



DEPARTMENT OF
ENVIRONMENT
CAYMAN ISLANDS GOVERNMENT



MIT BE
BIOLOGICAL ENGINEERING



THE UNIVERSITY of EDINBURGH
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PROJECT PROPOSAL

PRELIMINARY PROJECT TITLE

Investigations into green iguana (*Iguana iguana*) as a potential reservoir for a novel strain of *Helicobacter* spp, pathogenic for the endangered blue iguana (*Cyclura lewisi*).

INTRODUCTION

Cyclura lewisi is an iguana species indigenous to Grand Cayman. They were once classified as Critically Endangered with under 25 individuals left in the wild (Burton, 2004). However, thanks to intensive conservation efforts, they were re-classified as Endangered by IUCN in 2012. Current threats to the species include predation from feral dogs and cats, habitat conversion, traffic accidents, and indirect influences from the invasive green iguana (*Iguana iguana*) (IUCN, 2012).

Due to a previously undescribed, "mystery illness," the Queen Elizabeth II Botanic Park (QEIBP), located in Grand Cayman, encountered a series of casualties in both its wild population and captives within the breeding facility. Symptoms are unspecific and include lethargy, inappetence, weakness of the hind quarters, collapse and sudden death. Necropsies did not reveal any pathognomonic signs. After intensive investigations, a novel *Helicobacter* spp has been isolated from blood and faecal samples and linked to approximately half of the cases recorded.

Presently there is no information regarding this bacteria, and nothing is known about its pathogenicity, epidemiology, geographical distribution or whether it is species specific or a multi-host pathogen.

PROJECT RATIONALE

This master's thesis project is aimed at investigating the presence of the pathogenic *Helicobacter* spp. in green iguana populations adjacent to the QEIBP wild and breeding facility populations in order to establish a primary disease reservoir. If sufficient funding is available, an island-wide survey could be conducted. Due to close contact and phylogenetic similarity of the two species, it is hypothesised that green iguanas present a high probability of harbouring and disseminating this novel pathogen to blue iguanas. Establishing a reservoir would greatly improve our understanding of this pathogen's epidemiology and would provide a broader information base for decision making regarding biosecurity of the facility. It could also provide a practical disease model, helpful in establishing an appropriate treatment protocol and evaluating faster, cheaper and more accurate diagnostic tools. Furthermore, green iguanas have the potential to invade other islands, posing a risk for native iguana species, such as sister isles rock iguana (*Cyclura nubila caymanensis*) in Cayman Brac and Little Cayman.

SCIENTIFIC BACKGROUND

Helicobacter is a Gram-negative, spiral shaped bacteria belonging to the phylum *Spyrochaetes*. It is known to be a comensal pathogen in many species (including humans), and has been reported to cause gastritis,

celulitis and septicaemia in a variety of hosts (Solnik and Schauer, 2001). However reports of pathogenic *Helicobacter* in reptiles are extremely limited (Jacobson et al., 1980, Stacy and Wellehan, 2010). Case under-reporting could be explained by the difficulty in diagnosis: *Helicobacter* requires extremely specific conditions to be cultured, and there are no readily available tests for the veterinary practitioner. Other testing includes fluorescent in situ hybridisation (FISH) and polymerase chain reaction (PCR) that targets the 16S ribosomal RNA gene.

For the present survey, a 16S rRNA PCR method has been developed by the Massachusetts Institute of Technology, Department of Biological Engineering, using samples of the originally infected blue iguanas.

METHODOLOGY

The Cayman Islands Department of Environment (DoE) launched an island-wide invasive green iguana (*Iguana iguana*) cull, where animals are humanely trapped and euthanised by trained personnel. Culled iguanas located inside and in the proximity of Queen Elizabeth II Botanical Park would be selected randomly for post-mortem examination. This particular location is chosen due to the fact that the endangered blue iguana (*Cyclura lewisi*) reproduction facility is located here, and furthermore, the *Helicobacter* strain has been associated with blue iguana deaths in the captive and wild populations only around this park. The number of green iguanas selected for the study will ultimately depend on the funding available, however a minimum of 100 animals will be sampled.

During the post-mortem examinations, any gross pathologic changes will be recorded in order to correlate with the presence of *Helicobacter*. Additionally, the following sampling protocol will be followed:

- *Helicobacter* culture media: whole blood and feces in separate media vials, followed by freezing at low temperature
- Cytology
 - Blood smear (heart blood)
 - Impression smear of liver on a glass slide
 - Impression smear of spleen on a glass slide
- Frozen samples
 - Whole blood (collected from the heart, not spun/separated)
 - Spleen
 - Liver
 - Feces or cloacal swab
 - Additional samples at the prosector's discretion (These are not essential for *Helicobacter* diagnosis/confirmation, but can be helpful if other diseases are suspected)
- Formalin samples
 - Full set of tissues
 - Endoparasites
- Ectoparasites in alcohol

Additional information, such as sex, size, weight, approximate age, etc. would be recorded, as well as environmental and spatial parameters: GPS coordinates, temperature, rain fall and proximity to stagnant waters. Anecdotally, it has been noticed that *Helicobacter* outbreaks in blue iguanas during the past years have been associated with heavy, prolonged rain fall, therefore any relationship between the presence of *Helicobacter* and these covariants will be investigated.

Collected samples will be processed at the Massachusetts Institute of Technology, Department of Biological Engineering, mainly through polymerase chain reaction (PCR) that targets the 16S ribosomal RNA gene. Specific primers have been developed using material from infected blue iguanas. The cytological examination will be performed on island, and the frozen and formalin fixed samples will be banked for follow-up studies.

PROJECT OUTCOME

After completion of tests, the data would be statistically analysed, prepared for publication and presented to the University of Edinburgh, DOE, National Trust and all relevant stakeholders together with a set of suggestions regarding further actions.

COSTS

The funding necessary for this project can be estimated as following (all estimates given in USD):

- Laboratory PCR testing: approx. \$30/sample x 100 samples = \$3000 (Discussions are being conducted with MIT to obtain a discounted price)
- Shipping and transport costs: approx. \$350
- Protection gear (gloves, masks, disinfectants, overalls): approx. \$200
- Overhead costs: \$250
- TOTAL = \$3800

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