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BASELINE HEALTH PARAMETERS FOR A NEWLY ESTABLISHED POPULATION OF LONG-NOSED POTOROO (*POTOROUS TRIDACTYLUS*) AT BOODEREE NATIONAL PARK, AUSTRALIA

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ABSTRACT: Over two field seasons during 2014–15, 35 long-nosed potoroos (*Potorous tridactylus*) were captured in state forests in South Eastern New South Wales for translocation to Booderee National Park, Jervis Bay Territory, Australia. Animals were anesthetized for physical examination and collection of samples to assess general health and screen for select diseases identified during a disease risk assessment. Morphologic, hematologic, and biochemical parameters were determined, and parasites were identified where possible. *Trypanosoma gilletti*, *Trypanosoma vegrandis*, and novel genotypes most similar to a *Trypanosoma* wallaby-derived isolate (ABF) were identified from blood samples by PCR; the first time *Trypanosoma* has been described in this species. Also reported is the first confirmation of the Australian paralysis tick, *Ixodes holocyclus*, from the long-nosed potoroo. Surveillance showed that *Cryptococcus* sp. may form part of the normal nasal flora for long-nosed potoroo. *Salmonella enterica* serotype Dublin and *Salmonella enterica* subsp. *enterica* was identified from rectal swabs of otherwise healthy animals. The data provide baseline health and disease parameters for this newly established population and the source population and will inform future translocation and conservation management activities. These data expand current knowledge on aspects of the biology and microbiology of the long-nosed potoroo, both locally and nationally.

Key words: *Cryptococcus*, *Ixodes holocyclus*, long-nosed potoroo, *Potorous tridactylus*, *Salmonella*, translocation health, *Trypanosoma*.

INTRODUCTION

The long-nosed potoroo (*Potorous tridactylus*) is an elusive, medium-sized nocturnal marsupial with scattered populations along Australia's eastern seaboard from southern Queensland to Tasmania (Norton et al. 2010; Frankham et al. 2011). The status of the population in New South Wales (NSW) is poorly understood but is considered vulnerable under the Biodiversity Conservation Act 2016 (NSW Government 2021). Population declines have been attributed to predation by introduced feral predators, environmental degradation, and population fragmentation (Frankham et al. 2011). The threat to long-nosed potoroo populations from disease is uncertain because of very few long-term studies and a general lack of baseline health data.

In the Jervis Bay area of NSW, historical evidence from Aboriginal middens suggest long-nosed potoroos were present in this area before European settlement (Lampert 1971). Booderee National Park (Booderee; 35°10′S, 150°40′E) is a protected area located in Jervis Bay Territory, 200 km south of Sydney, NSW, Australia, that boasts a range of habitats and a mild year-round climate of 9–24 C (Lindenmayer et al. 2014). Environmental managers of Booderee sought to repopulate the park with long-nosed potoroos through the translocation of wild animals from monitored

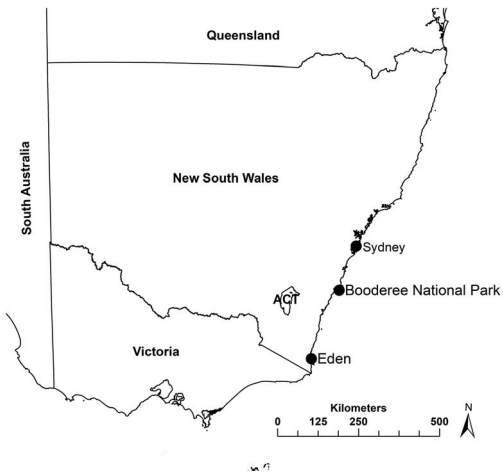

FIGURE 1. Long-nosed potoroo (*Potorous tridactylus*) trapped at Nadgee, Timbillica, and East Boyd State Forests in South Eastern New South Wales near Eden, Australia, were transported by road approximately 310 km north to Booderee National Park, Jervis Bay Territory, Australia. ACT denotes the Australian Capital Territory.

populations in state forests of South Eastern NSW.

A comprehensive disease risk assessment with the use of published and unpublished data was carried out to develop a pretranslocation diagnostic plan (Jacob-Hoff et al. 2014). We evaluated the health of individual long-nosed potoroos and conducted surveillance for a range of disease agents to support translocation of a healthy founding population to Booderee and to inform ongoing management of these populations and future conservation management activities at this site. The data will also expand the current body of knowledge for the health of free-ranging, long-nosed potoroo which may benefit other conservation actions around the country.

MATERIALS AND METHODS

Animals were trapped at Nadgee, Timbillica, and East Boyd State Forests south of Eden in South Eastern NSW (Fig. 1) during Spring 2014–15. During each field season a total of 155 traps, monitored over four consecutive nights, were deployed at sites with known robust populations on the basis of long-term monitoring data from two of the authors (P.K., R.B.). Wire 20×30×50-cm bait-suspension pedal traps covered with plastic and shade cloth were baited with a mixture of rolled oats, peanut butter, and golden syrup. Traps were cleared at first light each day. Nontarget species were released directly from the trap. Long-nosed potoroos were transferred to soft, dark holding bags, which were secured with a trap label and placed into transport boxes before being driven to a central processing location.

On arrival at the processing facility, animals were anesthetized with Isoflurane™ (Veterinary Companies of Australia, Kings Park, NSW, Australia) in oxygen by mask. Once anesthetized, a heat pack was placed over the tail for 3–5 min to increase blood flow and facilitate blood collection. Animals were physically examined by a veterinarian to assess general body condition, molar wear, and respiratory function with a visual examination of skin and mucocutaneous junctions. Any abnormalities, injuries, or lesions were further investigated.

Blood was collected before other samples and measurements to increase the probability of successfully obtaining a sample. A small area of fur overlying the lateral tail vein was clipped, and the skin was cleaned with a preoperative preparation of Chlorhex-C as per manufacturer's instructions (Jurox Pty. Ltd., Rutherford, NSW, Australia). Up to 2 mL of blood was collected from the vein with a 25-gauge needle, and divided between pediatric-sized ethylenediaminetetraacetic acid (EDTA), non-gel lithium-heparin and non-gel serum separator tubes (BD Vacutainer, Becton, Dickinson, and Company, Plymouth, UK). Ectoparasites removed from the pelage with blunt forceps were placed into 70% ethanol. Feces were collected, if produced, directly from the animal or the holding bag. Swabs were collected from the nasal airway, conjunctiva, rectum, and urogenital ostium and placed into individual sterile 2-mL cryotubes (Interpath Services Pty. Ltd., Melbourne, Australia) stored at –20 C before culture. A pinna biopsy was taken from the right ear in males and left ear in females with a sterile, disposable 3-mm biopsy punch (Kai Medical, Tokyo, Japan), and the tissue was placed in 70% ethanol for genetic studies not reported in this article.

Morphologic data recorded included weight, body condition determined by muscle and fat overlying the thoracolumbar spine and hips, tooth wear, head length, right pes length, and ear length. Pouch young were recorded, when present, as small, if pink and undeveloped; medium, if developed, but ears flat; and large, if fully developed, furred, eyes open, and ears upright.

Individuals were marked with a passive induction transponder tag (Trovan, Microchips Australia, Keysborough, Australia) injected subcutane-

ously between the shoulder blades, in accordance with the *Guidelines for Transponder Placement and Recording* (Association of Zoos and Aquariums Institutional Data Management Advisory Group 2010). After physical examination and sample collection was completed, isoflurane delivery was terminated, and animals were maintained on oxygen until visible signs of recovery from anesthesia were noted, such as increased respiratory rate and paddling of limbs. Diazepam (Troy Animal Healthcare, Glendenning, Australia) was administered (1 mg/kg intramuscular) before the individual was placed into a well-ventilated dark bag along with a quarter of an apple. Each animal was monitored by direct observation until they became sufficiently alert to position themselves upright and maintain their own airway; then, the bags were sealed with string.

Once an individual was determined by physical assessment and preliminary hematologic and biochemical analysis to be suitable for translocation, the bagged animal was placed into a well-ventilated transport box before being driven by sealed road approximately 310 km to Booderee National Park and released at dusk on the same day (Fig. 1).

Blood collected into EDTA was assessed for white blood cell count and hematocrit and by hand-held refractometer for total protein (Bacto Laboratories, Mt. Pritchard, Australia) within 15 min of blood collection. Multiple blood films were prepared and stained with Diff-Quik (Thermo Fisher Scientific, Scoresby, Victoria, Australia) and subjected to differential cell analysis and examination for hemoparasites within 1 h of collection. Residual EDTA whole blood was frozen at –80 C then transported on dry ice to Murdoch University, Perth, Western Australia, for molecular characterization of blood parasites.

For blood parasite detection by PCR, DNA was extracted from EDTA whole blood (200 μL) with a MasterPure™ DNA Purification Kit (Epicentre Biotechnologies, Madison, Wisconsin, USA) following manufacturer recommendations, eluted in 35 μL of Tris-EDTA buffer, and stored at –20 C until use. *Trypanosoma* spp. DNA was amplified from partial regions of the 18S rRNA locus with primers previously described (Noyes et al. 1999; Austen et al. 2009). Piroplasma DNA was amplified from partial regions of the 18S rRNA locus with primers previously described (Jefferies et al. 2007). All controls (no-template, extraction-reagent blanks, and known positive PCR controls) produced appropriate PCR results. The PCR product was run on a 1.5% agarose gel stained with SYBR Safe (Invitrogen, Waltham, Massachusetts, USA) and viewed under ultraviolet light. Amplified PCR products of the appropriate size were excised from the gel with a sterile scalpel blade for each band to prevent cross-contamination, purified by an in-house filter tip method, and used for sequencing without any further purification, as previously described (Yang et al. 2013). Samples were sent for Sanger sequencing at the Australian Genome Research Facility (Perth, Western Australia) on an Applied Biosystems 3730 DNA sequencer (Thermo Fischer Scientific) by BigDye Terminator v3.1 chemistry (Thermo Fisher Scientific). Chromatograms were imported into Geneious v10 (Biomatters Ltd., Auckland, New Zealand; Kearse et al. 2012) for quality inspection and primers were trimmed. Sequences were then subject to basic local alignment search tool (BLAST) analysis by BLASTN 2.10.0+ (Zhang et al. 2000) against a nonredundant nucleotide collection database (Morgulis et al. 2008; Benson et al. 2017) to identify the most similar species and genotypes.

Plasma was analyzed with a VetScan VS2 clinical chemistry analyzer with the use of a Comprehensive Diagnostic Profile for glucose, urea, creatinine, calcium, phosphate, sodium, potassium, total protein, albumin, globulin, total bilirubin, amylase, alanine transaminase, and alkaline phosphatase (REM Systems, Sydney, NSW, Australia). Residual plasma was diluted 1:10 for creatine kinase and aspartate aminotransferase analysis on a Reflotron analyzer (DTS Diagnostics, Wetherill Park, NSW, Australia).

Sera were transported on dry ice to the Animal Health Laboratory (Department of Primary Industries, Parks, Water, and Environment, Tasmania, Australia) for a *Toxoplasma gondii* modified agglutination test.

One nasal swab from each individual was cultured with Bird Seed Agar incubated at 25 C for 2 d for *Cryptococcus* spp. surveillance. Purity plates and an API-ID32C yeast identification system (bioMerieux, North Ryde, Sydney, NSW, Australia) were used to identify growth of colonies morphologically consistent with yeast by Gram stain. Samples showing a strong morphologic similarity to *Cryptococcus* were forwarded to the University of Sydney for further characterization. Fresh subcultures were used for DNA extraction. Samples were tested in PCR with MyTaq™ Red Mix (Bioline [Aust] Pty. Ltd., Eveleigh, Australia) by cycling at 95 C for 3 min followed by 40 cycles of 95 C for 15 s, 60 C for 15 s, and 72 C for 20 s. DNA was amplified with universal internal transcribed spacer (ITS) primers ITS1 and ITS4, as described (Kumar and Shukla 2005). The PCR products of expected size were sequenced after purification at Macrogen Inc. (Seoul, Korea). *Cryptococcus* sp. isolates were further identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonik MALDI Biotyper, Bruker Biosciences, Victo-

ria, Australia) by Pathology North (St. Leonards, NSW, Australia).

Rectal swabs were subject to PCR for *Salmonella* and *Campylobacter* spp. surveillance at Melbourne University. The PCR primers used to detect the presence of *Campylobacter jejuni* and *Campylobacter coli* were based on the *cadF* gene (Konkel et al. 1999). The reaction mixture contained 5 µL of *Taq* 5X buffer, 1.5 µL of 25 mM $MgCl^{2+}$, 1.6 µL of 1.25 mM deoxyribonucleotide triphosphates, 0.25 µL of *Taq* polymerase at 5 U/µL, 1 µL each of the F2B (forward) and R1B (reverse) primers at 10 µM, 9.65 µL of water, and 5 µL of template DNA extracted from pooled swabs (Konkel et al. 1999). The positive control used *C. jejuni* genomic material extracted from laboratory stocks. The thermocycler program used was 30 cycles of 60 s at 94 C, 45 s at 45 C, and 35 s at 72 C. The cycles were preceded by a 120-s interval of 95 C and followed by a 120-s interval of 72 C.

To detect *Salmonella* spp., a primer pair that detects a 132-base pair (bp) product of the *invA* gene (Pusterla et al. 2010) was used. The reaction mixture contained 4 µL of *Taq* 5X buffer, 2 µL of 25 mM $MgCl^{2+}$, 0.4 µL of 1.25 mM deoxyribonucleotide triphosphates, 0.16 µL of *Taq* polymerase, 0.1 µL of *invA* forward and reverse primers at concentrations of 100 µM, 4.24 µL of water, and 5 µL of template extracted from pooled swabs (Pusterla et al. 2010). The positive control used a previously sequenced, confirmed sample of *Salmonella*. The thermocycler was programmed for 45 cycles of 30 s at 95 C, 30 s at 60 C, and 30 s at 72 C. This set of 45 cycles was preceded and followed by 120-s intervals of 95 C and 72 C, respectively. The PCR products were run on a 1.5% agarose gel stained with SYBR Safe (Invitrogen) and viewed under ultraviolet illumination. Samples with detectable PCR product bands were processed with the QIAquick® Gel Extraction Kit (Quiagen, Chadstone, Victoria, Australia). Their DNA concentrations were measured with a NanoDrop spectrophotometer (Thermo Fischer Scientific). The samples were then sent to the Centre for Translational Pathology at the University of Melbourne for sequencing. Sequence results were analyzed by Geneious and National Center for Biotechnology Information BLAST software as described earlier.

Ticks in 70% ethanol were transported to the Department of Medical Entomology, Centre for Infectious Diseases and Microbiology Laboratory Services (Westmead Hospital, NSW, Australia), where they were identified by medical entomologists according to Roberts (1970). Fecal samples were examined fresh, on the same day as collection, for the presence of intestinal parasites. A wet preparation of feces and saline was examined at 10× magnification. Remaining feces were placed in an Ovitector© system (BGS Medical Products Inc., Venice, Florida, USA) and examined for fecal parasites at 10× magnification.

A urogenital, nasal, and conjunctival swab from each individual were sent to the University of Melbourne for herpesvirus PCR testing by a nested PCR method (Chmielewicz et al. 2003).

Morphology and hematologic and biochemical reference intervals, to compare adult males and females by a Welch two-sampled independent *t*-test, were obtained by analyzing all data with the statistical package R version 4.0.3 (R Core Team 2020). Where analysis of hematologic and biochemical parameters showed no significant difference between sexes, data were pooled. According to weight, as suggested by Norton et al. (2010) and Frankham et al. (2011), males >800 g, and females >700 g were considered adult.

RESULTS

Over two field seasons, 51 long-nosed potoroo (14 adult males, four independent juvenile males, 17 adult females, 15 pouch young at various stages of development, and one at-foot juvenile) were trapped and processed for release in Booderee National Park. Pouch young and at-foot dependent animals were not handled for sample collection. An additional adult female was euthanatized at the time of examination because of luxation and fracture of the left hock, probably sustained while in the trap. Gross postmortem examination and histopathology of representative sections of each tissue stained with H&E confirmed the traumatic injuries identified antemortem, with no further lesions. Samples were collected from this individual before euthanasia and are included in this analysis.

Mild to moderate hyperkeratosis, with or without erythema and alopecia, was identified in seven female and five male potoroos, generally around the inguinal area, at the base of the testicles or around the pouch, and inner thighs. Some animals had old abrasions over the hips, rump, sternum, or tail. One female had a small lesion on the mucocutaneous junction of the lower eyelid, which was swabbed for herpesvirus PCR despite appearing traumatic in nature.

TABLE 1. Comparison of morphologic measurements for adult male (>800 g) and adult female (>700 g) long-nosed potoroos (*Potorous tridactylus*) sampled during translocation from Nadgee, Timbillica, and East Boyd State Forests in South Eastern New South Wales, Australia, to Booderee National Park, Jervis Bay Territory, Australia, 2014–15.[a]

	n	Mean	SD	Min	Max	SE	Reference interval (95% CI)
Weight (g)							
Male	12	1,116	140	890	1,375	40.3	1,027–1,205
Female	18	1,013	145	729	1,150	34.2	941–1,085
Head length[b] (mm)							
Male	12	91.0	4.38	83.1	99.3	1.27	88.2–93.8
Female	18	84.6	3.74	76.7	90.0	0.88	82.7–86.4
Pes length (mm)							
Male	12	58.9	13.3	49.2	83.8	3.85	50.4–67.4
Female	18	57.8	10.5	49.0	77.4	2.48	52.6–63.0
Ear length (mm)							
Male	12	38.6	5.16	23.6	43.3	1.49	35.3–41.9
Female	18	38.9	2.50	33.2	42.5	0.589	37.7–40.2

[a] *n* = number of individuals; Min = minimum value; Max = maximum value; CI = confidence interval.
[b] Significant difference ($P<0.05$).

Morphologic measurements are reported for adult male and female long-nosed potoroos only, because the data were insufficient for meaningful analysis of other age classes (Table 1). All female potoroos were >700 g or had pouch young, thus confirming adult classification. Female weights include pouch young, if present. Approximately 90% of females had pouch young at various stages of development. One female was trapped with an "at-foot" juvenile. The smallest female with pouch young weighed 729 g; the pouch young was classified as small. One female weighing 980 g was captured without pouch young. Male potoroos were considered adult at weights >800 g. The smallest independent animal, a male, weighed 410 g. Differences between sexes were not significant for weight [$t(24)=1.94$, $P=0.0628$], pes length [$t(19)=0.24$, $P=0.8154$], or ear length [$t(14)=0.22$, $P=0.8298$]. The difference in head length between male and female potoroo was significant [$t(21)=4.19$, $P=0.0004$], with males having consistently longer heads than females. The weight of one male was not recorded because of handling error; because it had a head length measurement consistent with that of other adult males, it was classified as such.

Hematologic and biochemical analyses are reported for adult male and female long-nosed potoroos only (Table 2), because the data were insufficient for meaningful analysis of other age classes. Four females, all with medium-sized pouch young, had a creatinine result below the range of the analyzer. Two females and one male returned an alkaline phosphatase result beyond analyzer capabilities (>2,400 U/L), and one male returned a result below detectable levels (0 U/L). One male had insufficient sample volume for creatine kinase analysis.

Occasional Howell–Jolly bodies were present in 60% of blood smears, and hemoglobin crystals were present in low numbers in 65% of smears. Mild anisocytosis, polychromasia, and poikilocytosis were common. No blood parasites were detected visually. Neither hematologic nor biochemical parameters when comparing adult male and female potoroos were significantly different, except that neutrophils ($\times 10^9$/L) [$t(20.2)=2.48$, $P=0.02$] and serum calcium [$t(27)=2.31$, $P=0.03$] were both consistently higher in males.

TABLE 2. Hematologic and biochemical parameters for adult male (>800 g) and adult female (>700 g) long-nosed potoroos (*Potorous tridactylus*) sampled during translocation from Nadgee, Timbillica, and East Boyd State Forests in South Eastern New South Wales, Australia, to Booderee National Park, Jervis Bay Territory, Australia, 2014–15.[a]

Parameter	*n*	Mean	SD	Min	Max	SE	Reference interval (95% CI)
White blood cells ($\times 10^9$/L)	29	5.66	2.8	1.43	12.1	0.52	4.59–6.73
Hematocrit (%)	29	39.1	3.8	33.0	49.0	0.7	37.7–40.6
Neutrophils (%)	29	48.1	12.8	19.0	66.0	2.38	43.3–53.0
Neutrophils ($\times 10^9$/L)[b]							
Males	12	3.57	1.64	1.46	6.78	0.47	2.53–4.61
Females	17	2.16	1.30	0.59	5.71	0.32	1.49–2.83
Lymphocytes (%)	29	44.9	12.7	27.0	72.0	2.36	40.1–49.8
Lymphocytes ($\times 10^9$/L)	29	2.52	1.47	0.66	6.91	0.27	1.96–3.08
Monocytes (%)	29	2.76	2.32	0.00	8.00	0.43	1.87–3.64
Monocytes ($\times 10^9$/L)	29	0.17	0.22	0.00	0.97	0.04	0.08–0.26
Eosinophils (%)	29	2.52	2.37	0.00	9.00	0.44	1.62–3.42
Eosinophils ($\times 10^9$/L)	29	0.14	0.15	0.00	0.46	0.02	0.09–0.20
Basophils (%)	29	1.62	1.37	0.00	5.00	0.25	1.10–2.14
Basophils ($\times 10^9$/L)	29	0.09	0.10	0.00	0.42	0.02	0.05–0.14
Total protein (g/L)[c]	27	66.10	5.32	58.00	78.00	1.02	64.0–68.3
Glucose (mmol/L)	30	8.48	2.35	4.20	15.90	0.43	7.60–9.36
Urea (mmol/L)	30	4.05	1.32	2.60	8.20	0.24	3.56–4.55
Creatinine (μmol/L)	26	33.7	7.16	20.00	46.00	1.40	30.8–36.5
Calcium (mmol/L)[b]							
Males	12	2.28	0.06	2.15	2.39	0.02	2.24–2.32
Females	18	2.21	0.11	1.85	2.36	0.03	2.15–2.26
Phosphate (mmol/L)	30	2.15	0.42	0.92	2.85	0.07	1.99–2.31
Sodium (mmol/L)	30	141.0	2.50	135.00	147.00	0.45	140–142
Potassium (mmol/L)	30	3.63	0.30	3.10	4.30	0.05	3.51–3.74
Total protein (g/L)	30	64.40	5.58	56.00	76.00	1.02	62.3–66.5
Albumin (g/L)	30	43.90	3.26	38.00	51.00	0.59	42.7–45.1
Globulin (g/L)	30	20.40	5.12	13.00	32.00	0.93	18.5–22.3
AST (U/L)	30	175.0	89.80	8.10	364.00	16.40	141–208
ALT (U/L)	30	74.40	28.40	13.00	183.00	5.19	63.8–85.0
Total bilirubin (μmol/L)	30	6.83	1.46	5.00	10.00	0.26	6.29–7.38
Amylase (U/L)	30	260.0	65.10	157.00	415.00	11.90	236–285
ALP (U/L)	27	820.0	431.00	0.00	2,068.00	82.90	649–990
Creatine kinase (U/L)	29	4,801.0	3,231.00	756.00	16,200.00	600.00	3,572–6,030

[a] *n* = number of individuals; Min = minimum value; Max = maximum value; CI = confidence interval; AST = aspartate aminotransferase; ALT = alanine transaminase; ALP = alkaline phosphatase.

[b] Significant difference ($P<0.05$) between male and female values.

[c] Total protein (g/L) measured by refractometer.

With the use of molecular techniques, all blood samples were negative for piroplasms; however, 44% (16/36) of long-nosed potoroos tested positive for *Trypanosoma* spp. Eight samples were positive for *Trypanosoma gilletti* (GU966589; 100% similarity). A representative 1,157-bp sequence was submitted to GenBank (accession no. MT898511). One sample was positive for *Trypanosoma vegrandis* G6 (KC753535; 98.8%) (597-bp fragment submitted to GenBank, MT898512). Five samples were most similar to *Trypanosoma* sp. wallaby ABF (AJ620564; 99.0%–99.8% similarity) (four unique 512–514-bp fragment sequences submitted to GenBank, MT895514–MT895516, MT895518). One

sample was most similar to *Trypanosoma* sp. TL.AV.43 cl 101E (AJ620571; 98.6%) (512-bp fragment submitted to GenBank, MT898513). One sample was most similar to *Trypanosoma* sp. TL.AQ.45 (AJ620575; 97.3%) (517-bp fragment submitted to GenBank, MT898517).

Nasal swabs from three of the 36 potoroos grew *Cryptococcus* spp. in culture. The organism cultured from one individual was identified as *Cryptococcus flavescens*, with the organism from another potoroo identified as *Cryptococcus laurentii*, by PCR and sequence analysis. Organisms cultured from a third animal could not be confirmed by PCR or sequence analysis despite several attempts but was strongly positive for *C. laurentii* by API-ID32C. Other nasal fungi identified during this process included *Millerozyma farinosa*, *Candida norvegica*, and *Acremonium kiliense*.

From the rectal swabs of 36 potoroos, one tested positive for *Salmonella enterica* serotype Dublin, and *Salmonella enterica* subsp. *enterica* was identified from two others. *Campylobacter* spp. were not detected. *Toxoplasma gondii* modified agglutination test was negative in all 35 serum samples tested. Herpesviruses were not detected by PCR from nasal, oral, or urogenital swabs from 36 animals.

We found mites, fleas, and ticks in the pelage of most animals. Ticks were identified as *Haemaphysalis bancrofti* or *Ixodes holocyclus*. Singular species were recovered from most animals, regardless of capture location; however, both *H. bancrofti* and *I. holocyclus* were recovered from two animals. Feces collected from nine potoroos showed mild infection with nematodes (five animals) and coinfections of nematodes and coccidia (four animals). Mite ova were also identified in feces. Identification of fecal parasites to genus level was not possible.

DISCUSSION

Thirty-five long-nosed potoroos (14 adult males, four independent juvenile males, and 17 adult females and their dependent offspring) were found to be clinically healthy and were successfully translocated to Booderee National Park between 2014–15. All but two females were carrying pouch young at varying stages of development at the time of capture and transport. One female was captured with a young at foot; both animals were transported to Booderee. No incidents occurred in which a joey was ejected from the pouch during these activities.

We considered male potoroos weighing >800 g and females weighing >700 g to be adult. All females weighed >700 g, including pouch young, and were confirmed as sexually mature from reproductive evidence. The single female that did not have pouch young was also adult by weight; therefore, we are unable to determine at what body size long-nosed potoroos in this population may reach sexual maturity. Only 13 males were considered adult by weight, with an additional male classified as adult from head length. In some populations, long-nosed potoroos display significant sexual dimorphism, with males being larger than females in weight, head length, and pes length (Norton et al. 2010; Frankham et al. 2011). In our study, only head length was found to be significantly different between sexes, with adult males having larger heads than adult females. We had insufficient data to conduct statistical analysis of immature animals. Interpretation of hematologic and biochemical analyses needs to acknowledge this and should also take the small sample size of our study into consideration.

Except for the single animal with significant trauma, presumably sustained in the trap, the potoroos showed no physical or clinical signs of stress on removal from traps or capture bags, and all appeared calm upon physical restraint. For some animals, obtaining blood samples was noticeably more difficult than for others, which was alleviated by the use of a warmed heat pack to the vein before blood collection and by carrying out the blood draw before any other sample collection activities.

Blood cell morphology for this population appeared normal when compared with previous studies (Clark 2004; Vaughan et al. 2009). Hemoglobin crystals may be considered indicators of hemoglobin C disease or sickle

cell anemia in humans (Barger 2010); however, they are rarely reported in veterinary medicine, and their presence is not understood. There was no indication of anemia in the blood smears; therefore, the presence of hemoglobin crystals in these animals is likely of little significance.

We have no explanation for the sex-related differences in neutrophil concentrations and serum calcium. Individuals that produced analyte readings beyond the range of the analyzer had variable body condition scores, tooth wear (suggesting various ages), and assorted parasite burdens; therefore, these results probably reflect the limitations of the automated analyzers designed for companion animal biochemistry.

This article reports the first record of *Trypanosoma* spp. from long-nosed potoroos. We identified *T. gilletti*, *T. vegrandis* (G6), and novel genotypes of the *Trypanosoma cyclops* clade, similar to *Trypanosoma* sp. wallaby ABF, TL.AQ.45, and TL.AV.43 (Hamilton et al. 2005; McInnes et al. 2011b). The lack of trypomastigotes observed in blood smears is consistent with previous studies that have shown molecular tools are more sensitive for the detection of these blood parasites (Rodrigues et al. 2019) and is most likely due to low levels of parasitemia. *Trypanosoma* species have been identified in a variety of Australian mammals, including northern brown bandicoot (*Isoodon macrourus*), eastern barred bandicoot (*Perameles gunnii*), quenda (*Isoodon fusciventer*), brush-tailed bettong (woylie, *Bettongia penicillata*), and Gilbert's potoroo (*Potorous gilbertii*; Austen et al. 2009; Thompson et al. 2014). *Trypanosoma copemani* in the critically endangered brush-tailed bettong has been associated with smooth and cardiac muscle pathologies (Botero et al. 2013), and a statistical association between *T. gilletti* and a variety of concurrent diseases has been described in koalas (McInnes et al. 2011a). No direct correlation between *Trypanosoma* sp. infection and ill health was observed in long-nosed potoroos in the present study, but further investigation of this relationship is warranted.

Several ubiquitous environmental fungi were identified from nasal swabs during surveillance for *Cryptococcus*, including *C. laurentii* and *C. flavescens*. Generally regarded as nonpathogenic, *C. laurentii* has been described as an opportunistic human pathogen, causing meningeal, pulmonary, ocular, and skin lesions in immunocompromised patients (Molina-Leyvaa et al. 2013). *Cryptococcus flavescens* has been identified as the causative agent for subcutaneous abscessation in a dog (Kano et al. 2012) and within the cerebrospinal fluid of an AIDS patient (Kantarcioğlu et al. 2007). Fatal cryptococcosis caused by *Cryptococcus neoformans* and *Cryptococcus gattii* has been described in captive Gilbert's potoroos and long-nosed potoroos (Vaughan et al. 2007), respectively. Although all potoroos in our study appeared clinically normal, it is interesting to note the potential for *Cryptococcus* spp. to become opportunistic pathogens in this species.

The individual and population-level significance of identifying *Salmonella* spp. from rectal swabs of two potoroos is questionable, but wildlife has been shown to be an important reservoir for multiple *Salmonella* species that can cause clinical disease in both agricultural and domestic animals and humans (Staff et al. 2012; Simpson et al. 2018). *Salmonella enterica* has been cultured from wild long-nosed potoroo previously (Parsons et al. 2011). Further characterization to determine the subspecies and serovars is warranted.

Low-level burdens of both internal and external parasites were detected. Nematodes could not be speciated in this study, but endoparasitic nematodes including *Hymenolepis* spp. (Ladds 2009) and *Potoroxyuris potoroo* (Hobbs and Elliot 2016) have been reported in potoroos in the past and are not considered pathogenic. *Haemaphysalis bancrofti*, also known as the wallaby tick, is well described from a variety of mammals along the east coast of Australia, including the long-nosed potoroo (Barker and Walker 2014). Similarly, the Australian paralysis tick *I. holocyclus* has been reported from a wide variety of Australian mammals, although not

previously from the long-nosed potoroo. Various *Ixodes* spp. have been theorized to be a vector for trypanosome infections (Austen et al. 2009); this relationship warrants further investigation. Our microbial and parasitic findings highlight the continued need for appropriate hygiene and personal protective equipment when handling any wildlife.

This study has provided crucial data to inform disease risk assessments related to current and future conservation activities for the long-nosed potoroo. By documenting health parameters for the new population founded at Booderee National Park and extant populations near Eden, NSW, conservation managers will be better placed to understand the potential risk of disease in both populations and identify changes in this baseline into the future. Ongoing monitoring of the Booderee population through health assessment of live animals and full postmortem examination of fresh remains will be vital in assessing risks that could threaten the success of this program and the ongoing health and welfare of both populations.

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LITERATURE CITED

Austen JM, Jefferies R, Friend JA, Ryan U, Adams P, Reid SA. 2009. Morphological and molecular characterization of *Trypanosoma copemani* n. sp. (Trypanosomatidae) isolated from Gilbert's potoroo (*Potorous gilbertii*) and quokka (*Setonix brachyurus*). *Parasitology* 136:783–792.

Association of Zoos and Aquariums Institutional Data Management Advisory Group. 2010. *Guidelines for transponder placement and recording*. https://assets.speakcdn.com/assets/2332/guidelines_for_transponder_placement_and_recording_aza_branded1.pdf Accessed July 2016.

Barger AM. 2010. Erythrocyte morphology. In: *Schalm's veterinary hematology*, 6th Ed., Weiss DJ, Wardrop KJ, editors. Wiley-Blackwell, Ames, Iowa, pp. 114–151.

Barker SC, Walker AR. 2014. Ticks of Australia: The species that infest domestic animals and humans. *Zootaxa* 18:1–144.

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2017. GenBank. *Nucleic Acids Res* 45:D37–D42.

Botero A, Thompson CK, Peacock CS, Clode PL, Nicholls PK, Wayne AF, Lymbery AJ, Thompson RC. 2013. Trypanosomes genetic diversity, polyparasitism and the population decline of the critically endangered Australian marsupial, the brush tailed bettong or woylie (*Bettongia penicillata*). *Int J Parasitol Parasites Wildl* 2:77–89.

Chmielewicz B, Goltz M, Lahrmann KH, Ehlers B. 2003. Approaching virus safety in xenotransplantation: A search for unrecognized herpesvirus in pigs. *Xenotransplantation* 10:349–356.

Clark P. 2004. *Haematology of Australian mammals*. CSIRO Publishing, Melbourne, Australia, 250 pp.

Frankham GJ, Reed RL, Fletch TP, Handasyde KA. 2011. Population ecology of the long-nosed potoroo (*Potorous tridactylus*) on French Island, Victoria. *Aust Mammal* 33:73–81.

Hamilton PB, Stevens JR, Gidley J, Holz P, Gibson WC. 2005. A new lineage of trypanosomes from Australian vertebrates and terrestrial bloodsucking leeches (Haemadipsidae). *Int J Parasitol* 35:431–443.

Hobbs RP, Elliot AD. 2016. A new species of *Potoroxyuris* (Nematoda: Oxyuridae) from the woylie *Bettongia penicillata* (Marsupialia: Potoroidae) from southwestern Australia. *Int J Parasitol* 5:211–216.

Jakob-Hoff RM, MacDiarmid SC, Lees C, Miller PS, Travis D, Kock R. 2014. *Manual of procedures for wildlife disease risk analysis*. World Organisation for Animal Health, Paris, France, 160 pp.

Jefferies R, Ryan UM, Irwin PJ. 2007. PCR-RFLP for the detection and differentiation of the canine piroplasm species and its use with filter paper–based technologies. *Vet Parasitol* 144:20–27.

Kano R, Ishida R, Nakane S, Sekiguchi M, Hasegawa A, Kamata H. 2012. The first reported case of canine

subcutaneous *Cryptococcus flavescens* infection. *Mycopathologia* 173:179–182.

Kantarcioğlu AS, Boekhout T, de Hoog GS, Theelen B, Yücel A, Ekmekci TR, Fries BC, Ikeda R, Koslu A, Altas K. 2007. Subcutaneous cryptococcosis due to *Cryptococcus diffluens* in a patient with sporotrichoid lesions case report, features of the case isolate and in vitro antifungal susceptibilities. *Med Mycol* 45:173–181.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649.

Konkel ME, Gray SA, Kim BJ, Garvis SG, Yoon J. 1999. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the cadF virulence gene and its products. *J Clin Microbiol* 37: 510–517.

Kumar M, Shukla PK. 2005. Use of PCR targeting of internal transcribed spacer regions and single-stranded conformation polymorphism analysis of sequence variation in different regions of rRNA genes in fungi for rapid diagnosis of mycotic keratitis. *J Clin Microbiol* 43:662–668.

Ladds P. 2009. *Pathology of Australian native wildlife*. 1st Ed. CSIRO Publishing, Melbourne, Australia, 648 pp.

Lampert RJ. 1971. Burrill Lake and Currarong: Coastal sites in southern New South Wales. In: *Terra Australis 1*, Golson J, editor. Canberra Publishing and Printing Co. Pty. Ltd., Canberra, Australia, pp. 1–86.

Lindenmayer D, MacGregor C, Dexter N, Fortescue M. 2014. *Booderee National Park: The jewel of Jervis Bay*. 1st Ed. CSIRO Publishing, Collingwood, Australia, 152 pp.

McInnes LM, Gillett A, Hanger J, Reid SA, Ryan UM. 2011a. The potential impact of native Australian trypanosome infections on the health of koalas (*Phascolarctos cinereus*). *Parasitology* 138:873–883.

McInnes LM, Hanger J, Simmons G, Reid SA, Ryan UM. 2011b. Novel trypanosome *Trypanosoma gilletti* sp. (Euglenozoa: Trypanosomatidae) and the extension of the host range of *Trypanosoma copemani* to include the koala (*Phascolarctos cinereus*). *Parasitology* 138:59–70.

Molina-Leyva A, Ruiz-Carrascosa JC, Leyva-Garcia A, Husein-Elahmed H. 2013. Cutaneous *Cryptococcus laurentii* infection in an immunocompetent child. *Int J Infect Dis* 17:e1232–e1233.

Morgulis A, Coulouris G, Raytselis Y, Madden TL, Agarwala R, Schaffer AA. 2008. Database indexing for production MegaBLAST searches. *Bioinformatics* 24:1757–1764.

Norton MA, Claridge AW, French K. 2010. Population biology of the long-nosed potoroo (*Potorous tridactylus*) in the Southern Highlands of New South Wales. *Aust J Zool* 58:362–368.

Noyes HA, Stevens JR, Teixeira M, Phelan J, Holz P. 1999. A nested PCR for the ssrRNA gene detects *Trypanosoma binneyi* in the platypus and *Trypanosoma* sp. in wombats and kangaroos in Australia. *Int J Parasitol* 29:331–339.

NSW Government. 2021. *Biodiversity Conservation Act 2016*. https://www.legislation.nsw.gov.au/view/html/inforce/current/act-2016-063#. Accessed April 2021.

Parsons SK, Bull CM, Gordon DM. 2011. Substructure within *Salmonella enterica* subsp. *enterica* isolates from Australian wildlife. *Appl Environ Microbiol* 77: 3151–3153.

Pusterla N, Byrne BA, Hodzic E, Mapes S, Jang SS, Magdesian KG. 2010. Use of quantitative real-time PCR for the detection of *Salmonella* spp. in fecal samples from horses at a veterinary teaching hospital. *Vet J* 186:252–255.

R Core Team. 2020. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org/. Accessed December 2020.

Roberts FHS. 1970. *Australian ticks*. CSIRO Publishing, Melbourne, Australia, 267 pp.

Rodrigues MS, Lima L, Xavier SCC, Herrera HM, Rocha FL, Roque ALR, Teixeira MMG, Jansen AM. 2019. Uncovering *Trypanosoma* spp. diversity of wild mammals by the use of DNA from blood clots. *Int J Parasitol Parasites Wildl* 8:171–181.

Simpson KMJ, Hill-Cawthorne GA, Ward MP, Mor SM. 2018. Diversity of *Salmonella* serotypes from humans, food, domestic animals and wildlife in New South Wales, Australia. *BMC Infect Dis* 18:623.

Staff M, Musto J, Hogg G, Janssen M, Rose K. 2012. Salmonellosis outbreak traced to playground sand, Australia, 2007–2009. *Emerg Infect Dis* 18:1159–1162.

Thompson CK, Godfrey SS, Thompson RCA. 2014. Trypanosomes of Australian mammals: A review. *Int J Parasitol Parasites Wildl* 3:57–66.

Vaughan RJ, Vitali SD, Eden PA, Payne KL, Warren KS, Forshaw D, Friend JA, Horwitz AM, Main C, Krockengerger MB, et al. 2007. Cryptococcosis in Gilbert's and long-nosed potoroo. *J Zoo Wildl Med* 38:567–573.

Vaughan RJ, Warren KS, Mills JS, Palmer C, Fenwick S, Monaghan CL, Friend AJ. 2009. Hematological and serum biochemical reference values and cohort analysis in the Gilbert's potoroo (*Potorous gilbertii*). *J Zoo Wildl Med* 40:276–288.

Yang R, Murphy C, Song Y, Ng-Hublin J, Estcourt A, Hijjawi N, Chalmers R, Hadfield S, Bath A, Gordon C, et al. 2013. Specific and quantitative detection and identification of *Cryptosporidium hominis* and *C. parvum* in clinical and environmental samples. *Exp Parasitol* 135:142–147.

Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. *J Comput Biol* 7:203–214.

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