ATTEMPTED ISOLATION OF CRYPTOCOCCUS SPECIES FROM HUMAN ORAL CAVITIES

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Cryptococcosis is a potentially serious endemic fungal infection, caused by *C. neoformans* and *C. gattii*. Primarily within immunocompromised patients, and acquired through inhalation of aerosolized yeast.

Previous understanding that *Cryptococcus* was dependent on geographic location and climate, has since been challenged from the increase in case documentation and migration of *C. gattii*.

Environmental associations of these fungal species include certain trees and soils for both species (perhaps influenced by certain climatic or anthropic factors), and bird guano for *C. neoformans*. 

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**INTRODUCTION**

**Cryptococcus cells**

**Cryptococcal meningitis**

**Spores or Yeast**

1. Cross BBB gaining access to CNS
2. Dissemination from extracellular cells or Trojan horse
3. Brain parenchyma
4. Brain parenchyma

**Mating/fruiting and sporulation**

**Spore/yeast inhalation**

**Lung infection**

**Dissemination**

**Brain infection**

**Niches**
INTRODUCTION: IS IT AROUND HERE?

• Virtually nothing is reported in the literature regarding the frequency or distribution of human cryptococcosis in Wisconsin. A Medline search for English language articles, without any other restriction, including year of publication, of ‘Cryptococcus, Cryptococcus neoformans, or Cryptococcus gattii’ crossed with ‘Wisconsin’ yielded only one 1966 article which demonstrated Cryptococcus neoformans in a variety of samples of pigeon droppings throughout various areas of Metropolitan Milwaukee.

• In the case of C.gattii, new endemic areas for this fungus are currently being recognized, including in bordering Lake County, Illinois.
INTRODUCTION: IS IT AROUND HERE?

- Retrospective analysis of 1465 Cryptococcal Ag tests on 1211 unique patients (50.2% female, 73.9% White, mean age 53.7+/−16.5) Jan 2013-Apr 2017:
- At least one Cryptococcal Ag was positive in 23/1211 patients (1.9%); From these, 21/23 were immunocompromised. Positive patients were disproportionately male (82.6%) and non-white (3.8% of those tested vs. 1.2%) [p values <0.01].
- Positive patients were more prevalent in the ZIP codes that included Milwaukee (11/377 [2.9% of those tested] vs. 12/834 [1.4% of all those tested in the remaining area of the state]), but this was not significant.
- No other case clustering or close proximity to waterways was observed (41% were <162m from green space, similar to historical controls).
INTRODUCTION

• Historically, cryptococcal species were not thought to be part of the human microflora, but have recently been identified via molecular testing within the human oral cavity:

• Cryptococcus species have been described as occasional members of human oral biofilms

• Dupuy and colleagues, utilizing expectorated saliva samples and DNA extraction, demonstrated Cryptococcus and related species represented 0.22% of the identified fungal DNA sequences from 6 young healthy non-smoking human volunteers recruited in Connecticut

• Ghannoum and coinvestigators demonstrated Cryptococcus species in 20% of 20 healthy, nonsmoking adults from the Cleveland, Ohio area, utilizing a PBS swish/gargle oral rinse for one minute, DNA extraction, and PCR analysis
INTRODUCTION

• Could *Cryptococcus* species present in the oral cavity could lead to opportunistic infections?
  – Lung infection by aspiration?
  – Brain infection by direct extension via nasopharynx/sinuses???

• To our knowledge, no one has attempted to culture *Cryptococcus* species from human oral cavities.
• Isolation of the live organism via culture would all further characterization not possible with molecular identification only.
PURPOSE

This pilot study aimed to determine if potentially pathogenic Cryptococcus species may be demonstrated, by culture technique, in the oral cavities of patients and volunteer caregivers.
METHODS — SUBJECT POPULATION

Included

- Pts attending primary care clinic for routine health maintenance, chronic illness or minor illness episodic visits
- Staff of Family Medicine practice
- Staff of Center for Urban Population Health
- Aged 18 years or more, able to read English

Excluded

- Serious acute medical illness, or unstable vital signs
- Clinical assessment of distress
- Inability to safely submit to oral sampling or unable to give informed consent
- Requiring immediate transfer to the hospital or emergency department or stat laboratory assessment
- Oral or systemic anti-fungal agents within 4 weeks (non-oral topical anti-fungal agents permitted)
METHODS — SAMPLING

Forms of Sampling:
- Running tip of eSwab across the upper lip sulcus
- Oral rinse consisting of sterile phosphate buffer saline
- All samples were plated within 4 hours

First plated on Staib (Birdseed) agar, and incubated at 34 - 36°C for 4 - 6 days

Tan or darker yeast-like colonies were isolated on Sabouraud Dextrose agar (SDA), examined microscopically, and biochemically
METHODS — DATA ANALYSIS

Social history/Demographic data collected:
- Age, gender, race, ethnicity, BMI, current smoking status, home address ZIP code, time of last food and fluid intake, and time of last tooth brushing and mouth washing

Medical history data collected:
- Chronic kidney disease (CKD), Diabetes mellitus, Hypertension, Coronary artery disease (CAD), Heart failure, Cancer, Immunosuppression, Asthma, Chronic lung disease, Chronic or current antibiotic, systemic steroid, or inhaled steroid use

Power calculation:
- 80% confidence level (with 5% percentage point margin of error) for 59 subjects [design effect=1.0], for a true rate of positives of 10%

Summary statistics of our subject population were calculated.
RESULTS — POPULATION DESCRIPTION

- Sample (N=61) was primarily female, white, non-Hispanic, non-smokers

- Most common current medical condition was hypertension

- Wide range of time of last food and fluid intake, and time of last tooth brushing and mouth washing

### Demographic of Total Population (N=61+)

<table>
<thead>
<tr>
<th>Demographic Factor</th>
<th>Mean Value (N=61+)</th>
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<tbody>
<tr>
<td>Age</td>
<td>44.0 ± 17.1 years</td>
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<tr>
<td>BMI</td>
<td>27.6 ± 6.7 kg/m²</td>
</tr>
<tr>
<td>Time of last food</td>
<td>4.9 ± 5.9 hours</td>
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<tr>
<td>Time of last fluids</td>
<td>2.3 ± 3.2 hours</td>
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<tr>
<td>Time of last tooth brushing</td>
<td>6.2 ± 3.8 hours</td>
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<tr>
<td>Time of last mouth washing</td>
<td>20.9 ± 22.7 hours</td>
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### Significant Medical History

<table>
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<tr>
<th>Medical History</th>
<th>Number of subjects (N=61)</th>
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<tbody>
<tr>
<td>CKD</td>
<td>6.6% (n=4) 93.4% (n=57)</td>
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<tr>
<td>Diabetes</td>
<td>8.2% (n=5) 91.8% (n=56)</td>
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<tr>
<td>Hypertension</td>
<td>21.3% (n=13) 78.7% (n=48)</td>
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<tr>
<td>CAD</td>
<td>4.9% (n=3) 95.1% (n=58)</td>
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<tr>
<td>Cancer</td>
<td>6.6% (n=4) 93.4% (n=57)</td>
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<tr>
<td>Asthma</td>
<td>11.5% (n=7) 88.5% (n=54)</td>
</tr>
<tr>
<td>Chronic Lung Disease</td>
<td>4.9% (n=3) 95.1% (n=58)</td>
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<tr>
<td>Chronic/Current Antibiotic Use</td>
<td>3.3% (n=2) 96.7% (n=59)</td>
</tr>
<tr>
<td>Chronic/Current Inhaled Steroids</td>
<td>8.2% (n=5) 91.8% (n=56)</td>
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RESULTS

Of the 61 subjects sampled, none yielded Cryptococcus from their oral cavities.

Growth of *Exophiala dermatitidis* from 1 subject:
- Grew in 4 days on birdseed agar at 35°C
- Over 25-30 days became black, with minimal appearance of typical septate hyphae and conidia
- MALDI-TOF: very high score for *Exophiala dermatitidis*
Presumed *Exophiala dermatitidis* at 35°C X 1 month
Exophiala dermatitidis at 35°C for 1 month, X400
**Exophiala dermatitidis: Ecology**

- Ubiquitous; may have originated in tropical rainforest
- Many virulence factors allows to survive hot & harsh
- May be particularly in man-made environments:
  - Especially dishwashers
  - Also saunas, steam baths, drinking water, sewage, gas stations, creosote-treated railroad ties, railway stations
- May contaminate “sterile” medical products
Exophiala dermatitidis: Disease

- (Rarely) may cause a variety of diseases
- Mostly among immunocompromised, unless Asian
- Colonizes up to 19% of those with cystic fibrosis
- Can be present in a variety of clinical specimens
- Isolated once from the oral cavity: nasopharyngeal CA Txd with chemo, then radiation
CONCLUSION

After sampling 61 subjects, there was no culture-based identification of Cryptococcus species from within the human oral cavity*

- Population sample was primarily female, white, non-Hispanic, non-smokers

*We incidentally isolated Exophiala dermatitidis: perhaps the first known isolation from the oral cavity of a normal human host

Limitations:
- With limited time and resources, we did not do DNA-analysis, and only re-streaked and looked microscopically at colonies typical of Cryptococcus or black yeast

Future directions:
- Consider multi-site study with much larger sample, perhaps with targeted inclusion of chronic steroid and antibiotic use and immunosuppression (for both Cryptococcus and Exophiala dermatitidis)
REFERENCES


I would like to thank our team, Caroline Toberna, undergraduate research intern and Jessica Kram, MPH for their hard work and dedication to this project; and Eric Beck, PhD, ACL laboratories for MALDI-TOF and *Exophiala dermatitidis* isolate confirmation.