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CAUSES OF NEONATAL MORTALITY
IN THE NEW ZEALAND SEA LION (*PHOCARCTOS HOOKERI*)

A Thesis
presented in partial fulfilment of
the requirements for the degree of

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Abstract
CAUSES OF NEONATAL MORTALITY
IN THE NEW ZEALAND SEA LION (*PHOCARCTOS HOOKERI*)

As part of a health survey of New Zealand sea lions (*Phocarctos hookeri*) on Enderby Island, Auckland Islands (50° 30'S, 166° 17'E), neonatal mortality was continuously monitored at the Sandy Bay Beach rookery, from 1998/1999 to 2004/2005. The primary causes of death were categorised as trauma (35%), bacterial (24%) and hookworm (13%) infections, starvation (13%) and stillbirth (4%). During the 2001/2002 and 2002/2003 breeding seasons, bacterial epidemics caused by *Klebsiella pneumoniae* increased mortality by three times the mean in non-epidemic years.

Uncinaria spp. from New Zealand sea lion (NZSL) pups was described for the first time using morphometric criteria. It differed from the two species already described in pinnipeds, *Uncinaria lucasi* and *Uncinaria hamiltoni*, suggesting the existence of a different morphotype in NZSLs. A study on the epidemiology of hookworm infection showed that all pups up to at least three months of age harboured adult hookworms in their intestines and transmammary transmission was identified as the route of infection of NZSL pups. Uncinariosis as a primary cause of mortality was generally associated with anaemia, haemorrhagic enteritis and frank blood in the lumen. The relationship between hookworm burden and clinical disease could not be clearly established.

The 2001/2002 and 2002/2003 bacterial epidemics at Sandy Bay Beach rookery were caused by a clonal strain of *Klebsiella pneumoniae* as verified by pulse-field gel electrophoresis and antimicrobial testing. Suppurative arthritis was the most common post-mortem diagnosis during the two epidemic seasons. Internal lesions were consistent with septicaemia, which explained the wide range of organs from which the pathogen was grown in pure culture. A serological test investigating the exposure of NZSLs to *Klebsiella* spp. showed that the large majority of pups up to two months of age did not have any anti-*Klebsiella* antibodies, even after the epidemics, but that almost all the adults were seropositive. In addition, passive immunoglobulin (Ig) transfer from lactating females to neonates was examined by measuring IgG levels in pups and was very low compared to terrestrial mammals although similar to other pinniped neonates.

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Preamble

The research reported in this thesis aimed at identifying and pondering the causes of neonatal mortality in New Zealand sea lion (NZSL) (*Phocarctos hookeri*) pups at Sandy Bay Beach on Enderby Island (New Zealand's Sub-Antarctic Islands, between 48°S and 53°S). Investigation into neonatal mortality commenced in 1998/1999 after an unusual mass mortality of NZSLs was noted during the 1997/1998 breeding season. A variety of personnel were involved in undertaking necropsies and collection of samples up until the commencement of these PhD studies in 2003/2004. However analysis of all these samples and consequent data are part of this PhD research. The thesis is articulated around two major themes, hookworm infection and bacterial epidemics caused by *Klebsiella pneumoniae*. These were investigated in the course of parallel studies conducted both in the field and in several laboratories on mainland New Zealand. Therefore the thesis appears as a succession of discrete chapters that have been, or will be, submitted to various journals for publication. To facilitate some uniformity of presentation, all chapters have been presented here in the same style and format. Where applicable, cross-referencing to other chapters or sections in chapters has also been included. A set of complementary documents including the standard operating procedures developed and used for this research is provided for each chapter in the Appendices section. These are numbered as for the chapter they refer to and are generally in addition to the documents presented to the various journals for publication and are also referred to in the relevant section of these chapters. A list of abbreviations commonly used in the text is presented immediately before the literature review.

A detailed literature review of the causes and circumstances of mortality in neonates of other pinniped species than the New Zealand sea lion is given in Part One (Chapter 1). Part Two includes Chapter 1 that presents the causes of neonatal mortality observed at Sandy Bay Beach for seven consecutive years. Prevalence of the main causes of death and necropsy findings are detailed in this section. Part Three is dedicated to studies on hookworm infections in New Zealand sea lion pups. It comprises Chapters 3 to 6. The first

chapter gives a morphometric description of the species found in NZSLs and compares it to the hookworm species occurring in otariids around the world. This has been published in *Parasitology Research* (Appendix 3.1). A morphological description of larval stages and immature adults can be found in Appendix 3.2 as a series of drawings and microscopic pictures. This supplement provides some important and innovative data on the anatomical development of the parasite in its immature forms. Chapter 4 consists of a brief study on the weather conditions at Sandy Bay Beach and the possible influence of temperature fluctuations on hookworm free-living stages. Chapter 5 considers the life cycle and the descriptive epidemiology of hookworms in NZSLs. Chapter 6 describes the clinical pathology associated with this parasitic infection. It also investigates its effects on some biological parameters of pups. Part Four includes three chapters describing the 2001/2002 and 2002/2003 *Klebsiella pneumoniae* epidemics on the Auckland Islands. The bacterial agent is first characterized using phenotypical and molecular analyses (Chapter 7). Then some aspects on the epidemiology and clinical pathology of these epidemics are investigated (Chapter 8), followed by a chapter on a serological survey covering 7 years of population monitoring at Sandy Bay Beach (Chapter 9). A general discussion concludes this study on neonatal mortality in the New Zealand sea lion (Part Five, Chapter 10).

The elements of histopathology associated with *Klebsiella pneumoniae* epidemics and presented in Chapter 8 correspond to the research achieved within the frame of this PhD thesis. However, further analyses are recommended before submitting this work for publication in a scientific journal. Other experimental chapters have been or will be submitted for publication as follows: *Journal of Wildlife Diseases* (Chapter 2); *Parasitology Research* (Chapter 3, published; Chapter 5); *Journal of Comparative Pathology* (Chapter 6) and *Veterinary Microbiology* (Chapters 7 and 9).

List of Abbreviations

Ab	Antibody
Ag	Antigen
BA	Blood Agar
CPE	Cytopathogenic Effect
DOC	Department Of Conservation (New Zealand)
ELISA	Enzyme-Linked ImmunoSorbent Assay
ENSO	El Niño Southern Oscillation
ESBL	Extended Spectrum Beta-Lactamases
G.A.L.T	Gut-associated Lymphoid Tissue
Gram+/Gram-	Gram stain positive/ negative
Hct	Hematocrit
H&E	Hematoxylin & Eosin
Ig	Immunoglobulin
IgG	Immunoglobulin G
L₁, L₂, L₃	Larvae first-stage, second-stage and third-stage
LPS	Lipopolysaccharides
MAC	MacConkey agar
MHC	Major Histocompatibility Complex
MGG	May-Grunwald Giemsa
MIC	Minimum Inhibitory Concentration
NZSL	New Zealand sea lion
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
RBC	Red blood cell
SOP	Standard Operating Procedure
WB	Western Blot
WBC	White Blood Cell
XLD	Xylose-lysine desoxycholate agar

Most frequently cited Pinniped species

Otariids

- New Zealand sea lion.....*Phocarctos hookeri*
New Zealand fur seal.....*Arctocephalus forsteri*
Australian sea lion.....*Neophoca cinerea*
Australian fur seal.....*Arctocephalus pusillus doriferus*
Northern fur seal.....*Callorhinus ursinus*
California sea lion.....*Zalophus californianus*
Steller's sea lion.....*Eumetopias jubatus*
Antarctic fur seal.....*Arctocephalus gazella*
Sub-Antarctic fur seal.....*Arctocephalus tropicalis*
Juan Fernandez fur seal.....*Arctocephalus philippii*
South American sea lion.....*Otaria flavescens* (= *O. byronia*)
South American fur seal.....*Arctocephalus australis*
Galapagos fur seal.....*Arctocephalus galapagoensis*
South African fur seal.....*Arctocephalus pusillus pusillus*

Phocids

- Grey seal.....*Halichoerus grypus*
Harbour seal.....*Phoca vitulina*
Crabeater seal.....*Lobodon carcinophagus*
Northern elephant seal.....*Mirounga angustirostris*
Southern elephant seal.....*Mirounga leonina*