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Topical ivermectin is a highly effective seal 'spot-on': A randomised trial of hookworm and lice treatment in the endangered Australian sea lion (*Neophoca cinerea*)

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ABSTRACT

The Australian sea lion (*Neophoca cinerea*) is an endangered and declining otariid species, with a high rate of pup mortality associated with endemic hookworm (*Uncinaria sanguinis*) infection a suspected contributor to this decline. Injected ivermectin is an effective treatment for *Uncinaria* sp. in otariids, with optimal outcomes achieved by the early treatment of pups prior to disease development. This randomised controlled trial evaluated the effectiveness of the novel use of a topical ivermectin formulation against hookworm infection and lice (*Antarctophthirus microchir)* infestation, in comparison with injected ivermectin. During the 2017 breeding season at Dangerous Reef, South Australia, pups ≤ 70 cm in standard length (≤ 2 weeks of age; n = 85) were randomised to single dose topical (500 μg/kg spot-on; n = 27) or injected (200 μg/kg subcutaneous; n = 29) ivermectin treatment groups, or to an untreated control group (n = 29). Topical ivermectin was highly effective for *U. sanguinis* elimination, and not significantly different to the injected formulation (estimated effectiveness 96.4% and 96.8%, respectively; $P > 0.05$). Its application resulted in an 81.6% reduction and 62.7% additional clearance for *A. microchir* infestation by 15–24 days post-treatment, compared with untreated control pups (also not significantly different to injected ivermectin; 83.1% and 59.4%, respectively; $P > 0.05$). Treatment with either ivermectin formulation significantly ameliorated increases in inflammatory markers detected in the blood of untreated control pups – peripheral blood eosinophil counts (persisting to 36–41 days post-recruitment $P < 0.05$) and increased plasma protein concentrations (15–24 days post-recruitment; $P < 0.05$). Further, an initial short-term decrease in body condition in the control group was not observed in either of the treatment groups. This study demonstrates that topical ivermectin is an effective antiparasitic treatment in *N. cinerea.* It offers an alternative administration method for ivermectin delivery to a young pup cohort in this species, and an alternative, minimally invasive management tool for species conservation.

1. Introduction

The Australian sea lion's estimated population of 10,000 animals continues to decline, with the species having been listed as endangered by the International Union for Conservation of Nature since 2008 (Goldsworthy and Gales, 2008) and up-listed from threatened to endangered status under the Australian Government's *Environment Protection and Biodiversity Conservation Act* in 2020 (Threatened Species Scientific Committee, 2020). Contributors to this decline are multifactorial, with suggested anthropogenic factors that include fisheries interaction (by-catch, resource competition) (Hamer et al., 2013), marine debris entanglement (Byard and Machado, 2019; Page et al., 2004), and pollution (such as human-source microbiota and chemical pollutants) (Fulham et al., 2018; Taylor et al., 2021), being additional to habitat degradation, climate alteration (Kovacs et al., 2012; Schumann et al., 2013) and disease (Lindsay and Gray, 2021; Marcus et al., 2014). Despite ongoing research, the role of known pathogens and their resultant disease in modulating population recovery remains an important knowledge gap for this species (DSEWPC, 2013; Threatened Species Scientific Committee, 2020).

Disease investigation is limited by the species' population structure – currently 80 temporally asynchronous breeding sites (Goldsworthy et al., 2021) dispersed across more than 3000 km of Australia's southern and southwestern coastline from the Houtman Abrolhos (Easter Island:

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28.468°S, 113.814°E) to The Pages Islands (35.756°S, 138.300°E) (Gales et al., 1994). Just four of these sites, including Dangerous Reef, Spencer Gulf, exceed 100 births each breeding season and contribute nearly 40% of the species total pup abundance, currently estimated at 2739 individuals (Goldsworthy, 2020; Goldsworthy et al., 2021). This represents a 64% reduction over three generations (Goldsworthy et al., 2021). Limited recruitment to the breeding population from fewer pup births is compounded by a high rate of preweaning pup mortality, which varies substantially by season and between breeding colonies (Goldsworthy et al., 2015). At the extreme of wide-ranging mortality rates, during the highest mortality seasons at each of the three largest breeding colonies pup deaths have reached 40–50%: The Pages Islands (55.6%, 1995-96) (Shaughnessy et al., 2013), Seal Bay (41.8%, 2011-12) (Goldsworthy et al., 2019) and Dangerous Reef (44.6%, 2002) (Goldsworthy et al., 2007). Gross observations have attributed pup death to trauma from conspecific animals (31.6%), emaciation (starvation) (10.4%) and stillbirth or prematurity (7.6%), with cause of death being undetermined in half of the cases (Higgins and Tedman, 1990; McIntosh and Kennedy, 2013). More recent investigations indicate that hookworm (*Uncinaria sanguinis*) infection could contribute to up to 40% of pup deaths (Gray, unpublished), and a direct correlation has been shown between increased hookworm infection intensity and higher seasonal colony pup mortality (Marcus et al., 2014).

Uncinaria sanguinis is a blood-feeding small intestinal nematode with demonstrated 100% prevalence in Australian sea lion pups at Dangerous Reef (Marcus et al., 2014). Pup infection occurs soon after birth by ingestion of infective third-stage larvae in colostrum, and patency is detectable from 11 days of age, with adult hookworms cleared from the intestine by 2–3 months of age (Marcus et al., 2014). Resultant disease ranges from mild, subclinical, and detectable antemortem by changes in blood parameters, to severe haemorrhagic enteritis and emaciation grossly apparent at necropsy (Marcus et al., 2015a). Adult hookworm feeding causes the direct intestinal loss of red blood cells and protein, as well as local intestinal inflammation that reduces intestinal absorptive capacity. Consequently, hookworm infection results in anaemia and hypoproteinemia and elicits a systemic host inflammatory response – the latter reported as eosinophilic and lymphocytic in the Australian sea lion (Marcus et al., 2015a, b), with an additional neutrophilic component reported in South American fur seals infected with an *Uncinaria* species of hookworm (Seguel et al., 2019).

Ivermectin is a potent broad-spectrum anthelmintic available commercially in oral, topical (pour-on, spot-on) and injectable formulations (Papich, 2016). To date, its off-label use for the treatment of hookworm in free-ranging otariid (fur seal and sea lion) populations has utilised the injected formulation, commonly administered as a single 200 μg/kg subcutaneous dose, with effectiveness approaching or achieving 100% for elimination of adult hookworms from pups in the Northern fur seal (*Callorhinus ursinus*) (Beekman, 1984; DeLong et al., 2009), New Zealand sea lion (*Phocarctos hookeri*) (Castinel et al., 2007a; Chilvers et al., 2009; Michael et al., 2021b), Australian sea lion (Marcus et al., 2015b) and South American fur seal (*Arctocephalus australis*) (Seguel et al., 2019). Optimal treatment outcomes, including a survival benefit, have resulted from the early treatment of young pups in populations experiencing an increased seasonal pup mortality rate – either as a direct result of hookworm infection or from an unrelated comorbidity (Chilvers et al., 2009; DeLong et al., 2009; Lyons et al., 2001; Michael et al., 2021b). In the northern fur seal, treatment of endemic hookworm infection contributing to 50% pup mortality significantly reduced pup deaths, with a survival benefit attributed to the treatment of pups younger than 2 weeks of age (DeLong et al., 2009). In the New Zealand sea lion, early ivermectin treatment also significantly improved pup survival during an epizootic outbreak of *Klebsiella pneumoniae* septicaemia (Chilvers et al., 2009) that caused a doubling of the mean seasonal pup mortality rate (Castinel et al., 2007b). The benefit occurred despite more than half of the pup deaths still being attributed to bacterial septicaemia (Castinel et al., 2007b). The treatment benefit was not apparent in the following low mortality seasons (Castinel et al., 2007a; Chilvers et al., 2009), but was again seen subsequent to the bacterial disease becoming endemic within the population (Michael et al., 2021b).

As well as demonstrated effectiveness approaching 100%, the first ivermectin hookworm treatment trial in the Australian sea lion (Dangerous Reef, 2011-13; injected ivermectin, 200 μg/kg sc) showed treatment-related haematological benefits – an increased absolute erythrocyte count and decreased eosinophil count (Marcus et al., 2015b). A lack of survival benefit was partly attributed to the practical limitations of early (<2 weeks old) pup recruitment in this species, including the prolonged 10-day post-natal period of maternal attendance with a male mate-guard (Higgins and Gass, 1993). Consequently, the present study planned for colony visits approximately 2 weeks apart to maximise the opportunity to detect and treat the younger pup cohort. Further, a topical formulation of ivermectin was evaluated as a minimally invasive hookworm treatment option for these pups. Ivermectin was selected in preference to newer generation macrocyclic lactones due to its demonstrated effectiveness and safety in multiple otariid species in the aforementioned trials, and the adequacy of a short-acting product for treating a parasite acquired at a single timepoint without the risk of pup reinfection. The infestation of Australian sea lion pups with sucking lice (*Antarctophthirus microchir*) occurs from conspecific animals at any pup age (McIntosh and Murray, 2007), has a limited pathological impact on the host and is associated with a mild anaemia and hyperproteinemia (Marcus et al., 2015a, b). Evaluation of topical ivermectin effectiveness against lice was included in the present study as an additional comparator with the injected formulation.

The primary objective of the current study was therefore to assess the effectiveness of the topical formulation of ivermectin in treating hookworm and lice parasitism in Australian sea lion pups less than 2 weeks of age. Equivalence to the injected formation was hypothesised. Intrinsically, the availability of a minimally invasive formulation requiring limited pup handling or operator expertise for application, was anticipated as the next step in the development of a disease management tool for the Australian sea lion.

2. Materials and methods

All samples in this study were collected with approval of the Government of South Australia, Department of Environment and Water (Permit/Licence Number: MR00073-2-R; Scientific Research Permit Number: A25088-12) and the study protocol was approved by The University of Sydney Animal Ethics Committee (Protocol Number 2014/726). Trial reporting was guided by the recommendations in the CONSORT (Consolidated Standards of Reporting Trials) statement (Schulz et al., 2010).

2.1. Study site and population

This study was conducted at Dangerous Reef (34.817°S, 136.217°E), a small reef system of rocky substrate within the Sir Joseph Banks Group Conservation Park in Spencer Gulf, South Australia. The colony produces approximately 400 pups over a 7-month breeding season (Goldsworthy et al., 2007, 2021). Sample collection was undertaken at three colony visits of 4–6 days duration separated by approximately two weeks during the 2017 winter breeding season (19–22 July, 8–13 August and 27–30 August).

2.2. Study design and sample collection

A controlled trial allocated recruited pups to one of three experimental groups – ‘untreated control’, ‘topical ivermectin’, or ‘injected ivermectin’. Randomisation (blocked in groups of 30) was achieved by assigning a random number (Microsoft Excel for Mac 2016) against pup capture number, sorting those numbers by size and then assigning each

sequential group of 10 pups to one of the three trial groups. Treatment allocation was concealed from assessors until preliminary capture data were recorded, and for the entirety of pup recapture and sample processing. Topical ivermectin (IVOMEC® Pour-on for Cattle, 5 mg/ml, Boehringer Ingelheim Animal Health Australia Pty. Ltd., North Ryde NSW) was administered at 500 μg/kg to the skin surface by parting the pelage of the dorsal interscapular region. The injected ivermectin (IVOMEC® Antiparasitic Injection for Cattle, 10 mg/ml, Boehringer Ingelheim Animal Health Australia Pty. Ltd., North Ryde NSW) was administered at 200 μg/kg subcutaneously in the same region. Topical and injected ivermectin treatment of Australian sea lion pups represented off-label use, with due consideration given to potential risks. The topical ivermectin dose rate was chosen based on the generic recommendation for use of this formulation in domestic cattle (Vercruysse and Claerebout, 2014) as was the dose for the injected formulation, the latter dose rate having previously been shown to be effective in the Australian sea lion (Marcus et al., 2015b).

Protocols for pup health assessment, specimen collection and processing followed those of the earlier study at this site (Marcus et al., 2015b); summarised and differing details are as follows. Recruited pups were manually restrained within a purpose-made canvas bag to cover the head and minimise potential capture stress and injury. Initial capture state (sleeping, awake or mobile), sex and standard morphometrics – bodyweight (nearest 0.1 kg), standard length (linear distance between extremities of nose and tail (nearest 0.5 cm), moult status and subjective four-scale body condition assessment (poor, fair-thin, good, excellent) – were recorded. A subsequent physical examination determined the presence of macroscopic lesions such as hair loss, dermatitis, skin ulceration, wounds or swellings. Lice presence and subjective four-scale intensity assessment (0-none, 1-low, 2-medium or 3-high) were evaluated at three body locations – the ventral thorax, ventral abdomen and dorsal lumbosacral area – and later summed to provide a semi-quantitative measure of infestation intensity (lice intensity score; range: 0–9). A faecal sample was collected using a rayon-tipped dry swab (Copan Diagnostics, Murrieta, USA) introduced within a lubricated open-ended polyethylene sheath (modified 1–3 mL transfer pipette, Livingstone International, Sydney, Australia). A blood sample was collected from the brachial vein using a 21-gauge x 1-inch needle and 5 mL syringe and transferred to 1.3 mL EDTA-anticoagulated tubes (Sarstedt, Nümbrecht, Germany). When allocated, treatment was applied just prior to pup release. Respiration rate and effort were assessed at multiple timepoints during restraint and prior to release for signs of distress, and all pups were monitored from a distance for 1–2 min after release for signs of an immediate adverse event.

The inclusion criterion for the study was standard length $\leq$ 70.0 cm, used as an age proxy for recruiting pups less than 2 weeks of age (McIntosh and Kennedy, 2013). Only pups likely to meet the size criterion based on remote visual assessment and whose mothers were absent were approached for capture. To enable resight and recapture of recruited pups a unique hair clip and bleach mark were applied to the dorsosacral pelage, permitting remote pup identification from approximately 20-30 m and which remained for the duration of the study (being lost at the first moult occurring 4–5 months of age).

Pups were recruited during the first and second colony visits and opportunistically recaptured during the second and third colony visits (Fig. 1). Consequently, two time periods were available for analysis – period 1 (P1): recruitment-to-first recapture; and period 2 (P2): first recapture-to-second recapture. Given that the low recruitment number and short follow-up period were unlikely to provide relevant survival data, recruited pups found dead were examined using standard gross necropsy technique (degree of decomposition permitting) to exclude treatment-related death, and for assessment of hookworm infection and lice infestation status. Resights of recruited pups unable to be recaptured were also noted.

2.3. Haematological analyses

Anticoagulated blood samples were stored at 4 °C prior to initial processing in a field laboratory within 10 h of collection. Following centrifugation at 13,700g for 120s (StatSpin MP, StatSpin Technologies, Norwood, USA) in microhaematocrit tubes (IRIS Sample Processing, Westwood, USA), the packed cell volume (PCV; L/L) was measured, and total plasma protein (TPP; g/L) was estimated by hand-held refractometer (Reichert TS Meter, Cambridge Instruments, Buffalo, USA). Comment was made of any degree (mild, moderate or marked) of sample haemolysis or lipaemia. Duplicate blood smears were prepared and fixed by immersion in 100% methanol (Chem-Supply Pty Ltd, Port Adelaide, South Australia) for 4 min. A 200 μL aliquot of EDTA anticoagulated blood was preserved with an equal volume of Streck Cell preservative (Streck, Omaha, USA) and stored at 4 °C.

Automated haematological analysis (Sysmex XT-2000iV, Sysmex, Kobe, Japan) at the Veterinary Pathology Diagnostic Service, Sydney School of Veterinary Science, The University of Sydney, was performed within 2–8 days of sample collection to determine the total erythrocyte count (RBC, x10^12/L), routine red cell parameters (haemoglobin [Hb, g/L]; haematocrit [HCT, %]; mean cell volume [MCV, fL]; and, mean cell haemoglobin concentration [MCHC, g/L]), platelet count (PLT, x10^9/L) and total nucleated cell count (TNCC, x10^9/L). Methanol-fixed duplicate blood smears were stained at the Veterinary Diagnostic Laboratory, School of Animal and Veterinary Sciences, The University of

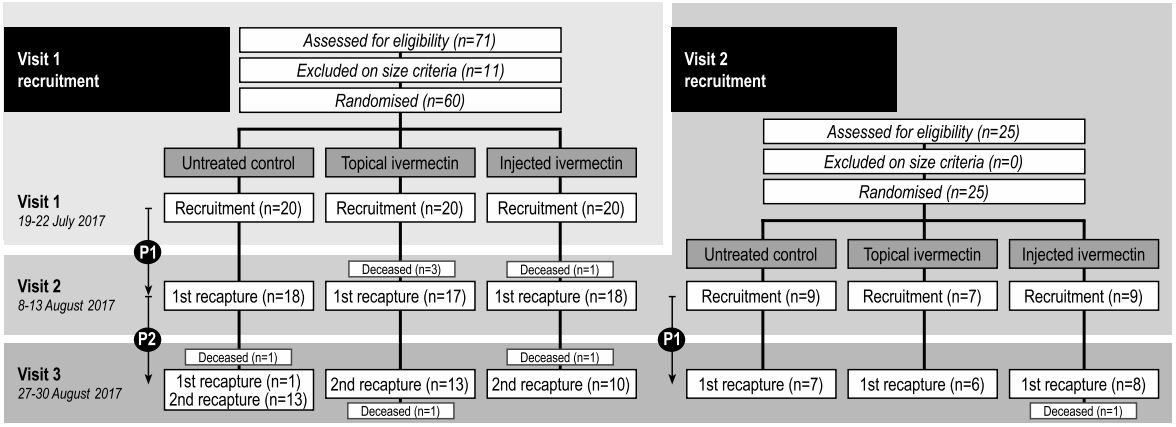

Fig. 1. Flow diagram of the trial course showing pup recruitment and recapture count for the three experimental groups for each of the three colony visits. P1 = time between recruitment and first recapture; P2 = time between first recapture and second recapture. Observation of deceased pups is shown relative to (i.e., before or after) the pup's sampling at that visit.

Adelaide using a Wrights-Giemsa protocol: 0.26% Wrights-Giemsa stain (Kinetik Pty Ltd, Narangba, Queensland) for 2 min; 1:5 ratio of 0.26% Wrights-Giemsa in Sorensen's buffer pH 6.8 (Fronine Pty Ltd, Riverstone, New South Wales) for 6 min; Sorensen's buffer rinse for 5 dips. Stained smears were reviewed (Leica N PLAN 100x/1.25/FN26.5 oil objective) to determine the differential leukocyte count (100 leukocytes were counted for every 10x10^9/L TNCC) and nucleated red cell (nRBC) count per 100 WBC. The corrected leukocyte count (cWBC, x10^9/L), and absolute neutrophil, lymphocyte, monocyte and eosinophil counts (x10^9/L) were subsequently determined for each sample.

2.4. Hookworm infection assessment

Faecal swabs were stored at 4 °C in the field and −20 °C prior to processing within 14 days of collection. Faecal specimens were collected from all captured pups, both at recruitment and recapture, and from all necropsied pups. Patent hookworm infection (positive ≥ one ova) was detected by examining a faecal smear on a glass slide. A single drop of deionised water was used to liberate faecal material from dry swabs. Negative smears were repeated to confirm the absence of any parasite ova. Based on a previous reporting of 100% prevalence of hookworm infection in Australian sea lions at this colony (Marcus et al., 2015b) and the young age of recruited pups, a negative faecal smear at recruitment was interpreted as likely pre-patent infection.

2.5. Statistical analyses

All statistical analyses were performed using the software package Stata version 16.1 (StataCorp LLC., College Station, Texas, USA). Statistical test results were interpreted at the 5% level of significance (type-I-error or α). A Bonferroni correction was used to adjust for multiple comparisons between pairs of experimental groups.

2.5.1. Evaluation of successful random allocation

Distribution of morphometrics and health records at recruitment were compared across the three experimental groups to assess the success of the predetermined random allocation. Pairs of experimental group means were compared using simple linear regression for continuous variables. Frequencies across categorical variables were compared among experimental groups using the Fisher's Exact test.

2.5.2. Standard multifactorial modelling

A standard multifactorial model building process was used across this study unless described otherwise. Using fundamental biological understanding of the investigated parasitoses, a base additive model was set up as the foundation of the model construction including the experimental groups allocation as the primary investigation aim. Even if one or more factors of the base model was not significant all remained in the final model. Then a forward selection process was used to select additional factors, retaining only factors significantly associated to the outcome of interest at the 5% significance level. If some predictors were strongly collinear, the most meaningful factor was retained. Potential first-degree interactions were explored among all possible pairwise combinations of single predictors retained in the model.

2.5.3. Evaluation of treatment effectiveness against hookworm and lice

The therapeutic effectiveness of ivermectin on hookworm was assessed by comparing across experimental groups the (i) apparent prevalence of hookworm at first recapture and (ii) apparent incidence of hookworm clearance during P1 (between recruitment and first recapture). The base additive model for effectiveness against hookworm included by default the duration of the time period in days until first recapture, the pup's standard length at recruitment (proxy of age) and its experimental group allocation. The hookworm prevalence and the incidence of clearance at first recapture were modelled using the Firth's variant (Firth, 1993) of multifactorial logistic regression which is based on a penalized maximum likelihood estimation to overcome the problem of 'separation' (i.e. covariate factors where all observations are the same, either all positive or all negative, perfectly predict the outcome and become inestimable using the conventional maximum likelihood estimation approach). The model construction followed the process described previously. Marginal estimates of hookworm frequencies (either prevalence or incidence) in each treatment group from the final models were used to calculate the treatment effectiveness as follow:

$$Effectiveness = \frac{infection\,frequency_{Control} - infection\,frequency_{Treatment}}{infection\,frequency_{Control}} \quad \text{(Eq.1)}$$

The boundaries of the 95% CI for the effectiveness were calculated using the lower and upper limits of the 95% CI of the hookworm frequency estimates, respectively.

The effectiveness of ivermectin against lice was assessed by comparing across experimental groups the (i) apparent incidence of lice clearance during P1 in pups infested at recruitment (therapeutic effectiveness), (ii) apparent incidence of lice infestation during P1 and P2 in lice-free pups at recruitment or first recapture, respectively (persistent effectiveness), and (iii) apparent lice prevalence at first and second recapture in any pups (combined therapeutic and persistent effectiveness). All base additive models included the length of the time period in days until recapture, the pup's standard length at recruitment and its experimental group allocation. The base models for therapeutic and combined effectiveness also included the lice intensity score at recruitment. Conventional multifactorial logistic regressions were built following the described standard process. Effectiveness estimates and their 95% CIs were calculated as per those for hookworm (Eq. (1)).

2.5.4. Evaluation of treatment impact on blood analyte levels

The impact of treatment on pup haematological profiles was evaluated by comparing the means of each measured blood analyte across experimental groups at first and second recaptures. For each analyte, the base additive model included the time duration in days until recapture, the pup's standard length at recruitment and its experimental group allocation. Additional factors included sex, analyte measure at recruitment, presence of lice at recruitment and at recapture and presence of hookworm at recapture. All factors were fitted within a multifactorial regression model (full model) and a stepwise backward elimination process was utilised to remove ancillary factors not associated with the outcome or those highly collinear with another predictor to build the final model. A Box-Cox transformation analysis was conducted on each analyte's full model to identify potential transformation of the analyte values to suit assumptions of a linear regression. When the model outcome required transformation, model estimates and their 95% CI boundaries were back-transformed for reporting. Back-transformed means must be interpreted as medians.

2.5.5. Evaluation of treatment impact on growth

The impact of treatment on pup growth was evaluated on the changes in three morphometrics – body mass (kg), standard length (m) and body condition index (BCI). The BCI was calculated using the slope-adjusted ratio index approach (Jakob et al., 1996):

$$Body\ condition\ index\ (BCI) = \frac{body\ mass\ (kg)}{standard\ length\ (m)^{slope}} \quad \text{(Eq.2)}$$

where the slope is the least-squares estimates from the linear regressing of ln (body mass) against ln (standard length), and ln is the natural log of the data.

To be comparable, the bodyweight, standard length and BCI growth were compared using a daily specific growth rate (SGR) calculated at each study period (P1, P2 and P1+P2) as follows:

$$Specific\ growth\ rate\ (SGR)_{daily} = \frac{ln\ (morphometric_{t+1}) - ln\ (morphometric_t)}{time_{t+1}(day) - time_t\ (day)} \quad \text{(Eq.3)}$$

Estimates of SGRs were compared across experimental groups using multifactorial linear regressions. The base additive model for the morphometric SGRs included by default the length of the time period in days, the pup's absolute morphometric measurement at the start of the period (standard length used for BCI) and its experimental group allocation.

3. Results

3.1. Recruitment and success of random allocation

There was no evidence of difference in demographic or health distribution across the three experimental groups, supporting a successful random group allocation (Bonferroni adjusted *P*-values > 0.05 for Fisher's exact test; similar adjustment applied to *P*-values for all subsequently reported pair-wise comparisons) (Supplementary Table 1). A total of 96 pups were captured and assessed for eligibility during the first and second visits (71 pups and 25 pups, respectively). Eleven of the pups from the first visit were not recruited as they did not meet the inclusion criterion ($\leq$ 70 cm in standard length). Variations from the inclusion criterion were exclusion of two pups (69 cm and 70 cm and weighing 10.0 kg) in visit 1 and inclusion of a one pup (71 cm and weighing 9.8 kg) in visit 2, due to an early intension to minimise cohort age and a later need to balance cohort numbers, respectively. Overall, 85 pups were recruited and randomly allocated to the untreated control (n = 29), topical ivermectin (n = 27) and injected ivermectin (n = 29) experimental groups (Fig. 1). One pup allocated to the topical ivermectin group was excluded from the treatment impact analysis because it was diagnosed with severe anaemia, considered to be unrelated to hookworm disease, which deteriorated during the trial, and it died shortly after the second recapture. A second pup with severe anaemia allocated to the injected ivermectin group was excluded from treatment impact analysis due to the lack of a second capture event.

Of the 85 pups, n = 75 (88.2%) were recaptured once 15–24 days after recruitment (excepting a single pup recruited at visit 1 and recaptured 39 days later at visit 3). Of the n = 53 pups recruited during visit 1 and recaptured during visit 2, 36 (67.9%) were recaptured a second time during visit 3, 36–41 days after recruitment. Excluding animals found dead, the recapture rates for first and second recaptures were 91.4% and 70.6%, respectively.

3.2. Treatment effectiveness against hookworm and lice

Compared to the control group, both ivermectin treatment groups demonstrated a similar significant reduction in hookworm prevalence at first recapture (topical 96.4%; injected 96.8%; $P < 0.05$) and additional hookworm clearance during P1 (topical 94.2%; injected 95.5%; $P < 0.05$) (Table 1). There was no significant difference in effectiveness between the two ivermectin treatment groups ($P > 0.05$) for either outcome. None of the factors ancillary to the base additive models (visit, sex, bodyweight or BCI) were associated with the therapy effectiveness against hookworm. Standard length at recruitment (OR = 2.80, $P < 0.001$), P1 duration (OR = 33.0, $P < 0.001$) and their interaction (OR = 0.95, $P < 0.001$) were significantly associated with hookworm prevalence at recapture. However, neither factor explained the incidence of hookworm clearance during P1 ($P = 0.795$, $P = 0.639$, respectively).

Irrespective of infection status at recruitment, patent hookworm infection was not detected in any pup in either of the ivermectin treatment groups at first or second recapture. In the untreated control group, 17/21 (81.0%) of pups with patent hookworm infection at recruitment had patent hookworm infection at first recapture, and 4/5 (80%) of pups without patent hookworm infection at recruitment

Table 1
The estimated prevalence and incidence of hookworm infection and lice infestation at recapture (with 95% confidence interval) in Australian sea lion (*N. cinerea*) pups, and the adjusted effectiveness of topical and injected ivermectin treatments.

Condition of interest	Incidence or prevalence estimate (95%CI)						Treatment effectiveness estimate (95%CI)	
	n	Control	n	Topical ivermectin	n	Injected Ivermectin	Topical ivermectin	Injected Ivermectin
Hookworm								
Prevalence at first recapture	25	82.7% (69.2%–96.2%)	22	2.9%^{A} (0.0%–10.4%)	26	2.6%^{A} (0.0%–9.2%)	96.4% (89.2%–100.0%)	96.8% (90.5%–100.0%)
Incidence of clearance during P1	20	19.5% (2.4%–36.5%)	15	95.3%^{A} (83.4%–100.0%)	17	96.4%^{A} (86.9%–100.0%)	94.2% (82.9%–100.0%)	95.5% (86.6%–100.0%)
Lice								
Prevalence at first recapture	25	71.8% (54.5%–89.1%)	22	22.0%^{A} (5.2%–37.3%)	26	24.3%^{A} (8.0%–40.5%)	69.4% (56.5%–90.4%)	66.2% (54.5%–85.3%)
Prevalence at second recapture	13	83.8%^{A} (56.5%–100.0%)	12	43.1%^{A,B} (16.0%–70.3%)	10	30.5%^{B} (5.4%–55.7%)	48.5% (29.7%–71.7%)	63.5% (44.3%–90.5%)
Incidence of clearance during P1	14	23.3% (0.1%–46.4%)	14	71.4%^{A} (47.7%–95.0%)	17	68.8%^{A} (46.0%–91.7%)	62.7% (47.7%–90.7%)	59.4% (45.9%–84.6%)
Incidence of infestation during P1	11	64.6% (36.9%–92.3%)	8	11.9%^{A} (0.0%–34.3%)	9	10.9%^{A} (0.0%–31.3%)	81.6% (62.9%–100.0%)	83.1% (66.1%–100.0%)
Incidence of infestation during P2	4	74.7%^{A} (32.1%–100.0%)	10	34.5%^{A,B} (6.6%–62.4%)	7	11.7%^{B} (0.0%–33.8%)	53.8% (37.6%–79.3%)	84.3% (66.2%–100.0%)

^{A,B}For incidence and prevalence estimates, experimental groups that share the same superscripted letter within the same parameter row are not significantly different at the 5% level after accounting for multiple pairwise comparisons using the Bonferroni adjustment. The incidence of clearance of patent hookworm infection or detectable lice infestation in pups positive at recruitment reflects the therapeutic effectiveness. The incidence of lice infestation in pups without detectable lice infestation at recruitment or first recapture reflects the persistent effectiveness during P1 and P2, respectively. The adjusted effectiveness of each treatment is calculated using the relative risk reduction of each treatment compared with the control group.

developed patent hookworm infection by first recapture.

Both ivermectin treatment groups achieved a significant reduction in lice prevalence at first recapture (topical 69.4%; injected 66.2%) compared to the control group, resulting from a reduction in lice infestation (topical 81.6%; injected 83.3%) and additional lice clearance during P1 (topical 62.7%; injected 59.4%; all $P < 0.05$). There was no significant difference between the two treatment groups ($P > 0.05$) (Table 1). The impact of the treatment on lice during P2 could only be investigated in a subset of the pups recaptured a second time (n = 35). While the sample size was too small for sufficient evidence to differentiate between the topical ivermectin treatment and untreated control groups ($P > 0.05$), longer-term effectiveness in the injected ivermectin treatment group during the second period ($P < 0.05$) was seen. Injected ivermectin reduced the lice prevalence at second recapture by 63.5% and the lice infestation during P2 by 84.3%, compared to control pups. There was no significant difference between the two ivermectin treatment groups' longer-term (P2) effectiveness ($P > 0.05$). None of the potential ancillary factors or the factors included in the base additive models were associated with the treatment effectiveness against lice, regardless of outcome or time period.

3.3. Treatment impact on blood analyte levels

A comparison of the model predictions for haematological parameters for each experimental group at first and second recapture are reported in Table 2. Both topical and injected ivermectin groups showed significantly lower total plasma protein concentration compared with the untreated control group at first recapture but not at the second recapture (topical −13.1 g/L, 95%CI: −18.9–7.4 and injected −14.8 g/L, 95%CI: −20.5–9.2). The total plasma protein concentration at first recapture was also associated with the presence of lice at recruitment (+5.2 g/L, 95%CI: 0.9–9.5) and hookworm at first recapture (−16.7 g/L, 95%CI: −26.6–6.9); however, these effects were no longer evident at second recapture. None of the other base additive model factors were significantly associated with total plasma protein concentration (Supplementary Table 2).

Topical and injected ivermectin treatment groups showed significantly lower absolute eosinophil counts compared with the untreated control group at both the first (topical -0.73×10^9/L, 95%CI: −0.93–0.51 and injected -0.77×10^9/L, 95%CI: −1.10–0.51) and second recaptures (topical -1.05×10^9/L, 95%CI: −1.93–0.50 and injected -1.09×10^9/L, 95%CI: −2.03–0.51) (Table 2), and none of the base additive model factors were significant (Supplementary Table 2).

The injected ivermectin group showed significantly higher red cell parameters (PCV, RBC, Hb and HCT) compared with the control group at the first but not the second recapture (e.g., PCV +0.052 L/L, 95%CI: 0.020–0.084) (Table 2) and none of the base additive model factors were significant (Supplementary Table 2). While the topical ivermectin group also showed higher red cell parameters compared with the control group at first recapture, the difference between the groups was not significant ($P > 0.05$). Likewise, there was no significant difference in red cell parameters between either of the two ivermectin treatment groups themselves at first recapture. There were no significant differences in red cell parameters between any of the experimental groups by second recapture.

3.4. Treatment impact on growth

Using study pup measurements for BCI calculation, the slope between bodyweight and standard length on the logarithm scale was estimated at approximately 2.5 (slope = 2.52, 95%CI: 2.28–2.76). Regardless of group allocation, pup standard length increased faster during P1 (0.300% per day) than during P2 (0.230% per day), while pup weight increased faster during P2 (0.676% per day) than during P1 (0.572% per day) translating into a relative loss of BCI during P1 (−0.163% per day) and a relative gain during P2 (0.125% per day)

Table 2
The predicted mean or median values (with 95% confidence interval) of haematological parameters of Australian sea lion (*N. cinerea*) pups at first and second recapture.

Parameters	n	Predicted means or medians[a] (95% CI) Control	Topical ivermectin	Injected ivermectin
At first recapture				
Field blood parameters				
PCV (L/L)	58	0.307[A] (0.288–0.324)	0.332[A,B] (0.313–0.349)	0.359[B] (0.344–0.372)
Total plasma protein (g/L)	65	85.2 (82.2–88.3)	72.1[A] (68.2–75.9)	70.4[A] (66.7–74.0)
Laboratory blood parameters				
RBC ($x10^{12}$/L)	48	3.93[A] (3.71–4.14)	4.03[A,B] (3.79–4.27)	4.33[B] (4.12–4.54)
Haemoglobin (g/L)	48	111.9[A] (105.2–118.5)	115.2[A,B] (107.8–122.6)	127.2[B] (120.6–133.7)
Haematocrit (L/L)[b]	48	0.348[A] (0.327–0.368)	0.359[A,B] (0.336–0.380)	0.391[B] (0.373–0.409)
MCV (fL)	48	86.4[A] (84.2–88.5)	88.3[A] (85.9–90.7)	89.4[A] (87.3–91.6)
MCHC (g/L)	48	324.0[A] (318.3–329.7)	327.5[A] (321.2–333.8)	327.1[A] (321.4–332.8)
Platelets (10^9/L)	59	336.0[A] (278.2–393.7)	289.4[A] (227.0–352.0)	297.4[A] (242.6–352.1)
WBC (10^9/L)	59	17.9[A] (14.4–21.9)	13.6[A] (10.3–17.2)	15.6[A] (12.4–19.1)
Neutrophils (10^9/L)	60	11.6[A] (8.8–14.8)	9.0[A] (6.4–12.0)	10.3[A] (7.8–13.2)
Lymphocytes (10^9/L)	60	4.0[A] (3.2–4.8)	2.9[A] (2.2–3.7)	3.7[A] (3.0–4.5)
Monocytes (10^9/L)	60	1.1[A] (0.8–1.4)	1.0[A] (0.7–1.3)	1.2[A] (0.9–1.4)
Eosinophils (10^9/L)	60	0.77 (0.51–1.12)	0.04[A] (0.00–0.19)	0.00[A] (0.00–0.02)
nRBC/100WBC[b]	60	0.09[A] (0.00–1.14)	0.00[A] (0.00–0.02)	0.02[A] (0.00–0.24)
At second recapture				
Field blood parameters				
PCV (L/L)	31	0.306[A] (0.280–0.333)	0.329[A] (0.297–0.361)	0.328[A] (0.289–0.366)
Total plasma protein (g/L)	35	75.5[A] (72.1–78.8)	76.1[A] (72.5–79.6)	76.4[A] (72.2–80.7)
Laboratory blood parameters				
RBC ($x10^{12}$/L)	26	4.10[A] (3.66–4.53)	4.18[A] (3.74–4.61)	4.23[A] (3.65–4.80)
Haemoglobin (g/L)	26	112.8[A] (98.3–127.3)	113.6[A] (98.6–128.6)	119.0[A] (100.2–137.7)
Haematocrit (L/L)	26	0.332[A] (0.290–0.374)	0.341[A] (0.298–0.385)	0.352[A] (0.298–0.407)
MCV (fL)	26	79.4[A] (76.3–82.5)	82.6[A] (79.5–85.8)	81.4[A] (77.5–85.3)
MCHC (g/L)	33	340.7[A] (333.2–348.2)	333.6[A] (326.8–340.4)	329.1[A] (321.4–336.8)
Platelets (10^9/L)	33	357.8[A] (282.6–433.1)	324.9[A] (244.6–405.1)	351.4[A] (260.0–442.8)
WBC (10^9/L)	33	17.2[A] (13.9–21.3)	15.6[A] (12.4–19.5)	16.8[A] (12.9–21.7)
Neutrophils (10^9/L)	34	11.0[A] (8.2–13.8)	10.1[A] (7.2–12.9)	10.4[A] (7.1–13.8)
Lymphocytes (10^9/L)	26	4.56[A] (3.31–6.30)	4.37[A] (3.15–6.06)	4.7[A] (3.10–7.12)
Monocytes (10^9/L)[b]	33	0.871[A] (0.681–1.114)	0.915[A] (0.704–1.190)	1.063[A] (0.788–1.433)
Eosinophils (10^9/L)	34	1.12 (0.51–2.18)	0.07[A] (0.01–0.25)	0.03[A] (0.00–0.15)
nRBC/100WBC[b]	34	0.002[A] (0.000–1.129)	0.000[A] (0.000–0.004)	0.000[A] (0.000–0.044)

RBC: total red blood cell count; MCV: mean cell volume; MCHC: mean cell haemoglobin concentration; WBC: total leukocyte count, corrected for presence of nucleated red blood cells (nRBC).

[A,B]Experimental groups that share the same superscripted letter within the same parameter row are not significantly different at the 5% level after accounting for multiple pairwise comparisons using the Bonferroni adjustment. Manual PCV

was included in addition to haematocrit to overcome any impact on the latter from the delay to processing.

[a] Median reported for back-transformed model estimates.

[b] Sub-optimal compliance with linear regression assumptions.

Table 3
The predicted mean or median values (with 95% confidence interval) of daily specific growth rate (SGR) for the bodyweight, standard length and body condition index in Australian sea lion (*N. cinerea*) pups during the first and second treatment trial period.

Parameters	n	Predicted means or medians[a] (95% CI)		
		Control	Topical ivermectin	Injected ivermectin
Recruitment-to-first recapture (P1)				
Bodyweight daily SGR (%)	73	0.342[A] (0.112–0.573)	0.717[A] (0.472–0.961)	0.671[A] (0.446–0.897)
Standard length daily SGR (%)	73	0.281[A] (0.229–0.334)	0.338[A] (0.283–0.394)	0.307[A] (0.256–0.358)
Body condition index daily SGR (%)	73	−0.357[A] (−0.573–0.141)	−0.090[A] (−0.318-0.138)	−0.039[A] (−0.250-0.172)
First recapture-to-second recapture (P2)				
Bodyweight daily SGR (%)	35	0.619[A] (0.316–0.923)	0.852[A] (0.536–1.167)	0.538[A] (0.199–0.878)
Standard length daily SGR (%)	35	0.197[A] (0.123–0.271)	0.286[A] (0.209–0.363)	0.207[A] (0.123–0.292)
Body condition index daily SGR (%)	35	0.127[A] (−0.160-0.415)	0.132[A] (−0.168-0.433)	0.113[A] (−0.217-0.442)

[A]Experimental groups that share the same superscripted letter within the same parameter row are not significantly different at the 5% level after accounting for multiple pairwise comparisons using the Bonferroni adjustment.

[a] Median reported for back-transformed model estimates.

(Table 3). A comparison of the model predictions for bodyweight, standard length and BCI SGR in each experimental group during P1 and P2 is reported in Supplementary Table 3. During P1, the bodyweight SGR of treated pups (topical and injected) was approximately twice that of the control pups while standard length SGR was equivalent across the three experimental groups. This resulted in a decreased BCI in the control pups but not in treated pups (BCI SGR estimate not significantly different from zero). However, after Bonferroni correction for multiple pairwise comparison, the observed evidence was not sufficiently strong to discriminate between groups ($P > 0.05$).

Within the final bodyweight SGR models, the duration in days of the period (+0.088% per day, 95%CI: 0.025–0.150) and the presence of hookworm at recruitment (+0.338% per day, 95%CI: 0.030–0.646) impacted the bodyweight SGR during P1; while only bodyweight at first recapture (start of P2) was associated with the bodyweight SGR during P2 (−0.135% per day, 95%CI: −0.242–0.028) (Supplementary Table 3). Standard length at recruitment (−0.058% per day, 95%CI: −0.071–0.044), bodyweight at recruitment (+0.098% per day, 95%CI: 0.065-0.013) and male sex (+0.101% per day, 95%CI: 0.065-0.013) influenced standard length SGR during P1; bodyweight at the first recapture was marginally associated (+0.040% per day, 95%CI: 0.000–0.080) with standard length SGR during P2. Duration in days of the period (+0.084% per day, 95%CI: 0.026–0.143), standard length at recruitment (+0.137% per day, 95%CI: 0.082–0.193), bodyweight at recruitment (−0.325% per day, 95%CI: −0.461–0.189) and the presence of hookworm at recruitment (+0.353% per day, 95%CI: 0.063–0.643) impacted the BCI SGR during P1; both standard length (+0.100% per day, 95%CI: 0.004–0.192) and bodyweight at the first recapture (−0.371% per day, 95%CI: −0.504–0.188) impacted the standard length SGR during P2.

3.5. Monitoring of pup survival

Of the 85 pups recruited, eight were found dead (9.4%: control n = 1; topical n = 4; injected n = 3) during 14 visit days across the 42-day study period, with seven cadavers suitable for necropsy. Based on gross examination, the cause of death was determined to be conspecific trauma in three pups (topical n = 2; injected n = 1), and starvation with conspecific trauma in two pups (control n = 1; injected n = 1). The cause of death was undetermined in one injected ivermectin treatment pup. The cause of death during visit three of the topical ivermectin pup excluded from treatment impact analysis was progression of the previously detected severe anaemia. This pup's health status was monitored during all three visits – the cause of the anaemia was undetermined based on gross necropsy review (and additional microscopic tissue specimen review). None of the pups necropsied from any experimental group were determined to have died from hookworm infection, based on the absence of haemorrhagic enteric pathology. At necropsy, all treated pups had a negative faecal hookworm smear, while the untreated control pup had a positive faecal hookworm smear. In addition to these known deaths, four pups (control n = 1; topical n = 2; injected n = 1) were not resighted at subsequent visits.

4. Discussion

This study reports the first use of topical ivermectin treatment for hookworm infection and lice infestation in a free ranging otariid population and found a high level of effectiveness not significantly different to that of the injected ivermectin formulation. There were no treatment related deaths detected with either topical or injected ivermectin administration. The Dangerous Reef colony was an appropriate site for this study, based on adequate pup recruitment and a short-term recapture rate providing statistically significant effectiveness outcomes. Inclusion of two recapture events 15–24 days and 36–41 days post-recruitment provided further short-term temporal detail to the earlier study observations in this species, which had showed an absence of a growth benefit, a trend for decreased total plasma protein concentration and a significant reduction in eosinophil count at 27–67 post-treatment (Marcus et al., 2015b). Treatment with either formulation in the present study ameliorated a decrease in body condition (based on BCI) and elevation of plasma protein present in untreated pups present 15–24 days post-recruitment, while a beneficial reduction in a systemic eosinophilic inflammatory response persisted to 36–41 days post-recruitment in line with ongoing patent hookworm infection in untreated animals. The topical formulation provided an alternative method of ivermectin administration that was minimally invasive with little requirement for operator expertise. In the field these benefits could translate to a reduction in the restraint time necessary to treat a pup and offer an additional management tool for the early elimination of endemic hookworm infection in Australian sea lion pups.

4.1. Treatment effectiveness against hookworm and lice

In the present study, estimated hookworm prevalence at 15–24 days post-treatment with topical ivermectin treatment (2.9%) did not differ significantly to that of the injected formulation (2.6%), and both were comparable to that reported 27–67 days after injected ivermectin treatment (2.4%) in the previous study at this colony (Marcus et al., 2015b). Clearance of hookworm within this shorter time period and the absence of re-infection post-treatment were anticipated based on the exclusive lactogenic transmission (in colostrum) of hookworm in otariid species (Lyons et al., 2011; Olsen and Lyons, 1965), and demonstrated clearance of adult worms from the intestine by 16 h (DeLong et al., 2009) and 47 h (Marcus et al., 2015b) after injected ivermectin treatment (DeLong et al., 2009; Marcus et al., 2015b). The absence of re-infection following elimination of infection was one justification for the use of an older generation, shorter-acting anthelmintic of known

safety profile in the current study. The assumption of prepatent infection in pups with a negative faecal smear at recruitment in our young cohort was supported by 80% (n = 5) of such animals in the untreated control group subsequently displaying patent hookworm infection at first recapture. This finding combined with that of 81.0% (n = 21) of pups in the untreated control group with patent hookworm infection at recruitment maintaining that status at first recapture, suggested minimal transference of topical ivermectin treatment from conspecific animals.

As an additional comparator against the injected formulation, inclusion of effectiveness against lice achieved the objective of showing no significant differences between the formulations for prevalence, incidence of clearance or incidence of infestation at either of the measured time points. Given possible reinfestation at any age, the lower incidence of infestation during period 2 (from 15-24 days to 36–41 days post-recruitment) for the treatment groups compared with the untreated control group (significance reached only for the injected group) indicated some persistent activity during this period. This complements findings from the earlier site study of injected ivermectin, which showed the persistent treatment benefit for lice prevalence was no longer apparent by 60 days post-treatment (Marcus et al., 2015b).

4.2. Treatment impact on haematological parameters

In the present study hookworm elimination contributed to increases in measures of red cell quantity (PCV, RBC, Hb, HCT) by 15–24 days post treatment (reaching significance only for the injected group), a benefit that did not persist to 36–41 days post-treatment. This short-term benefit with elimination of a *U. sanguinis* explains the absence of a similar treatment-associated difference for PCV in the earlier site study (recaptures 27–67 days post-recruitment), although in that study a significantly higher RBC count was still apparent with treatment (Marcus et al., 2015b). A similar treatment benefit has been documented in the South American fur seal, with higher haemoglobin levels at 3–7 weeks of age compared with hookworm-infected pups (Seguel et al., 2019). Any treatment-associated increase in red cell parameters is superimposed on the rapid physiological decline reported in the first weeks of life in otariid pups, which must be considered when contemplating treatment-related temporal changes during this post-natal period (Michael et al., 2021a; Seguel et al., 2019; Trillmich et al., 2008).

A significantly lower peripheral eosinophil cell count in ivermectin-treated Australian sea lion pups persisting to the second recapture 36–41 days post-treatment is consistent with previous reports in this and other otariid species (Marcus et al., 2015b; Michael et al., 2021a; Montalva et al., 2019; Seguel et al., 2019), and indicates the elimination of parasite-associated eosinophilic tissue inflammation.

Both topical and injected treatment resulted in a significantly lower total plasma protein at 15–24 days post-treatment, a short-term difference that did not persist to 36–41 days post-treatment. This result is contrary to that expected with elimination of a blood-feeding intestinal parasite and with observations in ivermectin-treated New Zealand sea lions (Michael et al., 2021a). A short-term protein elevation in untreated animals resulting from positive acute phase inflammatory response to parasitism (supported by the eosinophilic inflammatory response) and temporal differences in sampling could explain this finding. Significantly higher globulin levels have been reported in South American fur seal pups compared with adult animals, albeit in pups not showing patent hookworm infection, while albumin levels did not differ significantly to those in adult animals (Seguel et al. 2016). Further investigation (serial serum protein analysis) is necessary to further define the cause of the protein elevation observed short-term in untreated Australian sea lions.

4.3. Treatment impact on growth

Although between group differences did not reach significance, pups treated with either of the ivermectin formulations showed bodyweight specific growth rates approximately double that of the control pups for the period to 15–24 days post-recruitment. This benefit in the treated pup groups ameliorated the decrease in body condition (based on BCI) observed in the untreated control group during this period. The absence of a similar benefit to second recapture at 36–41 days post-treatment indicates that any growth rate benefit from hookworm elimination is short-term in the Australian sea lion, similar to the finding in the previous study at this site (Marcus et al., 2015b). In other otariid species ivermectin hookworm treatment has contributed to significant growth rate improvements measured over various time periods, including in northern fur seal pups (absolute growth rate over 2.5 months) (DeLong et al., 2009), in New Zealand sea lion pups (absolute and relative growth rates over 10–93 days [mean 68 days]) (Chilvers et al., 2009), and in South American fur seal *(Arctocephalus australis)* pups (absolute growth rate over >20 days versus a severely infected pup group) (Montalva et al., 2019). Varying parasite impact on host (parasite species, hookworm infection intensity), differences in methodology for measurement of growth rate and time to resampling may explain these differences (Chilvers et al., 2009; DeLong et al., 2009). In humans, suboptimal nutrition, growth and stunting during gestation and the first 24 months of a child's life are associated with irreversible, life-long detrimental health impacts, including reduced stature (height, body-mass index), decreased learning ability, increased susceptibility to infection and chronic disease, greater mortality risk and reduced off-spring birthweight (Black et al., 2013; Victora et al., 2008). Not surprisingly, early interventions during this critical period of development to remove disease negatively impacting growth (including hookworm parasitism) (Mofid et al., 2017) have a substantial impact on health outcomes over a life time (Victora et al., 2008). Longer term follow up is necessary to determine if short-term growth rate benefits in ivermectin-treated Australian sea lion pups also translate into the desired long-term goals of improved health and survival benefits.

4.4. Monitoring of pup survival

There were no pup deaths in either of the treatment groups attributed to the treatment interventions. Additionally, the mortality rate for the recruited cohort did not exceed that expected for this population during a higher mortality winter (versus summer) breeding season, during which the incidence of pup mortality averages 37% (observed range 30–45% for the 1996–2005 seasons) (Goldsworthy et al., 2007). Conspecific trauma was a contributing factor in all five pup deaths for which a grossly detectable cause of death was apparent. Dangerous Reef is a high density colony of Australian sea lions (Marcus et al., 2014), a likely contributor to this finding. These results are consistent with recent reporting from the largest controlled trial of injected ivermectin in a free-ranging otariid population (New Zealand sea lions), in which there were no reports of treatment-related adverse events in any pup treated across two breeding seasons (Michael et al., 2021b). Nonetheless, any risk posed by anthelmintic use in otariids requires ongoing review and monitoring, and the small sample size in this study limits recommendations for the more widespread or routine use of topical ivermectin in any of these species.

The present study was not powered or intended to detect a treatment-associated survival benefit. However, this remains the primary objective of any treatment intervention in the Australian sea lion – improved pup survival and a subsequent increase in recruitment to the breeding population necessary to arrest the current declining population trend. Rather, the validation of a minimally invasive disease management tool was intended as a first step to the treatment of a young cohort of Australian sea lion pups, particularly for times when strategic use could counter additional stressors decreasing their survival and that of this endangered species generally (Chilvers, 2015; DeLong et al., 2009; Lyons et al., 2001; Michael et al., 2021b).

To this end, this study confirmed topical ivermectin to be a highly

effective anthelmintic for treatment of endemic hookworm disease in the Australian sea lion, comparable to the injected formulation. The next step in understanding hookworm disease impact on modulating population growth will require a longitudinal study conducted across low and high mortality breeding seasons, at a site free of the logistical constraints of Dangerous Reef. These pertain to easier colony access to increase resighting and mortality investigations, and long-term animal identification (not currently implemented at Dangerous Reef) to permit the collection of resight-recapture data for long-term survival investigations.

Declaration of competing interest

All authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2021.11.002.

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