IJP: Parasites and Wildlife 7 (2018) 274–279

Contents lists available at ScienceDirect

IJP: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Debilitating disease in a polyparasitised woylie (*Bettongia penicillata*): A diagnostic investigation

Amy S. Northover[a,*], Aileen D. Elliot[a], Sarah Keatley[a], Ziyuan Lim[a], Adriana Botero[a], Amanda Ash[a], Alan J. Lymbery[a], Adrian F. Wayne[b], Stephanie S. Godfrey[c], R.C. Andrew Thompson[a]

[a] School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch, Western Australia, 6150, Australia
[b] Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions, Brain Street, Manjimup, Western Australia, 6258, Australia
[c] Department of Zoology, University of Otago, 362 Leith Street, Dunedin, 9016, New Zealand

ARTICLE INFO

Keywords:
Bettongia penicillata
Fauna translocation
Paraustrostrongylus sp.
Polyparasitism
Sarcocystis sp.
Trypanosoma copemani

ABSTRACT

During monitoring of critically endangered woylie (*Bettongia penicillata*) populations within the south-west of Western Australia, an adult female woylie was euthanased after being found in extremely poor body condition with diffuse alopecia, debilitating skin lesions and severe ectoparasite infestation. *Trypanosoma copemani* G2 and *Sarcocystis* sp. were detected molecularly within tissue samples collected post-mortem. *Potorostrongylus woyliei* and *Paraustrostrongylus* sp. nematodes were present within the stomach and small intestine, respectively. Blood collected ante-mortem revealed the presence of moderate hypomagnesaemia, mild hypokalaemia, mild hyperglobulinaemia and mild hypoalbuminaemia. Diffuse megakaryocytic hypoplasia was evident within the bone marrow. We propose various hypotheses that may explain the presence of severe ectoparasite infection, skin disease and poor body condition in this woylie. Given the potential deleterious effects of parasite infection, the importance of monitoring parasites cannot be over-emphasised.

1. Introduction

Critically endangered woylie (brush-tailed bettong, *Bettongia penicillata*) populations have declined by more than 90% since 1999, and are now restricted to three indigenous wild populations within the south-west of Western Australia (Wayne et al., 2015). In June 2014, as part of the ongoing conservation management of this species, 182 woylies were translocated from Perup Sanctuary, a 423-ha predator-proof enclosure located 50km east of Manjimup (34.2506°S, 116.1425°E), to supplement two natural populations. During monitoring within one of these populations (Walcott, situated 20km north-west of Perup Sanctuary; 34.0592°S, 116.3859°E; Fig. 1) six months after translocation, an adult female resident (i.e. non-translocated) woylie was found in extremely poor body condition with diffuse alopecia and skin lesions predominantly affecting the head, hindlimbs, tail base and tail. Severe ectoparasite infestation was also apparent. Veterinary assessment deemed this animal unsuitable for release and the woylie was euthanased in the field via barbiturate injection while under inhalant anaesthesia. A post-mortem examination was carried out within seven hours of death.

For threatened species such as the woylie, in which only small fragmented wild populations remain, parasites may have a significant impact on population dynamics and host health (Thompson et al., 2010). With regard to woylie population declines, a clear spatio-temporal pattern of decline in population size between 1999 and 2006 was identified, which suggests the potential role of an infectious disease agent (Wayne et al., 2015). Field monitoring carried out immediately prior to, and during this period, found a high prevalence of woylies with moderate to severe alopecia, skin thickening, skin excoriations and scale with a predilection for the head (periocular region and ears) and dorsal tail base/rump region (Wayne et al., 2013). Clinical signs were strikingly similar to those described here. Despite investigation into this 'skin condition', a causative disease agent could not be identified. Since then, the focus of investigation has shifted toward the potential role of other disease agents. Trypanosomes have been of particular interest, given the demonstrated pathogenicity of *Trypanosoma copemani* genotype 2 (G2) and the association between *T. copemani* and declining woylie populations (Smith et al., 2008; Botero et al., 2013; Thompson et al., 2014; Godfrey et al., 2018). While previous investigation into the decline has focused on the effects of individual parasite species, the

* Corresponding author. 90 South Street, Murdoch, Western Australia, 6150, Australia.
E-mail address: A.Northover@murdoch.edu.au (A.S. Northover).

https://doi.org/10.1016/j.ijppaw.2018.07.004
Received 14 March 2018; Received in revised form 1 June 2018; Accepted 12 July 2018

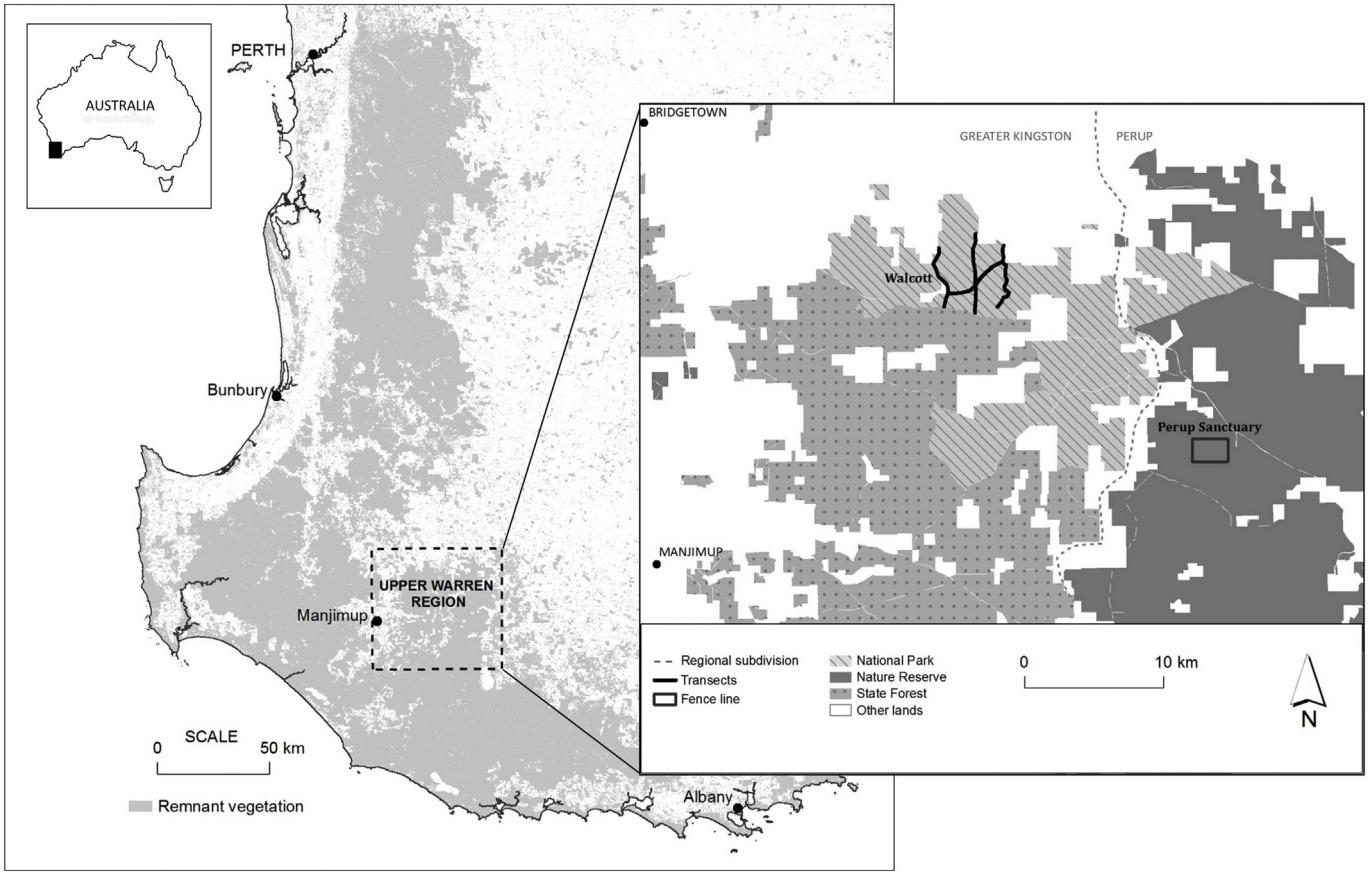

Fig. 1. Map depicting our study sites within the Upper Warren Region. As shown on the right, Walcott is located approximately 20km north-west of Perup Sanctuary.

effect of coinfection has not been evaluated. Here we explore various hypotheses that may explain the presence of severe ectoparasite burdens, debilitating skin disease and poor body condition in this woylie.

2. Materials and methods

2.1. Trapping regime

Trapping was conducted in December 2014. Woylies were captured using Sheffield cage traps (Sheffield Wire Products, Welshpool, WA), which were set along multiple transects (60 traps/night, 200m spacing) at dusk and baited with universal bait (rolled oats, peanut butter and sardines). Newspaper was placed beneath each trap to collect faeces, which were stored chilled prior to examination. Traps were cleared within 3 hours of sunrise.

2.2. Parasitological analysis

2.2.1. Gastrointestinal parasites

Fresh faeces (2.6g) were examined for eggs/oocysts using simple faecal flotation with sodium nitrate (NaNO3) as described by Northover et al. (2015). The entire gastrointestinal tract was also examined for the presence of endoparasites, and specimens were morphologically identified using keys developed by Mawson (1973), Beveridge and Durette-Desset (2009), and Smales (1997, 2005).

2.2.2. Haemoparasites

Blood was collected ante-mortem (under anaesthesia) from the lateral caudal (tail) vein into EDTA MiniCollect tubes (Greiner Bio-One, Germany) for molecular analyses and stored at −20 °C prior to processing. DNA was extracted from 200 µl aliquots of blood using the QIAamp 96 DNA blood kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. A nested polymerase chain reaction (PCR) targeting the 18S rDNA gene region was carried out using generic trypanosome primers, as described by Maslov et al. (1996) and McInnes et al. (2009). Positive samples were subsequently screened for the presence of different *Trypanosoma* spp. using clade-specific primers designed by Botero et al. (2013) and McInnes et al. (2011). PCR reactions were performed as outlined in Cooper et al. (2018) with the exception that 2 µl of DNA was added to a 24 µl master mix.

2.2.3. Tissue parasites

Eleven tissue samples (spleen, liver, lung, heart, kidney, brain, oesophagus, tongue, skeletal muscle, anal glands, and bone marrow) were collected post-mortem, extensively washed with phosphate buffered saline, and stored in 100% ethanol for DNA isolation, and fixed in 10% formalin for histopathological analysis. Genomic DNA was obtained using the QIAamp tissue DNA MiniKit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA samples were screened by PCR for the presence of trypanosomes and coccidian parasites. Trypanosome PCR was performed as described above. For the detection of coccidian parasites a fragment of about 800bp from the 18SrDNA gene was amplified using the coccidia generic primers 1L and 3H as described previously (Yang et al., 2001). Sequencing was carried out to confirm parasite genotype/species using the generic coccidian primers in both directions, and using an ABI Prism TM Terminator Cycle Sequencing Kit on an Applied Bio-systems 3730 DNA Analyser (Applied Biosystems, California, USA). Sequences were aligned against reference libraries generated from GenBank®.

2.2.4. Ectoparasites

Prior to euthanasia, the woylie was examined in a systematic