Proposal and Methodology for New Zealand Sea Lion Disease Research, Auckland Islands 2016-17

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Pup mortality at Enderby Island

• NZ sea lion pup production at Auckland Islands has almost halved within last 15 years (Childerhouse et al., 2016)
• Pup mortality recognised as important contributing factor to decline of species (NZSL TMP, 2016)
• Approximately 60% of pup mortality at Enderby Island is attributed to infection with a virulent strain of the bacteria *Klebsiella pneumoniae*
• Investigation into risk factors for mortality and infection with *K. pneumoniae* critical for management and potential mitigation
Pup deaths at Enderby Island by season 2014-2016

Number of dead pups

Date

Approximate dates of monitoring

2014
2015
2016
Project components

- **Case control study**
  - To understand risk factors for pup mortality and interaction between predisposing factors

- **Ivermectin treatment trial**
  - To understand impact of hookworm carriage on mortality and *K. pneumoniae* infection

- **Prospective cohort study**
  - To understand pup health status and body’s response to progression of disease
**CAPTURE 1 (INITIAL ID)**
All pups at ~3-7 days of age
- Hand capture
- Weigh and measure
- Check sex, physical exam
- Implant microchip
- Oral/rectal *K. pneumoniae* swabs
- Apply vinyl ID cap to rump

**CAPTURE 2 (TAGGING)**
Mid-January, all pups
- Hand capture
- Weigh
- Check sex, physical exam
- Scan microchip
- Flipper tagging
- Collect faeces for hookworm assessment

**FURTHER CAPTURES**
Throughout season
December - March
For every pup that dies, two control live pups are randomly selected

**Ivermectin trial**
- 50% of pups randomly selected for ivermectin administration
- Every 6th pup also joins prospective cohort study

**CASE (DEAD) PUPS:**
- Scan microchip or read tags for ID
- GPS coordinates
- Collect data on risk factors
- Weigh and measure
- Full necropsy and sample collection
- Oral/rectal *K. pneumoniae* swabs

**CONTROL PUPS:**
- Scan microchip or read tags for ID
- Hand capture
- Weigh and measure
- GPS coordinates
- Collect data on risk factors
- Physical examination
- Oral/rectal *K. pneumoniae* swabs
- Collect faeces for hookworm assessment
- Collect blood from brachial vein

**COHORT PUPS:**
Hand captured approximately fortnightly
- Weigh and measure
- Physical examination
- Oral/rectal *K. pneumoniae* swabs
- Collect faeces for hookworm assessment
- Collect blood from brachial vein
- Measure temperature

**Cohort pups may also be selected as controls or become cases**
Field logistics 2016/17

- On island from approximately 12 December 2016 – 16 March 2017
- Team of minimum 3 project personnel at a time throughout season
Identification of pup date of birth

- Monitoring of colony from arrival at island to identify pups as they are born
- Initial mark of day old pups where possible with non-toxic stock paint on fur with colour or pattern specific to date of birth
- Applied from end of ~3m pole to minimise disturbance to pup and mother
First capture

• All pups, hand capture at ~3-7 days of age or when unattended by mother
• Determine date of birth based on colour mark on fur
• Measurement of weight, length, girth and check sex
• Physical examination for wounds, injuries, signs of infection
• Implantation of microchip in dorsal lumbar region and ID cap glued to rump
• Oral and rectal swabs to detect *K. pneumoniae* carriage
• 50% pups randomly chosen to join ivermectin treatment group
• Every 6th pup (to a total of 50) joins prospective cohort study
Ivermectin treatment trial

- Broad spectrum anti-parasitic
- NZ sea lion pups have up to 100% prevalence of hookworm (*Uncinaria* sp.) carriage at Sandy Bay (Castinel et al., 2007)
- Ivermectin is safe and effective in NZ sea lions and other pinnipeds
- Treatment efficacy tested by collection of faeces at tagging
- Results will feed into case control study to determine associations between hookworm infestation and *Klebsiella* infection
Flipper tagging

- All pups, permanent external identification
- Routinely carried out approximately 16 January at Sandy Bay, Enderby Is.
- Correlate tags applied with already implanted microchip identification
- Weigh and confirm sex
- Collect faeces from all pups where possible
  - Faecal smear examined with microscope to determine presence or absence of hookworm eggs, and therefore ivermectin treatment efficacy
Case control study

• For every pup that is found dead throughout the season, 2 live pups will be randomly selected through a grid system within 24 hours
• A full necropsy will be conducted on all dead pups
• Risk factors and samples will be collected from the dead pup and the two controls
• Grid squares identified on the ground by GPS waypoints at the southwest corner
• 10x10m squares of Sandy Bay area where pupping occurs
• Move to island-wide 20x20m grid once animals have dispersed from the Sandy Bay grid
Grid selection outside Sandy Bay
Random control pup selection

- Pups that are nursing are excluded from selection
- If one pup is present in the square, it is selected as a control
- If more than one pup is present in the square, a fraction will be randomly generated and multiplied by the number of pups present to select the control
- If no pups are present in the square, either:
  - The nearest pup to the GPS waypoint (within 40m radius) is selected
  - Another square is randomly selected and the process repeated
PUP IS FOUND DEAD

Dead pup data collected eg. GPS coordinates, habitat, substrate and circumstances of death

Pup brought back to base for necropsy

Pup identified by tag and/or chip

Pup weighed, sexed and measured

Necropsy undertaken with collection of routine samples as well as any abnormal lesions

Intestine flushed to quantify hookworm infection intensity

Preliminary diagnosis assigned

TWO LIVE CONTROL PUPS RANDOMLY SELECTED

Pups identified by tag and/or chip and captured by hand or net

Pups weighed, sexed and measured, given physical examination

Throat/rectal swabs for later culture to determine *K. pneumoniae* carriage

Faeces collected for assessment of hookworm presence or absence

Data collected about risk factors eg. location, weather, pup and maternal variables
## Risk factor data collected from all pups

<table>
<thead>
<tr>
<th>Environmental variables</th>
<th>Pup variables</th>
<th>Location</th>
<th>Maternal variables</th>
<th>Clinical variables</th>
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</thead>
<tbody>
<tr>
<td>Air temperature</td>
<td>Colony of origin</td>
<td>GPS</td>
<td>Age of mother</td>
<td>Physical examination</td>
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<td>Rainfall</td>
<td>Age</td>
<td>Site</td>
<td>Parity of mother</td>
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<td>Wind speed</td>
<td>Sex</td>
<td>Habitat type</td>
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<td>Hookworm infestation</td>
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<td>Clinical signs</td>
<td>Clinical sign type</td>
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<td>Recent suckling</td>
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<td></td>
<td>Rescue from sink hole</td>
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<td></td>
<td>Handling events</td>
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<table>
<thead>
<tr>
<th>Pup morphometrics</th>
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<td>Axillary Girth</td>
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<td>Body condition</td>
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</table>

## Notes
- Environmental variables include factors such as air temperature, rainfall, wind speed, tourist interactions, and skua presence.
- Pup variables include colony of origin, age, sex, pup status, recent suckling, rescue from sink hole, and handling events.
- Maternal variables include age of mother and parity of mother.
- Clinical variables include physical examination, K. pneumoniae carriage, ivermectin treatment, hookworm infestation, clinical signs, and clinical sign type.

## Examples
- Example of environmental variables: Air temperature, Rainfall, Wind speed, Tourist interactions, Skua presence
- Example of pup variables: Colony of origin, Age, Sex, Pup status, Recent suckling, Rescue from sink hole, Handling events
- Example of maternal variables: Age of mother, Parity of mother
- Example of clinical variables: Physical examination, K. pneumoniae carriage, Ivermectin treatment, Hookworm infestation, Clinical signs, Clinical sign type
Data management

Database being developed by Ahmed Fayaz, mEpiLab, Massey University
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Comments:
Tag wounds moderate purulent discharge. Bite wound present dorsal thoracic region - mild swelling but no associated discharge.
Prospective Cohort Study

- Up to 50 pups selected at first capture
- Pups then caught approximately fortnightly (two groups in alternate weeks)
- Risk factor data and samples collected
  - Blood: health status, anaemia, inflammatory response, immune function
  - Faeces: hookworm carriage
  - Oral and rectal swabs: *K. pneumoniae* carriage
  - Temperature: body’s inflammatory response to disease
Sub-Antarctic skuas commonly scavenge pup faeces and sea lion carcasses. Sampling in 2014/15 showed that skuas can carry the same form of *K. pneumoniae* that causes disease in pups, in their intestinal tracts. No apparent clinical effect on skua health. Skuas could potentially spread the bacteria to other NZ sea lion colonies and potentiate numbers of environmental bacteria at high density sites. 5/33 (15.1%) samples positive on Enderby Island in 2014/15 but birds not individually identified. Sampling in 2016/17 will include leg banding of skuas to more accurately determine prevalence of *K. pneumoniae* carriage.
Substrate reservoirs of *K. pneumoniae*

- Testing of substrate around Enderby Island in February 2014 showed 46% of samples positive for *K. pneumoniae*
- The environment may be the source of infection for many pups
- **Sampling in 2016/17 will take place in early, mid and late season time points from a suite of sites around Sandy Bay and other opportunistic locations**
- Results will feed into case control study
Acknowledgments

- Blue Planet Marine: Simon Childerhouse
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- DOC Bird Banding Office: Michelle Bradshaw, Sandy Taylor
- DOC Geospatial Services: Andrea Sward for mapping and grid development
- Massey University: Ahmed Fayaz for database development, Tony Russell, Mike Hogan, Anne Midwinter, Kristene Gedye, Peter Wildbore for logistic support and procurement