Mode of Transmission of *Mycobacterium ulcerans*:
Rationale, Current Status of Research, and
What Needs to Be Done

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In order to successfully prevent this agent of disease from affecting people, we have to understand its reservoirs, hosts, and mode of transmission in nature.
CURRENT STATUS OF RESEARCH
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RESEARCHERS: Merritt, Benbow, Boakye
COUNTRY: USA (Michigan State University)
Ghana (Noguchi, University of Ghana)

FINDINGS TO DATE (SUMMARY):
1. Site Selection & Measurements
In Ghana we sampled 20 sites in Greater Accra and Ashanti region (10 of these endemic, 10 non-endemic. Each site (i.e., waterbody) was sampled for standardized collections of the following: macroinvertebrates, macrophyte community cover and diversity, suspended sediment, and for *Mycobacterium ulcerans* in the environment (suspended sediments, filtered water, detritus and other microhabitats).
2. Water quality

Water quality variables were measured *in situ* (e.g., oxygen, pH, turbidity, conductivity, etc.) using a YSI Data Sonde logger, and additional samples were collected for laboratory measurements of nutrient load (e.g., phosphates and nitrates, etc.). Sites were sampled based on Buruli ulcer (BU) incidence in nearby villages associated with water bodies.

3. Landsat imaging

Using Landsat ETM+ images of Ghana, we are also running spatial analyses to quantify land use, land cover (LULC) with water quality and biological community metrics (i.e., macroinvertebrates, etc.) at the 20 sites that were sampled.

4. Conclusion

These results will provide a preliminary understanding of the potential ecological factors important for *M. ulcerans* environmental distribution and associations with nearby communities. We have also been compiling village/community level BU incidence data for other West African nations (Benin, Cameroon).
WHAT NEEDS TO BE DONE:

1. The initial finding is that land use/cover is not the only driving factor, as the occurrences are on different land use/covers among the three countries. A regression analysis seem to indicate that a combination of various factors are driving this, but we need to identify and quantify them.

2. Other factors that will be included in future analyses are the following: wetness, humidity, temperature, and vegetative cover.

3. Higher resolution analyses of sets of individual communities with proximate (several km) land use/cover, water body size and ecological condition, and population density.

4. Better data are needed on disease incidence in villages to assist with correlation with water quality parameters and other environmental determinants.

5. Resampling water sites and adding new sites to obtain a representative data base are scheduled for this year, along with collecting associated aquatic flora and fauna.
RESEARCHERS: Pam Small, R. Merritt

COUNTRY: USA (Univ. of Tennessee & Michigan State University)

FINDINGS TO DATE (SUMMARY):

1. Productive infection in a species of American naucorid bug (Pelocoris femoratus) with M. ulcerans, that have been fed lab-infected with blow fly maggots (Calliphoridae: Protophormia terranovae). Basically, repeated Laurent Marsollier’s experiments that he conducted with French naucorids in the laboratory.

2. Transmission of M. ulcerans to uninfected maggot by infected naucorids, resulting in increasing number of M. ulcerans.

3. Using M. marinum as a control, we do not appear to get productive infection (supporting Marsollier’s studies), but just transient colonization.

4. African belostomatids (Appasus nepoides) do not appear to support the amplification of this bacteria in the environment.
WHAT NEEDS TO BE DONE:

1. Need to use African naucorids and repeat the above experiments to show a productive infection can occur in Buruli endemic area.

2. Look for other potential reservoirs or vectors using *M. ulcerans* specific probes.
RESEARCHER: Paul Johnson

COUNTRY: Australia (Austin Health, Melbourne)

FINDINGS TO DATE (SUMMARY):

1. Pools of mosquitoes (Ochleratus camptorhynchus, Coquillitidia linealis) at Point Lonsdale, Victoria positive for IS2404 PCR, and some for IS2606.

2. Positive soil samples for IS2404/IS2606 at bottom of storm water drainage system and in mud from endemic areas.

3. Significant association between mosquito bites and risk of M. ulcerans.

4. Mosquito larvae fed M. ulcerans results in PCR positive adults, but may be passive contamination rather than productive infection.

5. M. ulcerans transmission studies to mice by mosquitoes ongoing.

6. Potential nutrient enrichment of coastal environments may be causing blooms of M. ulcerans (hypothesis).
WHAT NEEDS TO BE DONE:

1. Further work on mosquitoes and their potential to transmit *M. ulcerans* through adults biting or trophic means.
CURRENT STATUS OF RESEARCH

RESEARCHERS: P. Couppié, et al.

COUNTRY: French Guyana (Cayenne Hospital Centre)

FINDINGS TO DATE (SUMMARY):
1. Macroinvertebrates, fish, and plant samples (312 specimens) taken in west coast region of country. All samples were negative for PCR (IS2404). This area had highest incidence of *M. ulcerans* infection in country. No naucorids were collected.

WHAT NEEDS TO BE DONE:
1. More sampling of endemic areas.
FINDINGS TO DATE (SUMMARY):
1. Studies were conducted on feeding by naucorids in the laboratory and the movement of bacteria into the main cavity. Bacilli are phagocytosed by the plasmatocytes which carry bacteria to salivary glands. Mycolactone plays essential role at this time.

WHAT NEEDS TO BE DONE:
1. Further work on movement of *M. ulcerans* into salivary glands so it can be transmitted to host.