Coccidioidomycosis STUDY GROUP

69th Annual Coccidioidomycosis Study Group Meeting Abstracts

April 4-5, 2025 | Health Futures Center | Phoenix, AZ

Meeting Agenda
Day 1: Friday, April 4, 2025

7:30 am – 4:00 pm	**Registration Open**
7:30 am – 8:00 am	**Continental Breakfast**
8:00 am – 8:15 am	**Opening Remarks:** *President: Bridget Barker, PhD* *Local Hosts: Douglas Lake, PhD & Mitch Magee, PhD*
8:15 am – 10:15 am	**Scientific Section I | Clinical Advances** *Moderators: Neil Ampel, MD & Mitch Magee, PhD*
8:15 am – 8:30 am	**Immune and Metabolic Biosignatures and Associated Pathogenic Mechanisms of Coccidioides Infection and Restoration During Recovery Phase** Ramona Abbattista, Ikaika Loque, Clarissa Rocha, Sumanthi Sankaran-Walters, Elise Buser, Kelly Crucillo, Elizabeth Robison, Marie Nearing, Mame Ndiaye, Erik Settles, George Thompson, Satya Dandekar UC Davis, Davis, CA, USA
8:30 am – 8:45 am	**Paradoxical Reactions to Olorofim Therapy in Patients Undergoing Treatment** George Thompson[1], Anke Bruns[2], Christopher Heath[3], Jannik Helweg-Larsen[4], Philip Koehler[5], Jeffrey Jenks[6], Bennett Penn[1], Martin Hoenigl[7], Johan Maertens[8], Andrej Spec[9], Tom Walsh[10], Mark Bresnik[11], John2 Rex[11] [1]UC-Davis, Sacramento, USA. [2]University Medical Center, Utrecht, The Netherlands, Utrecht, Netherlands. [3]Department of Medicine, University of Western Australia, Western Australia, Australia. [4]Copenhagen University Hospitals, Copenhagen, Denmark. [5]University of Cologne, Cologne, Germany. [6]Durham County Department of Public Health, Durham, USA. [7]Medical University of Graz, Graz, Austria. [8]University Hospitals Leuven, Leuven, Belgium. [9]Washington University, St. Louis, USA. [10]Weill Cornell Medicine, New York, USA. [11]F2G, Manchester, United Kingdom
8:45 am – 9:00 am	**Comparing Diagnostic Values of Fungal Flip-Flop Sign Versus Serology in FDG Avid Pulmonary Nodules in Coccidioides-Endemic Regions** Jingjing Chen[1], Nikita Ashcherkin[2], Joe Zein[1], Neil Ampel[1], Anisha Shetty[1], Dakota McNierney[1], Felipe Martinez[1], Natalya Azadeh[1], Cyril Varghese[1], Kenneth Sakata[1] [1]Mayo Clinic, Scottsdale, USA. [2]Duke University, Durham, USA
9:00 am – 9:15 am	**Developing a Flow Cytometry-based Assay to Diagnose and Monitor Valley Fever Cellular Responses** Mrinalini Kala[1], Mame Diarra Bousso Ndiaye[2], Erin Kelley[3], Maxx Harvey[2], Farhan Babur[1], Garrett Grischo[1], Jessica Marshall[2], Jinhee Yi[2], John Altin[3], Bridget Barker[2], Paul Keim[2], Kenneth Knox[1], Erik Settles[2] [1]Department of Internal Medicine, University of Arizona College of Medicine Phoenix, Phoenix, USA. [2]Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, USA. [3]3The Translational Genomics Research Institute (TGen), Flagstaff, USA
9:15 am – 9:45 am	**Break/Visit Our Sponsors**

Day 1: Friday, April 4, 2025

9:45 am – 10:00 am	**Pediatric Coccidioidomycosis in an Integrated Managed Care System** Sean Fitzwater[1], Chiara Michienzie[1], John Galgiani[2] [1]Kaiser Permanente Southern California, Los Angeles, USA. [2]University of Arizona, Tucson, USA
10:00 am – 10:15 am	**Pearls and Pitfalls of Intrathecal Amphotericin B Therapy for Refractory Coccidioidal Meningitis in Children: An Illustrative Pediatric Case Series** Sanchi Malhotra[1], Kristina Adachi[1], Aria Fallah[1], Royce Johnson[2], Ishminder Kaur[1], James McCarty[3], Lawrence Ross[4], Paul Krogstad[1] [1]University of California Los Angeles, Los Angeles, USA. [2]Kern Medical Center, Bakersfield, USA. [3]Stanford University, Stanford, USA. [4]Keck School of Medicine, Los Angeles, USA
10:15 am – 10:45 am	**Break/Visit Our Sponsors**
10:45 am – 12:15 pm	**Scientific Section II | Immunology** *Moderators: Chiung-Yu Hung, PhD & Janis Blair, MD*
10:45 am – 11:00 am	**B Cell Depletion Damages Granuloma Formation in a Mouse Model of Coccidioidomycosis** Lisa Shubitz, Daniel Powell, Christine Butkiewicz, Hien Trinh, John Galgiani University of Arizona, Tucson, USA
11:00 am – 11:15 am	**Identification of Coccidioides spp. Specific T Cell Clones in Naturally Exposed Pig-tailed Macaques (Macaca Nemestrina)** Allison Harmon[1], Mame Ndiaye[1], Jessica Marshall[1], Paul Phillips[1], Megan Fredericks[2], Deborah Fuller[2,3], Bridget Barker[1], Paul Keim[1], Erik Settles[1] [1]Pathogen and Microbiome Institute; Northern Arizona University, Flagstaff, USA. [2]Department of Microbiology; University of Washington, Seattle, USA. [3]Washington National Primate Research Center, Seattle, USA
11:15 am – 11:30 am	**Single-cell Transcriptomic Analysis Reveals Unique Patterns of Myeloid Cell Differentiation and Potential Therapeutic Targets During C. Posadasii Infection** Dina Weilhammer, Oscar Davalos, Margarita Rangel, Deepa Murugesh, Nicole Leon, Ashlee Phillips, Aimy Sebastian, Nicholas Hum Lawrence Livermore National Laboratory, Livermore, USA
11:30 am – 11:45 am	**Single-Cell Analysis and Functional Exploration of Human Airway Epithelial Responses to Coccidioides Posadasii Infection** Olivia W Hepworth[1,2], Alfred T Harding[3,4], Arianne J Crossen[1], Jennifer L Reedy[1,2], Kyle J Basham[1], Yanting Zhang[5], Viral S Shah[6,7,8,9], Hannah Brown Harding[1,2], Manalee V Surve[6,7,10], Patricia Simaku[1], Geneva N Kwaku[1], Kirstine Nolling Jensen[1,2], Yohana Otto[7], Rebecca A Ward[1], George R Thompson 3rd[11], Bruce S Klein[12,13,14], Jayaraj Rajagopal[6,7,8,9], Pritha Sen[2,15,16], Adam L Haber[5], Jatin M Vyas[1,2]

Day 1: Friday, April 4, 2024

10:45 am – 12:15 pm	**Scientific Section II | Immunology** *Moderators: Chiung-Yu Hung, PhD & Janis Blair, MD (continued)*
	[1]Division of Infectious Diseases, Department of Medicine, Massachusetts General Hospital, Boston, USA. [2]Department of Medicine, Harvard Medical School, Boston, USA. [3]Institute for Medical Engineering and Sciences, Massachusetts Institute of Technology, Cambridge, USA. [4]Department of Microbiology, Harvard Medical School, Cambridge, USA. [5]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, USA. [6]Harvard Stem Cell Institute, Cambridge, USA. [7]Center for Regenerative Medicine, Massachusetts General Hospital, Boston, USA. [8]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Massachusetts General Hospital, Boston, USA. [9]Klarman Cell Observatory, Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, USA. [10]Broad Institute of MIT and Harvard, Cambridge, USA. [11]Division of Infectious Diseases, and Departments of Internal Medicine and Medical Microbiology and Immunology, University of California-Davis, Sacramento, USA. [12]Department of Pediatrics, School of Medicine and Public Health, University of Wisconsin Madison, Madison, USA. [13]Department of Medicine, School of Medicine and Public Health, University of Wisconsin Madison, Madison, USA. [14]Department of Medical Microbiology and Immunology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, USA. [15]Transplant, Oncology, and Immunocompromised Host Group, Division of Infectious Disease, Department of Medicine, Brigham and Women's Hospital, Boston, USA. [16]Dana-Farber Cancer Institute, Boston, USA
11:45 am – 12:00 pm	**Diabetic Mice are More Susceptible to Coccidioides Posadasii Infection, but Protected by a Subunit Vaccine** Matthew Mendoza Barker, Althea Campuzano, Sofia Lozano, Nawal Abdul-Baki, Reimi Navarro, Austin Negron, Jieh-Juen Yu, Astrid Cardona, Chiung-Yu Hung Department of Molecular Microbiology and Immunology and South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, San Antonio, USA
12:00 pm –12:15 pm	**Multi-Valent DNA Vaccines Encoding Coccidioides Proteins and Potent Genetic Adjuvants Induce Mucosal and Th1 Immune Responses, Afford Protection Against High Dose Challenges with C. Posadasii and Enable the Discovery of Novel Immunogens** Deborah H Fuller[1,2], James T Fuller[1], Daniel Kollath[3], Ana Braga[3], Phillip M Bohn[1], Sanford E Leake IV[1], Erik Settles[3], Bridget Barker[3] [1]University of Washington, Seattle, USA. [2]Washington National Primate Research Center, Seattle, USA. [3]Northern Arizona University, Flagstaff, USA
12:15 pm – 1:30 pm	**Lunch and Networking,** *Sponsored by* MiraVista DIAGNOSTICS
1:30 pm – 3:00 pm	**Symposium Panel Discussion: Cocci Risks in the Immunomodulators Era** *Moderator: Fariba Donovan, MD, PhD* *Invited Speaker: Sharon Chen, MD, "*Does This "Biologic" Increase the Risk of Cocci Infection? Connecting the Biologic Target to the Host Immune Response to Cocci*"* *Panelists: Neil Ampel, MD, Janice Blair, MD, John Galgiani, MD, George Thompson III, MD, Fariba Donovan, MD, PhD*

Day 1: Friday, April 4, 2024

3:00 pm – 3:30 pm	**Break/Visit Our Sponsors**
3:30 pm – 4:45 pm	**Scientific Section III | Ecology** *Moderators: Rebecca Sunenshine, MD & Douglas Lake, PhD*
3:30 pm – 3:45 pm	**Updated Ecological Niche Model of Coccidioides Using Compiled Soil Samples** Morgan Gorris[1], Cari Lewis[1], Bridget Barker[2], Daniel Kollath[2], Marieke Ramsey[2], Heather Mead[3], Antje Lauer[4], Hanna Oltean[5], Adriana Romero-Olivares[6], Jovani Catalán-Dibene[6], Yahaira Álvarez-Gandía[1], Kimberly Kaufeld[1], Andrew Bartlow[1] [1]Los Alamos National Laboratory, Los Alamos, USA. [2]Northern Arizona University, Flagstaff, USA. [3]The Translational Genomics Research Institute, Flagstaff, USA. [4]California State University Bakersfield, Bakersfield, USA. [5]Washington State Department of Health, Shoreline, USA. [6]New Mexico State University, Las Cruces, USA
3:45 pm – 4:00 pm	**Leveraging Elevation Gradients to Characterize the Distribution of Coccidioides in Southern Utah: A Field and Modeling Study** Emanuel Fonseca[1,2], Katrina Derieg[3], Eric Rickart[4], Evelyn Adams[1], Ammon Miles[1], Bridget Barker[5], Kevin Perry[1], Katharine S. Walter[1] [1]University of Utah, Salt Lake City, UT, USA. [2]Elfa Analytics, Ponte Nova, Brazil. [3]Philip L. Wright Zoological Museum, Missoula, MT, USA. [4]Natural History Museum of Utah, Salt Lake City, UT, USA. [5]Northern Arizona University, Flagstaff, AZ, USA
4:00 pm –4:15 pm	**Temporal Surveillance of Coccidioides in Aerosols and Soils at a Single Location in Arizona** Amelia Stout[1], Marieke L. Ramsey[2], Daniel R. Kollath[2], Megan C. Ruby[2], Bridget M. Barker[2], Pierre Herckes[1], Matthew Fraser[1] [1]Arizona State University, Tempe, USA. [2]Northern Arizona University, Flagstaff, USA
4:15 pm –4:30 pm	**Multiplexed qPCR Lab-on-Chip Technology and Personal Exposome Tracker for Scalable Environmental Surveillance of Coccidioides** Jeremy Woods[1], Fanqing Chen[1], Allison Zhang[2], Michael Snyder[2] [1]Kelliop, Newark, USA. [2]Stanford, Palo Alto, USA
4:30 pm –4:45 pm	**Transcriptomic Atlas of the Morphologic Development of the Fungal Pathogen Coccidioides Reveals Key Phase-Enriched Transcripts** Christina Homer, Mark Voorhies, Keith Walcott, Elena Ochoa, Anita Sil University of California, San Francisco, San Francisco, USA
4:45 pm – 5:00 pm	**Break/Visit Our Sponsors**

5:00 pm – 7:00 pm	Poster Session and Networking, *Sponsored by* MiraVista DIAGNOSTICS

Day 2: Saturday, April 5, 2025

7:30 am – 8:30 am	Continental Breakfast
8:30 am – 9:30 am	Keynote Address: *Insights from Talaromycosis That May Be Inferred for Coccidioidomycosis,* **Thuy Le, PhD, DPhil,** Associate Professor of Medicine, Molecular Genetics and Microbiology at Duke University School of Medicine, Co-Director of the Clinical Core of the Duke Center for AIDS Research, and Co-Director of the Tropical Medicine Research Program for Talaromycosis in Vietnam
9:30 am – 9:45 am	Break/Visit Our Sponsors
9:45 am – 10:45 am	Scientific Section IV | Basic Science *Moderator: GR Thompson III, MD*
9:45 am – 10:00 am	**Macrophages Promote the Development of Coccidioides Spores into the Parasitic Morphology** Jane Symington, Apoorva Dabholkar, Bevin English, Mark Voorhies, Anita Sil UCSF, SF, USA
10:00 am – 10:15 am	**Novel Peptide Drug Demonstrates Anti-Coccidioides Activity** Sofia Lozano[1], Sarah Saeger[1], Matthew Mendoza Barker[1], Guiseppe Buda de Cesare[2], Michael Lorenz[2], Jieh-Juen Yu[1], Chiung-Yu Hung[1] [1]University of Texas at San Antonio, San Antonio, USA. [2]University of Texas Health Science Center at Houston, Houston, USA
10:15 am – 10:30 am	**The Coccidioides Tri-factor: Innate Immune Detection of Fungal Conidia, Spherules and Endospores by Macrophages** Ka Pui Sharon Yau[1], Jonathan Rodrigo Erlich[1], Matthew Tate[2], Janset Onyuru[1], Cameron J. Nowell[3], Aaron Carlin[1], Theo Kirkland[1], Josh Fierer[1,4], Hal M. Hoffman[1], Sinem Beyhan[2], Ben A. Croker[1] [1]University of California San Diego, La Jolla, USA. [2]J. Craig Venter Insitute, La Jolla, USA. [3]Monash Institute of Pharmaceutical Sciences, Parkville, Australia. [4]VA Medical Center, San Diego, USA
10:30 am – 10:45 am	**Discovery of Novel Seroreactive Antigens for Coccidioides** Megan Koehler[1], Kenta Reilly[2], Francisca Grill[2], Lusheng Song[1], Yunro Chung[1], Vel Murugan[1], Joshua LaBaer[1], Marc Orbach[3], Janis Blair[2], Thomas Grys[2], Douglas Lake[1], D Mitch Magee[1] [1]Arizona State University, Tempe, USA. [2]Mayo Clinic, Phoenix, USA. [3]The University of Arizona, Tucson, USA
10:45 am – 11:15 am	Break/Visit Our Sponsors

Day 2: Saturday, April 5, 2025

11:15 am – 12:15 pm	**Scientific Section V | Molecular Biology** *Moderator: Bridget Barker, PhD*
11:15 am – 11:30 am	**Expanding the Known Genomic Diversity of Coccidioides Posadasii Through Whole Genome Sequencing and Comparative Genomics** Jason Sahl[1], Bridget Barker[1], John Galgiani[2], David Wagner[1], Paul Keim[1], Dawn Birdsell[1], Dan Kollath[1], Irene Ruberto[3], Thomas Williamson[3] [1]Northern Arizona University, Flagstaff, USA. [2]University of Arizona, Tucson, USA. [3]Arizona Department of Health Services, Phoenix, USA
11:30 am – 11:45 am	**Ancestry-aware GWAS Identifies Novel Common Variants Associated with Susceptibility to Disseminated Coccidioidomycosis** Samantha L. Jensen[1], Sarah J. Spendlove[2], Alexis V. Stephens[1], Zhenjie Jin[1], Kangcheng Hou[1], Christa Caggiano[3], Ruhollah Shemirani[3], Rachel Mester[1], George R. Thompson[4], Royce H. Johnson[1,5], Arash Heidari[6,7], Rasha Kuran[1,5], Bogdan Pasaniuc[1], Harold Pimentel[1], Manish J. Butte[1], Valerie A. Arboleda[1] [1]UCLA, Los Angeles, USA. [2]Kaiser Permanente Molecular Genetic Pathology Laboratory, Los Angeles, USA. [3]Icahn School of Medicine at Mount Sinai, New York City, USA. [4]UC Davis, Davis, USA. [5]Kern Medical, Bakersfield, USA. [6]Bakersfield Memorial Hospital, Bakersfield, USA. [7]Morehouse School of Medicine, Atlanta, USA
11:45 am – 12:00 pm	**Assessing Hierarchical Population Structure Among Coccidioides spp. Across the Americas** Cari Lewis, Morgan Gorris, Kimberly Kaufeld, Andrew Bartlow Los Alamos National Laboratory, Los Alamos, USA
12:00 pm – 12:15 pm	**Coccidioides Genomes from Low-Incidence States Reveal Complex Migration History Across the Western US** Emanuel M. Fonseca[1,2], Adrienne L. Carey[3], Bridget Barker[4], Megan Hirschi[3], Kimberly E. Hanson[3,5], Katharine S. Walter[1] [1]Division of Epidemiology, University of Utah, Salt Lake City, USA. [2]Elfa Analytics, Ponte Nova, Brazil. [3]Department of Medicine, Division of Infectious Diseases, University of Utah School of Medicine, Salt Lake City, USA. [4]Department of Biological Sciences, Northern Arizona University, Flagstaff, USA. [5]Department of Pathology, Division of Clinical Microbiology, University of Utah and ARUP Laboratories, Salt Lake City, USA
12:15 pm – 1:30 pm	**Lunch Break/Business Meeting** - *Sponsored by* MiraVista DIAGNOSTICS
1:30 pm – 2:00 pm	**CDC & NIAID Update**

Day 2: Saturday, April 5, 2025

2:00 pm – 4:15 pm	**Scientific Section VI | Epidemiology** *Moderators: Lisa Shubitz, DVM & Adrienne Carey, MD*
2:00 pm – 2:15 pm	**Testing for Coccidioidomycosis in Hospitalized Patients with Pneumonia Within the Coccidioides-Endemic Area** Janis Blair, Gretchen Taylor Mayo Clinic, Phoenix, AZ, USA
2:15 pm – 2:30 pm	**Increasing Cases of Severe Pediatric Coccidioidomycosis From 2000 to 2024: Description and Outcomes at a Tertiary Care Center** Sanchi Malhotra, Kristina Adachi, Paula Arribas, Ishminder Kaur, Paul Krogstad University of California Los Angeles, Los Angeles, USA
2:30 pm – 2:45 pm	**Characteristics of Disseminated Coccidioidomycosis in Pediatric Patients** Sarah Zhang[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, Phoenix, USA
2:45 pm – 3:00 pm	**Owner Economic Burden of Canine Coccidioidomycosis in Endemic States** Christine Butkiewicz[1], Jane Sykes[2], Lisa Shubitz[1] [1]University of AZ - VFCE, Tucson, USA. [2]School of Veterinary Medicine, University of California-Davis, Davis, USA
3:00 pm – 3:15 pm	**Break**
3:15 pm – 3:30 pm	**Delayed Diagnosis and Treatment of Coccidioidomycosis Identified Through Enhanced Surveillance — Riverside County, California, 2024** Bethan Swift[1,2], Amanda Mitry[2], Wendy Hetherington[2], Jennifer Chevinsky[2], Marshare Penny[2], Barbara Cole[2] [1]Centers for Disease Control and Prevention, Atlanta, USA. [2]Riverside County Department of Public Health, Riverside, USA
3:30 pm – 3:45 pm	**Forecasting the Impact of Hydroclimatic Swings on Coccidioidomycosis Incidence in California** Simon Camponuri[1], Alexandra Heaney[2], Gail Sondermeyer-Cooksey[3], Duc Vugia[3], Seema Jain[3], Daniel Swain[4,5], John Balmes[1,6], Justin Remais[1], Jennifer Head[7] [1]University of California, Berkeley, Berkeley, USA. [2]University of California, San Diego, San Diego, USA. [3]California Department of Public Health, Richmond, USA. [4]University of California, Los Angeles, Los Angeles, USA. [5]NSF National Center for Atmospheric Research, Boulder, USA. [6]University of California, San Francisco, San Francisco, USA. [7]University of Michigan, Ann Arbor, USA

2:00 pm – 4:15 pm	**Scientific Section VI | Epidemiology** *Moderators: Lisa Shubitz, DVM & Adrienne Carey, MD (continued)*
3:45 pm – 4:00 pm	**Coccidioidomycosis Cases at a Regional Medical Center in West Texas, 2021-2024** 34 Chun Ho Szeto[1], Gloria Erazao Motalvan[1], Thao Do[2], Trisha Singh[1], Sai Siva Mungara[1] [1]Texas Tech University Health Sciences Center at Permian Basin, Odessa, USA. [2]Medical Center Hospital, Odessa, USA
4:00 pm – 4:15 pm	**Refractory Disseminated Coccidioidomycosis with Musculoskeletal Involvement in a 14-year-old Effectively Treated with Adjunctive Interferon Gamma** Sarah Zhang[1], Keith Sacco[2,1], Michell Lozano Chinga[2,1], Jessica Burns[2,1], Kathryn King[2,1], Scott Osdiek[2,1], Wassim Ballan[2,1], Matthew Smith[2,1], Michelle Ratkiewicz[2,1], James Woodward[2,1], Holly Miller[2,1], Brenna LaBere[2,1] [1]University of Arizona, College of Medicine-Phoenix, Phoenix, USA. [2]Phoenix Children's, Phoenix, USA
4:15 pm – 4:30 pm	**Concluding Remarks, Poster & Travel Awards Recognition**
6:00 pm – 9:00 pm	**CSG Optional Networking Dinner** – **Separate ticket required* *The Sicilian Butcher | 15530 N Tatum Blvd, #160 | Phoenix, AZ*

Coccidioidomycosis
STUDY GROUP

Podium Presentations

Scientific Section I | Clinical Advances

Immune and Metabolic Biosignatures and Associated Pathogenic Mechanisms of Coccidioides Infection and Restoration During Recovery Phase

Ramona Abbattista, Ikaika Loque, Clarissa Rocha, Sumanthi Sankaran-Walters, Elise Buser, Kelly Crucillo, Elizabeth Robison, Marie Nearing, Mame Ndiaye, Erik Settles, George Thompson, Satya Dandekar
UC Davis, Davis, CA, USA

Paradoxical Reactions to Olorofim Therapy in Patients Undergoing Treatment

George Thompson[1], Anke Bruns[2], Christopher Heath[3], Jannik Helweg-Larsen[4], Philip Koehler[5], Jeffrey Jenks[6], Bennett Penn[1], Martin Hoenigl[7], Johan Maertens[8], Andrej Spec[9], Tom Walsh[10], Mark Bresnik[11], John2 Rex[11]
[1]UC-Davis, Sacramento, USA. [2]University Medical Center, Utrecht, The Netherlands, Utrecht, Netherlands. [3]Department of Medicine, University of Western Australia, Western Australia, Australia. [4]Copenhagen University Hospitals, Copenhagen, Denmark. [5]University of Cologne, Cologne, Germany. [6]Durham County Department of Public Health, Durham, USA. [7]Medical University of Graz, Graz, Austria. [8]University Hospitals Leuven, Leuven, Belgium. [9]Washington University, St. Louis, USA. [10]Weill Cornell Medicine, New York, USA. [11]F2G, Manchester, United Kingdom

Comparing Diagnostic Values of Fungal Flip-Flop Sign versus Serology in FDG Avid Pulmonary Nodules in Coccidioides-Endemic Regions

Jingjing Chen[1], Nikita Ashcherkin[2], Joe Zein[1], Neil Ampel[1], Anisha Shetty[1], Dakota McNierney[1], Felipe Martinez[1], Natalya Azadeh[1], Cyril Varghese[1], Kenneth Sakata[1]
[1]Mayo Clinic, Scottsdale, USA. [2]Duke University, Durham, USA

Developing a Flow Cytometry-based Assay to Diagnose and Monitor Valley Fever Cellular Responses

Mrinalini Kala[1], Mame Diarra Bousso Ndiaye[2], Erin Kelley[3], Maxx Harvey[2], Farhan Babur[1], Garrett Grischo[1], Jessica Marshall[2], Jinhee Yi[2], John Altin[3], Bridget Barker[2], Paul Keim[2], Kenneth Knox[1], Erik Settles[2]
[1]Department of Internal Medicine, University of Arizona College of Medicine Phoenix, Phoenix, USA. [2]Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, USA. [3]3The Translational Genomics Research Institute (TGen), Flagstaff, USA

Does Coccidioides Affect the Lung Mycobiome in Small Mammals? Using Molecular Methods to Examine the Prevalence of Coccidioides in Wild Mammals and Describe their Lung Fungal Mycobiome

Ana Fabio-Braga, Jaida Salois, Daniel Kollath, Bridget Barker
Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, USA

Pediatric Coccidioidomycosis in an Integrated Managed Care System

Sean Fitzwater[1], Chiara Michienzie[1], John Galgiani[2]
[1]Kaiser Permanente Southern California, Los Angeles, USA. [2]University of Arizona, Tucson, USA

Pearls and Pitfalls of Intrathecal Amphotericin B Therapy for Refractory Coccidioidal Meningitis in Children: An Illustrative Pediatric Case Series

Sanchi Malhotra[1], Kristina Adachi[1], Aria Fallah[1], Royce Johnson[2], Ishminder Kaur[1], James McCarty[3], Lawrence Ross[4], Paul Krogstad[1]
[1]University of California Los Angeles, Los Angeles, USA. [2]Kern Medical Center, Bakersfield, USA. [3]Stanford University, Stanford, USA. [4]Keck School of Medicine, Los Angeles, USA

Immune and metabolic biosignatures and associated pathogenic mechanisms of Coccidioides infection and restoration during recovery phase

Ramona Abbattista, Ikaika Loque, Clarissa Rocha, Sumanthi Sankaran-Walters, Elise Buser, Kelly Crucillo, Elizabeth Robison, Marie Nearing, Mame Ndiaye, Erik Settles, George Thompson, Satya Dandekar

UC Davis, Davis, CA, USA

Abstract

Introduction: Coccidioides infection leads to acute or chronic pulmonary disease (Valley Fever, VF) with a wide spectrum of disease severity. 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 to determine metabolic and immune correlates of Coccidioides infection and determine the mechanisms contributing to recovery from the infection. An understanding of the functional networks underlying the recovery process will identify candidates for diagnostic and therapeutic targeting.

Methods: We performed metabolomic profiling and immunologic analysis of peripheral blood and/or CSF samples from Individuals with Coccidioides infection during the stages of acute infection, disseminated infection and recovery from infection and correlated with clinical data as available (UCD IRB approved). The samples were divided among following Groups: (1): patients with Coccidioides infection; (2): Coccidioides infected patients with and without diagnosed meningitis; (3): Longitudinal assessment of Individuals with Coccidioides infection during the disease course; (4): patients with Coccidioides infection during anti-fungal therapy. Immunological parameters (Coccidioides antibody titers) and metabolomic changes (by untargeted metabolomic profiling) were analyzed and correlated with clinical data. Samples from uninfected healthy individuals served as controls. Multiplex Cytokine secretion assay was performed to determine peripheral blood immune cell responses to Coccidioides antigens.

Results: Coccidioides infection induced substantial changes in the metabolomic profile as compared to uninfected healthy controls. The most altered functional pathways included lipid metabolism, energy metabolism and inflammation. Metabolic changes linked *Coccidioides* infection to cellular energy starvation and consequent mitochondrial dysfunction. Alteration of acylcarnitine profile and ω-oxidation of fatty acids constitute an early signature of *Coccidioides* infection highlighting mitochondrial stress and peroxisomal activity for energy production. *Coccidioides* infection severely impacted androstanes steroids production in adrenal glands, suggesting endocrine dysfunction during the disease. Hypoxia affecting steroidogenesis and adrenal insufficiency may contribute to fatigue in VF disease. Individuals recovering from Coccidioides infection showed partial recovery of lipid and energy metabolism that was coupled with increased levels of androstenediols, suggesting the crucial role of adrenal glands in the recovery. Process. Changes in IL-2 cytokine responses in VF patients and recovered patients correlated with changes in androstanes. Longitudinal analysis validated the metabolic biosignature and identified cellular pathways contributing to disease pathogenesis and were independent of anti-fungal treatment effects.

Conclusion: Our study identified most dysregulated cellular functional pathways during *Coccidioides* infection and provided insights into pathogenic mechanisms and clinical manifestations of the infection and reveal new opportunities for the therapeutic approaches and monitoring of the outcomes.

Paradoxical Reactions to Olorofim Therapy in Patients Undergoing Treatment

George Thompson[1], Anke Bruns[2], Christopher Heath[3], Jannik Helweg-Larsen[4], Philip Koehler[5], Jeffrey Jenks[6], Bennett Penn[1], Martin Hoenigl[7], Johan Maertens[8], Andrej Spec[9], Tom Walsh[10], Mark Bresnik[11], John2 Rex[11]

[1]UC-Davis, Sacramento, USA. [2]University Medical Center, Utrecht, The Netherlands, Utrecht, Netherlands. [3]Department of Medicine, University of Western Australia, Western Australia, Australia. [4]Copenhagen University Hospitals, Copenhagen, Denmark. [5]University of Cologne, Cologne, Germany. [6]Durham County Department of Public Health, Durham, USA. [7]Medical University of Graz, Graz, Austria. [8]University Hospitals Leuven, Leuven, Belgium. [9]Washington University, St. Louis, USA. [10]Weill Cornell Medicine, New York, USA. [11]F2G, Manchester, United Kingdom

Abstract

Introduction: Olorofim (formerly F901318) is an orally available first-in-class antifungal currently in development for the treatment of invasive fungal infections (IFIs). Recently, an open-label, single-arm Phase IIb study (FORMULA; NCT03583164), designed to assess safety, tolerability, pharmacokinetics, and efficacy of oral olorofim in patients with IFIs for which there were limited, or no other treatment options was completed [6]. During the conduct of this trial several investigators noted a localized, paradoxical and transient worsening of disease in a subset of treated patients despite subsequent improvement

Methods: Detailed methods and results of the FORMULA trial have been previously described. Briefly, patients with hyaline moulds, endemic fungi, or other fungi requiring sponsor approval were enrolled in an open label Phase IIb study of olorofim therapy

Results: In the overall population of 203 patients, 202 patients had data review committee adjudicated IFIs, and 156 (76.5%) completed the main phase (Day 84-90) while 114 (55.9%) received extended treatment beyond day 90. Of this group, five patients were identified as fulfilling criteria for a paradoxical response to therapy. The demographic and baseline clinical characteristics are shown in Table 1. The majority of these patients were immunocompetent and 4/5 (80%) did not have evidence of underlying immunosuppression.

Conclusions: Paradoxical reactions to appropriate antimicrobial therapy have not been previously described in coccidioidomycosis or fungal diseases. Jarish-Herxheimer is well known following the treatment of spirochetes, and paradoxical responses are seen in the treatment of both tuberculosis (TB) (worsening of lymphadenitis) and leprosy (reversal reactions) suggesting a similar pathophysiologic mechanism may be responsible given the overlapping immune response between mycobacterial and fungal pathogens.

Comparing Diagnostic Values of Fungal Flip-Flop Sign versus Serology in FDG Avid Pulmonary Nodules in Coccidioides-Endemic Regions

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Abstract

Introduction: Pulmonary granulomatous disease can mimic metastatic lung cancer on FDG PET-CT due to FDG avidity, leading to unnecessary biopsies in fungal infection-endemic areas. The fungal flip-flop (FFF) sign has been observed in benign granulomatous disease, but its diagnostic utility remains understudied. The aim of the study is to compare the diagnostic performance of FFF sign and serology in PET-CT avid pulmonary nodules in Coccidioides-endemic areas.

Methods: FDG PET-CT performed between 2/2016-12/2021 for pulmonary nodules or mass were reviewed. Inclusion required ≥ one hilar or mediastinal lymph node with FDG avidity. Exclusion criteria included: 1) known current malignancy, 2) lack of pathologic confirmation or ≥ two years follow-up, and 3) solid and growing pulmonary nodule. Positive FFF sign criteria included: 1) ≥ one FDG-avid solid or partial-solid pulmonary nodule in the absence of necrosis, invasion, or calcification, 2) ≥ one FDG-avid draining lymph node with SUVmax ≥ that of the pulmonary lesion, and 3) no extrathoracic malignancy. A board-certified thoracic radiologist reviewed all PET-CT images. Final diagnoses were based on pathology or the absence of malignancy after two years of follow-up if biopsies were non-diagnostic. Patients were grouped as: 1) positive serology only (cocci EIA or complement fixation), and 2) both positive serology and FFF sign. The benignity was assessed using logistic regression models adjusted for age and gender. The primary outcomes were the sensitivity and specificity of FFF sign and serology in diagnosing coccidioidomycosis in the setting of FDG avid pulmonary nodule.

Results: There were no significant differences in demographic characteristics between the FFF-positive (n=6) and the serology-positive (n=24) group. Maximal SUV measurements of nodules and lymph nodes significantly differed between the above two groups. All patients with a positive FFF sign also had ≥ one positive serology. Compared to serology, positive FFF sign had lower sensitivity (41.67% vs. 81.82%) but higher specificity (94.44% vs. 50.00%) in identifying coccidioidomycosis. Logistic regression models showed that a benign diagnosis was significantly associated with positive serology (OR = 8.18, 95% CI [1.01, 66.46], p = 0.049) and lower nodule SUV levels (OR = 0.73, 95% CI [0.54, 0.97], p=0.032), indicating that for each unit decrease in SUV, the likelihood of benignity increased by approximately 37.7%. Adjusting for serolgoy results, positive FFF sign was not significantly associated with coccidioidomycosis (OR=2.06, 95% CI [0.20, 20.96], p=0.54). Though the positive predictive value (PPV) of FFF sign significantly differed from the PPV of serology (16.67% vs. 18.18%, p<0.0001), the absolute difference was minimal.

Conclusion: Positive serology and lower nodule SUV level, but not the FFF sign, remain key predictors of coccidioidomycosis in FDG-avid pulmonary nodules. These findings suggest that while FFF sign may support clinical decision-making in fungal-infection endemic regions, serology and SUVmax levels remain the most reliable diagnostic indicators of pulmonary coccidioidomycosis. Larger studies are needed to further assess the diagnostic value of FFF in this context.

Developing a Flow Cytometry-based Assay to Diagnose and Monitor Valley Fever Cellular Responses

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Abstract

Introduction: Valley Fever (VF) is caused by the endemic fungus *Coccidioides* and is a common cause of community acquired pneumonia in the American Southwest. Serologic detection of antibodies is useful in the diagnosis of VF during acute infection, but these *Coccidioides*-specific antibodies become undetectable after a few months. A standardized blood test that measures the long-lived T cell response would improve occupational health efforts, help define the expanding endemic area, allow physicians to personalize treatment regimens, and stratify who will be eligible for future vaccines. Flow cytometry is increasingly used to diagnose malignancies and to monitor responses to immunotherapy. We aim to leverage existing flow cytometry protocols in clinical laboratories to advance diagnostics in VF. Utilizing our novel immunostimulatory peptide cocktail (NAUpep108), we developed a flow cytometry-based assay to aid in VF diagnosis and vaccine response monitoring.

Methods: We identified VF specific T-cell epitopes by using their ability to bind MHC II molecules and stimulate T-cells. Additionally, we performed an MHC binding prediction algorithm and empirically tested the predictions on unknown antigens with an *in vitro* multiplex MHC binding analysis. PBMCs from subjects with a known diagnosis of VF, endemic (EHC) and nonendemic healthy controls (NEHC) were stimulated with the identified peptides and evaluated for immunologic memory responses by surface and intracellular flow cytometry. These subjects were further characterized by ex-vivo cytokine secretion (IFN-g, IL-2, IL-10, IL-6, TNF-a).

Results: A total of 108 *Coccidioides* peptides were identified. The 108 peptides (NAUpep108) were synthesized, pooled, and jointly evaluated for immunogenicity in VF positive individuals. The peptides stimulated memory $CD4^+$ T cells in VF confirmed and endemic control specimens when compared to PBMCs from non-endemic control subjects as determined by activation marker and cytokine secretion. Reactive VF specific T-cells were identified as responders, as indicated by $CD69^+$, $CD137^+$, $CD69^+/CD137^+$ co-expression and IFN-g secretion in $CD4^+$ memory T-cells. IFN-g accumulation in CD4 memory cell was significant between VF and NEHC (*p-value* = 0.041). $CD4^+CD137^+$ and $CD4^+CD137^+CD69^+$ activated memory cells were higher in VF patients compared to NEHC (respectively *p-value* = 0.0087, *p-value* = 0.047). $CD8^+CD69^+$ and

$CD8^+CD137^+CD69^+$ activated memory cells are higher in VF patients compared to NEHC (respectively *p-value* = 0.049, *p-value* = 0.034). IFN-g accumulation in CD4 and CD8 memory cell was significant between VF and EHC (*p-value* = 0.012). $CD8^+CD137^+$ activated memory cells are higher EHC compared to NEHC (*p-value* = 0.019). Ex-vivo cytokine analysis showed the proinflammatory cytokine IL-2 was significantly higher in VF group compared to NEHC (*p-value* = 0.0002). While IL-10 expression was significantly different in VF patients compared to NEHC and EHC (*p-value* = 0.0026 or 0.0485)

Conclusion: This study identified VF specific peptides capable of stimulating T cell memory responses in a flow cytometry-based assay. NAUpep108 was able to distinguish known infected individuals from endemic area healthy controls and non-exposed individuals residing outside the endemic area. Stimulation of PBMCs enhanced expression of T cell surface markers routinely assayed in clinical laboratories. Using routine flow cytometry to measure T cell responses represents a real-world approach to development of VF diagnostics.

Does Coccidioides Affect the Lung Mycobiome in Small Mammals? Using Molecular Methods to Examine the Prevalence of Coccidioides in Wild Mammals and Describe their Lung Fungal Mycobiome

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Abstract

Introduction: Lung mycobiome studies are limited, especially in wildlife. Studies looking at microbial communities in other systems report a decrease in diversity associated with active infection (Jani and Briggs, 2014, Allender *et al.*, 2018, Lloyd and Pespeni, 2018). The lung microbiome is challenging to study, due to low organism abundance and potential for detection of organisms that are not established in the microbiome, but transiently in the lung. According to the endozoan hypothesis (Taylor and Barker, 2019), *Coccidioides* could be present in wildlife's lungs without causing disease, behaving as a commensal. In this study, we describe the mycobiome in lungs of wildlife and compare communities between *Coccidioides* positive and negative samples. Based on the endozoan hypothesis, if *Coccidioides* is not causing infection, we expect no significant differences in fungal communities of positive and negative samples.

Methods: Small mammals used in this study were trapped in Tucson, AZ (n=26) and Mesa, AZ (n=14). Lung samples were donated to Northern Arizona University postmortem. Lung samples were screened for *Coccidioides* presence through CocciDx assay (Bowers *et al.*, 2019) and mycobiome communities were obtained through an Illumina-based amplicon sequencing of ITS2 (Taylor *et al.*, 2016). We compared alpha and beta diversity to identify effects associated with *Coccidioides* presence and host species differences. Additionally, we researched the literature and used the FunGuild database (Nguyen et al., 2016) to identify fungal genera that were previously known to be associated with vertebrates.

Results: In Mesa samples, we found 384.9±953.5 sequence reads per sample with 16.8±8.8 genera identified per sample. The top five genus were *Coccidioides* (78.7%), *Epicoccum* (5.5%), *Toxicocladosporium* (2.0%), *Alternaria* (1.9%), and *Neodidymelliopsis* (1.8%). In Tucson samples, we found 114706.4±57945.8 sequence reads per sample with 12±7.8 genera identified per sample. The top five genus were *Ajellomyces* (34.8%), *Chaetomium* (10.9%), *Rhizopus* (7.1%), *Naganishia* (6.7%) and Thelebolus (5.2%). We found that most of the fungal genera in our samples were previously associated with vertebrate hosts: 89.2% of the sequence reads in Tucson samples and 88.3% in Mesa samples. Samples from Mesa were not used for diversity comparisons since all samples were positive for *Coccidioides*. No difference in alpha and beta diversity was found between *Coccidioides* positive and negative samples from Tucson. Host species had a significant effect on beta diversity, but not on alpha diversity. Our results support the endozoan hypothesis, since no differences associated with *Coccidioides* presence were found. We speculate that the effect of host species on beta diversity is due to behavioral and physiological differences that help shape the mycobiome in the lungs.

Conclusion: In this study we described the mycobiome of wildlife lung samples from two locations in Arizona where *Coccidioides* is endemic. We found no difference in alpha and beta diversity between *Coccidioides* positive and negative samples, supporting the endozoan hypothesis (Barker & Taylor, 2019). We recognize our study is limited due to sample size and cross-sectional design. Future observational studies would benefit from more comprehensive datasets with more samples as well as more information on general health and *Coccidioides* status for study subjects. This could be achieved by looking at animal samples coming from zoos, rehabilitation centers, and farms.

Pediatric Coccidioidomycosis in an Integrated Managed Care System

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Abstract

Introduction: Management of pediatric coccidioidomycosis (CM) is highly variable. There are no pediatric specific guidelines and there are few comprehensive pediatric studies. Those studies that exist tend to focus on severe cases seen at tertiary care hospitals and specialty clinics, and appropriate management is often extrapolated from adult CM management. Common management difficulties include difficulty interpreting serology results, uncertainty regarding treatment duration, and fear of developing disseminated disease. The aim of this study is to evaluate the diagnosis, management, and outcomes of pediatric CM to assist the management of CM in the pediatric population, with a specific focus on non-disseminated CM.

Methods: This study is a retrospective chart review of patients aged 0-17 years old in the Kaiser Permanente Southern California system with active CM between 1/2015 and 12/2021. All patients in whom a CM ELISA was sent or had an ICD-10 code for CM were screened for possible CM. Patient with laboratory findings, clinical finding, and a diagnosis of CM were included in the full analysis. Chart reviews were performed to extract relevant CM laboratory results, date of symptom onset, symptomatic prestation, radiographic findings, management, complications, and long-term outcomes.

Results: 3107 patients were identified based lab testing or ICD-10 codes, of which 339 were found to have labs suggestive of CM, of which 229 met clinical criteria for CM. Of these, 39 did not have a CM diagnosis (but many were clinically suspicious for CM). 190 had a final diagnosis of CM, with average follow up time of 4.6 years. Among these patients there were 441 paired IgG and IgM and 973 complement fixation (CF) results. IgG positive tests without a positive IgM was rare before 29 days of symptoms (4% of tests), while IgM positivity without IgG positivity was rare after 29 days (1%). 97% of test sent between 15 and 21 days after symptom onset were positive for IgG or IgM. The highest proportion of tests with positive CF occurred between 4-6 months after symptom onset (74%). Most patients,180 (95%) were found to have non-disseminated CM. Pneumonia (158) and isolated erythema nodosum (20) were the most common presentations. Ten patients had disseminated CM. Most non-disseminated cases were treated;143 (79%). Two (1.4%) of the treated patients required repeated treatment after stopping antifungals due to concern for recurrence. One of the 37 untreated cases eventually required treatment (2.7%). No disseminated disease occurred during the follow up of patients who were not initially diagnosed with dissemination. Duration of treatment for non-disseminated CM ranged from 19 days to indefinite, with an average treatment of 202 days. Serial antibody monitoring in the absence of symptoms lead to numerous blood draws and were often cited as the reason for prolonging treatment in asymptomatic children.

Conclusion: Management of pediatric CM was highly variable within this population. Understanding of the evolution of CM serology can help determine likely false positive IgM results and non-diagnostic IgG results. Observation off antifungals is reasonable in non-severe cases, as the rate of failure of observation without treatment was low. Prolonged treatment for non-disseminated CM was common. Long term antibody monitoring in the absence of symptoms did not positively affect the management of non-disseminated CM cases. These findings highlight the importance of thoughtful diagnostic and management guidance for pediatric CM patients.

Pearls and Pitfalls of Intrathecal Amphotericin B Therapy for Refractory Coccidioidal Meningitis in Children: An Illustrative Pediatric Case Series

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Abstract

Introduction: The incidence of pediatric coccidioidomycosis has risen over the last 20 years and coccidioidal meningitis is the most severe form of disseminated disease. Intrathecal (IT) amphotericin B deoxycholate has historically improved outcomes and remains a mainstay of therapy when response to azole therapy alone is inadequate. However, the dosing, route of administration and duration of therapy in young pediatric patients poses unique challenges due to their anatomy, inability to communicate and the need for hospital based administration. We present four new cases over 30 years at our institution, in addition to comparison with prior published pediatric cases of intrathecal amphotericin B therapy and the associated outcomes and challenges.

Methods: Case information was abstracted from the electronic health record at University of California Los Angeles with institutional review board approval. Literature review was conducted to look for additional cases on PubMed with search terms: “pediatric coccidioidomycosis”, "coccidioidomycosis in children", "pediatric *Coccidioides*", “coccidioidal meningitis”, “*Coccidioides* meningitis”, “intrathecal amphotericin”.

Results: Four cases are presented describing the unique challenges and courses of pediatric patients with IT amphotericin B including two patients with successful outcomes and two patients whom did not survive. Starting doses, methods of administration, complications, and neurosurgical challenges are described. A table comparing these cases to the 2 others presented in the literature including reason for initiation, starting dose, final dose, concurrent steroid (intrathecal and systemic) use, duration of therapy and outcome. Patients had prolonged hospitalizations as subspecialists were not available to administer outpatient IT amphotericin B in needed areas, with one patient successfully transitioning to once weekly 24 hour hospitalizations to prolong administration. Therefore, dose escalation and subsequent weaning of IT medication was done more rapidly than described for adults to allow for shorter courses. IT treatment was considered an initial stabilizing measure while attempting to optimize azole monotherapy. Cisternal administration remains a challenge and mortality remains significant.

Conclusion: Intrathecal amphotericin B remains an important treatment option in pediatric patients with refractory Coccidioidal meningitis, however due to challenges with administration and tolerance may be more of a temporizing measure rather than definitive prolonged therapy. Further experience and multidisciplinary care is needed to reduce mortality.

Scientific Section II | Immunology

B Cell Depletion Damages Granuloma Formation in a Mouse Model of Coccidioidomycosis

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Identification of Coccidioides spp. Specific T Cell Clones in Naturally Exposed Pig-tailed Macaques (Macaca Nemestrina)

Allison Harmon[1], Mame Ndiaye[1], Jessica Marshall[1], Paul Phillips[1], Megan Fredericks[2], Deborah Fuller[2,3], Bridget Barker[1], Paul Keim[1], Erik Settles[1] [1]Pathogen and Microbiome Institute; Northern Arizona University, Flagstaff, USA. [2]Department of Microbiology; University of Washington, Seattle, USA. [3]Washington National Primate Research Center, Seattle, USA

Single-cell Transcriptomic Analysis Reveals Unique Patterns of Myeloid Cell Differentiation and Potential Therapeutic Targets During C. Posadasii Infection

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Single-Cell Analysis and Functional Exploration of Human Airway Epithelial Responses to Coccidioides Posadasii Infection

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Scientific Section II | Immunology (continued)

Diabetic Mice are More Susceptible to Coccidioides Posadasii Infection, but Protected by a Subunit Vaccine

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Multi-Valent DNA Vaccines Encoding Coccidioides Proteins and Potent Genetic Adjuvants Induce Mucosal and Th1 Immune Responses, Afford Protection Against High Dose Challenges with C. Posadasii and Enable The Discovery of Novel Immunogens

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B Cell Depletion Damages Granuloma Formation in a Mouse Model of Coccidioidomycosis

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Abstract

Introduction: B cells have received little attention in studies of coccidioidomycosis. Neither serum nor immune B cell fractions are capable of adoptively transferring immunity to naïve mice, and humans with immunoglobulin defects are not a population that suffers complications with coccidioidomycosis. A recent RNAseq experiment demonstrated that lung granulomas in mice are tertiary lymphoid structures, and emerging data from tuberculosis in mice and macaques demonstrates B cells are important in maintaining granulomas.

Methods: Genetically engineered heterozygous C57BL/6-LSD mice (B6-LSD), which are more resistant to coccidioidomycosis, were treated with 250 µg of anti-CD20 antibody (clone MB20-11) 3 days prior to infection and every 14 days throughout the study. Mice were infected IN on day 0 with 50 spores of *C. posadasii* strain 1038 and sacrificed on day 42 post-infection. Depletion of B cells was verified by flow cytometry of a blood sample the day before infection and before each subsequent anti-CD20 injection. At sacrifice, the lung granulomas and spleens from half the mice were incubated overnight in medium and the supernatant assayed for cytokines (13-plex mouse bead assay – Biolegend, San Diego CA). For the other half of the mice, the lungs were perfused and placed in 4% paraformaldehyde. Serial sections were stained with H&E, periodic acid-Schiff, and anti-CD3 antibody. T cells were enumerated from photomicrographs of the CD3-stained slides using QuPath.

Results: Histopathological examination of the slides showed a lack of organized lymphoid aggregates in the mantle region of granulomas in B cell depleted mice. On CD3 stained slides, the T cells were more scattered and less likely to be clustered in lymphoid aggregates and they also appeared fewer in number. Enumeration of 5 representative 20x microscope fields revealed a statistically significant decrease in T cells in the mantle region of B cell depleted mice (mean 637.6 ±374.6/field) compared to controls (mean 1,049.2 ±386.2/field (p=0.001). Analysis of the cytokines showed very diminished IFNγ (Control - 836.9 ±1,168.9 pg/ml vs CD20 antibody 13,442.1 ± 9,604.3 pg/ml [Mean ± STDEV]) and IL-17A (Control - 581.3 ± 902.2 pg/ml vs CD20 antibody 12,770.0 ± 6,185.7 pg/ml) in the supernatants of lung granulomas from these mice. Spleen supernatants did not show any differences.

Conclusion: B cells appear to be required for full structural organization of the *Coccidioides* granuloma in its stable, controlled state. These preliminary findings support the value of determining the role of B cells in *Coccidioides* granulomas, which B cells subsets and functions are important, the T cell pathways that B cells influence in coccidioidal granulomas, and if ongoing abrogation of B cells leads to increased mortality in the chronic mouse model of infection.

Identification of Coccidioides spp. Specific T Cell Clones in Naturally Exposed Pig-tailed Macaques (Macaca Nemestrina)

Allison Harmon[1], Mame Ndiaye[1], Jessica Marshall[1], Paul Phillips[1], Megan Fredericks[2], Deborah Fuller[2,3], Bridget Barker[1], Paul Keim[1], Erik Settles[1]

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Abstract

Introduction: Coccidioidomycosis (Valley fever) is frequently misdiagnosed and mistreated as bacterial or viral pneumonia due to similar clinical presentations. While infection in endemic areas is common, severe Valley fever manifests in <5% of symptomatic cases and can be life-threatening. Currently, there are no means to prevent this disease, so developing a vaccine is desirable to improve public health outcomes in regions associated with Valley fever. 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 Valley fever disease and vaccination. Our focus on the pig-tailed macaque (PTM) model is due to their established Valley fever susceptibility and the availability of naturally infected colony animals.

Methods: Peripheral blood mononuclear cells (PBMCs) were collected from pig-tailed macaques 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). The PBMCs were non-specifically expanded and 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 markers (CD3 and CD4), as well as T cell activation markers (CD137 and CD69), and supernatants from stimulated cells were collected for cytokine secretion assays. The activated T cells were sequenced, and the data were demultiplexed to associate T cell receptor (TCR) clonotypes with their stimulating Coccidioides antigens. Additionally, PBMCs stimulated with the total pool of peptides underwent single-cell sequencing and were screened for cytokine secretion.

Results: Thus far, our results have confirmed reactivity of previously known Coccidioides antigens while also identifying 12 new antigens not previously associated with T cell immunogenicity in mice or humans. Updated results of these studies will be discussed.

Conclusions: T cell-reactive antigens will be prioritized for nucleic acid vaccinations. In addition, we will use this clonotype information and additional samples from ongoing PTM challenge and vaccination studies to track the circulating or lung-localized T cell clones in response to infection or vaccination. These antigens and their epitopes can be further investigated for potential diagnostic tools.

Single-cell Transcriptomic Analysis Reveals Unique Patterns of Myeloid Cell Differentiation and Potential Therapeutic Targets During C. Posadasii Infection

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Abstract

Introduction: While most Coccidioides infections are asymptomatic, a subset of patients develop severe, chronic, or disseminated disease, yet the mechanisms underlying disease progression and predictive indicators of progression to severe disease remain poorly defined. We previously employed single-cell RNA sequencing (scRNA-seq) to profile the lung immune response during Coccidioides infection in a mouse model of severe infection [1], revealing extensive myeloid infiltration and upregulation of immune checkpoint molecules, including PD-L1. Here, we extend this approach to analyze immune responses in the blood, with the goal of identifying circulating biomarkers of severe disease. Comparative analysis was performed to similar lung and blood data sets generated from Influenza A (IAV) infected mice to determine the specificity of identified myeloid populations to Coccidioides infection. To assess the translatability of our sequencing-based findings, we assessed the impact of PD-L1 blockade on survival outcomes in C. posadasii infected mice.

Methods: C57BL/6 mice were infected intranasally with 500 C. posadasii Silveira arthroconidia (n = 5-6 mice/group). Lungs and blood were collected from infected mice at 5, 9, and 14 days post-infection, as well as from uninfected controls and single cell suspensions were generated from each tissue type. scRNA-seq was performed using 10x Genomics Fixed RNA kit followed by sequencing on the Illumina NextSeq 2000 platform. Data were analyzed using Cell Ranger and the Seurat R toolkit. Myeloid infiltration into the lung and upregulation of PD-L1 was confirmed via flow cytometry (n = 6 mice/group). To assess the impact of PD-L1 on survival, mice (n = 20 mice/group) were infected as before and treated with 200 µg of anti-PD-L1 or isotype control antibody starting at 4 days post-infection and every 2-3 days subsequently through 26 days post-infection. Inhibition of PD-L1 expression by antibody treatment was assessed via flow cytometry at 7 and 13 days post-infection (n = 4-6 mice/group).

Results: Comparative analysis of sequencing data revealed distinct patterns of myeloid cell prevalence in the blood and lungs of C. posadasii and IAV infected mice. Spp1+ macrophages were highly abundant in the lungs of both C. posadasii and IAV infected mice yet were specifically highly abundant in the blood of C. posadasii infected mice only. Similarly, CD5L+ macrophages were enriched in the lungs and blood of C. posadasii infected mice, yet present at very low levels in IAV infected blood and absent from IAV infected lungs, suggesting unique myeloid cell differentiation and trafficking during representative severe fungal and viral infections. In lethal C. posadasii infection, PD-L1 blockade resulted in significant downregulation of surface PD-L1 expression on neutrophils as well as a modest yet significant improvement in survival.

Single-cell Transcriptomic Analysis Reveals Unique Patterns of Myeloid Cell Differentiation and Potential Therapeutic Targets During C. Posadasii Infection (continued)

Conclusions: Our findings highlight the power of scRNA-seq in uncovering immune mechanisms of Coccidioides pathogenesis and identifying potential blood-based biomarkers of severe disease. The expansion of Spp1+ and CD5L+ macrophage populations in circulation may serve as predictive markers of disease outcome. Furthermore, the observed therapeutic benefit of PD-L1 blockade suggests that myeloid-mediated immune suppression contributes to severe infection. These results provide new insights into host-pathogen interactions and support a role for immune checkpoint regulation in host susceptibility to infection, suggesting potential strategies for therapeutic intervention.

1. Davalos OA, Sebastian A, Leon NF, Rangel MV, Miranda N, Murugesh DK, et al. Spatiotemporal analysis of lung immune dynamics in lethal Coccidioides posadasii infection. mBio. 2024:e0256224. Epub 20241129. doi: 10.1128/mbio.02562-24. PubMed PMID: 39611685.

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.

Single-Cell Analysis and Functional Exploration of Human Airway Epithelial Responses to Coccidioides Posadasii Infection

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Abstract

Introduction: *Coccidioides posadasii* (*Cp*) is the causative agent of coccidioidomycosis, which is a pulmonary infection resulting from the inhalation of arthroconidia. *Cp* morphologically transitions into spherules and eventually the release of endospores in the host. Despite the clinical relevance of *Coccidioides* infections, the early immune responses and host-pathogen interactions, particularly within the airway epithelium, are poorly understood. This study focuses on defining the role of human airway epithelial cells (hAECs) in early infection, specifically regarding innate immune responses.

Methods: We modeled the human lung environment using primary hAECs cultured at an air-liquid interface and infected them with *Cp*. Single-cell RNA sequencing (scRNA-seq) was employed to characterize cell-type-specific transcriptional responses. To further investigate innate immune activation, we assessed the transepithelial migration of neutrophils in response to fungal infection using primary and immortalised epithelial transwell assays. Differentially expressed genes from scRNA-seq were used to identify key pathways involved in epithelial responses and immune cell recruitment, which were further interrogated using functional assays.

Single-Cell Analysis and Functional Exploration of Human Airway Epithelial Responses to Coccidioides Posadasii Infection (continued)

Results: scRNA-seq revealed distinct activation of hypoxia-related pathways in secretory cells, while other epithelial subtypes displayed upregulated stress responses and chemoattractants. Techniques such as neutrophil transepithelial migraton assays and CRISPR knockout models combined with hAECs are being used to determine the functional roles of these genes identified by scRNA-seq in mediating early innate immune responses and inflammation in response to pulmonary *Cp* infections.

Conclusions: This study provides novel insights into the early host response to *Cp* at the airway epithelium, highlighting the role of innate immune activation in driving neutrophil recruitment. The combination of scRNA-seq and functional assays offers a comprehensive approach to dissecting fungal pathogenesis in the lung and identifying targetable host pathways that may influence disease progression.

Diabetic Mice are More Susceptible to *Coccidioides Posadasii* Infection, but Protected by a Subunit Vaccine

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Abstract

Introduction: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, dyslipidemia, and other metabolic changes that present major health challenges. This chronic metabolic disorder impairs immune function and can exacerbate inflammatory responses. Diabetic patients are three times more likely to develop disseminated or cavitary CM. With DM being a well-known risk factor for CM and the growing prevalence of DM in the U.S. underscores the urgent need to address how DM impacts CM pathogenesis and to develop effective preventive strategies. Developing a vaccine against *Coccidioides* infection presents a promising approach for controlling CM. Our lab has developed an effective subunit vaccine (GCP-rCpa) which confers protective immunity against pulmonary coccidioidomycosis. In this study, we evaluate the impact of DM on *Coccidioides* pathogenesis and the protective efficacy of the GCP-rCpa vaccine in diabetic mice.

Methods: C57BL/6 mice (n=10) consisted of a diabetes-induced group (DI), in which diabetes was induced through daily intraperitoneal injections (IP) of Streptozotocin (STZ) 60 mg/kg in citrate buffer for 5 days and a control group that received a citrate buffer injection (IP). After confirming hyperglycemia via blood glucose measurement, the diabetic mice were maintained for four weeks. They were then transferred to the BSL-3 facility and challenged by the oropharyngeal route with a potentially lethal dose (80-100 spores) of *Coccidioides posadasii* clinical isolate C735. Disease progression was monitored daily by recording body weight. At 10 days post challenge (dpc) lung cell suspensions were cultured at various dilutions on chloramphenicol-containing GYE agar plates to determine pulmonary, splenic and cerebral fungal burden in each mouse. Supernatant from cell suspension was analyzed through Bio-Plex Pro™ Mouse Cytokine Th17 Panel A 6-Plex. In the immunization study, non-diabetic and DI C57BL/6 mice (n=12) were then vaccinated subcutaneously two times at a 14-day interval with GCP-rCpa5 or GCP-MSA as an adjuvant control. Four weeks following the last vaccination, mice were challenged. Body weight, fungal burden and cytokine production were evaluated.

Diabetic Mice are More Susceptible to *Coccidioides Posadasii* Infection, but Protected by a Subunit Vaccine (continued)

Results: C57BL/6 DI mice experienced significant weight loss at 7 and 8 dpc compared to the mock group. Due to the severity of disease progression in the DI mice, they were euthanized at 10 dpc. Notably, DI mice exhibited significantly higher CFU levels in the lungs, the spleen, and the brain. This data is in agreement with the clinical findings that DI patients develop severe CM disease. Furthermore, our BioPlex cytokine analysis revealed elevated levels of TNF-α, IL-1β, and IL-10 in the infected DI mice compared to the mock group, no differences in IL-6 and IL-17a levels. In investigation of vaccination efficacy, our data showed GCP-rCpa5-vaccinated DI mice significantly reduced the fungal burden in all three tested organs compared to non-vaccinated DI mice. Interestingly, the vaccinated DI mice and vaccinated non-diabetic mice showed comparable reduction of fungal burden and reduced dissemination. We will furthermore characterize the survival of the DI mice through histopathology and other immune responses compared to non-diabetic mice.

Conclusion: Our results indicate that DI mice have an increased susceptibility to *Coccidioides* infection exhibited by increased fungal burden and reduced body weight. Additionally, we demonstrate the GCP-rCpa5 vaccine is protective against a lethal pulmonary challenge with the clinical isolate C735 for DI mice comparable to wild type mice. The protective efficacy is demonstrated with reduce fungal burden, while the survival is currently ongoing. Altogether, we have established a DI mouse model for the study of immune responses to *Coccidioides* infection. This DI mouse model recapitulates human disease, and it appears to be useful for evaluation of immune protective mechanisms, pathogenesis, vaccine efficacy, and antifungal drug treatment.

Multi-valent DNA vaccines encoding Coccidioides proteins and potent genetic adjuvants induce mucosal and Th1 immune responses, afford protection against high dose challenges with C. Posadasii and enable the discovery of novel immunogens

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Abstract

Introduction: Nucleic acid (NA) vaccines, including DNA and RNA vaccines, induce robust CD4+ and CD8+ T cell responses including IFN-γ and Th-17 responses. In addition, unlike recombinant protein vaccines, constructing a NA vaccine is rapid and simple, requiring only the genetic sequence of a given antigen. The ease and speed in making NA vaccines makes them an ideal tool to rapidly screen many potential fungal antigens to identify new antigens for vaccines. Here, we investigated the immunogenicity and protective efficacy of gene gun delivered multivalent DNA vaccines encoding known *Coccidioides* immunogens formulated with two potent genetic adjuvants designed to enhance Th1 and mucosal immune responses and the role of antibody vs T cell responses induced by these vaccines in protection. We also used DNA vaccines encoding multiple fungal proteins to rapidly screen for new candidate vaccine immunogens based on their ability to express protein, induce immune responses and afford protection.

Methods: DNA vaccines expressing 3 known *Coccidioides* immunogens (PMP1, ELI-1, PRA-Ag2) were delivered by gene gun into the skin (2mg doses) either separately or in combination as a trivalent vaccine in mice. To increase mucosal and Th1 T cell responses, the DNA vaccines were co-formulated with two plasmids expressing two genetic adjuvants that increase mucosal and systemic T cell responses. Mice were primed and boosted 4 weeks apart. Antibody and T cell responses were measured two weeks after the boost and then, mice were challenged with a high dose (500 conidia) of *C. Posadasii* Silveira. To determine the contribution of antibody vs T cell responses to protection, prior to challenge, additional groups of mice were depleted of T cells or received passive immunization using sera from DNA vaccinated mice with high titers of antibody. To screen for novel antigens, DNA vaccines encoding 26 fungal proteins were constructed. Groups of mice were immunized with pools of 4-5 antigens and then challenged with C. *Posadasii*. Groups exhibiting protection were further analyzed for protein expression and induction of immune responses against each antigen to identify ones contributing to protection.

Multi-valent DNA vaccines encoding Coccidioides proteins and potent genetic adjuvants induce mucosal and Th1 immune responses, afford protection against high dose challenges with C. Posadasii and enable the discovery of novel immunogens (continued)

Results: Monomeric and trivalent DNA vaccines induced robust antibody responses and systemic and mucosal IFN-γ and Th17 responses in the spleen and lung against each immunogen. Interestingly, T cell responses were higher in the lung than in the spleen. Following challenge, the trivalent DNA vaccine afforded 100% protection from weight loss and mortality and superior protection when compared to live attenuated vaccines. Fungal burden showed the trivalent DNA vaccine reduced fungal burden in the lungs by 2.5 logs, and CFU were undetectable in the spleen and brain only in this group, indicating the vaccine blocked fungal dissemination from the lung. Notably, T cell depletion prior to challenge abrogated protection and additionally, 50% of naïve mice passively immunized were protected indicating a role for both antibody and T cell responses in protection. The DNA vaccine encoding 3 antigens afforded superior protection when compared to live attenuated vaccine. Using our rapid screening with DNA vaccine, we also identified 8 novel immunogens that expressed protein, induced significant immune responses, and contributed to protection.

Conclusions: These results show that a multi-antigen DNA vaccine affords superior protection to live attenuated vaccines in protection from a fungal disease, and co-delivery of multiple antigens targeting different stages of the fungal life cycle may afford a synergistic effect in protection. We show antibody and T cell responses contribute to protection and that DNA vaccines can be used to rapidly screen and identify novel vaccine immunogens that could enhance protection. These results show nucleic acid vaccines offer a pathway toward development of effective vaccines against fungal diseases. Additional studies in progress that will be discussed are testing the same antigens in the context of self-amplifying RNA vaccines.

Scientific Section III | Ecology

Updated Ecological Niche Model of Coccidioides Using Compiled Soil Samples

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Leveraging Elevation Gradients to Characterize the Distribution of Coccidioides in Southern Utah: A Field and Modeling Study

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Temporal Surveillance of Coccidioides in Aerosols and Soils at a Single Location in Arizona

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Multiplexed qPCR Lab-on-Chip Technology and Personal Exposome Tracker for Scalable Environmental Surveillance of Coccidioides

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Transcriptomic Atlas of the Morphologic Development of the Fungal Pathogen Coccidioides Reveals Key Phase-Enriched Transcripts

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Updated Ecological Niche Model of *Coccidioides* Using Compiled Soil Samples

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Abstract

Introduction: Estimating coccidioidomycosis endemicity has been challenging due in part to limited *Coccidioides* samples from the environment and biases with using clinical case data as a proxy for *Coccidioides* presence. Recent soil sampling campaigns for *Coccidioides* have increased both the number of *Coccidioides* presence data points and the spatial extent of this data. With an increasing body of evidence suggesting the endemic area for *Coccidioides* may extend across the western US, we created an updated species distribution map using contemporary soil sampling data.

Methods: After deduplicating and spatially thinning the data to reduce spatial bias, we used 44 *Coccidioides* occurrence data from prior research campaigns and the National Center for Biotechnology Information (NCBI) database to create a species distribution map of *Coccidioides* spp. For our environmental drivers, we included seven climate variables, 16 land cover types, 22 soil property variables, and a measure of rodent richness. We used the Maxent ecological niche model to develop the species distribution map.

Results: Our model suggests the distribution of *Coccidioides* extends across much of the western United States. Known endemic areas in California, Arizona, and southeastern Washington state are depicted as high habitat suitability. There is decreased relative habitat suitability further east, with the easternmost states with moderate to low endemicity being North Dakota, South Dakota, Nebraska, Kansas, Oklahoma, and Texas. The most important variables structuring this spatial distribution were precipitation, rodent richness, shrub land cover, soil temperature regime, and soil order. These results suggest there may be three additional states endemic to coccidioidomycosis where the disease is currently not reportable: Idaho, Oklahoma, and Texas.

Conclusion: Our species distribution map suggests 17 states across the western US may be endemic to coccidioidomycosis. This map can be a resource to identify areas for future epidemiologic and soil surveillance efforts. It can also serve as a justification for adding coccidioidomycosis as a reportable disease for states not currently reporting.

Leveraging Elevation Gradients to Characterize the Distribution of Coccidioides in Southern Utah: A Field and Modeling Study

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Abstract

Introduction: While both climatic and soil characteristics are associated with the distribution of *Coccidioides* in the environment, the key determinants of its distribution—and therefore of human risk of Valley fever—are not fully understood. Further, systematic environmental sampling for *Coccidioides* has not been conducted in many low-incidence states, including Utah, and current models of human risk of infection are often based on reported Valley fever cases rather than the environmental distribution of *Coccidioides*. High-resolution *Coccidioides* distribution models are critically needed to develop public health interventions to reduce infection risk and to project areas of future risk.

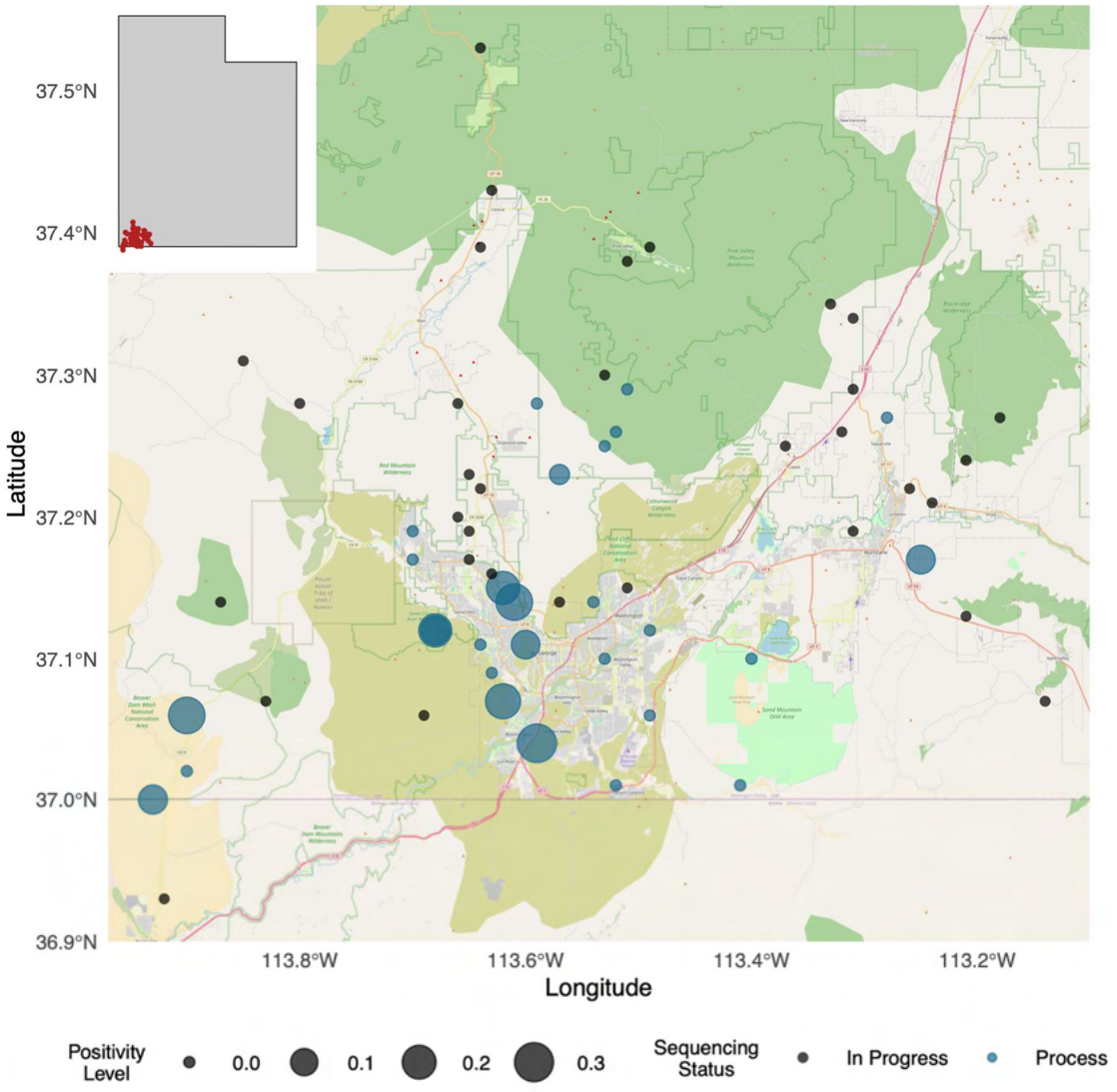
Methods: To identify climatic and environmental factors associated with the current distribution of *Coccidioides,* we conducted spatially stratified random sampling along an 8,000-foot elevation gradient in Washington County, Utah, where incidence is highest in the state, 43 per 100,0000 in 2020. We sampled soil from small mammal burrows, extracted DNA from soil samples, and tested soil with a *Coccidioides*-specific qPCR. We additionally collected data on 16 variables, including elevation, soil properties, and climate covariates. We modeled site-level *Coccidioides* presence using boosted regression trees. To mitigate multicollinearity, we excluded variables with correlations exceeding 70%. We assessed model performance using leave-one-out cross-validation.

Results: We sampled 539 small mammal burrows across 31 sites and detected *Coccidioides* in 6.9% of samples (37 positive based on qPCR) (Figure 1). Site-level prevalence ranged from 0% to 31%. A boosted regression tree model identified minimum vapor pressure deficit (43.4% relative influence) and soil coarse fragments (24.3%) as the most important predictors of *Coccidioides* presence, with elevation (20.6%) and silt content (11.6%) also contributing. The model achieved 75% accuracy, with 72.7% sensitivity and 76.5% specificity.

Conclusion: We detected *Coccidioides* in soils sampled across a rapidly growing metropolitan area in southern Utah, evidence of a moderate local risk of infection. Our analysis identified soil properties and climatic variables as key predictors of *Coccidioides* presence, with minimum vapor pressure deficit and soil coarse fragments exerting the strongest influence, suggesting that these factors may shape the dispersal patterns and environmental persistence of *Coccidioides*.

Leveraging Elevation Gradients to Characterize the Distribution of Coccidioides in Southern Utah: A Field and Modeling Study (continued)

Figure 1. Map of the sampling area in Washington County, Utah. Sites where soil testing using qPCR has been conducted are shown in blue, while sites pending results are marked in black. The size of the tested sites represents prevalence of *Coccidioides* in mammal burrows. The inset map in the top left shows the location of sampling sites (red points) in Washington County, Utah.



Temporal Surveillance of Coccidioides in Aerosols and Soils at a Single Location in Arizona

Amelia Stout[1], Marieke L. Ramsey[2], Daniel R. Kollath[2], Megan C. Ruby[2], Bridget M. Barker[2], Pierre Herckes[1], Matthew Fraser[1]

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Abstract

Introduction: The primary transport mechanism of *Coccidioides*, the causative agent of coccidioidomycosis or Valley Fever, into humans is inhalation of the fungal spores through aerosol dust. The purpose of this study is to look at the presence of *Coccidioides* in the air and soil temporally at a single site within the hyper-endemic region of Valley Fever. This study aims to relate detection of *Coccidioides* in the air and soil with various chemical and meteorological parameters at a highly positive site in Mesa, Arizona.

Methods: *Air sample collection:* Two Tisch Environmental high volume PM_{10} samplers were deployed at the site. Aerosol samples were collected for 24 hours every 6 days, following the Environmental Protection Agency (EPA) one in six-day sampling schedule. Samples were collected from 12:00 am – 11:59 pm. The samplers collected particulate matter with an aerodynamic particle diameter less than or equal to 10 µm. One of the samplers was equipped with pre-fired (550°C overnight) quartz fiber filters, and the other sampler with cellulose filters (as received). After sample collection, the QFF filters were used for gravimetric analysis, ion chromatography (IC), organic and elemental carbon (OC/EC) analysis, and biological analysis (presence/absence of *Coccidioides*). The cellulose filters were used for elemental analysis via inductively coupled plasma mass spectrometry (ICP-MS).

Gravimetric Analysis: Prior to sampling, the QFF filter was weighed three times on Denver Instrument APX-100 Analytical Balance to obtain gravimetric mass under controlled temperature and relative humidity. After sampling, the QFF filter was equilibrated to controlled temperature and relative humidity and weighed three more times, to obtain a total mass of particulate matter on the filter. This mass was converted into an air mass concentration of $\mu g/m^3$ of collected PM_{10}, normalized based on length of sampling time and volume of air flow.

Ion Analysis: Ion analysis was performed for key ions using ion chromatography (IC). Ions were extracted from QFF filters. Key anions extracted were fluoride, chloride, nitrite, bromide, nitrate, phosphate, and sulfate. Key cations extracted were lithium, sodium, ammonium, potassium, magnesium, and calcium. Pieces of the QFF filter were added to a container with 10 mL of ultrapure water and sonicated for 15 minutes. After sonication, the sample was filtered through a pre-wet 0.22µm Millex GP PES membrane syringe filter. Anion and cation analysis were performed on the Metrohm 930 Compact IC Flex. Columns used for anion and cation analysis were A Supp 5 150/4.0 and Metrosep C6 – 150/4.0, respectively.

Carbon Analysis: Carbon analyses were performed using an Organic carbon – Elemental Carbon (OC-EC) Analyzer (Sunset Laboratory, Inc., Forest Grove, OR, USA). Organic carbon and elemental carbon are differentiated based on their distinct thermal characteristics (Birch and Cary, 1996). During analysis, the filter is first exposed to an inert helium atmosphere, and a stepped temperature ramp increases the temperature of the oven, that is immediately

Temporal Surveillance of Coccidioides in Aerosols and Soils at a Single Location in Arizona (continued)

followed by exposure to an oxidizing environment. A Flame Ionization Detector (FID) detects CH_4 produced. The samples on filters were analyzed with variable time steps lasting between 80 and 110 seconds during the evolution of OC at temperature plateau of 310°C, 475°C, 615°C, and 870°C. The temperature profile during the evolution of EC included 45 second time step plateaus at 550°C, 625°C, 700°C, 775°C, and 800, with a final hold at 870°C for 110 seconds.

Elemental Analysis: Elemental analysis of samples was performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). ICP-MS analysis included the following 30 elements: Na, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Co, Cu, Zn, Ga, As, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Ba, Ce, W, Pt, Au, Pb. Samples were prepared for ICP-MS by hydrofluoric acid (HF) microwave digestion of cellulose filters in a MARS5 microwave digestion oven. After digestion, samples were evaporated on a hot plate and then diluted with 2% nitric acid to a volume of 50mL. The 30-element analysis was performed using the Perkin Elmer NexION 1000 ICP-MS. Results were converted to air concentrations, as $\mu g/m^3$.

Quartz Fiber Air Filter Extraction: After gravimetric analysis was complete, each QFF filter was cut into eight pieces. Four of the eight pieces were sent to Northern Arizona University for DNA extraction and qPCR detection of *Coccidioides*. Phenol chloroform extractions were performed on the QFF filters (Bryant et al., 2022). Due to potential infectious propagules in the filters, DNA extractions were performed in a biological safety level two laboratory inside a BSC. Appropriate PPE was used consisting of a front gown and gloves (primary and secondary). Handling of phenol was performed in a fume hood or ducted BSC. DNA was stored in a -20°C freezer. QFF were used for DNA extraction and *Coccidioides* detection due to higher DNA yield outcomes compared to the cellulose filters.

Molecular Detection using RT-qPCR Coccidioides Detection Assay: QFF filters were screened using the CocciDx real-time qPCR-based assay to detect *Coccidioides* (Litvintseva et al., 2014). For the purpose of this study, if any one of four pieces of air filter came back as positive, the entire sample is considered to be positive. These TaqMan-based assays are highly sensitive and target a repetitive region of DNA that is only known to occur in the two species within the *Coccidioides* genus (Bowers et al., 2019). The CocciDX assay targets a 106-bp sequence present in multiple copies within a transposable element in the genome of both *Coccidioides* species and is specific to this genus (Saubolle et al., 2018; Litvintseva et al., 2014). The assay was performed on the Applied Biosystems QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific). Each reaction was performed in 20 µl-volume containing a mixture of 2X TaqMan Environmental Master Mix 2.0 (Thermo Fisher Scientific), 1x of CocciDX at 100µM concentration of Cocci Assay oligo/probe mix, and two µl of DNA template. PCR cycling conditions were performed as follows: Two minutes of activation step at 50° C, 10 minutes at 95° C for denaturation step, followed by 45 cycles of 15 seconds at 95°C and one minute at 60° C. Each sample was performed in triplicate and the average Cycle threshold (Ct) value was taken if amplification occurred. Control samples included purified DNA from Coccidioides posadasii strain Silveira as a positive control and molecular grade sterile water was used as negative no-template DNA control. Soils were considered positive if the Ct value was < 40 and negative for a Ct value >40. A sample was

Temporal Surveillance of Coccidioides in Aerosols and Soils at a Single Location in Arizona (continued)

considered “low positive” if Ct value ranged from 38-40, “moderately positive” if Ct value ranged from 35-37, and “high positive” if Ct value ranged from 25-34. Any Ct value <25 is considered a “very high positive” detectability.

Results: *Coccidioides* were detected frequently in the air. The detection was more likely to occur during high PM_{10} days. Overall PM_{10} concentrations at the site were low for the region. The chemical measurements showed little anthropogenic impact. Probability of occurrence in the soil did not match the probability of occurrence in the air, leading us to investigate the relationship between various meteorological and chemical parameters and detection of *Coccidioides* in the air. There are statistically significant relationships between PM_{10} concentrations and detection of *Coccidioides* in the air, as well as between wind speeds and detection of *Coccidioides* in the air.

Conclusion: This is a unique study that gives insight into the temporal occurrence of *Coccidioides* in both the soil and air at a known positive site within the hyper-endemic region of Valley Fever. Chemical parameters from soil and air samples indicate whether *Coccidioides* present in the air are from local or regional sources. Genome sequencing can be used to further verify the source of the *Coccidioides* present in the air at this site.

REFERENCES

1. Birch, M. E., Cary, R. A. (1996). “Elemental Carbon-Based Method for Monitoring Occupational Exposures to Particulate Diesel Exhaust.” Aerosol Sci. Technol. 25(3):221-241.
2. Mitchel Bryant, H. M., Bridget M. Barker, Austin Blackmon (2022). "Phenol Chloroform DNA Extraction Optimized for HMW gDNA from Coccidioides spp." protocols.io.
3. Bowers, J., et al. (2019). "Direct detection of Coccidioides from Arizona soils using CocciENV, a highly sensitive and specific real-time PCR assay." Medical mycology 57(2): 246-255.

1. Litvintseva, A. P., et al. (2015). "Valley fever: finding new places for an old disease: Coccidioides immitis found in Washington State soil associated with recent human infection." Clinical Infectious Diseases 60(1): e1-e3.
2. Saubolle, M. A., et al. (2018). "Multicenter clinical validation of a cartridge-based real-time PCR system for detection of Coccidioides spp. in lower respiratory specimens." Journal of Clinical Microbiology 56(2): 10.1128/jcm. 01277-01217.

Multiplexed qPCR Lab-on-Chip Technology and Personal Exposome Tracker for Scalable Environmental Surveillance of Coccidioides

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Abstract

Introduction: Environmental surveillance of *Coccidioides* is crucial in endemic regions, especially as climate change is expected to expand its range across the western U.S. Traditional airborne soil sampling programs are labor-intensive, geographically limited, and require manual data entry. We present a multiplexed qPCR lab-on-chip system integrated with the Personal Exposome Tracker (PET) and drone technology, enabling scalable, automated surveillance of Coccidioides.

Methods: The Kelliop RapidSeq126, a compact qPCR system, detects up to 144 microbial targets with results in under an hour. Designed for rugged environments, it can function autonomously with drones for sample collection or be manually operated without specialized training. Its single-use cartridges integrate DNA extraction and qPCR processing, while built-in satellite and IoT connectivity allow real-time data transmission. The PET, developed by Exposomics, is a wearable or drone-mounted device that collects airborne samples via a polyethersulfone (PES) filter, coupled with particulate matter, humidity, and temperature sensors. PET samples are processed and analyzed using the RapidSeq126, with ongoing development for fully automated drone-based sample collection as well as farm equipment and farmworker-based deployment of PET devices.

Results: The RapidSeq126 and PET system have been analytically validated, with RapidSeq126 demonstrating high sensitivity and specificity (>0.99) and processing times of 20 minutes for single targets and 40 minutes for multiplexed detection. Automation development is ongoing with a plan to begin environmental surveillance with the system in California in 2025.

Conclusion: The integration of RapidSeq126 with PET enables a cost-effective, scalable, and automated surveillance system for *Coccidioides*. Multi-modal sample collection—including drone, equipment, and individual-level sampling—overcomes limitations of static surveillance and provides real-world exposure data. IoT connectivity facilitates real-time data collection, functioning as an early warning system for the spread of coccidioidomycosis. PET deployment with agricultural workers enhances individual exposure monitoring, enabling early detection and intervention.

Transcriptomic atlas of the morphologic development of the fungal pathogen *Coccidioides* reveals key phase-enriched transcripts

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Abstract

Introduction: *Coccidioides spp.* are highly understudied but significant dimorphic fungal pathogens that can infect both immunocompetent and immunocompromised people. In the environment, they grow as multicellular filaments (hyphae) that produce vegetative spores called arthroconidia. Upon inhalation by mammals, arthroconidia undergo a process called spherulation. They enlarge and undergo numerous nuclear divisions to form a spherical structure, and then internally segment until the spherule is filled with multiple cells called endospores. Mature spherules rupture and release endospores, each of which can form another spherule, in a process thought to facilitate dissemination. Spherulation is unique to *Coccidioides* and its molecular determinants remain largely unknown. Earlier transcriptomic studies of spherules have been limited to 1-2 timepoints. Here, we report the first high-density transcriptomic analyses of dynamic *Coccidioides* development, defining morphology-dependent transcripts and those whose expression is dependent on Ryp1, a major regulator required for spherulation and virulence.

Methods: We profiled the transcriptome of *Coccidioides* development from arthroconidia to spherules over 7 timepoints and germination into hyphae over 5 timepoints for both the wildtype Silveira strain and a *ryp1Δ* deletion mutant. We defined important transcript subsets based on morphology-correlated expression and *RYP1* dependence. To determine direct Ryp1-binding targets, we implemented ChIP-Seq of Ryp1 in *Coccidioides* in both spherules and hyphae. To validate that this transcriptomic resource allowed us to define genes of interest, we selected a cluster of 6 *RYP1*-dependent, arthroconidia-associated transcripts and used CRISPR-Cas9 to create the corresponding mutant. We used transmission electron microscopy to characterize the cell wall of this mutant compared to wild-type arthroconidia.

Results: Of approximately 9000 predicted transcripts, we discovered 273 transcripts with consistent spherule-associated expression, 82 of which are *RYP1*-dependent, a set likely to be critical for *Coccidioides* virulence. ChIP-Seq revealed 2 distinct regulons of Ryp1, one shared between hyphae and spherules and the other unique to spherules. Spherulation regulation was elaborate, with the majority of 227 predicted transcription factors in *Coccidioides* displaying spherule-enriched expression. We identified provocative targets, including 20 transcripts whose expression is endospore-enriched and 14 putative secreted effectors whose expression is spherule-enriched, of which 6 are secreted proteases. To highlight the utility of these data, we selected a cluster of *RYP1*-dependent, arthroconidia-associated genes for deletion and found that they play a role in arthroconidia cell wall biology.

Conclusion: We present an important transcriptomic resource for the *Coccidioides* field, characterizing spherule and hyphal development over many timepoints. We identify a cluster of transcripts involved in cell wall biology, demonstrating the power of this resource in illuminating *Coccidioides* biology and virulence.

Scientific Section IV | Basic Science

Macrophages Promote the Development of Coccidioides Spores into the Parasitic Morphology

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Novel Peptide Drug Demonstrates Anti-Coccidioides Activity

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The Coccidioides Tri-factor: Innate Immune Detection of Fungal Conidia, Spherules and Endospores by Macrophages

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Discovery of Novel Seroreactive Antigens for Coccidioides

Megan Koehler[1], Kenta Reilly[2], Francisca Grill[2], Lusheng Song[1], Yunro Chung[1], Vel Murugan[1], Joshua LaBaer[1], Marc Orbach[3], Janis Blair[2], Thomas Grys[2], Douglas Lake[1], D Mitch Magee[1]

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Macrophages Promote the Development of Coccidioides Spores into the Parasitic Morphology

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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 arthroconidia per 10 macrophages or 1 arthroconidia 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. We assessed the role of phagocytosis with cytochalasin D. 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 utilize 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 *Coccidioides posadasii* Silveira arthroconidia at MOI 1 or MOI 0.1 at multiple timepoints over the course of 48hrs. 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. This process is dependent on phagocytosis by macrophages and is not recapitulated with dead macrophages or macrophage supernatants. To further evaluate the response of arthroconidia to macrophages, we analyzed the transcriptomes of arthroconidia grown with macrophages compared to those grown alone otherwise similar conditions. Spherule specific genes are enriched from samples of *Coccidioides* exposed to macrophages. In the presence of macrophages, *Coccidioides* spherules express a unique set of genes compared to in vitro spherules.

Conclusion: Phagocytosis by macrophages strongly promotes the ability of *Coccidioides* arthroconidia to transition to the parasitic form of *Coccidioides* (spherule). Analyzing the transcriptome of *Coccidioides* as arthroconidia develop into spherules in the presence of macrophages we have identified a key set of enriched genes that may represent virulence factors or responses to macrophage induced stress. This work creates a foundation for better understanding the initial interactions between macrophages and *Coccidioides*.

Novel Peptide Drug Demonstrates Anti-Coccidioides Activity

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Abstract

Introduction: Clinical isolates of *Coccidioides* have exhibited a concerning trend of increasing resistance to fluconazole. To address this challenge, it is essential to develop new antifungal drugs with novel targets. Dr. Lorenz's laboratory has discovered the EntV peptide produced by *Enterococcus faecalis* with *in vitro* and *in vivo* efficacy against *C. albicans* infection. An iterative peptide library based on EntV sequence was investigated for antifungal activity. These peptides are neither fungicidal nor fungistatic. They are thought to bind to lipids and inhibit the secretion of extracellular vesicles (EVs). It is proposed that this type of peptide may play an immunomodulation role during fungal infections. Here we evaluate one of the most effective peptides (Peptide X) for anti-*Coccidioides* activity using both *in vitro* spherule culture and an insect model of *Coccidioides* infection. We aim to evaluate the broad-spectrum activity of this peptide.

Materials and Methods: In a BSL-3 laboratory setting, arthroconidia of the fluconazole-resistant strain *C. posadasii* (#C735) were cultured in Converse medium at 39°C, 10% CO_2, and 180rpm for a duration of 24 hours to facilitate their conversion into spherules. To evaluate the *in vitro* susceptibility of spherules to Peptide X, spherules were dispensed into a 96-well plate containing 2-fold serial dilutions of drug over the following concentrations: 40 – 0.04 µM Amphotericin B, 4 – 0.004 % DMSO, or Peptide X (100 – 0.1µM; 40 – 0.04 µM; 1 – 0.0001 µM; 500 – 0.0005 nM; or 100 – 0.0001 nM). After incubating in the drugs for 24-hours, XTT, a metabolic indicator, was added to the cells for an additional 24-hours to determine the metabolic capacity of the spherules as an indicator of growth. Spherules incubated with DMSO (drug vehicle; <1%) served as the normalization control. To assess the *in vivo* efficacy of Peptide X, a total of 50 *Galleria mellonella* larvae were infected with 4.5×10^5 arthroconidia via injection. Two hours post-infection, five groups of larvae (n=10) were treated with 10 µL of one of the following: PBS alone, 258 µM Amphotericin B, or Peptide X (0.5 µM, 0.1 µM, or 0.01 µM). *Galleria* survival was monitored daily over 5 days and culminated in quantifying the fungal burden by CFU. In parallel, a separate cohort of uninfected *Galleria* larvae (n=5) were injected with each drug alone to evaluate potential toxicity.

Results: Peptide X did not show antifungal activity up to 40 µM concentration against 24-hour spherules using a metabolic assay with XTT substrate. This result is in agreement with inhibition data from *C. albicans*. The peptide did not show toxicity in the *Galleria* model at the highest concentration of 0.5 µM. Interestedly, one dose injected via the hemocoel route of this peptide two hours post-infection at a

Novel Peptide Drug Demonstrates Anti-Coccidioides Activity (continued)

concentration of 0.01 μM yielded a significantly higher survival rate of 80% compared to the PBS-treated control larva. Notably, the survival of Peptide X was significantly higher compared to the group treated with 258μM of Amphotericin B control. Surprisingly, the fungal burden of the PBS-treated group (mean log10 CFU: 4.76±0.64), compared to the 0.01 μM Peptide X was significantly reduced (mean log10 CFU: 3.75±0.72), though not to the extent of Amphotericin B (mean log10 CFU: 1.89±0.80).

Conclusion: Peptide X demonstrates the greatest survival rate at 0.01 μM, with a significantly reduced fungal burden compared to the PBS negative control group, but it was higher compared to the Amphotericin B treatment. This may be due to the differing mechanisms of action of the peptide compared to the controls. Similar to previous studies in *C. albicans*, *Coccidiodes* growth was also not inhibited by Peptide X as supported by the XTT study. This may suggest that the peptide does not kill the spherules but possibly inhibits their ability to germinate or segment, however further *in vitro* and *in vivo* investigation is needed to fully understand these mechanisms.

The *Coccidioides* Tri-factor: Innate Immune Detection of Fungal Conidia, Spherules and Endospores by Macrophages

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Abstract

Background: In the mammalian host, the human fungal pathogen *Coccidioides* species undergo morphological transition, switching from its infectious arthroconidia form to parasitic spherules and endospores that drive chronic infection known as coccidioidomycosis or Valley Fever. Little is known about the nature of the innate immune response to inhaled arthroconidia, or the larger spherules which play a role in chronic infection and organ dissemination through the release of endospores. Prior studies implicate IL-1R signaling in restricting fungal dissemination from the lung. However, the pathogen sensing apparatus and innate immune effector mechanisms engaged by arthroconidia, spherules, and endospores to promote IL-1α/β release and restrict fungal dissemination have not been investigated. In this study, the role of inflammasomes and inflammatory cell death was examined in murine macrophages exposed to *Coccidioides* arthroconidia, spherules, and endospores.

Methods: Live arthroconidia of the *C. immitis* RS strain was used to infect bone marrow-derived macrophages from mice deficient in NLRP3, pyrin, ASC, Caspase-1, Caspase-11, Dectin-1, GSDMD, GSDME, MLKL, or Caspase-8. The ferroptosis pathway and iron-dependent lipid peroxidation of arthroconidia was examined using the ferroptosis inhibitors Liproxstatin-1 and Ferrostatin-1, and by supplementing cultures with ferric citrate or the polyunsaturated fatty acid DHA. Macrophage death was monitored using live cell microscopy and automated image analysis. The fungicidal activity of macrophages was evaluated by plating colony forming units onto 2X glucose yeast extract agar plates 24 hours post-infection. Spherules were generated from live arthroconidia in macrophage cultures supplemented with formalin-killed conidia over a duration of 3.5 days. FunGal Finder, a custom-scripted deep learning algorithm, was used to quantify the number and size of spherules. Endospores released from ruptured spherules were collected by filtering supernatant through a 4μm filter. IL-1β release from infected macrophages was measured by ELISA.

Results: Arthroconidia triggered the death of macrophages without IL-1β release, and this was not impacted by loss of pyroptosis, necroptosis, or apoptosis signaling. However, ferroptosis inhibition further decreased macrophage viability upon arthroconidia exposure, suggesting that iron-dependent lipid peroxidation actively suppresses arthroconidia in macrophages and impairs fungal cytotoxicity directed towards macrophages. Additionally, loss of ferroptosis signaling by inhibition of iron-dependent lipid peroxidation

The *Coccidioides* Tri-factor: Innate Immune Detection of Fungal Conidia, Spherules and Endospores by Macrophages (continued)

reduced killing of arthroconidia by macrophages. In the presence of macrophages, arthroconidia transform into large (~100um) spherules. While intact spherules fail to stimulate IL-1β, ruptured spherules trigger IL-1β processing and secretion by macrophages. Macrophages from *Nlrp3*$^{-/-}$, *Mefv*$^{-/-}$, *Casp1*$^{-/-}$, *Asc*$^{-/-}$, and *Gsdmd*$^{-/-}$ *Gsdme*$^{-/-}$ mice displayed a reduction in IL-1b production in response to ruptured spherules. FunGal Finder was used to monitor spherule number and size revealing enhanced spherule growth in *Gsdmd*$^{-/-}$ *Gsdme*$^{-/-}$ macrophages. Endospores also trigger IL-1β production and activate pyroptosis in macrophages but do so exclusively via the NLRP3 inflammasome.

Conclusions: The innate immune response utilizes diverse pathogen sensors and deploys different effector mechanisms to direct the inflammatory response at each stage of the *Coccidioides* developmental lifecycle. The findings from our study provide new insight into the role of NLRP3 and Pyrin inflammasomes and gasdermin pore formation in driving cytokine release during initial infection and in the context of chronic and disseminated disease. These data improve our understanding of the initial anti-fungal response to arthroconidia and the role of inflammasomes and inflammatory cell death to spherules and endospores. Future studies will help define therapeutic strategies that impair the viability and developmental transition of *Coccidioides*, while also utilizing targeted biologics to reduce chronic inflammation and enhance the host innate and adaptive immune responses.

Discovery of Novel Seroreactive Antigens for *Coccidioides*

Megan Koehler[1], Kenta Reilly[2], Francisca Grill[2], Lusheng Song[1], Yunro Chung[1], Vel Murugan[1], Joshua LaBaer[1], Marc Orbach[3], Janis Blair[2], Thomas Grys[2], Douglas Lake[1], D Mitch Magee[1]

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Abstract

Introduction: A clinical diagnosis of probable Valley Fever (VF) requires a combination of compatible symptoms, radiology, and positive serology. However, current commercial serologic assays utilize complex fungal antigen preparations, which can differ between manufacturers and may cause variation in test results and reduced sensitivity. Importantly, assays such as immunodiffusion chiefly detect reactivity to a single antigen, the immunodominant CF/CTS1. We hypothesize that additional seroreactive antigens exist that would improve diagnostic ability of enzyme immunoassay (EIA) serological tests for Valley Fever. Our aim was to assess the reactivity of human serum samples against an array of >800 *Coccidioides* spherule expressed proteins utilizing Nucleic Acid Programmable Protein Arrays (NAPPA).

Methods: NAPPA is a protein micro-array allowing high-throughput serum screening against hundreds of proteins. DNA containing the open reading frame of *Coccidioides* genes are printed individually onto a microarray slide. Incubation with an in-vitro transcription and translation mixture prints proteins on the slide. Serum is then incubated on the slide and antibodies that bind the expressed proteins are detected with fluorescent secondary anti-IgG and IgM. Sero-reactive proteins are scored using cutoff values and odds ratios. The array also has a number of viral antigens such as influenza antigens, to which most humans have been exposed, serving as positive controls and host proteins serving as negative controls.

Human serum samples were obtained from Mayo Clinic and included patients seropositive by one or more VF antibody tests (n=101) and seronegative patients (n=50). All clinical serology was performed by Mayo Clinic, including complement fixation (CF), immunodiffusion (ID), and enzyme immunoassay (EIA) testing. Among the 101 positive samples, 39 were positive by all three assays; 21 were positive by a combination of any two assays; and 41 were positive by only one assay.

Results: Positive and negative controls confirm a high serological result quality. For the *Coccidioides*-specific proteins, CF / CTS1 was immunodominant as expected, identified in 81/101 (80%) seropositive samples. In addition to CF/CTS1, 9 other proteins were reactive in seropositive patients, including endo-1,3-betaglucanase, identified in 52/101 (51%), and peroxisomal matrix protein, identified in 57/101 (56%). Additionally, four novel proteins on the array were reactive by patient IgM (with the most seroreactive

Discovery of Novel Seroreactive Antigens for *Coccidioides (continued)*

protein identified in 38/101 seropositive samples) but non-reactive by IgG. In seronegative patients, we detected antibodies to two or more of these novel proteins in 10 (20%) out of the 50 patient samples.

Conclusion: Utilizing the protein array, human patient serum samples were most frequently reactive to CF / CTS-1. Nine other antigens were also highly reactive, even in patients who were CF/CTS-1 non-reactive. The NAPPA platform allowed for the discovery of additional antigens that have potential use in a future multi-protein diagnostic platform. By improving the classification of VF patients, particularly in those classified as seronegative, we could prevent delays in initiation of a proper therapeutic plan.

Scientific Section V | Molecular Biology

Expanding the Known Genomic Diversity of Coccidioides Posadasii Through Whole Genome Sequencing and Comparative Genomics

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Ancestry-aware GWAS Identifies Novel Common Variants Associated with Susceptibility to Disseminated Coccidioidomycosis

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Assessing Hierarchical Population Structure Among Coccidioides spp. Across the Americas

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Coccidioides Genomes from Low-Incidence States Reveal Complex Migration History Across the Western US

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Expanding the Known Genomic Diversity of Coccidioides Posadasii Through Whole Genome Sequencing and Comparative Genomics

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Abstract

Introduction: Understanding the exposure and risk of *Coccidioides posadasii* to humans is vital in the control and mitigation of this potentially deadly pathogen. Whole genome sequencing (WGS) is a powerful tool to address questions of pathogenesis, environmental distribution, and exposure risk. To date, the sequencing efforts for *C. posadasii* are relatively limited and geographically diverse. The focus of this study was to sequence a large number of clinical isolates collected from patients diagnosed in Arizona from 2022-2024, to better understand the diversity of gene content, the geographical distribution of virulent isolates, and genomic regions associated with poor patient outcomes.

Methods: Isolation, DNA extraction, and sequencing of clinical isolates. Isolates were collected from clinical specimens using standard isolation methods (Teixeira et al., 2019) and confirmed using established molecular assays (Bowers et al., 2018); all isolates were collected in Phoenix as part of routine diagnosis and care. Confirmed *Coccidioides* isolates were grown on standard laboratory media and DNA was extracted and prepared for sequencing.

DNA library construction for enrichment sequencing was performed using the KAPA Hyper Prep Kits for Illumina® NGS platforms and KAPA UDI Primer Mixes per manufacturer's protocol with double-sided size-selection performed after sonication. Final libraries were quantified on an Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System using the KAPA SYBR® FAST ROX Low qPCR Master Mix for Illumina platforms. The libraries were pooled together at equimolar concentrations and quality was assessed with Agilent Technologies TapeStation 4150 System. Final quantification by qPCR preceded sequencing of the final library. Samples were sequenced on the Illumina NextSeq1000 using the NextSeq1000/2000 P1 XLEAP-SBS Reagent Kit using the standard Illumina protocol.

Whole genome sequencing (WGS), assembly, and analysis. Isolates were sequenced on the Illumina NextSeq1000, returning >100x genome coverage per sample. Genomes were assembled with SPAdes v3.15.5 (Bankevich et al., 2012), producing contiguous assemblies of ~300 contigs per genome. For SNP discovery and comparison, reads were aligned against *C. posadasii* str. Silveira (de Melo Teixeira et al., 2022) with minimap2 v2.26 (Li, 2018) and single nucleotide polymorphisms (SNPs) were called with the UnifiedGenotyper method in GATK v4.5.0.0 (McKenna et al., 2010). For pairwise comparisons, SNPs were aligned against each genome assembly from each pair to maximize the relatedness of strains.

To understand the population structure of the species, GATK output files were combined with NASP v1.2.0 (Sahl et al., 2016) to generate a complete SNP matrix. A maximum-likelihood phylogeny was inferred on the concatenated SNP

Expanding the Known Genomic Diversity of Coccidioides Posadasii Through Whole Genome Sequencing and Comparative Genomics *(continued)*

alignment with IQ-TREE v2.3.6 (Minh et al., 2020) in conjunction with the integrated ModelFinder (Kalyaanamoorthy, Minh, Wong, von Haeseler, & Jermiin, 2017).

Results: To date, we have sequenced >400 genomes from clinical isolates in this study, greatly expanding our understanding of the genomic diversity of *C. posadasii*. The population structure demonstrates that most genomes cluster within the "Phoenix" clade, although we also see genomes grouping with isolate genomes from Tucson. A few genomes were typed genomically as *C. immitis*, suggesting that travel to California was likely the origin on the infection.

The phylogeny exhibited long and deeply branching nodes, indicative of an extensive and ingrained recombination signal. In more than 30 cases, we saw 2-3 genomes exhibiting clonal behavior and focused additional investigation. In most cases, clonal isolates were obtained from the same patient at different visits. Even in those cases, SNPs were identified and may help better understand the rate at which mutations occur over time during an active infection. These data could be useful to track back infections to singular events and focus epidemiological efforts.

In more than 15 cases, we observe closely related genomes obtained from different patients. As direct transmission does not occur, these results suggest environmental exposure from a common location and/or source material. As part of this work, we are also characterizing the genomic diversity of environmental isolates, with the hopes of identifying environmental hot-spots that are capable of multi infection events. These cluster results are focusing public health agencies to conduct patient interviews, which may identify common exposure sources, and help focus risk mitigation efforts.

Once the full genomics dataset has been generated (~700), we will address virulence, antifungal resistance, and shared exposure. This dataset represents an important resource to address long-standing questions in *Coccidioides'* ecology and infection.

Conclusion: Clinical isolates collected in Arizona from 2022-2024 are spread across the phylogenetic tree of *C. posadasii*, exhibiting a recombination signal supporting sexual reproduction, which has been suggested in several publications (Burt, Carter, Koenig, White, & Taylor, 1996; Laux, Teixeira, & Barker, 2023) . In this study, we not only drastically expand the known diversity of this species, especially in Arizona, but provide genomics datasets that can be used to answer additional questions vital to understanding the ecology, virulence, and environmental acquisition of *C. posadasii*.

References

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., . . . Pevzner, P. A. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol., 19*(5), 455-477. doi:10.1089/cmb.2012.0021

Expanding the Known Genomic Diversity of Coccidioides Posadasii Through Whole Genome Sequencing and Comparative Genomics *(continued)*

Bowers, J. R., Parise, K. L., Kelley, E. J., Lemmer, D., Schupp, J. M., Driebe, E. M., . . . Barker, B. M. (2018). Direct detection of Coccidioides from Arizona soils using CocciENV, a highly sensitive and specific real-time PCR assay. *Med. Mycol., 57*(2), 246-255. doi:10.1093/mmy/myy007

Burt, A., Carter, D. A., Koenig, G. L., White, T. J., & Taylor, J. W. (1996). Molecular markers reveal cryptic sex in the human pathogen Coccidioides immitis. *Proc Natl Acad Sci U S A, 93*(2), 770-773. doi:10.1073/pnas.93.2.770

de Melo Teixeira, M., Stajich, J. E., Sahl, J. W., Thompson, G. R., Brem, R. B., Dubin, C. A., . . . Barker, B. M. (2022). A chromosomal-level reference genome of the widely utilized Coccidioides posadasii laboratory strain “Silveira”. *G3 Genes/Genomes/Genetics, 12*(4), jkac031. doi:10.1093/g3journal/jkac031

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods, 14*(6), 587-589. doi:10.1038/nmeth.4285

Laux, K., Teixeira, M. M., & Barker, B. (2023). Love in the time of climate change: A review of sexual reproduction in the order Onygenales. *Fungal Genet Biol, 167*, 103797. doi:10.1016/j.fgb.2023.103797

Li, H. (2018). Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics, 34*(18), 3094-3100. doi:10.1093/bioinformatics/bty191

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., . . . DePristo, M. A. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res., 20*(9), 1297-1303. doi:10.1101/gr.107524.110

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol., 37*(5), 1530-1534. doi:10.1093/molbev/msaa015

Sahl, J. W., Lemmer, D., Travis, J., Schupp, J. M., Gillece, J. D., Aziz, M., . . . Keim, P. (2016). NASP: an accurate, rapid method for the identification of SNPs in WGS datasets that supports flexible input and output formats. *Microb Genom, 2*(8), e000074. doi:10.1099/mgen.0.000074

Teixeira, M. M., Alvarado, P., Roe, C. C., Thompson, G. R., 3rd, Patané, J. S. L., Sahl, J. W., . . . Barker, B. M. (2019). Population Structure and Genetic Diversity among Isolates of in Venezuela and Surrounding Regions. *MBio, 10*(6). doi:10.1128/mBio.01976-19

Ancestry-aware GWAS Identifies Novel Common Variants Associated with Susceptibility to Disseminated Coccidioidomycosis

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Abstract

Introduction: Coccidioidomycosis is a fungal infection endemic to the Americas and disease prevalence and severity have been associated with environmental and biological factors. Most individuals who contract coccidioidomycosis have minimal respiratory symptoms, however fewer than one percent develop a severe disease in which the fungal pathogen spreads beyond the lungs to brain, bones, or other tissues, called disseminated coccidioidomycosis (DCM). Over the past 70 years, numerous epidemiological studies have shown an increased risk of DCM in individuals who self-identify as African-American, Latino, Filipino and Native American. However, self-identified race and ethnicity (SIRE) reflects social determinants of health and biological factors that are impossible to disentangle. Techniques relying on genetic ancestry allow identification of genomically encoded risk factors.

Methods: To better understand the contribution of biological factors on risk of disseminated disease, we obtained DNA and sequencing data from a cohort of 635 individuals with coccidioidomycosis from endemic regions of California. We calculated local and global genetic ancestry estimates to assess the influence ancestry-specific common variants may have on susceptibility to DCM. We then conducted genome-wide association (GWAS) testing with admixture mapping and other ancestry-aware methods.

Results: We identified 10 loci where patient genotype or local ancestry was significantly associated with DCM by at least one GWAS method. Admixture mapping (ADM) identified a 99 kilobase-pair region of chromosome 2 where patients carrying an African or South Asian haplotype were significantly more likely to have DCM ($p = 8.2 \times 10\text{-}9$). This region includes the genes *TTC7A*, *STPG4*, and *CALM2*. Other variants significantly associated with DCM include several in the gene *PRELID2* and eQTLs for immune-related genes *IFI44*, *IFI44L*, *DENND3*, and *TMEM41B*.

Conclusion: This analysis is the largest yet genome-wide analysis of host susceptibility to coccidioidomycosis dissemination and identified ten regions that may confer disease risk. Further research into the pathways of disease progression will enable better treatment and prevention of serious coccidioidomycosis.

Assessing Hierarchical Population Structure Among Coccidioides spp. Across the Americas

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Abstract

Introduction: As whole-genome sequencing has become more accessible in diagnosing and studying *Coccidioides* isolates, the number of samples with whole genome sequence data on NCBI has grown. However, the metadata for WGS samples varies in completeness, making it challenging to use a population genetics approach. However, combining genome-wide single nucleotide polymorphisms (SNPs) with available metadata in a data-driven hierarchical network analysis can identify population clusters and provide valuable high-resolution information. For example, by exploring the topology of the network we can infer broad-scale admixture, patterns of infection, and fine-scale population structure. Samples with incomplete metadata can be inferred by their topology in the network in association with other isolates with more complete metadata. This methodology can be useful in the future as the number of environmental and animal-associated WGS samples increases to provide a more inclusive dataset than what is currently available.

Mothods: Publicly available whole-genome sequence data was used for *C. posadasii* (n=108) and *C. immitis* (n=79) to identify hierarchical population structure across their respective ranges. Genome-wide SNPs were isolated among both species using a series of bioinformatics tools and filtering steps. Hierarchical population structure was assessed using a combination of a genomic network analysis and sequential edge pruning and community detection algorithm implemented in R. A kinship analysis identified the presence of identical isolates that were removed, which reduced the dataset down to 102 *C. posadasii* and 48 *C. immitis* isolates. A phylogenetic tree was used to validate the population clusters identified in the network and compared to previous studies.

Results: A dataset of 169,101 SNPs were used for 183 samples, and an optimum of 19 clusters were identified. However, the kinship analysis flagged 11 groups containing 2-18 individuals that shared >99% of SNPs, which indicates clones and/or close relatives, and can inflate population estimates and subsequent statistics. Selecting a single representative for each group reduced the dataset to 150 samples with 168,211 SNPs. The optimum number of genetic clusters were 15, which corroborates well with the IQTREE phylogenetic tree topology. The reported clusters correspond to clades previously published among the two species but identify additional fine-scale populations in their respective regions and associated patterns of structure

Conclusion: Using publicly available data and a novel method to identify hierarchical population structure, we identified patterns that are consistent with previous publications but also identify new fine-scale characteristics of each species. Exploring a genetic network at different cluster resolutions provides support for the divergence of *Coccidioides* into two species, the hypothesized spread of each species, the presence of travel-associated infections, and patterns of population isolation.

Coccidioides Genomes from Low-Incidence States Reveal Complex Migration History Across the Western US

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Abstract

Introduction: The evolutionary history of *Coccidioides immitis* and *posadasii*, the fungal pathogens responsible for coccidioidomycosis, remains largely unknown in low-endemicity states where genomic and phylogenetic data are limited. Understanding the genetic diversity and evolutionary trajectories of these pathogens in regions outside of highly endemic areas is critical for improving epidemiological surveillance and disease prediction. This study aimed to characterize the evolutionary history of *Coccidioides spp.* in Utah, Colorado, and Nevada, states with no previously characterized *Coccidioides* genomes.

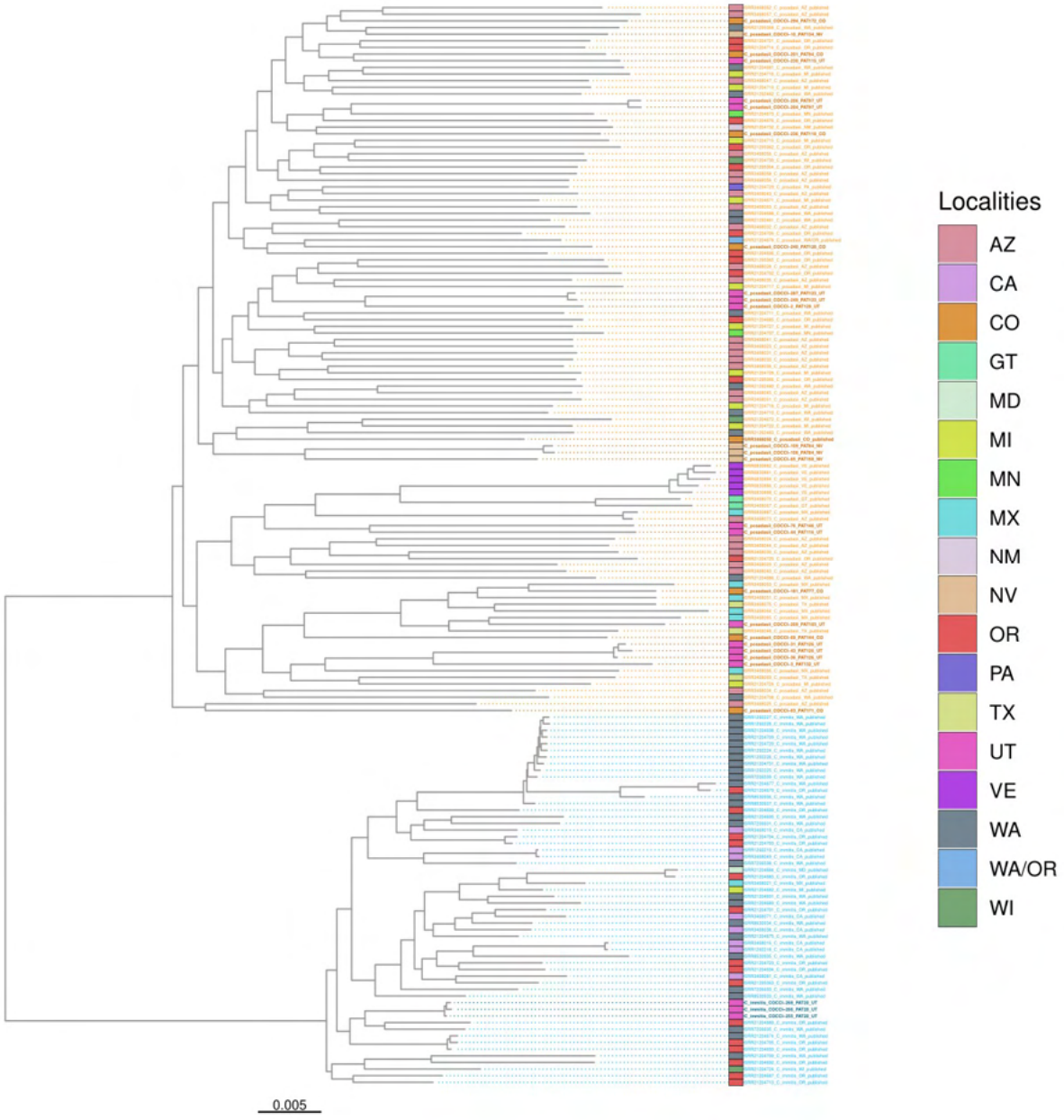
Methods: We prospectively sampled and sequenced the genomes of 24 *Coccidioides*-positive isolates submitted to ARUP, a national diagnostic laboratory from January 2023 to November 2024. We used the Illumina platform to perform the whole genome sequencing on 16 isolates from Utah (UT), 7 isolates from Colorado (CO) and 4 isolates from Nevada (4).We identified genomic variation in newly generated *Coccidioides* sequences and 139 previously published sequences using *cocci-call*, a novel genotyping tool tailored for *Coccidioides*; inferred maximum likelihood phylogenies; and performed ancestral state reconstruction on geographic location of samples.

Results: We assigned species identities to the newly sequenced isolates, identifying 3/27 as *C. immitis* and 24/27 as *C. posadasii*. We identified both *C. immitis* (3/16, from 1 individual patient) and *C. posadasii* (13/16, from 9 individual patients) in UT, whereas only *C. posadasii* was found in CO and NV. Isolates from UT, CO, and NV did not form distinct clades in maximum likelihood phylogenies (Figure 1). Instead, they fell into diverse lineages, suggesting multiple independent introduction events rather than a single, large-scale invasion. Specifically, we identified seven independent introductions of *C. posadasii* into UT, two into NV, and seven into CO (Figure 1)

Conclusion: The history of multiple *Coccidioides* introductions into states that are not considered “highly endemic” suggests that these regions may function as unrecognized reservoirs of the fungus. Further genomic and environmental sampling is needed to clarify the evolutionary trajectories of *Coccidioides* species and its long-term establishment in regions outside highly endemic areas. These results have important implications for disease surveillance and public health strategies in low-incidence areas.

Coccidioides Genomes from Low-Incidence States Reveal Complex Migration History Across the Western US *(continued)*

Figure 1. Maximum likelihood phylogenetic tree of *Coccidioides* isolates, including samples from Utah, Colorado, and Nevada (highlighted in bold), alongside previously published genomes. Bootstrap support values are shown at the nodes. Isolates classified as *C. posadasii* are labeled in orange, while *C. immitis* isolates are in blue.



Scientific Section VI | Epidemiology

Testing for Coccidioidomycosis in Hospitalized Patients with Pneumonia Within the Coccidioides-Endemic Area

Janis Blair, Gretchen Taylor
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Increasing Cases of Severe Pediatric Coccidioidomycosis From 2000 to 2024: Description and Outcomes at a Tertiary Care Center

Sanchi Malhotra, Kristina Adachi, Paula Arribas, Ishminder Kaur, Paul Krogstad
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Characteristics of Disseminated Coccidioidomycosis in Pediatric Patients

Sarah Zhang[1], James Woodward[2,1], Wassim Ballan[2,1], Katherine Perry[2,1], Keith Sacco[2,1]
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Owner Economic Burden of Canine Coccidioidomycosis in Endemic States

Christine Butkiewicz[1], Jane Sykes[2], Lisa Shubitz[1]
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Delayed Diagnosis and Treatment of Coccidioidomycosis Identified Through Enhanced Surveillance — Riverside County, California, 2024

Bethan Swift[1,2], Amanda Mitry[2], Wendy Hetherington[2], Jennifer Chevinsky[2], Marshare Penny[2], Barbara Cole[2]
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Scientific Section VI | Epidemiology (continued)

Forecasting the Impact of Hydroclimatic Swings on Coccidioidomycosis Incidence in California

Simon Camponuri[1], Alexandra Heaney[2], Gail Sondermeyer-Cooksey[3], Duc Vugia[3], Seema Jain[3], Daniel Swain[4,5], John Balmes[1,6], Justin Remais[1], Jennifer Head[7]
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Coccidioidomycosis Cases at a Regional Medical Center in West Texas, 2021-2024

Chun Ho Szeto[1], Gloria Erazao Motalvan[1], Thao Do[2], Trisha Singh[1], Sai Siva Mungara[1]
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Refractory Disseminated Coccidioidomycosis with Musculoskeletal Involvement in a 14-year-old Effectively Treated with Adjunctive Interferon Gamma

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Testing for Coccidioidomycosis in Hospitalized Patients with Pneumonia Within the Coccidioides-Endemic Area

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Abstract

Introduction: Within the *Coccidioides*-endemic area, coccidioidomycosis (cocci) has been identified as the cause of pneumonia in 15-29% of patients with community-acquired pneumonia (CAP). Although efforts to improve testing CAP patients for cocci are underway in the outpatient settings in Arizona, we do not know whether testing is common among patients admitted to the hospital with pneumonia. We undertook the current study to assess the frequency of testing for cocci among hospitalized patients with pneumonia in our institution, and to understand whether positive serologic testing led to a reduction in antibiotic use.

Methods: We commissioned the creation of an electronic tool by our Information Technology colleagues to create a report to identify inpatients of Mayo Clinic Hospital with a pneumonia diagnosis (J18.0 – J18.9), then collected information regarding testing for *Coccidioides* by any serology (enzyme immunoassay, immunodiffusion or complement fixation) and patient receipt of antibiotics or antifungal treatment (name, inclusive dates).

Results: Between January 1, 2024 and December 31, 2024, 1162 patients were admitted to the Hospital Internal Medicine service at Mayo Clinic Hospital, Phoenix AZ with a pneumonia diagnosis. Among these, 771 (66%) were tested serologically for coccidioidomycosis, and 87/771 (11%) were found to be positive. By calendar month, the percent of patients tested ranged 59 – 73%, and percent test positivity ranged 3% (March) - 19% (December). The mean time from admission to test order was 0.5 days (range 0 – 6 days) and mean time from admission to positive result was 3.0 days (range 0.4 – 12.0 days). However, among seropositive patients, the duration of antibiotics was 4.2 days, not different when compared with patients who were either seronegative or not tested, (4.1 days, p=NS). 173 (14.9%) received antifungal treatment during hospitalization, among whom 55 (31.2%) had positive coccidioidal serology and 118 (68.2%) did not.

Conclusions: Coccidioidomycosis is an important cause of pneumonia within the inpatient setting. However, one third of patients were not tested, representing an opportunity to understand and overcome barriers to testing. Positive tests were not associated with a reduction of antibiotic use.

Increasing Cases of Severe Pediatric Coccidioidomycosis From 2000 to 2024: Description and Outcomes at a Tertiary Care Center

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Abstract

Introduction: California had a record high case rate of coccidioidomycosis in 2024. However, there is limited published data from pediatric patients in the last 10 years. The purpose of this study was to describe clinical aspects of pediatric coccidioidomycosis cases at a tertiary care referral center including the increasing volume of cases, cases with disseminated disease or other complications, and relevant treatment practices and hypothesize case severity increased in the 2024 season. This study also describes rates of fluconazole resistance and/or treatment failure, particularly among those with disseminated or severe coccidioidomycosis.

Methods: We performed a retrospective observational study looking at pediatric coccidioidomycosis cases seen both inpatient and outpatient at the University of California Los Angeles. We searched our EMR for patients with ICD-10 codes B38.1 to B38.9, ICD-9 codes 114.0-114.9, positive microbiologic culture for *Coccidioides immitis/posadasii*, or positive *Coccidioides* complement fixation titer. We included patients age 0-18 years from January 2000 to December 2024 with a diagnosis of coccidioidomycosis. We excluded patients who were considered to have false positive testing, negative testing, had most of their management at a different institution, or were receiving prophylaxis against *Coccidioides* without evidence of true infection. In this preliminary review of our presented data, descriptive analysis was performed. Future analysis by chi square and multivariate analysis will be used to determine if age, race, or year of diagnosis were significantly associated with disease severity, disease outcome, and if year of diagnosis was associated with fluconazole resistance/refractory disease. Disease severity will also be classified according to a previously proposed classification system.

Results: We reviewed 105 patient charts, and 67 patients met inclusion criteria. Of our study population, 52% were male, most were adolescents (52%), and the most common identified race was white (40%), with 30% identifying as Hispanic/Latinx/Mexican ethnicity. A significantly increased number of patients were seen in 2024 (13) and 2018 (10) whereas a range of 0 to 4 cases were seen every other year. Preliminary analysis showed that 8.8% of patients were immunocompromised. 6 of 13 cases seen in 2024 (46%) had disseminated disease, with two-thirds having bone/joint and/or skin involvement as the most common site. In the 23 years prior, 15 (28%) had disseminated disease, with isolated lymphadenopathy being the most

Increasing Cases of Severe Pediatric Coccidioidomycosis From 2000 to 2024: Description and Outcomes at a Tertiary Care Center (continued)

common extrapulmonary manifestation. 88% of patients were started on treatment, often by their primary pediatrician or upon referral to our hospital. Of these, 39% required a change in medication, which was more

common for patients with disseminated disease (70%). Fluconazole was the initial treatment for 86% of patients. Susceptibility testing was available for 9 unique patient specimens (6 of them collected in 2024), with fluconazole MICs ranging from 8-32. The relationship between fluconazole susceptibility and clinical outcomes will be presented on further analysis. Outcome data and further analysis for statistically significant variables will be completed prior to the meeting.

Conclusion: The pediatric coccidioidomycosis case incidence and dissemination rate significantly increased for patients seen at our institution in 2024, with musculoskeletal disease becoming a more prominent site of dissemination. Consistent with prior reports, pediatric patients have a higher rate of disseminated disease than described in the adult literature, although this may be confounded by being a tertiary care referral center. Pediatricians often treat patients with uncomplicated pulmonary coccidioidomycosis and this may be an opportunity for stewardship and education for those in endemic areas.

Characteristics of Disseminated Coccidioidomycosis in Pediatric Patients

Sarah Zhang[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, Phoenix, USA

Abstract

Introduction: Coccidioidomycosis is endemic to the southwestern United States with Maricopa County, Arizona reporting more than 7,000 cases per year. Coccidioidomycosis commonly presents with pulmonary disease but may disseminate to extrapulmonary systems. Disseminated coccidioidomycosis is not well understood in the pediatric population. This study characterizes the epidemiology of pediatric patients with disseminated coccidiomycosis based on demographics and clinical data.

Methods: We conducted a retrospective chart review of patients diagnosed with disseminated coccidioidomycosis and seen from 2019-2022 at a pulmonology clinic of an urban pediatric hospital in Arizona. Approval for the study was granted by the Phoenix Children's Institutional Review Board.

Results: 35 patients (ages 0-17; median age 13) were identified with no mortalities. There is an even distribution by sex (18 males). 13 patients were Caucasian, 12 African American, 7 Hispanic, 2 Native American and 1 Pacific Islander. Diagnosis was made by positive result of serological tests (n=13) including detection of IgM, IgG to *C. immitis/posadasii*, immunodiffusion or complement fixation, culture (n=15), or histopathology (n=7). The spectrum of disease included meningitis (n=12), osteomyelitis (n=16), pneumonia (n=10), pleural effusion (n=4), skin lesion (n=3), soft tissue lesion (n=15), and lymphadenopathy (n=5) (Figure 1). 1 patient had a cytidine triphosphate synthetase 1 deficiency. 9 patients had secondary immunodeficiencies. All patients were initially treated with fluconazole; 5 switched to posaconazole, and 9 also received liposomal amphotericin B. Median absolute lymphocyte count was 2.1 K/μL (0.2-9.6) and median absolute eosinophil count was 0.2 K/μL (0-1.5).

Conclusion: Our study of disseminated coccidioidomycosis in the pediatric population contributes to the limited literature by further characterizing the affected patients' demographics, clinical data, and management. The results provide a framework to further examine risk factors, preventative measures, and potential treatments of pediatric disseminated coccidioidomycosis infection.

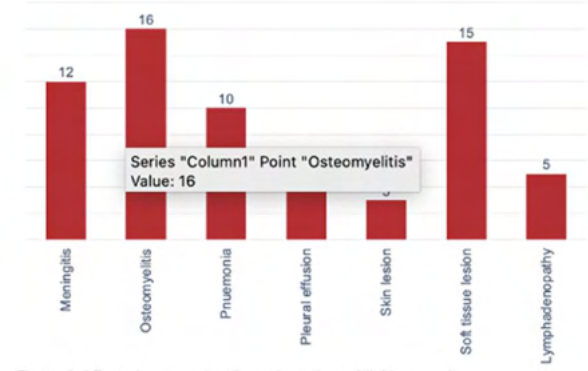

Figure 1. Affected systems by disseminated coccidioidomycosis.

Owner Economic Burden of Canine Coccidioidomycosis in Endemic States

Christine Butkiewicz[1], Jane Sykes[2], Lisa Shubitz[1]
[1]University of AZ - VFCE, Tucson, USA. [2]School of Veterinary Medicine, University of California-Davis, Davis, USA

Abstract

Introduction: Coccidioidomycosis, the disease caused by the dimorphic fungi *Coccidioides immitis* or *C posadasii*, causes disease in dogs that is similar in scope and range of illness to humans. The disease requires diagnosis and often extended treatment, a cost usually borne by the dog's owner. We used nationwide serological data to estimate this cost for the six most endemic states (AZ, CA, NV, NM, TX, UT).

Methods: Dog populations/state were estimated using U.S. census household data with publicly available dog ownership statistics (American Veterinary Medical Association). The number of new canine coccidioidomycosis cases in 2022 was obtained from a study of all laboratories known to participate in serologic testing. Median prices for examination and diagnostic testing were obtained from publicly available data sources for 2019. Drug prices were estimated using online pharmacy discounted pricing. Costs in 2024 were determined using industry-based inflation rates.

Results: The total dog population in endemic states in 2022 was 20,344,734. There were 34,673 unique seropositive dogs, with 95% of these dogs from AZ. Costs to an owner for diagnosis ranged from $306-$775, depending on state and level of care. The cost to dog owners for diagnosis and treatment of dogs with pulmonary coccidioidomycosis in the endemic states for one year was $68M-$84M. These costs represent about 3% of median income and 10% of discretionary income in each state, with higher costs for severe/disseminated coccidioidomycosis. Adjusting for inflation from 2019 to 2024, the cost of diagnosis and treatment for one year in all six states was $97M-$112M.

Conclusion: The cost of diagnosis and treatment of canine coccidioidomycosis represents a significant financial burden to dog owners residing in the endemic region; this is likely to rise further with the impact of climate change on disease incidence.

Delayed Diagnosis and Treatment of Coccidioidomycosis Identified Through Enhanced Surveillance — Riverside County, California, 2024

Bethan Swift[1,2], Amanda Mitry[2], Wendy Hetherington[2], Jennifer Chevinsky[2], Marshare Penny[2], Barbara Cole[2]
[1]Centers for Disease Control and Prevention, Atlanta, USA. [2]Riverside County Department of Public Health, Riverside, USA

Abstract

Introduction: Coccidioidomycosis is primarily a lung disease caused by the *Coccidioides* fungus that grows in soil. In California, coccidioidomycosis is endemic and reportable. During 2019–2023, age-adjusted coccidioidomycosis rate in Riverside County (California) increased from 13.0/100,000 persons to 17.0/100,000 persons. Because of reporting volume, cases are confirmed using laboratory criteria without further investigation. Prompt diagnosis and clinical management can reduce risk for severe disease. We piloted enhanced surveillance to identify opportunities to improve prevention and clinical management efforts.

Methods: Coccidioidomycosis cases among Riverside County residents during January–September 2024 were identified from the California Reportable Disease Information Exchange. Residents aged <18 years or with a correctional facility address were excluded. We conducted enhanced surveillance interviews for consenting persons with a confirmed coccidioidomycosis case 1–4 weeks after their positive laboratory test collection date. Interviews collected demographic information; exposure, illness, and treatment history; and knowledge of test result.

Results: We interviewed 112 of 248 persons with a coccidioidomycosis case reported (45.2% response rate). Median interviewee age was 57.5 years (interquartile range [IQR]: 43.8–67.0). A majority were male (52.7%), employed (53.6%), and most common race and ethnicity was Hispanic (38.4%). Forty percent reported working outdoors daily or most days during the 2 months preceding illness or testing. Sixty-eight percent reported symptoms; 57 (50.9%) were hospitalized and 10 (17.5%) required intensive care unit treatment. Interviewees had a median of 4 interactions with healthcare providers before coccidioidomycosis testing; 46 (41.1%) were prescribed antibiotics. Median time from first healthcare encounter to testing was 55 days (IQR: 12.0–194.5). When interviewed, 50 (44.6%) had not received notification of their positive result.

Conclusion: Enhanced surveillance revealed that persons with coccidioidomycosis had delayed diagnosis and inappropriate treatment with antibiotics. Educating medical providers and the community regarding symptoms and treatment might improve health outcomes and promote antimicrobial stewardship.

Forecasting the Impact of Hydroclimatic Swings on Coccidioidomycosis Incidence in California

Simon Camponuri[1], Alexandra Heaney[2], Gail Sondermeyer-Cooksey[3], Duc Vugia[3], Seema Jain[3], Daniel Swain[4,5], John Balmes[1,6], Justin Remais[1], Jennifer Head[7]

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Abstract

Introduction: Coccidioidomycosis, or Valley fever, is an infectious disease caused by inhalation of *Coccidioides* spp., fungi found primarily in soils of the southwestern United States. Prior work showed that coccidioidomycosis cases in California sharply increase by nearly 2-fold following wet winters that occur one- and two-years following drought. Statewide drought between 2020-2022 followed by heavy precipitation during the 2022-2023 winter raised concerns over potential increases in coccidioidomycosis cases in the fall of 2023, prompting California Department of Public Health (CDPH) to issue public health alerts. As anticipated, California saw a near record number of cases in 2023, with 9,054 provisional cases reported (as of July 2024). During the 2023-2024 California wet season, precipitation was 115% the long-term average, furthering concerns about continued high coccidioidomycosis risk.

Methods: We developed an ensemble prediction model consisting of five candidate prediction algorithms, including generalized linear models and a random forest, that related monthly reported cases per census tract in California to climatological or environmental predictors. We applied our model to historical and extrapolated temperature and precipitation data to generate forecasts of coccidioidomycosis cases from January 2023 through March 2025.

Results*:* Using this model, we predicted a total of 11,846 cases (90% PI: 11,224–12,456) in California between April 1, 2023, and March 31, 2024, closely aligning with the preliminary state report of 10,593. Our model forecasted 12,244 cases (90% PI: 11,638–12,917) statewide between April 1, 2024, and March 31, 2025 — a 62% increase over the cases reported during the same period two years prior, and on par with the high incidence seen in 2023. The Southern San Joaquin Valley (5,399 cases, 90% PI: 4,993–5,902), Southern Coast (3,322, 90% PI: 3,172–3,494), and Central Coast (1,207 cases, 90% PI: 1,071–1,378) regions are expected to see the largest number of infections. Our model forecasts that disease incidence will exhibit pronounced seasonality, particularly in endemic regions, with cases beginning to rise in June and peaking in

Forecasting the Impact of Hydroclimatic Swings on Coccidioidomycosis Incidence in California (continued)

November at 1,411 (90% PI: 1,076–1,396) cases statewide – 98% higher than the peak two years prior (714) and nearly as high as the peak in 2023 (1,462).

Conclusions*:* Near-term forecasts have the potential to inform public health messaging to enhance provider and patient awareness, encourage risk reduction practices, and improve recognition and management of coccidioidomycosis. Future work is needed to develop an automated coccidioidomycosis forecasting system that can reliably be incorporated directly into public health practice in California and other endemic regions.

Coccidioidomycosis Cases at a Regional Medical Center in West Texas, 2021-2024

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Abstract

Introduction: Coccidioidomycosis is endemic to West Texas; however, epidemiological studies remain limited as the disease is not a reportable condition in the state [1]. This study aims to characterize the demographic distribution and clinical presentations of patients diagnosed with coccidioidomycosis at a regional medical center in West Texas.

Methods: A retrospective analysis was conducted using medical records from a 402-bed regional hospital in Ector County, Texas. Patients diagnosed with coccidioidomycosis between December 1, 2021, and April 30, 2024, were identified based on International Classification of Diseases, 10th Revision (ICD-10) code B38. The diagnosis was confirmed through pathology reports, radiological findings, or physician documentation. Rural counties were defined as those with a population of less than 50,000. Institutional Review Board's approval was obtained from Texas Tech Health Sciences Center (IRB-FY2024-339).

Results: A total of 71 patients with coccidioidomycosis were identified, all of whom were Texas residents. Of these, 25.3% (18/71) resided in rural counties in West Texas at the time of diagnosis. The majority of patients (45.1%, 32/71) were between 20–59 years old, while 31% (22/71) were over 60 years of age (Table 1). 57.7% (41/71) of cases were diagnosed during the study period. Pulmonary manifestations were predominant, with 11.2% of patients presenting with meningitis. The most common comorbidities were a history of smoking (49.2%, 35/71) and diabetes mellitus (54.9%, 39/71). 26.7% of patients had underlying chronic lung disease.

Conclusions: Our findings suggest a higher number of coccidioidomycosis cases compared to previous retrospective studies in West Texas [2]. Further epidemiological research is warranted to assess disease outcomes, particularly in rural populations, and to inform public health strategies in this endemic region.

Coccidioidomycosis Cases at a Regional Medical Center in West Texas, 2021-2024 (continued)

Demographics	No./total (%)
Age at diagnosis, y	
<20	4/71 (5.63%)
20-29	3/71 (4.22%)
30-39	6/71 (8.45%)
40-49	9/71 (12.6%)
50-59	14/71 (19.7%)
60-69	17/71 (23.9%)
>70	5/71 (7.0%)
Unknown	13/71 (18.3%)
Sex	
M	48/71 (67.6%)
F	23/71 (32.3%)
Race and ethnicity	
Hispanic	40/71 (56.3%)
Non-Hispanic White	19/71 (26.7%)
Non-Hispanic Black	11/71 (15.4%)
Asian/Pacific Islander	1/71 (1.40%)
Associated factors	
Smoking history	35/71 (49.2%)
Diabetes	39/71 (54.9%)
Immunocompromised	8/71 (11.2%)
Chronic lung disease	19/71 (26.7%)
Lung pathology	
Nodule or mass	29/71 (40.8%)
Pneumonia or consolidation	19/71 (26.7%)
Cavitation	19/71 (26.7%)
Effusion	10/71 (14.0%)
CNS pathology	
Meningitis	8/71 (11.2%)

Refractory Disseminated Coccidioidomycosis with Musculoskeletal Involvement in a 14-year-old Effectively Treated with Adjunctive Interferon Gamma

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Abstract

Introduction: Coccidioidomycosis commonly presents with pulmonary disease but may disseminate and cause extrapulmonary disease. First-line treatment is triazoles, with liposomal amphotericin B being reserved for severe cases. There is increasing interest in using adjunctive interferon gamma (IFN-γ) for refractory infections.

Case Presentation: A 14-year-old African American male presented with fever, weight loss, and back swelling. He had no personal or family history of severe infections. Imaging showed a miliary lung pattern, lymphadenopathy, brain lesions, bony destruction in ribs and lower extremities, paraspinal abscesses, and retroperitoneal fluid. Pelvic biopsy fungal culture grew Coccidioides species. He also had positive Coccidioides-specific serologies with a complement-fixation (CF) titer of 1:512. The patient was started on fluconazole, with a 2-week induction with liposomal amphotericin B. He also had to undergo multiple surgical debridements of the left calcaneus and cuboid where he had the most destructive bone lesions. His musculoskeletal disease progressed, and the CF titer remained elevated at 1:256 two months later. Therapy was changed to posaconazole, micafungin, and subcutaneous IFN-γ at 50mcg/m^2 thrice weekly.

Four months later, the CF titer was unchanged with recurrent drainage from the foot. Foot amputation was considered as a possible next step but was eventually avoided. IFN-γ was increased to 150mcg/m^2, causing only transient fevers. His clinical progression subsequently slowed, with improvement in CF titer to 1:128 within one month, and to 1:32 six months later. IFN-γ was discontinued after nine total months but he continues posaconazole, with planned life-long treatment. He is doing well clinically, with weight gain and normal ambulation.

Refractory Disseminated Coccidioidomycosis with Musculoskeletal Involvement in a 14-year-old Effectively Treated with Adjunctive Interferon Gamma (continued)

Conclusion: Immunomodulatory therapy with IFN-γ is well-tolerated and could be considered as an adjunctive therapy in disseminated coccidiomycosis with musculoskeletal involvement. This case demonstrates interferon gamma's potential in controlling disseminated coccidioidomycosis, highlighting the role of the Th1 response in fungal disease. Further research is necessary to elucidate the mechanism of immune dysregulation in disseminated coccidioidomycosis.

Table 1. Immunologic and hematologic evaluation at time of diagnosis and after treatment with interferon-γ for greater than nine months

	Initial	IFN-γ for >9 Months	Reference Range
WBC (K/µL)	17.4	10.6	4.5-13.0
ANC (K/µL)	13.6	7.9	1.8-8.0
ALC (K/µL)	2.1	1.6	1.2-5.8
AEC (K/µL)	0.3	0.4	0.0-0.5
CRP (mg/dL)	4.9	0.7	0.08-3.1
ESR (mm/Hr)	105	n/a	0-15
Ferritin (ng/mL)	419	184	12.7-82.8
Albumin (g/dL)	2.2	3.1	3.5-5.2
IgG (mg/dL)	3337	1344	700-1648
IgA (mg/dL)	192	207	53-287
IgM (mg/dL)	n/a	80	15-260
IgE	n/a	221	<629
CD3 abs (cells/µL)	1038	n/a	570-2400
CD4 abs (cells/µL)	787	n/a	430-1800
CD8 abs (cells/µL)	194	n/a	210-1200
CD19 abs (cells/µL)	204	n/a	91-610
NK (cells/µL)	59	n/a	78-470
Cocci IgG (IV)	10.5	n/a	<0.9
Cocci IgM (IV)	1.5	n/a	<0.9
Cocci CF Titers	1:512	1:32	

Poster Presentations

Poster Presentations

1. From Headache to Vasculopathy: Unraveling Moyamoya Syndrome in Coccidioidal Meningitis

Yousef Abdulrahman, Emilie No, William Byrne-Quinn, Kareem Shehab, Nathan Price
University of Arizona, Tucson, USA

2. Assessing Pediatric Provider Knowledge, Attitudes, and Practices Regarding Coccidioidomycosis in California

Ruzan Adams, Talal Seddik, Sharon Chen
Stanford, Palo Alto, USA

3. Treatment Outcomes for Chronic Coccidioidomycosis Pulmonary Colonization in a Patient with Cystic Fibrosis

Mohammed Al Mairi, Lienna Chan, Kareem W. Shehab, MD
Department of Pediatrics, Division of Infectious Diseases, University of Arizona College of Medicine, Tucson, USA

4. Understanding Soil Properties Conducive for *Coccidioides* ssp. Presence in the United States

Yahaira Alvarez-Gandia[1], Cari Lewis[1], Bridget Barker[2], Jovani Catalán-Dibene[3], Kimberly Kaufeld[1], Daniel Kollath[2], Antje Lauer[4], Heather Mead[5], Hanna Oltean[6], Marieke Ramsey[2], Adriana Romero-Olivares[3], Andrew Bartlow[1], Morgan Gorris[1] [1]Los Alamos National Laboratory, Los Alamos, NM, USA. [2]The Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ, USA. [3]New Mexico State University, Las Cruces, NM, USA. [4]California State University Bakersfield, Bakersfield, CA, USA. [5]The Translational Genomics Research Institute, Flagstaff, AZ, USA. [6]Washington State Department of Health, Shoreline, WA, USA

5. Description of *Coccidioides Posadasii* in Soil Samples From Southern New Mexico

Justin Best, Michael Woods
Burrell College of Osteopathic Medicine, Las Cruces, USA

6. Temperature and Density Affect Conidiation, Conidia Viability, and Thermotolerance in *Coccidioides* Species

Matthew Tate, Ka Pui Sharon Yau, Melanie Jimenez, Ben Croker, Sinem Beyhan
University of California San Diego, La Jolla, USA

7. Scaling Up Production of High-quality Coccidioides Genomes Via High Throughput Culture and DNA Extraction of Coccidioides Patient Isolates from Arizona

Dawn Birdsell[1], Ashley Itogawa[1], Daniel Kollath[1], Amber Jones[1], Rebecca Ballard[1], Roxanne Nottingham[1], Paul Keim[1,2], John Galgiani[3], Jason Sahl[1,2], Bridget Barker[1,2,3], Dave Wagner[1,2]
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8. The Correlation of Social Determinants of Health in Outcomes of Coccidioidomycosis: Preliminary Results

Niriksha Ravi, Nathan Delafield, Matthew Buras, Jaxon Quillen, Janis Blair
Mayo Clinic, Phoenix, USA

9. Epidemiological Profile of Coccidioidomycosis in Mexico and Baja California: Analysis of Hospital Discharges (2004-2023)

Nydia Castillo-Martinez, Ofelia Candolfi-Arballo, Jose Chavez-Mendez, Juliana Baez-Elizalde, Daniel Ramirez-Diaz
Universidad Autonoma de Baja California, Tijuana, Mexico

10. Frequency, Interval, and Patient Factors Associated with Recurrence of Cutaneous Coccidioidomycosis

Nathan Chow, Janis Blair
Mayo Clinic, Scottsdale, USA

11. Soil Nitrogen Dynamics at a Long-Term Coccidioides-Positive Site

Christopher Cobos[1,2], Hector Valencia[1], Joseph Burke[1,2], Simon Camponuri[3], Jennifer Head[4], Katie Lewis[1,2]
[1]Texas A&M AgriLife Research, Lubbock, USA. [2]Texas A&M University, College Station, USA. [3]University of California-Berkeley, Berkeley, USA. [4]University of Michigan, Ann Arbor, USA

12. Coccidioidomycosis Awareness, Diagnosis, and Symptom Severity: Enhanced Surveillance of Patients with Laboratory-Confirmed Coccidioidomycosis — Maricopa, Pima, and Pinal County Health Departments, Arizona, January 16–August 17, 2024

Jennifer Collins[1], Brandon Howard[1], Ellen Santos[2], Anissa Taylor[2], Kaeli Lugo[3], Zia Helgeson-Budrys[3], Megan Jehn[4], Camila Tompkins[4], Olivia Omura[5], Thomas Moore[5], Christopher Bratsman[1], Rebecca Sunenshine[1], R. Nicholas Staab[1], Shane Brady[6], Irene Ruberto[6], Thomas Williamson[6], Ariella Dale[1]
[1]Maricopa County Department of Public Health, Phoenix, USA. [2]Pima County Health Department, Tucson, USA. [3]Pinal County Public Health Services District, Florence, USA. [4]Arizona State University, Phoenix, USA. [5]University of Arizona, Tucson, USA. [6]Arizona Department of Health Services, Phoenix, USA

13. Functional Annotation and Computational Analysis of the Coccidioides Proteome in Virulence and Host Immunity

Arianna D. Daniel, Katrina K. Hoyer
UC Merced, Merced, USA

14. Epidemiology and Clinical Course of Pediatric Coccidiomycosis in a Tertiary Care Children's Hospital in Los Angeles

Priya Edward, Thomas Barter, Jean Meadows, Melissa Montague, Elissa Singson, Sindhu Mohandas
Children's Hospital Los Angeles, Los Angeles, USA

15. A Machine Learning Model to Forecast Case Counts in Arizona Using Historical Case Count, Genetic, and Environmental Data

Cally Erickson, Cari Lewis, Yahaira Alvarez-Gandía, Tenley Housler, Andrew Bartlow, Morgan Gorris, Kimberly Kaufeld
Los Alamos National Laboratory, Los Alamos, USA

16. Seasonal Dynamics of Airborne Microbial Communities in San Joaquin Valley and its Implications for Climate-Driven Disease Transmission and Respiratory Health

Adeola Fagbayibo, Maya Perez, Estrella Molina-Herrera, Asa Bradman, Katrina Hoyer
University of California, Merced, USA.

17. A Review of Hospitalizations for Coccidioidal Meningitis from 2020-2024

Michelle Fang[1], Jigar Patel[1], Jenessa Olson[2], Bianca Torres[1], Carlos D'Assumpcao[1], Rasha Kuran[1], Royce Johnson[1], Shikha Mishra[1]
[1]Valley Fever Institute, Bakersfield, USA. [2]Western University, Pomona, USA

18. Development of an Experimental Infection Model for Valley Fever in Pigtail Macaques

Megan Fredericks[1,2], Oliver Mauer[1,2], Jason Dufour[3], Kasi Russell-Lodrigue[3], Sandra Dross[1,2], Charlotte Hotchkiss[2], Bridget Barker[4], Deborah Fuller[1,2]
[1]University of Washington, Seattle, USA. [2]Washington National Primate Research Center, Seattle, USA. [3]Tulane National Primate Research Center, Covington, USA. [4]Northern Arizona University, Flagstaff, USA

19. Managing Coccidioidomycosis in Patients with Autoimmune Disease on Biological Response Modifiers

Leah Friedman[1], Karolina Perez[2], Rachel Wu[1], Mehraneh Mohseni Zadeh Harouzi[1], Fariba Donovan[3,2]
[1]University of Arizona, Tucson, USA. [2]University of Arizona College of Medicine-Tucson, Tucson, USA. [3]The Valley fever Center for Excellence, Division of Infectious Diseases, University of Arizona, Tucson, USA

20. Evaluation of an Anti-CTS1 Antibody Lateral Flow Assay to Monitor Antibody Response in *Δcps1*-Vaccinated Dogs Challenged with *Coccidioides*

Francisca Grill[1], Megan Koehler[2], Lisa Shubitz[3], Thomas Grys[4,1], D Mitchell Magee[5], Edward Robb[6], Douglas Lake[2,1]
[1]Cactus Bio, LLC, Phoenix, USA. [2]School of Life Sciences, Arizona State University, Tempe, USA. [3]University of Arizona,

Tucson, USA. [4]Department of Laboratory Medicine and Pathology, Mayo Clinic, Phoenix, USA. [5]Center for Personalized Diagnostics, Arizona State University, Tempe, USA. [6]Anivive Life Sciences, Inc, Long Beach, USA

21. Considering Coccidioides in the Environment: Where, How, and Why?

Thomas Grys
Mayo Clinic Arizona, Phoenix, USA

22. Evaluation of The Cactus Bio Immunodiffusion Reagents, And the Non-Utility Of IgM Testing By Immunodiffusion

Thomas Grys[1,2], Francisca Grill[2], Barbara Ronz[1], Anisha Misra[3], Douglas Lake[4,2], Erin Kaleta[1]
[1]Mayo Clinic Arizona, Phoenix, USA. [2]Cactus Bio, Phoenix, USA. [3]Cleveland Clinic, Cleveland, USA. [4]Arizona State University, Tempe, USA

23. The Coccidioides Complex Longitudinal Abstraction Tool (CLAT) for Integration and Harmonization of Complex Multimodal Data

Crystal Grys[1], Kenta Reilly[1], Douglas Lake[2], Neil Ampel[1], Janis Blair[1], Thomas Grys[1]
[1]Mayo Clinic Arizona, Phoenix, USA. [2]Arizona State University, Tempe, USA

24. To Biofilm or Not to Biofilm: Investigation of *Coccidioides* ability to Produce Biofilms and the Clinical Implications

Ashwarya Handa[1], Ashley Itogawa[1], Nawarat Somprasong[1], Robert Zarnowski[2], GR Thompson III[3], David Andes[2], Bridget Barker[1]
[1]Northern Arizona University, Flagstaff, USA. [2]University of Wisconsin Madison, Madison, USA. [3]UC Davis, Sacramento, USA

25. Evaluating Community-acquired Pneumonia Management in the Endemic Region for Coccidioidomycosis: A Continuous Quality Improvement Model

Justin Hayes[1], Ahmet Gungor[2], Neil Ampel[1]
[1]University of Arizona, Tucson, USA. [2]Banner University Medical Center-Tucson, Tucson, USA

26. Coccidioides (Spherulin) Skin Test Performance and Patterns of Prior Immunity by Age Among Men Entering State Prisons in California, 2015-2024

Jennifer Head[1], Andrew Brouwer[1], Arundhati Rajan[1], Marlena Scherer[2], Monica Williams[2], Kimberley Lucas[2]
[1]University of Michigan, Ann Arbor, USA. [2]California Correctional Health Care Services, Elk Grove, USA

27. Development of a Coccidioides Cytokine Release Assay

Eric Holbrook[1], Danielle Turner[1], Cody Banks[1], Ellen Johnson[1], Nicole Bridges[1], Joshua Malo[2], Althea Campuzano[3], Garrett Grischo[4], Richard De Armond[2], Mrinalini Kala[4], Neil Ampel[2], Heidi Erickson[2], Fariba Donovan[5], ChiungYu Hung[3], Kenneth S. Knox[4], L. Joseph Wheat[1]

[1]MiraVista Diagnostics, Indianapolis, USA. [2]University of Arizona, College of Medicine, Tucson, USA. [3]University of Texas San Antonio, San Antonio, USA. [4]Department of Medicine, University of Arizona College of Medicine, Phoenix, USA. [5]Valley Fever Center for Excellence, College of Medicine-Tucson, University of Arizona, Tucson, USA

28. Evaluating the diagnosis and reporting of coccidioidomycosis cases in an endemic region—Maricopa County, 2024–2025

Brandon Howard[1], Lia Koski[1], Christopher Bratsman[1], Megan Jehn[2], Camila Tompkins[2], R. Nicholas Staab[1], Rebecca Sunenshine[1], Jennifer Collins[1]
[1]Maricopa County Department of Public Health, Phoenix, USA. [2]Arizona State University, Tempe, USA

29. Characterization of Transcriptomic Changes Across Coccidioides Morphologies Using RiboMarker®-enhanced RNA Sequencing

Jonathan Howard[1], Aidan Manning[1], Rachel Clark[1], Tahirah Williams[2], Clarissa Nobile[2], Sergei Kazakov[1], Sergio Barberan-Soler[1]
[1]RealSeq Biosciences, Santa Cruz, USA. [2]University of California, Merced, Merced, USA

30. Differing Peripheral Leukocyte Response Between Pulmonary and Disseminated Coccidioidomycosis Identifies Key Biological Processes and Pathways

Amy Hsu[1], Nima Pouladi[2,3], Daniel Powell[2,4], Glennys Reynoso[1], Gary Ostroff[5], Lisa Shubitz[2], Steven Holland[1], Jeffrey Frelinger[2], Yves Lussier[3], John Galgiani[2]
[1]LCIM/DIR/NIAID/NIH, Bethesda, USA. [2]VFCE, University of Arizona, Tucson, USA. [3]University of Utah, Salt Lake City, USA. [4]BIO5 Institute, Tucson, USA. [5]University of Massachusetts, Chan Medical School, Worcester, USA

31. Clinical Isolates of *Coccidioides posadasii* Cause Rapid Mortality or Subclinical Disease in C57BL/6 Mice

Katrina Jackson[1], Ana Fabio-Braga[1], Daniel Kollath[1], Ashely Itogawa[1], John Galgiani[2], Jason Sahl[1], Bridget Barker[1]
[1]Northern Arizona University, Flagstaff, USA. [2]University of Arizona, Tucson, USA

32. Time Series Forecasting of Valley Fever Infection in Maricopa County, AZ, Using LSTM

Xueting Jin, Fangwu Wei, Srinivasa Kandala
Decision Theater, ASU, Tempe, USA

33. Understanding the effect of Agricultural Practices on Valley Fever through a Dual-Model Approach using Environmental Factors

Shubham Kale
Paradise Valley High School, Phoenix, USA

34. Assessing Virulence of *Coccidioides posadasii* Clinical Isolates Using the *Galleria mellonella Model*

Kiana Khalighi, Daniel Kollath, Bridget Barker
The Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, USA

35. Tracking Coccidioidomycosis in Arizona: Regional Trends and Seasonal Cycles of a Climate Sensitive Disease

Wenxin Chen[1], Sophia E. Kruger[2], Jennifer R. Head[2], Alexandra K. Heaney[1]
[1]University of California San Diego, San Diego, USA. [2]University of Michigan, Ann Arbor, USA

36. The Coccidioides Proteome Contains Undiscovered Antigenic Epitopes with Diagnostic Potential

Evan Elko[1], Luke Ryle[1], Nathan Sarbo[2], Georgia Nelson[2], George Thompson[3], Mrinalini Kala[4], Erik Settles[1], Bridget Barker[1], Kenneth Knox[4], John Altin[2], Jason Ladner[1], Heather Mead[2]
[1]Pathogen and Microbiome Institute, Flagstaff, USA. [2]TGen, Flagstaff, USA. [3]Department of Internal Medicine, Division of Infectious Diseases, and Department of Medical Microbiology and Immunology, University of California-Davis, Sacramento, USA. [4]University of Arizona College of Medicine, Phoenix, USA

37. Can Eosinophilia Aid in the Prediction of Coccidioidal Severity?

Shikha Mishra[1,2], Michelle Fang[1,2], Bianca Torres[2], Kelly Ayabe[3], Jigar Patel[1,2], Carlos D'Assumpcao[1,2], Rasha Kuran[1,2], Royce Johnson[1,2]
[1]Kern Medical, Bakersfield, USA. [2]Valley Fever Institute, Bakersfield, USA. [3]Thomas J. Long School of Pharmacy, University of Pacific, Stockton, USA

38. A Rare Case of Markedly Elevated CSF Protein in a Coccidioides Meningitis Case on Fluconazole. A Marker of Fluconazole Failure?

Jigar Patel[1,2], Jenessa Olson[1], Shikha Mishra[1,2], Michelle Fang[1,2], Bianca Torres[2], Carlos D'Assumpcao[1,2], Rasha Kuran[1,2], Royce Johnson[1,2]
[1]Kern Medical, Bakersfield, USA. [2]Valley Fever Institute, Bakersfield, USA

39. Disseminated Coccidioidomycosis with Triplet Gestation Pregnancy

Jigar Patel[1,2], Christine Peng[1], Shikha Mishra[1,2], Gabriella Busco[1], Jenessa Olson[1], Rohini Bilagi[1], Carlos D'Assumpcao[1,2]
[1]Kern Medical, Bakersfield, USA. [2]Valley Fever Institute, Bakersfield, USA

40. Ophthalmic Findings among Hospitalized Patients with Coccidioidomycosis at a Tertiary Referral Center

Karla Murillo[1,2], Adrian Au[3], Justin Hanson[4], Edmund Tsui[2], Irena Tsui[5], Colin A. McCannel[3], Gary N. Holland[2]
[1]Program in Medical Education - Los Angeles (PRIME-LA), University of California Los Angeles David Geffen School of Medicine,, Los Angeles, USA. [2]Cornea and Uveitis, University of California Los Angeles Jules Stein Eye Institute, Los Angeles, USA. [3]Retina, University of California Los Angeles Jules Stein Eye Institute, Los Angeles, USA. [4]University of California Los

Angeles David Geffen School of Medicine, Los Angeles, USA. [5]Ophthalmology, Stein and Doheny Eye Institutes, University of California, Los Angeles, USA

41. Cavitary Pneumonia: Tracing Causes in the Arid Landscapes of Southern Arizona

Rawan El Kurdi[1], Krish Nayar[2], Michael Gotway[2], Janis Blair[2], Sandhya Nagarakanti[2]
[1]University of Phoenix, Phoenix, USA. [2]Mayo Clinic Arizona, Phoenix, USA

42. Identification of *Coccidioides*-specific Human T Cell Epitopes Using an Immunoinformatic Approach

Reimi Navarro[1], Austin Negron[1], Nawal Abdul-Baki[1], Althea Campuzano[1], Nicolas Prather[1], Sofia Lozano[1], Christina Homer[2], Anita Sil[2], Jane Homan[3], Chiung-Yu Hung[1]
[1]The University of Texas at San Antonio, San Antonio, USA. [2]University of California San Francisco, San Francisco, USA. [3]ioGenetics LLC, Madison, USA

43. Identification of Coccidioidomycosis Specific and Reactive T-cell Receptors Clonotypes Expressed During Infection

Mame Diarra Bousso Ndiaye[1], Mrinalini Kala[2], Jessica Marshall[1], Allison Harmon[1], Connor Morgan[1], Paul Phillips[1], Bridget Barker[1], George R. Thompson[3], Kenneth Knox[2], Paul Keim[1], Satya Dandekar[3], Erik W. Settles[1]
[1]Northern Arizona University, Flagstaff, USA. [2]University of Arizona, Phoenix, USA. [3]University of California-Davis, Davis, USA

44. Organ Transplant Screening Identifies Nearly One-third of Reported Coccidioidomycosis Cases in Utah: Key Findings from Surveillance Data, 2009–2024

BreAnne Osborn[1], Clay Esplin[2], Travis Langston[3]
[1]Utah Department of Health and Human Services, Salt Lake City, USA. [2]Southwest Utah Public Health Department, St. George, USA. [3]Salt Lake County Health Department, Salt Lake City, USA

45. Disseminated Coccidioidomycosis in an African American Woman with Invasive Ductal Carcinoma, Dichotomy of Double Demons

Konstantino Papatheodorou[1], Arash Heidari[2,3]
[1]Touro University, California, College of Osteopathic Medicine, Vallejo, USA. [2]Internal Medicine, Bakersfield Memorial Hospital, Bakersfield, USA. [3]Internal Medicine, Morehouse School of Medicine, Atlanta, USA

46. Two Cases of Severe Pulmonary Coccidioidomycosis Concomitant with Cardiovascular Diseases, Anchoring on One, Forgetting the Other.

Konstantino Papatheodorou[1], Muhammad Ashraf-Alim[2,3], Arash Heidari[2,3]
[1]Touro University, California, College of Osteopathic Medicine, Vallejo, USA. [2]Internal Medicine, Bakersfield Memorial Hospital, Bakersfield, USA. [3]Internal Medicine, Morehouse School of Medicine, Atlanta, USA

47. Disseminated Cutaneous Coccidioidomycosis in a Renal Transplant Patient, 24 Years of Hiding

Konstantino Papatheodorou[1], Arash Heidari[2,3]
[1]Touro University, California, College of Osteopathic Medicine, Vallejo, USA. [2]Internal Medicine, Bakersfield Memorial Hospital, Bakersfield, USA. [3]Internal Medicine, Morehouse School of Medicine, Atlanta, USA

48. Coccidioidomycosis Pulmonary Nodule, A Master of Disguise

Sukhjinder Sandhu[1], Konstantino Papatheodorou[2], Arash Heidari[3,4]
[1]Bakersfield College, Bakersfield, USA. [2]Touro University, California, College of Osteopathic Medicine, Vallejo, USA. [3]Internal Medicine, Bakersfield Memorial Hospital, Bakersfield, USA. [4]Internal Medicine, Morehouse School of Medicine, Atlanta, USA

49. Steroid in a Case of Coccidioidomycosis Meningoencephalitis with Complicated Hydrocephalus, When the Left and Right Stopped Talking

Sukhjinder Sandhu[1], Konstantino Papatheodorou[2], Arash Heidari[3,4]
[1]Bakersfield College, Bakersfield, USA. [2]Touro University, California, College of Osteopathic Medicine, Vallejo, USA. [3]Internal Medicine, Bakersfield Memorial Hospital, Bakersfield, USA. [4]Internal Medicine, Morehouse School of Medicine, Atlanta, USA

50. Internet-based Decentralized Clinical Trial for Oteseconazole as a Salvage Consolidation Therapy for Those Intolerant of First-line Therapy

Matthew Pullen[1], Fariba Donovan[2], George Thompson[3], Neil Ampel[2], Nathan Bahr[1], Geetha Sivasubrahmanian[4], Peter Pappas[5], Janis Blair[6], David Boulware[1]
[1]University of Minnesota, Minneapolis, USA. [2]University of Arizona, Tuscon, USA. [3]UC Davis, Davis, USA. [4]UCSF, San Francisco, USA. [5]University of Alabama - Birmingham, Birmingham, USA. [6]Mayo Clinic, Scottsdale, USA

51. HVAC-style Air Filters Attached to Evaporative Coolers Outside San Joaquin Valley Homes Trap *Coccidioides* Spores, Minimizing Indoor Pathogen Exposure Risk

Molly Radosevich[1], Jeff Wagner[2], Gina Solomon[3], Lisa Couper[1], John Taylor[1], Justin Remais[1]
[1]University of California Berkeley, Berkeley, USA. [2]California Department of Public Health, Richmond, USA. [3]University of California San Francisco, San Francisco, USA

52. A Case of Reaction to Liposomal Amphotericin B as the last resort for treatment of spinal disseminated Coccidioidomycosis: A desperate Situation

S Sandhu[1], Nosheen Hasan[2], Arash Heidari[3]
[1]Bakersfield college, Bakersfield, CA, USA. [2]Morehouse School of Medicine, Atlanta, GA, USA. [3]Internal Medicine, Bakersfield Memorial Hospital, Bakersfield, CA, USA

53. A Unique Presentation of Primary Cutaneous Coccidioidomycosis, By Infecting Burn Wounds with Extensive Contiguous Local Spread.

Anhad Sarang[1,2], Arash Heidari[2,3]
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54. Intra-abdominal Pseudocysts Complicating Ventriculo-Peritoneal Shunts in Coccidioidal Meningitis

Geetha Sivasubramanian
University of California, San Francisco, Fresno, USA

55. Intersecting Epidemiology: A 2020 Focus on When Valley Fever Meets Tuberculosis

Cherie Stafford
Arizona Department of Health Services, Phoenix, AZ, USA

56. Genetically Diverse *Cocciodiodes posadasii* Identified at a Single Site in Arizona with Evidence of Transmission Among Non-Human Hosts and the Environment

Daniel Kollath[1], Nathan Stone[1], Marieke Ramsey[1], Jason Sahl[1], Amelia Stout[2], Ashley Itogawa[1], Amber Jones[1], Charlotte Hotchkiss[3], Deborah Fuller[3], Pierre Herckes[4], Matthew Fraser[2], David Wagner[1], Bridget Barker[1]
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57. Pediatric Disseminated Coccidioidomycosis Inpatient Cases at UCLA

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58. Withdrawn

59. Immuno-phenotyping of the T-cell Compartment in Coccidioidomycosis

Timothy Thauland, Alexis Stephens, Miguel Moreno, Maria Garcia-Lloret, Manish Butte
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60. Silent Osseous Dissemination with Cutaneous Coccidioidomycosis: What You See and What You Don't See

Shikha Mishra[1,2,3], Patrick Betadam[2,4], Robert Collins[2,4], Safa Mousavi[1], Chirag Aulakh[2], Bianca Torres[1], Rupam Sharma[2], Jigar Patel[1,2,3], Michelle Fang[1,5], Carlos D'Assumpcao[1,2,3], Royce Johnson[1,3], Rasha Kuran[1,3]
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61. Paralysis Associated with Coccidioidal Meningitis: A Review of 12 Cases

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62. Fine Mineral Dust Exposures as a Potential Mediator of the Association Between Racialized Residential Segregation and Coccidioidomycosis Incidence in California 2010 – 2017

Amanda Weaver[1], Jennifer Head[2], Simon Camponuri[1], Abinash Bhattachan[3], Ellen Eisen[1], Gail Cooksey[4], Duc Vugia[4], Seema Jain[4], Justin Remais[1], David González[1]
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63. RiboMarker®-Enhanced RNA Sequencing Reveals Small RNA Profiles and Transcriptomic Dynamics in *Coccidioides posadasii*

Jonathan Howard[1], Aidan Manning[1], Rachel Clark[1], Tahirah Williams[2,3], Clarissa Nobile[2,3], Sergei Kazakov[1], Sergio Barberan-Soler[1]
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64. Investigation Into a Sudden Increase in Valley Fever Cases in Arizona from January to March 2024

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

65. Uncovering Wild Rodent Lung Fungal Community Dynamics in a Madrean Sky Island"

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1. From Headache to Vasculopathy: Unraveling Moyamoya Syndrome in Coccidioidal Meningitis

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Abstract

Introduction: Coccidioidomycosis is an endemic fungal infection that can present with severe extrapulmonary manifestations, including central nervous system (CNS) involvement. Coccidioidal meningitis and associated complications, such as vasculitis and moyamoya-like syndrome, remain rare but life-threatening manifestations. Moyamoya vasculopathy, with an average annual incidence of 0.54 per 100,000 population in the US, is a progressive cerebrovascular disorder characterized by narrowing of the intracranial arteries, leading to the formation of abnormal collateral vessels, which increases the risk of ischemic or hemorrhagic strokes. There have not been published cases of moyamoya vasculitis secondary to coccidioidal meningitis. Herein we present a case of coccidioidal meningitis complicated by moyamoya.

Case Presentation: A 16-year-old girl, previously diagnosed with coccidioidal pneumonia, presented with progressive fever, weight loss, headaches, syncope, and behavioral changes. Eight months prior, she developed primary coccidioidal pneumonia with fever, weight loss and CF titer of 1:8 whose symptoms spontaneously resolved over weeks. In view of her new neurologic symptoms, she was hospitalized for additional evaluation. Cerebrospinal fluid (CSF) examination revealed lymphomonocytic pleocytosis, with a CSF *Coccidioides* CF titer of 1:4 (serum 1:16) and positive CSF and urine *Coccidioides* antigens. Magnetic resonance imaging (MRI) demonstrated diffuse leptomeningeal enhancement throughout the brain and brainstem, with enhancement of the basal ganglia that was difficult to discern due to dental hardware artifacts. An MRA at that time was unremarkable, with no obvious vascular abnormality detected. A second MRI during this initial admission revealed diffuse leptomeningeal enhancement throughout the brain and multifocal infarcts in the basal ganglia, again obscured by her dental hardware.

The patient was treated with fluconazole 800 mg daily and was discharged after symptomatic improvement. A repeat MRA performed after the removal of her dental hardware showed increased leptomeningeal enhancement and stable basal ganglia lesions, believed to be infarctions. She was doing well clinically, with eventual recurrence of headaches and occasional short-term memory loss one month after hospital discharge. She was hospitalized for evaluation and management of possible increased intracranial pressure and was admitted for external ventricular drainage (EVD) placement.

From Headache to Vasculopathy: Unraveling Moyamoya Syndrome in Coccidioidal Meningitis (continued)

Repeat brain MRI findings were notable for persistent leptomeningeal enhancement, chronic-appearing multifocal infarcts in the basal ganglia bilaterally, and new infarcts present in the thalamus. A repeat MRA revealed new-onset multifocal arterial stenosis, raising concerns for CNS vasculitis. The EVD revealed a normal intracranial pressure (ICP) and was removed after 24 hours, and the patient was discharged home. Over the course of five months, serial MRI/MRAs revealed the progression of severe bilateral M1 segment stenosis, partial occlusion, and the development of moyamoya-type collateralization. A cerebral angiogram confirmed moyamoya syndrome. Repeat lumbar puncture showed no pleocytosis and CSF CF titer was negative. Overall, surgical intervention was deemed unnecessary, and corticosteroids were avoided due to the unclear benefit and the lack of active vascular inflammation, as the perfusion map from her recent MRI did not show significant changes compared to previous imaging.

Fluconazole therapy was continued, and the patient demonstrated improved cognition, nearing her baseline, with no recurrent headaches and a return to her normal activity levels, and with no further complications over a 7-month follow-up period.

Results:

Table 1. Key Diagnostic and Monitoring Results:

Date	CSF WBC (cells/mm³)	CSF Monocyte Count (%)	CSF Glucose (mg/dL)	CSF Protein (mg/dL)	CSF CF Cocci Titer	Serum CF Cocci Titer	MRI/MRA Findings
12/2023	595	14	18	185	1:4	1:16	Pineal cyst, basal ganglia infarcts, nodules
2/2024	82	0	64	56		1:16	Mild ventriculomegaly, basal ganglia improvement
5/2024	4	0	50	31	neg	1:8	Moyamoya-like stenosis, improved enhancement

Conclusion: This case highlights the critical need for early detection and management of extrapulmonary coccidioidomycosis with CNS involvement, given its potential for vascular complications and serious outcomes. The patient experienced early cerebral infarcts in typical areas like the basal ganglia, along with thalamic involvement.

Moyamoya vasculitis secondary to coccidioidal meningitis has not been previously reported as a complication of this disease. She recovered to her neurological baseline with fluconazole alone. There is little data to guide the use of corticosteroids in pediatric coccidioidal meningitis complicated by vasculitis. In this patient, corticosteroids were avoided due to the chronic and stable nature of her infarcts without evidence of ongoing vascular inflammation. It is unclear if administration of corticosteroids early in her course might have prevented onset of moyamoya vasculopathy.

2. Assessing Pediatric Provider Knowledge, Attitudes, and Practices Regarding Coccidioidomycosis in California

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Abstract

Introduction: Pediatric coccidioidomycosis accounts for only 10% of symptomatic cases in California, leading to reduced clinical familiarity among pediatric providers and potential delays in diagnosis and treatment. Frontline pediatric providers play a critical role, particularly in regions with limited subspecialty services, which correspond with regions of high disease incidence. This study aims to assess the knowledge, attitudes, and practices (KAP) of pediatric providers and identify variability and gaps in provider knowledge and confidence. Findings will guide targeted educational interventions to improve early detection, management, and ultimately, patient outcomes.

Methods: This mixed-methods, cross-sectional survey study examines licensed pediatric providers in California. The survey, adapted from validated KAP tools used by the Arizona and Washington Departments of Public Health, was refined through pilot testing with local pediatricians. Participants were anonymously recruited via convenience and snowball sampling through professional associations, medical groups, and health networks. Providers were categorized by county-level coccidioidomycosis incidence into high-, medium-, and low-incidence regions. Composite scores were calculated for each participant: knowledge scores from 9 questions (reported as a percentage) and confidence scores from 6 Likert-scale questions (1 = strongly disagree, 5 = strongly agree). Data analysis was conducted in R Studio. Descriptive statistics were reported for demographics. Kruskal-Wallis and ANOVA were used to compare knowledge and confidence scores across incidence tiers, with post hoc tests applied for significant findings. IRB approval was granted by Stanford University.

Results: Sixty-four licensed pediatric providers completed the survey, with recruitment ongoing. Most (89%) specialize in pediatrics and work in outpatient settings. Over two-thirds have lived and practiced in California or Arizona for over 10 years. Among respondents, 31% practice in high, 36% in medium, and 33% in low coccidioidomycosis incidence regions. More than half reported seeing ≤5 pediatric coccidioidomycosis cases in their careers. In high-incidence regions, 40% reported seeing ≥6 cases, compared to <20% in medium and low-incidence regions.

Knowledge and confidence scores significantly differ across incidence tiers ($p=0.001$ for knowledge, $p=0.01$ for confidence). Providers in high-incidence regions scored highest in both. Post hoc Dunn's test identified significant knowledge score differences between the high and medium incidence groups (mean 77.5% vs

Assessing Pediatric Provider Knowledge, Attitudes, and Practices Regarding Coccidioidomycosis in California (continued)

57.6%, p=0.00065). The low-incidence group's knowledge score (66.4%) was not significantly different from the other groups. Providers in medium and low-incidence regions showed gaps in recognizing clinical signs, diagnostic tests, and first-line treatment. All groups struggled with identifying risk factors for disseminated disease.

Post hoc Tukey's test revealed significant confidence score differences between high vs. medium (3.69 vs. 3.01, p=0.03) and high vs. low (3.69 vs. 2.99, p=0.03) incidence groups. Providers in high-incidence regions were significantly more likely to recognize coccidioidomycosis as a problem in California and their county. While not statistically significant, those in medium and low-incidence regions had lower confidence in diagnosing (p=0.07) and treating (p=0.075) coccidioidomycosis. Few providers across all groups expressed confidence in their knowledge of diagnostic tests (means: 3.2 in high, 2.39 in medium, 2.67 in low; p=0.072).

Conclusion: Despite greater exposure in endemic regions, most participants had limited direct experience with pediatric coccidioidomycosis. Providers in high-incidence regions demonstrated significantly higher knowledge and confidence, particularly compared to those in medium-incidence areas, which include emerging endemic counties. Gaps persist in recognizing clinical signs, selecting diagnostic tests, and identifying first-line treatments. With limited medical and public health resources, targeted regional training and education are essential to ensure early diagnosis and effective management of pediatric coccidioidomycosis, especially as climate change drives shifts in disease distribution and incidence.

Table 1. Demographics

		Cocci Incidence in County of Practice*			
	Overall (N=64)	High (N=20)	Medium (N=23)	Low (N=21)	p-value**
Participant Age					0.06
31-40	18 (28.1%)	3 (15.0%)	8 (34.8%)	7 (33.3%)	
41-50	20 (31.3%)	12 (60.0%)	4 (17.4%)	4 (19.0%)	
51-60	18 (28.1%)	2 (10.0%)	8 (34.8%)	8 (38.1%)	
61-70	5 (7.8%)	2 (10.0%)	2 (8.7%)	1 (4.8%)	
Greater than 70	3 (4.7%)	1 (5.0%)	1 (4.3%)	1 (4.8%)	
Number of years living in CA or AZ					0.02

Assessing Pediatric Provider Knowledge, Attitudes, and Practices Regarding Coccidioidomycosis in California (continued)

1 or less	5 (7.8%)	2 (10.0%)	1 (4.3%)	2 (9.5%)	
2-5	4 (6.3%)	3 (15.0%)	1 (4.3%)	0 (0%)	
6-10	8 (12.5%)	6 (30.0%)	2 (8.7%)	0 (0%)	
11-19	6 (9.4%)	2 (10.0%)	2 (8.7%)	2 (9.5%)	
20 or more	41 (64.1%)	7 (35.0%)	17 (73.9%)	17 (81.0%)	
Number of years practicing in CA or AZ					0.1
1 or less	5 (7.8%)	2 (10.0%)	1 (4.3%)	2 (9.5%)	
2-5	7 (10.9%)	4 (20.0%)	3 (13.0%)	0 (0%)	
6-10	14 (21.9%)	6 (30.0%)	5 (21.7%)	3 (14.3%)	
11-19	13 (20.3%)	5 (25.0%)	5 (21.7%)	3 (14.3%)	
20 or more	25 (39.1%)	3 (15.0%)	9 (39.1%)	13 (61.9%)	
Received any education/training in CA or AZ					0.0004
No training	25 (39.1%)	15 (75.0%)	6 (26.1%)	4 (19.0%)	
Some training***	39 (60.9%)	5 (25.0%)	17 (73.9%)	17 (81.0%)	
Degree					0.2
MD	56 (87.5%)	18 (90.0%)	18 (78.3%)	20 (95.2%)	
DO	5 (7.8%)	2 (10.0%)	3 (13.0%)	0 (0%)	
NP	2 (3.1%)	0 (0%)	2 (8.7%)	0 (0%)	
PA	1 (1.6%)	0 (0%)	0 (0%)	1 (4.8%)	
Specialty					0.1
Pediatrics	57 (89.1%)	18 (90.0%)	21 (91.3%)	18 (85.7%)	
Combined Med/Peds	3 (4.7%)	2 (10.0%)	1 (4.3%)	0 (0%)	
Family Medicine	1 (1.6%)	0 (0%)	1 (4.3%)	0 (0%)	
Other****	3 (4.7%)	0 (0%)	0 (0%)	3 (14.3%)	
Received education on coccidioidomycosis in the last 3 years					0.6
No	59 (92.2%)	19 (95.0%)	20 (87.0%)	20 (95.2%)	
Yes	5 (7.8%)	1 (5.0%)	3 (13.0%)	1 (4.8%)	
Number of pediatric coccidioidomycosis cases seen in last 12 months					0.1
None	45 (70.3%)	10 (50.0%)	19 (82.6%)	16 (76.2%)	
1-5	17 (26.6%)	8 (40.0%)	4 (17.4%)	5 (23.8%)	

Assessing Pediatric Provider Knowledge, Attitudes, and Practices Regarding Coccidioidomycosis in California (continued)

6-10	1 (1.6%)	1 (5.0%)	0 (0%)	0 (0%)	
11-20	1 (1.6%)	1 (5.0%)	0 (0%)	0 (0%)	
More than 20	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Number of total pediatric coccidioidomycosis cases seen during practice					0.3
None	17 (26.6%)	3 (15.0%)	10 (43.5%)	4 (19.0%)	
1-5	31 (48.4%)	9 (45.0%)	9 (39.1%)	13 (61.9%)	
6-10	6 (9.4%)	3 (15.0%)	2 (8.7%)	1 (4.8%)	
11-20	6 (9.4%)	3 (15.0%)	2 (8.7%)	1 (4.8%)	
More than 20	4 (6.3%)	2 (10.0%)	0 (0%)	2 (9.5%)	
Average Knowledge Scores					0.001
Mean (SD)	66.7 (17.5)	77.5 (15.7)	57.6 (16.5)	66.4 (14.7)	
Median [Min, Max]	67.0 [33.0, 100]	78.0 [56.0, 100]	56.0 [33.0, 100]	67.0 [33.0, 100]	
Average Confidence Scores					0.01
Mean (SD)	3.22 (0.890)	3.69 (0.832)	3.01 (0.782)	2.99 (0.917)	
Median [Min, Max]	3.17 [1.00, 5.00]	3.92 [1.50, 4.83]	3.00 [1.67, 4.67]	3.00 [1.00, 5.00]	

*High incidence includes counties reporting >=30 cases per 100,000 in 2023, Medium 10 to <30 cases per 100,000 in 2023, and Low <10 cases per 100,000 in 2023. Incidence rates based on publicly available reports from California Department of Public Health.

**To compare distribution of demographic factors between groups, we used Fisher's Exact Test. Kruskal Wallis Rank Sum test was used to compare average knowledge scores between groups given data was not normally distributed (based on Shapiro-Wilk test for normality). ANOVA was used to compare average confidence scores between groups given data was normally distributed (based on Shapiro-Wilk test for normality). P-values <0.05 considered statistically significant.

***Including medical, PA, or nursing school, residency, and/or fellowship

****Pediatric subspecialists including Pediatric Neurology and Pediatric Rheumatology

3. Treatment Outcomes for Chronic Coccidioidomycosis Pulmonary Colonization in a Patient with Cystic Fibrosis

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Abstract

Introduction: Primary pulmonary coccidioidomycosis is typically an asymptomatic and self-limiting infection, with treatment generally reserved for patients at risk for severe or disseminated disease. Individuals with cystic fibrosis (CF) have a perceived risk of disseminated disease due to factors related to altered immunity and structural lung abnormalities; however, no established guidelines exist on managing asymptomatic colonization or infection in this patient population. Recent evidence suggests a potentially lower prevalence and severity of coccidioidomycosis infection in CF patients. This further complicates treatment decisions particularly for patients where colonization is persistent and not clearly linked to worsening pulmonary function. Here, we present the treatment challenges and outcomes of a young CF patient with chronic Coccidioides colonization who ultimately received treatment before undergoing solid organ transplantation.

Case presentation: A 12-year-old female with cystic fibrosis complicated by liver cirrhosis, portal hypertension, esophageal varices, hypersplenism, pancreatic insufficiency, and CF-related diabetes mellitus presented for evaluation of a positive sputum culture for *Coccidioides* spp. and *Mycobacterium avium* complex (MAC) during a hospital admission for esophageal variceal bleeding. *Coccidioides* was first isolated from a bronchioalveolar lavage (BAL) sample obtained a year prior and continued to grow on multiple subsequent sputum samples. Her serology was positive for *Coccidioides* IgG (titer 1:16) but negative for IgM by enzyme immunoassay. Treatment was initially deferred given her stable pulmonary status with predicted FEV1 at baseline in the mid-90s% and concerns over hepatotoxicity of azole therapy in the setting of her known liver disease.

Treatment was reconsidered during this admission due to declining pulmonary function, with her most recent FEV1 at 88% predicted, and in anticipation of imminent liver transplantation due to her cirrhosis. Repeat BAL sampling showed persistent growth of *Coccidioides* spp. with a complement fixation titer of <1:2. She was started on fluconazole therapy 400mg PO daily to reduce colonization burden before transplantation. Treatment for MAC was deferred at that time due to hepatotoxic potential, especially when combined with fluconazole.

Treatment Outcomes for Chronic Coccidioidomycosis Pulmonary Colonization in a Patient with Cystic Fibrosis (continued)

The patient continued to experience a steady decline in her pulmonary function (FEV1 ~65% predicted) despite being on therapy for approximately 18 months, but this was thought to be likely related to frequent CF exacerbations and colonization with other resistant organisms including ciprofloxacin-resistant *Pseudomonas aeruginosa* and with *Stenotrophomonas maltophilia*. Complement fixation titers during this period increased to 1:8 and repeat BAL sampling showed regrowth of *Coccidioides* spp. and MAC. Consequently, treatment for MAC was initiated with azithromycin, rifabutin, and ethambutol. Her liver function was closely monitored with weekly testing initially and remained relatively stable.

The patient had her first negative sputum culture for *Coccidioides* and MAC one month later, with reduced cocci CF titers of 1:2. BAL sampling after 1 year of combined therapy was clear of *Coccidioides* and MAC but demonstrated growth of *Aspergillus fumigatus*. MAC therapy was discontinued after 12 months of treatment, and she was continued on fluconazole. The patient showed no further growth of cocci on repeat samplings with serology that was negative by ID and variably positive by EIA with stable CF titers at 1:2. She was eventually transitioned to a prophylactic fluconazole 100mg PO daily after 2.5 years of treatment. She tolerated therapy well with no significant side effects or hepatotoxicity.

The patient underwent a living-related donor liver transplantation from her cousin in 2018. She remained on fluconazole prophylaxis in the peri and post-transplant period with no reported regrowth of cocci on subsequent sputum cultures. She was maintained on tacrolimus for long-term immunosuppression and level was monitored closely with no major alterations in serum level reported with concurrent fluconazole.

Conclusion: This case highlights the complexities of managing chronic cocci colonization in CF patients especially ones with complicated disease and multiple comorbidities. While primary pulmonary coccidioidomycosis is often self-limited, CF patients may be at higher risk for persistent colonization due to impaired mucociliary clearance, altered immunity, and frequent co-infections. Given the prolonged treatment course and potential associated risks, treatment should be considered early for patients with impending transplantation to optimize their post-transplant course.

4. Understanding Soil Properties Conducive for *Coccidioides* ssp. Presence in the United States

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Abstract

Introduction: The specific soil characteristics that support the growth of *Coccidioides* are not well understood, including whether these characteristics differ between the two species, *C. immitis* and *C. posadasii*. Recent advances in the detection of *Coccidioides* from soil samples over the last decade have provided new opportunities to investigate this question.

Methods: We compiled *Coccidioides* occurrence data from prior research campaigns and the National Center for Biotechnology Information (NCBI) database to explore the soil properties associated with the presence of these pathogens. We examined 13 soil properties from the California Soil Resource Lab at the University of California Davis, using data derived from USDA-NCSS. This included numerical variables such as pH, water holding capacity, and calcium absorption as well as categorical variables, such as hydrologic group and soil texture. Lastly, we analyzed the defined ecoregion of the occurrence data from the US Environmental Protection Agency. We conducted non-parametric statistical tests (i.e., Chi-squared test, Mann-Whitney U-test) to examine whether there were differences in the soil properties between *C. immitis* and *C. posadasii*.

Results: We compared 35 samples of *C. immitis* to 25 samples of *C. posadasii* collected from California, Washington, and New Mexico. We found that *C. immitis* was present in soils with higher levels of calcium carbonate than *C. posadasii* ($p<0.001$). Also, *C. immitis* was present in soils that were higher in clay content and silt content than *C. posadasii* ($p = 0.061$; $p < 0.001$). When specifically testing soil types, *C. immitis* was mostly found in clay and silty loamy soils, while *C. posadasii* in loamy sand and sandy environments ($x^2=18.95$; $p = 0.004$). *C. immitis* was associated with both higher water holding capacities ($p < 0.001$) and higher soil organic content ($p = 0.006$) than *C. posadasii*. *C. immitis* was more associated with the Mediterranean California ecoregion (e.g. wetter winters, drier summers) and *C. posadasii* was more associated with North American deserts (e.g., very arid areas; $x^2=19.69$; $p<0.001$).

Understanding Soil Properties Conducive for *Coccidioides* ssp. Presence in the United States (continued)

Conclusion: These findings suggest that C. *immitis* may prefer more productive, wetter soils than C. *posadasii*. We analyzed soil conditions at the ecosystem level, so there may be differences between our results and microecosystem soil conditions researchers have reported concurrently with their *in situ* soil samples. By identifying the soil properties favorable for each species, we can better predict areas at risk for the pathogen presence, contributing to the development of more precise risk maps and targeted public health strategies for coccidioidomycosis prevention.

5. Description of *Coccidioides Posadasii* in Soil Samples From Southern New Mexico

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Abstract

Introduction: *Coccidioides posadasii*, the soil-dwelling fungus responsible for coccidioidomycosis, is traditionally considered endemic to arid regions of the southwestern United States. While historically associated with California and Arizona, the disease is gaining attention in states such as Utah, Nevada, New Mexico, and Texas. Despite this, limited soil sampling has been conducted in southern New Mexico. This project aims to definitively establish the presence of *Coccidioides* in the region's soil and explore ecological factors influencing its growth. Confirming its presence could have significant implications for a rapidly growing population, emphasizing the need for increased awareness among healthcare providers and public health officials.

Methods: Recruitment involved flyers posted at a local infectious disease clinic inviting individuals diagnosed with Valley Fever to participate. Additionally, residential and commercial properties with reported wood rat middens but no known history of *Coccidioides* exposure were included. Targeted soil sampling was conducted at suspected exposure sites, including the backyard of a local resident whose dog was diagnosed with coccidioidomycosis, suggesting potential environmental exposure.

At each site, 20–30 soil samples (1 mL each) were collected from various locations at a depth of 5–10 cm. Samples were transferred to disruptor tubes and transported to the Burrell College of Osteopathic Medicine BioScience Laboratory (BSL-2) for processing. DNA extraction and purification followed the Omega BioTek E.Z.N.A. Soil DNA Kit protocol, yielding approximately 50 µL of eluted DNA, which was stored at -20°C.

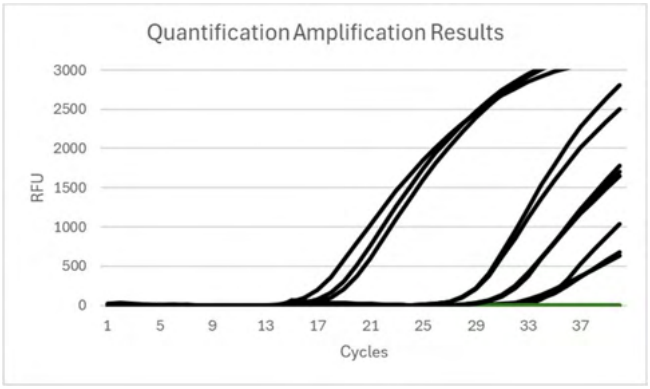
Quantitative PCR (qPCR) analysis was performed on the CFX Opus 96 using the SsoAdvanced Universal Probes Supermix and the CocciDx assay to detect *Coccidioides* DNA. Positive and negative controls were included, with positive controls consisting of soil spiked with *Coccidioides* DNA. A Ct value < 35 was considered positive. Data was collected, stored, and analyzed to assess the presence of *Coccidioides* in the sampled locations.

This study was approved by the Burrell College of Osteopathic Medicine Institutional Review Board for conducting electronic surveys, storing participant data, and publishing results. Proper safety trainings were completed, and all laboratory procedures adhered to established biosafety protocols. Personal protective equipment was used at all times, including gloves, lab coats, N95 masks, and eye protection. Standard

Description of *Coccidioides Posadasii* in Soil Samples from Southern New Mexico (continued)

molecular biology techniques were followed for DNA extraction and qPCR analysis, and all biohazardous waste was disposed of according to institutional and federal regulations.

Results: Over 300 soil samples were collected from various locations in Doña Ana County. The table above highlights positive samples identified at a residential property where canine exposure to coccidioidomycosis was suspected. Of the 26 samples collected from this site, three tested positive and showed consistent amplification in triplicate. Subsequent resampling of the area confirmed additional positive results, reinforcing the presence of *Coccidioides* DNA in the local environment.



Conclusions: Despite a lower-than-expected rate of positive samples, this study successfully detected *Coccidioides* DNA at a suspected exposure site, demonstrating its environmental persistence in southern New Mexico. These findings provide direct evidence of the fungus in the region, supporting the need for increased awareness among healthcare providers and the public. Further research and targeted surveillance are warranted to better understand the distribution of *Coccidioides* and its implications for human and animal health.

6. Temperature and Density Affect Conidiation, Conidia Viability, and Thermotolerance in *Coccidioides* Species

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Abstract

Introduction: The understanding of the fundamental biology underlying Coccidioides arthroconidia development and their differentiation to spherules remain limited, as there are many unexplored environmental and biological factors influencing these processes. Temperature is the only known signal that controls the morphological transitions from arthroconidia to spherule or from arthroconidia to hyphae transition. In addition, previous studies on *Aspergillus* species suggested that temperature can be an important signal in determining the responses of conidia to stressors such as heat and oxidative stress. Here, we further investigated the roles of temperature, seeding density, and the duration of hyphal growth in the formation of arthroconidia. Lastly, we examined whether these conditions affect the thermotolerance of arthroconidia.

Methods: Mycelial cultures were seeded in a series of 10-fold dilutions from previously harvested *Coccidioides posadasii* C735 arthroconidia in T225 tissue culture flasks with 2X glucose yeast extract agar (GYE) + penicillin/streptomycin (P/S). Cultures were incubated at either room temperature (RT) or 30℃ and harvested every two weeks for up to 10 weeks. We assessed the effects of these growth conditions on arthroconidia development by acquiring arthroconidia yield using two methods: (a) total arthroconidia yield was obtained by staining arthroconidia with lactophenol blue and counted using a hemocytometer. (b) Viable arthroconidia yield was obtained by performing serial dilutions and plating on 2X GYE + P/S agar plates. Colonies were counted 4-5 days after incubation at 30℃. Percent viability was determined by the viable arthroconidia of colonies grown in plates out of the total arthroconidia from hemocytometer counts.

The heat stress assay to examine the thermotolerance of the arthroconidia was performed by diluting the arthroconidia stocks to 1000 CFU/ml, then subjecting them to 42℃ for 30 or 60 minutes. After incubation, 100 µl of the heat-treated or untreated (control) arthroconidia were plated on 2X GYE + P/S agar plates. Colonies were counted 4-5 days after incubation at 30℃. Percent survival was defined as the number of viable heat-treated arthroconidia over the untreated arthroconidia.

Results: We demonstrate that arthroconidia harvested from mycelia cultures grown at RT produce more total arthroconidia. In addition, arthroconidia from RT-grown mycelia are more viable than those harvested from 30℃-grown mycelia. Interestingly, while arthroconidia from 30℃-grown mycelia have low viability in

Temperature and Density Affect Conidiation, Conidia Viability, and Thermotolerance in *Coccidioides* Species (continued)

general, they show increased thermotolerance compared to the arthroconidia from RT-grown mycelia when exposed to 42℃ for 30 or 60 minutes. This phenotype is further pronounced in arthroconidia harvested after 8- or 10-week-old mycelia.

Conclusion: Coccidioides species are fungal pathogens that are exposed to varying environmental conditions to propagate in the environment and survive in the mammalian hosts. However, little is known about the environmental factors controlling their persistence, virulence, and morphological transitions from the environmental arthroconidia to the parasitic host form. Our study investigates the factors required for making arthroconidia with high viability rates and thermotolerance. The results obtained from this study can help shed light on the temperature- and density-dependent phenotypes of Coccidioides species.

7. Scaling Up Production of High-quality Coccidiodes Genomes Via High Throughput Culture and DNA Extraction of Coccidiodes Patient Isolates from Arizona

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Abstract

Introduction: *Coccidioides* are highly infectious pathogenic soil dwelling fungi that cause Valley Fever. Understanding the population structure of pathogenic strains has been limited by the fact that only a few hundred high-quality genomes are publicly available. High-throughput generation of new *Coccidioides* genomes is hampered by the challenges around growing *Coccidioides* isolates and extracting their DNA. The high infectivity hazard of *Coccidioides* requires that their manipulation be done in high containment facilities (BSL3), which are limited throughout the world. *Coccidioides* species possesses a tough exterior cell membrane that results in inefficient DNA capture. Other factors, including the slow growth rate of isolate cultures, their vulnerability to contamination by environmental fungi, and the labor-intensive efforts to harvest sufficient biomass, further compounds the delay in large-scale production of new genomes. To meet the demands to rapidly expand our understanding of *Coccidioides*, we devised a methodological strategy that enabled the generation of high-quality genomes on a large scale.

Methods: Human *Coccidioides* clinical isolates collected in 2022-2023 were provided to the University of Arizona through partnership with Sonora Quest laboratories. Glycerol stocks were transported to Northern Arizona University for culture and DNA extraction conducted in a BSL3 facility. To obtain sufficient DNA amounts using a commercial kit, we cultured large quantities of *Coccidioides* biomass for DNA extraction. To achieve this, isolates were cultured on agar media in T75 vented flasks, a fully enclosed container to protect cultures from environmental fungus contamination. To reduce labor requirements of biomass harvest, isolate cultures were aged to the arthroconidia stage (spore lifecycle), which took between 6-8 weeks. After biomass harvest, DNA was efficiently extracted using a commercially available kit (Promega Wizard) after undergoing an enzymatic digestion step. The DNAs then underwent processing for library generation for whole-genome sequencing. Genomes were sequenced with Illumina NextSeq instruments.

Results: Our methodological strategies reduced both the time and costs traditionally required to obtain *Coccidioides* DNA, enabling us to generate genomes from >500 clinical isolates within a ten-month time window. Improvements included identifying a strategy that allowed for successful use of a commercially

Scaling Up Production of High-quality Coccidiodes Genomes Via High Throughput Culture and DNA Extraction of Coccidiodes Patient Isolates from Arizona (continued)

available DNA extraction kit instead of the Phenol-Chloroform method, as the latter is highly labor intensive and not scalable. Because we grew large quantities of biomass, the commercial kit captured enough high-quality DNA for successful whole genome sequencing and allowed for higher throughput sample extractions (i.e., multiple samples at once). We found that removal of biomass from agar media surface (harvesting) was less labor intensive when *Coccidioides* was in an arthroconidia (spore forming) state compared to mycelia (vegetative state). The use of T75 vented flasks, a fully enclosed culture container, allowed ample growth of *Coccidioides* biomass while successfully prevented contamination of our pure culture from environmental fungi. These flasks also increased the biosafety of sample handling compared to traditional petri dishes by securely containing any *Coccidioides* culture overgrowth and preventing escape through aerosolization. Used collectively, these methods allowed the DNA extraction process prior to generation of new *Coccidioides* genomes to be less time intensive and more scalable while still performed under increased biosafety conditions.

Conclusion: The need to scale up the production of high-quality genomes has become more pressing with the increase in human cases of Valley Fever combined with rapid real estate development in *Coccidioides* endemic regions. Methodologies described here makes this possible and will be instrumental to expanding new knowledge of this pathogen that can be determined from genome sequences, including antifungal resistance mechanisms, genetic basis of virulence, and other relevant genotypes.

8. The Correlation of Social Determinants of Health in Outcomes of Coccidioidomycosis: Preliminary Results

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Abstract - Declined to Publish

9. Epidemiological Profile of Coccidioidomycosis in Mexico and Baja California: Analysis of Hospital Discharges (2004-2023)

Nydia Castillo-Martinez, Ofelia Candolfi-Arballo, Jose Chavez-Mendez, Juliana Baez-Elizalde, Daniel Ramirez-Diaz
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Abstract

Introduction: Coccidioidomycosis, a systemic fungal infection caused by *Coccidioides* spp., is endemic to arid regions of North and South America. Despite its clinical relevance, the disease is not reportable in Mexico, limiting epidemiological surveillance. Baja California, bordering highly endemic U.S. regions, presents a significant burden of disease, yet data on its impact remain scarce. This study aims to analyze hospital discharge records from 2004 to 2023 to better understand the epidemiology of coccidioidomycosis in Mexico, focusing on Baja California.

Methods: A retrospective study was conducted using national hospital discharge data to identify cases of coccidioidomycosis (ICD code B38). The analysis employed descriptive statistics to examine demographic characteristics, length of hospitalization, and seasonal trends, utilizing STATA 13 for data analysis. ***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.

Results: A total of 2,425 hospital discharges were recorded in Mexico from 2004 to 2023, with 338 cases (13.94%) in Baja California. The mean age of hospitalized patients was 35.5 years (SD = 21.0), with 60.65% of cases in males. The median hospital stay was 7 days in Baja California, with severe cases requiring prolonged hospitalization. A seasonal trend was observed, with peak hospitalizations from June to September likely linked to environmental conditions favoring fungal dispersion. The most common clinical presentation was pulmonary coccidioidomycosis (44.08%), followed by meningitis (10.65%) and disseminated disease (7.1%).

Epidemiological Profile of Coccidioidomycosis in Mexico and Baja California: Analysis of Hospital Discharges (2004-2023) (continued)

Conclusion: Coccidioidomycosis poses a significant health burden in northern Mexico, particularly in Sonora, Nuevo Leon, and Baja California. The findings highlight the need for enhanced surveillance, early diagnosis, and targeted prevention strategies. Mandatory disease reporting, increased clinician awareness, and improved diagnostic capacity are essential to mitigate the impact of this disease in endemic regions.

10. Frequency, Interval, and Patient Factors Associated with Recurrence of Cutaneous Coccidioidomycosis

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Abstract

Introduction: Coccidioidomycosis (cocci) is a fungal infection caused by *Coccidioides* and a common cause of community acquired pneumonia in endemic regions. Disseminated cocci occurs in ≤ 1% of persons with cocci, and among these, disseminated cutaneous coccidioidomycosis (DCC) is estimated to occur in 15 to 67% of cases, however little data exists to describe its frequency and timing of recurrence. Limited small studies show recurrence rates from 17 to 50% and often do not distinguish skin dissemination from other soft tissues. Our aim was to determine the frequency, timeframe and risk factors for relapsed DCC.

Methods: Potential cases of disseminated cocci were identified by ICD-10 codes 38.3, 38.7, 38.9 (& ICD-9 equivalents) and keyword-based search of pathology reports from 1/1/2008 - 3/31/2024. We included patients with proven or probable DCC. We excluded patients with primary cutaneous cocci or reactive rash. We performed a retrospective review to identify episodes of recurrence, comorbidities, and antifungal regimen.

Results: We identified 44 proven and 3 probable cases of DCC (33 males, 14 females), median age 48 years, 66.0% white, 21.3% black, 5.4% Hispanic. 18 (38.3%) were immunosuppressed and 6 (13.6%) had diabetes mellitus. Median follow-up duration was 68 months; 12 (25.5%) patients had ≥1 recurrence, 7 (14.9%) of whom relapsed ≥2 times. Median interval off of antifungals until recurrence was 14 months (range: 1 - 96). 3 (6.4%) patients had recurrence within three months of diagnosis, and 6 (13.0%) and 11 (23.9%) had a recurrence by the 1st and 5th follow-up year, respectively. 21 (87.5%) recurrences were at the same site as a prior occurrence, 4 (18.2%) occurred on antifungal therapy. There were no significant differences in recurrence rates by race or diabetes. Of the 18 immunosuppressed cases, there was 1 (5.6%) recurrence, though 14 (77.8%) continued antifungal therapy throughout follow-up.

Conclusion: Once antifungal treatment was discontinued, recurrence of disseminated cutaneous cocci was common, and among those whose DCC relapsed, multiple relapses were noted. Longer follow up is needed to better characterize the risk of long-term recurrence.

11. Soil Nitrogen Dynamics at a Long-Term Coccidioides-Positive Site

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Abstract

Ammonium- and nitrate-nitrogen represent inorganic nitrogen pools microbially available within the soil ecosystem. Correlating these factors with *Coccidioides* presence are the first steps into elucidating key soil ecological parameters necessary for *Coccidioides* growth in the soil. Differences in ammonium- and nitrate-nitrogen levels within active and non-active burrows and elevated ammonium- and nitrate-nitrogen levels in burrows compared to surface soil samples indicate a strong relationship with animal activity. These findings suggest that animal activity potentially plays a significant role in the availability of soil nutrients, soil microbiome, and soil C/N cycling within the ecosystems. Increased levels of soil inorganic nitrogen could be impacted by concentrated urea levels in urine commonly found within xeric mammalian species, such as Kangaroo Rats. Subsequent amminization and ammonification of urea to nitrate could provide *Coccidioides* with a nitrogen-rich soil environment and increase its presence within semi-arid animal burrow entrances, a hypothesis we aim to test next in this study.

12. Coccidioidomycosis Awareness, Diagnosis, and Symptom Severity: Enhanced Surveillance of Patients with Laboratory-Confirmed Coccidioidomycosis — Maricopa, Pima, and Pinal County Health Departments, Arizona, January 16–August 17, 2024

Jennifer Collins[1], Brandon Howard[1], Ellen Santos[2], Anissa Taylor[2], Kaeli Lugo[3], Zia Helgeson-Budrys[3], Megan Jehn[4], Camila Tompkins[4], Olivia Omura[5], Thomas Moore[5], Christopher Bratsman[1], Rebecca Sunenshine[1], R. Nicholas Staab[1], Shane Brady[6], Irene Ruberto[6], Thomas Williamson[6], Ariella Dale[1]
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Abstract

Introduction: In Arizona, patients with laboratory-confirmed coccidioidomycosis are rarely interviewed during routine surveillance. Therefore, information on patient demographic characteristics, length of illness, treatment, and health outcomes are limited. Arizona Department of Health Services collaborated with Maricopa, Pima, and Pinal County health departments to conduct a multicounty enhanced surveillance effort to characterize coccidioidomycosis epidemiology in Arizona and guide future public health interventions.

Methods: Eligible participants had a laboratory-confirmed coccidioidomycosis case reported to the statewide infectious disease surveillance system, were a resident of a participating county, were not residing on tribal lands, and aged ≥18 years. During January 16, 2024–January 31, 2025, 10–20% of eligible participants with a laboratory-confirmed incident case were randomly selected and contacted for survey participation within 1–2 months following the first report to public health. Surveys, available in English and Spanish, were administered by phone and included questions on demographics, awareness of coccidioidomycosis, diagnosis, testing, and disease severity. Participants who reported symptoms were asked about symptom onset date and whether symptoms resolved. A diagnosis interval was calculated based on symptom onset and diagnosis dates; Mann-Whitney U tests were used to compare this interval, stratified by symptom resolution status.

Results: During January 16–August 17, 2024, of 701 eligible participants contacted, 201 (28.7%) completed the survey; median age was 63 years (range: 19–91 years), 112 (55.7%) identified as female, 128 (63.7%) as White or Caucasian, and 52 (25.9%) as Hispanic, Latino/a/x, or Spanish. There were 149 (74.1%) participants who heard of coccidioidomycosis before their illness, most commonly from family and friends (63/149 [43.0%]) or veterinarians (23/149 [15.4%]). Despite positive laboratory test results, 60 (29.9%) participants reported not being aware of their test results. Among 147 (73.1%) symptomatic participants, 76 (51.7%) were treated for coccidioidomycosis and 36 (24.5%) recovered from their symptoms by the time of

Coccidioidomycosis Awareness, Diagnosis, and Symptom Severity: Enhanced Surveillance of Patients with Laboratory-Confirmed Coccidioidomycosis — Maricopa, Pima, and Pinal County Health Departments, Arizona, January 16–August 17, 2024 (continued)

interview. Among treated participants with symptom onset dates (58/201 [28.9%]), the median diagnosis interval was 26 days (range: 3–485 days); there was no difference in median diagnosis interval (*P* value = 0.32) between participants who recovered from their symptoms (18/58 [31.0%]) and those who did not (40/58 [69.0%]) at the time of interview.

Conclusions: Almost one third of participants lacked awareness of their positive coccidioidomycosis laboratory test result. Further study is necessary to identify whether this lack of awareness is due to a gap in patient understanding, follow-up, or provider practices.

13. Functional Annotation and Computational Analysis of the Coccidioides Proteome in Virulence and Host Immunity

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Abstract - Declined to Publish

14. Epidemiology and Clinical Course of Pediatric Coccidiomycosis in a Tertiary Care Children's Hospital in Los Angeles

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Abstract

Introduction: With rising rates of Coccidioides infections in California, we sought to evaluate our institutional experience in caring for children with coccidiomycosis to better understand epidemiology, presentations, and clinical courses of this infection. In addition, some variation in clinical practice exists in terms of treatment choice, duration of treatment, and interim evaluation, which we hope to better characterize in order to identify strategies for improving the care of pediatric patients.

Methods: Approval for this study was obtained from the Children's Hospital Los Angeles institutional review board. We performed a retrospective chart review of pediatric patients ≤18 years old from April 2023 to October 2024 who were seen by the pediatric infectious diseases service at CHLA in both inpatient and outpatient consults with a diagnosis of coccidiomycosis. Patients were identified by ICD-10 code search for "coccidiomycosis".

Results: Fifty patients were identified, 47 (94%) of these with pulmonary coccidiomycosis, and 3 patients (6%) with extrapulmonary/disseminated disease. Average age of patients was 12.5 years (range 5 to 18 years) with 25 (50%) male and 25 (50%) female patients. Fifteen (30%) patients required hospitalization at some point in their course and of these hospitalizations, 7 (47%) were primarily for diagnostic purposes, 3 (20%) were primarily for IV antifungal treatment, and 5 (33%) were primarily for respiratory support, such as supplemental oxygen or chest tube management.

Thirty-nine of these patients were treated with antifungals. The majority, 34 patients (87 %) were treated initially with fluconazole. Two of these patients were changed to posaconazole due to increase in complement fixation titers while on fluconazole therapy. All 3 patients with disseminated disease were treated with Ambisome initially. Initial complement fixation titers ranged from <1:2 to 1:512 overall with a range of 1:128 to 1:256 in the patients with disseminated disease. Of the 36 patients who had more than one repeat complement fixation titer sent, 18 (50%) patients demonstrated decreasing titers, 8 (22%) without change, 5 (14%) with increasing titers and 5 (14%) with fluctuating titers during course of ID follow-up to date.

Epidemiology and Clinical Course of Pediatric Coccidiomycosis in a Tertiary Care Children's Hospital in Los Angeles (continued)

Conclusion: This study provides important insights into pediatric coccidiomycosis at our institution, underscoring the complexity of managing this infection in children. We noted a high proportion of pulmonary coccidioidomycosis, significant hospitalization rate, and variable complement fixation titer responses, highlighting the importance of deepening our understanding of disease progression in pediatric patients. By examining the epidemiological patterns and clinical challenges, our findings emphasize the importance of developing more standardized management approaches to improve clinical outcomes for pediatric coccidiomycosis. We hope to expand our study to include more of our pediatric patients with coccidiomycosis in the future.

Age	Average 12.5 years (range 5-18)	
Sex	25 male	25 female
Past Medical History	43 previously healthy	7 with prior medical history
Referred from Outside Hospital	42 patients	
Coccidiomycosis diagnosis prior to ID visit/consult	43 patients	
Extent of Infection (number of patients)		
Pulmonary Coccidiomycosis	47	
Disseminated Disease	3	
		1: Lymph node, mediastinal mass, lung
		1: Lymph node, lung
		1: Soft tissue mass, osteomyelitis, lung
Hospitalization Required (number of patients)	15	
Reason for hospitalization		
Primarily IV antifungal treatment	3	
Primarily respiratory support (oxygen, chest tube, etc)	5	
Primarily diagnostic	7	
Length of Hospitalization	13 days (range 1-58)	
Initial Complement Fixation Titer	**Pulmonary**	**Disseminated**
< 1:2-1:8	29	0
1:16-1:64	14	0
1:128- 1:512	1	3
Initial titer unavailable	3	0
Initial Antifungal Treatment (number of patients)	39 on antifungal therapy	11 never received antifungal therapy
Fluconazole	34	
Posacoanzole	2	
Isavucoanzole	1	
Ambisome	4	
Dual (Ambisome +Azole)	2	

15. A Machine Learning Model to Forecast Case Counts in Arizona Using Historical Case Count, Genetic, and Environmental Data

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Abstract

Introduction: Accurate diagnosis of coccidioidomycosis depends on regional public health knowledge and practices. We aim to improve capacity in these areas by leveraging machine learning to forecast Arizona case counts one to two months in advance using historical case count, environmental, genomic, and socioeconomic data. This information can increase clinical preparedness by acting as an early warning for a potential rise in cases while also providing insights into the influence various factors have on case counts for future research.

Methods: We collected case count data, environmental time series data (surface temperature, surface runoff, snowmelt runoff, subsurface runoff, rainfall rate, total rainfall, snow depth, soil moisture, soil temperature, air temperature), soil characteristics data (pH and concentrations of CaCO, sodium, clay, sand, silt), *Coccidioides* SNPs data, and socioeconomic data for the years 2000 to 2015. Using case count data as our predictor, we created a model using gradient boosting to predict case counts one to two months in advance for Arizona.

Results: In this project, we train our model on Arizona data from 2000 to 2010 and test it on data from 2011 to 2015. We expect that lagged temperatures contribute to higher case counts and case counts from previous years are indicative of future case counts. Using these statistical relationships, we are able to build a model to predict future cases of coccidioidomycosis case burden in Arizona, while providing a better understanding of the magnitude of the environmental, genetic, and socioeconomic factors.

Conclusion: This work aims to increase clinical awareness and preparedness for coccidioidomycosis by forecasting case counts one to two months in advance.

16. Seasonal Dynamics of Airborne Microbial Communities in San Joaquin Valley and its Implications for Climate-Driven Disease Transmission and Respiratory Health

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Abstract - Declined to Publish

17. A Review of Hospitalizations for Coccidioidal Meningitis from 2020-2024

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[1]Valley Fever Institute, Bakersfield, USA. [2]Western University, Pomona, USA

Abstract

Introduction: Coccidioidal meningitis (CM) is a form of disseminated coccidioidomycosis that is associated with significant morbidity and mortality. Based on anecdotal observations of increased numbers and complexity of hospitalizations for CM by infectious diseases clinicians at Kern Medical (KM), a community teaching hospital in Bakersfield, CA, we sought to characterize recent hospitalizations at KM for CM.

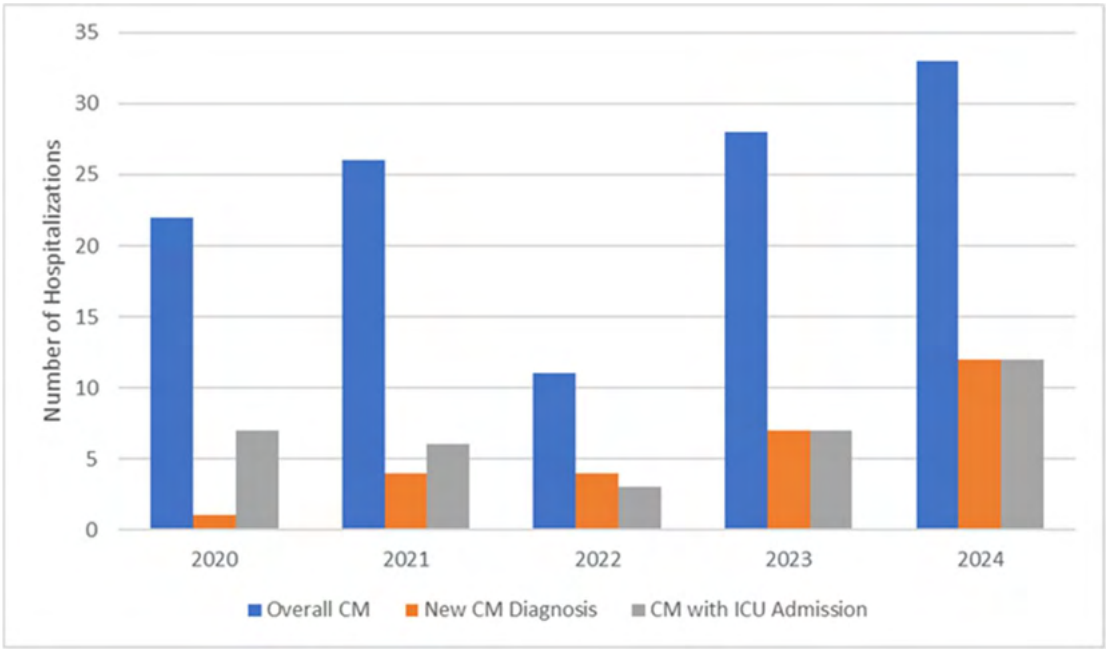
Methods: This retrospective review was approved by the KM Institutional Review Board and included hospitalizations for CM at KM from January 1, 2020 to December 31, 2024. Hospital admissions with associated International Classification of Diseases, tenth revision (ICD-10) codes related to coccidioidomycosis and CM (B38.0-B38.9) were extracted from the electronic medical record. These encounters were manually reviewed to exclude admissions unrelated to management of CM and to gather additional demographics and outcomes data. Characteristics of interest for both the overall population and the subgroup of hospitalizations for a new diagnosis of CM included length of stay (LOS), intensive care unit (ICU) admission, disposition, and trends over time.

Results: Across the five-year time frame at KM, 1702 hospital admissions were identified with coccidioidomycosis-associated ICD-10 codes, with 203 admissions (99 unique patients) specifically associated with the ICD-10 code for CM (B38.4). Of these, 120 admissions (73 unique patients) were determined to be primarily for management of CM, with 28 admissions for new CM diagnoses. Mean hospitalizations per patient during the specified time frame was 1.6 (range 1-6 admissions). Mean age at time of admission was 48 years, with male patients accounting for 101 hospitalizations (84%). Mean LOS was 10 days (range 1-80 days). Admission to the ICU was required during 35 encounters (29%), including 14 endotracheal intubations (12%). Discharge disposition included home (63%), rehabilitation/nursing care facility (23%), death/hospice (10%), and other (4%). Of the hospitalizations for a new diagnosis of CM, mean LOS was 15 days (range 2-66 days), 12 (43%) required ICU admission, and 7 (25%) required intubation. Discharge disposition of these patients included home (50%), rehabilitation/nursing care facility (25%), death/hospice (21%), and other (4%). Trends in admissions for CM over time are described in Figure 1.

Conclusion: CM is a devastating manifestation of coccidioidomycosis, and this review highlights the burden of CM on clinical outcomes and healthcare resource utilization in an endemic setting. The apparent increase

in hospitalizations for both new diagnoses and exacerbations of CM over a 5-year period is alarming and calls for further investigation to validate this trend and identify potential contributing factors.

Figure 1. CM Hospitalizations at KM from 2020-2024



18. Development of an Experimental Infection Model for Valley Fever in Pigtail Macaques

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Abstract

Introduction: Animal models of Valley Fever (VF) 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. 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. To develop an experimental challenge PTM model of VF infection that closely mirrors human disease, we compared two methods that achieved inhaled exposure to *Coccidioides* and their impact on clinical disease and the induction of immune responses following challenge.

Methods: Eight male PTMs naïve to *Coccidioides spp.* were challenged with a dose of 500 arthroconidia *C. posadasii* Silveira. Four (n=4) animals were challenged intratracheally via droplet and the remaining n=4 were challenged intratracheally via fine atomized spray. Blood and bronchoalveolar lavage (BAL) were collected at several timepoints post infection to analyze immune responses and fungal burden. Clinical signs of disease were measured through 37 days post-infection.

Results: Upon necropsy at 37 days post-infection, 7/8 (87.5%) of animals PTM displayed granulomatous/pyogranulomatous pneumonia and lymphadenitis upon necropsy, of which 3/8 (%37.5) displayed *severe* pyogranulomatous pneumonia, indicating a productive infection with *Coccidioides*. Analyses of fungal burden and humoral and cellular immune responses post challenge are in progress and will be reported.

Conclusions: Our results show that pigtail macaques challenged with *Coccidioides* show pathology that aligns similarly to that of humans that develop Valley Fever. Limitations of this work include the lack of female PTM within this cohort and the short duration of this study (37 days) that may not capture later disease manifestations. Despite these limits, these findings show that infection of PTMs leads to measurable clinical disease and supports further development of this model as a valuable preclinical tool to investigate new vaccines and therapies for the prevention and treatment of Valley Fever.

19. Managing Coccidioidomycosis in Patients with Autoimmune Disease on Biological Response Modifiers

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Abstract

Introduction: Coccidioidomycosis (CM) is an endemic fungal infection that poses significant risks to immunocompromised individuals, particularly autoimmune patients undergoing treatment with biologic response modifiers (BRMs). While BRMs effectively manage autoimmune diseases, their immune suppression increases susceptibility to CM and can potentially lead to disseminated CM (DCM). In this study, we developed two clinical algorithms to guide CM management in BRM-treated patients.

Methods: Our research team reviewed literature to address knowledge gaps in managing CM patients with autoimmune diseases requiring BRMs. We developed two algorithms: one for patients not yet on BRMs and another for those already undergoing BRM therapy. We focused on BRM use in CM patients, and the risks of CM reactivation or progression with BRM therapy.

Results: Management recommendations for each CM clinical presentation are based on the IDSA guidelines. Treatment strategies vary based on CM disease status, symptoms, and the risk of disease progression. Patients residing in endemic areas should undergo coccidiodal screening before initiating BRM. The first algorithm is for autoimmune patients not yet on a BRM. Antifungal treatment is not recommended for asymptomatic patients with positive serology; however, delaying BRM for at least six months is advised. Pulmonary CM may require 3–6 months of antifungal therapy with a similar BRM delay. Chronic cavitary CM should be managed based on symptoms. Asymptomatic patients should be followed, and symptomatic cases may require antifungal treatment. DCM requires long-term antifungal therapy with a delay in BRM treatment. The second algorithm is for autoimmune patients currently on BRM, if asymptomatic with positive serology, they may continue BRMs without antifungal treatment but require routine follow-ups and education on the potential risk of CM reactivation/dissemination. Pulmonary CM patients may require antifungal therapy, with BRMs paused and resumed for at least three months if symptoms are resolved. Asymptomatic cavitary CM patients can continue with BRM, while symptomatic cases require antifungal treatment and may continue BRM therapy. Disseminated CM requires indefinite antifungal therapy and cessation of BRMs for at least six months. Patient education and periodic monitoring are essential.

Managing Coccidioidomycosis in Patients with Autoimmune Disease on Biological Response Modifiers (continued)

Conclusion: In this study, we developed algorithms to guide healthcare providers in treating autoimmune conditions while considering CM-related risks. The proposed management strategies emphasize the importance of early serological screening, judicious use of antifungal treatments, and close follow-up for patients on or off BRM therapy. These algorithms aim to optimize patient outcomes by reducing the risk of CM progression and ensuring the appropriate BRM and antifungal use.

20. Evaluation of an Anti-CTS1 Antibody Lateral Flow Assay to Monitor Antibody Response in *Δcps1*-Vaccinated Dogs Challenged with *Coccidioides*

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Abstract

Introduction: Natural infection with *Coccidioides* spp. results in a strong antibody response against a conserved immunodominant glycoprotein called chitinase 1 (CTS1, also termed CF), which has been used clinically for decades to aid in diagnosis and monitoring of coccidioidomycosis (Valley Fever, VF). Using this knowledge, our group produced an antibody lateral flow assay (LFA) that detects anti-CTS1 antibodies in any species rapidly (10 mins), providing a substantial improvement in versatility, simplicity, and speed over current antibody detection methods such as complement fixation and immunodiffusion (ID) testing. Here, we used sera from dogs who received an experimental live avirulent VF vaccine (Δ*cps1*) and later infected with virulent spores to investigate anti-CTS1 antibody response to vaccination and subsequent challenge with *Coccidioides*.

Methods: We hypothesized that little to no anti-CTS1 antibodies would be produced by the host against the live avirulent *Δcps1* vaccine. To investigate this, archived sera that were collected every 2 weeks from dogs during the vaccination period (weeks 0-6) and every two weeks from immediately prior to infection with a virulent spore challenge until study end (weeks 8-16) (Shubitz et al. *Vaccine* 2021) were tested by the CTS1 LFA. Groups included prime/boost at 3 different potencies (n=6 per group), a prime only group (n=6), and an unvaccinated control group (n=6). Terminal ID antibody titers were compared with the semi-quantitative output of the LFA.

Results: In general, the LFA detected either no or minimal and transient anti-CTS1 antibodies during the vaccination phase, regardless of the potency of the *Δcps1* vaccine or whether the dogs received one (prime) or two (prime/boost) doses. All unvaccinated control dogs mounted an antibody response detectable by the CTS1 LFA within 4 weeks of intratracheal challenge with *Coccidioides* (ID titers ranging from negative to 1:32). When data from the dogs in the 3 prime/boost groups that showed protection are combined (n=18), antibody responses after challenge were similar; only 7 of 18 dogs developed an antibody response (ID titers ranging from negative to 1:8). For dogs that were given only a prime vaccine, in which protection was

Evaluation of an Anti-CTS1 Antibody Lateral Flow Assay to Monitor Antibody Response in *Δcps1*-Vaccinated Dogs Challenged with *Coccidioides* (continued)

not significantly better than controls, 4 of 6 dogs mounted an antibody response, and the titers overlapped considerably with controls (range 1:4-1:64).

Conclusion: Similar to EIA and ID test results reported in the initial study, the LFA detects little to no anti-CTS1 antibody response in dogs during vaccination with the *Δcps1* live avirulent vaccine. Considering that antibody detection is a mainstay diagnostic aid for VF, it is important that antibody detection techniques are still effective and dependable in a VF-vaccine setting. Thus, since the LFA can still detect natural disease antibody response for VF-vaccinated animals who may not have developed full protection, the rapid LFA may be useful during VF-vaccine development studies in animal models to assess protective efficacy of a vaccine prior to terminal evaluations, which may take several weeks to months.

21. Considering Coccidioides in the Environment: Where, How, and Why?

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Abstract

Abstract: The endozoan small animal hypothesis is the current paradigm for the maintenance of Coccidioides in the environment. While the paradigm explains may aspects of persistence, transmission, and human exposure, there are also gaps in the mechanisms that are unsatisfactory. For instance, if dead rodents are a nidus for fungal outgrowth and arthroconidia formation, are there enough carcasses that persist without being scavenged by other animals and birds of prey? Intriguingly, there are aspects of the fungal genome and hints from older literature that may support alternative or additional environmental reservoirs, including in birds, lizards, and insects. The genome of Coccidioides suggests it is most prepared for animal hosts, and keratin-rich niches in particular. Early in vitro culture work also suggests there are key factors for spherule formation that may include surfactants and certain fatty acids like oleic and linoleic acid, present in many insects. There are other yeasts (Cryptococcus) and dimorphic fungi (Histoplasma) that are closely associated with birds. The keratin of birds or their distant relatives, lizards, may provide dermal niches or gastrointestinal/cloacal niches for Coccidioides. In fact, an egg yolk sacs culture model was shown to produce pure spherule cultures. Here, a collection of attributes and previous findings will be considered to stimulate further investigations into the environmental reservoir for Coccidioides and in vitro culture conditions, which could provide additional data to reduce human exposure and to improve in vitro spherule culture models to support drug discovery and anti-fungal susceptibility testing.

22. Evaluation of The Cactus Bio Immunodiffusion Reagents, And The Non-Utility Of IgM Testing By Immunodiffusion

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Abstract

Introduction: Immunodiffusion (ID) is a moderately sensitive and highly specific method for detection of antibodies against Coccidioides. In recent decades, it is often used only after a plate-based EIA assay to confirm presence of antibodies, especially in populations where coccidioidomycosis is not highly endemic. The value of IgM by ID is not fully characterized in the current setting of widely available Enzyme immunoassay (EIA) testing to detect early disease, and availability of CF and ID to confirm presence of antibodies.

Methods: EIA (Meridian Premier, Meridian Diagnostics, Cincinnati, OH) is performed at MCA, and complement fixation (CF) and ID were sent to a reference laboratory (Mayo Clinic Laboratories, Rochester, MN). All specimens from September 2021 (n=1058) from the Mayo Clinic Arizona (MCA) serology laboratory were reviewed in detail to understand the impact of ID IgM on a clinical diagnosis. Separately, samples from MCA during fall of 2023 and 2024 (n=1541) that were sent for CF/ID were also tested in-house using ID reagents from Cactus Bio (Phoenix, AZ).

Results: The 1058 specimens from September 2021 contained only 30 positive results by ID IgM. Most of those were also positive by ID IgG (17) or were false-positive (10) based on chart review. Of the three remaining, one patient later tested positive by ID IgG, one had previous positive by CF testing, and one had been treated based on the ID IgM and was never positive again by serology. Given the lack of utility for IgM, we then undertook an evaluation of a newly available set of reagents from Cactus Bio for ID IgG testing. Using 1541 serum samples, the Cactus Bio reagents were found to be 96.9% sensitive and 85.9% specific on direct comparison to ID performed at the reference laboratory. A chart review determined that some of the apparent Cactus Bio false positive results represented true positives, and the final determination was that the results using the Cactus Bio ID IgG test reagents were 98% sensitive and 94.6% specific.

Conclusions: Performing IgM by ID adds little value when EIA and CF are also being performed on a specimen. The Cactus Bio ID IgG reagents produced results equivalent to reference laboratory testing.

23. The Coccidioides Complex Longitudinal Abstraction Tool (CLAT) for Integration and Harmonization of Complex Multimodal Data

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Abstract

Introduction: Coccidioidomycosis is a complex fungal disease that may require prolonged treatment. Assessment of clinical, laboratory, and radiological features are used to manage disease because a reliable and responsive prognostic indicator is lacking. Abstraction of longitudinal, comprehensive, multimodal data is required to investigate the correlation of clinical features and treatment decisions over time to identify prognostic indictors.

Methods: To accomplish this task efficiently and accurately, a Complex Longitudinal Abstraction Tool (CLAT) was constructed using the REDCap platform. The approach to data collection was intended to enable harmonization across healthcare centers and facilitate increased understanding of coccidioidomycosis or other chronic diseases. Data fields were coded to SNOMED code and built in a way to minimize manual entry.

Results: Three REDCap instruments were created to capture 1) demographics and initial visit to Infectious Disease, 2) subsequent visits, and 3) laboratory/radiological data. Each instrument was intended to balance efficient and comprehensive documentation of patient history and data. For the 41 patients which were abstracted using the REDCap instruments, the number of antibody titers and associated office visits varied from 5 – 17 (median = 8.2). On average, abstraction of a patient's initial and subsequent visits and all the associated laboratory data took 3-4 hours.

Conclusion: The CLAT is a tool that can be used to abstract longitudinal multimodal data from patients with complex disease. The CLAT could be easily adapted to facilitate the study of other chronic diseases with multimodal data features.

24. To Biofilm or Not to Biofilm: Investigation of *Coccidioides* ability to Produce Biofilms and the Clinical Implications

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Abstract

Introduction: Coccidioidomycosis or Valley Fever is caused by the dimorphic fungus *Coccidioides* which is found in the soil in the Southwestern region of the United States. Both humans and animals are at risk of this illness, which occurs if they inhale dust containing fungal infectious arthroconidia from the environment. This disease primarily affects the lungs and causes valley fever. Recently, possible biofilms have been observed in clinical cases. Biofilm production has been associated with antifungal resistance making this something of potential concern.

Methods: *C. albicans* colonies were inoculated in YPD liquid media and adjusted to 92%T which measures the amount of light that passes through a material and is reported as a percent, instead of counting cells leading to overnight incubation. *Coccidioides* spp. were inoculated into 1xGYE broth leading to a sixteen hour incubation period. Biofilm production was tested in both the arthroconidia and germling life stages using an XTT assay. A hemocytometer was used to check germlings and arthroconidia under the microscope after adjusting the cell suspension to 92%T . Suspension of cells were measured within 10^6 cells. After two days of incubation, the biofilms were detected by measuring their metabolic activity with tetrazolium (XTT) salt. A plate reader was used to measure the biofilm production after 48 hour incubation.

Results: The *Coccidioides* spp. tested were shown to produce biofilms in the germling life stage but not the arthroconidia life stage. The presence of biofilm in the CSF or on invasive devices may also represent a potential source of relapsing infection.

Conclusions: Through this study, we are able to show that *Coccidioides* spp. are capable of producing biofilms using *C. albicans* as a comparative control. This could have clinical implications and might indicate drug resistance.

25. Evaluating Community-acquired Pneumonia Management in the Endemic Region for Coccidioidomycosis: A Continuous Quality Improvement Model

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Abstract

Introduction: Coccidioidomycosis (CM) is an infection caused by the fungal pathogens *Coccidioides*, which are endemic in the Southwestern United States. CM is known to cause a presentation similar to bacterial community-acquired pneumonia (CAP) and so presents unique challenges to antimicrobial stewardship programs in the coccidioidal endemic region. The objective of this project was to establish a continuous quality improvement model to collect information regarding CAP management practices at a major medical center in Arizona.

Methods: A daily reporting system was established to identify all adult patients admitted to Banner University Medical Center-Tucson that were coded for pneumonia with ICD-10 code J18.9 from December 1, 2024 – January 24, 2025. Patient demographic information as well as the severity of CAP cases (i.e., intensive care unit (ICU) status) were collected. CAP management was further assessed by reviewing CM serologic testing and antimicrobial prescription patterns.

Results: 78 patients were included for analysis. 40 (51.3%) patients were female, and the mean age (SD) was 62 (24) years. 23 (29.5%) patients were admitted to the ICU. 52.6% (41/78) patients had CM serologic testing performed with 65.2% (15/23) patients having CM testing performed in the ICU. Of the 41 patients that had CM testing performed, 6 (14.6%) had positive results. Additionally, only 51.3% (40/78) patients received CAP guideline recommended antibiotic therapy.

Conclusion: The study demonstrated that only 52.6% patients presenting with CAP at a major medical center in Arizona receive CM serologic testing even though approximately a quarter of CAP cases in Arizona are due to CM based on published data. It was notable in our cohort that nearly 15% of the patients that were evaluated for CM had positive results. The results also demonstrated that in addition to increased focus on diagnostic testing for CM in the inpatient setting, antimicrobial stewardship efforts for CAP are urgently needed.

26. Coccidioides (Spherulin) Skin Test Performance and Patterns of Prior Immunity by Age Among Men Entering State Prisons in California, 2015-2024

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Abstract

Introduction: In 2015, in an effort to reduce high incidence rates of coccidioidomycosis within facilities, the California Department of Corrections and Rehabilitation began testing incarcerated residents for history of prior infection. The purpose of the testing was to identify those susceptible to coccidioidomycosis so that they would not be housed in the prisons with the highest risk of transmission—Pleasant Valley and Avenal State Prisons, both of which house only men. Spherulin-based skin tests were offered to male residents statewide during a single screening in 2015, and thereafter were offered to most men 18-64 years old upon entry into the prison system. Understanding skin test performance and immunity within this population can inform baseline risk of infection among individuals entering state prisons, which is needed to evaluate the effectiveness of the skin test placement program.

Methods: We analyzed skin test and medical record data from 154,450 individuals incarcerated within California's prisons between January 11, 2015, and September 15, 2024. We computed test sensitivity as the probability of a positive test among those who had a coccidioidomycosis diagnosis prior to test receipt. For each county or region of residence at time of admittance into prison, we plotted the probability of having a positive test against age, and we fitted models to the resulting curve. We estimated the county-level population-level immunity by age and the force of infection (FOI), defined as the rate at which susceptible individuals become infected.

Results: 8401 (5.4%) individuals tested positive, suggesting prior history of infection. Test positivity was highest among American Indian/Native Hawaiian (5.9%, 95% CI: 4.9 – 7.1), non-Hispanic White (5.8%, 95% CI: 5.5 – 6.0), and Hispanic (5.7%, 95% CI: 5.5 – 5.9) individuals, and lowest among non-Hispanic Black (4.7%, 95% CI: 4.5 – 5.0) and Asian (3.0%, 95% CI: 3.4 – 3.7%) individuals. Among those tested, 688 had a confirmed coccidioidomycosis diagnosis prior to the test, of whom 336 tested positive, corresponding to a sensitivity of 48.8% (95% CI: 45.1 – 52.6%). Sensitivity did not vary significantly by time since diagnosis, county of prior residence, race/ethnicity, or age. Sensitivity was higher during the initial screening in 2015 (60%) than in 2016-2024 (40%). In immunity-vs-age analyses, we observed inverted U-shaped curves that peaked typically between ages 45 and 55 years of age, a pattern consistent with either strong cohort effects or waning in the ability of the skin test to detect prior infection. Models allowing age-varying FOIs and waning of skin test detection by time since infection best fit the observed data. We estimated that the prevalence of immunity among men entering state prisons from Kern County ranged from 34% at 20 years of age to 82% by 65 years. Less endemic regions in California also demonstrated meaningful prevalences of

Coccidioides (Spherulin) Skin Test Performance and Patterns of Prior Immunity by Age Among Men Entering State Prisons in California, 2015-2024 (continued)

immunity. We estimated that the prevalence of immunity among men entering state prisons from San Francisco Bay Area County ranged from 3% at 20 years of age to 11% by 65 years.

Conclusion: While the skin test may not be a reliable indicator of immunity at the individual level due to potential waning of detection, this study has yielded important insights into the probability that an individual entering the carceral system is susceptible to infection.

27. Development of a Coccidioides Cytokine Release Assay

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Abstract

Introduction: Coccidioidomycosis (CM) is an endemic fungal infection common in the Southwestern U.S. causing a range of illnesses from asymptomatic to life threatening disseminated disease. A growing challenge in endemic regions involves an increasing population undergoing organ transplantations and receiving immunosuppressive therapies, putting them at increased risk for severe CM. The current standard of care is to screen individuals before transplants for the presence of anti-*Coccidioides* antibodies; however, the sensitivity of these methods is variable and not indicative of a protective cellular immune response. Here we describe the development of a *Coccidioides* cytokine release assay (CRA) to measure cytokine release after stimulation of whole blood with coccidioidal antigens measuring the cellular immune response. This CRA aims to provide a better tool for risk stratification in this population for prophylaxis and/or monitoring. It could additionally provide a tool in monitoring protective cellular immune responses in *Coccidioides* vaccine developments.

Methods: From March 2024 to December 2024 CM+ donors were recruited from Banner University Medical Centers in both Phoenix and Tucson based on chart review. Whole blood (20mLs) was collected in Na heparin tubes from 48 donors with prior resolved CM (≥ 3 months after active infection). Subjects were excluded if immunosuppressing therapies or conditions were present. All donors were ≥ 18 years of age (University of Arizona IRB: 2105781507). Whole blood from donors outside of the *Coccidioides* endemic area was purchased from StemCell (n=69). All specimens were coded at the participation site or purchased deidentified. Blood was stimulated at the draw site with a positive mitogenic control (PHA-L) and a negative control (media only) in addition to proprietary coccidioidal antigens. Blood was shipped to MiraVista Diagnostics (MVD) and stimulated with antigens at 37°C with 5% CO_2 before plasma was separated by centrifugation. Cytokine concentrations were determined by ELISA and results analyzed using MedCalc and/or Analyze-It for Microsoft Excel version 2.30 (Leeds, UK) software. Blood specimens (CM+ or CM-) had to demonstrate reactivity with PHA-L to be considered a viable sample for evaluation. A 95% confidence was used for statistical significance for evaluations.

Development of a Coccidioides Cytokine Release Assay (continued)

Results: Coccidioidal antigens demonstrated a significant ability to stimulate IFNγ production from whole blood (CM+) compared to the nil control. Given potential loss in viability during transit cytokine production was evaluated before and after shipping. Blood stimulated with coccidioidal antigens before shipping were observed to have significantly higher IFNγ concentrations compared to blood stimulated after shipping, median IFNγ concentrations of 349.7 pg/mL before shipping compared to 14.4 pg/mL after shipping ($p<0.0001$). Transit times varied from about 12 hours to 96 hours, with longer transit times associated with lower IFNγ concentrations. A time course study indicated that after about 30 hours of transit time IFNγ production begins to drop significantly. The performance of the CRA was evaluated by stimulating CM+ and CM- blood with various antigen(s), the best combination resulted in a sensitivity of 92.0% (95% CI: 74.0-99.0) and specificity of 93.0% (95% CI: 76.5-99.1) with an AUC of 0.961. The removal of donors with suspected prior histoplasmosis, based on reactivity with *Histoplasma* antigens, resulted in sensitivity remaining at 92.0% and specificity improving to 100.0%. The variability of IFNγ concentration produced from whole blood was also evaluated by stimulating donor blood with antigens at two separate time points. IFNγ concentrations were found to be consistent between stimulations with R^2 values ranging from 0.82 to 0.92, depending on the antigen.

Conclusions: The CM CRA demonstrated the ability to detect a T-cells response to CM infection and differentiate it from those without prior CM infection. Some cross-reactivity was observed in donors reacting to *Histoplasma* antigens. This data demonstrates feasibility and potential utility of identifying past *Coccidioides* exposure.

28. Evaluating the diagnosis and reporting of coccidioidomycosis cases in an endemic region—Maricopa County, 2024–2025

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Abstract

Introduction: Coccidioidomycosis is an endemic, yet underdiagnosed, disease in southern and central Arizona. Suspected and confirmed coccidioidomycosis cases associated with Arizona residents, including all positives labs, are required to be reported to public health by laboratories and providers. Coccidioidomycosis cases are classified solely based on laboratory results according to the CDC and Arizona case definitions for high-incidence jurisdictions. Cases are “confirmed” if there is a positive anti-coccidioidal serology (IgG or IgM reactivity), including enzyme immunoassay (EIA), immunodiffusion (IMDF), and complement fixation (CF), or another positive CLIA-approved test for coccidioidomycosis.

Coccidioidomycosis cases are not routinely investigated; however, beginning in January 2024, Maricopa County Department of Public Health (MCDPH) began interviewing case-patients as part of a multi-county enhanced surveillance effort. Using data from this enhanced surveillance effort, we aimed to characterize serological results in the Arizona communicable disease database by whether the case-patient self-reported being diagnosed with coccidioidomycosis by their provider.

Methods: During the enhanced surveillance project, case-patients were randomly sampled as 10-20% of eligible laboratory-confirmed cases reported to MCDPH during January 16, 2024 – January 31, 2025, and interviewed during February 19, 2024 – March 3, 2025 (future). Eligibility criteria included: being a confirmed case, being a Maricopa County resident, not residing on tribal land, and being age ≥18 years at the date of testing. Cases were assigned for investigation 15-31 days following the first report to public health and were prioritized for investigation within 14 days of assignment. Data were included in this analysis if case-patients answered the question on whether they were diagnosed by their provider (“yes”, “no”, “unknown/unsure”) and had a record of a positive serological test result with differentiated immunoglobulin type (i.e., IgM, IgG). Case-patients who responded “no” or “unknown/unsure” to being aware of a coccidioidomycosis diagnosis were grouped together as not being aware of a diagnosis.

Cases were categorized into three groups using all reported serological results (IgG positive only, IgM positive only, or both IgG and IgM positive), agnostic of test type, to evaluate potential differences in patient-reported awareness of a coccidioidomycosis diagnosis by serological immunoglobulin result. Sensitivity analyses were performed to evaluate the impact of including case-patients with only EIA results

Evaluating the diagnosis and reporting of coccidioidomycosis cases in an endemic region—Maricopa County, 2024–2025 (continued)

and restricting to case-patients with reflex or confirmatory serological testing results (IMDF, CF, etc.), regardless of other testing. Groups were compared using a chi-square test or Fisher's exact test depending on counts.

Results: Of 339 confirmed cases interviewed, 328 (96.8%) had differentiated IgG and IgM anti-coccoidal serological results and were included in this analysis. Of the 328 case-patients included in this analysis, 77 (23.5%) had IgM only positive results, 127 (37.6%) had IgG only positive results, and 124 (36.7%) had positive results for both tests. One hundred and four (31.7%) case-patients self-reported not being diagnosed by their provider, of which 60 (47.2%) had only an IgG positive lab result, 25 (32.4%) had only an IgM positive result, and 19 (15.3%) had both an IgG and IgM positive lab result, $x^2(2, N = 328) = 28.5$, $p < 0.0001$. Those with both positive IgM and IgG results were more likely to have been made aware of their diagnosis by a provider than those with either only IgM or IgG positive results. Sensitivity analyses maintained the finding of a higher proportion (53.9%) of those with IgG-only positive EIAs (n = 178) self-reported not being diagnosed, $x^2(2, N = 178) = 7.4$, $p = 0.025$. Among case-patients with records of reflex or confirmatory testing (n = 146), IgG-only positive case-patients made up a higher proportion (31.6%) of those self-reporting not being diagnosed (Fisher's exact test; p-value = .001).

Table 1: Patient Awareness of Diagnosis by Serological Result—Maricopa County, 2024–2025

	IgG + Only n (col %)	IgM + Only n (col %)	IgM & IgG + n (col %)	*Total*	X^2 p-value
Not Aware of Diagnosis	60 (47.2%)	25 (32.5%)	19 (15.3%)	*104*	<.001
Aware of Diagnosis	67 (52.8%)	52 (67.5%)	105 (84.7%)	*224*	
Total	*127*	*77*	*124*	*328*	

Note: Includes all individuals with at least one differentiated serological result (i.e. IgG vs. IgM), regardless of additional testing.

Conclusions: Although public health surveillance data cannot capture the individualized medical decision-making by a healthcare provider, MCDPH surveillance data show that over 30% of confirmed surveillance cases interviewed did not report being diagnosed by their healthcare provider. Case-patients with both IgM and IgG positive results were most likely to self-report having been diagnosed, followed by those with IgM positive only results, and lastly those with IgG positive only results. These findings suggest a need for additional education to providers regarding testing for coccidioidomycosis, including when to pursue additional testing and how to improve patient compliance with confirmatory or reflex testing collection.

29. Characterization of Transcriptomic Changes Across Coccidioides Morphologies Using RiboMarker®-enhanced RNA Sequencing

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Abstract

Introduction: As of 2023, the World Health Organization's (WHO) inaugural list of "Fungal Priority Pathogens" included Coccidioides as a microbe of growing concern, requiring increased attention concerning basic research and the development of clinical tools (both diagnostic and pharmacological). In order to elucidate genetic drivers of life cycle progression, researchers have interrogated the modulation of transcriptional programs that support the saprobic-to-parasitic phase transition of *Coccidioides* using next-generation sequencing (NGS) approaches. While these data have been informative to our work herein, they overlook or select against sequencing of the small noncoding (sRNAs) and fragmented pools of RNA.

Methods: To better characterize the small RNA profile of *Coccidioides,* we performed small RNA sequencing using an enhanced RealSeq-RiboMarker® protocol to generate the most complete "small RNA atlas" of **C. posadasii.** We profiled across a 96-hr growth time course of cultures for the saprobic mycelia and arthroconidia as well as from parasitic spherules. Additionally, small RNAs were purified and profiled from mycelia-, arthroconidia-, and spherule-conditioned media to ascertain small RNAs that might be found in the extracellular space of each of these stages.

Results: 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), in-line with previous mRNA sequencing results across the same morphologies.

Additionally, we characterize a number of unannotated RNA loci, (validated with additional total RNA sequencing), which appear to also show morphological-specific expression. While some of these loci appear to be unannotated, but canonical, non-coding RNAs, the majority are inconsistent with characterized RNA sub-types.

Moreover, characterization of the small RNA transcriptome using the RiboMarker® platform revealed distinct profiles of RNA fragmentation in both small RNAs (e.g. tRNAs, snRNAs) but also protein-coding genes. Curiously, these fragmentation profiles modulate between saprobic and parasitic stages, and further analysis of these fragmentation profiles suggest morphologically-unique fragmentation patterns of a sub-

Characterization of Transcriptomic Changes Across Coccidioides Morphologies Using RiboMarker®-enhanced RNA Sequencing (continued)

set of RNAs. These patterns may be indicative of stage-specific RNA secondary structure or RNA-protein interactions (among others), resulting in unique fragmentation of target RNAs.

Finally, we noted evidence of RNA export to the extracellular space, particularly regarding snRNA and tRNA-derived fragments, as well as mRNA-derived transcripts, during the transition from arthroconidia to either mycelia or spherules, which may play roles in cell-cell, and/or host-pathogen communication.

Conclusion: 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 novel RNA genes that require identification and characterization, but also putative RNA biomarkers for Coccidioidomycosis diagnostics and a stepping-stone to combat the increasing disease incidence and expanding geographic range of Valley Fever. Also, the use of fragmentation pattern analysis of pathogen-derived RNAs may become a key tool in both biological identification/characterization of unknown pathogens, but also use in characterization of infections from biofluids for diagnostic purposes. 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.

30. Differing Peripheral Leukocyte Response Between Pulmonary and Disseminated Coccidioidomycosis Identifies Key Biological Processes and Pathways

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Abstract

Introduction: Coccidioidomycosis, infection by the dimorphic fungus, *Coccidioides spp*, is a geographically isolated disease, with highest infection rates occurring in the desert Southwest United States. Extrapulmonary dissemination (DCM) to bone, brain, skin, or other soft tissues, is a severe complication, occurring in <1% of infected individuals. Despite ~600-1000 DCM cases/year, only 15 DCM patients have been reported with Mendelian mutations. More recently, common (<10%) population variants impacting fungal recognition (DECTIN-1, PLCγ2) and response (DUOX1/DUOXA1) have been shown to be overrepresented in DCM versus isolated pulmonary (PUL) patients or healthy controls. We hypothesize that a patient's immunologic response to infection drives differences in disease severity.

Methods: Peripheral blood mononuclear cells from DCM (n=6) or PUL (n=11) patients were stimulated (β-glucan-chitin particles [GCP]) or not (NS) followed by bulk RNASeq. For each subject, we compared GCP/NS to identify differentially responsive transcripts (DRT) which were then prioritized by adjusted p-value. We then collapsed the DRT into curated biological process pathways to identified differentially responsive pathways. This N-of-1 single subject analysis was repeated with a validation cohort (DCM n=3, PUL n=4). Ingenuity pathway analysis was used to identify upstream regulators of DRT. RNA-Scope in mouse lungs 5 days post *C. posadasii* infection was used to assess *Il1b* transcript levels.

Results: Analysis of the discovery cohort discovered DRT between the two groups; inclusion of the validation cohort resulted in 55 DRT which retained both significance (FDR<0.05) and directionality. Unsupervised clustering revealed a pattern in which DCM patients exhibit a dampened response, either failing to respond or responding to a lesser degree (up or down) compared to PUL. Multiple differentially responsive pathways were identified including IL1β production, negative regulation of NFκB activity, cell chemotaxis, and response to lipopolysaccharide. Further analysis implicates STAT3 and IL1β as key drivers of the DRT observed. Consistent with these findings, RNA-Scope of mouse lungs revealed high *Il1b* shortly after primary spherule rupture.

Differing Peripheral Leukocyte Response Between Pulmonary and Disseminated Coccidioidomycosis Identifies Key Biological Processes and Pathways (continued)

Conclusion: Together these data suggest individuals with DCM have a decreased innate response compared to PUL. Differences between the two groups appear to be modulated by the transcription factor, STAT3, known to be mutated in several DCM patients, and IL1β, a previously unreported contributor to human DCM. These studies will further inform genetics and immunologic pathways underlying dissemination susceptibility.

#This work was supported with funds from the Division of Intramural Research, NIAID, and grants R01AI132140 to JNG, R21AI152394 to YAL, and U01AI122275 to SMH and JNG.

31. Clinical Isolates of *Coccidioides posadasii* Cause Rapid Mortality or Subclinical Disease in C57BL/6 Mice

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Abstract

Introduction: *Coccidioides* causes infection following the inhalation of arthroconidia from the environment. While most persons exposed to arthroconidia experience subclinical disease, others have a range of disease outcomes, from acute or chronic pneumonia to disseminated coccidiomycosis and meningitis. The underlying mechanisms behind this spectrum of disease are poorly understood, though patient immune status and inoculum size are both known to be associated with worse outcome. It is not known whether strain genotype impacts patient outcome or if patients experience strain-specific immune responses.

Methods: We infected C57BL/6 mice with the *C. posadasii* laboratory reference strain, Silveria, and tracked survival, CFUs, and circulating cytokines at days 3,5,7, and 10. We have an in-house collection of clinical isolates that were collected recently, have matched metadata, and have not been serially passaged. We infected mice with nine of those isolates and tracked survival. The mouse median survival was compared to isolate site of origin. Finally, we collected lungs from mice at terminal endpoint and determined the levels of three cytokines: IFNγ, IL-4, and IL-17A.

Results: Mice infected with the *C. posadasii* laboratory reference strain, Silveria, experienced rapid mortality paired with a 4.5x fold increase in fungal burden. The amounts of circulating cytokines were unchanged from the uninfected control at days 3,5,7, and 10. Surprisingly, C57B/6 mice infected with clinical isolates had a bimodal outcome, with the isolates causing either mouse mortality or no apparent disease. The origin of the isolate, either geographically or patient site of collection, did not appear to correlate with virulence in mice. We measured the levels of IFNγ, IL-4, and IL-17A in mouse lung homogenate, three cytokines selected for their importance in driving the Th1, Th2, or Th17 arms of the immune response. Interestingly, the cytokine levels in the lungs were all below the limit of detection, though IL-17A was increased compared to the uninfected control. The lack of cytokine response at the murine terminal endpoint could indicate that *Coccidioides* is masking to prevent immune detection, other cytokines are more relevant in the murine immune response, or that the early immune response is more applicable to understanding strain-specific variations in disease.

Clinical Isolates of *Coccidioides posadasii* Cause Rapid Mortality or Subclinical Disease in C57BL/6 Mice (continued)

Conclusions: In this study, we screened clinical isolates through mice and measured cytokine response. Surprisingly, the strains were either highly virulent or avirulent in C57BL/6 mice, without an intermediate outcome. We also detected a surprising lack production of three cytokines measured at the terminal endpoint. Overall, this study acts as an initial foray into further understanding the impact of strain genotype on disease, the *Coccidioides* mouse model, and the immune response of the mouse model to *Coccidioides.*

32. Time Series Forecasting of Valley Fever Infection in Maricopa County, AZ, Using LSTM

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Abstract

Introduction: Coccidioidomycosis (CM), also known as Valley fever, is a respiratory infection. Recently, the number of confirmed cases of CM has been increasing. Precisely defining the influential factors and forecasting future infection can assist in public health messaging and treatment decisions.

Methods: We utilize Long Short-Term Memory (LSTM) networks to forecast CM cases, based on the daily pneumonia cases in Maricopa County, Arizona from 2020 to 2022. Besides weather and climate variables, we examine the impact of people's lifestyle change during COVID-19. Factors, including temperature, precipitation, wind speed, PM10 and PM2.5 concentration, drought, and stringency index, are included in LSTM networks, considering their association with CM prevalence, time-lag effect, and correlation with other factors.

Results: LSTM can predict CM prevalence with accurate trend and low mean squared error (MSE). We also found a tradeoff between the length of the forecasting period and the performance of the forecasting model. The models with longer forecasting periods have less accurate trends over time and higher MSEs. Two models with different lengths of forecasting periods, 10 days and 30 days, are identified with good prediction.

Conclusion: LSTM algorithms, combined with traditional statistical methods, could help with the forecasting of CM cases. By predicting the CM prevalence, our results can inform researchers, epidemiologists, clinicians, and the public in order to assist public health

33. Understanding the effect of Agricultural Practices on Valley Fever through a Dual-Model Approach using Environmental Factors

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Abstract

Introduction: Valley Fever (VF), or coccidioidomycosis, is a fungal infection endemic to the Southwestern United States, primarily transmitted through inhalation of airborne *Coccidioides* spores (Valley Fever: Timely Diagnosis, 2017). Given the increasing incidence of VF and its significant economic burden, effective preventive strategies are crucial. Environmental factors, particularly agricultural practices (APs) as proposed by ADEQ, have been implicated in the spread of VF (Agricultural Dust Program | ADEQ, n.d.). This study aims to assess the influence of APs on VF epidemiology using a novel dual-model computational approach. By integrating environmental data with VF case trends, the research seeks to identify the most effective agricultural interventions to mitigate VF incidence.

Methods: To understand the impact of agricultural practices on Valley Fever (VF) incidence, this study employs a data-driven, dual-model approach integrating machine learning and environmental data. The project utilizes four key stages, each building upon the last to ensure a comprehensive analysis of VF epidemiology. The first stage involves scraping and cleaning environmental and VF case data from the environmental database, CDC Wonder, for Maricopa County, Arizona by month and year from 2000-2011. CDC Wonder, an initiative directed by the CDC, provides a vast amount of environmental data for public use, and extensive VF case data was found through GitHub (https://github.com/valleyfever/valleyfevercasedata/blob/master/coccidioidomycosis_m2000_2015_v0.1.csv). During data extraction, the researcher decided to focus on Maricopa County only, since it contains the most comprehensive VF data and represents the Arizona population the best. Following data extraction, data cleaning was needed to ensure alignment of VF case data with the environmental factors utilized like Fine Particulate Matter, Sunlight, Precipitation, Daily Air Temperatures & Heat Index, and Daily Land Surface (Day & Night) temperatures. The data cleaning was done for the extracted environmental CSV files to drop unnecessary columns and add the corresponding VF case value for month code (float value for month: MM), year code (int value for year: YY), and county (string value for county: Maricopa only). With a refined dataset, the second stage focuses on building and calibrating a combined Gradient Boosting Regressor (GBR) and Multilayer Perceptron (MLP) model. GBR captures steady, structured trends through a grid search for parameters, effectively handling seasonal and temporal variations in VF cases. Meanwhile, MLP identifies nonlinear relationships, detecting fluctuations and interactions between environmental variables that might

Understanding the effect of Agricultural Practices on Valley Fever through a Dual-Model Approach using Environmental Factors (continued)

be overlooked by traditional models. This hybrid approach maximizes predictive accuracy, offering a well-rounded representation of VF case dynamics. Along with this, a random split of the data was conducted to create train, test and validation subsets. The third stage extends beyond computational modeling by incorporating an Arduino-based sensor system, including the CO_2 Humidity and Temperature Sensor (SCD30) and the Optical Dust Sensor (GP2Y1010AU0F), to collect real-world agricultural practice (AP) data. Using the Arduino Giga R1, the system monitors dust levels, temperature, and humidity across different AP conditions. The SCD30 sensor needed to be soldered to be usable by the Arduino Giga R1 as well. Finally, in the fourth stage, the trained model is tested against environmental data from APs, using three identical planter boxes filled with MiracleGro Soil and covered with either mulch or organic material. By comparing changes in environmental factors across these conditions, the study identifies the most beneficial agricultural interventions for reducing VF cases.

Results: The results highlight the predictive accuracy of the GBR and MLP approach, the effectiveness of different APs, and key environmental factors influencing VF cases. The combined GBR & MLP model achieved an R^2 score of 0.903, meaning it explains 90.03% of the variance in VF case numbers. The model's Mean Absolute Error (MAE) was 97.197 cases, indicating that monthly VF case predictions are within ~100 cases of actual values. The Mean Squared Error (MSE) was 15,799.909, showcasing the model's robustness in capturing overall VF trends. A Shapiro-Wilk test on residuals yielded a test statistic of 0.9390 and a p-value of 0.4435, confirming that the residuals were normally distributed, meaning the model's errors were random rather than biased. This test was essential to confirm the model was not overfitting the data, as well as preventing biases in model evaluation. 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: This study establishes a novel dual-model computational framework integrating Gradient Boosting Regression (GBR) and Multilayer Perceptron (MLP) to predict Valley Fever (VF) incidence based on environmental and agricultural factors. By combining machine learning with real-world sensor validation, the findings demonstrate that mulch cover is the most effective agricultural intervention, reducing VF cases by 21.32%, followed by organic material cover at 15.39%. The model's high predictive accuracy (R^2 = 0.903, MAE = 97.197 cases) supports its potential application in public health decision-making. These results emphasize the critical role of agricultural dust control measures in mitigating VF transmission and provide a data-driven foundation for future policy recommendations and disease intervention strategies (Comrie, 2021; McCotter et al., 2019). Future work will focus on integrating population density metrics, expanding

Understanding the effect of Agricultural Practices on Valley Fever through a Dual-Model Approach using Environmental Factors (continued)

datasets (CDC, NOAA, NASA), and exploring advanced predictive models (LSTM, XGBoost, SARMIA, ARIMA) to enhance real-world applicability and accuracy.

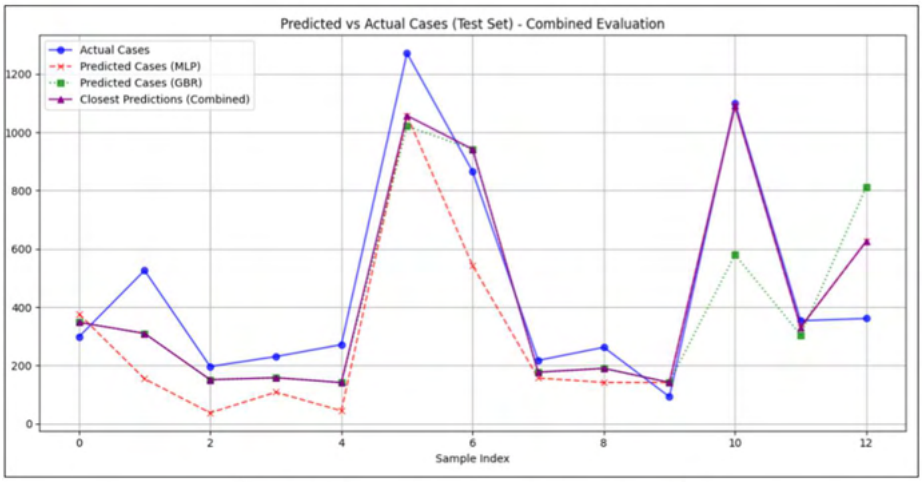

Figure 1: Graph Showing Predicted vs. Actual Cases of GBR & MLP Combined Model

References:

Agricultural Dust Program | ADEQ. (n.d.). Azdeq.gov. Retrieved December 19, 2023, from https://azdeq.gov/AgriculturalDust

Centers for Disease Control and Prevention (CDC). (2017, May 22). Valley Fever: Timely Diagnosis, Early Assessment, and Proper Management. Www.youtube.com. https://www.youtube.com/watch?v=M9GZ9MJ2-Ig&t=480s

Comrie, A. C. (2021). Climate and coccidioidomycosis: Interactions and implications for disease spread. Environmental Health Perspectives, 129(4), 1-10.

McCotter, O. Z., et al. (2019). Coccidioidomycosis: A review of recent advances. The Journal of Infectious Diseases, 220(3), 295-302.

Valley Fever 2020 Annual Report. (2020). Arizona Department of Health and Safety.

34. Assessing Virulence of *Coccidioides posadasii* Clinical Isolates Using the *Galleria mellonella Model*

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Abstract

Introduction: To create effective control strategies, it is essential to gain a better understanding of Coccidioides spp. The primary model for assessing virulence in *Coccidioides spp.* has traditionally relied on mice; however, this model presents limitations, particularly in the context of rapid evaluation and testing of multiple strains. Determining **Galleria mellonella** as a model provides many advantages, including low cost, short life cycle, small size, easy handling and maintenance, and no ethical constraints (Pereira et al., 2020). This research aimed to evaluate this model and develop a better understanding of the virulence of *Coccidioides* species strains

Methods: Twenty-two *Coccidioides posadasii* clinical isolates collected from patients in southern Arizona with the help of the Arizona Department of Health Services and Dr. John Galgiani, were studied. In addition to the clinical isolates, the common *C. posadasii* laboratory strain Silvera, and the *C. posadasii* soil strain CPA0032 strains were used. These were compared to a mock infection group (PBS). Arthroconidia from each strain were harvested and quantified following the procedure described by Mead et al. (2020).

Galleria mellonella larvae in the final instar stage were sorted by weight and appearance, selecting only those with no signs of melanization. Then each larva was infected with 10 µL of 102,10^3, or 10^4 viable spores of the selected strain. *G. mellonella* were observed for survival and melanization for 7 to 10 days after infection. Each *G. mellonella* was scored from 0-5 following the same scoring as described by Mendoza Barker et al. (2024).

Statistical analyses were conducted with GraphPad Prism 10. Survival curves for each group of Galleria mellonella were created using the Kaplan-Meier method, and overall and intergroup comparisons were performed using the log-rank (Mantel-Cox) test.

Results: Eleven strains were found to be significantly different when compared to the control group, with a significance level (α) of 0.05.

Assessing Virulence of *Coccidioides posadasii* Clinical Isolates Using the *Galleria mellonella Model (continued)*

Conclusion: This approach offered practical advantages such as cost-effectiveness, rapid turnaround, and ethical feasibility. Using this model allowed us to investigate multiple strains, including wildtypes and clinical isolates, within a shorter period. We have identified potentially hypervirulent strains, which with further study can help advance our understanding of infectious diseases and their management.

Mead, H. L., Van Dyke, M. C. C., & Barker, B. M. (2020). Proper Care and Feeding of Coccidioides: A Laboratorian's Guide to Cultivating the Dimorphic Stages of C. immitis and C. posadasii. *Curr Protoc Microbiol*, *58*(1), e113. https://doi.org/10.1002/cpmc.113

Mendoza Barker, M., Saeger, S., Campuzano, A., Yu, J. J., & Hung, C. Y. (2024). Galleria mellonella Model of Coccidioidomycosis for Drug Susceptibility Tests and Virulence Factor Identification. *J Fungi (Basel)*, *10*(2). https://doi.org/10.3390/jof10020131

Pereira, M. F., Rossi, C. C., da Silva, G. C., Rosa, J. N., & Bazzolli, D. M. S. (2020). Galleria mellonella as an infection model: an in-depth look at why it works and practical considerations for successful application. *Pathog Dis*, *78*(8). https://doi.org/10.1093/femspd/ftaa056

35. Tracking Coccidioidomycosis in Arizona: Regional Trends and Seasonal Cycles of a Climate Sensitive Disease

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Abstract

Introduction: Coccidioidomycosis, a fungal disease caused by inhalation of spores of Coccidioides species, has increased across the southwestern US in association with increasing aridity, warming temperatures, and precipitation volatility. In Arizona, changes in incidence within distinct climatological regions remain to be characterized. Here, we investigate regional trends in and seasonal patterns of coccidioidomycosis incidence over time.

Methods: Using surveillance data reported to Arizona Department of Health Services since 2005, we calculated incidence rate ratios (IRRs) to describe increases in incidence within six ecoregions. To identify region-specific periodicity in incidence, we performed wavelet analyses on regional monthly incidence rates normalized in each year.

Results: Between 2005 and 2022, the annual incidence rate (IR) more than doubled in Arizona, with the greatest median annual IR in the southern Sonoran Desert ecoregion (112.13 per 100,000) and the lowest in the northern Plateaus ecoregion (19.91). From 2005-2007 to 2020-2022, the two northernmost regions – the Plateaus and the Mojave Desert – reported the highest relative increases in incidence rate, with the IR in 2020-2022 6.6 (95% CI: 4.0, 10.9) and 4.5 (95% CI: 3.3, 6.1) times that in 2005-2007, respectively. All other regions reported IRRs between 1.44-3.32. IRs also increased between 2011-2013 to 2020-2022 within all regions, with the greatest IRR observed in the Plateaus region (IRR: 1.81, 95% CI: 1.33, 2.45). Wavelet analyses revealed consistent annual and intermittent semi-annual cycles within the Sonoran Desert region, with bi-annual periodicity occurring in years with wetter Monsoon seasons.

Conclusion: Overall, while the periodicity of coccidioidomycosis incidence is most pronounced within the highly endemic Sonoran Desert region, incidence is increasing most rapidly within northern regions, including those considered less suitable (i.e., cooler, wetter) for Coccidioides species, necessitating targeted public health messaging in these regions.

36. The Coccidioides Proteome Contains Undiscovered Antigenic Epitopes with Diagnostic Potential

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Abstract

Introduction: 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.

Methods: We designed the first proteome-wide peptide library, which covers both species of *Coccidioides*. Then epitopes discovered using this method were used to create a pilot ELISA diagnostic. Serum from known Coccidioidomycosis patients and healthy controls from outside the endemic region were assayed against a 244,000 plex 30mer library; then, antibody reactivity was mapped at the protein and epitope levels. A pilot ELISA was designed based on epitopes which were significantly reactive in Coccidioidomycosis patients sample set compared to the controls.

Results: We observed 48 epitopes from six proteins that were uniquely recognized by the disease cohort. An ELISA containing 16 of the epitopes was able to discriminate between Coccidioidomycosis patients and healthy controls (AUC 0.94).

Conclusion: The *Coccidioides* proteome contains novel antigens recognized by Coccidioidomycosis patient antibodies, a subset of which demonstrate diagnostic potential.

37. Can Eosinophilia Aid in the Prediction of Coccidioidal Severity?

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Abstract

Introduction: The severity of disease in coccidioidomycosis, a potentially devastating infection endemic to the southwestern United States, is primarily determined by the host immune response. Previous studies suggest that eosinophils may play a role in this initial response, potentially affecting the acuity and chronicity of coccidioidal disease. The aim of this study is to analyze the relationship between initial eosinophilia and severity of coccidioidomycosis.

Methods: This IRB-approved retrospective cohort study evaluated patients treated at the Valley Fever Institute in Bakersfield, CA for a new diagnosis of non-miliary pulmonary coccidioidomycosis from 2020-2021. Diagnosis was made or confirmed by C*occidioides spp.* IgG immunodiffusion tests at the Kern County Public Health Department. Patients were excluded if a complete blood count with differential and chest imaging from initial presentation (index date) were unavailable for review. Peripheral eosinophilia was defined as an absolute eosinophil count of ≥350 cells/microliter and further categorized as mild (350-1000 cells/mcL), moderate (>1000-1500 cells/mcL), or severe (>1500 cells/mcL). The incidence of disseminated coccidioidomycosis diagnosed within 3 years of index was compared in patients with peripheral eosinophilia (PE) versus insignificant eosinophilia (IE) at index. Other endpoints included hospitalization, length of hospital stay (LOS), oxygen requirement, and intensive care unit (ICU) admission. Clinical categories (Table 1) were used to describe disseminated disease.

Results: Fifty-five patients with newly diagnosed pulmonary coccidioidomycosis from 2020-2021 were identified; of these, 27 had peripheral eosinophilia at index. Mean age at index was 37 years (IE 34 years, PE 41 years); 58% were male (IE 50%, PE 67%); 24% had diabetes mellitus with a mean hemoglobin A1c of 9.0% (IE 25% HbA1c 10.0%, PE 22% HbA1c 7.7%); and mean body mass index was 30 kg/m^2 (IE 30 kg/m^2, PE 30 kg/m^2). Disseminated coccidioidomycosis was diagnosed in 2 patients (IE) vs. 1 patient (PE). Both patients in the IE group had onset of dissemination within 3 months of index and included category 4B and 5A disease. In the PE group, dissemination with category 4B disease occurred at 2.9 years. In total, 20 patients (36%) were hospitalized at index (IE 29%, PE 44%); of these, mean LOS was 9 days (IE) vs. 4 days (PE), oxygen was required for 38% (IE) vs. 25% (PE), and ICU care was required for 13% (IE) vs. 0% (PE).

Can Eosinophilia Aid in the Prediction of Coccidioidal Severity? (continued)

Conclusions: In this study, peripheral eosinophilia at initial presentation with pulmonary coccidioidomycosis appeared to be associated with higher frequency of acute severity requiring hospitalization, although patients with PE generally had a shorter and less complicated hospital course compared to those with IE. The early identification of and initiation of antifungal treatment for pulmonary coccidioidomycosis likely explains the low frequency of disseminated coccidioidomycosis in our study population, although the difference in onset of dissemination between the IE and PE groups is interesting and should be explored in a larger study. These findings support further investigation of the relationship between peripheral eosinophilia and coccidioidal disease, with the hope of discovering insights that eventuate an improved clinical understanding of disease progression.

38. A Rare Case of Markedly Elevated CSF Protein in a Coccidioides Meningitis Case on Fluconazole. A Marker of Fluconazole Failure?

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Abstract

Introduction: Coccidioidal meningitis (CM) is a rare but serious manifestation of Coccidioides infection, typically presenting cerebrospinal fluid (CSF) findings of lymphocytic pleocytosis, low glucose levels, and elevated protein. Levels of CSF protein may be markedly elevated in complications such as hydrocephalus, with levels reported previously as high as in the thousands. Here we report a rare case of CM complicated by hydrocephalus with CSF protein levels rising to 10,000 mg/dL, despite therapy with fluconazole. We are unaware of any CSF protein reported at this or greater value.

Methods: This case study was approved by the Kern Medical Institutional Review Board (IRB). Patient data was collected by chart review, and all identifying information was removed to ensure confidentiality.

Results: A 50-year-old male initially presented to the Emergency Department with a 14-day history of subjective fever, nonproductive cough, fatigue, with some mild chest discomfort, and was found to have a miliary pattern on chest X-ray and positive Coccidioides serology with positive immunodiffusion (ID) for IgM and IgG with compliment fixation titer (CF) of 1:32. The patient was discharged on fluconazole 400 mg daily with referral for outpatient follow up, but returned four months later with headaches after running out of medication three weeks prior.

Lumbar puncture (LP) revealed an opening pressure (OP) of 480 mm H_2O, CSF protein of 311 mg/dL, a CSF Coccidioides IgG positive by ID and CF titer of 1:1 with cultures showing *Coccidioides immitis*. Fluconazole 1000 mg daily was initiated, and despite compliance, the patient had two additional admissions within the following month for worsening headaches, ataxia, and emesis.

Symptoms improved post-LP with normal OPs of 140mm H_2O and 155 mm H_2O. Protein levels with these LPs markedly increased from 4100 mg/dL to 5700 mg/dL respectively. CT head without contrast showed mild periventricular hypodensities and prominent ventricles, concerning for now normal pressure hydrocephalus, but remained stable, requiring no intervention. Fluconazole was increased to 1200 mg daily, yet CSF protein continued to rise, reaching 10,000 mg/dL at outpatient follow-up five months later, which was interpreted as fluconazole failure. Fluconazole was discontinued, and isavuconazonium 372 mg daily was initiated after the loading dose.

A Rare Case of Markedly Elevated CSF Protein in a Coccidioides Meningitis Case on Fluconazole. A Marker of Fluconazole Failure? (continued)

Three months after switching therapy, CSF protein levels significantly decreased to 2265 mg/dL, and the patient remained asymptomatic without signs of relapse.

Conclusion: This case highlights the importance of serial LPs in coccidiodal meningitis patients and monitoring CSF parameters even with normal OP. Increasing CSF protein may indicate treatment failure or new pathology which should be addressed promptly. Alternative azole therapy like isavuconazonium may be beneficial in these cases and should be considered.

39. Disseminated Coccidioidomycosis with Triplet Gestation Pregnancy

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[1]Kern Medical, Bakersfield, USA. [2]Valley Fever Institute, Bakersfield, USA

Abstract

Introduction: Coccidioidomycosis is a fungal infection caused by *Coccidioides spp*. While most infections are self-limited, severe or disseminated disease can occur, particularly in immunocompromised individuals and pregnant women. The treatment of coccidioidomycosis in pregnancy is well documented. However, the optimal approach for high-risk pregnancies, such as multifetal gestations, remains poorly researched and lacks standardized guidelines. Here, we present a case of a 30-year-old woman with disseminated osseous axial skeleton coccidioidomycosis who became pregnant with triplets while on fluconazole.

Methods: This case study was conducted at Kern Medical Center and was approved by the Institutional Review Board (IRB). Patient data was collected through a retrospective chart review, and all identifying information was removed to ensure confidentiality.

Results: A 30-year-old African-American woman initially presented to an outside hospital with a persistent left-sided headache for one week after experiencing a ground-level fall with head trauma. CT head without contrast revealed multiple calvarial lytic lesions. She was discharged the same day. Her headaches persisted and she presented to Kern Medical one month later. She underwent Lumbar puncture (LP) and was found to have normal opening pressure with no evidence of meningitis. MRI brain with/without contrast found pachymeningeal enhancement with calvarial osseus lesions and subsequent biopsy showed granulomatous inflammation with spherules and endospores consistent with coccidioidomycosis.

Coccidioidomycosis serology revealed positive Immunodiffusion with reactive IgM and IgG antibodies with complement fixation (CF) titer of 1:256. She was discharged on fluconazole 800 mg daily and instructed to establish care with Infectious disease (ID) outpatient. Due to social barriers, she could not attend her appointments.

CT chest abdomen pelvis two months from initial presentation found right upper lobe nodular consolidation with miliary dissemination, mediastinal lymphadenopathy, 8th rib lytic lesion and left posterior iliac bone lesion.

Disseminated Coccidioidomycosis with Triplet Gestation Pregnancy (continued)

She endorsed formation of boils on her head, back, buttock, and right arm which worsened since her hospitalization. Culture from the back abscess grew Coccidioides immitis. She was finally able to follow up with ID clinic seven months after diagnosis. She reported compliance to fluconazole. Due to failure to clinically improve on fluconazole and sheer fungal burden, the decision was made to start Liposomal Amphotericin B (AmBisome). She was found to be pregnant with triplets one month after starting AmBisome.

She has been following with high-risk obstetrics and the most recent ultrasound showed a 19-week intrauterine dichorionic triamniotic triplet gestation with no gross fetal anomalies. Current plan is to continue AmBisome through her pregnancy which she is receiving at the Infusion clinic with weekly ID follow ups. The CF titer remains at 1:256 at this time.

Conclusion: The IDSA recommends management of coccidioidomycosis infection based on fetal maturity. For women with infection diagnosed prior to pregnancy, current recommendation is cessation of azole therapy for the first trimester due to teratogenic effects, and treatment with AmBisome in early pregnancy. However, there are currently no specific guidelines regarding management of coccidioidomycosis in high-risk pregnancies, including multiple gestations, as in this patient. Continuing to follow the management and outcomes of this patient will contribute to what we know about coccidioidomycosis in high-risk pregnancy. This case also highlights the importance of educating patients about risks of fluconazole use during pregnancy and using contraception.

40. Ophthalmic Findings among Hospitalized Patients with Coccidioidomycosis at a Tertiary Referral Center

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Abstract

Introduction: Ocular involvement with *Coccidioides immitis* infections is thought to be rare. Traditionally, ophthalmic examinations have not been included in the evaluation of infected individuals unless there were ophthalmic signs or visual symptoms. This project aims to evaluate ophthalmic findings among patients hospitalized with active or histories of systemic coccidioidomycosis at a tertiary referral center, whether or not they have ophthalmic signs or symptoms.

Methods: We performed a retrospective chart review of patients hospitalized with coccidioidomycosis at the Ronald Reagan UCLA Medical Center from January 2014 through June 2024, who had been referred to the Ophthalmology Service for examination during hospitalization. Data collected included age, sex, race and ethnicity, travel or residence in endemic areas, date of coccidioidomycosis diagnosis, and treatment history. Infection was confirmed with serologic tests, including IgG EIA, IgM EIA, and complement fixation. Ophthalmic data included visual acuity, intraocular pressure, anterior chamber findings, and slit lamp biomicroscopic and dilated fundus examination findings.

Results: We reviewed medical records of 66 patients. A total of 34 (51.5%) had clinical evidence of active systemic coccidioidomycosis; 32 had dilated fundus examinations. Ophthalmic disease was found in 8 (25%) patients (11 eyes); 4 of the 8 patients did not have concurrent signs or symptoms of inflammatory eye disease. Findings of ophthalmic disease included chorioretinitis (10 eyes; 8 patients), some also having anterior chamber inflammation (2 eyes; 2 patients) and vitreous inflammatory reaction (1 eye). One patient with unilateral symptoms had a subretinal mass in the same eye, but also had focal retinal lesions in the opposite, asymptomatic eye. The 32 patients with histories of coccidioidomycosis had been admitted for other diseases; 2 (6.3%) of these patients had chorioretinal scars (2 eyes), but no known history of inflammatory eye disease. Patients with ophthalmic involvement were predominantly male (75%); age ranged 1-80 years (median 29).

Ophthalmic Findings among Hospitalized Patients with Coccidioidomycosis at a Tertiary Referral Center (continued)

Conclusion: Disseminated coccidioidomycosis may result in severe intraocular disease, including chorioretinitis, anterior uveitis, and endophthalmitis. Asymptomatic chorioretinal lesions can also occur and heal without serious sequelae. Patients with histories of coccidioidomycosis, but without active disease, may present with chorioretinal scars, suggesting past occult intraocular disease. This study underscores a need for dilated fundus examinations for all patients with coccidioidomycosis, regardless of ophthalmic signs or symptoms.

41. Cavitary Pneumonia: Tracing Causes in the Arid Landscapes of Southern Arizona

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Abstract

Introduction: Cavitary lung disease is characterized by gas-filled spaces within lung tissue. Bacteria such as *Staphylococcus*, *Streptococcus*, and *Pseudomonas* species are frequent culprits of cavitary pneumonia. This study aimed to determine whether bacterial pathogens remain the most common cause of cavitary pneumonia in southern Arizona, where coccidioidomycosis is endemic.

Methods: A retrospective study of adults (≥18 years) admitted with cavitary lung disease, identified by chest CT from January 2018 to December 2023, was conducted. Patients with known active lung malignancy or lung metastasis or non-cavitary lesions were excluded. Clinical, microbiological, and radiological data were analyzed, and fungal versus bacterial cavitary pneumonia was compared using chi-square and t-tests. Pulmonary coccidioidomycosis was classified as proven, probable, or possible based on laboratory and radiological findings. Statistical analysis included multiple regression with LASSO regularization to predict fungal pneumonia.

Results: Of 1,123 patients initially identified, 382 were included after exclusions. Fungal infections accounted for 66% of cases with a known etiology, with *Coccidioides* responsible for 39% of all cavitary pneumonias and 82% of fungal pneumonias. Bacteria were the etiology in 30% of patients, with *Staphylococcus aureus (12%)*, *Streptococcus species (12%)*, and *Pseudomonas species (11%) as* most common bacterial organisms. Seven percent of patients had synchronous fungal and bacterial infections. Of the 148 patients with possible cavitary Coccidioides infection, 24% proven coccidioidomycosis, 51% probable, and 25% had possible coccidioidomycosis. Serological tests were significant, EIA IgG was positive in 85%, EIA IgM was positive 58%. Immunodiffusion (ID) IgG was positive in 58%, and ID IgM was positive in 38%. Complement fixation (CF) testing was positive in 45%. Antibiotic and antifungal treatments were administered in 96.5% and 95% of patients. Surgical intervention was performed in 7%. Overall mortality was 25%, with 34% of deaths attributed to cavitary pneumonia.

Conclusion: Cavitary pneumonia remains a serious condition. *Coccidioides* species are leading causes, followed by bacterial infections in southern Arizona. Co-infection with bacterial and fungal organisms is relatively rare. Despite strong adherence to treatment protocols, the mortality rate remains high, highlighting the need for continued research into more effective and early therapeutic strategies.

42. Identification of *Coccidioides*-specific Human T Cell Epitopes Using an Immunoinformatic Approach

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Abstract

Introduction: *Coccidioides* species can cause respiratory illness and life-threatening fungal meningitis. Currently, no FDA-approved vaccines exist for this fungus. T cell epitopes, short peptide fragments recognized by the immune system, are essential for developing vaccines and diagnosis tools, particularly against *Coccidioides* infections. Protective immunity relies on activating mixed Th1/Th17 memory responses through major histocompatibility complex II (MHC-II)-mediated antigen presentation. Our goal is to identify immunogenic coccidioidal peptides that are highly conserved across *Coccidioides* spp. and contain epitopes capable of binding to prevalent human MHC-II molecules to stimulate protective immune responses.

Methods: *Coccidioides* spherules differ in their protein expression profiles compared to arthroconidia. Therefore, we performed an RNA-seq analysis on *C. posadasii* strain Silveira arthroconidia and day 1 and 6 spherules to identify genes that are highly expressed during spherule development, are highly conserved in *C. posadasii* strain C735, and have no homology to human or murine proteins. We then applied immunobioinformatics algorithms (ioGenetics and Immune Epitope Databases) to predict whether the proteins encoded by these genes contained peptides capable of binding to multiple MHC-II alleles. These predicted peptides were synthesized to validate their immunogenicity in transgenic (Tg) mice expressing the human HLA-DRB1*04:01 (DR4) molecule. DR4 Tg mice were subcutaneously vaccinated twice at two-week intervals with a batch of 12-13 peptides emulsified in incomplete Freund' adjuvant with a Th1-skewing CpG oligonucleotides (ODN) adjuvant (1µg of peptide + 10µg of CpG ODN/100µL dose). Non-vaccinated mice were also included as a negative control. Four weeks following the final vaccination, splenocytes were isolated and restimulated with individual synthetic peptides to assess memory responses using IFN-γ ELISPOT assays. Splenocytes stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin or media will serve as a positive and negative control, respectively. Peptides that can stimulate significant IFN-γ production will be determined by comparing individual peptides to media via a Mann-Whitney U test.

Results: The RNA-seq analysis identified 25 proteins encoded by genes upregulated during spherule development, exhibit >98% identity in *C. posadasii* strain C735, and have no homology to human or murine proteins. The *in vivo* gene expression of these targets was confirmed by qRT-PCR using spherule mRNA

Identification of *Coccidioides*-specific Human T Cell Epitopes Using an Immunoinformatic Approach (continued)

isolated from mice infected with *C. posadasii* strain C735. From these 25 coccidioidal proteins, 110 peptides (15-mers) were predicted to bind with high affinity to multiple human HLA-DR alleles. We confirmed that 62 *Coccidioides* peptides (17-30AA) stimulated significant IFN-γ production in splenocytes harvested from vaccinated DR4 Tg mice, whereas unstimulated controls showed no detectable IFN-γ production. The 62 identified immunogenic coccidioidal peptides exhibit 90-100% amino acid sequence identity in *C. posadasii* and *C. immitis* strains.

Conclusion: Here, we describe the first identification and validation of human peptide epitopes derived from the *Coccidioides* genome. These highly conserved, immunogenic peptides represent promising candidates for vaccine and diagnostic tools development. Our next goals are to assess the immunoreactivity and recognition of these identified peptide epitopes in an *ex vivo* IFN-γ recall assay using peripheral blood mononuclear cells (PBMCs) isolated from healthy donors and coccidioidomycosis (CM) patients. Additionally, we will evaluate whether these *Coccidioides*-specific peptides can be used for detecting *Coccidioides* exposure through an IFN-γ release assay (IGRA) with whole blood samples from healthy donors and CM patients. Lastly, all identified peptides have been deposited into the IEDB databases.

43. Identification of Coccidioidomycosis Specific and Reactive T-cell Receptors Clonotypes Expressed During Infection

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Abstract

Introduction: Valley Fever (VF) also known as Coccidioidomycosis is a common cause of community acquired pneumonia in the American Southwest. The two species are generally spatially separated with *Coccidioides immitis* in California and *Coccidioides posadasii* in other American states. The VF T-cell response is important to control the disease and measuring and characterizing this response is critical to better understand disease outcome. We aim to identify *Coccidioides* specific reactive T-cell receptors (TCR) clonotypes, epitopes, and characterize the immune response in *Coccidioides spp.* infected individuals.

Methods: We previously identified a pool of VF specific T-cell peptides that activate T cells and secrete cytokines in VF patients. Recently, peripheral blood mononuclear cells (PBMC) collected from individuals infected with *C. immitis* with pulmonary and disseminated infections or *C. posadasii* with pulmonary infections were stimulated with a pool of 108 *Coccidioides* specific peptides to evaluate for cytokine responses, TCR clonotype diversity, and epitope identification by sequencing.

Results: The *Coccidioides* peptides induced in VF confirmed PBMC high inflammatory response compared to the healthy controls characterized by IL-2 secretion. IFN-g and TNF-α expression are elevated in pulmonary VF group compared to disseminated VF. After peptide stimulation, activated CD4 T-cells were sorted and sequenced for T-cell receptors (TCR) clonotypes and epitope identification. We identified 10 specific TCR clonotypes expressed in pulmonary VF patients, belonging to 2 *Coccidioides* antigens.

Conclusion: This study described immunogenic properties of *Coccidioides* peptides in VF patients. The peptides were immunoreactive in VF infected individuals with *C. posadasii and C. immitis,* but also in two clinical forms of the infection. Using TCR sequencing we identified specific TCR clonotypes that are expressed during VF infection. In the future, single-cell sequencing will be performed to better characterize VF specific T-cell phenotypes associated with specific TCRs and epitopes.

44. Organ Transplant Screening Identifies Nearly One-third of Reported Coccidioidomycosis Cases in Utah: Key Findings from Surveillance Data, 2009–2024

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Abstract

Introduction: Utah has conducted surveillance of coccidioidomycosis since 2009. Since then, incidence of coccidioidomycosis has increased throughout the southwestern United States. The Utah Department of Health and Human Services (DHHS), in conjunction with local health departments, attempts to interview all individuals with reported positive *Coccidioides* test results using a standardized case investigation form. DHHS began using an updated case definition to classify cases in 2023. We assessed surveillance data to identify trends in reported illnesses and evaluate exposures.

Methods: We summarized trends in the number of Utah residents with positive *Coccidioides* test results reported during 2009–2024, and calculated the proportion of individuals reporting each race and ethnicity. For individuals reported in 2024 who also met the updated case definition, we calculated the statewide incidence of coccidioidomycosis and its incidence among individuals living in the Southwest region of Utah (defined as the Southwest Utah Public Health jurisdiction), where *Coccidioides* is endemic. We also calculated the median age and the proportion of those who were male, hospitalized, had underlying health conditions, were solid organ transplant donors or recipients, participated in various recreational exposures, traveled, and reported certain occupations. This analysis was considered public health surveillance and did not require IRB review.

Results: Utah residents with reported positive *Coccidioides* test results increased by more than 250% from 52 in 2009 to 200 in 2024. Data on race were available for 1,634 individuals; 1,456 (89%) were White, 48 (3%) were Native Hawaiian or Other Pacific Islander, 47 (3%) were American Indian or Alaska Native, 37 (2%) were Asian, and 36 (2%) reported their race as “other”. Of 1,577 individuals with available ethnicity data, 179 (11%) identified as Hispanic or Latino. Of individuals reported in 2024, 196 met the updated case definition. Statewide incidence in 2024 was 5.67 per 100,000 population, and incidence in the Southwest region was 28.73 per 100,000 population. The median age was 57 years; 120 (61%) cases were male and 69 (35%) were reportedly tested during an inpatient hospital visit. Among interviewed cases with data available, 60/80 (75%) reported an underlying health condition; 24/83 (29%) were screened for coccidioidomycosis prior to either receiving or donating a solid organ; 37/74 (50%) reported travel, including 28 (38%) who reported out-of-state travel, 20 (27%) who reported in-state travel, and 4 (5%) who reported foreign travel; and 31/69

Organ Transplant Screening Identifies Nearly One-third of Reported Coccidioidomycosis Cases in Utah: Key Findings from Surveillance Data, 2009–2024 (continued)

(44%) reported recreational exposure. A summary of data regarding organ transplants and travel is available in the table below.

Reported travel and solid organ transplants among cases reported in 2024

	n(%)
Travel (N=74)	
Out-of-state travel	28 (38)
In-state travel	20 (27)
Foreign travel	4 (5)
Solid organ transplant screening (N=83)	
Transplant recipient	18 (22)
Living organ donor	5 (6)
Unknown	1 (1)

N = number of individuals with data available

n = number of individuals who reported each exposure or transplant screening

Conclusion: Reported coccidioidomycosis has increased dramatically since 2009. Although relatively few cases were interviewed in 2024, these data show that nearly one-third of cases are tested as part of the screening process prior to receiving or donating solid organs. This aligns with our understanding that many people who get coccidioidomycosis are either not symptomatic or have mild illness and do not seek treatment. Additionally, half of cases reported travel, and some may not be aware of health risks that come from visiting certain travel destinations. DHHS hopes to use surveillance data to inform public health messaging to higher-risk groups in the future.

45. Disseminated Coccidioidomycosis in an African American Woman with Invasive Ductal Carcinoma, Dichotomy of Double Demons

Konstantino Papatheodorou[1], Arash Heidari[2,3]

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Abstract

Introduction: There is a heightened risk of opportunistic coccidioidomycosis in the immunocompromised, but the severity of infection in solid-organ cancer patients may not be as well described. Although risk remains heightened in regions endemic to *Coccidioides*, disseminated infection may be overlooked by the presumption of malignancy metastasis. In this case report we highlight the course of a 50-year-old African American woman with invasive ductal carcinoma who developed extensive pulmonary coccidioidomycosis with osseous dissemination.

Methods: Retrospective Case Report

Results: A 50-year-old African American female with a history significant for hypertension, right breast invasive ductal carcinoma, status post two right breast lumpectomies. She presented to our institution with severe dyspnea, fatigue, and altered mental status. She was recently offered to be placed on hospice due to extensive pulmonary metastasis with a lack of response to treatment. She was admitted and intubated due to respiratory failure. Computed tomography (CT) head showed bilateral lytic lesions in the frontal and parietal regions. (Figure a) The CT chest showed inflammatory left breast malignancy and extensive bilateral infiltrates, ground glass, and cavitary nodules consistent with atypical pneumonia and acute respiratory distress syndrome (ARDS). (Figure b). Her serum coccidioidomycosis turned positive for IgM and IgG Immunodiffusion with complement fixation of 1:8. (ARUP). Her lumbar puncture was essentially negative. Sputum and bronchoscopy samples both grew coccidioides immitis. identified by MALDI-TOF. (ARUP). She was started on Liposomal amphotericin B (LAmB) for severe pulmonary coccidioidomycosis with presumption for osseous dissemination to the skull, and methylprednisolone for 21 days with taper due to ARDS. After obtaining records, previous PET/CT one year prior showed new peripheral consolidation with hypermetabolism in the right middle lobe with suspicion of pneumonia versus post-radiation changes. She moved to the Central Valley of California during that time and presumptively was indicative of her infection with pulmonary coccidioidomycosis. The patient is still in the intensive care unit on LamB with slow clinical response.

Disseminated Coccidioidomycosis in an African American Woman with Invasive Ductal Carcinoma, Dichotomy of Double Demons (continued)

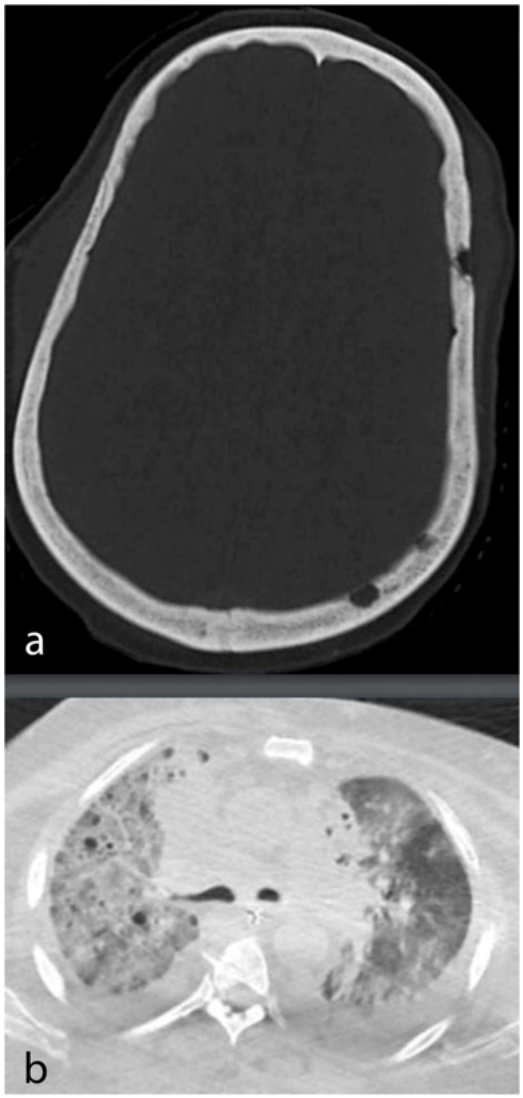

Figure a: CT brain showed multiple lytic lesions; Figure b: CT chest shows ground glass and alveolar extensive infiltration and cavitary nodules particularly in upper lobes.

Conclusion: The diagnosis of coccidioidomycosis in patients with malignancy is difficult as both may present similarly on PET-scan as metastasis. Clinicians in the endemic area should be vigilant about the chance of coexistence, particularly among African Americans and Filipinos who have an increased risk for dissemination of coccidioidomycosis.

46. Two Cases of Severe Pulmonary Coccidioidomycosis Concomitant with Cardiovascular Diseases, Anchoring on One, Forgetting the Other

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Abstract

Introduction: Our institution is located in the Central Valley of California endemic to coccidioidomycosis (CM) and we observed a recent increase in cases. Pulmonary CM is the most common manifestation of infection, which in severe cases makes the identification of concomitant cardiovascular disease increasingly difficult. We present two cases of severe pulmonary coccidioidomycosis masking cardiovascular disease to highlight the significance of thorough evaluation and workup to prevent the overlook of concurrent disease.

Methods: Retrospective Case Report

Results:

Case 1

A 66-year-old Hispanic female with hypertension, coronary artery disease, patent foramen ovale, status post MitralClip, and transcatheter mitral valve replacement one month prior on apixaban presented to our institution for dyspnea, cough, and fever. Workup on admission revealed left upper and lower lobe consolidation with pleural effusion and CM serology positive for IgM and IgG immunodiffusion (ID) with complement fixation (CF) of 1:16 (ARUP). She underwent thoracocentesis, was initiated on Liposomal amphotericin B (LAmB) due to hypoxemia transitioned to fluconazole immediately due to acute kidney injury (AKI) requiring temporary hemodialysis (HD), and was discharged upon stabilization of condition. She followed up with her primary tertiary care center, found to have mitral valve thrombosis on transesophageal echocardiography (TEE), where apixaban was replaced with coumadin. Three weeks later, the patient presented back to our institution for fever and lethargy; found to be in septic shock, with an international normalized ratio of 13.60, and a small subarachnoid hemorrhage (SAH) found on CT. Her supratherapeutic anticoagulation was reversed with Vitamin K. Blood cultures revealed Enterococcus faecalis for which she started on ampicillin alongside continued Fluconazole as chest x-ray and pleural effusion were improved. Due to concern for mitral valve vegetation and her complicated clinical history, the patient was transferred to her original institution for further cardiology workup. TEE was found to have thickened mitral prosthetic

Two Cases of Severe Pulmonary Coccidioidomycosis Concomitant with Cardiovascular Diseases, Anchoring on One, Forgetting the Other (continued)

valve leaflets with restricted motion, presumed vegetation, and infective prosthetic endocarditis (IE) given bacteremia. Treatment included ampicillin and ceftriaxone for 6 weeks, with lifelong amoxicillin suppression for prosthetic IE. She will remain on fluconazole and follow up at our institution for the management of her pulmonary CM.

Case 2

A 56-year-old Hispanic male with polysubstance abuse who developed cough, night sweats, fever, and ten-pound weight loss for two months presented to our institution as stroke alert with left-sided facial droop. Magnetic resonance imaging revealed bilateral patchy, punctate, and serpiginous acute ischemia within the frontal/parietal/occipital lobes. CT chest showed bilateral airspace infiltration and ground glass opacities with pleural effusions and prominent hilar and mediastinal lymphadenopathy. His CM serology returned positive for IgM and IgG ID with CF of < 1:2. He was initiated on LAmB due to extensive infiltration and hypoxemia, replaced with renally dosed fluconazole after developing AKI requiring HD and later continuous renal replacement therapy. There was suspicion of accompanying pulmonary edema seen in previous CT with ejection fraction of 35% characterized by echocardiogram, and BNP found to be 2,397 pg/mL. The patient developed non-ST segment myocardial infarction associated with cardiogenic shock which warranted cardiac catheterization, found to have 90% stenosis of the left circumflex and 99.99% of the D1 and D2 of left anterior descending arteries. The patient required multiple intubations for several failed extubations throughout his hospital course, thought to be originally only due to pulmonary coccidioidomycosis. His tracheal aspirate grew *Coccidiodes immitis* identified by MALDI-TOF (ARUP). The goal of care was discussed with the family, which resulted in a shared decision to proceed with comfort care if there was no major clinical improvement. The patient succumbed to his illness.

Conclusion: When patients have severe pulmonary CM in the endemic area clinicians may anchor and focus on the management of CM. This may delay or could potentially overlook the diagnosis or management of other concomitant diseases, particularly cardiovascular that could mimic and mask similar symptoms and radiological findings. This includes receiving a detailed history, identifying predisposing risk factors, completing a thorough physical examination, and considering several differential diagnoses in the workup.

Two Cases of Severe Pulmonary Coccidioidomycosis Concomitant with Cardiovascular Diseases, Anchoring on One, Forgetting the Other (continued)

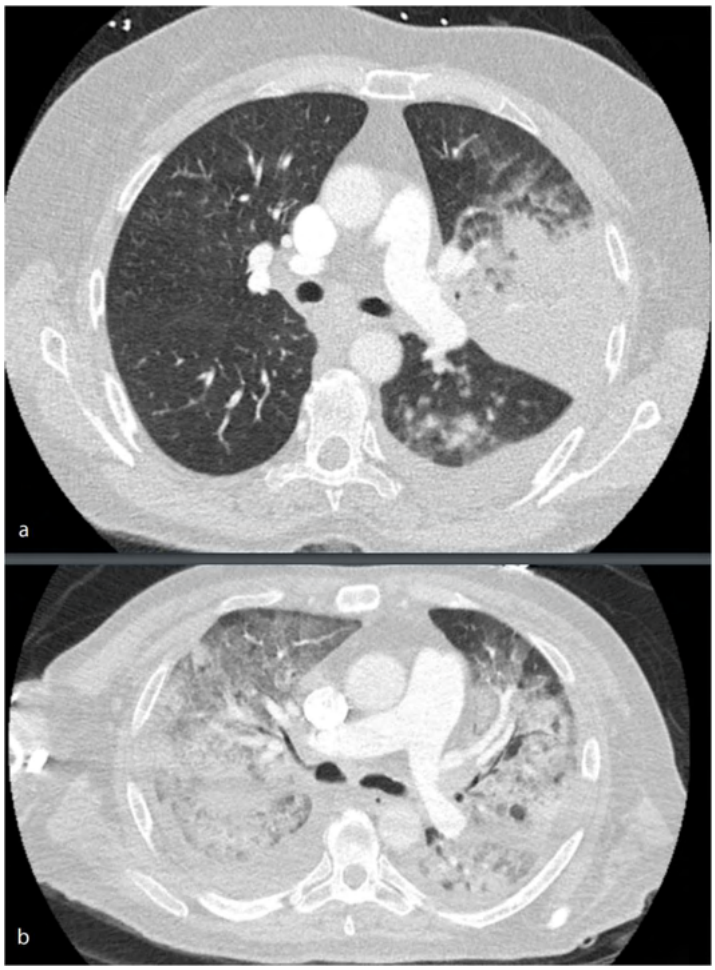

Figure a: case one, CT chest shows left upper and lower lobe consolidation with pleural effusion; Figure b: case two, CT chest shows bilateral airspace infiltration and ground glass opacities with pleural effusions.

47. Disseminated Cutaneous Coccidioidomycosis in a Renal Transplant Patient, 24 Years of Hiding

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Abstract

Introduction: The risk of coccidioidomycosis is increased in patients with solid-organ transplantation, this risk is even greater within the first year of transplantation as immunosuppression is maximal. Individuals undergoing transplantation should receive pre and post-transplantation screening along with donor screening to minimize the risk of endemic mycoses. We present a case of an individual with treated pulmonary coccidioidomycosis 24 years ago who underwent recent renal transplantation and developed disseminated cutaneous coccidioidomycosis.

Methods: Retrospective Case Report.

Results: A 65-year-old Hispanic male from the Central Valley of California with a previous right kidney transplant 26 years ago, pulmonary coccidioidomycosis 24 years ago, and end-stage renal disease previously on dialysis, status post left kidney transplant 8 months ago on immunosuppressive therapy who developed disseminated cutaneous coccidioidomycosis 24 years after primary infection. Four weeks before the presentation, he developed a painful, nonpurulent, oozing skin lesion on his superoposterior right shoulder (Figure a) with a similar lesion on his nose and headache shortly thereafter. At outpatient evaluation, he was diagnosed with presumptive shingles that failed to respond to empirical treatment in the following days. He presented to our institution with an enlarged right shoulder lesion, a crusted nasal lesion (Figure b), and a worsened headache. His wound cultures came back positive for *Coccidioides immitis* confirmed by MALDI-TOF (ARUP). His coccidioidomycosis serology showed reactive IgM immunodiffusion and negative IgG and complement fixation titer of <1:2 (ARUP). Due to headache and the location of infection on his nose, a lumbar puncture was performed which was essentially entirely negative. Nuclear medicine bone scan demonstrated 2 nonspecific, small foci of osteoblastic activity in the left iliac bone that raised suspicion for disseminated coccidioidomycosis. Upon questioning, the patient had a left pelvic fracture due to a car accident in the past and his MRI showed no evidence of infection. His condition improved within days of initiating fluconazole 400 mg, a dose to target therapeutic serum level due to drug-drug interactions with his immunosuppressive therapy. He was discharged and followed up in the clinic with significant improvement of his lesions. The duration of treatment is unknown but at least 3 years and until continuation of immunosuppression.

Disseminated Cutaneous Coccidioidomycosis in a Renal Transplant Patient, 24 Years of Hiding (continued)

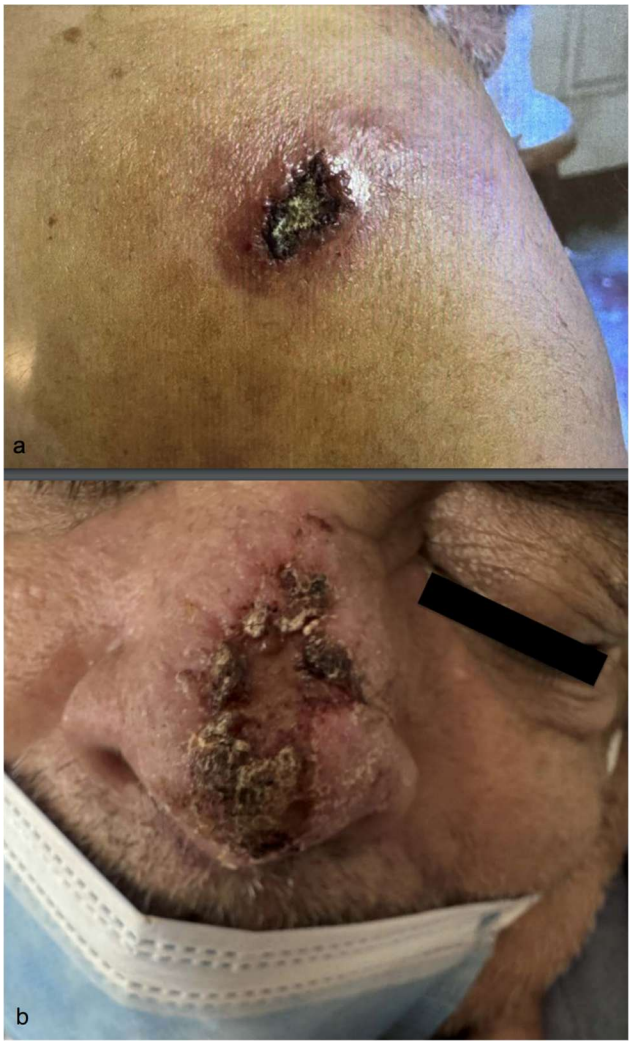

Figure a: oozing skin lesion on right shoulder; Figure b: crusted nasal lesion.

Conclusion: Coccidioidomycosis can manifest in various forms, either as primary or as disseminated form. Although the patient has a history of pulmonary coccidioidomycosis treated approximately 24 years ago, there was no documentation for pre-transplantation screening of Coccidioides as the transplantation was done in a nonendemic region. Clinicians should remain vigilant regarding this infection where the fungus is endemic, given the diverse presentations associated with coccidioidomycosis and the chance of reactivation due to immunosuppression.

48. Coccidioidomycosis Pulmonary Nodule, A Master of Disguise

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Abstract

Introduction: Pulmonary coccidioidomycosis is often a self-limiting disease. However, as one of the granulomatous infections may leave an asymptomatic residual pulmonary nodule that mimics malignancy, particularly in patients with risk factors. We report an interesting case of a 72-year-old African American male with a smoking history and right upper lobe (RUL) pulmonary nodule that evolved into cavitation and abscess who, presented with right hydropneumothorax, was found to be due to coccidioidomycosis.

Methods: Retrospective case report.

Results: A 72-year-old African American male with a history of hypertension, type 2 diabetes, chronic kidney disease (CKD), and a 50-pack-year tobacco smoking history presented to our institution with large right hydropneumothorax. (Figure a and b). Records indicated that 3 years before this presentation he was found to have a 2.2 cm RUL pulmonary nodule. This was biopsied later when it enlarged to 3.3 cm (Figure c). Histopathology showed caseating necrosis without evidence of malignancy. The nodule developed central cavitation with a central mass, 1 year before presentation. Upon admission, he underwent thoracentesis and chest tube placement and was placed on broad-spectrum antibiotics due to leukocytosis and suspicion of bacterial empyema. He was found to be hypercalcemic with elevated ionized Ca++. Due to his risk factor of living in central California and working as a truck driver, serum coccidioidomycosis serology was obtained and became positive for IgM and IgG immunodiffusion with complement fixation of 1:16 (ARUP). He was placed on posaconazole 400 mg to cover aspergillosis and other molds (presumptive fungal ball “mass” in the center of his cavity one year ago). His pleural fluid grew *Coccidioides immitis*, confirmed by MALDI-TOF (ARUP) and his treatment was switched to fluconazole 800 mg daily. He improved clinically and his hypercalcemia resolved during his 3 weeks of admission. His chest tube was taken from suction to water seal and eventually removed and was switched to a Heimlich valve which he was discharged with to follow up as an outpatient. Pleural fluid cytology came back negative for malignancy. He perhaps had a right upper lobe pulmonary infection with a residual nodule. Due to possible immunosenescence and his comorbidities, his nodule was caveated. Over several months, he must have developed an abscess inside his cavity which eventually ruptured into the pleural space and collapsed his lung due to the presence of a bronchopulmonary fistula.

Coccidioidomycosis Pulmonary Nodule, A Master of Disguise (continued)

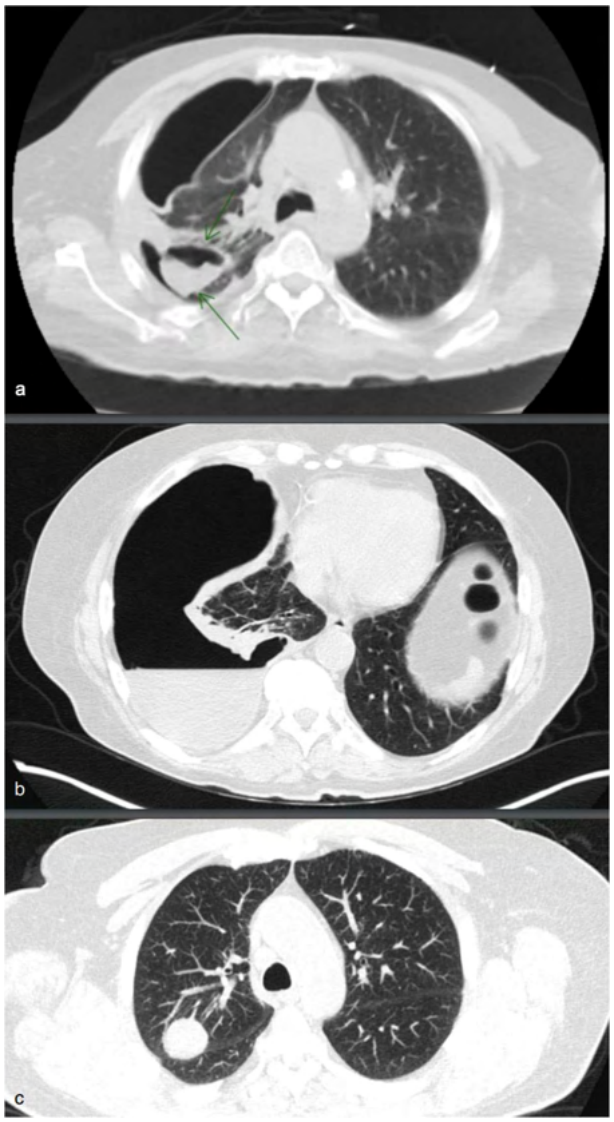

Figure a: CT chest indicating rupture of RUL abscess to pleural space; Figure b: CT chest with hydropneumothorax; Figure c: CT chest with RUL nodule 2 years prior to admission, enlarged to 3.3 cm.

Conclusion: The workup for pulmonary nodules in the endemic area of coccidioidomycosis should start with obtaining a detailed history and serology to avoid unnecessary invasive procedures such as biopsy and delay in diagnosis. African American and Filipino patients are particularly at risk for complications and dissemination. Making a prompt diagnosis and initiation of treatment is paramount.

49. Steroid in a Case of Coccidioidomycosis Meningoencephalitis with Complicated Hydrocephalus, When the Left and Right Stopped Talking

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Abstract

Introduction: Disseminated coccidioidomycosis occurs in only 1-5% of cases and spinal and meningoencephalitis by far are the most severe consequential form of dissemination. We present the case of a 24-year-old Hispanic male with 4 years of fentanyl abuse and malnutrition who was diagnosed with pulmonary coccidioidomycosis with spinal epidural abscess, meningoencephalitis, complicated non-communicating hydrocephalus who avoided bilateral shunting using steroids.

Methods: Retrospective case report.

Results: A 24-year-old Hispanic male with 4 years of fentanyl abuse and severe malnutrition presented to our institution after 5 months of weight loss, cough, headache, recent onset ataxia, and 3 days of daily seizures. Urgent computed tomography (CT) of the head showed severe hydrocephalus with transependymal resorption that was followed with immediate insertion of right frontal ventriculostomy (EVD). The ventricular fluid had 46 cells/mm^3. Further work-up included CT chest that showed bilateral pulmonary infiltration with a left upper lobe cavitary lesion. Coccidioides serology returned positive for IgM and IgG immunodiffusion (ID), with complement fixation (CF) of 1:512. (ARUP). He was initiated on 1000 mg of fluconazole. Lumbar puncture revealed yellow fluid, WBC count of 13 cells/mm^3, glucose of < 10 mg/dL, protein of 1,974 mg/dL, Coccidioides IgM and IgG ID with CF of 1:4. Magnetic resonance imaging (MRI) of brain and spine showed severe basilar leptomeningeal enhancement and an epidural abscess posterior to the thecal sac extending from the first thoracic vertebrae to the S1. Due to the inoperable epidural abscess, Liposomal amphotericin B (LamB) was added. The right Ventriculoperitoneal (VP) shunt was placed. The patient briefly improved but in the following days developed lethargy and left cranial sixth nerve palsy. CT of the head showed worsening hydrocephalus more on the left leading to revision of the right frontal VP shunt and placement of a new left EVD. MRI brain showed worsening of basilar leptomeningeal enhancement, so fluconazole was changed to isavuconazole. The neurosurgery team has changed the setting of the EVD and the VP shunt without success due to perhaps low-pressure hydrocephalus and severe basilar leptomeningeal enhancement creating a new functional noncommunicating between left and right ventricles. He was placed on 20 mg of dexamethasone and responded clinically. Left EVD was clamped and

Steroid in a Case of Coccidioidomycosis Meningoencephalitis with Complicated Hydrocephalus, When the Left and Right Stopped Talking (continued)

eventually removed and bilateral shunting was avoided. The patient was de-escalated from the neurology intensive care unit. After 7 days of dexamethasone 20 mg the plan is to taper by 4 mg every 4 days. He will remain in the hospital until he is stabilized for outpatient management to finish at least 12 weeks of LAmB concomitant with lifetime isavuconazole.

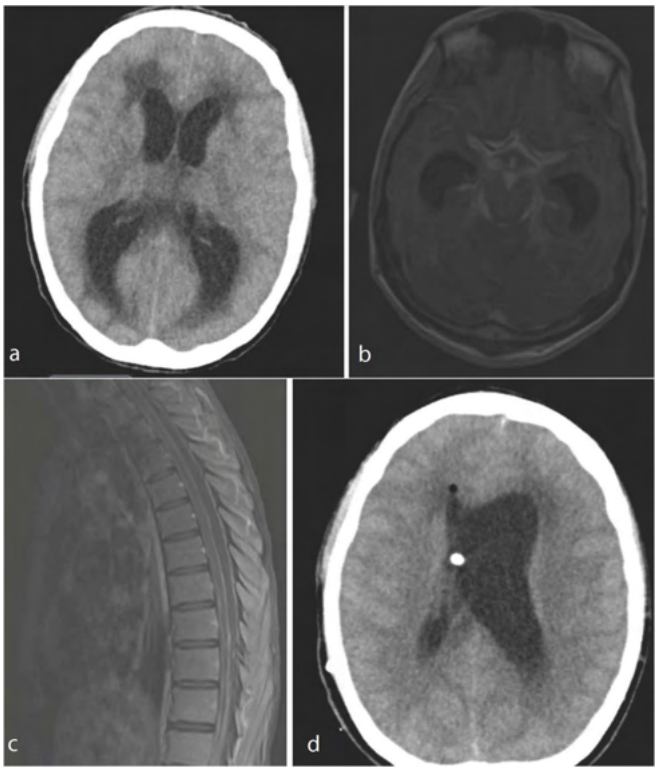

Figure a: CT brain showed severe hydrocephalus with transependymal resorption; Figure b: MRI brain shows extensive basilar enhancement; Figure c: MRI T-spine shows epidural abscess posterior to the thecal sac extending from the thoracic vertebrae to S1; Figure d: CT brain shows right VP shunt after revision and left ventricle hydrocephalus before left EVD.

Conclusion: There are multiple publications about the utilization of steroids in disseminated CNS coccidioidomycosis. To our knowledge, this is a unique case of hydrocephalus that steroids assisted in avoiding extra shunting.

50. Internet-based Decentralized Clinical Trial for Oteseconazole as a Salvage Consolidation Therapy for Those Intolerant of First-line Therapy

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Abstract

Introduction: A Mycoses Study Group Education & Research Consortium (MSGERC) clinical trial will evaluate the safety and efficacy of oteseconazole as a salvage consolidation therapy for patients with coccidioidomycosis who are intolerant of or fail standard therapies. Conducted via a decentralized, internet-based platform (fungalstudy.org), the trial will leverage a nationwide enrollment strategy and features two cohorts: 1) an intensive in-person cohort with detailed clinical follow-up and a 2) community cohort focused on patient-reported outcomes. The community-cohort will be internet-based and any U.S. healthcare provider can refer. Participants will receive oteseconazole according to coccidioidomycosis-specific regimens and undergo comprehensive monitoring for treatment tolerability, adverse events, and therapeutic outcomes.

Methods: Eligibility criteria include adults aged 18 years or older diagnosed with non-CNS coccidioidomycosis; intolerance, failure (worsening/recurrence of disease-related symptoms, re-admission for same infection, etc.), or unavailability of current first-line consolidation therapy; and an anticipated need for at least six additional months of antifungal treatment. Additional criteria include absence of renal and liver failure, no concurrent enrollment in other investigational trials of antifungal therapy, no history of hypersensitivity to azoles or study drug components, and no current pregnancy or breastfeeding and if able to become pregnant, the participant must agree to birth control for 24 months after the last dose. Participants must have reliable internet access to complete electronic follow-up surveys and provide informed consent.

Results: The primary endpoint, assessed using the Win Ratio, includes serious adverse events (e.g. death, all-cause re-hospitalization, permanent neurologic deficit, etc.), treatment discontinuation due to intolerance and therapeutic failure. Secondary endpoints examine liver function, symptom improvement, and functional status over 12 months via monthly follow-up with PROMIS-29 scoring and 10-point visual analogue scale questionnaires. Optional sub-studies will explore serum drug levels and genetic factors influencing drug metabolism.

Internet-based Decentralized Clinical Trial for Oteseconazole as a Salvage Consolidation Therapy for Those Intolerant of First-line Therapy (continued)

Conclusions: By addressing the limitations of current azole antifungal therapies, such as hepatotoxicity, poor bioavailability, and drug-drug interactions, this trial seeks to establish oteseconazole. a safe and effective alternative therapy. Data will be crucial for future antifungal treatment strategies.

51. HVAC-style Air Filters Attached to Evaporative Coolers Outside San Joaquin Valley Homes Trap *Coccidioides* Spores, Minimizing Indoor Pathogen Exposure Risk

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Abstract

Introduction: Some low-income residents of California's San Joaquin Valley are vulnerable to airborne contaminant exposure at home due to the utilization of low-cost evaporative (swamp) coolers, which draw unfiltered air indoors, to cool residences. Such airborne contaminants include ambient air pollution, wildfire smoke, and potentially *Coccidioides* spp. spores. Through the FRESSCA (Filtration for Respiratory Exposure to wildfire Smoke from Swamp Cooler Air) study, residents of Fresno and Kern Counties in the southern San Joaquin Valley (specifically, agricultural workers and their families) installed MERV 13 HVAC-style filters on the outside of their swamp coolers to trap air pollution before it was drawn indoors. The filters were in operation for 2.5 months during the summer/fall seasons of 2023. We assessed these filters for the presence of *Coccidioides* DNA, quantified spore concentrations, and estimated the infection risk and cases averted via entrapment of spores on filters.

Methods: We obtained 15 HVAC-style air filters from the California Department of Public Health and assessed the filters for *Coccidioides* DNA. Briefly, filter media was submerged in buffer and vortexed to remove all biomass from the filter. The biomass was pelleted via centrifugation, and total genomic DNA was extracted via the Qiagen DNeasy PowerSoil Pro kit. We assessed the presence of *Coccidioides* DNA using the CocciEnv qPCR assay. For a subset of filters, we ran digital PCR (dPCR) using an assay targeting a single-copy gene specific to *Coccidioides*, and we estimated the gene copy number present in each sample. Using this, we inferred the total *Coccidioides* spore count and spore concentration (spore counts per volume of air filtered) for each sample.

Results: Our full results are pending. Thus far, we have detected *Coccidioides* DNA in 5 out of 5 filters analyzed via qPCR, and plan to analyze the remaining samples over the next month.

Conclusions: *Coccidioides* spores can enter the indoor environment through swamp coolers in the southern San Joaquin Valley. However, HVAC-style filters attached to the outside of swamp coolers trap *Coccidioides* spores before they enter the home. This low-cost intervention has the potential to lower *Coccidioides* exposure risk and coccidioidomycosis infection rates.

52. A Case of Reaction to Liposomal Amphotericin B as the last resort for treatment of spinal disseminated Coccidioidomycosis: A desperate Situation

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Abstract

Case Report: Disseminated coccidioidomycosis (CM) occurs only in 1-5% of cases. Spinal CM particularly needs special attention and aggressive treatment with amphoteric to avoid contiguous spread to the central nervous system with devastating consequences. This is a case of 24-year-old Hispanic male who was diagnosed with pulmonary CM at age of 16 without proper access to care. He developed soft tissue abscess since of age of 22 and was being treated as bacterial soft tissue infection with antibiotics and frequent incision and drainages. He presented to our institution after a local surgeon noticed that the soft tissue abscess in his lumbar area was tunneling to deeper muscular anatomy and he would need imaging and further work up. His work up was consistent with osseous dissemination of CM to several ribs and T spine from T7 to T12 with multiple large paravertebral abscesses on both sides of the spine including psoas muscles (Attached figure). Fortunately, there was no neurological deficit without epidural involvement. He underwent multiple drainage placement with interventional radiology and purulent discharge of all drainage grew coccidiodal spp. He was started on 200 mg (5mg/Kg) of Liposomal Amphotericin B (LAmB). Within 5 minutes of the first infusion, he developed a severe drug reaction with tachycardia as high as 130 beats per minute, diaphoresis, and rigors.LAmB was immediately stopped and rapid response team resuscitated him with intravenous IV fluid with the resolution of symptoms over the next hour. After contacting the hospital pharmacy supply it was revealed that the other available lipid formulation of Amphotericin B, (Amphotericin B Lipid Complex, or ABLC has had manufacturing issues and has been on backorder in the United States for several months. Due to his immediate need, it was decided to desensitize him against LAmB. He was placed on hydration with 500 ml of normal saline (NS) starting 3 hours prior with 25 mg of diphenhydramine, 650 mg of acetaminophen, and 25 mg of meperidine Intramuscular all to be given 30 minutes before the infusion. The dose of LAmB was reduced to 0.2mg/ml (50mg/100 ml of D5W) and the infusion rate was reduced to over 4 hours (12.5mg/hr) and post-infusion hydration with another 500 ml of NS. He tolerated the first dose with no immediate or delayed reaction. The escalated dose of 75 mg (18.75mg/hr 100 mg 25mg/hr 150 mg (37.5mg/hr) and eventually back to 200 mg (50mg/hr) within the next 4 days with the same pre and post-infusion fashion was utilized and he tolerated the escalated dosing process with no reactions. He remained on daily LAmB dosing for the next 14 days inpatient and was discharged on a 3 times a week regimen infusion to the infusion center. Several stepwise protocols have been published for LAmB desensitization. In this case, a simple 4-step fashion escalating dose protocol was successfully utilized without the need for time assuming pharmacy compounding. (No Table Selected)

53. A Unique Presentation of Primary Cutaneous Coccidioidomycosis, By Infecting Burn Wounds with Extensive Contiguous Local Spread

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Abstract

Introduction: Coccidioidomycosis, also known as Valley Fever, is a fungal infection caused by the dimorphic fungus *Coccidioides* spp. Cases of primary cutaneous coccidioidomycosis are exceedingly rare as most cutaneous cases are usually a form of dissemination after pulmonary infection. Less than 100 cases have been reported to date.

Case Presentation: This is a case of a Hispanic 42-year-old male from Central California Valley who underwent extensive burn injury to his right upper arm and shoulder with an ongoing open wound on his right antecubital area due to burn contracture. He noticed the development of skin lesions around the chronic wound which slowly spread upwards towards his upper arm, shoulder, and upper back over his burn scar area. (Figure 1). He came to our institution due to the extension of his lesion with oozing blood and associated excruciating pain from lesions. Before admission a local clinic obtained a skin punch biopsy which came back positive for the presence of coccidioidomycosis spherules with endosporulation. 4 fungal culture swabs were collected from oozing lesions and all were identified as *Coccidioides* spp. His serology returned positive by precipitin IgG at our institution and positive immunodiffusion IgG with complement fixation of 1:2024 at the ARUP reference laboratory. He was started on liposomal amphoteric B daily for 14 days, with significant improvement in his lesions, less oozing blood, and the closing of open wounds. He was discharged to our infusion center to continue liposomal amphotericin B for another 12 weeks on a 3-times-a-week basis. After his 14 weeks total of liposomal amphotericin B infusions, the patient reported no constitutional symptoms, as well as significant resolution of his pain and lesions. His most recent CF titer level is 1:64, and he was then started on Posaconazole 400mg daily. He will continue on this oral dosage until further resolution of his CF titers, current literature recommends oral treatment for 1-3 years for cutaneous cocci infections.

A Unique Presentation of Primary Cutaneous Coccidioidomycosis, By Infecting Burn Wounds with Extensive Contiguous Local Spread (continued)

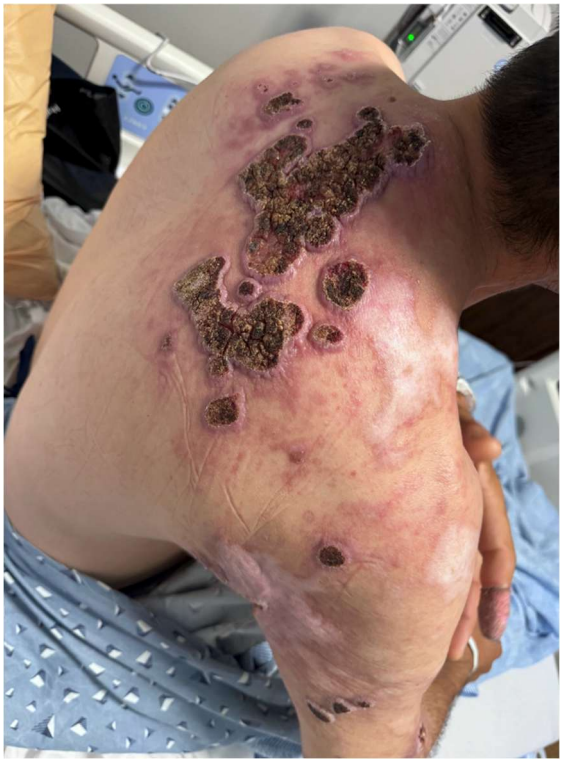

Figure 1

Conclusions: It is conceivable that he acquired primary cutaneous CM infection via an open wound by working on a dairy farm in an endemic area with significant exposure to soil and dust. The reason behind the local contiguous spread from the primary infection site towards his upper arm shoulder and upper back is unknown. Still, it could be hypothesized that the mounted tissue T cell immunity and interruption of lymphatics in the burn scar tissue may have played a role in the development and spread of these lesions. The spread did not go beyond the burn scar margin, and all his surface swabs of lesions grew Coccidioides spp. This brings up the question, are the extensive cutaneous cocci lesions potentially contagious due to the growth of mycelia at the surface of the skin lesions? Primary Cutaneous Cocci is rare and will need further investigation to understand the characteristics and pathophysiology. This rare dermatological manifestation can be mistaken for other cutaneous lesions, so it is important to consider this diagnosis in endemic areas.

54. Intra-abdominal Pseudocysts Complicating Ventriculo-Peritoneal Shunts in Coccidioidal Meningitis

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Abstract

Introduction: Ventriculoperitoneal (VP) shunt placement is the most common method of cerebrospinal fluid (CSF) diversion in patients with Coccidioidal meningitis (CM)-associated hydrocephalus. The distal end of the VP shunt catheter in the abdomen can cause complications such as infection, organ perforation, and CSF ascites (1). A rare complication is the formation of abdominal pseudocyst (APC). APCs form when CSF resorption at the distal site is impaired with the resultant collection of walled-off CSF surrounded by non-epithelial serosal lining (2). This may cause symptoms and further complications related to the shunt function. While APCs have been well described in pediatric patients requiring VP shunts for congenital hydrocephalus, literature describing this complication in patients with Coccidioidal meningitis is sparse (3,4). We aim to characterize the presenting clinical features, underlying causes, imaging findings, and management strategies in patients with Coccidioidal meningitis and VP shunt placement who developed abdominal pseudocysts. While APC is an uncommon complication, physicians managing patients with chronic infections needing VP shunts should be familiar with its presentation and management.

Methods: We conducted a retrospective chart review of patients with CM from 2010 to 2024 at a tertiary referral center in Fresno, California, using ICD-9 and ICD-10 codes for case identification. We identified patients who required VP shunt placement for the management of CM. Among these, patients who developed APCs were included in this analysis. Data were collected on demographics, underlying conditions, clinical findings, shunt characteristics, imaging findings, and management strategies. The Community Medical Centers Institutional Review Board approved the study.

Results: Our preliminary analysis identified 65 patients with CM who required VP shunt placement, of whom 10 (15%) developed APC as a complication (Table). APC development occurred between 6 months and 9 years after initial shunt placement. All patients were on antifungal therapy, though adherence could not be assessed. Five of the 10 patients had a history of prior shunt revision surgeries. The most common presenting symptoms included abdominal pain, headaches, and altered mentation. Ongoing active Coccidioidal infection, confirmed by CSF or cyst fluid cultures, serology, or PCR, was detected in 7 out of 10 patients. Secondary bacterial infection was identified in 2 of the 10 patients. Management in all cases required revision or removal of the distal catheter (or entire shunt), along with pseudocyst drainage.

Intra-abdominal Pseudocysts Complicating Ventriculo-Peritoneal Shunts in Coccidioidal Meningitis (continued)

Table: Characteristics of Coccidioidal meningitis (CM) patients with ventriculoperitoneal (VP) shunt-associated abdominal pseudocysts (APC) (n =10)

Patient	Age at diagnosis (years)/Race	Shunt duration	Symptoms	Abdominal Imaging	APC fluid cultures	Management	Current Status of Life
1	46/Hispanic	2 years	Abdominal pain	8 x 25 x 19 cm fluid collection	*Coccidioides immitis, Staphylococcus aureus*	Percutaneous drainage	Alive
2	25/Caucasian	2 years	Abdominal pain	13 x 13 x 11.8 cm fluid collection	*Coccidioides immitis*	Laparoscopic drainage and shunt revision	Alive
3	36/African American	3 years	Altered mentation	11.7 x 4.4 x 7.3 cm fluid collection	Not sent	Laparoscopic drainage and shunt revision	Deceased
4	49/Caucasian	1 year	Altered mentation	7.4 x 5.0 cm fluid collection	Not sent	Laparoscopic drainage and shunt revision	Deceased
5	21/African American	2 years	Altered mentation	No fluid collections seen on imaging	Negative	Laparoscopic drainage and shunt revision	Alive
6	31/Hispanic	6 months	Abdominal pain	12.2x13.3x2.6 fluid collections	*Staphylococcus aureus*	Percutaneous drainage	Alive
7	52/Caucasian	3 years	Abdominal pain, headaches and altered mentation	7.7 x 5.4 cm fluid collection	Not sent	Laparoscopic drainage and shunt revision	Alive
8	18/Caucasian	3 years	Abdominal pain, constipation and headaches	9.3 x 5.7 x 4.3 cm fluid collection	*Coccidioides immitis*	Laparoscopic drainage and shunt revision	Alive
9	40/Caucasian	5 years	Headaches and altered mentation	5.5x 9 and 6 x 2.5 cm fluid collection	*Coccidioides immitis*	Laparoscopic drainage and shunt revision	Deceased
10	42/Caucasian	9 years	Abdominal pain and altered mentation	16 x 7 cm fluid collection	Not sent	Laparoscopic drainage and shunt revision	Alive

Conclusions: Fifteen percent of patients in our study population developed APC complicating VP shunts. Patients either presented with abdominal pain or change in mentation due to shunt malfunction and all of

Intra-abdominal Pseudocysts Complicating Ventriculo-Peritoneal Shunts in Coccidioidal Meningitis (continued)

them needed surgical management with shunt revision or replacement. The pathophysiology of APC development in chronic VP shunts remains poorly understood. Potential mechanisms include infections, foreign body reactions to the shunt, elevated CSF protein levels, and peritoneal adhesions from prior shunt revisions. In patients with CM, persistent coccidioidal infection in the shunt and cerebrospinal fluid and the ensuing chronic inflammation-induced thickening of the peritoneal serous membranes may play a crucial role in pseudocyst formation. Further studies are needed to elucidate the mechanisms underlying APC development and to determine optimal management strategies for preventing this serious complication.

References

1). Johnson RH, Einstein HE. Coccidioidal meningitis. Clin Infect Dis. 2006 Jan 1;42(1):103-7

2). Fatani GM, Bustangi NM, Kamal JS, Sabbagh AJ. Ventriculoperitoneal shunt-associated abdominal cerebrospinal fluid pseudocysts and the role of laparoscopy and a proposed management algorithm in its treatment. A report of 2 cases. Neurosciences (Riyadh). 2020 Aug;25(4):320-326

3). Cummins DD, Morshed RA, Goldschmidt E, Kuo YH. Comparison of Shunt Outcomes for Nonbacterial Infection Hydrocephalus with Common Hydrocephalus Etiologies: A Retrospective Case-Control Study. World Neurosurg. 2022

4). Hanak BW, Bonow RH, Harris CA, Browd SR. Cerebrospinal Fluid Shunting Complications in Children. Pediatr Neurosurg. 2017;52(6):381-400

55. Intersecting Epidemiology: A 2020 Focus on When Valley Fever Meets Tuberculosis

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Abstract

Introduction: Following the disruptive events in 2020, reported tuberculosis (TB) fell to record lows, likely reflecting under detection of TB, to a ten year high in 2023. Cross matches between coccidioidomycosis (cocci) and TB prior to 2020 did not find a difference in outcome between TB patients with cocci and those without; however, delay in diagnosis can result in patients presenting with a higher burden of disease and may result in poor outcomes particularly in vulnerable populations. Diving into the subset of TB patients who have also been reported with cocci allows us to explore the intersection of these two diseases with the goal of informing public health strategies for managing these co-infections. Since 2020, the Arizona Department of Health Services (ADHS) TB program has incorporated cocci as a reported comorbidity among TB patients; however, data cross match analysis offers a more comprehensive snapshot of the trends in people reported with both TB and cocci.

Methods: Data cross match of reported TB in Arizona (2009-2023) with reported Valley Fever (2009-2023).

Results:

1. **TB patients reported with Cocci have trended upwards.** Since 2020, twelve percent of TB cases have also been reported to have cocci, an increase from nine percent found in the prior cross match (2009-2016). The vast majority appear to be dual diagnosis (78%), and all but fourteen percent overlap over a two year time period.
2. **Individuals reported with Cocci have a TB risk comparable to high burden countries.** From 2020 to 2023, 94 of 43,517 reported cocci cases also had been reported to have TB. While TB is rare in Arizona, ranging from 1.8 to 2.7 cases per 100,000 during the same time period, individuals with cocci have a much higher TB risk. A case rate of 216 TB cases per 100,000 is higher than Mexico (29/100,000) and comparable to India (195/100,000) and Nigeria (219/100,000).
3. **TB with concurrent cocci remains curable, despite concerning trends.** Since 2020, smear positivity was high with 55% presenting with respiratory smear positive results, indicating patients were diagnosed later in the disease continuum. During the same time period, an increased death rate was observed during TB treatment among those who were reported with cocci (17%) compared to those without cocci (9%). One year, 2023, accounted for the majority of deaths in this group. Despite higher mortality rates, only 54% of deaths in this cohort were attributed to complications due to TB.

Intersecting Epidemiology: A 2020 Focus on When Valley Fever Meets Tuberculosis (continued)

Conclusion: Working patients up simultaneously for cocci and TB has reinforced that concurrent diagnosis is not unusual. The majority of this cohort were evaluated for TB at the same time as cocci, suggesting that delays in diagnosis are affecting both morbidities. While there is a concerning trend in poor outcomes among this group since 2020, only a portion can be attributed to delayed TB diagnosis.

56. Genetically Diverse *Cocciodiodes posadasii* Identified at a Single Site in Arizona with Evidence of Transmission Among Non-Human Hosts and the Environment

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

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 complex environmental and animal samples. The system was initially designed to target all coding region sequences identified in Silveria, a laboratory strain with a completed genome. Using this approach, we attempted to obtain *C. posadasii* genomic data from PCR-positive soil, air filter, and rodent samples from a single site in Arizona.

Results: We generated partial *C. posadasii* genomes from a diverse set of DNA extracts obtained from complex samples, including two air filters, one soil sample, and one 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 genomes within a relevant phylogenetic framework of publicly available *C. posadasii* genomes. Soil, air filter, and mouse enrichments from a single Arizona field site over a similar time frame displayed multiple genotypes, suggesting that our understanding of local *C. posadasii* diversity and distribution in the environment and natural animal reservoirs is under characterized and highlighting the utility of culture independent genomic methodologies to enhance our understanding of Valley Fever risk and epidemiology.

Conclusions: We utilized the *C. posadasii* DNA capture and enrichment system that we developed to characterize the diversity of *C. posadasii* in four complex samples from a single sampling site in Arizona

Genetically Diverse *Cocciodiodes posadasii* Identified at a Single Site in Arizona with Evidence of Transmission Among Non-Human Hosts and the Environment (continued)

using ~50% of the core genome of this species. Use of this system on additional environmental and animal samples will provide researchers with a more comprehensive understanding of *C. posadasii* distribution and diversity circulating in the environment and natural animal reservoirs and inform epidemiology and risk assessment activities that can improve targeted strategies to reduce human exposure.

57. Pediatric Disseminated Coccidioidomycosis Inpatient Cases at UCLA

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Abstract

Introduction: Disseminated coccidioidomycosis (DCM) is a rare complication of Valley Fever, occurring in less than 1% of cases. Dissemination is even less frequent in pediatric cases. This reduced occurrence could be attributed to fewer risk factors, differences in immune system function, and limited exposure to soil, the primary reservoir for the fungus. The CDPH reports that 2024 had the highest ever number of cases with Valley Fever. Whether pediatric cases follow the same trends have not been determined. We investigated the prevalence of pediatric cases in California, analyzing clinical outcomes and management strategies to enhance patient care.

Methods: The University of California (UC) Data Warehouse was utilized to identify trends in pediatric inpatient cases across all UC hospitals. For pediatric cases admitted at UCLA, we obtained informed consent. Advanced immune assessment included intracellular cytokine staining on cultured CD4+ T cells. All samples were processed for sequencing.

Results: The frequency of pediatric coccidioidomycosis inpatient cases mirrored that of the adult reported cases. There was a 5-fold increase in cases admitted for coccidioidomycosis in 2024 versus 2023 or 2022. Our team was consulted on seven DCM patients admitted at our institution for immune work up (3 disseminated with meningitis, 4 disseminated without meningitis). The ages of the cases ranged from 10 months to 17 years. None had a history of immune compromise. T cell cytokine assays showed increased Th2 skewing, proportionately higher than we saw in adults. Genome and RNA sequencing identified interesting variants that could explain immune susceptibility.

Conclusion: While the number of pediatric cases with disseminated coccidioidomycosis continues to rise, there is a scarcity of research focusing on this patient population. Our results indicate that susceptibility to DCM in this population is driven by both T-cell and genetic factors.

58. Withdrawn

59. Immuno-phenotyping of the T-cell Compartment in Coccidioidomycosis

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Abstract - Declined to Publish

60. Silent Osseous Dissemination with Cutaneous Coccidioidomycosis: What You See and What You Don't See

Shikha Mishra[1,2,3], Patrick Betadam[2,4], Robert Collins[2,4], Safa Mousavi[1], Chirag Aulakh[2], Bianca Torres[1], Rupam Sharma[2], Jigar Patel[1,2,3], Michelle Fang[1,5], Carlos D'Assumpcao[1,2,3], Royce Johnson[1,3], Rasha Kuran[1,3]
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Abstract

Introduction: Disseminated coccidioidomycosis (CM) is rare, accounting for 0.5%–2% of all CM cases. Cutaneous involvement is a more common and mostly benign form of dissemination occurring in 15% to 67% of cases, often presenting as painful nodules, ulcers, abscesses, or unidentified skin lesions. Osseous involvement is present in 10% to 20% of cases, and typically presents with pain, swelling, or lytic lesions. Asymptomatic presentation of osseous disease has not been studied to our knowledge. It would not be readily recognized by routine medical care. We present a case of disseminated CM in a young, healthy patient who experienced cutaneous lesions and was also found to have asymptomatic osseous involvement.

Methods: This is a case report with patient and photo consent with approval from the Kern Medical Institutional Review Board. A literature search was performed on PubMed and Google Scholar using keywords such as "cutaneous or skin coccidioidomycosis", "osseous coccidioidomycosis", and "disseminated coccidioidomycosis".

Results: A 24-year-old African American woman with no significant past medical history from Bakersfield, California, was referred from dermatology clinic to the emergency room (ED) at Kern Medical due to concern for possible fungal infection based on growth of mold from culture of a lesional biopsy. The patient reported a 7-month history of chronic productive cough with clear sputum and a 2-month history of pustular lesions on her right jaw that progressed over 3 to 4 weeks into purplish lesions on her left cheek, upper chest, shoulder, and left mid-back. She had no other symptoms or complaints at that time. On physical examination in the ED, a 3 × 3 cm pustular mass at the right upper chest wall, along with an erythematous pustular rash over the right jaw (figure 1) and left cheek.

Chest X-ray at admission showed diffuse paratracheal and hilar soft tissue prominence with subsegmental left base airspace disease. Chest computed tomography (CT) revealed destructive pulmonary lesions, and CT of the abdomen and pelvis incidentally revealed lytic lesions of the left posterior 10th rib and the right

Silent Osseous Dissemination with Cutaneous Coccidioidomycosis: What You See and What You Don't See (continued)

anterior iliac bone (11 mm), along with multiple lytic lesions of bilateral pubic bone. Complete blood count with differential revealed microcytic anemia (MCV 73.6 fL, Hgb 8.6 g/dL) and eosinophilia (absolute eosinophils 600 cells/mcL). A comprehensive metabolic panel showed hyponatremia (Na 132 mEq/L) and mildly elevated liver enzymes (ALP 149 units/L, ALT 93 units/L, and AST 110 units/L), and an ultrasound of the liver was unremarkable. Sputum, tissue, and fungal cultures were all obtained, as well as Coccidioides antibody immunodiffusion with complement fixation (CF). Coccidioides IgM and IgG antibodies were reactive and CF titers ≥ 1:512.

Nuclear medicine whole-body bone scan showed increased uptake in the left posterior 10th rib although radiotracer accumulation in the bladder obscured the pubic and anterior iliac bones (figure 2), so a CT-guided core biopsy of the lytic lesion of the symphysis pubis was performed.

Pathological examination of a skin punch biopsy of the right superior lateral neck showed sporulating spherules, consistent with CM. The bone biopsy revealed numerous spherules containing endospores within the bone marrow, substantiating the CM diagnosis, and growth of *Coccidioides immitis* from the bone biopsy specimen confirmed the diagnosis. Sputum culture also grew *C. immitis*. The patient was discharged on fluconazole 800 mg daily.

The patient followed up two weeks after discharge at the Valley Fever Institute in Bakersfield, CA. She reported significant improvement in the lesions on both her face and chest after two weeks of fluconazole. At this time, the patient reported pain in her left mid-back and right anterior iliac bone. Two months later, after multiple missed appointments, a new enlarging fluctuant mass was found on her left mid-back which interfered with her sleep. A plan was made for an IR-guided aspiration, but due to insurance issues, the patient was subsequently lost to follow-up.

Silent Osseous Dissemination with Cutaneous Coccidioidomycosis: What You See and What You Don't See (continued)

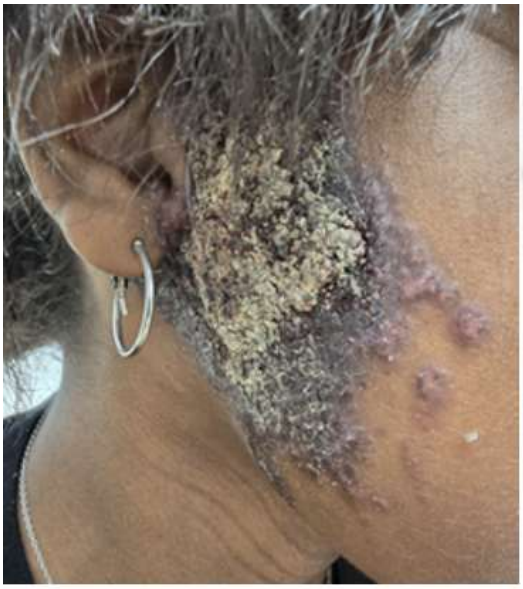

Figure 1: Cutaneous Manifestation

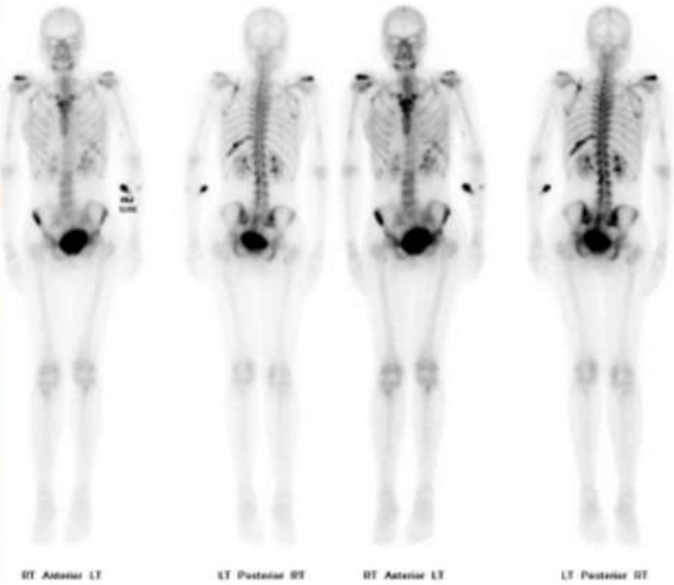

Figure 2: Whole Body Bone Scan

Conclusion: Osseous CM is a rare but insidious form of disseminated CM that can impact clinical management in the absence of early detection. This is particularly important with cases involving dissemination to the axial skeleton due to differences in possible complications and management. While diagnosis is most often based on the onset of osseous symptoms, this case demonstrates that osseous CM can initially present asymptomatically, which raises the question of whether osseous CM screening should be standard in patients with other forms of dissemination. Clinicians must remain alert for silent dissemination, as the identification of sites of involvement is significant to management, including choice and duration of therapy.

61. Paralysis Associated with Coccidioidal Meningitis: A Review of 12 Cases

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Abstract

Introduction: Disseminated coccidioidomycosis occurs in approximately 1% of coccidioidomycosis cases and can affect the skin, bone and central nervous system (CNS). Coccidioidal meningitis (CM) is a severe form and represents one third to one half of cases. CM typically presents with neurological symptoms, such as headache or altered mental status. In severe cases, CM can result in paralysis which could be due to vasculitic infarction or arachnoiditis. Hemiplegia is often due to vasculitic infarction. Paraplegia and quadriplegia are most often due to arachnoiditis. This study explores neuroradiology findings, and outcomes in patients with CM who develop paralysis. The purpose of this study is to provide insights into diagnosis evaluation and treatment of these patients.

Methods: This retrospective study reviewed the medical records of patients diagnosed with CM and associated paralysis seen by Infectious Disease (ID) physicians at the Valley Fever Institute in Bakersfield, CA. Institutional approval was obtained from Kern Medical's Institutional Review Board and patient consent was waived. Patients were included in the study if they had a compatible clinical illness, compatible cerebrospinal fluid (CSF), serum and CSF immunodiffusion and complement fixation or microbiologic evidence of *Coccidioides* and neuroimaging. Where available, data was analyzed at three key time points, time of CM diagnosis, onset of paralysis and time of the patients most recent outcome assessment.

Results: A total of 12 patients were included in this review, all of whom developed paralysis associated with CM despite antifungal therapy. Three of the patients did not have data at the time of CM diagnosis. Data was not available for two of the patients at onset of paralysis. The mean age was 51.8 years (range: 30 to 67 years old), with 8 males and 4 females. The majority of patients were of Hispanic ethnicity (66.7%, 8/12). Comorbidities included hypertension (33.3%, 4/12), diabetes mellitus type 2 (25%, 3/12) out of which, 1 was controlled (A1C: 6.6) and 2 uncontrolled (A1C: 7.6, 11), depression (25%,3 /12), and HIV (8%, 1/12).

Paralysis Associated with Coccidioidal Meningitis: A Review of 12 Cases (continued)

Eight patients had Ventriculoperitoneal (VP) shunt (66.6% (8/12), two patients at CM diagnosis, two at paralysis onset, and four after paralysis. Neuroradiology findings at the onset of paralysis revealed basilar leptomeningeal enhancement (58.3%, 7/12), hydrocephalus (41.6%,5/12), vasculitic infarctions (58.3%, 7/12), arachnoiditis (41.6%, 5/12), and subarachnoid hemorrhage (8%, 1/12). No patients had mass lesions in the brain. One patient had a right middle cerebral artery aneurysm, etiology uncertain. Neurologic findings corresponded to the infarction location. Patients with vasculitic infarction received antifungal therapy and dexamethasone. Majority of patients with arachnoiditis received antifungal therapy and glucocorticoids. Ten patients showed partial neurological improvement, while two patients died.

Conclusion: This study highlights the profound morbidity associated with CM in patients with paralysis due to vasculitic infarctions or arachnoiditis that is amendable to antifungal therapy with judicious use of glucocorticoids.

62. Fine Mineral Dust Exposures as a Potential Mediator of the Association Between Racialized Residential Segregation and Coccidioidomycosis Incidence in California 2010 – 2017

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Abstract: Persistent disparities in coccidioidomycosis incidence rates have been observed across racial and ethnic groups in California, with rates highest in Black and Hispanic/Latinx populations. Racialized residential segregation is a fundamental driver of poor health, limiting marginalized populations' access to healthy environments. Of relevance to coccidioidomycosis, highly segregated communities experience disproportionate exposures to mineral dust, which have been shown to be associated with coccidioidomycosis risk. It is unclear the degree to which disparities in incidence are mediated by dust exposure, limiting the targeting of interventions that could reduce disparities. We estimated the association between residential segregation using the Index of Concentration at the Extremes—which measures how people are concentrated into the most or least privileged group within a given area—and coccidioidomycosis incidence from 2010 to 2017 at the census tract-level, and determined the degree to which exposure to fine mineral dust concentrations mediates this association. Using monthly census tract-level incident cases of coccidioidomycosis reported in California, model-derived fine mineral dust concentrations, and demographic data from the American Community Survey, we conducted a longitudinal, g-computation based mediation analysis. Residential segregation was examined as the exposure and fine mineral dust concentration as a potential mediator. We estimated the total effect of residential segregation on coccidioidomycosis incidence, the pure direct effect, the total indirect effect that acts via dust exposure, and the proportion mediated. We found a strong, positive total effect of residential segregation on coccidioidomycosis incidence, estimating that the incidence rate for census tracts consisting of all Hispanic/Latinx would be 2.96 (95% CI: 1.63, 5.56) times that of the counterfactual scenario of those census tracts consisting of all White people. The pure direct effect was 2.74 (95% CI: 1.52, 5.23) and the total indirect effect was 1.08 (95% CI: 1.02, 1.16). We estimated that 6.69% (95% CI: 1.34%, 22.01%) of the effect of residential segregation on coccidioidomycosis incidence was attributable to higher fine mineral dust concentrations within segregated census tracts. We found that fine mineral dust concentrations are a modest, but significant, mediator of the relationship between residential segregation and coccidioidomycosis incidence in California. While other potential mediators, including occupational exposures, underlying comorbidities, and access to health resources, warrant further research, near-term, season-specific reductions in exposure to ambient fine mineral dust via occupational and planning interventions—particularly in areas burdened by other environmental threats—could help prevent cases of coccidioidomycosis while reducing racial disparities in incidence.

63. RiboMarker®-Enhanced RNA Sequencing Reveals Small RNA Profiles and Transcriptomic Dynamics in *Coccidioides posadasii*

Jonathan Howard[1], Aidan Manning[1], Rachel Clark[1], Tahirah Williams[2,3], Clarissa Nobile[2,3], Sergei Kazakov[1], Sergio Barberan-Soler[1]

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Abstract

Introduction: *Coccidioides* is a dimorphic fungal pathogen responsible for Valley fever, a lung infection with significant public health implications in endemic regions. While transcriptomic studies have characterized transitions between its saprobic and pathogenic life stages, small RNA (sRNA) profiles, which have been shown to play crucial roles in host-pathogen communication and virulence in other pathogens, remain underexplored in *Coccidioides*. This study aimed to investigate the sRNA expression and RNA fragmentation patterns across the three major morphologies of *Coccidioides posadasii*—arthroconidia, mycelia, and spherules—to provide new insights into the sRNA profiles governing fungal morphological transitions and pathogenesis.

Methods: sRNA and RNA fragments were isolated from intracellular and extracellular fractions of *C. posadasii* strain NR-166 in the arthroconidia, mycelia, and spherule forms. Libraries were prepared using RealSeq RiboMarker® sRNA and RNA fragment library preparation to enhance transcriptomic coverage by incorporating typically excluded RNAs. Using a computational pipeline, the sequencing data were analyzed to identify differentially expressed sRNAs and RNA fragmentation patterns during life stage transitions in *C. posadasii*. In the pipeline, reads were trimmed with Cutadapt, aligned to the reference genome with annotated tRNA isodecoders (tRNAScan-SE), novel sRNA transcripts were identified with ShortStack, and differential expression was analyzed using DESeq2 to uncover sRNA regulatory dynamics.

Results: RiboMarker®-enhanced RNA sequencing improved the detection of diverse sRNA populations across *Coccidioides* morphologies by overcoming limitations associated with conventional library preparation methods. Principal component analysis (PCA) of expression profiles confirmed that sRNA profiles and RNA fragments were distinct at each stage of the fungal life cycle. Differential expression analysis revealed ecotype-specific transcriptomic shifts, with hierarchical clustering identifying morphology-dependent sRNA expression patterns. Notably, there was a high upregulation of INDA1 in mature mycelia, HSK1 in spherules, and elevation of key metabolic and protein transport-related transcripts during the arthroconidia-to-mycelia transition. tRNA fragments (tRFs/tDRs) displayed distinct abundance patterns, with lower overall expression levels across all morphologies.

RiboMarker®-Enhanced RNA Sequencing Reveals Small RNA Profiles and Transcriptomic Dynamics in *Coccidioides posadasii* (continued)

Transcriptomic analysis further identified a previously unannotated population of sRNA-producing loci, suggesting the existence of novel RNA species with potential regulatory roles in fungal adaptation. Figure 1 illustrates a clustered heatmap of these unannotated loci, displaying significant differential expression of sRNAs across *C. posadasii* morphologies. Cluster 1 corresponds to arthroconidia, Cluster 2 to mycelia, and Cluster 3 to spherules, highlighting dynamic transcriptomic shifts during fungal life cycle transitions. Additionally, differential fragmentation patterns of annotated and unannotated noncoding RNAs (ncRNAs) were morphology-specific. Notably, tRNA fragments were more abundant in arthroconidia and mycelia than spherules, suggesting a potential role of tRNAs in fungal adaptation and morphology-specific regulatory processes. These findings indicate that sRNA and RNA fragmentation profiles may serve as useful molecular markers of *Coccidioides* life stages.

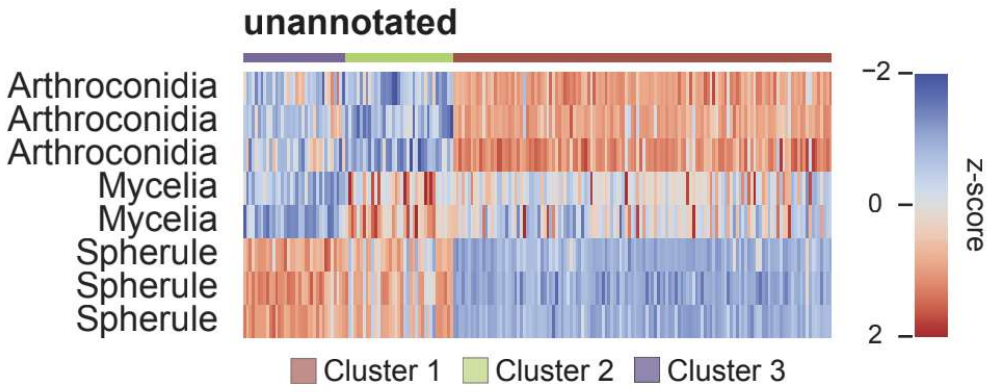

Figure 1. Clustered heatmap of unannotated ShortStack loci displaying significant differential expression of sRNAs across distinct *C. posadasii* morphologies. Cluster 1 corresponds to arthroconidia, Cluster 2 to mycelia, and Cluster 3 to spherules.

Conclusion: This study presents the first comprehensive intra- and extracellular sRNA atlas for *C. posadasii*, providing evidence of potential regulatory roles of sRNAs during fungal life cycle transitions. These findings enhance our understanding of *Coccidioides* pathogenesis and establish a foundation for biomarker discovery, with implications for developing future diagnostic and therapeutic strategies targeting fungal life stages.

64. Investigation Into a Sudden Increase in Valley Fever Cases in Arizona from January to March 2024

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Abstract

Introduction: The Arizona Department of Health Services (ADHS) collects population health and laboratory data on reportable conditions, such as *Coccidioidomycosis* (or Valley fever). During the 2024 surveillance year, a spike in Valley fever cases among Arizona residents was recorded from January to March. We sought to understand if it represented a real increase in disease incidence.

Methods: Laboratory data reported to our Medical Electronic Disease Surveillance Intelligence System (MEDSIS) from January to March 2024 was analyzed. Valley fever is a laboratory-reportable condition, only positive results are mandated to be shared with ADHS. A total of 9,987 positive laboratory results across 4,439 cases were evaluated. Observations were excluded if the case resided outside of Arizona or if the individual had been reported to public health before the 2024 surveillance year.

Results: From January to March 2024, 4,439 cases reported at least one positive Valley fever test to MEDSIS, an 89.8% increase as compared to the five-year median of 2,339 cases. Following expected demographic trends, these cases predominantly resided in Maricopa County (76.5%), were male (50.8%), and had a median age of 59 years.

To investigate the rise in the number of reported cases, our team contacted the largest reporting laboratory of positive coccidioidal tests (reporting ~55% of all annual positive coccidioidal lab results on average). While they assured that no changes in test type or testing procedures were made, the lab identified an increase in reactivity from enzyme immunoassay (EIA) kits which they reported to the manufacturer. Upon further investigation, the manufacturer identified an issue with a test component found in a single batch that was sold in December 2024, to four laboratories in Arizona. On average, these facilities report a cumulative 65% of all annual positive coccidioidal lab results. Our team then analyzed testing data to determine if this faulty batch could explain the increase in the number of cases.

After separating the laboratories by testing kit manufacturer, the four facilities that received the affected kits reported a 42.1% cumulative increase in monthly positive testing across all types of tests during the three-month period (from 1,651 labs in January to 2,346 labs in March). When broken down by test type, the increase in reported positives among affected laboratories was attributed to EIA (73.1%; 1,017 labs in Jan. to 1,760 labs in Mar.) and complement fixation (CF) (5.9%; 286 labs in Jan. to 303 labs in Mar.) testing; the

Investigation Into a Sudden Increase in Valley Fever Cases in Arizona from January to March 2024 (continued)

greatest increase was reported specifically for EIA IgG (118.2%; 669 labs in Jan. to 1,460 labs in Mar.). Moreover, those affected laboratories reported a 52.4% increase in new Valley fever cases from January to March (965 and 1,471 cases, respectively).

In comparison, laboratories whose testing kits were not purchased from the affected manufacturer recorded a cumulative decline (-17.4%; 1,566 labs in Jan. to 1,293 labs in Mar.) in the frequency of positive testing across all types. The largest reported decline occurred among immunodiffusion (ID) (-28.1%; 146 labs in Jan. to 105 labs in Mar.) and CF (-19.9%; 181 labs in Jan. to 145 labs in Mar.) testing, with the number of positive EIAs reported remaining near consistent across the three-month timespan (-1.4%; 705 labs in Jan. to 695 labs in Mar.). Specific to Valley fever case counts, other facilities witnessed a combined 43.2% decline from January to March (461 and 262 cases, respectively) 2024.

The largest reporting facility indicated that the affected kits were in use until March 2024. Immediately after, in the month of April, a statewide decline in both positive laboratory reports (-26.8%; 3,639 labs in Mar. to 2,665 labs in April) and Valley fever cases (-46.6%; 1,733 cases in March to 926 cases in April) was recorded. Laboratories affected by the manufacturing mishap reported 38.4% fewer positive tests (2,346 labs in March to 1,445 in April) and 57.0% fewer Valley fever cases in April; reported positive EIA testing fell by 54.5% with the largest decline among EIA IgG (-68.5%). In contrast, the rate of decline for reported positive testing among unaffected laboratories nearly plateaued (-5.6%, 73 fewer labs), but the frequency of Valley fever cases increased (11.8%, 31 more cases) in April.

Conclusion: The surge of Valley fever cases from January to March 2024, most likely stems from false-positive results reported by a compromised batch of EIA testing kits. The increased frequency of positive reports by a single test type (EIA IgG) from four reporting facilities in Arizona, highlights the importance of closely monitoring laboratory results and case reports as aberrations are detected as has happened in the past. Prompt notification on changes made to laboratory testing procedures or the efficacy of testing kits, ensures the accuracy of public health data as these may artificially inflate positive results. Therefore, it's important to continuously monitor disease trends and investigate unusual increases, which greatly impacts public health resource allocation and intervention.

65. Uncovering Wild Rodent Lung Fungal Community Dynamics in a Madrean Sky Island

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Abstract

Microbial communities play critical roles in the fitness, evolution, and community structure of wildlife hosts. Moreover, wildlife pathogens can pose threats to biodiversity, humans and domestic animals, as well as create significant economic losses. While bacteria and viruses in microbiomes are comparatively well studied, few systematic studies have focused on the impact of host genetics on mycobiome (fungal microbiome) composition. Furthermore, less is known about the lung mycobiome in general. Using ribosomal RNA ITS2 sequences from the lungs of 30 individuals across 4 rodent species (Family: Heteromyidae, Cricetidae) collected in the Santa Catalina Mountains (north of Tucson, AZ), we investigated hhow the rodent host's (1) phylogenetic ancestry and (2) habitat affects its lung mycobiome. The three target habitats (desertscrub, desert grassland, and interior chaparral) were chosen in part to determine the presence and elevational range of *Coccidioides*, the causative agent of coccidioidomycosis (Valley Fever). Heteromyid rodents specifically have been shown to have a particularly high incidence of *Coccidioides*. Our preliminary results have shown diverse fungi in the lungs of rodents, including the orders Pleosporales, Eurotiales, and Caplodiales. Given that mammalian microbiomes have been shown to closely parallel the phylogeny of their host species – a hypothesis termed "phylosymbiosis" – and that fungal species tend to have a high responsiveness to environmental influences, we expect a joint interaction of host genetics and habitat to ultimately determine mycobiome composition. Our findings will provide insights relevant to future regional wildlife management and to understanding the role of small mammals as reservoirs for emerging fungal pathogens.