Knowing where to look: environmental sources of cryptococcal disease in the Pacific Northwest

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Vancouver Island outbreak ...

- Annual incidence of all cryptococcosis (including AIDS associated) ~ 3-5/10^6
- As of July 2001 B.C. cryptococcosis cases:
  - Humans (n=38)
  - Pets (n=34)
  - Porpoises (n=2)
- Incidence on Vancouver Island ~ 20/10^6

Source: BC CDC
What is cryptococcosis?

- *Cryptococcus* is an encapsulated yeast and primary pathogen.
- Cryptococcosis may involve lungs, CNS, or other organ systems.
- Usually a disease of immunocompromised hosts.
- The fourth most common cause of life threatening infections in persons with AIDS.
Typical cryptococcosis …

Encapsulated yeast, can easily identify with microscope

Distributed world-wide

Associated with aged pigeon faeces & soil contaminated with faecal material
Pathogenic Cryptococcus

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)
Cryptococcus gattii!

Canavanine-glycine-bromthymol blue (CGB)

*C. gattii* = grows & turns medium blue 24 h – 5 days
*C. neoformans* = no growth, medium yellow or green
Cryptococcus neoformans primer:

- Pathogenic cryptococci
  - C. neoformans var. grubii
    - Serogroup A/ VN I & VN II
  - C. neoformans var. neoformans
    - Serogroup D/ VN IV
  - C. neoformans hybrid
    - Serogroup AD/ VN III
Cryptococcus gattii primer:

- C. gattii (formerly known as C. neoformans var gattii)
  - Serogroups B or C
  - RFLP VG I, VG II, VG III, VG IV
  - URA-5 VG IIa, VG IIb, VG IIc
  - MLST multiple sequence types
URA5-RFLP unique genotypes

Source: S. Kidd
Outbreak …continues

- Annual incidence of all cryptococcosis (including AIDS associated) $\sim 3-5/10^6$
- As of 2009 B.C. cases *C. gattii*:
  - Humans (n=263)
  - Pets (estimate = 3 – 4 times diagnosed human cases)
- Incidence on Vancouver Island $\sim 24/10^6$
- Case fatality ratio 8.7 %

Source: BC CDC
Cryptococcus gattii:

- Geographic distribution
  - Exposure directly from the environment
  - Restricted distribution (e.g. formerly known as tropical or sub-tropical)
Distribution of Human and Animal Cryptococcal Cases on Vancouver Island, 1999-2001*
Environmental niche: Australia, California

*Eucalyptus camaldulensis*  
*Eucalyptus tereticornis*
Environmental niche
British Columbia

Douglas fir

Red Alder
Veterinary environmental risk factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>Feline</th>
<th>Canine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OR</td>
</tr>
<tr>
<td>Soil disturbance within 10 km</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Logging within 10 km</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>Animal travel to Van. Island</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td>Know another crypto. case</td>
<td>20</td>
<td>4.3</td>
</tr>
<tr>
<td>Owners hiking within 6 months</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Owner administered supplements</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Owners visiting a botanical garden</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>

Source: Duncan 2006
Distribution Map of Human and Animal Cryptococcosis in BC by Place of Residence 1999-2009

Note: Cases are mapped by address of residence at a geographic scale that does not identify individuals. The majority of mainland cases are due to travel and exposure to Cryptococcus gattii on Vancouver Island. Animal data provided by Dr. Karen Bartlett, SOEH UBC and Dr. Stephen Ravery, AHC MAL. Map created February 2019.
Source:
Datta et al.
2009 Emerg Infect Dis
15(8): 1185 - 1191
BC Host tree species

Non-native

Native, deciduous & non-deciduous
positive tree by geographic location

- Campbell River < 1%
- Courtenay 6%
- Parksville 32%
- Port Alberni 19%
- Cowichan 8%
- Victoria 3%
C. gattii soil concentration (range CFU/g)

- Campbell River: < LOD
- Courtenay: < to $4 \times 10^3$
- Parksville: < to $3 \times 10^4$
- Port Alberni: < to $5 \times 10^2$
- Cowichan: < to $4 \times 10^4$
- Gulf Island: < to $2 \times 10^5$
- Victoria: < to $2 \times 10^3$
Airborne Cryptococcus by month

<table>
<thead>
<tr>
<th>Month</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>18</td>
</tr>
<tr>
<td>Feb</td>
<td>8</td>
</tr>
<tr>
<td>Mar</td>
<td>6</td>
</tr>
<tr>
<td>Apr</td>
<td>8</td>
</tr>
<tr>
<td>May</td>
<td>16</td>
</tr>
<tr>
<td>Jun</td>
<td>24</td>
</tr>
<tr>
<td>Jul</td>
<td>18</td>
</tr>
<tr>
<td>Aug</td>
<td>9</td>
</tr>
<tr>
<td>Sep</td>
<td>7</td>
</tr>
<tr>
<td>Oct</td>
<td>14</td>
</tr>
<tr>
<td>Nov</td>
<td>19</td>
</tr>
<tr>
<td>Dec</td>
<td>7</td>
</tr>
</tbody>
</table>
Aerosolization of *Cryptococcus*

<table>
<thead>
<tr>
<th>Activity</th>
<th>CFU/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>$4 \times 10^2$</td>
</tr>
<tr>
<td>Limbing</td>
<td>$2 \times 10^3$</td>
</tr>
<tr>
<td>Chainsawing</td>
<td>$2 \times 10^4$</td>
</tr>
<tr>
<td>Chipping</td>
<td>$5 \times 10^4$</td>
</tr>
</tbody>
</table>
Mobility of *Cryptococcus*

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

<table>
<thead>
<tr>
<th>Direction</th>
<th>n</th>
<th>Positive C. gattii</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>From BC Mainland</td>
<td>50</td>
<td>2</td>
<td>VG IIa 100%</td>
</tr>
<tr>
<td>From Vancouver Island</td>
<td>70</td>
<td>21</td>
<td>VG IIa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VG IIb</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VG I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30%</td>
<td>86% 5% 10%</td>
</tr>
</tbody>
</table>
### C. gattii genotype by sample type

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>VG IIa</th>
<th>VG IIb</th>
<th>VG I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>99</td>
<td>89%</td>
<td>7%</td>
<td>4%</td>
</tr>
<tr>
<td>Air</td>
<td>50</td>
<td>70%</td>
<td>15%</td>
<td>7%</td>
</tr>
<tr>
<td>Soil</td>
<td>81</td>
<td>67%</td>
<td>18%</td>
<td>6%</td>
</tr>
<tr>
<td>Swab</td>
<td>144</td>
<td>58%</td>
<td>16%</td>
<td>9%</td>
</tr>
<tr>
<td>Water</td>
<td>8</td>
<td>63%</td>
<td>38%</td>
<td>0</td>
</tr>
</tbody>
</table>

Typing: Sarah Kidd
Environmental *C. gattii* genotype by location

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>VG IIa</th>
<th>VG IIb</th>
<th>VG I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Courtenay</td>
<td>10</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Parksville</td>
<td>90</td>
<td>87%</td>
<td>13%</td>
<td>0</td>
</tr>
<tr>
<td>Port Alberni</td>
<td>10</td>
<td>90%</td>
<td>10%</td>
<td>0</td>
</tr>
<tr>
<td>Nanaimo</td>
<td>11</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duncan</td>
<td>120</td>
<td>37%</td>
<td>23%</td>
<td>14%</td>
</tr>
<tr>
<td>Victoria</td>
<td>9</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gulf Islands</td>
<td>23</td>
<td>91%</td>
<td>0</td>
<td>9%</td>
</tr>
</tbody>
</table>

Typing: Sarah Kidd
Veterinary *C. gattii* genotype by location

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>VG IIa</th>
<th>VG IIb</th>
<th>VG I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Courtenay</td>
<td>25</td>
<td>80%</td>
<td>20%</td>
<td>0</td>
</tr>
<tr>
<td>Parksville</td>
<td>12</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Port Alberni</td>
<td>2</td>
<td>50%</td>
<td>50%</td>
<td>0</td>
</tr>
<tr>
<td>Nanaimo</td>
<td>28</td>
<td>79%</td>
<td>21%</td>
<td>0</td>
</tr>
<tr>
<td>Duncan</td>
<td>17</td>
<td>66%</td>
<td>35%</td>
<td>0</td>
</tr>
<tr>
<td>Victoria</td>
<td>34</td>
<td>82%</td>
<td>18%</td>
<td>0</td>
</tr>
<tr>
<td>Gulf Islands</td>
<td>4</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mainland BC</td>
<td>18</td>
<td>94%</td>
<td>6%</td>
<td>0</td>
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</table>
### First evidence: *C. gattii* in the Pacific Northwest

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>Sample</th>
<th>Location</th>
<th>VG IIa</th>
<th>VG IIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>1</td>
<td>Air</td>
<td>Metro Van BC</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>2</td>
<td>Vet clinical</td>
<td>Fraser Valley BC</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>2</td>
<td>Air</td>
<td>Fraser Valley BC</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>2</td>
<td>Vet clinical</td>
<td>Washington USA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2005</td>
<td>2</td>
<td>Swab + soil</td>
<td>Washington USA</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>1</td>
<td>Vet clinical</td>
<td>Metro Van BC</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>2</td>
<td>Vet clinical</td>
<td>Metro Van BC</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>3</td>
<td>Vet clinical</td>
<td>Fraser Valley BC</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2007</td>
<td>2</td>
<td>Vet clinical</td>
<td>Metro Van BC</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
Ecological niche modeling of *Cryptococcus gattii* in the Pacific Northwest

Forecasted ecological niche:
Composite of 50 model runs

- 46 - 50
- 41 - 45
- 36 - 40
- 31 - 35
- 26 - 30
- 21 - 25
- 16 - 20
- 11 - 15
- 6 - 10
- 1 - 5
- 0

Model based on 181 human cases, 150 animal cases, and 452 positive environmental samples in British Columbia, 1998-2008. The data was split into 50% training and 50% testing subsets, and applied against 15 predictor environmental data layers: elevation, temperature (°C), and precipitation (mm). The mean training and testing accuracy of the model was 96.3% (p<0.0001). Map created June 16’10 by Sunny Mak, CDC Centre for Disease Control.

Source: Sunny Mak 2010
Conclusions:

- Cryptococcus gattii is endemic on the east coast of Vancouver Island
Conclusions:

- Cryptococcus gattii is endemic on the east coast of Vancouver Island

- Cultured from a wide range of native tree species
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 (limit of detection → hot spots).
Conclusions:

- Human cases preceded by animal cases, veterinarians most likely to see sentinel cases
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.
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
Conclusions:

- Best response
  - Education
    - (MDs, DVMs, Public)
  - Cooperation
    - (MDs, DVMs, Epidemiologists, Environmental hygiene)
- Knowledge Translation/Transfer
Team Crypto once and future …

Vancouver

BC CDC

  Epidemiology
  
  L. MacDougall (ex), S. Mak, E. Galanis, Colette Gaulin, Min Li, Marsha Taylor

  Laboratory
  
  M. Moreshed, Min Lee, L. Hoang, S. Mithani

UBC

  • Sarah Kidd (ex), Yat Chow (ex), 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 (ex), Edmond Byrnes, Joseph Heitman, Mira Leslie, Shelley Magill, Nicola Marsden-Hoag, Ron Whorle

- CDC *Cryptococcus* group
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- BC Parks employees, Vancouver Island EHOs
Publications:


