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Epidemiology of Coccidioidomycosis in Missouri

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EPIDEMIOLOGY OF COCCIDIOIDOMYCOSIS IN MISSOURI

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ABSTRACT

Aggu-Sher, Ravi K. M.P.H., Purdue University, August 2014. Epidemiology of Coccidioidomycosis in Missouri. Major Professor: Gerald C. Hyner.

Introduction. Incidence of Coccidioidomycosis has been increasing nationally, from 2,271 cases in 1998 to 17,802 cases in 2012. Missouri is not endemic to Coccidioidomycosis but the incidence has been increasing since becoming reportable in 2003. To describe epidemiology of Coccidioidomycosis in Missouri we conducted a retrospective review of surveillance data at Missouri Department of Health & Senior Services (DHSS) for the years, 2004-2013.

Methods. Data was obtained from Missouri Health Surveillance Information System (WebSurv), the statewide reporting system for notifiable diseases. All cases that were "Confirmed" were included in the study.

Results. There were a total of 93 confirmed cases eligible for the study, of which 67 (72%) were male and 26 (28%) were female. The incidence rate of Coccidioidomycosis increased from 0.05 per 100,000 population in 2004 to 0.28 per 100,000 in 2013. The age groups, > 70 yrs (24%) and 60-69 years (23%) were most affected. The predominant race was white accounting for 54 % of cases and the race of 37 % was unknown.

Pneumonia (23%) and Flu-like illness (22%) were the most common presentations. Culture (26%) and Complement Fixation (20%) were the most common diagnostic tests. Median time from symptom onset to diagnosis was 25 days (range 3 – 304 days). A total of 43 (46%) patients required hospitalization and 5 of these were admitted to an ICU. Of the 69 patients with known travel history, 45 had history of travel to endemic regions and 24 had no travel history. Mapping of cases with and without history of travel to the endemic areas outside the state revealed that cases were occurring in all regions of Missouri. Those with history of travel were significantly more likely to be diagnosed based on positive culture and/or PCR testing compared to those who did not travel, who were more likely to be diagnosed with serological tests.

Conclusions. Our study demonstrated significant increase in the incidence of Coccidioidomycosis in Missouri during 2004-2013. Majority of cases were related to travel to endemic areas. There was a similar distribution of cases with or without travel to endemic areas across the state. Additional studies will be required to ascertain whether true endemic cases exist in Missouri.

CHAPTER 1. INTRODUCTION

AGENT

Coccidioidomycosis, or Valley Fever, is a systemic disease caused by the fungus *Coccidioides*, which is endemic to southwestern region of the United States, Mexico, Central and South America (Figure 1).[1] This fungus normally resides in the soil and people get infected through the inhalation of airborne spores. *Coccidioides* genetically comprises of two species, *C immitis*, which is limited to the San Joaquin Valley of California, and *C posadasii*, which is found throughout the rest of the endemic areas.[2] However, despite the genetic speciation, there is minimal to no difference in their morphology and no clinical or immunologic difference in the infection caused by either species.[3]

Figure 1. Geographic distribution of Coccidioidomycosis.

Coccidioides is a dimorphic fungus that exists in both a mold/hyphae and a yeast form (Figure 2).[4]

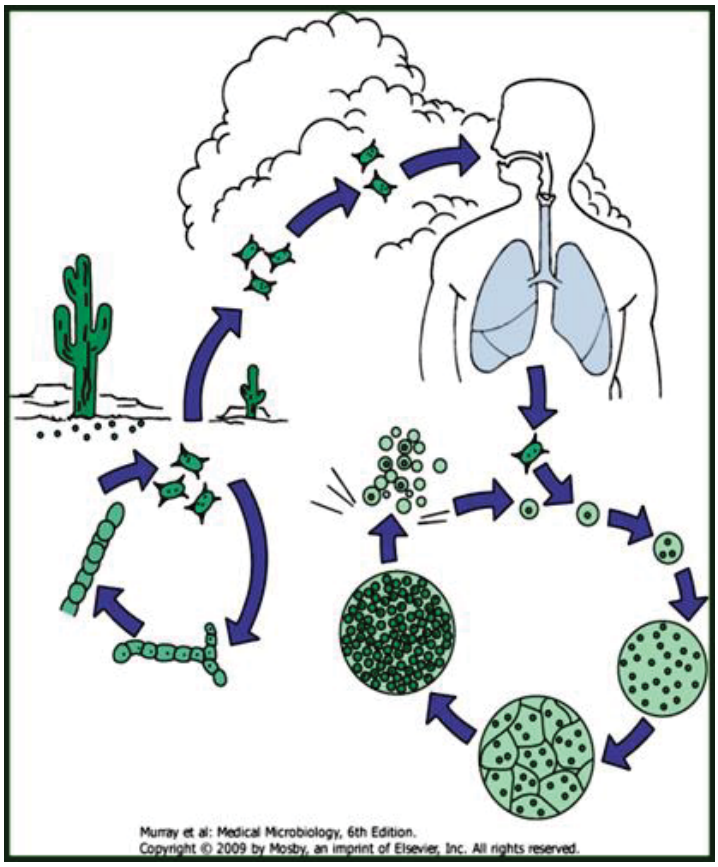

Figure 2. Life cycle of Coccidioides

The fungus grows in the soil as mold and reproduces asexually by forming arthroconidia (spores) from the hyphae that disperse into the atmosphere by wind in dry conditions. Infection in humans and animals occurs through inhalation of the dust-borne spores. In the lungs, the spores change into spherules, which then form multiple endospores (progeny) inside. Once the spherule ruptures, endospores are released and each is capable of forming a spherule.

EPIDEMIOLOGY

Exposure to infected dust is the main risk factor for acquiring infection; human-to-human transmission is extremely rare.[1] In the United States, the endemic areas for Coccidioidomycosis are California, Arizona, Nevada, Utah, New Mexico and Texas.[5] It is notifiable nationally and is reportable to the CDC in the following states: Arizona, California, Delaware, Louisiana, Maryland, Michigan, Minnesota, Missouri, Montana, Nebraska, Nevada, New Hampshire, New Mexico, North Dakota, Ohio, Rhode Island, Utah, Washington, and Wyoming.[6] Coccidioidomycosis is endemic but not reportable in Texas. The majority of cases occur in Arizona and California. During the years, 1998-2011, the total reported cases to the CDC from 28 states and the District of Columbia were 111,717 and of these 66% were from Arizona, 31% from California, 1% from rest of the endemic states, and less than 1% from non-endemic states.[7]

The number of reported cases of Coccidioidomycosis in the United States has been increasing over the past several years. In the endemic areas (excluding Texas), the age-adjusted incidence rose from 5.3 cases per 100,000 population in 1998 to 42.6 per 100,000 in 2011.[7]. There were 240 cases reported in 2011 in non-endemic areas compared with 6 cases in 1998.[6] Missouri is not considered an endemic state for Coccidioidomycosis, but it became a reportable condition in 2003, and since then the rate has increased from 0.02 per 100,000 in 2003 to 0.25 per 100,000 in 2010.[8]

CLINICAL MANIFESTATIONS

An estimated 150,000 new infections occur annually in the United States.[9] Of these, 60% are asymptomatic and do not come to medical attention.[10] Symptomatic individuals develop a flu-like illness—fever, headache, cough, sore throat, night sweats, body aches, dyspnea, and pleuritic chest pain—approximately 7 to 21 days after exposure. Other manifestations are anorexia, weight loss and skin rashes. The majority of symptoms resolve themselves within 2 to 3 weeks, but some patients may suffer with arthralgias, myalgias, and fatigue that can last for months; hence the synonym, "desert rheumatism."[11]

Community Acquired Pneumonia is a common presentation at healthcare settings and is usually indistinguishable from bacterial or other causes of Pneumonia. Patients can have infiltrates, adenopathy or effusion on chest radiographs. In a study that evaluated patients presenting with symptoms of Community Acquired Pneumonia in Arizona, 29% of the patients were found to have Coccidioidomycosis.[12] In the majority of patients, the infection is mild and resolves itself without specific antifungal treatment. Some patients may develop chronic pulmonary nodules or cavities that are asymptomatic or that cause symptoms such as chest pain, dyspnea, or hemoptysis.[13]

In a few groups of patients, the infection is severe and causes diffuse pneumonia and respiratory failure leading to extrapulmonary dissemination. The immunocompromised, such as those dealing with HIV/AIDS, malignancy, organ transplant (solid organ and hematopoietic cell transplant), autoimmune disorders, inflammatory conditions, diabetes,

corticosteroid therapy, chemotherapy, or anti-TNF medications, are particularly at risk.[10] Fewer than 5% of the general population develops persistent pulmonary infection or disseminated Coccidioidomycosis.[14] In a study of Coccidioidomycosis in patients on renal replacement therapy the rate of disseminated infection was 75% with a mortality rate of 63% for those receiving immunosuppressive therapy.[15] Dissemination can affect any organ, but the most common sites are skin, soft tissues, bones, joints, and meninges.[16]

DIAGNOSIS

Laboratory tests available to diagnose Coccidioidomycosis include serologic, direct microscopy, histopathology, culture, Polymerase Chain Reaction (PCR), urine antigen, and skin testing. Serologic response to Coccidioidomycosis is the production of two types of antibodies, IgM and IgG. The IgM antibodies are seen early during the course of infection and wean off rapidly, whereas IgG antibodies are produced later and persist for much longer.[17] The most commonly used tests for IgG are Enzyme Immunoassay (EIA), Immunodiffusion (ID) and Complement Fixation (CF); those for IgM are EIA and ID.[18] Both ID and EIA are reported as quantitative and/or qualitative, whereas CF is reported as titer.

EIA is considered the most sensitive test but is prone to false positives, particularly for IgM. Hence, it is recommended that ID testing, which is the most specific, be used to confirm a positive EIA.[19, 20] Immunodiffusion (ID) tests for the presence of precipitin (IgM) or CF (IgG) antibodies and can be done both as qualitative and quantitative test. Serum CF titer is not as sensitive as EIA or ID, but it does provide several benefits. It

supports the diagnosis made by EIA/ID, helps assess the severity of the disease, and can be used to follow its course over time and measure its response to treatment.[21] A low CF titer can be the result of a new infection or one from years ago. If the test results change on repeat measurement, it indicates a recent infection. The higher the titer, the more severe the disease and titers greater than 1:16 indicate the possibility of disseminated disease. In addition, the CF test is the primary serologic test to diagnose meningitis and its presence in cerebrospinal fluid (CSF) is diagnostic.[22]

Since Coccidioides is not part of the normal flora of humans, identification of the organism in clinical specimens is considered the gold standard for diagnosis.[23] Common specimens are sputum, tracheal aspirate, bronchoalveolar lavage (BAL), lung/pleural biopsy, blood, CSF or other body fluids and tissue biopsy. The organism can either be identified by direct microscopy or after growth in fungal cultures. Direct microscopy can also reveal spherules, which are diagnostic, although not definitive as seeing organism itself since spherules can be mistaken for other fungi or artifacts.[24] Histopathology using special stains such as Grocott methenamine silver (GMS), Periodic acid Schiff (PAS) and Hematoxylin-eosin (H&E) is an important direct microscopy method.[25]

Coccidioides are not very fastidious and grow on most any bacterial or fungal culture media. The commonly used fungal media are brain-heart infusion agar (BHI), Sabouraud dextrose agar (SDA), and potato dextrose agar (PDA).[24] Once there is growth on the culture media, Coccidioides can be rapidly identified by a DNA probe test (AccuProbe) developed by Gen-Probe, Inc. (San Diego, CA).[26]

The test is highly sensitive and specific and takes less than 1 hour. In vitro susceptibility testing is then carried out to identify susceptibility to the common antifungal drugs.

Culture can take days to weeks and cause delays in diagnosis and treatment. A Real-Time PCR assay has been developed that can detect *Coccidioides* directly in clinical specimens.[27] It is highly sensitive and specific and has the potential to diagnose within hours; however, it is still not the standard diagnostic test. Another rapid way to diagnose Coccidioidomycosis is antigen testing. A *Coccidioides* Antigen Enzyme Immunoassay (EIA) was developed that detects urinary antigen and had sensitivity of 71% in cases of severe Coccidioidomycosis.[28]

The cellular immunity to Coccidioidomycosis can be tested by Coccidioidin skin test, which is a delayed-type hypersensitivity reaction.[29] Patients develop dermal reactivity to the infection that lasts a lifetime, therefore, a positive test is not always helpful to diagnose current illness unless the patient is known to have had a negative test in the past. However, it is an important epidemiological test to study prevalence geographically.

TREATMENT

Treatment of Coccidioidomycosis depends on the severity of infection and a patient's risk factors for complications, such as immunosuppression or pregnancy. Patients with mild symptoms and no risk factors can be managed without antifungal drugs but need to be frequently monitored for worsening conditions and resolution. Although most patients can be managed as outpatients, those with severe illness may need hospitalization.

Several drugs are used in practice both in inpatient and outpatient settings. Intravenous Amphotericin B is typically used for severe infections. Other available drugs are the antifungal Azoles, Ketoconazole, Fluconazole, Itraconazole, Voriconazole and Posaconazole. Sometimes in severe infections Amphotericin B is combined with an Azole. Duration of treatment ranges from several months to lifetime in some cases.[9, 30]

CHAPTER 2. STUDY AND METHODS

STUDY

OBJECTIVE

Describe current epidemiology of Coccidioidomycosis in Missouri using state's electronic surveillance database of reportable conditions.

STUDY PERIOD

Study was conducted for the period of 2004-2013. This study period included years when the complete Coccidioidomycosis surveillance data was available.

STUDY POPULATION

The study population included the entire Missouri state population during the study period. Population estimates from US census bureau were used for rate calculations.

METHODS

We conducted a retrospective review of communicable disease surveillance at the Missouri Department of Health & Senior Services (DHSS). Data was obtained from Missouri Health Surveillance Information System (WebSurv), the statewide reporting

system for notifiable diseases. All cases that were "Confirmed" were included in the study. The study was done at the Missouri DHSS as a part of surveillance data evaluation. The study was approved by Institutional Review Board of Purdue University, West Lafayette, IN.

Following variables were analyzed: Age, Sex, Race, Co-morbid conditions (HIV/AIDS, Cancer, Diabetes, Heart disease, Lung disease, Organ transplant, Immunosuppressive disease, and Immunosuppressive drugs), Residential address, Travel to endemic areas, Occupational risk, Serology (Immunodiffusion, Complement Fixation, Enzyme-linked immunoassay), Histopathology, Culture, Polymerase Chain Reaction (PCR), Urine antigen, and Skin testing.

Cases were compared using their travel to endemic area status using above mentioned variables.

CASE DEFINITION [31]

"Confirmed case" of Coccidioidomycosis in the study was based on the case definition consistent with the definition used by the CDC's National Notifiable Diseases Surveillance System (NNDSS) and the Council of State and Territorial Epidemiologists (CSTE).

CLINICAL DESCRIPTION

Infection may be asymptomatic or may produce an acute or chronic disease. Although the disease initially resembles an influenza-like or pneumonia-like febrile illness primarily involving the broncho-pulmonary system, dissemination can occur to multiple organ systems. An illness is typically characterized by one or more of the following:

- Influenza-like signs and symptoms (e.g., fever, chest pain, cough, myalgia, arthralgia, and headache)
- Pneumonia or other pulmonary lesion, diagnosed by chest radiograph
- Erythema nodosum or erythema multiforme rash
- Involvement of bones, joints, or skin by dissemination
- Meningitis
- Involvement of viscera and lymph nodes

LABORATORY CRITERIA FOR DIAGNOSIS

A confirmed case must meet at least one of the following laboratory criteria for diagnosis:

- Cultural, histopathologic, or molecular evidence of presence of *Coccidioides* species, OR
- Positive serologic test for coccidioidal antibodies in serum, cerebrospinal fluid, or other body fluids by:
    - Detection of coccidioidal immunoglobulin M (IgM) by immunodiffusion, enzyme immunoassay (EIA), latex agglutination, or tube precipitin, OR
    - Detection of coccidioidal immunoglobulin G (IgG) by immunodiffusion, EIA, or complement fixation, OR
    - Coccidioidal skin-test conversion from negative to positive after onset of clinical signs and symptoms

CASE CLASSIFICATION-CONFIRMED

A case that meets the clinical criteria and is laboratory confirmed.

STATISTICAL METHODS

All data was analyzed using R statistical software with significance defined as $P < 0.05$ level. Poisson regression analysis was used to model Coccidioidomycosis trend over time. Shapiro test was used to determine if studied groups follow normal distribution. Mann-Whitney U test was used to compare groups with no normal distribution. Hypothesis tests for proportions was conducted to evaluate the difference between the proportions of patients with a specific symptom, proportion of patients with positive result for a specific test, and hospitalization proportion in each group.

CHAPTER 3. RESULTS

There were a total of 93 reported cases during the years, 2004-2013. (Figure 3) Of these 67 (72%) were male and 26 (28%) were female. The age distribution showed no cases less than 10 years, 1 case 10-19 years, 6 cases 20-29, 12 cases 30-39, 15 cases 40-49, 16 cases 50-59, 21 cases 60-69 and 22 cases greater than or equal to 70 years. With regard to

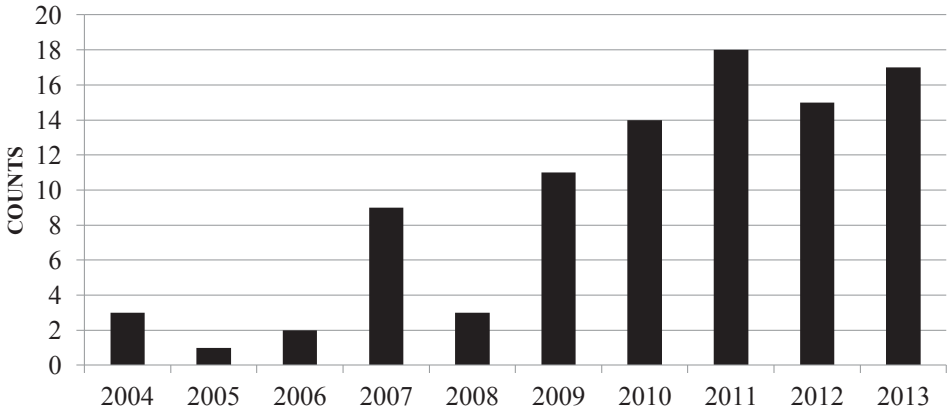

Figure 3. Reported cases of Coccidioidomycosis in Missouri, 2004-2013

race, 50 (54%) were white, 7 were black, 1 was a Pacific Islander, 1 was Asian and the races of the remaining 34 (37%) were unknown. (Table 1 and Figures 3, 4, 5, 6, 7, 8)

Table 1. Demographics-Sex, Age, and Race

Year	Total Reported Cases	Male	Female	10-19	20-29	30-39	40-49	50-59	60-69	≥70	White	Black	Pacific Islander	Asian	Unknown
2004	3	2	1	0	0	0	1	0	1	1	2	0	0	0	1
2005	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1
2006	2	2	0	0	0	0	0	2	0	0	1	0	0	0	1
2007	9	8	1	0	1	1	2	2	2	1	5	1	0	0	3
2008	3	2	1	0	2	0	0	0	0	1	3	0	0	0	0
2009	11	6	5	0	0	1	1	5	1	3	9	0	0	1	1
2010	14	7	7	0	0	4	2	1	3	4	7	2	0	0	5
2011	18	17	1	0	1	3	3	2	3	6	8	1	1	0	8
2012	15	12	3	1	1	2	2	2	6	1	7	2	0	0	6
2013	17	10	7	0	0	1	4	2	5	5	8	1	0	0	8
TOTAL (%)	93	67 (72%)	26 (28%)	1 (1%)	6 (6%)	12 (13%)	15 (16%)	16 (17%)	21 (23%)	22 (24%)	50 (54%)	7 (8%)	1 (1%)	1 (1%)	34 (37%)

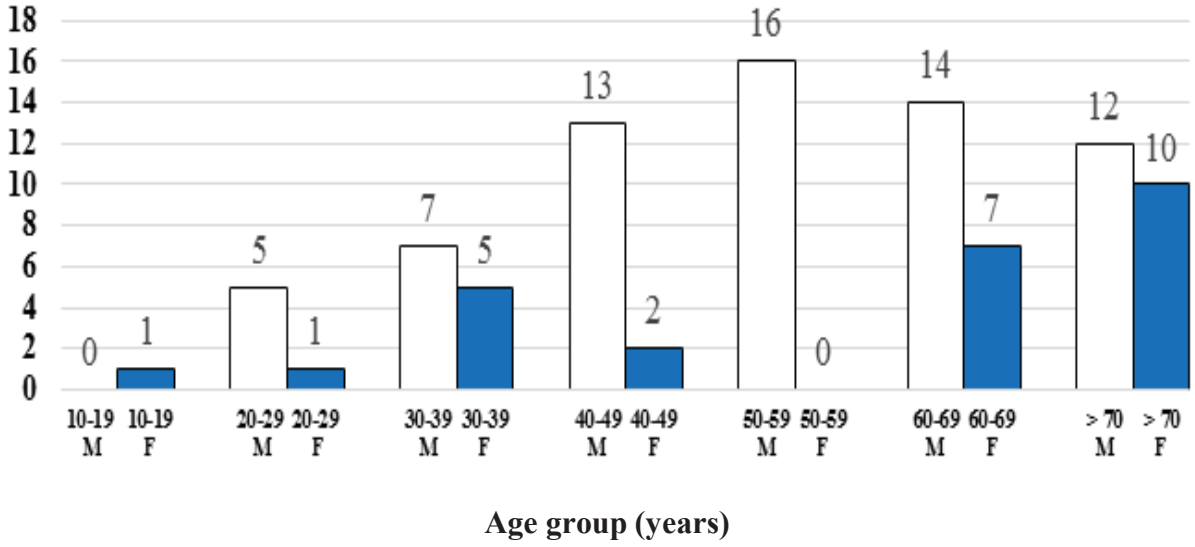

Figure 4. Cases of Coccidioidomycosis by sex and age group, Missouri, 2004-2013.

Table 2. Cases of Coccidioidomycosis according to Missouri highway patrol regions, 2004-2013.

Year	Total Reported Cases	Region A	Region B	Region C	Region D	Region E	Region F	Region G	Region H	Region I	Unknown Region
2004	3	0	0	3	0	0	0	0	0	0	0
2005	1	0	0	1	0	0	0	0	0	0	0
2006	2	1	0	1	0	0	0	0	0	0	0
2007	9	0	1	3	2	1	0	0	1	0	1
2008	3	0	0	2	0	1	0	0	0	0	0
2009	11	0	0	3	5	0	2	0	0	1	0
2010	14	2	1	5	4	0	1	0	0	0	1
2011	18	8	2	4	3	1	0	0	0	0	0
2012	15	3	0	7	0	0	2	0	1	2	0
2013	17	5	0	4	2	1	1	2	1	1	0
TOTAL (%)	**93**	**19 (20%)**	**4 (4%)**	**33 (35%)**	**16 (17%)**	**4 (4%)**	**6 (6%)**	**2 (2%)**	**3 (3%)**	**4 (4%)**	**2 (2%)**

Figure 5. Missouri highway patrol regions

Figure 6. Mapping of Missouri Coccidioidomycosis cases, 2004-2013.

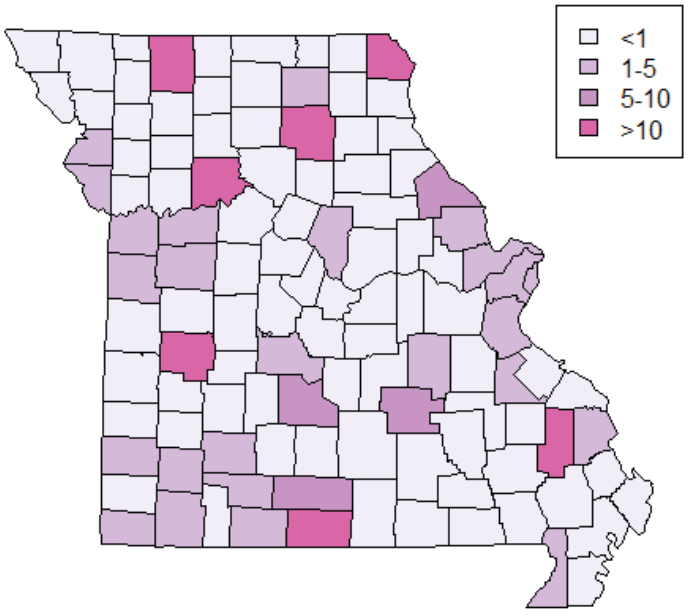

Figure 7. Cases of Coccidioidomycosis in Missouri by county, 2004-2013.

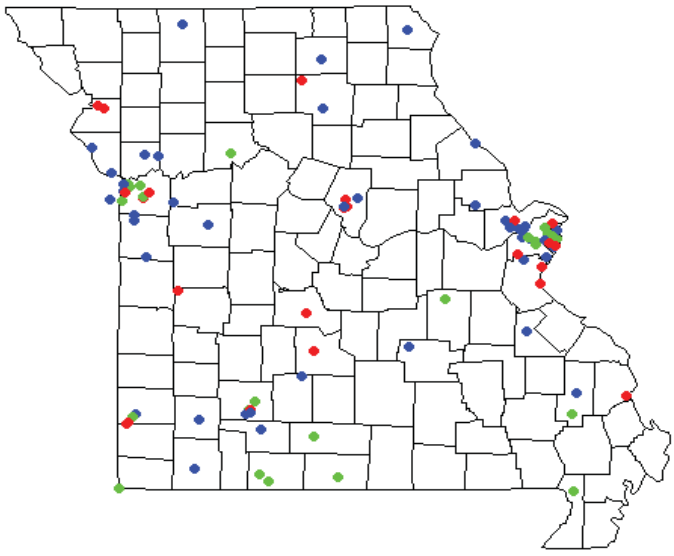

Figure 8. Mapping of Coccidioidomycosis cases by travel to endemic areas, MO, 2004-2013 Red: not traveled to endemic, Blue: traveled, Green: unknown

Clinical presentation of cases, for whom data was available (86/93), included: 2 cases with asymptomatic lung lesions, 14 (15%) with symptomatic lung lesions, 20 (22%) with flu-like illness, 4 with flu-like illness and skin lesions, 5 with Flu-like illness and night sweats, 2 with Flu-like illness and weight loss, 5 with hemoptysis, 21 (23 %) with Pneumonia, 2 with Arthritis/Arthralgia, 3 with headache/confusion, 3 with only skin lesions, 1 with Meningitis, 2 with Respiratory failure, 1 with sepsis, 1 presented with disseminated Cocci and 7 (8 %) cases had unknown symptoms. (Table 3)

Table 3. Clinical presentation of Coccidioidomycosis cases

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	TOTAL (%)
Total reported cases	**3**	**1**	**2**	**9**	**3**	**11**	**14**	**18**	**15**	**17**	**93**
Lung lesions, asymptomatic	0	0	0	0	0	0	0	0	2	0	**2 (2%)**
Lung lesions, symptomatic	0	0	0	0	1	1	4	2	3	3	**14 (15%)**
Flu like illness	2	0	1	1	1	3	4	3	3	2	**20 (22%)**
Flu like illness and skin lesions	0	0	0	0	0	1	1	0	0	2	**4 (4%)**
Flu like illness and night sweats	0	1	0	1	0	2	0	1	0	0	**5 (5%)**
Flu like illness and weight loss	0	0	1	0	0	1	0	0	0	0	**2 (2%)**
Hemoptysis	0	0	0	1	0	1	0	2	0	1	**5 (%)**
Pneumonia	0	0	0	2	0	1	1	7	3	7	**21 (23%)**
Arthritis/Arthralgia	0	0	0	0	0	1	1	0	0	0	**2 (2%)**
Headache/Confusion	0	0	0	1	0	0	0	0	1	1	**3 (3%)**
Skin lesions	0	0	0	1	0	0	1	0	1	0	**3 (3%)**
Meningitis	0	0	0	0	0	0	0	1	0	0	**1 (1%)**
Respiratory failure	0	0	0	0	1	0	0	0	0	1	**2 (2%)**
Sepsis	0	0	0	0	0	0	0	0	1	0	**1 (1%)**
Disseminated	0	0	0	0	0	0	0	0	1	0	**1 (1%)**
Unknown symptoms	1	0	0	2	0	0	2	2	0	0	**7 (8%)**

Patients were also diagnosed using several combinations of tests: 20 (22%) had Complement Fixation (CF), 15 (16%) had Immunodiffusion, 4 had CF and Immunodiffusion, 1 had CF and Culture, 1 had CF, Immunodiffusion and Culture, 1 had CF, PCR and Culture, 1 had CF and Histopathology, 1 had Histopathology, 9 had EIA/ELISA, 2 had EIA/ELISA and Immunodiffusion, 1 had EIA/ELISA and culture, 24

(2 %) had Culture, 1 had EIA/ELISA, Immunodiffusion and Culture, 3 had PCR and 9 (10%) had unknown serology. (Table 4)

Table 4. Laboratory diagnosis of Coccidioidomycosis cases part 1

Year	Cases	CF	Immunodiffusion	CF and Immunodiffusion	CF and Culture	CF, Immunodiffusion and Culture	CF, PCR and Culture	CF and Histopathology	Histopathology
2004	3	0	0	0	0	0	0	0	0
2005	1	0	0	0	0	0	0	0	0
2006	2	1	0	0	0	0	0	0	0
2007	9	2	0	0	0	0	0	0	1
2008	3	0	0	0	0	0	0	0	0
2009	11	1	2	0	0	1	0	1	0
2010	14	1	4	0	0	0	0	0	0
2011	18	6	3	1	0	0	0	0	0
2012	15	3	2	1	1	0	0	0	0
2013	17	6	4	2	0	0	1	0	0
TOTAL (%)	**93**	**20 (22%)**	**15 (16%)**	**4 (4%)**	**1 (1%)**	**1 (1%)**	**1 (1%)**	**1 (1%)**	**1 (1%)**

Table 5. Laboratory diagnosis of Coccidioidomycosis cases part 2

Year	Cases	EIA/ ELISA	EIA/ELISA and Immunodiffusion	EIA/ELISA and culture	Culture	EIA/ELISA, Immunodiffusion and Culture	Unknown Serology	PCR
2004	3	0	0	0	2	0	0	1
2005	1	0	0	0	0	0	1	0
2006	2	0	0	0	1	0	0	0
2007	9	1	0	0	5	0	0	0
2008	3	0	0	0	0	0	3	0
2009	11	0	0	1	5	0	0	0
2010	14	1	1	0	3	0	2	2
2011	18	2	0	0	4	0	2	0
2012	15	5	1	0	1	0	1	0
2013	17	0	0	0	3	1	0	0
TOTAL	**93**	**9 (10%)**	**2 (2%)**	**1 (1%)**	**24 (26%)**	**1 (1%)**	**9 (10%)**	**3 (3%)**

Out of 93 cases, 51 (55%) patients had the exact date of symptom onset documented. Time to diagnosis ranged from 3 to 304 days with a median of 25 days. A total of 43

(46%) patients required hospitalization and 5 of these were admitted to an ICU. With regard to available documentation on antifungal treatment, 29 patients received antifungal treatment and of these, 14 had outpatient therapy and 15 were inpatients. Fluconazole was the most used antifungal in 19 patients. 4 patients received Itraconazole, 2 received Fluconazole and Itraconazole, 1 received Voriconazole, 2 received Amphotericin B and 1 received Amphotericin B and Itraconazole. Of the 93 total cases, 8 died: 3 attributed to Coccidioidomycosis, 3 from other illnesses, and the cause of death in the remaining 2 was not known. Review of history of previous infection showed that 10 patients had a prior history of Coccidioidomycosis and the current infection was a recurrence.

On reviewing the travel history to endemic regions (States-CA, AZ, UT, NM, NV and TX. Countries-Mexico, South America), 45 (48%) had travel history, 24 (26%) had no travel history and the travel history of the remaining 24 (26%) was unknown. Among the 45 cases with travel history, 19 (20%) had recent travel (defined as development of symptoms while in endemic area or within 21 days of returning from travel to an endemic area), 20 had remote travel or history of residence in endemic regions, and the travel timeline of 6 cases was not known. There were 17 (18%) cases that were immunocompromised and 8 of these had travel history to endemic regions.

Of the 69 patients with known travel history, 45 had history of travel and 24 had no travel history (Table 6).

Table 6. Characteristics of Coccidioidomycosis cases by travel history

Clinical Feature	All Travel (n=45)	Recent Travel (n=19)	No Travel (n=24)
Age, years, median	63	67	50
Male sex	32/45 (71)	14/19 (74)	20/24 (83)
Race			
White	28/45 (62)	13/19 (68)	10/24 (42)
Black	4/45 (9)	0/19 (0)	1/24 (4)
Pacific Islander	0/45 (0)	0/19 (0)	1/24 (4)
Asian	0/45 (0)	0/19 (0)	1/24 (4)
Unknown Race	13/45 (29)	6/45 (13)	11/24 (46)
Presentation			
Asymptomatic lung lesions	0/45 (0)	0/19 (0)	1/24 (4)
Symptomatic lung lesions	7/45 (16)	2/19 (11)	5/24 (21)
Flu-like illness	13/45 (29)	7/19 (37)	10/24 (42)
Pneumonia	10/45 (22)	7/19 (37)	5/24 (21)
Hemoptysis	4/45 (9)	1/19 (5)	1/24 (4)
Arthralgia/Arthritis	2/45 (4)	1/19 (5)	0/24 (0)
Headache/Confusion	2/45 (4)	0/19 (0)	0/24 (0)
Skin lesions	2/45 (4)	0/19 (0)	0/24 (0)
Meningitis	1/45 (2)	0/19 (0)	0/24 (0)
Sepsis	0/45 (0)	0/19 (0)	1/24 (4)
Respiratory failure	1/45 (2)	0/19 (0)	0/24 (0)
Disseminated	1/45 (2)	0/19 (0)	0/24 (0)
Unknown	2/45 (4)	1/19 (5)	1/24 (4)
Days, symptoms to diagnosis, Median (range)	52 (3-304)	55 (6-123)	17 (6-108)
Type of test			
CF	10/45 (22)	4/19 (21)	7/24 (29)
Immunodiffusion	9/45 (20)	2/19 (11)	9/24 (38)
EIA/ELISA	6/45 (13)	3/19 (16)	5/24 (21)
Culture	18/45 (40)	7/19 (37)	4/24 (17)
PCR	3/45 (7)	3/19 (16)	0/24 (0)
Histopathology	2/45 (4)	0/19 (0)	0/24 (0)
Unknown serology	5/45 (11)	2/19 (11)	3/24 (13)
Hospitalization	27/45 (60)	11/19 (58)	9/24 (38)

All data are n/total (%).

Abbreviations: CF, compliment fixation; EIA, enzyme immunoassay; PCR, polymerase chain reaction

STATISTICAL ANALYSIS

Poisson regression analysis was used for the Coccidioidomycosis trend analysis during 2004-2013. By applying the Poisson regression model, we assume that the disease occurrence is rare and cases are independent; in other words, occurrence of Coccidioidomycosis does not increase or decrease the likelihood of other occurrences. Model $\hat{y} = e^{-486.21+0.243x}$ (where x is the year) was significant at $p<0.001$ level.

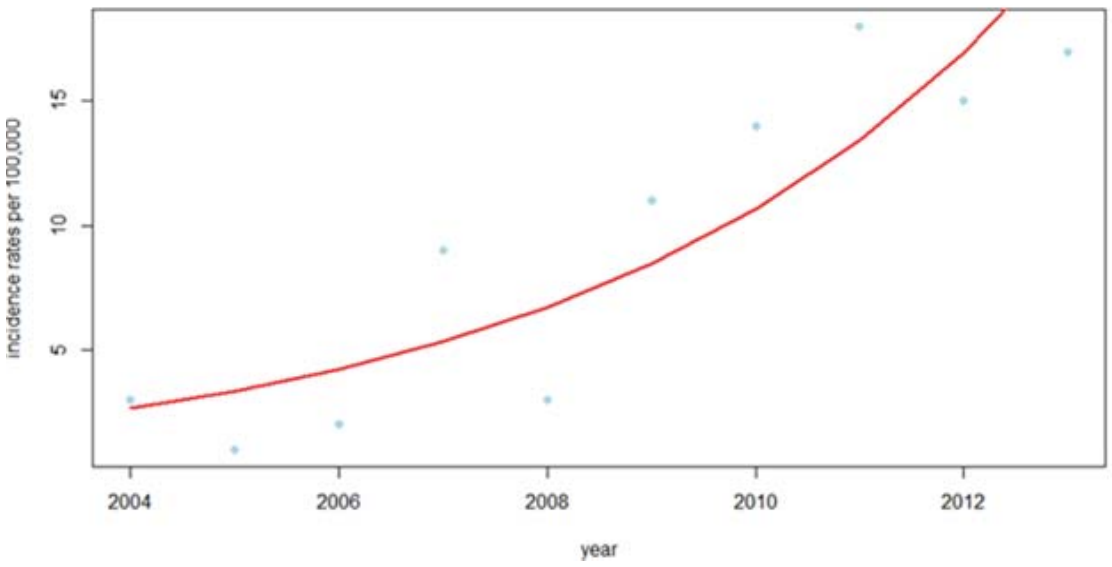

Figure 9. Poisson Regression model of Coccidioidomycosis incidence in Missouri (rate per 100,000 Population).

According to Hastie and Pregibon, for models with known dispersion (e.g., binomial and Poisson fits), the chi-square test is most appropriate, and for those with dispersion estimated by moments (e.g., gaussian, quasibinomial and quasipoisson fits) the F test is most appropriate.[32] Thus, analysis of variance was employed using chi-square to evaluate the Poisson regression model.

The patients with known travel history were categorized into three groups: 1) patients who have "not traveled" to the endemic areas, 2) patients who have "traveled" to endemic areas at any time and 3) patients who have "recently traveled" to such areas. According to the distributions (Figure 10), the age of patients who have "traveled" (either recently or not) is skewed to the left while the patients who have "not traveled" are skewed to the right. We used Shapiro test to analyze the age distribution data. The p-value for the "not traveled", "traveled", and "recently traveled" groups are 0.659, 0.052, and 0.002, respectively. The test result shows that "not traveled" groups are following a normal distribution while "recently traveled" are not, and the p-value for the "traveled" group is not decisive.

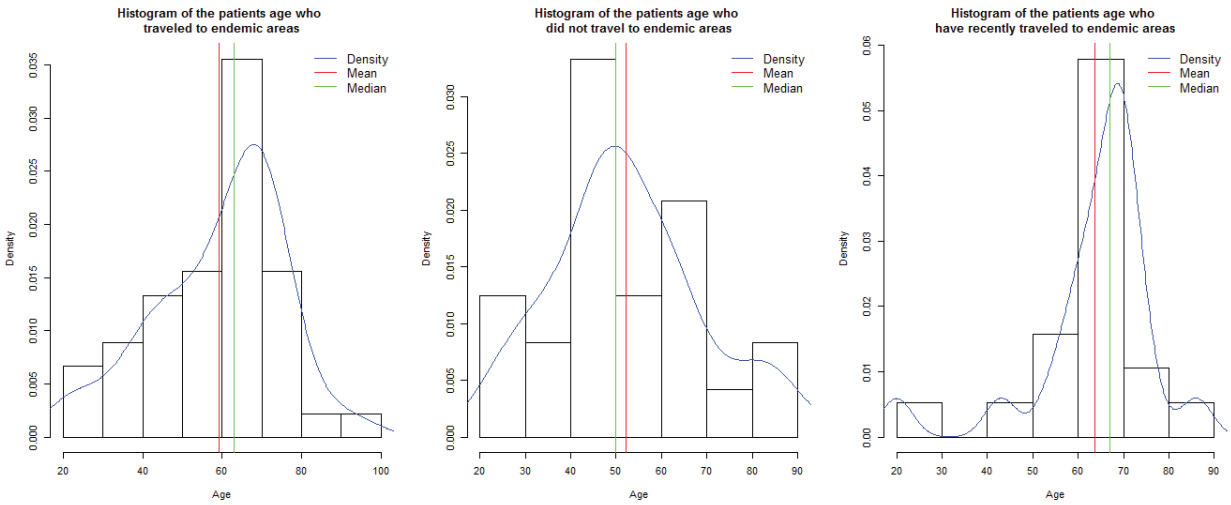

Figure 10. Probability density function of patient ages for "Traveled", "Not Traveled", and "Recently Traveled" groups

The p-value of the nonparametric Kruskal-Wallis test is 0.02603 that shows that at least distribution of one pair is not identical. Since the distributions are not normal, Pairwise Mann-Whitney-Wilcoxon tests are used to check whether the distributions of each pair are identical. We also applied Mood's nonparametric test to assess whether the median of each groups are identical. Test results (Table 7) reveal that the median of "not traveled" vs. "traveled" and "not traveled" vs. "recently traveled" are not identical. In addition not traveled patients and recently traveled patients do not follow identical age distribution but age distribution similarity of not traveled and traveled group are not decisive.

Table 7. Age comparisons of Not Traveled, Traveled and Recently Traveled groups

Comparisons	Mann-Whitney-Wilcoxon U Test		Mood's Median Test
	w	p-value	p-value
Not Traveled Vs. Traveled	692.5	0.05534	0.02595
Not Traveled Vs. Recently Traveled	341	0.005889	0.0139

The second variable analyzed between traveled, not traveled, and recently traveled is the days to diagnosis. Figure 11 shows the days to diagnosis distribution of each group. Kolmogrov-Smirnov test is applied to check whether the data follows exponential distribution. P-values for traveled, not traveled, and recently traveled patients are 0.2819, 0.2923, and 0.2982, respectively. Thus, the study finds that days to diagnosis variable of each group follows exponential distribution. Table 8 shows the mean and median information for each group.

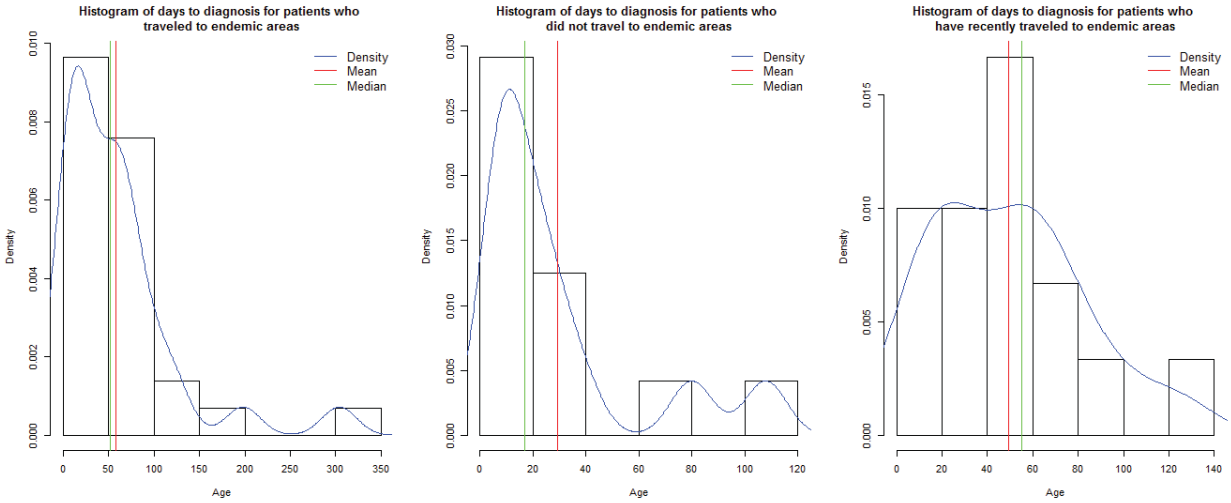

Figure 11. Probability density function of patient day to diagnosis for "Traveled", "Not Traveled", and "Recently Traveled" groups

Table 8. Mean and median days to diagnosis for "Traveled"," Not Traveled", and "Recently Traveled" groups

	Mean	Median
Not Traveled	29.33	17
Traveled	58.21	52
Recently Traveled	49.33	55
Not Recently Traveled	67.71	33.5

Since the time to diagnosis follows exponential distribution, we use Mann-Whitney-Wilcoxon U tests to check if days to diagnosis variable of different groups of patients follows an identical distribution. According to the test results, there is not a strong difference between the days to diagnosis distribution of not traveled and traveled patients,

however there is a difference between the days to diagnosis distribution of not traveled and recently traveled patients. (Table 9)

Table 9. Days to diagnosis comparisons of "Not traveled", "Traveled" and "Recently Traveled group"

Comparisons	Mann-Whitney-Wilcoxon U Test	
	w	p-value
Not Traveled Vs. Traveled	221	0.0912
Not Traveled Vs. Recently Traveled	53.5	0.03931

In addition to age and days to diagnosis variables, we tested whether diagnosis symptoms are different between "traveled" vs. "not traveled" and "not traveled" vs. recently traveled" patients. According to the F-test results (Table 10), the symptoms to diagnosis do not vary between different groups of patients.

Table 10. Patient proportions with a specific symptom and test results for comparing the proportions of "Traveled vs. Not Traveled" group of patients and proportions of "Not Traveled vs. Recently Traveled" groups

	Traveled Vs. Not Traveled			**Not Traveled Vs. Recently Traveled**		
	Traveled	**Not Traveled**	**p-value**	**Not Traveled**	**Recently Traveled**	**p-value**
Symptomatic lung Lesions	0.163	0.217	0.8312	0.217	0.111	0.6317
Flu-like illness	0.302	0.435	0.421	0.435	0.389	1
Pneumonia	0.232	0.217	1	0.217	0.389	0.3943
Hemoptysis	0.093	0.043	0.8129	0.043	0.055	1

Testing results are also compared between "traveled vs. not traveled" and "not traveled vs. recently traveled" patients (Table 11). According to the test results, the proportion of positive Culture + PCR results is larger for "traveled" (and recently traveled) patients than for "not traveled" ones. The proportion of other positive results do not significantly differ between "traveled and not traveled" or "not traveled and recently traveled" patients.

Table 11. Hypothesis test results for comparing proportion of patients whose specimens are positive under a special test

	Traveled Vs. Not Traveled			Not Traveled Vs. Recently Traveled		
	Traveled	**Not Traveled**	**p-value**	**Not Traveled**	**Recently Traveled**	**p-value**
CF	0.25	0.333	0.6971	0.333	0.235	0.762
Immunodiffusion	0.225	0.428	0.1735	0.428	0.118	0.08157
EIA/ELISA	0.15	0.238	0.6172	0.238	0.176	0.9496
Culture + PCR	0.525	0.19	0.024	0.19	0.588	0.02858

We also assessed the hospitalization variable between “traveled and not traveled” or “not traveled and recently traveled” groups of patients. The p-values of the comparisons are 0.1129 and 0.3103, respectively. Thus, the results show that the hospitalization proportions do not significantly change between “Traveled and Not Traveled” or “Not Traveled and Recently Traveled” groups of patients.

CHAPTER 4. DISCUSSION

Our study found a statistically significant increase in the incidence of Coccidioidomycosis in Missouri with rate going from 0.05 per 100,000 population in 2004 to 0.28 per 100,000 in 2013. Our findings are consistent with the national trend of increasing incidence of Coccidioidomycosis (from 2,271 cases in 1998 to 17,802 cases in 2012) that includes both endemic and non-endemic states.[6] Mapping of cases with and without history of travel to the endemic areas outside the state revealed that cases were occurring in all regions of Missouri. Since the study cases were distributed throughout the state, endemic foci of Coccidioidomycosis in Missouri seem unlikely.

One explanation for the increase in reported cases in Missouri could be the fact that it became reportable in 2003. There could be increased awareness in healthcare providers and the public leading to more testing. An increase in Coccidioidomycosis incidence has been observed in Arizona after it became reportable in 1997. The incidence increased from 21/100,000 in 1997 to 91/100,000 in 2006.[33]

For the resident of a non-endemic area, travel to an endemic state is a major risk factor for acquiring Coccidioidomycosis. In our study, those with no travel history were significantly younger compared to those who traveled, but there were no significant

differences regarding the most common clinical symptoms, rate of hospitalization, treatment with antifungals, or death. Those with history of travel were significantly more likely to be diagnosed based on positive culture and/or PCR testing compared to those who did not travel, who were more likely to be diagnosed with serological tests. Since the culture and PCR are more accurate tests for diagnosing recent Coccidioidomycosis infection compared to serological tests, it is difficult to say with certainty whether all cases with no travel history were truly experiencing this infection.

Current Coccidioidomycosis surveillance case definition makes no distinction between those with travel or residence in the endemic area and those without any of it, although performance of the diagnostic tests in detecting true cases is variable. Since people living in non-endemic areas have a much lower risk of having Coccidioidomycosis, a different, more stringent requirement for laboratory diagnosis of such cases seems prudent. It has been shown that laboratory tests may falsely categorize patients into Coccidioidomycosis cases since false positive tests are common when diagnosis is confirmed solely based on positive serology.[20] Also, it was not clear in some cases if the positive antibody test was IGG or IGM, and what was the titer for the Complement Fixation (CF) test.

Accurate history of travel to endemic areas is very important. In our study, the travel history of 26% of cases was unknown. It's possible that several of these patients traveled to endemic areas. Also, sometimes people may dismiss brief travels as insignificant and not report it but studies have shown that even changing planes or driving through an endemic area can increase risk.[5] Immunocompromised state has long been recognized as

risk factor for fungal diseases, including Coccidioidomycosis. [34] In our study 17 % of patients were immunocompromised and some patients' immunocompromised state, particularly diabetes, could have been missed due to incomplete documentation of past medical history.

The main limitation of our study is that the retrospective analysis was conducted on routine surveillance data, and no medical chart review or direct patient interviews were conducted. The surveillance data was incomplete for some cases in respect of demographics, travel history, medical history, clinical presentation, diagnosis, treatment, and follow up. The race of cases was missing in 37 % of cases, while it is known that African-Americans are at increased risk for severe infections and hospitalizations. In a study looking into hospitalizations in Arizona and California, the hospitalization rate for blacks in Arizona was 12-fold higher than the rate for whites.[35]

For future research, the data collection and documentation in the surveillance database should be more comprehensive. Demographics such as, race, occupation, travel history, and comorbid conditions are important in identifying at risk patients and need to be specifically addressed. It is important to document the exact diagnostic tests used for serology, whether it is IgG or IgM, and the exact titer for CF. Follow up of patients after the initial reporting by a lab or hospital is crucial to make sure no alternative diagnoses have emerged.

Also, Soil analysis for *Coccidioides* spores in various parts of Missouri can be extremely helpful to characterize the changing endemicity. We were unable to find any literature available to us documenting the presence of *Coccidioides* spores in Missouri. A cluster analysis of a larger sample of Coccidioidomycosis cases using SAT scan could be needed to provide a more accurate answer whether endemicity exists in Missouri. If further surveillance reveals more Coccidioidomycosis cases and/or disease clusters among Missouri residents without history of travel to endemic areas, an environmental study would also be warranted.

In conclusion, our study demonstrated significant increase in the incidence of Coccidioidomycosis in Missouri. Majority of cases are related to travel to endemic areas. Additional studies will be required to ascertain whether true endemic cases exist in Missouri.

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