5.5 *CRYPTOCOCCUS GATTII*, A TROPICAL PATHOGEN EMERGING IN A TEMPERATE CLIMATE ZONE

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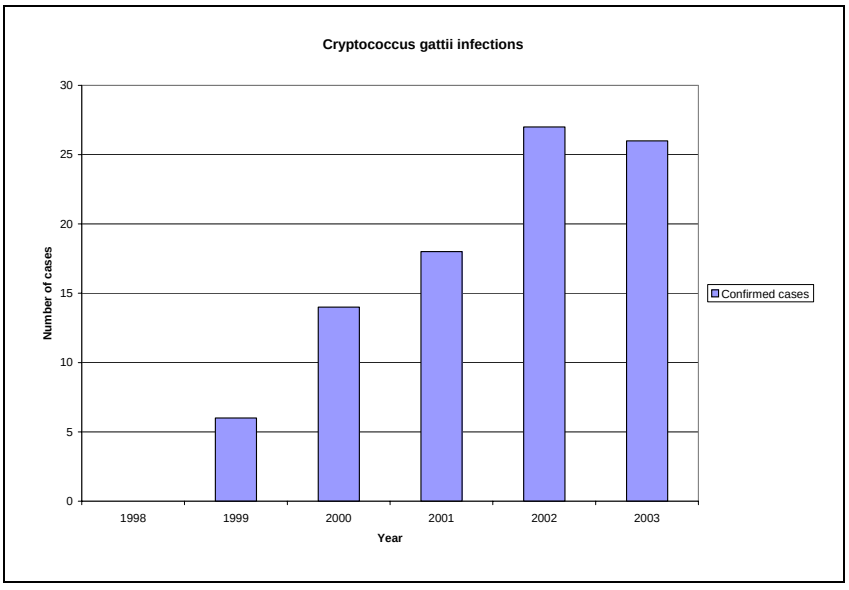
Introduction: Pathogenic cryptococci primarily belong to one of five serogroups: A (*Cryptococcus neoformans* var. *grubii*), B or C (*Cryptococcus gattii*), D (*Cryptococcus neoformans* var. *neoformans*) or the hybrid AD[1,2]. Cryptococci of serogroups A and D have a world wide distribution and are particularly associated with soil and weathered bird droppings, and are pathogenic for immunocompromised humans and animals [2-4]. The primary route of exposure to human disease is the lung with possible systemic spread to the central nervous system. Until recently, *C. gattii* was thought to have a limited habitat in the tropics or subtropics, and to inhabit specific tree hosts, the river gum Eucalypts. Beginning in 1999, increasing numbers of cases of human and animal disease associated with *C. gattii* were confirmed in hosts with no other travel history than to have lived or to have visited the eastern coast of Vancouver island, Canada. Vancouver Island is in a temperate climate zone.

Objective: As *C. gattii* is a basidiomycetous yeast associated with the environment, a search was initiated for sources of exposure to this organism. Particular emphasis was placed on documenting airborne exposures to account for the human and animal cases of cryptococcosis.

Methods: Samples were taken from the homes and environs of clinical cases (human and animal). Swab samples from the bark of trees, air samples from under the tree canopy, and soil samples from the loam surrounding trees were examined for the presence of *Cryptococcus* using a differential agar (Staib agar) for recovery of the organism. Trees which were culture positive by swab sample were mapped by GIS coordinates and repeatedly examined on a monthly basis to determine seasonal variation in air or soil concentration. Molecular fingerprinting (RFLP) was performed on human, animal, and environmental isolates.

Results:
The first cases of cryptococcosis in immunocompetent hosts were recognized by the Central Laboratory for Veterinarians, who informed the BCCDC. Cases of cryptococcosis in immunocompetent animals and humans were found to belong to serogroup B, *C. gattii*, an organism not previously described in British Columbia. The number of human cases through 2003 is shown in Figure 1.

Figure 1. Incidence of confirmed human cases of C. gattii infections (1998 – 2003).



Demographics: Mean case age at diagnosis was 59.7 years (range 20 – 82; SD 13.5). Fifty-eight percent of cases were male. Sixty percent were retired or unemployed at the time of their illness. Thirty matched case-control sets were interviewed. Risk factors for a diagnosis of cryptococcosis included: prior diagnoses of pneumonia (OR 2.7, 95% CI 1.05 – 6.98) or other lung problems (OR 3.2; 95% CI 1.08 – 9.52), or use of systemic corticosteroids (OR 8.1; 95% CI 1.74 – 37.8).

Trees in the environs of animal and human cases were examined for the presence of the yeast. Table 1 lists tree hosts found to date on the east coast of Vancouver Island.

Table 1. Trees or woody debris associated with positive *Cryptococcus gattii* colonization.

Tree name		**Number sampled**	**% positive**
Alder	(*Alnus rubea*)	206	14
Arbutus	(*Arbutus menziesii*)	110	11
Ash	(*Fraxinus*)	2	50
Birch	(*Betula*)	20	0
Bitter cherry	(*Prunus*)	8	25
Cherry (flowering or ornamental)		16	0
Cottonwood	(*Populus*)	10	0
Cedar	(*Thuja plicata*)	198	10
Dogwood	(*Cornus*)	5	0
Douglas fir	(*Pseudotsuga menziesii*)	618	13
Eucalyptus	(*Eucalyptus*)	23	0
Fir	(*Abies*)	6	33
Fruit trees (apple, pear, plum)		14	0
Garry Oak	(*Quercus garryana*)	81	17
Hemlock	(*Tsuga heterophylla*)	28	0
Maple	(*Acer macrophyllum*)	142	9
Meadows		7	14
Pine	(*Pinus*)	52	4
Poplar	(*Populus*)	6	0
Spruce	(*Picea*)	30	3
Stumps		11	9
Willow	(*Salix*)	11	0
Other (exotics, shrubs, cut lumber, straw, bark)		187	3
Total		**1791**	**12**

Air samples were taken in areas where colonized trees were found. Unlike the swab samples, which did not show a correlation with season, air samples were more likely to be a higher concentration in the summer than in the winter. Geometric mean concentrations of *C. gattii* are shown in Table 2.

Table 2. Airborne concentrations of *C. gatti*i on the east coast of Vancouver Island.

Season		**n**	**GM[a] CFU/m^3**	**GSD[b]**	**Range CFU/m^3**	**p-value[c]**
Summer	(June – August)	27	30.5	11.6	0 – 2692	*
Fall	(September – November)	23	6.7	5.5	0 – 436	* †
Winter	(December – February)	25	1.2	1.8	0 - 7	* † ‡
Spring	(March – May)	21	5.3	6.2	0 - 550	*

[a] geometric mean
[b] geometric standard deviation

[c]legend: *, †, ‡ group mean indicated significantly different from other means ($p < 0.001$) by Scheffe post hoc test.

Soil samples were taken from areas near colonized trees. Colonization of soil varied in concentration, with highest levels in the centre of the colonized areas and lower concentrations at what appears to be the extremes of the colonized zone. Table 3 lists soil concentrations and relative distances of sampling sites.

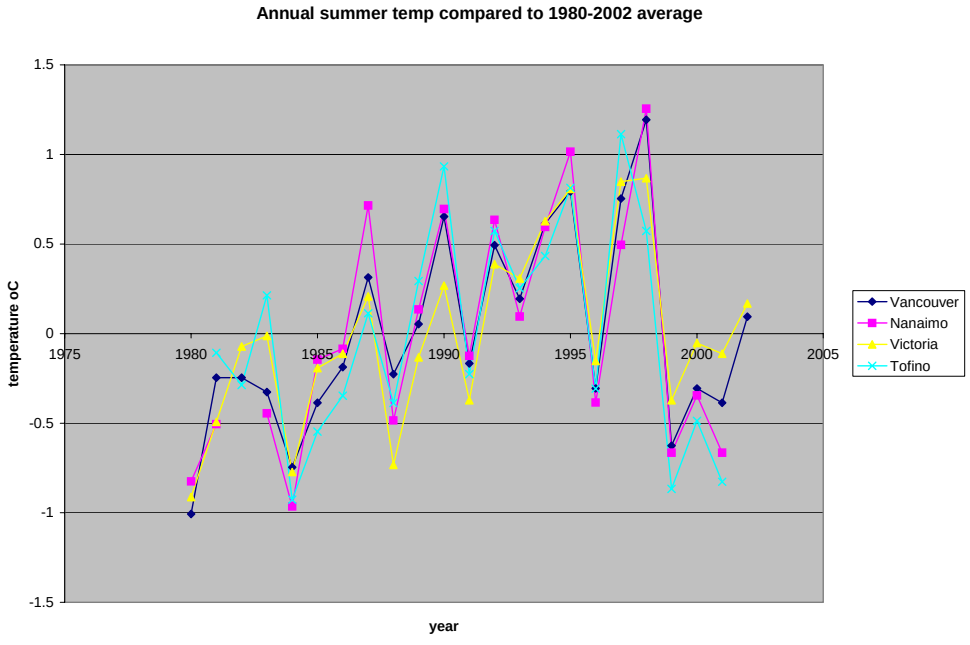
Table 3. *C. gattii* concentration in soil by geographic location.

Location	n	GM (GSD)		AM (SD) CFU/gram soil		Range
(1) Victoria* (southern tip of Vancouver Island)	101	5	(12.3)	125	(388)	0 – 3200
(2) Cowichan † (~ 50 km north of Victoria)	101	57	(44.2)	3850	(17033)	0 – 146980
(3) Nanaimo (~50 km north of Cowichan)	127	2	(4.2)	50	(390)	0 – 4325
(4) Parksville ‡ (~ 30 km north of Nanaimo)	266	12	(17.4)	1010	(6954)	0 – 86864
(5) Courtenay (~ 80 km north of Parksville)	133	3	(9.4)	135	(562)	0 – 4320
(6) Little Qualicum (~ 20 km west of Parksville)	39	29	(61.8)	10730	(33285)	0 – 161700
(6) Port Alberni (~ 40 km west of Parksville)	82	3	(7.8)	70	(277)	0 – 2080
(7) Gulf Islands (~ 10 – 25 km west of Cowichan)	64	0	0	0	0	0
(8) Mainland BC (~ 60 km east of Vancouver Island)	55	0	0	0	0	0

PCR:
Clinical cultures from immunocompetent humans and animals, and environmental isolates belonged to serogroup B. PCR-RFLP analysis of clinical and environmental isolates revealed two genetic variants, VGII (93%) and VG1 (7%). Clinical and environmental isolates were shown to be from the same clones.

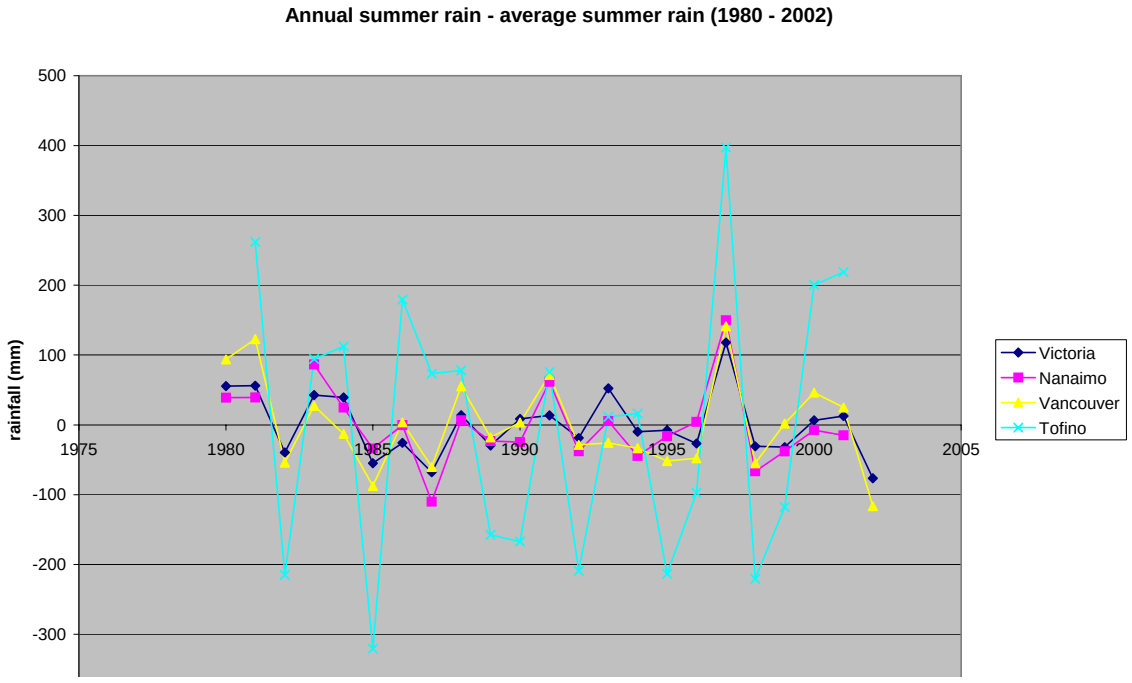
Climate:
The eastern coast of Vancouver Island has a unique climate in British Columbia. The summers tend to be very dry and the winters wet and mild (May - September mean rainfall 28.4 mm vs October – April 101.2 mm $p < 0.05$; summer temperature 14.7 ^{o}C vs winter 6.5 ^{o}C $p < 0.001$). There was a trend upward for summer temperatures compared to the 20 year average for a number of years preceding the appearance of cases of this disease.

Figure 2 illustrates summer temperatures from 1980 – 2002.



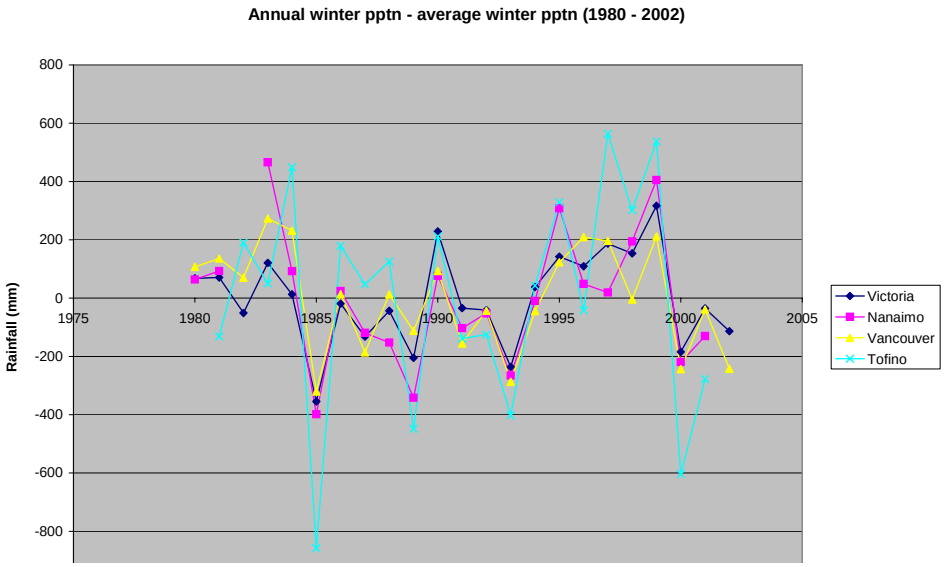
Annual summer rainfall was high in the strong El Niño year 1997 – 1998, followed by lower than average summer rainfall in the La Niña years 1998 – 2000 as illustrated in Figure 3.

Figure 3. Annual summer rainfall (mm) less average of years 1980 – 2002.



The annual winter precipitation was higher than average for the period 1994 – 1999 followed by drier than normal years 2000 – 2002 as illustrated in Figure 4.

Figure 4. Annual winter rainfall less average rainfall 1980 – 2002.



Notes for temperature and precipitation graphs: Victoria and Nanaimo are on the eastern coast of Vancouver Island. Vancouver is on the Mainland of British Columbia and is in the same biogeoclimatic zone as Victoria and Nanaimo. Tofino is on the western coast of Vancouver Island and is in a different biogeoclimatic zone. Tofino is included as a comparison to the zones where *Cryptococcus gattii* has been isolated.

Interestingly, a pattern has been observed in California, where *Coccidioidomyces*, a pathogenic yeast found in soil, is more likely to become airborne in summers after a rainy winter than after a dry winter.[5]

Discussion: This is the first description of recovery of airborne *Cryptococcus neoformans* from a stable environmental niche in a temperate climate zone. The appearance of *Cryptococcus neoformans* var. *gattii* as a clinical isolate in humans and animals can be established as late 1999, and may be related to climate. A series of wetter and then drier than normal winters may be related to the high air concentrations of *Cryptococcus* during the sampling period 2002-2003.

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