) or itraconazole MICs (i.e., ≥0.5 and 1 mg/ml) for each period were determined. Trends in elevated MIC values were also reviewed on an annual basis.

Results: Overall results for the three time periods are presented in the Table. Compared to the initial time-period (2001-2015), fluconazole MIC90 and GM MIC values increased between 2016-2019. The percentage of isolates with fluconazole MICs ≥32 and ≥64 mg/ml also increased between these two periods. Similar results were also observed with itraconazole between these two periods. In contrast, both fluconazole and itraconazole MIC90 and GM MIC values and the percentage of isolates with elevated MICs decreased markedly during the 2020-2023 time-period, although the number of isolates tested during between the different period remained relatively steady. When assessed on an annual basis, the percentage of isolates with elevated fluconazole MICs was highest between 2011-2019 and those for itraconazole between 2011-2017, with marked reductions observed for both azoles observed in the subsequent years.

Conclusion: Fluconazole and itraconazole MIC90 and GM MIC values and the percentage of isolates with elevated MICs (i.e., reduced susceptibility) fluctuate over time. The percentages of isolates with reduced susceptibility have recently decreased and have been at their lowest levels since 2010. The reasons for the fluctuations are currently unknown.

5. *In vivo* Effectiveness of Fluconazole and Posaconazole Against *Coccidioides posadasii* Meningitis Caused by Fluconazole Resistant Isolates

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Abstract

Introduction: Coccidioides species are endemic to the desert southwest of the United States, Northern Mexico, and in parts of Central and South America. *Coccidioides* meningitis is associated with significant morbidity and mortality. Treatment generally consists of long-term, high-dose fluconazole. However, reduced fluconazole *in vitro* susceptibility (MIC 16 mg/L) and fluconazole resistance (MIC ≥32 mg/L) has been reported to occur in the U.S. Correlations between *in vitro* susceptibility and *in vivo* outcomes are not well established. Our objective was to evaluate the *in vivo* effectiveness of fluconazole and posaconazole, including supratherapeutic doses, against fluconazole-resistant *C. posadasii* strains in an established murine model of CNS coccidioidomycosis.

Methods: *C. posadasii* clinical strains DI23-1 (cultured from a patient in Colorado) and DI23-1 (Texas) were used (fluconazole MICs ≥64 mg/L, posaconazole MICs ≤0.125 mg/L). Infection was established in immunocompetent mice via intracranial inoculation with arthroconidia. Oral therapy with vehicle control, fluconazole (25 mg/kg QD or 25 mg/kg BID) or posaconazole (10 mg/kg QD or 25 mg/kg BID) began 48 hours post-inoculation and continued for 7 days in the fungal burden arm and 14 days in the survival arm. Fungal burden was assessed by colony-forming unit (CFU) enumeration. In the survival arm, mice were followed for two weeks off therapy until day 30.

Results: Against infections caused by either *C. posadasii* strain, both median survival (>30 days) and percent survival to day 30 (range 80% - 100%) were significantly improved with either posaconazole dose compared to control (median survival 9 - 10.5 days, 0% percent survival; $p<0.001$ for all comparisons). Both fluconazole doses also enhanced median survival (13.5 - 25.5 days; $p<0.001$) compared to control, but percent survival (range 0-30%) was not improved. Fungal burden demonstrated greater variability between the strains and different doses of each antifungal. Against DI23-1, CFU counts on day 9 in mice treated with either fluconazole dose (range 3.87 - 4.40 $\log_{10}$ CFU/g) were not significantly different compared to the vehicle control group (4.91 $\log_{10}$ CFU/g) but were significantly lower with either posaconazole dose (0.0 - 1.09 $\log_{10}$ CFU/g; $p<0.01$). Against DI23-2 reductions in fungal burden were similar with both fluconazole doses and posaconazole 10 mg/kg QD (range 2.16-3.25 $\log_{10}$ CFU/g) and were lower than vehicle control (5.10 $\log_{10}$ CFU/g; $p<0.01$). The supratherapeutic dose of posaconazole had the lowest fungal burden (0.66 $\log_{10}$ CFU/g). In the survival arm, rebounds in fungal burden were observed for each azole against each isolate once therapy was stopped.

Conclusions: Survival was moderately enhanced with fluconazole as were reductions in fungal burden. However, the majority of mice succumbed to infection once therapy was stopped and rebounds in fungal burden were observed. Survival was markedly enhanced with posaconazole, which resulted in greater reductions in fungal burden with the supratherapeutic dose. Further studies are needed to determine if posaconazole therapy may be effective against coccidioidomycosis caused by fluconazole-resistant strains.

6. Toward Better Treatments: Investigating Fungal Secreted Proteases in Coccidioidomycosis

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Abstract

Introduction: *Coccidioides*, a dimorphic fungal pathogen endemic to the Southwest United States, Central, and South America, infects and kills immunocompetent individuals. Limited treatment options render coccidioidomycosis, the infection caused by *Coccidioides*, a cause of unacceptably high morbidity and mortality. We urgently need better treatments. *Coccidioides'* virulence stems from its unique and poorly understood host form, the spherule. Arthroconidia are inhaled by the host and develop into spherules. Once mature, a spherule is filled with hundreds of endospores. Spherules then rupture and disseminate endospores within the host in a process known as spherulation. As spherulation is unique to *Coccidioides*, most spherule and endospore biology remains unknown. Interestingly, the *Coccidioides* genomes encode two expanded families of proteases, the subtilases and the deuterolysins, that we hypothesize are involved in spherulation and are promising treatment targets for coccidioidomycosis. Protease inhibitors have been successfully developed as treatment for multiple diseases, from HIV to cancer, but their promise has not yet been leveraged for fungal infections.

Methods: To demonstrate the biologic significance of these protease families, we studied spherulation in the presence of AEBSF (a serine protease inhibitor) and 1,10-phenanthroline (a metalloprotease inhibitor) and used microscopy to characterize the developmental consequences of protease inhibition. We profiled the *Coccidioides* transcriptome and examined deuterolysin and subtilase expression over the entire spherulation cycle in triplicate samples, in Converse media, at 39˚C, 10% CO_2. This included timepoints with endospore release, whose transcriptome has only been sparsely sampled previously. We also examined deuterolysin and subtilase expression in mycelial growth in Converse media. Using multiplex substrate profiling-mass spectrometry on supernatants from protease deletion mutants or wildtype, we characterized total amount of secreted protease activity in these samples and amino acid preferences around sites of proteolysis.

Results: Treatment of *Coccidioides* with a protease inhibitor that blocks subtilase activity prevented spherule formation, indicating for the first time the importance of the subtilases in the development of the parasitic phase of *Coccidioides* biology. We have found that subtilase and deuterolysin expression is induced upon spherule formation, in four patterns: 1) during early formation of spherules, 2) continuous increase over the spherulation cycle, 3) two distinct peaks of expression in arthroconidia and endospore stages, or 4) dramatic increase in expression at the time of endospore release. One subtilase, Sub1, fits the fourth pattern, and is in fact one of the top 10 most abundant transcripts in the spherule at the time of endospore release. We have shown that Sub1 is a major contributor to spherule secreted protease activity and exhibits a preference for hydrophobic substrates. Phenotypic characterization of this mutant is underway, including virulence studies, and molecular work is ongoing to determine the substrates of these two protease families.

Conclusion: By linking protease activity to spherulation, we have demonstrated the importance of further dissecting the role of these two expanded protease families in *Coccidioides* virulence. Furthermore, these results support inhibition of the subtilase family, or possibly Sub1 itself, as a possible therapeutic strategy for coccidioidomycosis.

7. Creating a Precision Disease Risk Model For Coccidioidomycosis Using Historic and Current Genomics and Environmental Data

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Abstract

Introduction: Current maps used to estimate the endemic area for coccidioidomycosis are primarily based on clinical data, which suffers from underreporting and spatial biases. Few studies have used soil properties or limited environmental samples of *Coccidioides* to create niche maps. Our objective is to build upon that work to create a precision disease risk model that uses genomics and environmental modeling for both *Coccidioides* spp., and *C. immitis* and *C. posadasii* separately, comparing the results amongst models. To do so, we will use historical and newly collected rodent and soil data from Utah, a historically understudied area for coccidioidomycosis. This approach will help us identify differences in environmental drivers between the species, better identify which communities are at risk of contracting coccidioidomycosis, and enable projections of each *Coccidioides* species in response to climate change.

Methods: DNA will be extracted from historical rodent liver and spleen samples collected in an area hypothesized to have *Coccidioides*, the Great Basin Desert, Utah. We will resample soils and rodents from 2024-2026 at the same locations. PowerSoil® DNA isolation kit and PCR assay CocciDX will be used respectively, and ITS sequencing will be used to detect fungal pathogens. These sequences, and *Coccidioides* sequences from NCBI GenBank, will be processed in QIMME2 and EDGE to characterize the fungal communities. We will use the ecological niche model Maxent to create habitat suitability maps for both species, and statistical block design will determine differences in where *Coccidioides* exists. A Bayesian hierarchical model will combine soil and rodent genomic data to determine where coccidioidomycosis is endemic using environmental variables like climate, land cover, habitat, soil type, geology, and elevation. Phylogeography data will be used to create a multilayer spatio-temporal model to differentiate between the two *Coccidioides* species in a Bayesian hierarchical model framework. We will use these models to estimate the future geographical range of each *Coccidioides* species in response to climate change.

Results: This project began in October 2023 and we are beginning to process the historical data. Resampling will happen in Summer 2024. The historic rodent collection from 2014-2016 has approximately 680 individual rodent spleen and liver samples (16 species), collected from 12 locations in the Great Basin Desert in Utah. Habitats included sagebrush, pinyon-juniper, and the transition between sagebrush and pinyon-juniper. Locations were in an elevation range of 5100-7800 ft.

Conclusion: Changing climate poses the risk of extending the area of endemicity as well selecting for more virulent antimicrobial strains of fungal pathogens. A species-partitioned risk map of coccidioidomycosis that includes information on potential rodent reservoir species will more accurately estimate current and future areas of endemicity, specify dissimilarities between species, and may help inform spatially varying clinical outcomes.

8. Siloed Research Units: A Promising Strategy for Boosting Enrollment and Research Quality in Coccidioidomycosis Studies

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Abstract

Introduction: The UC Davis Infectious Diseases Research Unit (IDRU) supports both clinical trials and non-interventional research including several coccidioidomycosis studies. As part of our portfolio, many of our studies are investigator-initiated and as a Sponsor-Investigator, the Principal Investigator (PI) takes on the responsibilities of a Sponsor, which include monitoring the study data. The monitoring process ensures human research subject protections and verifies study data integrity, while also ensuring that the study site is compliant with external and internal regulations such as ICH GCP, IRB, protocol adherence, and institutional policies. In late 2022, our site restructured the IDRU by implementing a silo of duties that included the creation of a Regulatory and Quality Assurance team (RQA), a group of internal specialists. This transition removed regulatory pressure from our clinical team and resulted in a significant increase in enrollment numbers.

Methods: We obtained the enrollment numbers from continuing review packages of IRB-approved Coccidioidomycosis studies and compared the values between 2022 and 2023. The percent change was calculated using the formula $((V2-V1)/|V1|)\times100$ where V1 = enrollment numbers in 2022 and V2 = enrollment numbers in 2023.

Results: After 13 months of implementing the silo, our enrollment rate into investigator-initiated coccidioidomycosis studies increased by 142.857%.

Conclusion: Moving to a siloed research unit has increased productivity and boosted enrollment, maintaining a consistently high standard for all research in our division. A major component of the RQA role has been the development of Quality Assurance monitoring workflows that streamline the internal monitoring process and help keep it consistent across studies. The UC Davis IDRU can provide these workflows and other resources to assist research sites in developing a sustainable clinical research program that aims to improve coccidioidomycosis research quality.

9. Combining Unconventional Drugs with Current Treatments to Combat *Coccidioides*

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Abstract

Introduction: Clinical isolates of *Coccidioides* have displayed a trend of increasing resistance to fluconazole. To ameliorate this, we need new antifungal drugs with novel targets. We have discovered several new compounds that inhibit spherule-phase growth by screening FDA-approved drugs from four chemical libraries. By repurposing these drugs and subsequently leveraging synergistic or additive effects, we expect that low-dose combination therapy may ultimately be more efficacious than high-dose monotherapy. This approach minimizes individual drug toxicity, reduces adverse reactions, and allows clinicians to tailor treatments to enhance efficacy or avoid drug interactions. Diverse mechanisms of action in combination therapies may lower the risk of resistance compared to monotherapy, as seen in the case of flucytosine for invasive candidiasis, where concurrent use with amphotericin B (AmB) prevents rapid resistance acquisition and increases their antifungal activity. We aim to identify novel anti-*Coccidioides* compounds that can be repurposed and further developed into chemotherapy for use alone or in combination with other clinically approved drugs against coccidioidomycosis.

Materials and Methods: In a BSL-3 laboratory, arthroconidia of *Coccidioides* isolates (*C.p.*: C735, Silveira, and 3488; *C.i.*: RS and 2394) were cultured in Converse media for 24h to facilitate their conversion into spherules. Spherules of C735 were dispensed into each well of a drug plate containing up to 10μM of the library compounds then subject 24h later to an XTT assay for determining cell metabolic capacity as an indicator of growth. The metabolic capacity of spherules incubated with 1% DMSO (drug vehicle) served as a control. We have completed the screening of four drug libraries consisting of 7962 drugs total (Broad Repurposing, Prestwick Chemicals, Selleck L8200, and MCE CNS Penetrants). The potency of hit compounds was verified using dose-response curves followed by checkerboard synergy assays to assess the compound's therapeutic potential when combined with conventional antifungals including polyenes, triazoles, and echinocandins. To assess drug function and viability, the drug-treated spherules were stained for image flow cytometry using combinations of the following dyes: calcofluor white (cell wall), Syto9 (nucleic acids), propidium iodide (nucleic acids if compromised cell wall), and/or mitotracker red CMXros (oxidized mitochondrial membranes). Preliminary *in vivo* efficacy and dose optimization were performed in a *Galleria mellonella* over 7d with three hemocoel injection treatments following infection. Finally, the pan-isolate potency of the compounds was assessed in four additional clinical isolates, two from each species, in matrixed checkerboard synergy assays.

Results: Of the 7962 drugs screened 254 candidates exhibited ≥ 70% inhibition and 172 candidates had highly confident anti-*Coccidioides* activities with B-scores ≤ -3. A subset of 27 diverse compounds (0.53% hits) demonstrated both ≥ 70% inhibition and a B-score ≤ -3. We also examined three additional compounds that were effective against *Cryptococcus* and *Candida* for a total of 30 drugs to further characterize. Twelve drugs had IC50 < 5μM. Six of these drugs exhibited moderate synergy with AmB at concentrations < 10μM and reacted variably against the triazoles and echinocandins with moderate additivity. Image flow cytometry suggests that these six drugs are operating through alternative mechanisms inconsistent with AmB based on differences in spherule area, cell wall integrity, nucleic acid localization, and mitochondrial oxidation. Drugs were considered effective in *Galleria* if they increased survival by 50% compared to drug-free controls.

Conclusion: Our synergy assessments have successfully identified 6 diverse, novel antifungal compounds that demonstrate synergy with conventional antifungal treatments within therapeutically relevant ranges. Using combination therapy, these drugs could increase treatment efficacy while minimizing individual drug toxicity and reducing the risk of antifungal resistance. Pan-isolate activity and a literature review of the drugs in other fungi suggest these hits may have broad pan-fungal applications.

10. Prevalence of Atopic Disorders Among Pediatric Patients with Pulmonary and Disseminated Coccidioidomycosis

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Abstract

Introduction: Type 2 inflammatory responses, particularly elevated IgE and eosinophilia, are associated with worse prognosis in coccidioidomycosis. It is unclear whether patients with pre-existing type 2 inflammation and atopic disorders are predisposed to disseminated coccidioidomycosis.

Methods: We conducted a retrospective chart review of patients who were diagnosed with disseminated coccidioidomycosis and evaluated in the Phoenix Children's complex coccidioidomycosis clinic from 2019 to 2022. Approval for the study was granted by the Phoenix Children's Institutional Review Board. The study population included both sexes from birth to 17 years of age, at the time of diagnosis. Patients who visited the pulmonology clinic for a coccidioidomycosis diagnosis outside of the specified time range were excluded from the study. We collected demographic information and clinical data, including date of diagnosis, laboratory findings, treatment, body mass index (BMI), and history of atopic disorders. Laboratory findings were considered for analysis if obtained within 7 days of coccidioidomycosis diagnosis. Atopic disorders recorded included atopic dermatitis, classic immunoglobulin (Ig) E mediated food allergies, asthma, and/or allergic rhinitis. Pediatric patients with disseminated coccidioidomycosis were matched 1:1 by age, sex, and race to patients with isolated pulmonary coccidioidomycosis. Demographic and epidemiological characteristics were analyzed using basic descriptive statistics and non-parametric tests. Ninety-five percent confidence intervals (CIs) were calculated, and P-values less than 0.05 were considered statistically significant.

Results: 35 patients in each arm were identified (median age=13). There was an even distribution of sexes in the study cohort, with 18 male subjects and 17 female subjects. 36% of total patients self-identified as Caucasian, 33% African American, 24% Hispanic, 6% Native American, and 1% Pacific Islander. The prevalence of atopic diseases in the disseminated and non-disseminated cohorts were higher than the prevalence of reported atopic diseases in the general population (28.57% in disseminated vs 37.14% in non-disseminated versus 27.20% in general pediatric population1). There was no difference in the prevalence of atopic history between the disseminated and non-disseminated groups (odds ratio: 0.7, [95% CI, 0.2-1.8], p = 0.4) (Table 1). In the disseminated group, 3 out of 25 had elevated absolute eosinophil counts (AEC)>0.5K/μL(median=0.2, IQR, 0.1-0.3). In the pulmonary group, 5 out of 24 had elevated AEC (median=0.2, IQR, 0.1-0.7). The difference in AEC between the two groups was not significant (p=0.3). No IgE levels were drawn in the disseminated group. 2 out of 3 patients had elevated IgE in the pulmonary group. Absolute eosinophil count and IgE levels did not correlate with atopic history.

10. Prevalence of Atopic Disorders Among Pediatric Patients with Pulmonary and Disseminated Coccidioidomycosis *(continued)*

	Disseminated (n=35)	Non-Disseminated (n=35)	Odds Ratio	95% Confidence Interval	P value
Atopic Disorder	10	13	0.7	0.2-1.8	0.4
Food Allergies	2	2	1	0.1-7.5	1
Asthma	3	8	0.3	0.08- 1.3	0.1
Atopic dermatitis	4	2	2	0.4-12.5	0.4
Allergic rhinitis	3	6	0.5	0.1-2.0	0.3

Table 1. Proportions of allergic disorders between patients with disseminated versus non-disseminated coccidioidomycosis.

Conclusion: There was no significant difference in history of atopic disorders and eosinophilia on coccidioidomycosis diagnosis in pediatric patients. However, there is a trend for increased prevalence of atopy among all patients with coccidioidomycosis versus the general pediatric population. Tissue-specific type 2 responses may still play a role in coccidioidomycosis disease immune dysregulation.

Reference:

1. Zablotsky B, Black LI, Akinbami LJ. Diagnosed allergic conditions in children aged 0–17 years: United States, 2021. NCHS Data Brief, no 459. Hyattsville, MD: National Center for Health Statistics. 2023.

11. Real-World Diagnostic Performance in the Diagnosis of Coccidioidal Meningitis

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Abstract

Introduction: Coccidioidal meningitis is one of the most feared complications of coccidioidomycosis. Once diagnosed, it is considered an incurable infection requiring lifelong antifungal therapy and management. Making the diagnosis of coccidoidal meningitis is nuanced and can be easily missed or delayed. Due to the wide variation in presentation and workup, we sought to define which tests were the most often helpful in making the diagnosis.

Methods: Patients were identified by searching the ICD-10 and -9 codes for coccidioidal meningitis B38.4 and 114.2 respectively. Retrospective analysis was conducted by chart review. We included patients with proven and probable coccidioidal meningitis, and excluded those with an alternative cause of meningitis or any who did not meet the inclusion criteria. We recorded the results from the first cerebrospinal fluid (CSF) tests and the accompanying peripheral labs and summarized these labs as percentage positive.

Results: Between 6/1/1998 and 12/31/2023, we identified 106 patients for this study, with 85 (80.2%) proven and 21 (19.8%) probable coccidoidal meningitis. Of this cohort, 82 were male (77.5%) with a median age 60 years (range 18-90). Most patients included were white (78.3%) or black (9.4%) and not immunosuppressed (89.6%). 23.6% had diabetes mellitus. Direct coccidioidal testing with CSF PCR, CSF antigen and CSF culture were positive in 11/59 (18.6%), 6/17 (35.3%), and 4/84 (4.8%), respectively. CSF serologic testing for IgG was positive as follows: EIA IgG 39/51 (76.5%), immunodiffusion (ID) IgG 57/92 (62%), and complement fixation (CSF) 66/99 (66.7%) with a median CSF titer of 1:8 (1:2 - 1:256). Combined CSF testing with serologies, PCR, and coccidioidal antigen gave a combined sensitivity of 84/103 (81.6%). Peripheral serologies were positive for IgG by EIA (88.6%), ID (83.3%) and CF (78%), and adjunctive beta-D-glucan was positive in 55.5% of patients tested with a median value of 132.

Conclusion: Coccidioidal meningitis remains a difficult diagnosis to make despite advances with new testing modalities. We found that CSF serology still remains the best stand-alone diagnosic assay, however, a combined approach that utilizes CSF serology, antigen, PCR and culture offers the highest yield.

12. Screening the ChemDiv Antifungal Drug Library Against Parasitic Spherules of Coccidioides posadasii

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Abstract

Introduction: Coccidioides spp cause a severe and challenging fungal infection called coccidioidomycosis, commonly known as “Valley Fever”. This disease is often-overlooked but it is now getting increased attention due to its expanding geographic range and rising incidence rates. Current antifungal therapies are limited in their efficacy, and further complicated by the emergence of drug resistance. Thus, there is a growing urgency to explore the new treatment strategies. One of the promising approaches is high-content screening of drug libraries against *Coccidioides*, aiming to identify compounds with potent antifungal properties. In the present study we aimed to explore potential antifungal compounds from ChemDiv antifungal drug library against spherules, the parasitic form of Coccidioides.

Materials and Methods: A total of 10,000 compounds within the ChemDiv antifungal drug library were screened to identify those with antifungal activity. The screening of compounds was performed against the mutant strain (ΔT) of *Coccidioides posadasii* strain that can be cultured in BSL2 laboratory, using a metabolic XTT assay. Briefly, 24 hr spherules were incubated in the presence of a fixed concentration of drugs at 10 µM to identify those compounds with growth-inhibitory activity of the parasitic cells. Confirmatory dose-response assays were used to validate the activity of the initial hits and at the same time establish their potency.

Results: The primary screening identified a total of 35 initial hit compounds inhibiting >45% of growth and with a B score value of ≤ 4. These compounds were representative of a diverse range of chemical scaffolds including azoles, sulfonamides, 2-3-$_D$-pyrimidines, carboxamides, and triazines. The top active compounds are listed in the Table below.

12. Screening the ChemDiv Antifungal Drug Library Against Parasitic Spherules of Coccidioides posadasii *(continued)*

Table: List of highly active compounds against *Coccidioides posadasii* (ΔT)

S. No.	Scaffold	Compound ID	% Inhibition	IC_{50} (μM)	B-Score*
1	pyrazole	G657-0369	71.56	0.79	-6.52
2	pyrazole	D491-9916	47.70	1.21	-4.90
3	pyrazole	G657-0368	65.62	1.47	-5.80
4	pyrazole	G657-0345	71.56	1.63	-6.51
5	pyrazole	G657-0347	68.90	1.65	-6.25
6	triazole	G652-4168	69.84	1.70	-6.29
7	sulfonamide	D430-1300	83.32	1.85	-6.40
8	sulfonamide	D430-1301	88.25	1.98	-6.94
9	piperazine	V006-6647	72.51	1.98	-6.21
10	carboxamide	G656-1079	63.59	2.34	-5.47

*B-score represents a statistical analysis of confidence of relative potency from the raw sample values from primary screen.

Conclusion: The utilization of spherules isolated from the live-attenuated strain for the screening of compounds has proven to be effective for large-scale screenings. A total of 35 drug-like compounds with anti-*Coccidioides* activity have been identified with inhibitory activity against spherule growth. Further studies such as structural elucidation, toxicity assessment, pharmacokinetic studies, metabolism and biotransformation studies, and efficacy are underway to characterize them for potential clinical development.

13. Launching a Breath Analysis Study to Identify Volatile Biomarkers for Valley Fever

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Abstract

Introduction: In the United States alone an estimated 150,000 – 350,000 people contract Valley fever (VF) each year, with about 60% of those cases occurring in Arizona.[1] However, our ability to diagnose VF is limited due to the poor sensitivity and specificity of existing diagnostic tests, especially in early infection.[2] The result is an increased time-to-diagnosis for VF, inappropriate or delayed treatment, and poorer patient outcomes.[2] Our goal is to address the need for novel VF diagnostics through the development of sensitive, specific, and non-invasive breath-based diagnostic tests. Building off of preliminary data from mouse models[3] and human lung specimens from community-acquired pneumonia (CAP) patients, which demonstrated that VF infections have a unique profile of volatile organic compounds (VOCs) that distinguish coccidioidomycosis from other forms of CAP, we are initiating a pilot human subjects study to identify breath biomarkers for VF. We hypothesize that a panel of VOCs in breath will be able to discriminate subjects that are infected with primary pulmonary VF from subjects with other CAP, including subjects who had an initial false-positive EIA IgM for VF. To test this hypothesis, we will perform untargeted volatile analysis on human breath samples from patients with VF or CAP and utilize machine learning methods to identify discriminatory VOC biomarkers.

Methods: This study will be conducted with approval by the Mayo Clinic Institutional Review Board. Fifty six subjects, male or female and ≥18 years old, will be enrolled at Mayo Clinic Arizona and split into two diagnostic classes, confirmed or probable Valley fever (VF CAP; n = 28) and community-acquired pneumonia (non-VF CAP; n = 28). Breath samples will be collected and directly transferred onto two thermal desorption tubes (TDTs; Supelco Tenax GR) for storage and transport to Arizona State University for volatile analysis. The human breath samples will be analyzed by comprehensive two-dimensional gas chromatography linked to time-of-flight mass spectrometry (GC×GC–TOFMS). We will utilize Random Forest (RF) machine learning algorithm to identify putative VF biomarkers that discriminate VF CAP from non-VF CAP samples. Different RF models will be used to evaluate the sensitivity and specificity of the biomarker panel for classifying VF CAP from non-VF CAP samples.

13. Launching a Breath Analysis Study to Identify Volatile Biomarkers for Valley Fever *(continued)*

Expected Outcomes: We expect to identify a set of putative VF breath biomarkers capable of distinguishing VF from other forms of CAP. Further, for subjects who were diagnosed with possible VF based upon a positive EIA IgM only, and who did not seroconvert with antifungal treatment (i.e., false positive for VF), we expect that they will be classified as VF-negative by the breath VOC biomarkers. The pilot data gathered from this study will inform our next steps to develop a breath test to rule in or rule out VF in patients with CAP symptoms.

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14. The Unknown "Knowns" of *Coccidioides* Susceptibility

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Abstract

Coccidioidomycosis occurs after inhalation of airborne spores of the endemic, dimorphic fungus, *Coccidioides*. While the majority of individuals resolve the infection without coming to medical attention, the fungus is a major cause of community acquired pneumonia in the endemic region. Chronic pulmonary and extra-pulmonary disease poses significant personal and economic burdens. Frequently noted risk factors include immunosuppression, HIV+/AIDS, corticosteroid treatment, pregnancy, male sex, or ancestry, specifically African American or Filipino.

Commonly these risk factors are cited without recognizing their origins, putting them in context of newer, larger work, or questioning the strength of evidence. Using examination of historical reports coupled with recent cohort and epidemiology studies, themes surrounding risk factors impacting susceptibility to chronic pulmonary disease or dissemination are reviewed including immune suppression, genetic susceptibility, sex, pregnancy, and ancestry. The evidence for commonly reported risk factors is evaluated, leading to a suggestive link between male sex and pregnancy. Race/ethnicity is a convenient, but inaccurate, risk factor given the extensive admixture within the US population and the lack of disease prevalence among recently arrived individuals from African countries. Experimental approaches to address this issue are proposed as well as biologically relevant, but as yet untested, mechanisms underlying the race/ethnicity argument.

15. Pediatric Coccidioidomycosis – Experience at an Academic Pediatric Infectious Disease Clinic in Southern Arizona

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Abstract

Introduction: Coccidioidomycosis in children is not well described. Our current understanding of the clinical manifestations, laboratory findings, and radiographic characteristics of complicated pulmonary and extrapulmonary pediatric coccidioidomycosis is largely based on small case series and extrapolation from adults. Over 800 pediatric encounters for coccidioidomycosis were identified at the University of Arizona - Banner University Medical Center in Tucson, AZ (BUMCT), between 2013 – 2023. For the current study, we included children seen in 2023.

Methods: Criteria included: age under 18, seen in 2023 by at least one pediatric infectious diseases faculty member, either as an inpatient or outpatient at BUMCT, with a diagnosis of coccidioidomycosis in the consult note. Cases were excluded if the electronic medical record did not document at least one of the following supporting diagnostics: positive coccidioidal IgM or IgG by EIA, cultures positive for Coccidioides, or histopathological findings characteristic of coccidioidomycosis. IRB approval for retrospective chart review was obtained through the University of Arizona.

Results: Twenty-eight patients were included in the study. Sixteen (57%) were male, 20 (71%) were white or Caucasian, and 3 (11%) were black or African-American. Eleven (39%) were of Hispanic or Latino ethnicity. The youngest age at diagnosis was 4 months, and the oldest was 17 years, with a median age of 11.9 years.

Twenty-seven patients had a chest x-ray; 10 were normal while 17 had abnormal findings, including pleural effusion (n=6), consolidation/opacities (n=11), nodules (n=3), lymphadenopathy (n=3), cavitation (n=2), and pneumothorax (n=2).

The median white blood cell count was 8.9 k/uL, and median absolute eosinophil count was 200/uL (reference 0-600/uL). At time of diagnosis, 20 (71%) patients had a positive EIA IgG and 13 (46%) had a positive EIA IgM.

15. Pediatric Coccidioidomycosis – Experience at an Academic Pediatric Infectious Disease Clinic in Southern Arizona *(continued)*

Twenty (71%) patients had pulmonary disease only. Of these, 7 (35%) had a normal chest x-ray; other findings included consolidations, pleural effusions, nodules, lymphadenopathy, cavitation, and pneumothorax. The absolute eosinophil count was elevated (above 600/uL) in only 5 patients. Fourteen patients (70%) had a positive EIA IgG; 6 (30%) had a negative EIA IgG. The most common presenting symptoms for isolated pulmonary disease included respiratory symptoms (n=13), followed by fever (n=12), rash (n=7), poor feeding (n=6), and headache (n=4).

Of the 8 patients with disseminated disease, 5 had musculoskeletal involvement, 2 had central nervous system involvement, 3 had skin involvement, and 1 had pericardial involvement. Seven of the eight individuals with disseminated disease had chest x-rays performed; 3 chest x-rays were normal, 2 had nodules, 1 had consolidation only, and 1 had consolidation and a pleural effusion. Absolute eosinophil count was only elevated in one of the eight individuals at 1,340/µL. Six of 8 patients had EIA IgG performed; 3 were positive, 2 were negative, and 1 was indeterminate. The most common presenting symptoms for disseminated disease were discrete skin lesions (n=3) and respiratory symptoms (n=3), followed by musculoskeletal symptoms (n=2), headache (n=2), and poor feeding (n=2). Only one patient presented with fever.

Conclusion: Pediatric coccidioidomycosis affects children of all ages, from infants to adolescents. Findings distinct from those described for the adult population include normal chest radiographs in those with pulmonary disease and a higher frequency of negative serologies and normal eosinophil counts in both pulmonary and disseminated cases. Coccidioidomycosis should therefore be considered in children in the appropriate clinical setting, even with negative serologies, normal chest x-ray, or normal eosinophil count. This study is only a one-year subset of data from over 800 encounters; we plan to analyze these in future studies.

16. A Climate-driven, Seasonal Forecast of Relative Coccidioidomycosis Risk in Four Arizona Regions

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Abstract

Introduction: Interannual variability in climate conditions, especially precipitation levels, is known to affect the timing and number of coccidioidomycosis cases. Using climate conditions as drivers, we may be able to assess the relative risk of coccidioidomycosis cases. In this study, we analyzed previous climate conditions and disease incidence data from 2000 to 2019 to create a regional-level forecast model for coccidioidomycosis risk in Arizona, one of the highest endemic regions.

Methods: We collected coccidioidomycosis data at the county-level from 2000-2019 for Arizona from the US Centers for Disease Control and Prevention (CDC) National Notifiable Disease Surveillance System through a data use agreement with the CDC. We processed climate data from NASA's GLDAS dataset, including precipitation, dust mass, and temperature data based on biological hypotheses from literature review and performing a correlation analysis. We grouped Arizona counties into four regions based upon similar climate conditions and patterns of coccidioidomycosis incidence using time series decomposition and analyzing monthly quartiles of data. Based on patterns we identified, we grouped our monthly data into forecast seasons. We calculated the expected levels of coccidioidomycosis incidence in each season and region based on regional quartiles. Then, we created a model to predict relative coccidioidomycosis risk (normal versus higher than normal) for each region-season.

Results: We partitioned Arizona into four regions: North (Coconino, Navajo, and Apache Counties), South Central (Gila, Maricopa, Pima, and Pinal Counties), Southeast (Greenlee, Graham, Santa Cruz, and Cochise Counties), and West (Mohave, Yavapai, La Paz, and Yuma Counties). We divided the forecast into four seasons: winter (January, February, and March), spring (April, May, and June), summer (July, August, and September), and autumn (October, November, and December). Of the four regions, the South Central region had the highest mean incidence in each season. During the winter, South Central had a mean incidence of 9.4 cases per 100,000 population. The other three regions of Southeast, West, and North had lower mean winter incidences of 2.2 per 100,000 population, 1.9 per 100,000 population, and 1.7 cases per 100,000 population respectively. We are actively working on building the predictive models.

Conclusion: We are working with the Arizona Department of Health Services to best construct a meaningful forecast model. Our model may be useful for making public health decisions regarding the education and awareness of symptoms, testing, and risk factors for coccidioidomycosis for both healthcare professionals and the public.

17. Evaluating Immunogenicity of an Eisosome Component Identified in *Coccidioides posadasii*

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Abstract

Introduction: Protection against coccidioidomycosis is associated with the activation of Th1- and Th17-type $CD4^+$ T-cell responses following antigen-presenting cell (APC) presentation of peptides through the major histocompatibility complex class II (MHC-II). Identification of *Coccidioides* antigens with short peptide epitopes that can bind to human MHC-II molecules and stimulate T cell responses is essential for developing a human vaccine and diagnostic tools for coccidioidomycosis. Ideal antigen candidates should lack human or murine homologs, exhibit significant protein expression during spherule development, contain immunogenic peptide epitopes binding to MHC-II molecules, and stimulate the immune system to build a memory Th1 and Th17 response. Through immunoproteomic analysis, we have previously determined that an eisosome core protein known as Lsp1 is reactive with coccidioidomycosis patient sera, suggesting its potential as an antigen. Here, using glucan-chitin particles (GCPs) as a delivery system and adjuvant, we evaluate the T cell stimulating activity of Lsp1 and identify the key immunogenic epitopes within this protein using transgenic mice (Tg) expressing the human HLA-DRB1*04:01 (DR4) allele.

Materials and Methods: A multiple sequence alignment using available Lsp1 protein sequences of *Coccidioides immitis*(*Ci*) and *Coccidioides posadasii* (*Cp*) isolates was used to assess homology. *LSP1* gene expression in *Coccidioides* culture and infected murine lungs was assessed via qRT-PCR, and protein expression was subsequently confirmed through Western blot analysis. Recombinant Lsp1 (rLsp1) was produced using an *E. coli* pET expression system, purified via Ni-NTA affinity chromatography, and loaded into glucan-chitin particles (GCP) to create a subunit vaccine (10 μg rLsp + 200 GCPs /dose). We synthesized an overlapping peptide library derived from Lsp1, consisting of 15-mer peptides with an 8-amino acid overlap, totaling 49 peptides. Two groups of HLA-DR4 Tg mice were vaccinated subcutaneously three times at a 14-day interval with either the vaccine (GCP-rLsp1) or adjuvant alone (GCP-MSA). Four weeks post-final boost, splenocytes were isolated and restimulated *in vitro* with rLsp1 or the Lsp1 peptide library to determine the number of antigen-specific Th1 cells by IFN-γ ELISPOT and total IFN-γ production by ELISA.

17. Evaluating Immunogenicity of an Eisosome Component Identified in *Coccidioides posadasii (continued)*

Results: Lsp1 is conserved among *Cp* and *Ci* isolates with 100% identity. *In vitro LSP1* gene expression during various *Coccidioides* developmental stages was at least 2-fold lower than that of *GADPH*, while in the infected mouse samples it was less than a fold lower than *GADPH.* In HLA-DR4 mice, GCP-rLsp1 vaccination generated a significant amount of rLsp1-specific Th1 cells, averaging 992 CD4 T cells per $5x10^5$ splenocytes compared to mice immunized with GCPs alone, as determined by IFN-γ ELISPOT. In line with this, restimulation of splenocytes from GCP-rLsp1-vaccinated mice with rLsp1 resulted in robust IFN-γ production compared to control mice. Upon restimulation of splenocytes from GCP-rLsp1-vaccinated HLA-DR4 Tg mice with the Lsp1 peptide library, we found that 3 of the 49 peptides induced an average of 153 antigen-specific IFN-γ-secreting Th1 cells per $5x10^5$ splenocytes. In contrast, we could not detect IFN-γ-secreting cells in mice immunized solely with the adjuvant.

Conclusions: These findings demonstrate that rLsp1 contains immunogenic epitopes that can stimulate potentially protective Th1 memory responses. Our future goal is to determine whether these peptides encapsulated in GCPs can confer protection for an HLA-DR4 Tg mouse model of pulmonary coccidioidomycosis.

18. Evaluating the Efficacy of an mRNA Vaccine Against Coccidioidomycosis

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Abstract

Introduction: Development of a vaccine against *Coccidioides* infection is an attractive strategy to control coccidioidomycosis. Previous work in our lab using transgenic mice expressing the human MHC class II allele DRB1*04:01 (HLA-DR4 Tg mice) has shown that immunization with the multivalent antigen rCpa1 loaded into glucan-chitin particles (GCPs) as a delivery system and adjuvant is protective in mice intranasally challenged with a potentially lethal dose of *Coccidioides* spores. In this study, we further assess the durability of this GCP-rCpa1 vaccine, demonstrating longevity of roughly 1 year as shown by splenocyte $CD4^+$ T cell ELISPOT assay. We also intend to develop a mRNA vaccine similar to the rCpa1 antigen that can be manufactured quickly and is equally efficacious. Using a GCP-encapsulated mRNA construct encoding a multivalent *Coccidioides*-specific antigen (AgX), we have demonstrated that this delivery system results in uptake and expression of the AgX mRNA construct by antigen-presenting cells (APCs) in both C57BL/6 and HLA-DR4 Tg mice. In this study, we evaluate the protective efficacy of the GCP-AgX mRNA vaccine and the vaccine-induced T cell immunity.

Materials and Methods: C57BL/6 mice (n=3 mice per group) were immunized twice at 14-day intervals with either 5 μg of rCpa1 encapsulated in GCPs or empty GCPs as an adjuvant control by subcutaneous route. Eleven months following the initial vaccination, splenocytes were stimulated with 200 nM rCpa3 and the number of antigen-specific $CD4^+$ T cells was determined by IFN-γ and IL-17A ELISPOT assays. An mRNA construct comprised of the sequence encoding the *Coccidioides*-specific AgX was codon-optimized for expression in mammalian cells and the synthesis was subsequently carried out by TriLink. The resulting mRNA was encapsulated into GCPs at various doses. C57BL/6 or HLA-DR4 transgenic mice were then vaccinated subcutaneously 3 times at 14 day intervals with GCP-AgX mRNA or empty GCPs as an adjuvant control (n=15 mice per group). Four weeks following the last vaccination, vaccinated and unvaccinated mice were challenged with between 40 and 50 spores from the lethal C735 strain. Weight loss was monitored daily after challenge. At 7 and 14 days post-challenge, lung and spleen cell suspensions were cultured at various dilutions on chloramphenicol-containing GYE agar plates to determine pulmonary and splenic fungal burden in each mouse. Additionally, lung cells were mitogenically stimulated *in vitro* and the presence of IFN-γ- or IL-17A-producing T cells was determined by flow cytometry analysis. In HLA-DR4 Tg mice, the presence of Th1 and Th17 cells in the lung was also determined 7 days post-challenge by IFN-γ and IL-17A ELISPOT assays.

18. Evaluating the Efficacy of an mRNA Vaccine Against Coccidioidomycosis *(continued)*

Results*:* In C57BL/6 mice vaccinated with GCP-rCpa1, 11 months following the initial vaccination we were able to detect an average of 324 and 234 antigen-specific CD4 T cells per $1x10^6$ splenocytes by IFN-γ and IL-17A ELISPOT analysis, respectively. In comparison, no IFN-γ- or IL-17A-secreting cells were detected in unvaccinated mice or mice vaccinated with empty GCPs. The newly created mRNA vaccine is stable and easily synthesized. We determined that vaccination of C57BL/6 mice with 5 μg of AgX mRNA encapsulated in GCPs results not only in 30% higher body weight compared to unvaccinated mice but also results in greater than 3-fold reduction in both the pulmonary and splenic fungal burden. By flow cytometry analysis, we found a 5- and 3-times higher percentage of Th17 cells at day 7 and day 14 post-challenge, respectively, in the lungs of C57BL/6 mice vaccinated with GCP-AgX mRNA compared to unvaccinated controls. We achieved similar results upon repeating this study in HLA-DR4 Tg mice. Vaccinatation with GCP-AgX mRNA resulted in 15% higher body weight compared with unvaccinated controls, similar to what we observed in C57BL/6 mice, although this did not reach significance. However, there was a significant reduction in pulmonary and splenic fungal burden by roughly 2- and 5-fold, respectively, compared to unvaccinated controls. At day 7 post-challenge, we found that the percentage and number of Th17 cells was 4-times greater in the lungs of vaccinated mice compared to controls by both flow cytometry analysis and IL-17A ELISPOT analysis, respectively. Currently we are evaluating the impact of GCP-AgX mRNA vaccination on the survival of C57BL/6 or HLA-DR4 Tg mice challenged with the lethal C735 strain.

Conclusions*:* Our findings demonstrate that vaccination with GCP-rCpa1 induces durable memory Th1 and Th17 responses at least 1 year following the initial vaccination. Additionally, we show here in both C57BL/6 and HLA-DR4 Tg mice that our novel GCP-AgX mRNA vaccine is protective against lethal challenge with the C735 strain by reducing weight loss, significantly reducing the pulmonary and splenic fungal burden, and by inducing memory T cell responses associated with protection against *Coccidioides*. Though the current mRNA vaccine is protective, it does not achieve sterile immunity. Therefore, in addition to determining the impact of GCP-AgX mRNA vaccination on survival in these mouse models, our future goal is to further optimize the AgX mRNA construct design to improve protective efficacy of this anti-*Coccidioides* mRNA vaccine.

19. Identification of Coccidioidomycosis Specific Immunoreactive Peptides and the Associated Immune Response

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Abstract

Introduction: Coccidioidomycosis, known as Valley Fever (VF), is a fungal infection leading to notable regional mortality and substantial morbidity. T cell lymphocytes play a pivotal role in orchestrating the immune response against VF. Our objective was to identify immunogenic peptide sequences specific for *Coccidioides* spp. infections.

Methods: We employed two distinct methodologies to identify potential epitopes capable of binding to Major Histocompatibility Complex Class II (MHC II) molecules and stimulating T cells. Firstly, a bioinformatic epitope prediction analysis is conducted on five *Coccidioides* species antigens with known reactivity. Secondly an *in vitro* analysis on 121 *Coccidioides* spp. specific proteins from MHC binding study was performed using PepSeq technology. These peptides were synthesized and pooled. The pooled peptides were used to stimulate PMBC collected from endemic regions with or without current VF infection (University of Arizona sIRB#: STUDY00002062). PBMCs were incubated in the presence of pooled peptide overnight. Supernatants were collected and cells were analyzed by flow cytometry after staining for T cell markers and cytokines (IFN-g). Supernatants were screened for additional cytokines using a multiplex sandwich bead assay.

Results: The prediction analysis using MHC class II reference sets resulted in 79 peptides potentially reactive for *Coccidioides* spp. *In vitro* MHC: Pepseq binding and binding prediction identified 28 peptides from 121 proteins reactive for *Coccidioides*. From these antigens, a pool of 107 peptides has been generated from these two sets of peptides and evaluated for immunogenicity in endemic area individuals (EI, n=6) and non-endemic healthy control (NEHC, n=3). The stimulation resulted in higher activated $CD4^+$ memory T cells based on IFN-g expression compared to non-endemic control samples. The same observation was made comparing NEHC to VF patients (n=3).

Conclusion: This study has outlined a promising peptide pool with immunogenic properties in humans infected with VF that can be used to distinguish infected individuals from non-endemic area healthy controls. This approach holds relevance for the development of diagnostic assays for Coccidioidomycosis and screening individuals for participation in clinical trials.

20. Detection of *Coccidioides* Galactomannan With a Monoclonal Antibody-based Lateral Flow Immunoassay

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Abstract

Introduction: Accurate and rapid diagnosis of coccidioidomycosis is key for optimal patient care and recovery. However, most testing for coccidioidomycosis currently relies on serology, which requires a detectable antibody response from the patient after infection. Consequently, the sensitivity of serologic tests is often lower early in disease (*i.e.* right after symptom onset) *vs.* later in disease. Furthermore, many serologic tests require specialized equipment and user expertise, which can further delay diagnosis for patients in urgent care clinics or low-resource settings. The goal of our study was to address the unmet need for a rapid, accurate coccidioidomycosis diagnostic that could be used right after symptom onset and at or near the point of care.

Methods: Our approach is a monoclonal antibody-based immunoassay diagnostic that detects *Coccidioidies* spp. cell wall galactomannan in patient urine or serum.

Results: We successfully created a monoclonal antibody (mAb 7B12) with high inclusivity across galactomannans from four strains each of *C. immitis* and *C. posadasii*. The mAb also has high specificity to the *Coccidioides* genus (*i.e.* no detectable cross-reactivity with *Histoplasma, Aspergillus, Candida, Mucor, Rhizopus*, or *Fusarium*). We have incorporated the mAb into prototype lateral flow immunoassays (LFIAs) using both a gold-conjugate label, which enables visual readout with the naked eye, and a fluorescent europium-conjugate label, which enables extremely high sensitivity readout with a small, compact electronic reader or even a portable UV pen light. Both assays have a 15 minute runtime, which is similar to point-of-care antigen-detection immunoassays for other diseases.

Conclusion: Altogether, our mAb 7B12-based europium LFIA achieves high inclusivity, analytical specificity, and analytical sensitivity in an easy-to-use, rapid format. The assay has the potential to transform coccidioidomycosis diagnosis in the same way as the transition to point-of-care, antigen-detection COVID-19 testing (*i.e.* away from reference lab, PCR-based testing) simplified and greatly expanded access to COVID-19 diagnosis for patients regardless of clinic resources.

21. *Coccidioides* Species Hot Spots in the Arizona Landscape: Characteristics of Soils Harboring Valley Fever

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Abstract

Introduction: *Coccidioides* spp. are soil-borne fungi in the southwestern United States, living mainly in soils and dust of Arizona and California. *Coccidioides* spp., referred to as *Cocci*, are not evenly distributed in soil, and the factors controlling their spatial distribution are not yet well understood. Our study aims to investigate correlations between the detection of *Cocci* and soil chemical and physical properties regulating fungal growth, including texture, pH, EC, soluble and exchangeable ions, carbon and nitrogen content, and microbial biomass. We also tested the small mammal reservoir hypothesis (Taylor and Barker, 2019) which postulates a key role for burrowing rodents in the *Coccidioides* spp. lifecycle.

Methods: Soils were sampled at Mesa (Maricopa County), Tom Mix (Pinal County), and Catalina Foothills (Pima County). At each site, six burrows were sampled, and for each burrow, four non-burrow samples were collected at one meter from the burrow. Non-burrow soils were collected at the South, North, East, and West sides of horizontal surface burrow holes and the top, bottom, left, and right sides of wall burrow holes (i.e., each 90o from vertex burrow).

Prior to analysis, large stones, roots, and macrofauna were removed, and soils were sieved to 2 mm.

Soil total carbon and nitrogen were measured on oven-dried (110°C overnight) and ground soil (<100 µm) aliquot with an elemental analyzer instrument using gas chromatographic separation of CO_2 and N_2 after combustion.

Water-extractable carbon and nitrogen were subsequently measured on a 1:5 soil-to-water extraction. The solution was mixed on an end-over-end rotator (7 rpm) for 1 hour, followed by centrifugation (5000 g) and filtration using 0.7µm with GMF syringe filters. Water-extractable inorganic carbon, organic carbon, and total nitrogen were measured on the extracts by a Shimadzu TOC-L/TN analyzer. An aliquot of the solution was used to measure pH and electrical conductivity (EC) immediately following filtration.

Soil ammonium and nitrate were extracted with 2 M KCl at a 1:10 (w/v) soil-to-solution ratio. Slurries were shaken for 1 hour on a reciprocal shaker at 25 rpm and then centrifuged for 10 minutes at 5,000 g. Soil extracts were collected and filtered at 0.2 µm with nylon syringe filters.

Nitrogen species concentration was measured using colorimetric methods (Berthelot reagent for ammonium and the Griess reagent with added vanadium chloride for nitrate). Absorbance was measured at 650 and 540 nm for ammonium and nitrate, respectively, using a 96 well microplate photometer (accuSkan, Fisher Scientific)

Microbial biomass was estimated using the chloroform fumigation extraction method. Carbon and nitrogen were extracted with 0.5 M K_2SO_4 at a 1:5 (w/v) soil-to-solution ratio. Slurries were shaken for 1 hour on a reciprocal shaker at 25 rpm and then centrifuged for 10 minutes at 5,000 g. Soil extracts were collected and filtered at 0.7 µm with

21. *Coccidioides* Species Hot Spots in the Arizona Landscape: Characteristics of Soils Harboring Valley Fever *(continued)*

glass fiber syringe filters. Organic carbon and nitrogen concentration in fumigated and non-fumigated aliquots were measured with a Shimadzu TOC-L/TN analyzer, where after combustion, carbon dioxide is detected using an infrared gas analyzer, and nitrogen monoxide is detected using chemiluminescence. Carbon and nitrogen associated with microbial biomass were calculated as the differences in carbon and nitrogen concentration between fumigated and non-fumigated aliquots.

An aliquot of each sieved soil sample was sent to the Barker lab at Northern Arizona University for *Coccidioides* spp. detection. The presence of *Coccidioides* spp. was tested with the q-PCR assay CocciEnv with a cutoff count value of 42 for *Cocci* positivity. Significant differences between positive and negative samples will be tested using nested ANOVA in R package.

Results: At the Tom Mix site, *Cocci* was detected in two burrow hole samples out of six and one non-burrow sample out of 24. At the Mesa site, *Cocci* was detected in two burrow hole samples out of six and five non-burrow samples out of 24.

At the Mesa sites, the *Cocci* positive soil samples were within a similar range of values to the negative soil samples with respect to texture and organic nitrogen, ammonium, and nitrate content. However, the EC values of the negative soil samples ranged between 0.08 and 0.2 dS/m, while the EC values of the positive soil samples were up to 0.9 dS/m and above 0.1 dS/m. Additionally, the *Cocci* positive soil samples showed a narrower range of pH and organic carbon than the full set of soil samples. In general, the *Cocci* positive soil samples had a greater inorganic carbon content than the *Cocci* negative soil samples. Moreover, a comparison of EC and pH from our positive samples with those reported by Dobos et al. (2021) was not in agreement with respect to the range of suitable habitat (Fig. 1). Further analysis of the full set of samples will be completed in the coming months.

Conclusion: Our results showed that *Coccidioides* spp. were not solely found in rodent burrows at the sites sampled. These results are consistent with those reported in an earlier study, which found no association between rodent habitats and *Coccidioides* spp. (Chow et al. 2021). Our results also suggested an association between soil chemistry, such as inorganic carbon, and the presence of *Coccidioides* spp., which has never been reported before. Finally, we found new suitable soil habitats for *Coccidioides* spp. beyond those described in a previous study and provided additional characteristics (Dobos et al., 2021).

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22. Urban Versus Rural Hospital Outcomes for Coccidioidomycosis 2016-2020

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Abstract

Introduction: Rural populations exhibit a higher in-hospital mortality rate, with infectious diseases disproportionately affected [1]. Focusing on Coccidioides, we explored populations and outcome differences between rural and urban cases of coccidioidomycosis (CM) using the National Inpatient Sample (NIS) database. Given the absence of projects on CM using 2016-2020 NIS data, this research fills a critical gap in understanding outcomes in rural environments.

Methods: Data was obtained from the NIS, the largest USA inpatient database from the Healthcare Cost and Utilization Project. It has publicly-available, anonymized healthcare information from 1988 to 2020, including demographics, diagnoses, insurance, and related data [2]. The database was filtered by coccidioidomycosis ICD-10 codes and analyzed via R studio. Patients stratified to urban or rural designation using National Center for Health Statistics definitions. Metropolitan and micropolitan populations combined formed urban populations. Rural populations were defined as neither metropolitan nor micropolitan. Univariate analyses were performed via the “tableby” function for sex, age, race, mortality, hospital region, hospital division, insurance payer, median household income by ZIP Code, hospital transfers, mortality risk, and illness severity. Significance was defined as p-values of <0.05. Rural and urban populations were compared for mortality, length of stay (LOS), and total charges (TOTCHG) outcomes. Multivariable regressions were completed using the “glm” function. Beta coefficients were reported for continuous variables and odds ratios for categorical variables.

Results: From 2016 to 2020, we identified 8,743 hospitalized patients with CM, 8,609 in urban and 134 in rural areas. When comparing rural to urban hospitalization, differences existed in median age (55.6 vs 59, p<0.001), ethnic minorities (39% vs 52%, p<0.01), and CM-related mortality (8.2% vs 3.9%, p=0.011). The top location census in the urban population was Mountain (46%), Pacific (44%), and West South Central (4%), while rural was Mountain (40%), West South Central (15%), and West North Central (13%) (p<0.001). The top payers for urban populations were Medicare (39%), Medicaid (31%), vs Medicare (53%) and Medicaid (15%) (p<0.001) in rural locations.

Multivariate analysis identified elderly patients with higher mortality (p=0.01) compared to other ages and factors. Transferring from another facility was associated with increased mortality (p=0.05). Children had a significantly higher total cost compared with elderly populations.

22. Urban Versus Rural Hospital Outcomes for Coccidioidomycosis 2016-2020 *(continued)*

Multivariate analysis for LOS showed patients aged 0-14 years increased LOS at 11.79 days ($p<0.0001$), and ages 65+ decreased LOS by -1.23 days ($p<0.05$). Patients transferred into the hospital from another facility had significantly higher LOS. Hospitals that were private, non-profit, or invest own had significantly lower LOS vs government, nonfederal hospitals.

In all multivariate analyses, rural or urban population status did not significantly impact outcomes for death, LOS, total cost, and disposition.

Conclusion: Comparing differences between rural and urban patients hospitalized with CM, rural patients were older with less minority populations, and higher mortality. However, multivariate analyses did not find that rural status was a significant predictor for death, length of stay, and total cost outcomes, warranting further exploration.

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23. Coccidioidal Tenosynovitis - A Case Series

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Abstract

Introduction: Coccidioidomycosis (cocci) is an infection caused by *Coccidioides immitis or Coccidioides posadasii,* a dimorphic fungus that grows in the soil and is commonly found in southwestern California, Mexico, and Central America. It is also known as Valley fever due to the endemicity of *C. immitis* in the San Joaquin Valley. Most patients are asymptomatic, some present with pulmonary symptoms, and less than 3% have dissemination. Tenosynovitis is a rare manifestation of disseminated coccidioidomycosis with limited literature surrounding its diagnosis and treatment, which can lead to misdiagnosis or underdiagnosis. Our aim was to examine the clinical course of coccidioidal tenosynovitis via a case series of three patients.

Methods: This was a retrospective case series of three patients at the Valley Fever Institute in Bakersfield, California, with coccidioidal tenosynovitis of the hand and wrist between 2021 and 2024. Patient consent and Institutional Review Board approval were obtained for collection of demographic, clinical, laboratory, operative, and treatment data from electronic health records.

Results: We reviewed cases of coccidioidomycosis between 2021 and 2024 in the Valley Fever Institute database and identified 3 patients with coccidioidal tenosynovitis. All patients had an extended duration of intermittent wrist pain with a palpable growing mass lasting over a year prior to diagnosis of coccidioidal tenosynovitis. Two patients had confirmed pulmonary coccidioidomycosis. One had presumed pulmonary coccidioidomycosis based on incidental CT findings. All 3 patients had MRI of the wrist with and without contrast with the diagnosis of tenosynovitis and underwent surgery. Intraoperatively, 2 patients had inflammation and thickening of the synovium and 1 had dissolved flexor tendons. Intraoperative cultures of all 3 patients revealed *Coccidioides spp,* 2 of them identified as *C. immitis,* 1 pending identification. One patient had pathology showing rare fungus with spherules and 1 had fungus with spherules with endospores consistent with coccidioidomycosis.

One patient was continued on fluconazole, 1 had adverse reactions to fluconazole and posaconazole and was started on itraconazole, and 1 had failed fluconazole and was switched to isavuconazonium. Of the 3 patients, 1 required intravenous liposomal amphotericin B therapy for pulmonary coccidioidomycosis.

23. Coccidioidal Tenosynovitis - A Case Series *(continued)*

Patient sex, age at diagnosis, race	Comorbidities	History of previous cocci	Location of infection	Symptoms	Symptom duration before diagnosis	Serology/cultures	Treatment
M, 37, Hispanic	No known comorbid illness	Pulmonary cocci	Left wrist flexor tendon to index, middle, ring and fifth fingers, interosseous muscles, and the median nerve	Left hand and wrist intermittent swelling with growing mass, localized sharp pain, numbness, tingling, rigor, associated cellulitis	2yrs 7mos	Reactive IgM, IgG, CF titer (1:256 to 1:8) Intraop culture positive for *Coccidioides immitis*	Fluconazole and IV amphotericin. Currently on isavuconazonium Irrigation and debridement left wrist including skin, tissue, tendon, fascia
F, 63, Asian	Stage 3C cervical cancer s/p total abdominal hysterectomy with BSO, pelvic and paraortic LN dissection s/p adjuvant radiation; Diabetes Mellitus	Pulmonary cocci, presumed	Right wrist extensor synovium, possibly retinaculum	Right wrist intermittent pain with growing mass	1yr 6mos	Right wrist mass pathology with granulomatous inflammation with spherules and endospores of coccidioidomycosis Intraop culture positive for *Coccidioides immitis.* Weakly reactive IgM, IgG CF/QID 1:2	Currently on fluconazole Right dorsal wrist tissue tenosynovitis /mass excision and debridement of bursa, synovia of wrist and tendon sheaths
M, 44, Hispanic	OSA on BIPAP	Pulmonary cocci	Left wrist synovium	Left intermittent hand swelling with palpable mass waxing-and-waning in size, with long-standing left shoulder pain.	1yr	Reactive IgM, IgG, CF titer <1:2; Left wrist mass pathology with granulomatous inflammation with rare fungal organisms consistent with Coccidioides spherules. Intraop culture positive for *Coccidioides*, pending ID	Failed Fluconazole, posaconazole. On itraconazole Left dorsal wrist tissue tenosynovitis/mass excision and debridement of bursa,synovia of wrist and tendon sheaths

Conclusion: Tenosynovitis of the hand and wrist, although rare, is seen in disseminated coccidioidomycosis. Diagnosis is generally made based on clinical suspicion and intraoperative cultures and pathology. There is currently no consensus in the treatment guidelines on management of coccidioidal tenosynovitis, which is challenging and generally requires surgical debridement and extended antifungal treatment.

24. Acute Pulmonary Coccidioidomycosis with Bronchial Casts in Sputum

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Abstract

Introduction: Bronchial casts are rare circumstances, and their presence is not commonly associated with pulmonary coccidioidomycosis (cocci). The presence of bronchial cast is a notable finding, and cocci may be considered in differential diagnosis.

Methods: This is a retrospective case review from the Valley Fever Institute (VFI) in Bakersfield, California with approval from the Kern Medical Institutional Review Board and patient consent. A literature search was conducted with terms such as bronchial casts, pulmonary cocci and diagnostics from search engines PubMed and Google Scholar.

Results: Our patient was a 63-year-old male with history of diabetes mellitus and gastroesophageal reflux disease who developed flulike symptoms with sharp right anterior chest pain upon coughing and coughed up tree branch-like mucus that appeared to be bronchial casts. He was diagnosed with pneumonia and was given antibiotics, but did not respond to antibacterial therapy. Due to persistence of symptoms 3 months later, he was tested for coccidioidomycosis. Coccidioides serology was positive with IgM and IgG. Chest x-ray revealed a 12 cm area of opacification involving the right upper lobe. A CT chest was performed which showed a large confluent consolidation involving the right upper lobe. Numerous irregular patchy and nodular opacities were noted throughout the lungs, in addition to mediastinal lymphadenopathy, mild hepatomegaly, and 2 right adrenal gland nodules. A bone scan revealed activity in the right ribs and left hip. MRI of the hip suggested left gluteus minimus and maximus tendinosis involvement. The patient was started on fluconazole 800 mg daily. The Coccidioides immunodiffusion study was repeated 6 months from first presentation which revealed IgM reactive weakly, IgG reactive, and complement fixation titer of 1:8, along with a fluconazole level of 41.7 mcg/mL. A repeat bone scan showed likely post-traumatic uptake at the right anterior 4^{th} rib and no abnormal uptake suspicious for bony infection. One year later, patient developed rash with fluconazole and was switched to SUBA-Itraconazole. Patient tolerated SUBA-Itraconazole and completed two years of therapy. Off therapy, he had three CF titers <1:2 over a 9-month interval. Patient is doing well and has no complaints on follow-ups.

Conclusion: Although coccidioidomycosis most commonly presents with pulmonary disease, bronchial casts are very rare and have not been frequently reported with coccidioidomycosis in the literature. Our aim is to highlight this rare case of bronchial casts associated with coccidioidomycosis. More research is needed in this particular area as it is an uncommon association.

25. Disseminated Cocci Iridocyclitis: A Rare Presentation of Anterior Chamber Coccidioidomycosis in the Eye

Shikha Mishra[1], Kishan Ghadiya[2], Bianca Torres[1], Michelle Fang[1], Michael Valdez[1], Royce Johnson[1], Rasha Kuran[1]

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Abstract

Introduction: Ocular coccidioidomycosis (cocci) is a rare form of dissemination which occurs in roughly 0.5% of cases, but can have a significant impact on morbidity, especially if diagnosis or treatment is delayed. Ocular cocci can cause both posterior and anterior segment disease, and over half of the cases report severe loss of vision. Few cases have been reported of anterior chamber disseminated coccidioidomycosis with preservation of vision.

Methods: This is a retrospective case review with approval from the Kern Medical Institutional Review Board and patient consent. A literature search for similar reported cases was conducted within databases including PubMed, Google Scholar, and Investigative Ophthalmology and Visual Science (IOVS) with search terms such as ocular coccidioidomycosis, disseminated coccidioidomycosis, and iridocyclitis.

Results: A 51-year-old woman with a history of acute pulmonary coccidioidomycosis with miliary pattern on chest computed tomography (CT) on low dose fluconazole presented with redness in her left eye one year after her diagnosis of pulmonary cocci. She was treated with ophthalmic steroid drops with temporary improvement, but after discontinuing fluconazole one month after her index ocular presentation, the redness increased with diminished visual acuity. She was diagnosed with uveitis 2 months later, which worsened after oral methylprednisolone and ophthalmic steroids. She then underwent pars plana vitrectomy (PPV)/anterior chamber washout of left eye and vitreous and aqueous chamber biopsy 2 months later, with intraoperative cultures revealing *Coccidioides immitis.*

She was started on 100 ug of voriconazole intravitreal injections (100 ug/0.1 mL) twice a week and fluconazole 800 mg daily for one month without significant improvement. This was switched to 5 ug of amphotericin B intravitreal injection followed by PPV/anterior chamber washout and cataract extraction 6 months after her index presentation. She was continued on twice-a-week amphotericin intravitreal injections along with prednisone eyedrops.

Inflammation continued in the anterior chamber with the development of micro abscesses.

Three months later, she underwent PPV/pupillary membrane removal/debulking of iris abscesses.

Fourteen months after the index presentation and 7 months after the most recent procedure, the iris was still infected with retrodescematic deposit. Amphotericin injections were switched to once a week. Progression of iritis was noted on fluconazole and isavuconazonium in the setting of potentially diminished absorption due to previous Roux-en-Y gastric bypass, so the patient was later switched to SUBA-itraconazole as long-term therapy. A year later, intraocular injections were stopped, and the patient has since remained clinically stable with CF titers remaining <1:2 for 1 year.

Conclusion: The manifestation and management of ocular coccidioidomycosis with persistence of anterior chamber disease is not well documented in the literature. More studies are need for guiding the management and preservation of vision.

26. Coccidioidal Empyema in the Making

Bianca Torres[1], Shikha Mishra[2,1], Michelle Fang[3,1], Michael Valdez[2,1], Carlos D'Assumpcao[2,1], Royce Johnson[2,1,4], Rasha Kuran[2,1,4]

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Abstract

Introduction: Contrary to coccidioidal pleural effusions, cocidioidal empyemas are characterized by the development of hydropneumothorax and technically have an underlying leak. This case demonstrates a cavitary lesion leading to recurrent spontaneous right pneumothorax managed with right thoracoscopy with wedge resection of cavitary lesion of the right lower lobe.

Methods: This is a retrospective case report at the Valley Fever Institute (VFI) in Bakersfield, California with approval from Kern Medical's Institutional Review Board and patient consent. A literature search was conducted on PubMed and Google Scholar using search terms including coccidioidomycosis (cocci) and spontaneous pneumothorax, cavitary infections, and coccidioidal empyema.

Results: A 35-year-old male with history of pulmonary cocci diagnosed 3 years prior treated with one month of fluconazole prior to self-discontinuation presented to clinic with productive cough and generalized weakness. Chest computed tomography (CT) showed 2.4 cm cavitary lesion in the right lower lobe at the site of the original dense infiltrate with a thin fluid level indicating mild active infection, resulting in a diagnosis of cavitary coccidioidomycosis. Cocci IgM and IgG were weakly reactive with CF <1:2. The patient was scheduled for follow up but did not return. Ten months later, the patient presented to urgent care with hemoptysis, sputum production, pleuritic chest pain, fever, chills, chest pain, night sweats, with a small right lower lobe pneumothorax seen on imaging. The patient was admitted to Kern Medical and started on fluconazole 800 mg daily. Chest x-ray revealed right lobe cavitary lesion measuring 3.5 cm and a suggestion of a new nodular density inferiorly located measuring 3.1 cm with possible cavitation. Cocci IgG was reactive with an increased CF titer of 1:4. Patient underwent chest tube placement for right sided pneumothorax, with Coccidioides immitis on pleural fluid culture. Imaging revealed improved pneumothorax and chest tube was subsequently removed. Two months later, the patient reported adherence to fluconazole 800 mg (serum level 28.2 mcg/mL) and resolution of all previous symptoms aside from dyspnea but had new complaints of joint pain, so a bone scan was ordered, and fluconazole was increased to 1000 mg.

26. Coccidioidal Empyema in the Making *(continued)*

One month later, the patient reported worsening right pleuritic chest pain and subjective fever. Cocci serology revealed increase of CF titer to 1:8, fluconazole level was 29.1 mcg/mL, and chest x-ray showed right hydropneumothorax. Antifungal therapy was switched to SUBA-itraconazole 130 mg daily, with slight improvement of symptoms but still unresolved pleuritic chest pain and dyspnea on exertion. The patient was readmitted to Kern Medical and CT chest showed an increase in the right-sided hydropneumothorax and pleural effusion in the right lower lobe and a right sided chest tube was placed. After 72 hours of suction, the patient redeveloped a pneumothorax suggesting the cavitary lesion in the right lower lobe was active and the patient later underwent right lower lobe resection of cavitary lesion and apical mechanical pleurodesis with chest tube in place. Post procedure chest x-ray demonstrated right apical pneumothorax filled with pleural effusion, no demonstrable pneumothorax and some increase of the right chest wall emphysema with no additional interval changes. Two days after surgery, the chest tube output was minimal with no evidence of air leak and removed, post removal imaging showed no evidence of new pneumothorax or other interval changes. Currently, patient has no complaints with multiple CF titer <1:2 and SUBA-itraconazole level >4 mcg/mL over the most recent 18 months of follow-up.

Conclusion: A minority of patients with pulmonary cocci may develop pulmonary cavities, which have been shown to be more likely to close in patients who receive antifungal treatment. Without adequate treatment, complications such as coccidioidal empyemas may develop, which may require more invasive management that could potentially be avoided with better patient literacy and health systems support surrounding coccidioidomycosis.

27. Case Series of Coccidioidomycosis and Malignancies in an Endemic Area

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Abstract

Introduction: Coccidioidomycosis (CM) is a growing public health concern due to increased reported cases and evidence of geographic expansion. Coccidioidomycosis is an infection caused by the inhalation of airborne arthroconidia from the soil-dwelling fungi, Coccidioides spp. Since CM is a significant disease in the Central Valley of California, the aim of this study is to elucidate whether coccidiidial infections antecedent or post-malignancy are clinically different.

Methods: Retrospective chart review of patients at Kern Medical between January 2016 and March 2022 was conducted. ICD-10 codes were used to identify patients who were diagnosed with CM and malignant diseases. Inclusion criteria for coccidioidal diagnosis included evidence of IgG antibody serology and positive culture for CM at the Kern County Public Health Department or the University of California Davis Mycology laboratories. Qualifying patients' charts with the dual diagnosis were reviewed. Those charts were then abstracted for the following: patient demographic characteristics, cancer diagnosis by histopathology, and details of cancer therapy. These patients were further stratified by whether these cases were antecedent to cancer diagnosis or after.

Results: 3342 patients diagnosed with cancer and 1961 patients diagnosed with CM were identified. Of these patients, 53 met the inclusion criteria. 27 patients had a cancer diagnosis before a CM diagnosis with two patients who developed disseminated disease. 26 patients had a CM diagnosis after cancer diagnosis out of whom 13 patients developed disseminated CM disease.

Conclusions: Our study found that those who had a CM diagnosis antecedent to a cancer diagnosis had a much higher rate of disseminated cases. This warrants the use of further screening modalities and treatment regimens for controlling CM disease before initiating cancer treatment.

28. OPAT Liposomal Amphotericin B in an Immunocompetent Patient with Disseminated Coccidioidomycosis and Bone Involvement

Cynthia Ortiz-Lopez, Cristina Hernandez-Balboa, Emilio Castillo-Gonzalez, Laura Nuzzolo-Shihadeh, Eduardo Perez-Alba, Maria Teresa Ramirez-Elizondo, Reynaldo Lara-Medrano, Adrian Camacho-Ortiz

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Abstract

Introduction: Severe disseminated coccidioidomycosis involving bone structures requires an initial treatment of IV amphotericin B alone or in combination with azole therapy for several months, followed by a step down to oral azole therapy with a duration that may extend from years to a lifetime.

Case Presentation: A 20-year-old previously healthy Hispanic male was admitted with shortness of breath, dry cough, and disseminated skin abscesses that appeared during the past ten months. He had been living in northern Mexico for two years. He had neither chronic comorbidities nor allergies, and denied any animal contact, drug use, alcohol consumption, or smoking. He noticed a painful increase in volume in the neck region along with erythema and purulent discharge with intermittent fever and abscesses in the ankles, upper limbs, and thorax. His blood work at admission showed mild anemia, leukocytosis, and thrombocytosis, his HIV test was negative. A chest CT scan revealed an irregular opacity in the upper right lung with rib bone involvement. An arm abscess was drained, and samples were sent for bacterial, mycobacterial, and fungal cultures. Lactophenol blue stain and fungal culture were compatible with *Coccidioides sp.*. He was started on liposomal amphotericin B (LAmB) and fluconazole (FLU), and on day 52 of hospital admission, rib removal surgery was performed. The rib bone biopsy showed acute and chronic granulomatous inflammation with fungal spherules, morphologically compatible with *Coccidioides sp.*. He had a favorable clinical evolution. On day 95 of combination therapy with IV LAmB and FLU, the patient was discharged to the outpatient parenteral antimicrobial (OPAT) clinic with a peripherally inserted central catheter (PICC) to continue daily IV LAmB and oral FLU. He was closely monitored with biweekly infectious disease specialist consultations and serum creatinine and electrolytes determinations. Although his kidney function remained stable, he presented on several occasions with mild to moderate asymptomatic hypokalemia. IV potassium chloride infusion was safely administered as needed along with potassium gluconate tablets. The patient received six more weeks of LAmB OPAT. He was neither readmitted within the first 30 days after discharge nor had infusion-related reactions, and there were no PICC malfunctions or related infections. To date, he remains on follow-up and was started on oral itraconazole with good tolerability.

Conclusion: LAmB OPAT is an infrequent strategy in resource-limited settings due to side effects and challenging patient selection. Centers with real-life experience have reported that although almost half the patients treated with IV amphotericin B in OPAT were readmitted, the minority of readmissions were due to adverse events [1-3]. In our patient, LAmB OPAT proved to be a convenient approach with no need for readmission or severe side effects.

28. OPAT Liposomal Amphotericin B in an Immunocompetent Patient with Disseminated Coccidioidomycosis and Bone Involvement *(continued)*

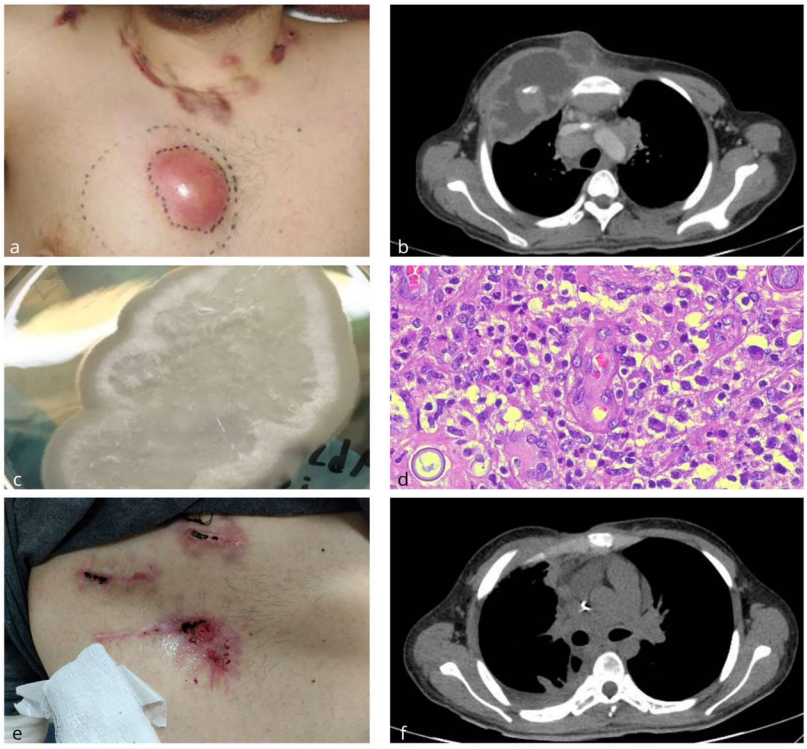

Figure a: thoracic abscess. Figure b: CT chest showed rib bone involvement. Figure c: Fungal culture on SDA with white colonies and cotton-like appearance. Figure d: Spherules found in rib bone biopsy with HE stain. Figure e: Six weeks follow up in OPAT. Figure f: CT chest following rib removal surgery.

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29. Immuno-phenotyping Reveals Dysregulation of the CD8 T Cell Compartment in Coccidioidomycosis

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Abstract

Introduction: Coccidioidomycosis is disseminated beyond the lungs in approximately 1% of infections, and these individuals suffer significant morbidity and must be on antifungal treatment for life. We are interested in determining the pathways and cell populations that distinguish patients with disseminated or pulmonary-only Cocci infections from healthy controls. Our long-term goal is to identify dysregulated cell populations in disseminated disease that have either diagnostic value or are amenable to therapeutic intervention.

Methods: We isolated PBMCs from ~180 patients with uncomplicated pulmonary, complicated pulmonary, and disseminated coccidioidomycosis. We performed FACS-based immunophenotyping on these samples to delineate T and B cell subsets.

Results and Conclusions: We find that a subset of Cocci patients have abnormal CD8 T cell compartments compared to controls. These abnormal CD8 populations are primarily characterized by large numbers of CD57+ senescent T cells. T cell senescence results in a hypo-proliferative state and are difficult to activate. We are currently testing the effector function of CD8 T cells in individuals with abnormal CD8 compartments. Importantly, several targeted treatments are currently being explored for modulation of senescent T cells.

30. Meta-Analysis of Laboratory Diagnostics for Coccidioidomycosis

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Abstract

Introduction: Although previous reviews of diagnostics for coccidioidomycosis have been conducted, they have been narrative in nature no meta-analysis has yet been performed to evaluate the diagnostic accuracy specifically of tests that are feasible in a primary care settings.

Methods: A systematic literature search was conducted for eligible diagnostic studies in Pubmed, (from 1970-2024) using Medical Subject Headings and free text words related to coccidioidomycosis and diagnostic accuracy. Our criteria for including a paper was: (1) sensitivity and specificity results reported, (2) statistical values reported, (3) comparison to a reference and, (4) reference to a specific company. exclusion criteria was: (A) case report,(B) review paper, (C) does not compare coccidioidomycosis test, (D) not human, (E) not coccidioidomycosis, (F) discovery/newly invented test, (G) clinical trial.

Results: We found 243 papers found that met our search criteria and after reading the abstracts, 206 were excluded and 37 included for a reading of the full text. After reading the full text of 37 papers, 18 papers were included in our review and 19 excluded. We found seven papers that used coccidioidomycosis enzyme immunoassays (EIA), three papers that used coccidioidomycosis enzyme-linked immunosorbent assay (ELIZA), two that used immunodiffusion (ID), one complement fixation (CF), one that combined ID and CF and one that used an antibody lateral flow assay (LFA). IgG antibody tests have a sensitivity of 79.0% (95% Confidence Interval 65.5-88.2) and specificity of 94.5% (92.7-95.9), IgM antibody tests have a sensitivity of 41.3% (25.3-59.4) and specificity of 93.5% (83.9-97.5) when compared to a reference test. EIA tests have a sensitivity of 66.7% (55.4-76.4) and specificity of 94.6% (90.7-96.9), ELISA tests have a sensitivity of 88.6% (80.1-93.8) and specificity of 93.2% (85.6-97.0), andID tests have a sensitivity of 84.4% (69.9-92.7) and specificity of 96.5% (95.2-97.5)Of the commercial tests available, Meridian has a sensitivity of 77.7% (62.3-88.0) and specificity of 96.0% (90.6-98.3), MiraVista has a sensitivity of 71.8% (53.5-84.9) and specificity of 94.1% (87.4-97.4), and IMMY has a sensitivity of 67.9% (49.9-81.8) and specificity of 95.8% (90.1-98.3).

31. Proteome Wide Antibody Profiling of Pig-tailed Macaques with Naturally Acquired Valley Fever Using PepSeq

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Abstract

Valley Fever (VF), a disease that is endemic to the southwestern United States, is associated with severe morbidity and mortality, and there is currently no vaccine available for the prevention of infection. Antibodies are important markers of past infections and have diverse roles in protecting against infectious disease, however, the enormous diversity of possible VF antibody targets poses a challenge for studying the antibody response to VF. To address this challenge, we have designed a highly-multiplexed serological assay utilizing the PepSeq platform to measure proteome-wide antibody reactivity profiles generated in response to VF. This assay will be used to screen serum from pig-tailed macaques (PTM) raised in the endemic VF region (Mesa, AZ) who have naturally acquired VF and a control PTM cohort raised in a non-endemic VF region (Seattle, WA). These results will offer insight into the antibody response to VF in PTM, which offers interesting comparisons to responses in humans. Additionally, these findings will provide valuable information on the antibody response to natural infection in PTM for future vaccine trials.

32. Improvement of a Colorimetric Cell Viability Test for Testing Novel Antifungals on *Coccidiodes* spp.

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Abstract

Introduction: Current antifungal treatments against Coccidioidomycosis are limited, with therapy further complicated by the emergence of resistance, leading to an urgent need for the development of novel antifungal agents. Here, we have refined a previous 96-well microtiter plate-based protocol using XTT-reduction for the assessment of the metabolic activity of spherule cells and optimized it for its use in high throughput screenings in search for novel compounds with antifungal activity against spherule cells.

Methods: The primary objective was to optimize the protocol, originally labor-intensive due to the nature of spherule harvesting and the number of steps involved. Experimental parameters were modified to obtain optimal conditions in the assay. These experiments used the avirulent triple mutant (Δcts2/Δard1/Δcts3) of *Coccidioides posadasii* C735 (ΔT C-735).

Results: Briefly, a suspension of spherules in non-phenol red RPMI-1640 was prepared to serve as the initial inoculum (1 x 10^6 spherules/mL). Subsequently, 100 µL of this inoculum was seeded into wells of a 96-well flat-bottom microtiter plate, resulting in a final concentration of 1 x 10^5 spherules/well. Wells from column 1 to column 11 served as growth control (seeded with inoculum), while inoculum in wells of column 12 was treated with Amphotericin B (final concentration of 10 µg/mL). After inoculating the plates, these were sealed with gas-permeable seals to avoid media evaporation and incubated at 39°C and 10% CO_2 for 24 hours. Following incubation, 100 µL of an XTT solution (0.5 mg/mL) supplemented with menadione (40 µM) was added to each well. For XTT reduction, plates were sealed with gas-permeable seals to avoid media evaporation and incubated at 39°C and 10% CO_2 for an additional 24 hours. Once XTT was reduced, gas seals were removed, the plate was read in a microtiter plate reader at 490 nm, and the percent inhibitory activity was determined. To further evaluate the reproducibility of the refined protocol, we monitored the intensity signal of reduced XTT after 6-hour and 24-hour incubations. A three-day uniformity study was conducted, utilizing distinct batches of ΔT C-735 spherules. Following the confirmation of protocol uniformity, the methodology's reproducibility was assessed through a dose-response assay. This experiment tested a reference compound, specifically Amphotericin B, at two-fold decreasing concentrations (final concentrations of 0.009 to 10 µg/mL) in 100 µL of spherule inoculum. Eight replicates were used per concentration point, and four replicates per the growth control. Plates were incubated and processed as previously described. Then, the inhibitory concentration required to inhibit 50% of the metabolic activity of the spherules (IC_{50}) was determined by fitting normalized results to the variable slope Hill equation (an equation that determines the nonlinear drug dose-response relationship). Additionally, the protocol was further evaluated by testing three compounds with characterized inhibitory activity on ΔT C-735 (Figure).

Conclusions: The resulting optimized protocol for antifungal activity assessment against ΔT C-735 spherules demonstrated significant improvements. In particular, increases the throughput of drug screening and minimizes the required steps, resulting in substantial savings of time, resources, and funds crucial for experimental endeavors.

33. Drug Discovery and Drug Target Identification in *Coccidioides posadasii*

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Abstract

Introduction: Current treatment for coccidioidomycosis typically requires 3-6 months of treatment with azoles (e.g., fluconazole), amphotericin B, and echinocandins. However, azole-resistant strains of *Coccidioides* species are becoming more common, and amphotericin B is toxic to the kidneys. These drawbacks leave a pressing need for a novel and improved medication to treat coccidioidomycosis. Here, we use both drug library screening and identification of potential drug target genes in *C. posadasii* to identify pharmaceutical compounds that can inhibit *C. posadasii* growth. These compounds have the potential to be developed as treatments for coccidioidomycosis.

Materials and Methods: Drug discovery: Ten thousand compounds from the ChemDiv Antifungal Library were screened for inhibition of *C. posadasii* C735 growth. These compounds are organized into families of molecules that are predicted to inhibit ergosterol biosynthesis, disrupt fungal membranes, interrupt fungal cell wall synthesis, impact sphingolipid biosynthesis, disrupt nucleic acid or protein synthesis, and inhibit microtubule biosynthesis. Spherule initials were incubated with 10μM of the ChemDiv Antifungal compounds for 24 hours. Inhibition was measured using a metabolic screening assay (XTT). Compounds that inhibited growth greater than 45% were tested for their minimum inhibitory concentrations at 50% inhibition (MIC50). The MIC50 was performed using 2X serial dilutions of the compounds at concentrations ranging from 50μM to 0.48μM. MIC50 results are presented in a dose-response curve using normalized XTT assay results. In both screening and MIC50 assays, Amphotericin B was used as a positive control, and colorless RPMI 1640 medium containing 1% DMSO was used as a negative control. **Drug target identification:** Potential drug targets were screened using a *C. posadasii* C735 mutant library generated using *Agrobacterium tumefaciens* and the Ti plasmid. *C. posadasii* mutants demonstrating potential attenuation in the *Galleria* model were analyzed using gene walking to identify the unknown disrupted genes. The disrupted gene in *C. posadasii* mutant 13 (Cp13) was identified using inverse PCR and plasmid sequencing. A plasmid construct for restoring virulence into mutant Cp13 was generated using Golden Gate assembly cloning methods.

Results: Our laboratory has previously completed a high-content screening of the ChemDiv Library for antifungal activity against spherules isolated from the attenuated strain *Δcts2/Δard1/Δcts3* that can be manipulated in a BSL-2 containment. Dr. Pradeep Kumar Singh identified a total of 35 compounds in this screening. We screened the BSL-3 strain using the ChemDiv Library and compared the MIC50 for those 35 compounds against the highly virulent strain, *C. posadasii* C735. We aim to compare the hits identified for both strains. If successful, we can use the BSL-2 strain as a surrogate for future discovery of novel antifungals. After we identify and characterize the disrupted gene of the Cp13 mutant, we plan to develop the Cp13 gene as a drug-screening target.

Conclusions: Potential drugs identified in the screening of the ChemDiv Antifungal Library will be further evaluated for both their toxicity and efficacy. By identifying the disrupted gene in the attenuated *C. posadasii* C735 mutant Cp13, we can also identify a potential target for pharmaceutical intervention.

34. Impact of Dust on Climate, Health, and Disease Transmission in the California San Joaquin Valley

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Abstract

Introduction: *Coccidioides* is the causative agent of the fungal infection known as Valley fever. This disease is caused by inhaling arthroconidia fungal spores that are aerosolized from the soil upon events such as dust storms, thunderstorms, and haboobs. Dust refers to fine particles of matter found on the earth's surface made up of pollen, ash, bacteria, dirt/rock and infectious components. Valley fever is on the rise within California and is beginning to spread across the southwestern United States, this is due in part to increased number of dust storms owing majorly to climate change. Our collaborative research seeks to understand how dust impacts climate, energy, agriculture, and health outcomes across the California San Joaquin valley. The fungal composition in aerosolized dust across the season within this region is unknown and this knowledge could allow strategies to mitigate the spread of diseases.

Methods: We aim to understand this by sampling dust, collecting air filter and sedimentation samples, isolating fungal DNA, and sequencing the filtrates to determine fungal diversity across different locations. Next, we will correlate this data with wind pattern and hospitalization data from the region.

Results/ Conclusion: An understanding of how dust affects climate, health outcomes, as well as disease transmission is crucial to fostering public health safety and mitigating disease spread. This research will go a long way in providing critical insights into how climate change plays a role in epidemiology and public health practices and will help us better understand ways of mitigating the adverse effects of climate change in California. This is work in progress and we will present our experimental procedure as well as findings.

35. Profiling Early Immune Responses in the Lung to *Coccidioides* Infection

Nadia Miranda[1,2], Oscar Davalos[2], Aimy Sebastian[2], Nicholas Hum[2], Margarita Rangel[2], Deepa Murugesh[2], Ashlee Phillips[2], Katrina Hoyer[1,2], Dina Weilhammer[2]

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Abstract

Introduction: Valley fever is an emerging respiratory disease caused by the fungal pathogen *Coccidioides*. Most cases (60%) are asymptomatic and resolve spontaneously, however ~40% of infections result in pulmonary disease ranging from a self-limited flu-like illness to more severe pneumonia, and 0.5–2% of cases progress to disseminated disease which can be fatal. Little is known about how the fungus interacts with the immune system and the lung microenvironment, hampering vaccine and therapy development. There is a critical need to identify which immune cells are interacting with the fungus in the lungs and how these cells respond to infection.

Methods: To investigate interactions between fungal spores and immune cells in the lung, we fluorescently labeled *C. posadasii Δcts2/Δard1/Δcts3* arthroconidia and confirmed their association with an alveolar macrophage cell line *in vitro*. Next, mice were infected intranasally with one million labeled arthroconidia and lungs were harvested at 24 hours. Association of arthroconidia association with $CD45^+$ cells *in vivo* was confirmed via flow cytometry and fluorescence imaging. We next sought to compare the response of immune cells that are specifically associated with fungal spores versus bystander cells. $CD45^+$ cells from the lungs of 3 infected mice were isolated via MACS bead sorting, followed by FACS sorting to separate cells into spore+ versus spore- populations. $CD45^+$ cells were also isolated from the lungs of uninfected control mice. Each population was then analyzed via single-cell RNA sequencing to identify gene expression patterns that correspond to infection as well as patterns specific to arthroconidia association.

Results/Conclusions: Our data demonstrates that diverse immune populations associate with *C. posadasii* arthroconidia *in vivo,* with enrichment noted in granulocyte and macrophage populations. Receptor-ligand interaction analysis indicates that neutrophils play a key role in coordinating the response to *C. posadasii* infection. Monocyte-derived macrophages associated with arthroconidia robustly expressed genes associated with fibrosis including *Spp1*, *Inhba*, and *Fn1*, while genes associated with foamy macrophage differentiation were enriched within alveolar macrophages. This analysis of how each population responds to infection will provide critical information to guide vaccine and therapeutic development aimed at preventing early infection.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Security, LLC, Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

36. Whole Genome and RNA Sequencing of Large Coccidioidomycosis Patient Cohort Enables Genetic Characterization of Uncomplicated and Severe Disease

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Abstract

Introduction: Coccidioidomycosis - a fungal infection by Coccidioides immitis and posadasii - affects an estimated 150,000 people in the United States yearly. While a majority of those infected have no symptoms or experience mild respiratory symptoms (uncomplicated Valley Fever, UVF), some have more severe pulmonary symptoms and require extended treatment with antifungals (chronic pulmonary coccidioidomycosis, CPC). In 1% of infections, the fungi spread to other parts of the body (disseminated coccidioidomycosis, DCM). Our consortium has recruited patients with coccidioidomycosis from across California to create the largest genetic cohort of patients to date.

Methods: We carried out whole genome sequencing (WGS) for 172 patients and RNA-sequencing (RNAseq) for 161 patients. We collected complete medical and family histories, as well as laboratory work-ups, and participants were classified by disease severity. We assessed the association of disease severity with demographic information using chi-squared and Fisher's exact tests. The sequencing data were subjected to quality control. With the addition of a cohort of 479 whole exome sequenced (WES) patients (previously described in Hsu et al. 2022) and a reference panel of 1000G samples, we assessed global genetic ancestry with principal components analysis (PCA) and an unsupervised ADMIXTURE model (k=5).

Results: We evaluated a total of 687 patients with WES, WGS, or RNAseq: 376 UVF, 100 CPC, and 211 DCM. Patients with dissemination were significantly more likely to identify as male (78% of DCM patients vs. 55% of UVF patients, $p<0.001$) and to identify as Black or African American (10% of DCM patients vs. 2% of UVF patients, $p<0.001$). In 555 patients with WES or WGS, those with DCM had significantly higher proportions of their genome deriving from African haplotypes than those with UVF ($\beta=22.5\%$, $p<2x10\text{-}16$). Patients with CPC also had significantly higher proportions of African genetic ancestry ($\beta=7.3\%$, $p=0.012$).

Conclusions: Demographic and genetic characterization of our cohort, including 219 new sequenced coccidioidomycosis patients, captured known dissemination risk factors, including sex and ancestry. Further analyses in this cohort will reveal novel genetic risk factors for disease severity and this independent cohort can be used to assess the validity of previously published risk variants.

37. Single-cell Transcriptomic Analysis Reveals the Dynamics of Immune Infiltration and Differentiation in the Lungs During C. Posadasii Infection

Oscar Davalos[1], Aimy Sebastian[1], Nicholas Hum[1], Nadia Miranda[2,1], Deepa Murugesh[1], Margarita Rangel[1], Ashlee Phillips[1], Gabriela Loots[3,1], Katrina Hoyer[2], Dina Weilhammer[1]

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Abstract

Introduction: Coccidioidomycosis is an emerging pulmonary infection caused by inhalation of *Coccidioides* fungi that are native to the arid soils of the southwestern United States. Cases of *Coccidioides* infection are estimated at 350,000 per year, a number which is anticipated to rise as climate change expands the geographic region where the fungus thrives. Most cases are asymptomatic and resolve spontaneously, however a subset of patients go on to develop long lasting and potentially life threatening illness. Understanding of basic mechanisms of fungal pathogenesis within the lungs is lacking, hampering vaccine and therapeutic development. To address this knowledge gap, we performed a thorough transcriptomic analysis of disease progression in the lungs over time using a mouse model of infection coupled with single cell RNA sequencing (scRNA-seq).

Methods: C57BL/6 mice were infected intranasally with 500 *C. posadasii* Silveira arthroconidia (5 mice/group). Lungs were harvested from infected mice at days 5, 9, and 14 post-infection as well as from uninfected control mice. Lungs were homogenized and processed for fungal burden determination and lung cells from 3 mice/group with comparable fungal burden were pooled and analyzed via scRNA-seq analysis. Sequencing libraries were generated using the 10x Genomics Fixed RNA kit and sequenced using an Illumina NextSeq 2000. Computational analysis of transcriptome data to determine cell-specific gene expression and cell identity was performed using Cell Ranger Single-Cell Software Suite and Seurat R toolkit.

Results: Disease progression over time was associated with a significant increase in cellularity within the lungs, with visible scarring of the lungs by day 14. Sequencing data identified 11 cell type clusters corresponding to immune and non-immune cell types. Massive infiltration of multiple myeloid cell populations was observed, including a significant increase in $Spp1^{+}$ macrophages by day 9 and neutrophil abundance by late-stage infection, in conjunction with a decrease in the relative abundance of fibroblasts, epithelial, and endothelial cells. Analysis of gene expression profiles indicated a shift towards a fibrotic phenotype in both infiltrating and tissue resident macrophages.

37. Single-cell Transcriptomic Analysis Reveals the Dynamics of Immune Infiltration and Differentiation in the Lungs During C. Posadasii Infection *(continued)*

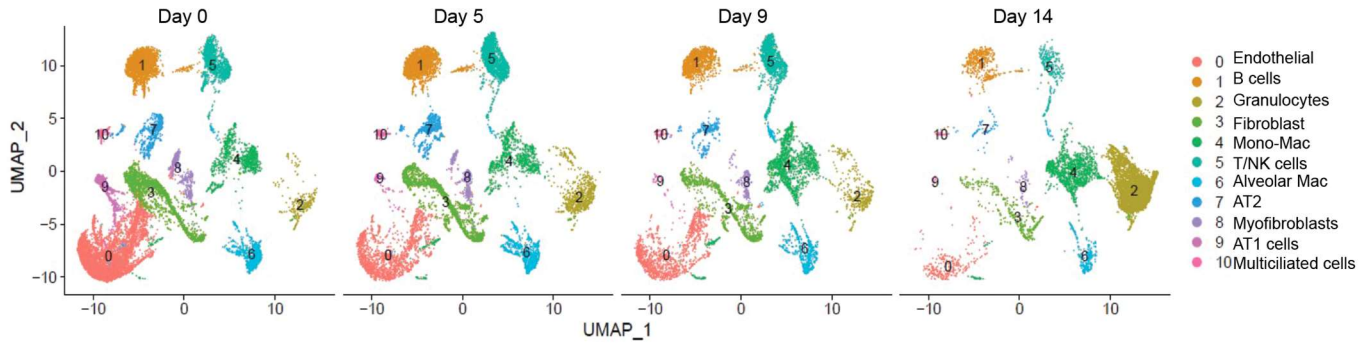

Fig 1. scRNA-seq reveals shifts in cell populations and immune infiltration in the lungs following *C. posadasii* infection. UMAP plots depicting cell clusters derived from the lungs of uninfected (Day 0) or infected mice over a time course of *C. posadasii* infection.

Conclusion: Employment of advanced sequencing technologies has enabled a thorough characterization of the response of diverse cell types within the lung to *Coccidioides* infection. Analysis of the response of each cell population will provide critical information about how the fungus establishes infection and evades immune clearance, which will be critical for the guidance of vaccine and therapeutic development.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Security, LLC, Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

38. Outcomes of Fluconazole Discontinuation in Solid Organ Transplant Recipients with at Least One *Coccidioides* Seropositivity

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Abstract

Introduction: Coccidioidomycosis (CM) in solid organ transplant (SOT) recipients is associated with high dissemination and mortality rates. Antifungal prophylaxis (PPX) for the suppression or prevention of CM in SOT recipients is an established practice that decreases CM in this population. Prior to SOT, all candidates are screened for CM using enzyme immunoassay (EIA), immunodiffusion (ID), and complement fixation (CF). However, when used as a screening tool, EIA IgM is frequently falsely positive. Following the first post-SOT year, low-risk, seronegative patients stop azole PPX; patients who had recently active pre-SOT CM, or who were seropositive prior to transplant (including potentially falsely positive EIA IgM-only), continue lifelong azole PPX. Fluconazole is the most utilized PPX for CM following SOT and is associated with several "minor" but disruptive adverse effects (such as alopecia or cheilitis) negatively affecting patient quality of life, and thus may be stopped by the patient. Such discontinuation may lead to relapsed CM. In this study, we sought to summarize the outcomes of SOT recipients with positive pre-SOT serology who stopped azole PPX.

Methods: We conducted a retrospective case-controlled study from a single high-volume SOT center located in Phoenix, AZ. Using transplant department records, we conducted retrospective chart reviews to identify SOT recipients who were seropositive at the time of SOT and were recommended to continue lifelong PPX but stopped. Case patients were seropositive at transplant and discontinued azole PPX. Control patients were SOT recipients with positive pre-SOT cocci serology (matched by age, race, and sex) who did not stop PPX. Primary and secondary outcomes were mortality and relapsed CM. We used summary statistics, compared proportions, and conducted univariate survival analysis, adjusting for several variables. A p value of <0.05 was considered significant. Mayo Clinic IRB approved and granted exempt status due to the low-risk nature of our study.

Results: From 1/1/2013-12/31/2020, there were a total of 4087 SOTs. During this time, 77 pre-SOT seropositive patients stopped their SOT-protocol-informed azole PPX (cases) and were matched with 77 controls. Cases and controls had median ages of 56 years, 55% male, 79% white, and 7% Black, with similar rates of comorbid diabetes and cancer; 88% lived in the *Coccidioides*-endemic area. Median follow-up time was 3 to 119 months. PPX discontinuation occurred between 16 and 1811 days post-SOT. 2/77 cases (versus 0/77 controls) experienced CM relapse (one of whom died), and one of these with relapsed CM occurred among the 27 EIA IgM-only cases. Among the cases, the consulting Infectious Disease physician's impression was that the pre-SOT serology was falsely positive (primarily EIA IgM-only) 31% of the time, compared to 12% of controls ($P<0.01$), which may have in part influenced eventual PPX discontinuation following repeat testing to confirm it was a false positive. Among cases and controls, 18 (11.7%) died in the follow-up period, and risk factors for death included diabetes ($p=0.02$) and relapsed CM ($p=0.51$). In cases with isolated EIA IgM-only positive serology, there was no increased mortality (HR 0.884, $p=0.887$).

Conclusion: Coccidioidomycosis and its prevention are important concerns in the SOT population, yet azole PPX is commonly accompanied by adverse effects. This study demonstrates that in SOT recipients with isolated EIA IgM seropositivity, interpreted as a falsely positive result, it may be safe to stop px after the first post-transplant year.

39. Spinal Cord Involvement with Coccidioidal Meningitis: A Case Series of 45 Adult Patients

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Abstract

Introduction: Central nervous system (CNS) involvement with coccidioidomycosis (cocci) is a serious infection that is universally fatal if not treated. Prior to the advent of magnetic resonance imaging (MRI), very few reports describe spinal cord involvement and autopsies often omitted spinal cord examination. Accurate and anatomic localization of areas affected by CNS cocci can be challenging due to mental status changes and the presence of brain abnormalities. Here, we describe radiologic and clinical characteristics of 45 cases of CNS cocci who had spinal MRI imaging performed.

Methods: This was a retrospective case series reviewing 45 adult patients at the Valley Fever Institute in Bakersfield, California with patients who had confirmed CNS cocci and MRI of at least one segment of the spinal image with and without contrast between 2011-2024. A waiver of consent was submitted, and approval was obtained by Kern Medical's Institutional Review Board. ICD 9 and ICD 10 codes were used to query electronic health record and cross referenced with completed imaging. Each record reviewed required inclusion criteria of age above 18 years, cerebrospinal fluid (CSF) abnormalities compatible with chronic meningitis and one of the following: positive CSF IgG antibody, CSF complement fixation (CF) for coccidioides or growth of coccidioides on CSF culture. MRI Imaging with and without contrast of at least one of the following: cervical, thoracic or lumbar spine.

Results: 80% of the patients were Latinx, 11% African American, 4% Asian and 4% Caucasian. Males make up the majority at 73%. Spinal cord abnormalities can be asymptomatic with the most common symptom of back pain followed by radiculopathy.

Conclusion: Coccidioidal meningitis frequently involves the spinal cord. Radiologic findings include leptomeningeal enhancement, adhesive arachnoiditis with nerve root clumping, myelitis and syringomyelia. Heightened awareness is required due to unpredictable symptomatology. This suggests potential benefit of performing MRI imaging on the entire neuro-axis at the time of diagnosis.

40. Elucidating Key Interactions Between Macrophages and the Fungal Pathogen Coccidioides

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Abstract

Introduction: In the environment, *Coccidioides* grows as hyphae, yet when the spores (arthroconidia) are inhaled by a host, they develop into spherules. Spherules can be elicited *in vitro* in the presence of specific media, elevated temperatures, and high CO2, yet what triggers this transition *in vivo* and the role of host immune cells in this transition remains largely unknown.

Methods: We performed live imaging of *Coccidioides posadasii* Silveira arthroconidia in the presence or absence of murine bone marrow derived macrophages isolated from wildtype (C57BL/6) mice) at different multiplicities of infection (MOI) of 0.1 or 1 (1 arthroconidium per 10 macrophages or 1 arthroconidium per macrophage). We noted the number of spherules or hyphae that develop over time. In transwell experiments, we separated *Coccidioides* and macrophages and examined the effect on morphogenesis. Images were taken every hour for the duration of the 3-day time course. We recorded the time at which hyphae first appeared, the number of spherules at the final time point, and average size of spherules at the final time point. We utilized fluorescent dyes to determine if arthroconidia has been phagocytosed and to assess host cell death. RNAseq was performed on RNA isolated from macrophages infected with Silveira arthroconidia at MOI 1 or MOI 0.1 at multiple timepoints over the course of 48 hrs. All experiments were conducted at 37C and 5% CO2.

Results: We observed that the presence of macrophages strongly promotes the ability of *Coccidioides* arthroconidia to transition to spherules at conditions that would otherwise promote formation of hyphae. In wells containing macrophages, there are higher number of spherules and they are larger in size. The presence of macrophages also delayed the time that hyphae first appear. Using a transwell system, we found that physical contact with macrophages was necessary to both promote spherule development and reduce hyphae formation. We observed that *Coccidioides* arthroconidia are phagocytosed by macrophages and that spherules can develop within macrophages. We are currently using transcriptional profiling to identify and characterize host pathways that play key roles in the macrophage response to *Coccidioides in vitro* as arthroconidia develop into spherules.

Conclusion: Macrophages strongly promote the ability of *Coccidioides* arthroconidia to transition to the parasitic form (spherules) in a contact-dependent manner. This work creates a foundation for better understanding the initial interactions between key immune cells and *Coccidioides*.