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The Outbreak of *Cryptococcus gattii* in Western North America: Epidemiology and Clinical Issues

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Abstract

Over the previous decade, we observed the emergence of the fungal pathogen, *Cryptococcus gattii*, as a cause of disease in humans and animals in a temperate climate. This outbreak, first documented on Vancouver Island, has since expanded throughout Western North America, with non-travel-associated cases now in British Columbia, Washington, Oregon, and California. Additionally, a secondary outbreak, originating in and still restricted to Oregon, has also occurred. During the past several years, several studies detailing molecular typing, virulence, antifungal susceptibilities, epidemiology, and clinical issues have been published. These studies begin to address the complex dynamics of this novel emergence of a rare and fatal fungus, outline clinical characteristics of human cases, and also opened several new areas that should be explored in the upcoming years.

Keywords

Cryptococcosis; Pacific Northwest; Fungal outbreak; Pneumonia; Meningitis

Introduction

Among the threats to human health, infectious diseases remain a significant cause of global morbidity and mortality [1-3]. Advances in healthcare, the onset of the global HIV/AIDS pandemic, the emergence of novel pathogens, and the re-emergence of diseases (ie, increasing incidence and geographic expansion) all contribute to a large worldwide percentage of those affected by bacterial, viral, and fungal diseases [2, 4]. A focal point of disease emergence in the fungal kingdom has been the unprecedented outbreak of *Cryptococcus gattii* in western North America. Although infections remain relatively rare,

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the incidences observed in this outbreak are higher than almost anywhere in the world. In fact, only two reports with higher overall incidences have ever been reported, and both were from tropical regions (Aboriginals in the Northern Territory of Australia, and a population in the central province of Papua New Guinea) [5•, 6, 7]. This is the first and only large outbreak documented to arise in a temperate climate to date.

C. gattii is a basidiomycetous yeast species and is estimated to have diverged from its sibling species *Cryptococcus neoformans* about 37.5 million years ago [8, 9]. Outside of the pathogenic *Cryptococcus* species complex (*C. neoformans* and *C. gattii*), most of the related species are nonpathogenic insect-associated saprophytes [10-12]. However, infections caused by these two species are significant globally, with the predominance of cases estimated to be attributable to *C. neoformans*. In fact, recent studies report that one million individuals are infected annually in Africa, with more than 620,000 attributable mortalities, accounting for about one third of all HIV/AIDS-associated deaths, surpassing tuberculosis mortality [13].

Historically, *C. neoformans* serotypes A, B, C, and D were considered one species; more recently, *C. gattii* was recognized as a unique species, which can be further subdivided into two serotypes (B and C) [14] and four molecular types (VGI-VGIV) [15].

The true prevalence of *C. gattii* as a cause of disease is not clear, because the organism is not routinely distinguished from *C. neoformans* in clinical microbiology laboratories. It can be identified by growth on CGB agar, in which *C. gattii* is canavanine resistant, and able to use glycine as a sole carbon source, triggering a bromothymol blue color reaction [16].

More detailed typing of *C. gattii* has demonstrated that outbreaks are often comprised of a predominance of one particular molecular type (eg, VGI in Australia and VGII in the North American Pacific Northwest) [17, 18]. Furthermore, unique clonal genotypes are frequently associated with outbreaks (eg, VGIIa, VGIIb, VGIIc) [19•, 20, 21]. Therefore, from clinical and surveillance perspectives, identification of species, molecular type, and genotype are important. In understanding the dynamics of cases in Western North America, such identification becomes especially relevant. As stated above, molecular type VGII accounts for about 95% of all cases in the Pacific Northwest, as far south as northern California. However, recent studies identified that more than 90% of cases from a cohort of patients in Southern California were molecular type VGIII, a type that has also been reported in Mexico [22-25]. The diversity observed in Southern California also indicates that VGIII may have been endemic and unrecognized in the region for longer than appreciated [24], highlighting the complexity of the North American outbreak. Because clinical presentation, optimal therapeutic approach, and outcomes may differ compared to *C. neoformans*, a thorough understanding of the epidemiology is needed. Recognition of this outbreak, and clinical aspects, are reviewed here.

Epidemiology

To fully appreciate the epidemiology of *C. gattii* in Western North America, one must examine not only clinical cases, but also veterinary cases and even isolates collected directly from the environment. Humans become infected through the inhalation of infectious

propagules (spores or desiccated yeast) from the environment. Therefore, monitoring the natural habitat is critical [26]. Furthermore, wide ranges of animals have been infected by isolates identical to those infecting humans [27, 28]. Veterinary cases serve as additional resources for data collection; because many animals are often nonmigratory, these cases are frequently sentinels for geographic expansion of outbreaks [29-31].

With the increasing discriminatory power of direct DNA sequence analysis and the continued reduction in costs, sequence analysis for molecular epidemiology has become increasingly applied in the analysis of *C. gattii* strains [19••, 20, 32-34]. The most widely used sequence-based typing method for the analysis of *C. gattii* is multilocus sequence typing (MLST) [35]. This method is robust and portable between laboratories. Most often, analysis of several genomic loci is conducted for each isolate [20, 32, 34].

One important application of molecular epidemiology was applied with the emergence of *C. gattii* in the North American Pacific Northwest. Amplified fragment length polymorphism (AFLP) analysis, then MLST, confirmed that two genotypes were largely responsible for the outbreak, VGIIa/major and VGIIb/minor [17, 21]. Subsequent studies on the outbreak were then employed to confirm these genotypes in the region and also to document that the outbreak had expanded into the United States [20, 27, 36]. Additionally, sequence-based analysis revealed that a third outbreak genotype, unique to Oregon (VGIIc/novel), was also contributing to this ongoing outbreak [19••, 28, 37]. The novel VGIIc genotype is thus far restricted to Oregon, with the exception of one travel-associated case (to Oregon) from Idaho that is slightly divergent from the other VGIIc isolates examined thus far [19••, 37].

Importantly, studies have shown that both the VGIIa and VGIIc genotypes are hypervirulent when compared to other closely related VGII genotypes that are not directly associated with the Pacific NW outbreak, both in macrophages and whole-animal murine models of infection [19••, 20, 38•]. To date, cases of all three of the outbreak genotypes have been reported in humans and other animals. However, there have been no cases of VGIIc in Canada, and VGIIc has yet to be isolated from the environment. Therefore, continued surveillance of human and veterinary cases, and further environmental sampling in the region, is warranted.

In addition to the expansion of the outbreak in the Pacific Northwest, studies now suggest that *C. gattii* molecular type VGIII is endemic in Southern California. A recent re-examination of *Cryptococcus* infections in patients with HIV/AIDS from Los Angeles County (collected prior to 2005) [25] demonstrated about 12% *C. gattii*; more than 90% of these isolates were VGIII. The diversity of genotypes observed was much greater than the levels seen in the Pacific Northwest outbreak region for VGII (> 25 VGIII genotypes). These findings suggest that *C. gattii* VGIII may have been endemic in Southern California for a longer period of time in comparison to the more clonal VGII population found in the Pacific Northwest, or that VGIII is more actively recombining and/or mutating [24]. More recent analysis of VGIII isolates collected from patients who do not have HIV/AIDS from the same region further indicates that *C. gattii* is endemic in the area of Southern California, and infecting humans (Byrnes, Marr, Filler, Heitman, unpublished data).

Another recent case of *C. gattii* infection that developed in a healthy person who lacked travel exposure was documented on Hawaii; the organism implicated in this infection was found to be a unique VGII strain, using MLST (Byrnes and Marr, data unpublished). Hence, these infections have been increasingly documented in Western North America, both in the United States and Canada. A summary of the documented emergence of different *C. gattii* types is presented in Fig. 1. It is important to recognize that many infections are established using antigen assays, thereby negating distinction of species. Even when the organism is isolated in culture, it has not been identified to species level in clinical microbiology laboratories, with most laboratories; hence, a significant burden of *C. gattii* infection is likely to be unrecognized globally, with potential prognostic and therapeutic implications.

Clinical Issues

Risks

One recent and comprehensive retrospective study from the British Columbia Center for Disease Control (CDC) analyzed demographic and clinical features of reported cases, hospitalizations, and deaths attributed to *C. gattii* during 1999–2007 [5•]. A total of 218 cases were reported with an average annual incidence of 5.8 per million persons. Most patients presented with a pulmonary finding (76.6%) or isolated lung cryptococcoma (75.4%). Overall, there were 19 deaths in the cohort, contributing to a mortality rate of 8.7%. About 40% of the patients had some underlying disease; 62% of those with *C. gattii* infection had no prior underlying health concerns [5•].

A study group in the United States initially described the first recognized cohort of 20 patients in Washington and Oregon, and noted that about half of the patients presented with only pulmonary disease, without central nervous system (CNS) findings, with about half of the patients described as “previously healthy”—having no prior medical diagnoses or immunocompromising states [39]. The US Centers for Disease Control and Prevention (CDC) has now summarized a larger cohort within the Pacific Northwest, reporting similarities, but also marked differences compared to what was observed in Canada [40•]. From 2004 to 2010, there were 52 documented cases, with more than 70 cases in the United States to date [40•]. In the United States, only 19% of patients were previously healthy hosts; underlying diseases in patients who presented with *C. gattii* infection include a diverse constellation of mildly to overtly immunocompromised states (eg, chronic obstructive pulmonary disease, hematologic malignancies, myelodysplastic syndromes, autoimmune disorders, and prior receipt of organ transplant). There have been few patients with HIV/AIDS who have developed documented *C. gattii* infection in this region, although these numbers are likely severely underestimated because of lack of routine isolate identification. In the US cohort, 20% of patients were thought to have died from *C. gattii* infection and 13% died with *C. gattii* infection [40•].

Most recently, a study from the British Columbia CDC documented that oral steroids, pneumonia, lung conditions, age ≥ 50 years, smoking, HIV infection, and history of invasive cancer were all significant factors for infection [41•]. This shows that although many without risk factors contract illness, several factors are associated with increased risk of

infection. This study should be used as a foundation for expanding upon the examination of risk factors throughout the region, specifically as it relates to infections in the United States.

These studies are relatively small, but they present some important clinical observations that should be further defined. Although this organism historically was noted to cause disease in primarily healthy hosts residing in endemic regions, in North America, it appears that 20% to 50% of patients who have developed this infection actually have an underlying immunosuppressive condition, or underlying pulmonary disease. The actual prevalence of *C. gattii* infection in patients with HIV/AIDS remains unclear, owing largely to lack of routine microbial diagnosis. It is likely that the diversity in hosts infected with *C. gattii* will at least partially mimic the population residing in the new endemic region; one can conclude that *C. gattii* causes invasive infection in both healthy hosts and patients with a multitude of immunocompromising conditions.

Presentation and Management

Most often, *C. neoformans* is recognized after infection disseminates from the lungs to involve the CNS, with typical findings of meningoencephalitis. A minority of patients are diagnosed with disease isolated to the lung. In contrast, *C. gattii* infection more frequently presents with isolated pulmonary disease, with about half of cases involving the lungs alone. In fact, several of the cases of isolated pulmonary disease were uncovered with biopsy and/or resection of focal lesions thought likely to be malignancy (Marr, unpublished observation).

Findings of meningoencephalitis, including fever, headache, mental status changes, and meningeal irritation develop in a proportion of patients who present with disease involving the CNS. Infection can be very inflammatory, with very high cerebrospinal fluid (CSF) opening pressures and cellular profiles denoting active infection. Radiography frequently demonstrates multiple small or large cryptococcomas, with biopsy typically revealing yeasts and active or granulomatous inflammation.

The current Infectious Diseases Society of North America (IDSA) treatment recommendations for *C. gattii* infection largely approximate the typical approach taken for *C. neoformans*, with induction courses of amphotericin B formulations, combined with 5-flucytosine, and maintenance fluconazole therapy being the primary treatment of infection involving the CNS; some patients with isolated pulmonary disease can be managed with azole therapy alone [42]. However, subtle differences are now just becoming apparent. Management of all CNS cryptococcal diseases requires aggressive management of intracranial hypertension, and this is especially true for *C. gattii* infection. It has been reported that intracranial hypertension occurs in more than 50% of immunocompromised patients, and in even more immunocompetent hosts [43]. During the course of therapy, it is not uncommon for these patients to evolve focal neurologic deficits secondary to inflammatory meningitis and ventricular obstruction, even causing a paradoxical deterioration in the setting of objective improvement of infection (eg, clearance of CSF cultures). In this setting, sterile arachnoiditis causing hydrocephalus can develop [44]. Pressure management, including placement of intraventricular shunts, is critical. Administration of corticosteroids has been reported to be associated with good outcomes,

especially in patients with sterile inflammation causing neurologic decline late in the course of infection [44, 45].

Recently, it has been reported that some *C. gattii* isolates have disparate susceptibilities to certain azole antifungals; specifically, it appears that some VGIIa and VGIIc isolates recovered from the Pacific Northwest outbreak have demonstrated relatively high minimum inhibitory concentrations to fluconazole [37, 46]. The clinical significance of this observation has yet to be documented, although we are personally aware of numerous cases in which fluconazole therapy, especially low-dose therapy, failed to elicit good clinical outcomes (Marr, personal observation). The optimal approach to treatment of *C. gattii* infection has not yet been defined.

Conclusions

In response to the North American *C. gattii* outbreak, a multidisciplinary *C. gattii* working group was established to address the epidemiology, clinical features, and basic science questions surrounding this emergence [40•, 47•, 48]. Very little is currently known about how or why humans develop disease. It likely involves unique host factors, including possible genetic predisposition(s). In addition, the origins of the outbreak genotypes remain elusive. Substantial progress has been achieved in addressing the molecular epidemiology and expansion of the outbreak, and the phenotypic characteristics that make these genotypes unique. However, many critical questions remain to be addressed in order to understand the dynamics of this unprecedented *C. gattii* emergence. Among these, expanded environmental sampling, further phenotypic characterization of associations with host animals and plants, and genome sequencing of *C. gattii* mitochondrial and nuclear genomes, in addition to those available for the VGI isolate WM276 and the VGIIa/major outbreak isolate R265 [49], should be conducted.

In addition, a pressing need exists for prospective clinical studies that capture more information than case description, clinical course, therapies, detailed underlying host risks, and the like. This would most likely be facilitated by the establishment of a network for prospective surveillance and reporting of clinical outcomes of *C. gattii* infections, to allow for follow-up during and after treatment, and the collection of fungal isolates and patient blood samples. A centralized clinical database and sample repository will allow analyses of epidemiology, therapeutic efficacy, clinical risks, and patient outcomes. Furthermore, a linked repository could serve as a critical resource for basic studies of fungal isolates and host immunity/genetic predisposition(s). Understanding aspects of this novel fungal emergence, via examinations of both host and pathogen, will foster translational research aimed at enhancing diagnosis, prognosis, and patient outcomes and serve as a paradigm for examinations of emerging pathogens. These studies, combined with basic research and environmental analyses, will allow for an enhanced understanding of this outbreak and generally how and why *C. gattii* infects hosts to cause life-threatening disease.

Why *C. gattii* emerged in a temperate climate for the first time remains unclear, and it is not known how far the outbreak will expand and if new risk areas may occur in other temperate regions. Furthermore, if the outbreak will inexorably expand down the coast or alternatively

discontinuously jump to a new region remains unclear. This makes the Pacific Northwest outbreak a focal point for studies and a paradigm for novel emergent fungal diseases. To this end, it is critical that clinical laboratories and veterinary diagnostic laboratories begin to assign species identity to clinical isolates, thereby distinguishing *C. neoformans* and *C. gattii*.

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Fig. 1.

Distribution of *Cryptococcus gattii* infections in Canada and the United States. From 1999–2011, *C. gattii* infections have been documented in British Columbia (BC), Alberta (AB), Washington (WA), Oregon (OR), California (CA), Hawaii (HI), and North Carolina (NC). Molecular types (VGI–VGIII) and outbreak genotypes (VGIIa, VGIIb, and VGIIc) are listed. All molecular types/genotypes were reported in human cases, with the exception of VGIIb-WA, VGIIb-CA, and VGI-HI. These types have only been found in animal cases to date but are presumed to be endemic in the respective regions and consequently could infect humans. All cases have occurred in western North America with the exception of well-documented travel-associated cases in Alberta and North Carolina