

Meet the new kid on the block
Cryptococcus gattii

Karen H. Bartlett, PhD
Associate Professor

kbartlet@interchange.ubc.ca
School of Environmental Health
University of British Columbia



The story as it unfolds ...

- In 2001, veterinary lab first to notice ↑ cases of cryptococcosis in pets on Vancouver Island
- About the same time, medical microbiologist also finds ↑ human cases ...

What is cryptococcosis?

- *Cryptococcus* is an encapsulated yeast and opportunistic primary pathogen.
- Cryptococcosis is the infection ... may involve lungs, CNS, or other organ systems.
- Almost exclusively a disease of immunocompromised hosts.
- It's the fourth most common cause of life threatening infections in persons with AIDS.

Typical cryptococcosis ...



Encapsulated yeast,
can identify from
microscope

“Normally”
associated with
pigeons, world
wide distribution



However!

- ↑ cases on Vancouver Island were NOT in immunocompromised humans or animals
- *Cryptococcus* isolates cultured from human cases were looked at again and subjected to serotyping
- Surprise! The *Cryptococcus* from these British Columbia cases were serogroup B.

Cryptococcus neoformans

- There are 4 serogroups of *Cryptococcus neoformans* that are pathogenic to mammals
- A and D are found world wide
- B and C are only supposed to be found in tropical or subtropical countries

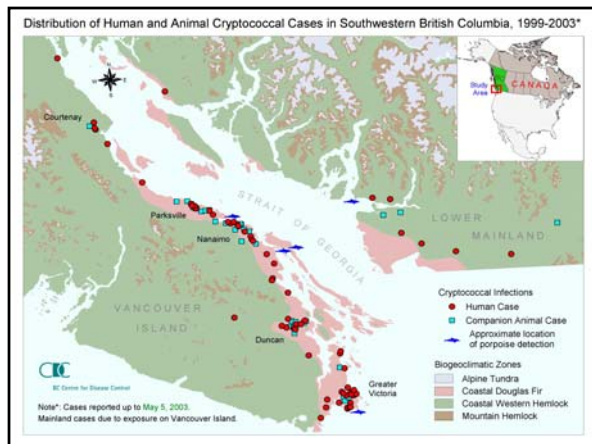
- BUT ... none of the new cases reported travel histories.

Relationship of pathogenic cryptococci

Variety	Serotype	Molecular type
<i>C. neoformans</i> var. <i>grubii</i>	A	VN I VN II
<i>C. neoformans</i> var. <i>neoformans</i>	D	VN IV
<i>Neoformans hybrid</i>	AD	VN III
<i>Cryptococcus gattii</i>	B	VG I VG II VG III VG IV
	C	VG I VG II VG III VG IV

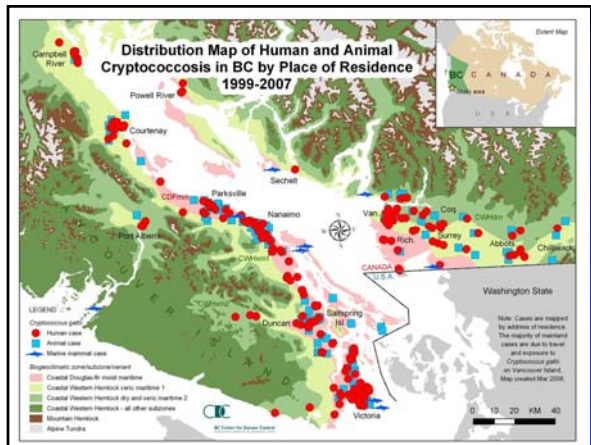
Outbreak ...

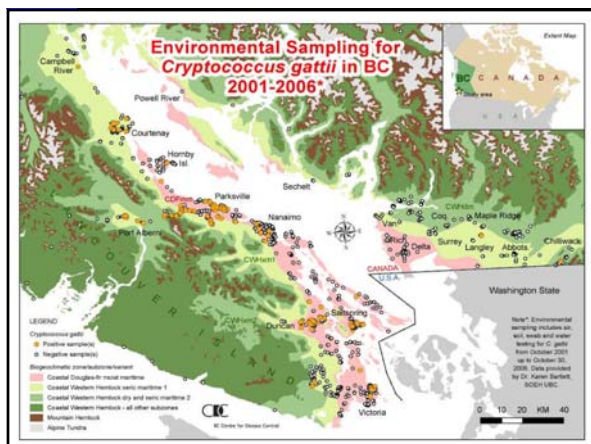
- Annual incidence of all cryptococcosis (including AIDS associated) ~ 3-5/10⁶
- As of July 2001:
 - Humans (n=38)
 - Pets (n=34)
 - Porpoises (n=2)
- Incidence on Vancouver Island ~ 20/10⁶

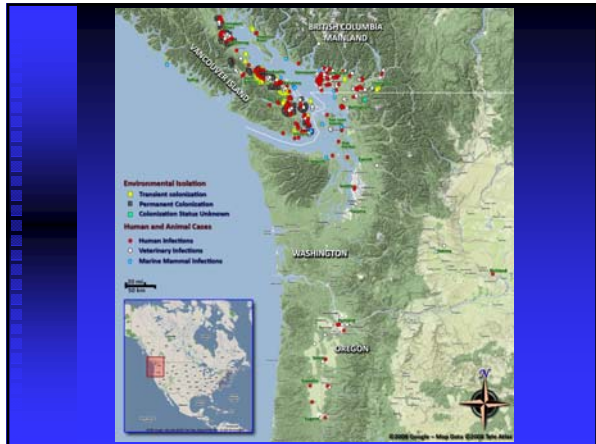


Outbreak ...continues

- Annual incidence of all cryptococcosis (including AIDS associated) $\sim 3\text{-}5/10^6$
- As of December 2008:
 - Humans (n=239)
 - Pets (estimate = 3 – 4 times diagnosed human cases)
- Incidence on Vancouver Island $\sim 24/10^6$
- Case fatality ratio 8.7 % (19 deaths)







Objective:
Identify *Cryptococcus gattii*

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Microscopic 1000x




India ink or calcofluor white
Capsule
Budding yeast with narrow base

Objective:
Identify *Cryptococcus gattii*
Cryptococcal antigen latex agglutination

- Very sensitive – 10 ng /mL biologic fluid
- Positive serum test (1:4) probable infection
- Positive test \geq 1:8 active infection
- Can be used to monitor effectiveness of tx
- Serum (pretreat with pronase)
- CSF (no pretreatment)


Does not differentiate between *C. gattii* and *C. neoformans*

Objective:
Identify *Cryptococcus gattii*

Culture techniques:


Primary plating as normal –
Cryptococcus grows well on Sabouraud
Dextrose Agar
Colonies visible at 48 – 72 hr
Optimal growth 30 – 35 °C (slower \geq 37)
Cream coloured, glistening or matte

Objective:
Identify *Cryptococcus gattii*



Add primary isolation media
Bird Seed Agar or Caffeic Acid Agar
(*C. gattii* = dark chocolate brown)
(*C. neoformans* = milk chocolate brown)

Objective:
Identify *Cryptococcus gattii*



Confirmation agar:

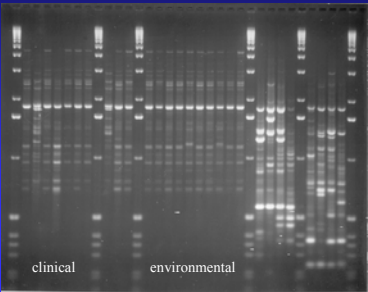
Canavanine-glycine-bromthymol blue (CGB)
C. gattii = grows & turns medium blue 24 h – 5 days
C. neoformans = no growth, medium yellow or green

Objective:
Identify *Cryptococcus gattii*

Physiological tests:
C. neoformans and *C. gattii* are indistinguishable:

- Germ tube test negative
- Hydrolysis of urea positive (rapid test available)
- Growth on cycloheximide media negative
- Carbohydrate assimilation tests
- Caffeic Acid disk (rapid test available)

URA5-RFLP unique genotypes



Source:
S. Kidd

C. gattii MLST genotyping scheme

URA5 - orotidine monophosphate pyrophosphorylase

CAP59 - capsular associated protein

GPD1 - glyceraldehyde-3-phosphate dehydrogenase

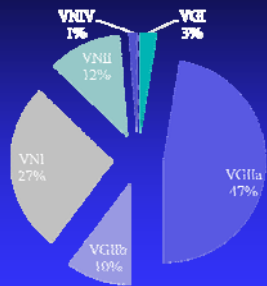
LAC1 - laccase

PLB1 - phospholipase

SOD1 - superoxide dismutase

IGS1 - rRNA intergenic spacer

Genotyping *Cryptococcus* at BCCDC



Source: M. Morshed

Susceptibility testing *Cryptococcus*

- Break points not established for crypto.
- E-test used by many laboratories
- Micro-broth dilution media not standardized (added glucose ±, shaking ±)

- Fluconazole ± Ampho B for uncomplicated pulmonary 3 – 12 months



Susceptibility testing *C. gattii*

Genotype	MIC µg/mL fluconazole			
	Range	Geo Mean	MIC ₅₀	MIC ₉₀
VG IIa	2 – 8	5.5	8	8
VG IIb	4 – 32	12.5	16	16
VG I & III	0.5 – 16	3.0	4	16
All	0.5 – 32	7.3	8	16

Source: Dr. Shawn Lockhart
Centers for Disease Control and Prevention

Susceptibility testing *C. gattii*

Genotype	MIC µg/mL Amphotericin B			
	Range	Geo Mean	MIC ₅₀	MIC ₉₀
VG IIa	0.125 – 0.25	0.19	0.19	0.19
VG IIb	0.06 – 0.25	0.16	0.19	0.25
VG I & III	0.05 – 0.19	0.10	0.125	0.19
All	0.05 – 0.25	0.16	0.19	0.19

Source: Dr. Shawn Lockhart
Centers for Disease Control and Prevention

Rates and demographics 1999 -2007

- Incidence
- 236 cases
- Mean 24.2 cases per year
- 25.1/million population Vancouver Island
- Demographics
- Mean age – 59 years
- Age range 2 – 92 y
 - ◆ 4 pediatric cases
 - ◆ 70 – 79 y highest incidence
- Male 56%

Source: BC CDC 2008

Mortality

- 19 deaths (case fatality ratio of 8.7%)
 - ◆ Underlying cause of death in 9, contributing cause in 10
- Mean age at death: 67.6 (range 26 – 91y)
 - ◆ Cases who died were older than those who did not
- Median time from diagnosis to death: 54 days (range 5 – 577 d)
- 12 (63.2%) had underlying medical conditions
 - ◆ 6 cancer
 - ◆ 3 COPD
 - ◆ 2 liver disease
 - ◆ 1 HIV, lung transplant, congestive heart failure, congenital heart malformation
- Cases who died were more likely to present with CNS disease

Source: Galanis 2008

Case-Control study 1999 – 2001 (n = 30)

Risk factors	Matched OR	95% CI
Corticosteroids	8.11	1.74, 37.80
Chronic lung disease	3.21	1.08, 9.52
Recurrent pneumonia	2.71	1.05, 6.98
Cancer	2.03	0.63, 6.81
Current or past smoker	1.18	0.44, 3.20
Diabetes	0.65	0.17, 2.50
Asthma	0.45	0.12, 1.66
Outdoor building repair	4.00	1.00, 16.00
Woods within mile of house	1.70	0.17, 2.02
Camping	1.23	0.23, 2.91
Gardening	1.15	0.47, 2.79
Branch clean-up	0.29	0.10, 0.76
Cutting/chopping wood	0.17	0.04, 0.76
Pruning	0.28	0.09, 0.88

Source: MacDougall & Fyfe 2008

Population-based risk factors 1999 – 2007 (n = 218)

Risk Factor	Prevalence in <i>C. gattii</i> cases	Prevalence in BC population	P-value
Age ≥ 50 years	72.4%	31.3%	< 0.001
Male	55.8%	49.6%	0.198
Current smoker	41.9%	17.8%	< 0.001
Invasive cancer	24.7%	3.6%	< 0.001
HIV Infection	3.7%	0.2%	< 0.001
COPD	4.1%	8.0%	0.09
Asthma	3.4%	7.8%	0.54
Corticosteroids	27.0%	unknown	

Source: Galanis 2008

Mobility of *Cryptococcus*

- Air
- Wood/sawdust
- Soil
- Shoes
- Car tires/wheel wells
- Water

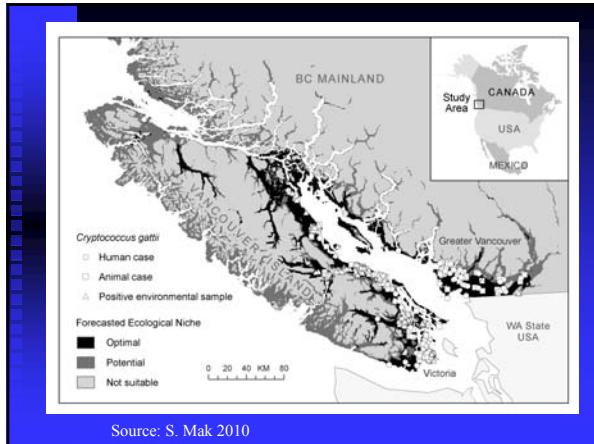


Mobility on car wheel wells

Source	n	Positive		Genotype	
BC Mainland	50	2/50	4%	VG IIa	100%
Vancouver Island	70	21/70	30%	VG IIa	86%
				VG IIb	5%
				VG I	10%

Environmental sampling: Mainland and beyond

Date	Sample type	Location	Related case	Genotype
October 2002	Air	Langley	Tapir	Tapir = VGIII Air = VG IIa
November 2003	Clinical	Langley	German Shepard	VG IIa
December 2003	Clinical	Columbia Valley	Llama	VG IIa
July 2004	Air	Langley	Mountain View	VG IIa
July 2004	Air	Columbia Valley	Llama	VG IIa
	Clinical	Lynden, WA	Cat	Cat = VG IIa
		Custer, WA		Cat = VG II b
October 2005	Swab	Lynden, WA	City park	VG IIa
October 2005	Soil	Lynden, WA	City park	VG IIa
December 2005	Clinical	Richmond	Cat	
January 2006	Clinical	Abbotsford	Cat	
February 2006	Clinical	Surrey	Dog	
June 2006	Clinical	New Westminster	Dog	
September 2006	Clinical	Chilliwack	Cat	
December 2006	Clinical	Chilliwack	Dog	
January 2007	Clinical	New Westminster	Dog	
June 2007	Clinical	Coquitlam	Dog	



Conclusions:

- *Cryptococcus gattii* is endemic on the east coast of Vancouver Island
- Cultured from a wide range of native tree species
- Not homogeneous in the environment (hot spots)

Conclusions:

- Human cases preceded by animal cases, veterinarians most likely to see sentinel cases
- *C. gattii* mis-identified as *C. neoformans* if additional laboratory tests not performed
- Multiple *C. gattii* genotypes in Cascadia

Team Crypto once and future ...

- Vancouver
 - ◆ BC CDC
 - ◆ Epidemiology
 - ◆ L. MacDougall (ex), S. Mak, E. Galanis , Colette Gaulin, Min Li
 - ◆ Laboratory
 - ◆ M. Moreshed, Min Lee, L. Hoang, S. Mithani
 - ◆ UBC
 - Sarah Kidd (ex), Yat Chow, Jim Kronstad

Team Crypto once and future ...

- Vancouver Island
 - Victoria and Health Authorities
 - Pam Kibsey, Murray Fyfe
 - Centre for Coastal Health
 - Craig Stephen, Colleen Duncan (ex)
- Animal surveillance
 - Central Laboratory (ex)
 - Canada West Critical Care (Vancouver)

Team Crypto once and future ...

- *Cryptococcus gattii* working group of the Pacific Northwest
 - Kieren Marr, Kausik Datta, Rebecca Baer, Edmond Byrnes, Joseph Heitman, Mira Leslie, Shelley Magill
- CDC *Cryptococcus* group
 - Tom Chiller, Shawn Lockhart, Julie Harris, Ron Wahrle

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Publications: in press or in preparation ...

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