Investigation of skin diseases observed in Woylies (*Bettongia penicillata ogilvyi*) from the Upper Warren Region, WA.

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Introduction

Numbers of woylies (*Bettongia penicillata ogilvyi*) in wild populations around the Upper Warren region have been undergoing dramatic declines (average 85-90%) over the last 10 years, with Keninup undergoing a rapid decline in 2007. Observations from field researchers have reported a high level of a skin condition prior to and during declines (Wayne et al. 2008), but this has not been investigated to date. Skin conditions have also been noted in animals trapped as part of population monitoring efforts, but also have not been investigated. This project will aim to investigate the nature of observed skin conditions in woylies and interpret potential significance in relation to population declines.

Methodology

Retrospective data analysis

Woylies from the Upper Warren region were trapped and monitored as part of a population monitoring program run by the Western Australian Department of Environment and Conservation (DEC). Woylies were examined by field workers, which included assessment of the health of the skin and pelage. Lesions were noted on a standardized health assessment form and data entered into a Microsoft® Access 2003 database. Data from two transects (Keninup and Warrup) was used in a cross-sectional study to investigate for differences in prevalence of skin disease between the populations of woylies from these transects and to assess for changes in prevalence over time. These two transects were chosen as representative of a high density population that underwent decline (Keninup) and a moderate density population that is considered stable (Warrup). Subjects were selected on the basis of transect (Keninup vs Warrup) and trapping period (Oct/Nov 06 vs Nov 08 – pre-decline and post-decline for Keninup based on population monitoring data). Data was transformed to indicate presence/absence of skin disease. Prevalence of skin disease was then determined for a given transect in a given year and comparisons made using Chi-squared tests in Microsoft® Excel 2003. Statistical significance was determined for p-values$\leq$0.05. Overall prevalences for the total database, all animals sampled in a given year and all animals sampled for a given transect were not established due to these being open populations sampled over a period of 2 years.

A subset of the above raw data was developed focusing on woylies that presented with evidence of skin disease. This data was analysed after transformation to assess for a pattern of distribution of skin lesions over the woylie. The body of the woylie was divided into a number of descriptive areas and a score of 1 given to a lesion being recorded in a particular area for a particular woylie. The results were then totalled for each body area and proportions calculated for numbers of animals with lesions in that area.

Field Health Assessment

During one monitoring session in February 2009, woylies from the Keninup area with evidence of skin disease were identified for further investigation. This involved general anaesthesia using

Isoflurane in oxygen, delivered via face mask. Once anaesthetized, each woylie underwent veterinary examination, including mapping of skin lesions on a standard diagram (Fig 1) to assess distribution. Photographs of typical lesions were taken where possible, and presence of ectoparasites was noted, identifying the type of parasite observed. Fungal culture samples were collected using a new, clean toothbrush, to brush the skin and coat, focusing on areas of lesions, around the inguinum and the axillae. Skin scrapes were collected from the margins of lesions using liquid paraffin and a fresh scalpel blade, and samples were examined under a microscope using 4x and 10x objectives. Skin biopsies of 6mm diameter were collected from lesions as well as one biopsy of normal skin from each individual. One biopsy was sectioned in half, with half being placed in sterile gauze soaked with sterile saline, to submit for bacterial culture, and the other half placed in an Eppendorf tube for freezing at -80ºC. Remaining biopsies were placed in 10% buffered neutral formalin for histopathology, which was performed by Phil Nicholls (Murdoch University). Biopsy site collection was standardized across woylies where possible.

Results

Retrospective data analysis

The prevalence of skin disease for given transects in a given year are presented in Table 1. Subjectively there appears to be a difference in prevalence between Keninup and Warrup as well as a change in prevalence over time for Keninup. Chi-squared tests comparing transects at a given time revealed statistically significant differences in prevalence between Keninup and Warrup for both October/November 2006 and November 2008 ($p<0.001$ and $p=0.029$ respectively). Chi-squared tests comparing changes in prevalence over time for a given transect also revealed statistically significant differences for Keninup between 2006 and 2008 ($p=0.001$), however this was not the case for Warrup ($p=0.2$).

	Keninup 2006	**Keninup 2008**	**Warrup 2006**	**Warrup 2008**
Skin disease absent (# cases)	63	22	14	10
Skin disease present (# cases)	21	25	23	31
TOTAL (# cases)	84	47	37	41
Prevalence % (skin disease absent)	75	47	38	24
Prevalence % (skin disease present)	**25**	**53**	**62**	**76**

Table 1. Prevalence of skin disease for a given transect in a given sampling period

Data analysis to assess for distribution of skin lesions in woylies is presented in Table 2. A number of animals presented with skin lesions in more than one location. This data suggests the observed skin lesions are noted particularly around the head, eyes and tail.

[redacted]	Head	Eye	Ear	Mouth	Nose	Throat	Forelimb	Chest	Abdomen	Back	Hindlimb	Rump	Tail	number of affected woylies
Keninup 2006	0	1	2	0	0	1	0	0	0	2	0	2	13	18
Keninup 2008	6	10	3	0	0	5	0	1	0	2	0	0	14	24
Warrup 2006	4	1	7	0	0	3	0	1	1	2	2	7	7	23
Warrup 2008	16	19	7	0	0	0	1	1	0	0	0	3	10	31
TOTAL	26	31	19	0	0	9	1	3	1	6	2	12	44	96
Proportion	**27**	**32**	**20**	**0**	**0**	**9**	**1**	**3**	**1**	**6**	**2**	**13**	**46**	[redacted]

Table 2. Distribution of skin lesions in affected woylies from Keninup and Warrup

Field Health Assessment

A total of nine woylies were examined for the field health assessment component of this study, with eight of these animals undergoing anaesthesia for sample collection and one animal (WC0878) examined under manual restraint (Table 3). Eight woylies showed evidence of skin lesions; one woylie was sampled as a reference for a 'healthy' woylie (WC 0879). Table 4 shows results of ectoparasite observations from examined woylies.

Sample Number	Woylie ID	Date	Skin exam + map	Photos	Skin scrape	Fungal culture	Skin Biopsy culture	Skin Biopsy Eppendorf	Skin biopsy Histo
WC 0729	DO8083/DN0853	3/02/2009	Y	-	pos	Y	Tail base	Tail base	R hip
WC 0730	DO8271/DO8272	4/02/2009	Y	-	neg	Y	Tail base	Tail base	L hip, Tail base
WC 0873	DO7974/DO7975	4/02/2009	Y	x8	pos	Y	Tail base	Tail base	L hip, R periocular, tail base
WC 0875	DO6669/DO6668	4/02/2009	Y	-	neg	Y	Tail base	Tail base	R hip, L periocular, tail base
WC 0876	DO1493/DO1494	4/02/2009	Y	-	neg	Y	Tail base	Tail base	R hip, tail base
WC 0735	DO3757/DO3756	5/02/2009	Y	-	neg	Y	Tail base	Tail base	L hip, tail base
WC 0736	DN0856/DN0857	5/02/2009	Y	y	neg	Y	N	N	R hip, tail base
WC 0878	DN0801/DN0802	5/02/2009	Y		neg	N	N	N	N
WC 0879	DO7985/DO7986	5/02/2009	Y	-	-	Y	Tail base	Tail base	R hip, tail base

Table 3. Summary of sampling of field health assessments of woylies, February 2009.

Woylie ID	Ectoparasite presence			
	Ticks	Fleas	Lice	Mites
DO8083/DN0853	X	X		
DO8271/DO8272				X
DO7974/DO7975	X	X	X	X
DO6669/DO6668	X		X	X
DO1493/DO1494				
DO3757/DO3756	X		X	
DN0856/DN0857	X		X	
DN0801/DN0802				
DO7985/DO7986	X		X	X

Table 4. Ectoparasite observations from field health assessments of woylies, February 2009

Lesion distribution typically involved the periocular skin and dorsal tail base (Table 5). Periocular lesions involved hair loss with minimal changes to the underlying skin; one woylie showed evidence of thickening of the skin at the medial canthus of both eyes. Lesions of the dorsal tail base typically showed evidence of fur loss, with thickening of the skin and varying degrees of scale formation. The extent of the lesions was variable – most were contained within the proximal quarter of the tail; one lesion extended approximately half way down the dorsal surface of the tail. Figures 2 and 3 show some typical lesions observed during field examinations.

Sample Number	Woylie ID	Date	Skin lesion distribution						
			R periocular	L periocular	R hip	L hip	Dorsal Tail base	Ventral neck	L stifle
WC 0729	DO8083/DN0853	3/02/2009	1	1	1	1	0	1	0
WC 0730	DO8271/DO8272	4/02/2009	1	1	0	0	1	0	0
WC 0873	DO7974/DO7975	4/02/2009	1	1	0	0	1	0	1
WC 0875	DO6669/DO6668	4/02/2009	1	1	0	0	1	0	0
WC 0876	DO1493/DO1494	4/02/2009	1	1	1	0	1	0	0
WC 0735	DO3757/DO3756	5/02/2009	1	1	0	0	1	0	0
WC 0736	DN0856/DN0857	5/02/2009	1	1	0	0	1	0	0
WC 0878	DN0801/DN0802	5/02/2009	0	0	0	0	1	0	0
WC 0879	DO7985/DO7986	5/02/2009	0	0	0	0	0	0	0
TOTAL			7	7	2	1	7	1	1

Table 5. Distribution of skin lesions (1 = present; 0 = absent); only affected areas are presented in the above table.

Two out of eight animals returned positive results for mite infections on skin scrapes. One woylie (WC 0729) was found to have a fur mite; another woylie (WC 0873) was found to have Chiggers mite infestation (Figure 4). No evidence of demodectic or sarcoptid mites were found in these woylies.

Bacterial culture and identification was undertaken for three of the affected woylies. Samples were selected from woylies with typical skin lesions. Culture results identified a range of bacteria, including alpha-haemolytic Streptococcus spp, Coryneform bacilli, Baccillus cereus, and Pasteurella spp.

Fungal culture was undertaken for the same three animals as above. Culture results identified evidence of *Microsporum gypseum* infection in one woylie; the other two woylies isolated mixed environmental fungi only.

Histopathology examination was undertaken for all animals that were biopsied. These revealed varying degrees of hyperkeratosis and acanthosis (thickening of the epithelium), some with mixed inflammatory infiltrates. Period Acid-Schifft staining did not reveal any evidence of yeasts or fungal hyphae. There was a lack of neutrophilic inflammation, suggesting bacterial infection is unlikely as a significant factor in these skin lesions. Neoplastic changes were not evident, however one woylie showed a focal area of excess collagen stroma – possibly recent scar tissue formation. There was no evidence of mites seen in any samples examined. Skin glands and hair follicles were all normal on histopathology, suggesting hair loss is secondary to rubbing or self trauma.

Discussion

Skin disease has been a factor in poor health and population declines of a range of wild terrestrial mammals. Well described examples include Sarcoptic mange in common wombats (*Vombatus ursinus*) in Australia (Skerratt et al 1998) and Parapoxvirus infection of red squirrels (*Sciurus vulgaris*) in the UK (Tompkins et al 2002). In both of these scenarios, the aetiological agent was identified in response to observation of skin lesions through pathology of affected cases fitting a particular case description, and then assessed to determine involvement in population declines. This study has taken a somewhat different approach, looking first at evidence of skin disease being a factor (causative or result) in woylie declines, then assessing particular individuals to determine the nature of the skin disease.

Statistical analysis of survey data indicates a significant difference in the prevalence of skin disease between woylies from Keninup and Warrup, as well as indicating a significant increase in the prevalence of skin disease for Keninup woylies from Oct/Nov 2006 to November 2008. Prior anecdotal reports have suggested a high prevalence of skin disease in woylies in the lead up to a decline; however these results do not support this. Skin disease appears more prevalent in the Warrup populations during both survey periods. Given this is considered a moderately stable population, it suggests that there can be a high prevalence of skin disease without evidence of population decline. An increase in the prevalence of skin disease at Keninup from 2006 to 2008 also suggests skin disease is not likely to be associated with causal factors for population declines, and is perhaps a symptom/result of these causal factors.

Distribution of skin lesions appears to typically affect the head region (including around the eyes and ears) as well as the tail base/rump region (Figures 2 and 3). This is supported with the results from field health assessment data. Although not typical for a particular aetiology, this information assists in developing a case definition for woylies seen with skin disease.

Ectoparasite infections were very common in woylies examined in the field, with 7 of 9 woylies infected with one or more types of ectoparasite. The most common parasites found were ticks and lice, with mites also being regularly found. Skin scrapes from one woylie were positive for a Chiggers-type mite infection (Figure 4). There is a large range of different species of Chigger's mites that have been identified from Australian marsupial hosts (Domrow and Lester, 1985), which can cause skin irritation in some species. Identification of the species of mite was not able to be performed due to deterioration of the sample. The significance of ectoparasite infections as a primary cause of disease in Australian marsupials is variable. Many marsupials may be infected with ectoparasites without overt clinical signs, however some ectoparasites, such as *Sarcoptes* spp of mites, can cause severe debilitation (Ladds 2009; Skerratt et al 1998). The authors were unable to find any reports in the literature of particular ectoparasites causing skin disease in woylies, however identification of ectoparasites from wild woylies of the Upper Warren region is being undertaken as part of a PhD project by Halina Burmeij.

Histopathology and culture results revealed a consistent pattern of thickening and hyperkeratosis of the skin, with mild to moderate inflammatory changes. There appears to be little to no involvement of the glands or hair follicles in the skin, suggesting hair loss is likely to be secondary to irritation and self trauma. A lack of neutrophilic inflammation, and culture of a range of bacterial isolates from different individuals, suggests primary bacterial infection is not a likely cause of skin disease in these woylies. *Microsporum gypseum* was isolated from one affected animal – this organism is a common saprophyte, and although capable of causing disease in its own right (Carter 1986), is likely to be a secondary invader in this particular case. These findings are non-specific and do not identify a particular aetiology for this condition, however would be consistent with hypersensitivity, self trauma or ectoparasite infections, the latter of which is supported by examination findings and skin scrape results. There does not appear to be any subjective correlation between the severity of skin changes on histopathology and the distribution of lesions, or the severity of ectoparasite infection, however the latter was not objectively assessed for these cases.

The term 'skin disease' encompasses a large array of aetiological agents and presenting signs, and it is beyond the scope of this investigation to examine all potential agents. The sample size included in this study, particularly in relation to the field health assessments, is also somewhat limited and does not accurately reflect the varying degrees of skin lesions anecdotally reported in wild woylies. However the results from this study help to define a case definition for this particular presentation of skin disease in these animals, and presents some of the potential aetiologies that may cause these lesions. Other agents that have been identified as causing skin lesions in woylies include a poxvirus, however the presentation of lesions associated with this infection are clinically quite different to the presentations described in this study, and have only been described in two cases to date.

Conclusion

Although skin disease has been anecdotally reported in high levels in the lead up to a population decline for woylies, data from this study does not support this, instead suggesting skin disease may occur at a high prevalence without signs of a population decline, and that prevalence of skin disease may change as a result of population decline. The nature and distribution of the skin lesions investigated in this study is non-specific, and may be the result of a range of causative agents, including ectoparasites. Affected woylies that underwent field examination tended to be infected with at least one type of ectoparasite, and it is likely that ectoparasites are involved with the presence of these skin lesions in wild woylies.

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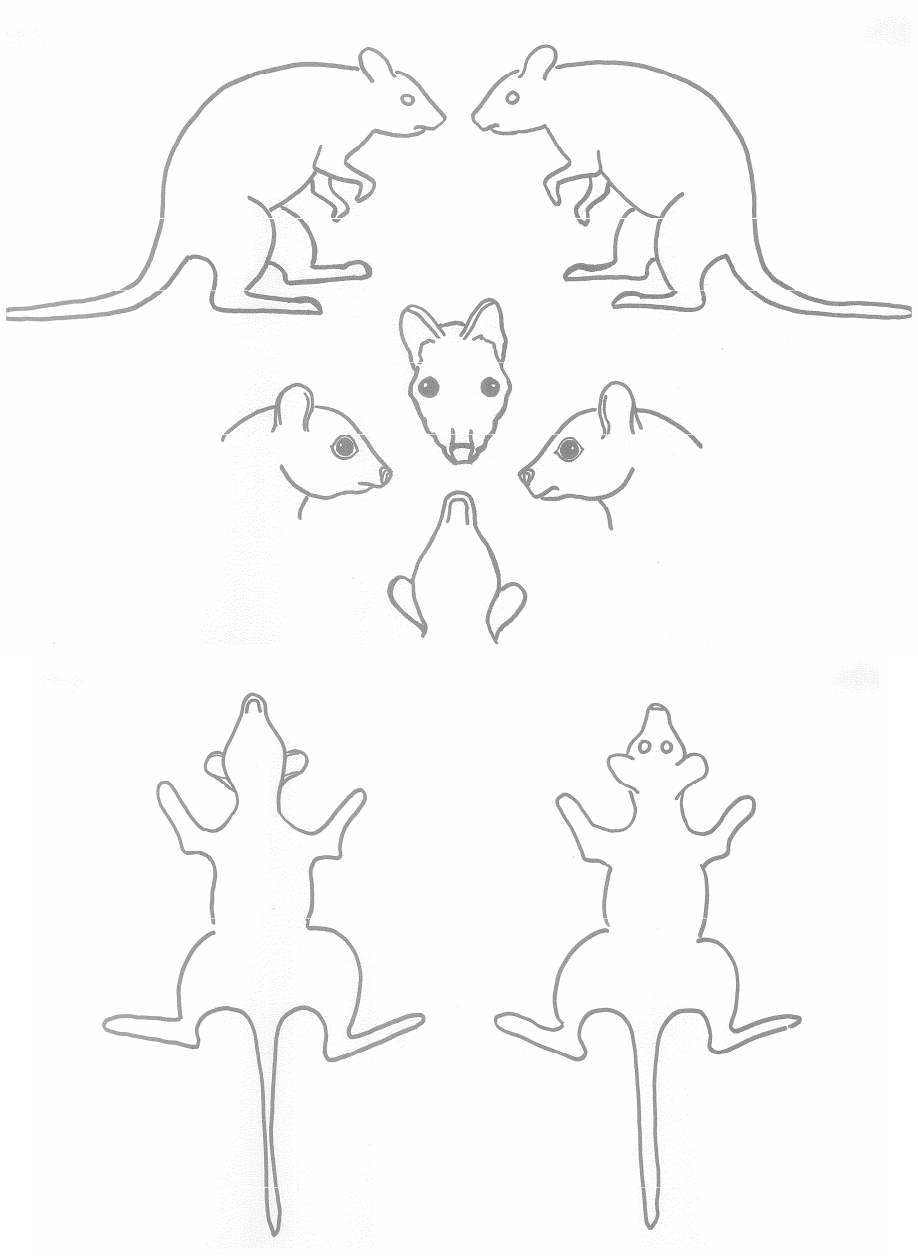

Figure 1. Standard diagram for mapping distribution of skin lesions in Woylies.

Figure 2. Tail lesion during field examination of a woylie, showing fur loss and hyperkeratosis (thickening of the skin).

Figure 3. This woylie presented with periocular fur loss and crusting, typical of anecdotal reports of skin disease observed in woylies in the lead up to a decline. Also note the crusting and ectoparasite infection of the pinna.

Figure 4. A Chiggers mite found on skin scrape from a woylie with skin disease.