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ORIGINAL ARTICLE

Blastomycosis in Children: An Analysis of Clinical, Epidemiologic, and Genetic Features

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Background. *Blastomyces* spp. are endemic in regions of the United States and result in blastomycosis, a serious and potentially fatal infection. Little is known about the presentation, clinic course, epidemiology, and genetics of blastomycosis in children.

Methods. A retrospective review of children with blastomycosis confirmed by culture or cytopathology between 1999 and 2014 was completed. *Blastomyces* sp. isolates were genotyped by using microsatellite typing, and species were typed by sequencing of *internal transcribed spacer 2* (*its2*).

Results. Of the 114 children with blastomycosis identified, 79% had isolated pulmonary involvement and 21% had extrapulmonary disease. There were more systemic findings, including fever ($P = .01$), poor intake ($P = .01$), elevated white blood cell count ($P < .01$), and elevated C-reactive protein level ($P < .01$), in children with isolated pulmonary disease than in children with extrapulmonary disease. Children with extrapulmonary disease had more surgeries ($P = .01$) and delays in diagnosis ($P < .01$) than those with isolated pulmonary infection. Of 52 samples genotyped, 48 (92%) were *Blastomyces gilchristii* and 4 (8%) were *Blastomyces dermatitidis*.

Conclusion. This is the first large-scale study of the clinical, epidemiologic, and genetic features of blastomycosis in children. The majority of the children had isolated pulmonary disease with systemic findings. Patients with extrapulmonary disease were less likely to have systemic symptoms or additional laboratory evidence of infection, which made delays in diagnosis more common. More than 90% of the pediatric cases were caused by *B gilchristii*.

Keywords. *Blastomyces dermatitidis*; *Blastomyces gilchristii*; blastomycosis; children; genotype.

BACKGROUND

Blastomyces spp. are thermally dimorphic fungi endemic to the Great Lakes region, Ohio and the Mississippi River valley, and the southeastern United States. Sporadic cases of blastomycosis have been reported worldwide. *Blastomyces* exists in the mycelial form in the environment. Disrupted mycelia release conidia that can be inhaled by animals and humans. Conidia rapidly change to the yeast form in vivo, which results in clinical blastomycosis. *Blastomyces* is prevalent in areas of high moisture and rich organic material; however, its environmental niche remains poorly understood [1–16]. The average annual incidence of blastomycosis in areas of endemicity ranges from 1 to 2 per 100 000, and the annual incidence in northern Wisconsin is estimated to be between 5 and 42 per 100 000 [1, 2, 4, 17]. Up to 10% of blastomycosis cases occur in children [18, 19].

Blastomyces produces a granulomatous infection in its host. Clinical characteristics of blastomycosis vary and can include asymptomatic infection, pulmonary infection, or extrapulmonary disease, including bone, genitourinary, cutaneous, and central nervous system infections [18–24]. Blastomycosis often mimics other diseases in humans, which may result in significant delays in diagnosis and poor clinical outcomes [18, 21]. Infection occurs in both immunocompetent and immunocompromised patients [18, 21, 22, 25, 26]. Host susceptibility plays a role in pathogenesis. Patients with immunodeficiency, including human immunodeficiency virus (HIV), have been shown to have more severe infections and an increased likelihood of dissemination [8, 27–31]. The number of alveolar macrophages has been shown to affect fungal load, and in animal studies, bronchoalveolar surfactant production impacted organism binding and host cytokine response [32, 33]. The mortality rate from blastomycosis is estimated to be 6% to 9% [4, 18, 21–24, 34]. Small studies and case series of blastomycosis in children have found pulmonary and extrapulmonary infection but have been limited by sample size, which has left clinical characteristics and significance of the infection in children largely unknown [18, 19, 35, 36].

Blastomycosis can be diagnosed by a variety of methods, including culture, evidence of broad budding yeast on smear or histopathology, serology, antigen testing, antibody testing,

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and polymerase chain reaction [24, 34, 37–42]. Blastomycosis in humans is reportable in several states, including Wisconsin, Minnesota, Michigan, Mississippi, and Louisiana. In Wisconsin, blastomycosis has been a reportable human disease since 1984, and clinical and exposure data for reported cases have been collected by the Wisconsin Division of Public Health (WDPH) since 2004. Blastomycosis must be confirmed by smear, histopathology, culture, or polymerase chain reaction to meet the WDPH case definition.

Although studies of adults have shown that exposure to soil, outdoor employment, and/or canine blastomycosis within the household may increase risk of infection, small pediatric studies have been unable to find these associations in children [3, 5, 7, 18]. In addition, racial and ethnic differences seem to play a role in the pathogenesis of disease, although it is unclear if these difference are present in the pediatric population [7, 22, 24, 43].

Recent advances in the genotyping of *Blastomyces* by microsatellite typing and species typing by *internal transcribed spacer 2* (*its2*) sequencing have shown 2 distinct species (*Blastomyces dermatitidis* and *Blastomyces gilchristii*) of the organism [43–45]. In a small study that compared *its2* sequencing with microsatellite typing, isolates were classified in the same genetic groups by both methods, but these results have not been validated by larger studies [45]. We recently demonstrated that among a cohort of 227 predominantly adult patients, clinical presentation and time to diagnosis varied significantly between group 1 and group 2 strains classified by microsatellite typing [43]. It is unknown which genotype predominates in pediatric cases or how the genotype may affect clinical presentation, duration, and severity of illness or time to diagnosis in children.

In this study, we assessed the clinical presentation, disease course, and environmental exposures for blastomycosis in children and explored if *Blastomyces* genotype affects clinical presentation and outcomes in this population.

METHODS

Patients with blastomycosis were identified from a query of the Marshfield Clinic Health System (MCHS) electronic health records using International Classification of Diseases 9th Revision code 116.0 and review of Marshfield Labs microbiology records from 1999 to 2014. The MCHS is located in northern Wisconsin and comprises more than 50 clinic sites. Additional cases were identified by the WDPH from 2004 to 2014. The WDPH has collected follow-up forms that included clinical and exposure data on patients with confirmed cases of blastomycosis since 2004.

Blastomycosis was defined as a disease that is clinically compatible with *Blastomyces* identified with culture or evidence of broad budding yeast on histopathology or smear. Patients aged 0 to 18 years at the time of diagnosis with clinical information available were included in the study. Patients were classified as having either isolated pulmonary or extrapulmonary infection, which included sites other than the lungs with or without lung involvement.

Research protocols were approved by the Marshfield Clinic Research Foundation Institutional Review Board. A waiver of informed consent was obtained for this retrospective review of clinical information and specimen genotyping.

Clinical and Epidemiologic Data Abstraction

Patient data, including demographics, diagnostic information, exposures, clinical presentation, radiographic and laboratory findings, clinical course, and outcomes, were abstracted from the MCHS electronic health records, state blastomycosis follow-up forms, or both into a RedCap database [46]. When information on follow-up forms and the electronic health records differed, electronic health record information was used, because it generally was more comprehensive and collected at the time of illness rather than retrospectively.

Deidentified epidemiologic data, including age, race, gender, type of infection, date of illness, and exposure history, from all children with confirmed blastomycosis and completed WDPH blastomycosis follow-up forms from 2004 to 2014 were analyzed. Race was either reported by the family or determined by public health officials. Exposure history was obtained by using a standardized WDPH form that included the most common exposures known to increase risk for disease in adults in the 3 months before infection. Exposure history was reported by the patient or a parent, reported by health care providers, or determined by public health officials.

Statistical analysis was completed using SAS version 9.3. Categorical data were compared by using the χ^2 or Fisher exact test. Continuous variables were compared by using 1-way analysis of variance or the Wilcoxon rank–sum test. Means were compared by using the t test. Significance was defined as a P value of $<.05$.

Genotyping and Genetic Analysis of Blastomycosis Isolates

Fifty-two *Blastomyces* isolates from previously identified pediatric patients were available for genotyping. All isolates were obtained as part of clinical diagnosis and identified as *Blastomyces* by using standard methods, which included culture of the mold form on brain-heart infusion agar with blood at 25°C and conversion to the yeast form when incubated in Middlebrook 7H9 broth at 35°C. Isolates were stored at –20°C.

DNA was extracted from frozen isolates using a QIAamp DNA minikit tissue protocol (Qiagen, Valencia, California) with modifications according to Meece et al [44]. For genetic typing of each isolate, 27 polymorphic microsatellite loci were evaluated as described previously [47]. DNA fragment sizes were grouped into their appropriate alleles manually by sing the fixed-bin method [48]. Genetically identical isolates were subsumed to a single unique haplotype; isolates

Table 1. Diagnostic and Genetic Testing of Patients With Blastomycosis

Location of Infection	n (%) (N = 114)
Isolated pulmonary only	90 (78.9)
Extrapulmonary and pulmonary	14 (12.3)
Isolated extrapulmonary	10 (8.8)
Bone	11 (9.6)
Skin	15 (13.2)
Other[a]	4 (3.5)
Diagnostic testing for blastomycosis[b]	
Fungal culture	95 (83.3)
Smear	92 (80.7)
Serology	16 (14.0)
Urine antigen positive[c]	26 (12.8)
Urine antigen negative	5 (4.4)
Isolates genotyped	52
B dermatitidis	4 (7.7)
Isolated pulmonary	1 (25.0)
Extrapulmonary	3 (75.0)
B gilchristii	48 (92.3)
Isolated pulmonary	42 (87.5)
Extrapulmonary	6 (12.5)

Abbreviation: CNS, central nervous system.
[a]Other locations include CNS (2), spleen (1), and abdomen (1).
[b]All patients had blastomycosis confirmed by fungal culture or smear. Patients may have had additional diagnostic testing completed.
[c]Urine antigen testing was completed for 31 patients.

that differed at 1 or more loci were considered unique. After haplotype construction, genetic structure among the individual samples was estimated by constructing an unrooted neighbor-joining tree, of unique haplotypes, based on an allele-sharing distance (D_A) using POPTREE2 [49–52]. Confidence in the resolved topology was based on 10 000 bootstrap replicates. Species typing was performed on each isolate by sequencing of the *its2* region as described previously [53]. Species assignment was based on a fixed nucleotide difference between *B dermatitidis* and *B gilchristii* at position 19 [45].

RESULTS

From 1999 to 2014, 114 confirmed cases of pediatric blastomycosis were identified. Twenty-three patients had data abstracted from state follow-up forms, 58 from the MCHS electronic health records, and 33 from both state follow-up forms and the MCHS electronic health records. All cases were confirmed with smear, culture, or both. Urine antigen testing was completed for 31 patients; the results were negative for 5 patients, and 2 tests were completed before the start of antifungal therapy (see Table 1 for diagnostic testing). Fifty-two patients had isolates available for genotyping. Eight additional patients were diagnosed with blastomycosis on the basis of urine antigen testing results but did not have a positive culture or smear. Given the test's high cross-reactivity with histoplasmosis, these patients were not included in the study [34, 37, 39, 42, 54].

Clinical Characteristics

Of the 114 pediatric patients, 90 had isolated pulmonary infection and 24 had extrapulmonary infection. Of the patients with extrapulmonary infection, 10 had isolated extrapulmonary infection and 14 had combined extrapulmonary and pulmonary infection. Extrapulmonary sites were predominantly skin and bone, although 2 central nervous system (CNS) lesions, 1 splenic lesion, and 1 abdominal mass were identified.

Demographic information is shown in Table 2. The mean age at diagnosis was 12.9 ± 4.6 years, and the youngest patient was 5 months old. Age, gender, and frequency of underlying medical problems did not differ significantly between patients

Table 2. Demographics and Underlying Medical Conditions of Children With Blastomycosis[a]

Demographic	Total (n = 114)	Isolated Pulmonary (n = 90)	Extrapulmonary (n = 24)[b]	*P* Value
Age (mean ± SD) (y)	12.9 ± 4.6	12.7 ± 4.5	13.7 ± 4.8	.34
Male (n [%])	76 (59.3)	52 (58.4)	15 (62.5)	.72
Race (n [%])				
White	73 (64.0)	58 (64.4)	15 (62.5)	.19
Asian	14 (12.3)	14 (15.6)	0 (0)	**.03**
Other/unknown	23 (20.2)	15 (16.7)	8 (33.3)	**.05**
Hispanic	3 (2.6)	3 (3.3)	0 (0)	.49
African American	1 (0.9)	0 (0)	1 (4.2)	.21
Underlying medical problems (n [%])[c]	28 (24.6)	23 (25.6)	5 (20.8)	.63
Pulmonary	17 (14.9)	15 (16.9)	2 (8.3)	.23
Neurologic	5 (4.4)	4 (4.5)	1 (4.2)	.45
Immunocompromised	2 (1.8)	1 (1.1)	1 (4.1)	.30
Other[d]	8 (7.0)	6 (6.7)	2 (8.3)	.31

[a]Categorical data were compared by using the χ^2 or Fisher exact test. Continuous variables were compared by using 1-way analysis of variance or the Wilcoxon rank-sum test. Means were compared by using the t test. Significance was defined as a *P* value of <.05 (shown in bold type).
[b]Includes isolated extrapulmonary and combined pulmonary and extrapulmonary infection.
[c]Some patients had more than 1 underlying medical problem.
[d]Includes 3 patients with endocrinopathy, 2 with a gastrointestinal disorder, 1 with congenital heart disease, 1 with chronic renal disease, and 1 with juvenile idiopathic arthritis not on immunosuppressant medication.

Table 3. Clinical, Laboratory, and Radiographic Findings of Children With Blastomycosis[a]

Clinical Course	Total (n = 114)	Isolated Pulmonary (n = 90)	Extrapulmonary (n = 24)[b]	*P* Value
Fatigue	77 (67.5)	64 (71.1)	13 (54.2)	.12
Fever	90 (79.0)	76 (84.4)	14 (58.3)	**.01**
Weight loss	47 (41.2)	39 (43.3)	8 (33.3)	.38
Cough	93 (81.6)	81 (90.0)	12 (50.0)	**<.01**
Difficulty breathing	45 (39.5)	41 (45.6)	4 (16.7)	**.01**
Hemoptysis	11 (9.7)	10 (11.1)	1 (4.2)	.21
Skin lesion	13 (11.4)	1 (1.1)	12 (50.0)	**<.01**
Bone or joint pain	43 (37.7)	26 (28.9)	17 (70.8)	**<.01**
Fracture	4 (3.5)	0 (0)	4 (16.7)	**<.01**
Chest pain	74 (64.9)	66 (73.3)	8 (33.3)	**<.01**
Poor oral intake	66 (57.9)	58 (64.4)	8 (33.3)	**.01**
Nasal congestion	26 (22.8)	20 (22.2)	6 (25.0)	.77
Pharyngitis	18 (15.8)	17 (18.9)	1 (4.2)	**.05**
Lymphadenopathy	14 (12.3)	11 (12.2)	3 (12.5)	.27
Crackles	37 (32.5)	34 (37.8)	3 (12.5)	**.02**
Wheezing	13 (11.4)	12 (13.3)	1 (4.17)	.15
Decreased breath sounds	43 (37.7)	39 (43.3)	4 (16.7)	**.02**
Abnormal neurologic examination	4 (3.5)	1 (1.1)	3 (12.5)	**.03**
Hypoxia	13 (11.4)	12 (13.3)	1 (4.2)	.16
Laboratory studies[c] (median)	81 (71.1)	65 (72.2)	16 (66.7)	.59
Initial WBC count (per 1000/μL)	13.7	15.1	10.2	**<.01**
Highest WBC count (per 1000/μL)	17.6	19.6	10.7	**<.01**
Highest CRP (mg/dL)	10.5	18.3	2.2	**<.01**
Highest ESR (mm/h)	60.0	75.0	51.0	.21
Chest radiograph obtained	103 (90.4)	84 (93.3)	19 (79.2)	**.04**
Interstitial markings	6 (5.3)	4 (4.4)	2 (8.3)	.26
Infiltrate	94 (82.5)	82 (91.1)	12 (50.0)	**<.01**
Effusion or empyema	30 (26.3)	29 (32.2)	1 (4.2)	**.01**
Mass	15 (13.2)	12 (13.3)	3 (12.5)	.27
Hospitalized[c]	76 (66.7)	58 (64.4)	18 (75.0)	.33
Length of hospitalization (days)	9.0	7.0	10.0	.74
ICU care	23 (20.2)	20 (22.2)	3 (12.5)	.14
Intubated	7 (6.1)	6 (6.7)	1 (4.2)	.38
Required oxygen	24 (21.1)	22 (24.4)	2 (8.3)	.09
Required surgery[d]	46 (40.4)	27 (30.0)	19 (79.2)	**.01**
Bronchoscopy	29 (25.4)	25 (27.8)	4 (16.7)	.27
Biopsy	14 (12.3)	3 (3.3)	11 (45.8)	**<.01**
Length of illness (days)	153.0	140.0	184.0	**.05**
Symptom onset to start of antifungal treatment (days)	19.0	15.0	46.5	**<.01**
Medications received				
Itraconazole	92 (80.7)	72 (80.0)	20 (83.3)	.22
Amphotericin B	39 (34.2)	31 (34.4)	8 (33.3)	.92
Fluconazole	16 (14.0)	14 (15.6)	2 (8.3)	.37
Voriconazole	11 (9.7)	8 (8.9)	3 (12.5)	.24
Ketoconazole	6 (5.3)	3 (3.3)	3 (12.5)	.09
Antibiotics	88 (77.2)	72 (80.0)	16 (66.7)	.17
Length of treatment (days)	140.0	140.0	140.0	.91
Mortality	5 (4.4)	5 (5.6)	0 (0)	.30

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ICU, intensive care unit; WBC, white blood cell.

[a]Categorical data were compared by using the χ^2 or Fisher exact test. Continuous variables were compared by using 1-way analysis of variance or the Wilcoxon rank-sum test. Means were compared by using the t test. Significance was defined as a *P* value of <.05 (shown in bold type). Values are n (%) unless stated otherwise.

[b]Includes isolated extrapulmonary and combined pulmonary and extrapulmonary infection.

[c]Continuous variables are shown as median values.

[d]Patients may have required more than 1 surgery.

with pulmonary and those with extrapulmonary infection. Underlying medical conditions were present in 24.6% of the patients. The most common underlying medical condition was asthma. Patients with isolated pulmonary disease were significantly more likely to be Asian than were patients with extrapulmonary infection (15.6% vs 0%, respectively; $P = .03$).

Clinical characteristics are shown in Table 3. As would be expected, pulmonary findings, including cough (90.0% vs 50.0%; $P < .01$), difficulty breathing (45.6% vs 16.7%; $P = .01$), chest pain (73.3% vs 33.3%; $P < .01$), decreased breath sounds (43.3% vs 16.7%; $P = 0.02$), and crackles (37.8% vs 12.5%; $P = .02$), were more common in patients with pulmonary infection than in those with extrapulmonary infection, respectively. Skin lesions (1.1% vs 50.0%; $P < .01$), bone or joint pain (28.9% vs 70.8%; $P < .01$), fracture (0% vs 16.7%; $P < .01$), and an abnormal neurologic exam (1.1% vs 12.5%; $P = .03$) were more likely in patients with extrapulmonary infection than in those with pulmonary infection, respectively. Fever (84.4% vs 58.3%; $P = .01$) and poor oral intake (64.4% vs 33.3%; $P = .01$) were more likely in patients with pulmonary infection than in those with extrapulmonary infection, respectively.

Laboratory values were obtained for 81 patients. Patients with isolated pulmonary disease had a significantly higher initial white blood cell (WBC) count (15.1 vs 10.2–1000/μL; $P < .01$), highest WBC count (19.6 vs 10.7–1000/μL, $P < .01$), and C-reactive protein (CRP) level (18.3 vs 2.2 mg/dL; $P < .02$) than those with extrapulmonary or combined pulmonary and extrapulmonary infection, respectively. Chest radiographs were obtained in 90.4% of patients and were most likely to show an infiltrate (82.5%) or effusion (26.3%), although 13.2% of these patients also presented with a mass. CNS imaging was obtained in 4 patients, and the results were abnormal for 2 (50%) of them.

Rates of hospitalization, critical care services, and need for intubation were similar between the groups. Patients with extrapulmonary infection were more likely to undergo surgery (30.0% vs 79.2%; $P = .01$), particularly biopsy (3.3% vs 45.8%; $P < .01$), than those with isolated pulmonary infection, respectively. The median length from symptom onset to the start of antifungal treatment was 19 days and was longer among patients with extrapulmonary infection (15.0 vs 46.5 days; $P < .01$) than among the others, respectively. The median length from symptom onset to resolution of illness was 153.0 days and was longer for patients with extrapulmonary infection (140.0 vs 184.0 days; $P < .01$) than for the others, respectively. Five (4.4%) patients received only amphotericin B, 34 (29.8%) received amphotericin B until symptoms improved or during worsening symptoms followed by an oral azole, and 75 (65.8%) received azoles (typically oral) alone. Itraconazole (80.7%) was the most common azole given. Itraconazole intolerance or insurance problems resulted in 17 patients being changed from being given itraconazole to being given a different azole during the treatment course. Antifungal medications and lengths of treatment did not differ between the groups. The median length of treatment was 140.0 days. There were 5 deaths that resulted from blastomycosis (4.4%). Each death occurred in a patient with isolated pulmonary infection who died as a result of respiratory failure. Of these patients, 3 had an underlying medical problem, including 21-hydroxylase deficiency, type 1 diabetes, or renal disease. The lengths of time from symptom onset to the start of treatment in patients who died were not significantly different than those of survivors.

Epidemiology

Epidemiologic data from state blastomycosis forms were available for 111 patients, as shown in Table 4. Epidemiologic surveillance data showed that, most cases were of pulmonary infection only (67.6%). Although less than 2.5% of the population in the reported area is Asian, 14.4% of the patients with blastomycosis were Asian [55]. More cases of blastomycosis occurred in the fall than in other seasons, although this finding was not statistically significant. Living near water was the most common exposure (61.3%), followed by camping, fishing,

Table 4. Exposure and Epidemiologic Data for Children From State Follow-Up Forms

Epidemiologic Data	Value (n = 111)
Age (mean ± SD) (y)	13.0 ± 4.2
Male (n [%])	67 (60.4)
Race or ethnicity (n [%])	
White	67 (60.4)
Black or African American	6 (5.4)
Asian	16 (14.4)
Latino or Hispanic	8 (7.2)
Other	22 (19.8)
Season of diagnosis (n [%])[a]	
Fall (Sep 23–Dec 20)	38 (34.2)
Winter (Dec 21–Mar 19)	26 (23.4)
Spring (Mar 20–Jun 20)	24 (21.6)
Summer (Jun 21–Sep 22)	23 (20.7)
Disease location (n [%])	
Isolated pulmonary	75 (67.6)
Extrapulmonary[b]	26 (23.4)
Unknown	10 (9.0)
Known exposures within 3 mo of diagnosis (n [%])[a]	
Hunting	13 (11.7)
Cabin	13 (11.7)
Camping, fishing, or hiking	50 (45.0)
All-terrain vehicle	21 (18.9)
Clearing brush	29 (26.1)
Excavation	16 (14.4)
Gardening	25 (22.5)
Beaver dam	9 (8.1)
Occupational	3 (2.7)
Travel	28 (25.2)
Dog owner	33 (29.7)
Ever had a dog with blastomycosis (n [%])	9 (8.1)
Household member with blastomycosis (n [%])	10 (9.0)
Lives near water (n [%])	68 (61.3)
<1 mi from water	58 (52.3)
<0.5 mi from water	44 (39.6)
<100 ft from water	22 (19.8)

[a]There was no statistical difference in seasonality or exposure characteristics between patients with disseminated, extrapulmonary, or pulmonary-only disease.
[b]Includes isolated extrapulmonary and combined pulmonary and extrapulmonary infection.

or hiking (45.0%). Of children with blastomycosis, 8.1% had a dog with blastomycosis, and 9.0% had a household member with current or previous blastomycosis. There were no statistical differences in seasonality or documented exposures between the patients with isolated pulmonary disease and those with extrapulmonary disease.

Genotyping

Fifty-two clinical pediatric *Blastomyces* isolates were genotyped. Genotyping revealed 26 unique haplotypes. Fifteen haplotypes were represented by a single isolate. The remaining 11 haplotypes were represented by multiple isolates. Six isolates from 2 different outbreaks were included in this data set [5, 7]; 2 of the haplotypes were observed exclusively in outbreaks. Assembly of an unrooted neighbor-joining tree revealed 2 main genetic groups separated by a deep node bootstrapped at 99%. Forty-eight (92%) isolates were identified as group 1, and 4 (8%) isolates were identified as group 2. There were 22 haplotypes in group 1 and 4 haplotypes in group 2. All group 1 isolates shared identical alleles at 11 of the 27 loci. There was no association with haplotype and disease severity or location of infection. Microsatellite group assignment and the results of *its2* species typing were 100% concordant; group 1 was equivalent to *B gilchristii*, and group 2 was equivalent to *B dermatitidis*. *B gilchristii* infections presented as isolated pulmonary disease in 42 (87.5%) cases (Table 1). *B dermatitidis* infections presented as extrapulmonary disease in 3 (75%) cases (Table 1).

DISCUSSION

This study is the first large-scale study to describe the clinical, epidemiologic, and genetic features of blastomycosis in children. As has been described previously, the majority of the children were previously healthy and presented with isolated pulmonary disease [18, 19, 36]. Our study included 1 infant (aged 5 months) with environmental exposure rather than vertical transmission. There are a few previous reports of infants being infected [35, 56].

As has been shown in studies of adults, a disproportionate number of Asian patients were infected for the geographic area, and only pulmonary disease was identified in this population [7, 43, 57]. We have shown previously that, in a cohort of predominantly adults, 75% of isolates from Hmong patients were from microsatellite group 1, which tends to be associated with isolated pulmonary disease and outbreaks [43]. Previous reports have also shown an increased incidence of blastomycosis among African Americans in Missouri and in aboriginal people in Manitoba, Canada [24]. Similar fungi, including *Coccidioides*, are also more likely to cause disseminated disease in Asians and African Americans, which suggests that host susceptibility or risk of exposure may vary among ethnic groups [58, 59]. Infection in many of the Asian patients in our cohort may be attributed to outbreaks of blastomycosis in a community with a large Hmong population, although this would not explain all cases in the Asian population [5, 7].

Patients with extrapulmonary disease had primarily skin or bone findings. CNS findings were rare (1.8%, which is less than the 3%–15% reported in previous, smaller studies [17–19, 21–23]). One patient had an isolated splenic lesion, and 1 patient had an abdominal mass. Splenic blastomycosis has been reported, but not in children [24]. To our knowledge, this is the first report of an isolated abdominal mass with blastomycosis infection in children or adults.

Similar to what has been described previously in both children and adults, systemic findings, including fever, poor oral intake, elevated WBC count, and elevated CRP level, were significantly more likely in patients with isolated pulmonary infection [20, 43]. Rates of hospitalization, need for ICU care, and rates of intubation were similar for both infection types, although the need for surgery was high among children with extrapulmonary disease (79.2%), likely as a result of difficulty in diagnosis and need for biopsy. The time from symptom onset to treatment start was significantly delayed (19 days), particularly for children with extrapulmonary disease (46.5 days). This time, however, was much shorter than that described previously for children and is comparable with that reported for adults [18, 43]. These findings reinforce the idea that physicians in areas of endemicity need to have a high index of suspicion for blastomycosis. Length of illness was also prolonged a median of 153 days in cases of extrapulmonary disease (184 days) versus isolated pulmonary disease (140 days), which would be expected given the differences in time to initiation of treatment between the groups. As is recommended, oral itraconazole was the predominant method of treatment, either initially or after parenteral amphotericin B was administered [24, 60]. Most patients received treatment for 20 weeks. Treatment lengths and antifungal regimens were similar among patients with isolated pulmonary disease and those with extrapulmonary disease. Five pediatric deaths from blastomycosis in this study are reported; each of these patients had isolated pulmonary disease and acute respiratory distress syndrome as a result of *Blastomyces* infection. This rate is similar to previously reported mortality rates in children. Two of these children had no underlying medical problems. All of them received parenteral amphotericin B. In addition, 1 patient received itraconazole, 1 patient received voriconazole, and 1 patient received both itraconazole and voriconazole concurrently. The length to diagnosis was not significantly delayed in these patients compared to that in the others.

The seasonal distribution of cases was similar to what has been reported previously. Disease occurred throughout the year with a slight fall predominance that was not statistically significant [2, 4, 18, 22, 23, 25]. Similar to adults, the most commonly reported exposures for these children were living near water and camping, fishing, or hiking [1–5, 18, 22–25]. Although we are

able to determine which exposures are most common in children, the study design cannot determine which exposures pose the greatest risk. A surprisingly large number of patients had a dog or household member with blastomycosis, which likely represents proximity to an environmental niche of the organism. There are no previously documented cases of blastomycosis transmission between animals and humans or between humans (aside from presumed vertical transmission from mother to infant). It is important to note that having a household member with blastomycosis is significantly associated with disease in outbreak situations and that outbreaks generally result in a rate of infection that is higher in children than rates from sporadic cases [5].

Genotyping of *Blastomyces* isolates by microsatellite typing and species typing by *its2* sequencing resulted in complete correlation. Of the pediatric isolates, 92% were *B gilchristii* (microsatellite group 1), which correlates with the high levels of pulmonary disease in this population. This finding is strikingly different than that in a predominantly adult cohort in the same clinical system and geographic region where 56% of the isolates were group 1 [43, 47]. Excluding outbreak-associated cases, 89% of the isolates in this study were *B gilchristii.* A smaller study of combined environmental, canine, and human isolates in the upper Midwest and Canada found that *B gilchristii* may be more prevalent in northern Wisconsin [45]. It is unclear if this difference in species that infect adults versus those that infect children is the result of variation in host susceptibility, underdiagnosis of extrapulmonary infections in children, which may be more likely to be *B dermatitidis* (group 2), or differences in exposures to environmental niches for children and adults. Additional clinical analysis for comparing these 2 species in children was limited by the small number of *B dermatitidis* isolates.

There were several limitations to this study. First, although culture and smear remain the gold standards for the diagnosis of blastomycosis, many children had only urine antigen testing completed and were not included in the study. Thus, the severity of disease in pediatric patients in general may differ from what we describe here. In addition, *Blastomyces* isolates were available for genetic analysis in only 52 (46%) pediatric cases. The retrospective nature of this study, particularly in regards to exposure history, makes it prone to recall bias. Finally, the genotypes and clinical findings described in this study were limited to patients from Wisconsin and may not represent other geographic regions where blastomycosis is endemic.

In summary, blastomycosis is a serious and life-threatening illness in children, and diagnosis is often delayed in this population. The majority of the children (>90%) in this study were infected with *B gilchristii* (group 1) and presented with isolated pulmonary involvement, systemic findings, and laboratory evidence of inflammation. Children with extrapulmonary disease were less likely to have laboratory evidence of systemic symptoms, which may result in delays in diagnosis. The etiologic species differed dramatically between children and adults in this study. Future studies are needed to further assess genotype and species distribution, host susceptibility, and environmental risk factors for infection in this population.

Notes

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