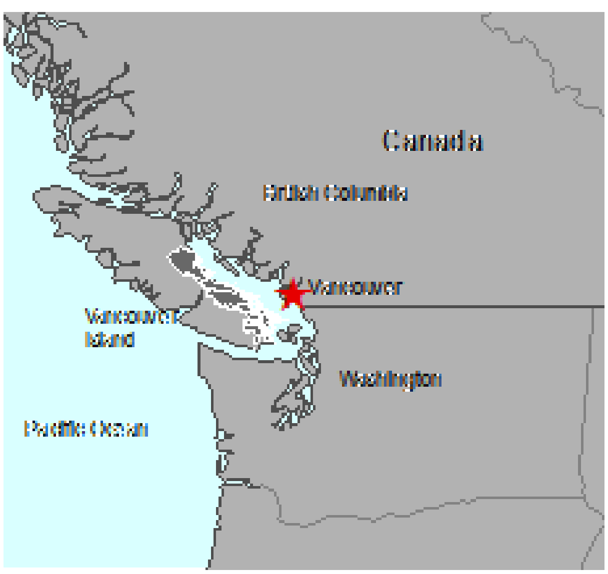
The emergence and colonization of *Cryptococcus gattii* in British Columbia

Emerging disease notice

December 22, 2004

Summary

During 1999, the eastern portion of Vancouver Island, British Columbia began experiencing an outbreak of a rare cryptococcal disease previously known to exist only in tropical and sub-tropical regions. It is thought that this outbreak on Vancouver Island is the world’s largest outbreak of cryptococcosis ever identified. It is not yet understood how this pathogen, widely regarded as a “tropical” fungus, emerged on Vancouver Island, however changes in the environment and the importation of tropical plants are among the suspected causes (APEC Emerging Infections Network 2004). The emergence of *Cryptococcus gattii* and its ability to colonize on Vancouver Island stresses the importance of worldwide monitoring of its distribution. Particular focus should be given to those areas that have climactic and ecological attributes similar to eastern Vancouver Island.



Since 1999, numerous animals and marine mammals on or offshore of Vancouver Island have been affected by disease caused by *C. gattii*. Diseased animals may present with signs of rhinitis and skin lumps and *C. gattii* can result in lung nodules, pneumonia, meningitis, and acute neurological disease. Over half of the affected companion animals diagnosed by veterinarians with *C. gattii* have died or been euthanized (Western College of Veterinary Medicine 2004).[1]

A case control study of diseased animals in 2001 helped scientists ascertain that these cases were part of a larger disease cluster narrowly focused on Vancouver Island's east side (Stephen, Lester, Black, *et al*. 2002). The shaded portion of the map shows the area where *C. gattii* has been isolated from environmental sources. Most diagnosed cases of *C. gattii* in the current outbreak have been linked to this area.

In addition to the affected wild and companion animals, over 100 humans have been diagnosed with *C. gattii*, four of which have died since the onset of the outbreak. Because the majority of affected human patients in the Vancouver Island outbreak were immunocompetent, the British Columbia Centre for Disease Control has listed cryptococcal infection as a notifiable disease.

Background

On June 6, 2002 the British Columbia Centre for Disease Control issued a health advisory alerting veterinarians, doctors, and residents to the emergence of *Cryptococcus neoformans var. gattii* (CNVG) on Vancouver Island and cautioning inhabitants to be on the alert for symptoms known to be associated with the disease (British Columbia Centre for Disease Control 2002). Since the onset of the outbreak on Vancouver Island, CNVG has been reclassified as a distinct species rather than a variety of *Cryptococcus neoformans* and is referred to in recent literature as *C. gattii* (Meyer, Boekhout, Kwon-Chung, *et al.* 2003). For convenience, the term *C. gattii* will be used in this disease notice in reference to studies prior to the reclassification from CNVG to *C. gattii*.

Of the 37 currently defined cryptococcus species, only *C. neoformans* and *C. gattii* are considered to be major pathogens for animals and humans. *C. gattii* and *C. neoformans* are structured as microscopic haploid and encapsulated (round- to oval-shaped) yeast-like fungi. These two species are comprised of five serotypes; serotype A (*C. neoformans* var. *grubii*), serotype D *(C. neoformans* var. *neoformans*), the hybrid serotype AD (corresponding to the teleomorph *Filobasidiella neoformans* var. *neoformans*), and serotypes B and *C* (*C. gattii*) (Meyer, Boekhout, Kwon-Chung, *et al.* 2003). The most common type that causes disease is serotype A followed by serotype D. Serotypes A and D cause disease mostly in temperate climates and in immunocompromised hosts and are considered to be opportunistic pathogens, while serotypes B and C, regarded as primary pathogens, generally infect immunocompetent hosts (Kibsey 2003).

The first case of animal cryptococcal disease in the current Vancouver Island outbreak was diagnosed as *C. gattii* in February 2000. Prior to this time, a major veterinary clinic servicing British Columbia and Alberta typically reported about five cryptococcosis cases annually (Stephen, Lester, Black, *et al*. 2002). By March 2002, 45 animal cases of cryptococcosis were found to be due to *C. gattii* and additional cases were suspected but not laboratory-confirmed. Principally, dogs and cats were affected. By the summer of 2004, almost 200 animal cases of *C. gattii* were documented as originating on Vancouver Island (Western College of Veterinary Medicine 2004).

Most cases in the current Vancouver Island outbreak have been identified as *C. gattii* serotype B, although researchers in Australia isolated *C. neoformans* var. *grubii* from two of Vancouver Island's diseased ferrets (Malik, Alderton, Finlaison *et al.* 2002).

A study of *C. gattii* cases on Vancouver Island between 1999 and 2001 found that 28 of 35 affected animals (80%) resided on the east coast of Vancouver Island. Four of the remaining terrestrial animal cases had visited the island within one year of diagnosis (Kidd, Hagen, Tscharke, *et al*. 2004). In another study, researchers tested over 300 healthy dogs and cats on Vancouver Island and found that about 2% were positive with *C. gattii*, but did not display clinical signs (Western College of Veterinary Medicine 2004).

Wild and companion animals that have died due to *C. gattii* include dozens of cats and dogs, eleven porpoises, one horse, and a llama (APEC Emerging Infections Network 2004, Stephen, Lester, Black, *et al*. 2002).[2] Other animals such as birds, cattle, foxes, and goats are susceptible to *C. gattii* infection, however none of these species on Vancouver Island has been diagnosed with the disease (APEC Emerging Infections Network 2002). A case series and case control study of animals did not find any significant risk factors for infection due to *C. gattii*, other than residing on or visiting the east coast of Vancouver Island (Stephen, Lester, Black, *et al*. 2002).

Clinical presentations

Animals infected with *C. gattii* present clinical signs of rhinitis and skin lumps and infection may result in lung nodules, pneumonia, and meningitis (British Columbia Centre for Disease Control 2004, Kibsey 2003). The incubation period for *C. gattii* is estimated to be from two to nine months and typically the infection is curable with anti-fungal drugs, however with progression to meningitis and CNS abnormalities successful treatment is rare (APEC Emerging Infections Network 2002, Western College of Veterinary Medicine 2004).

Epidemiology of *C. gattii*

Cryptococcal disease is caused by inhalation of cryptococcal spores, thought to be basidiospores or (less likely) dessicated yeasts. Animals may also acquire infection through open wounds (University of Wyoming 2004). The infection can not be spread through contact with animals or humans (British Columbia Centre for disease Control 2004).

Since it was initially characterized in 1970, isolation of *C. gattii* in animals has been relatively rare. When cryptococcal disease was identified in animals, serotyping procedures were usually not performed, however *C. gattii* was isolated in several dogs, cats, and horses in Australia during the early to mid 1990's (Campisi, Mancianti, Pini, *et al.* 2003). Also, during the early 1990's *C. gattii* was isolated from six goats with pulmonary disease in Spain . The six goats belonged to five different herds that experienced outbreaks due to *C. gattii*. Between 2.5 and 12% of the goats were affected and while the principal clinical signs were respiratory in nature, brain and liver infections were found in three of the five outbreaks (Baro, Torres-Rodriguez, Mendoza, *et al* 1998). Prior to 1990, fatal pulmonary cryptococcosis has also been described in two species of dolphins and in 1999 an Atlantic Bottlenose Dolphin in California was found to have been infected with *C. gattii* serotype B (Miller, W.G., A.A. Padhye, W. van Bonn, *et al*. 2002). While no environmental associations were described in the goat herd cases, it is thought that *Eucalyptus camaldulensis* trees in the local environments played a role in the Australian animal and California dolphin cases.

Although occurrences are relatively rare, disease due to *C. gattii* is endemic in Australia , Papua New Guinea , parts of Africa, the Mediterranean region, India , south-east Asia, Mexico , Brazil , Paraguay , and southern California (Mycology Online 2004). An Australian study found that all of the isolates gleaned from koalas and eucalypts exhibited the same genetic fingerprint. This finding is consistent with an epidemiological association between mammalian disease and exposure to host eucalypts (Mycology Online 2004).

In Australia, the rate of human disease is about one case per million per year, while on Vancouver Island the rate is estimated to be up to 37 cases per million per year for 2002 and 2003 (Kidd, Hagen, Tscharke, *et al*. 2004). According to researchers from the University of British Columbia and the British Columbia Centre for Disease Control, the Vancouver Island outbreak is the world's largest outbreak of cryptococcal disease ever identified (University of British Columbia--British Columbia Centre for Disease Control 2003).

Ecology of *C. gattii*

The first environmental isolation of *C. gattii* in the Barossa Valley, South Australia established a specific ecological association with *Eucalyptus camaldulensis*, a species of red gum eucalyptus tree widely distributed in mainland Australia (Ellis, Pfeiffer 1990). Since the initial isolations, four other species of eucalypts have been confirmed as natural reservoirs for *C. gattii*, three of which have been exported to countries where human *C. gattii* disease has been reported (Mycology Online 2004). Early investigations in Australia failed to reveal *C. gattii* hosts other than the five species of eucalypts (Sorrell, Ellis 1997).

In the endemic area of Vancouver Island, trees that have tested positive for *C. gattii* include alder, bitter cherry, cedar, Douglas Fir, and Garry Oak. Moreover, it is suspected that birds may carry the fungal spores from tree to tree (Thomson 2002). Surveillance for *C. gattii* also found that the spores can survive on driftwood in salt water, indicating a vector possibly affecting marine mammels (University of British Columbia 2002). More rarely, *C. gattii* has been isolated from bat feces, insect frass, a wasp's nest, and other substrata in other endemic areas (Baro, Torres-Rodriguez, Mendoza, *et al*. 1998).

Much effort is being given to determine the cause of *C. gattii*'s emergence on Vancouver Island. Analysis of weather patterns reveals that, compared to the 20 year average, the eastern coast of Vancouver Island has undergone a rising trend in summer temperatures over the past several years prior to the isolation of *C. gattii* on the island. In addition, Vancouver Island has experienced a series of wetter than normal winters followed by dryer than normal winters (Bartlett, MacDougall, Mak, *et al.* 2004). In California, an investigation into the diffusion of *Coccidioidomyces* (found in soil) indicates that this yeast is more likely to become airborne during summers after a rainy winter than after a dry winter (Bartlett, MacDougall, Mak, *et al.* 2004). Further research exploring the possible association of climate and the emergence of *C.gattii* on Vancouver Island is currently underway.

Molecular and genetic indications

Studies have documented the presence of four molecular types of *C. gattii* isolates; VGI, VGII, VGIII, and VGIV, and based on observed recombination outcomes between *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* it was postulated that speciation within the cryptococcal complex is a continuing process (Meyer, Boekhout, Kwon-Chung, *et al.* 2003). A recent examination of *C. gattii*-positive samples isolated on Vancouver Island found that 20 of 21 clinical isolates and 5 of 6 veterinary isolates were of the VGII molecular type, while the remaining isolates were identified as VGI. Of 67 environmental isolates (58, 8, and 1, respectively, from trees, air, and soil), all were of the VGII molecular type. A high similarity (>88%) was found between the Vancouver Island isolates and randomly chosen *C. gattii* database strains from Australia and the US and it has been suggested that this genotype may have the ability to colonize and cause disease in other temperate climes (Kidd, Hagen, Tscharke, *et al*. 2004).

Of the two mating cell types associated with *C. gattii*, MAT**a** and MAT**α**, the MAT**α** type is typically associated with virulence. While isolates from Australia have been found to be of the MAT**a** and MAT**α** types and not fertile, the majority of isolates on Vancouver Island are type MAT**α** and are fertile. Findings from a 2003 study led to speculation that a possibly recent serotype recombination event may have resulted in a *C. gattii* pathogen on Vancouver Island with increased virulence, an expanded host range, and an altered environmental niche (Fraser, Subaran, Nichols, *et al.* 2003). In a more recent study in which a mating analysis of VGII subtypes from Vancouver Island isolates was performed, it is postulated that major recombination between subtypes was unlikely to have occurred on Vancouver Island and that the differing strains of isolates were most likely introduced to the island. The authors suggest that their investigation results may indicate that *C. gattii* has been present on the island for a longer period and that a recent unknown event may have resulted the pathogen's release into local environs, intersecting with humans and animals (Kidd, Hagen, Tscharke, *et al*. 2004).[3]

Concluding thought

Regardless of the recombination ability of *C. gattii* strains on Vancouver Island or the length of time that *C. gattii* has been present on the island, the emergence of this "tropical" fungus and its ability to colonize on Vancouver Island stresses the importance of worldwide monitoring of its distribution. Particular focus should be given to those areas that have climactic and ecological attributes similar to eastern Vancouver Island.

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[1] -Often animal owners opt for euthanization due to the cost of required antifungal medicine.

[2] - Two llamas were found to have died from cryptococcosis, however only one was serotyped and found positive for *C. gattii* Also, one of nine porpoises stranded on the San Juan Islands, WA, USA in 2002 was found positive for cryptococcosis, however the serotype in this case is not known.

[3] - A case of cryptococcal disease in Seattle in the early 1970’s was retroactively identified as *C. gattii*, perhaps supporting this theory.