5.10. Bacteriology

Yazid Abdad[1], Peter Adams[1], L. Pallant[1], Adrian Wayne[2], Halina Burmej[1], Stan Fenwick[1]

[1]Murdoch University
[2]Department of Environment and Conservation

Abstract

Faecal and ectoparasite samples from woylies and other co-habiting native species were analysed for the presence of the potential bacterial pathogens *Salmonella* and *Rickettsia.* This work constitutes a component of the disease investigations within the Woylie Conservation Research Project (WCRP) - diagnosing recent declines in woylie populations in southwestern Australia. The significance of the bacterial isolates will be discussed in relation to declining woylie populations and zoonotic infections.

5.10.1. Introduction

After consideration by the Woylie Disease Reference Council (WDRC), and without any evidence to support the possibility of bacterial infections as the cause of the woylie decline, a decision was made to concentrate on two relatively non-invasive sample types to investigate the carriage of potential bacterial pathogens by woylies. The decision was influenced by samples already being collected as part of the current research, which included (i) faecal samples and (ii) ectoparasite samples. In particular, the enteric samples were to be analysed for the presence of *Salmonella*, which are potentially pathogenic bacteria responsible for disease in a wide range of domestic and wild animals. The ectoparasite samples (principally ticks) were to be analysed for the presence of members of the genus *Rickettsia*, which although not generally believed to be pathogenic for wildlife, are nonetheless zoonotic, i.e. they can potentially infect humans and which might under certain circumstances cause disease in other species.

5.10.2. Results

5.10.2.1. *Rickettsia*

Trapped woylies and other co-habiting species were examined carefully for ectoparasites, which were removed using forceps and/or flea combs and placed alive in sealed, plain vials. These were subsequently identified using taxonomic keys.

A total of 23 tick samples were screened for *Rickettsia* DNA via PCR, resulting in four positives in one tick species, and two other tick species coming up negative. 4/16 *Amblyomma triguttatum* were positive for a spotted fever *Rickettsia*, most likely candidatus "*Rickettsia gravesii*" which has been found in a number of animal species in the region. Six *Ixodes australiensis* and one *Ixodes fecialis* were also screened but were negative (Table 5.10.1).

Rickettsia screening summary

Prevalence in *A. triguttatum*

Total number screened = 16

Total positives = 4

Prevalence = 25%

Rickettsia species = Candidatus "*Rickettsia gravesii*" – to be confirmed

Samples tested were from woylie, koomal (common brushtail possum, *Trichosurus vulpecula*) and human

Woylie Samples = 16
- 2/11 *A. triguttatum* (~20%)
- 0/5 *I. australiensis*

Koomal Samples = 6

1/4 *A. triguttatum* (25%)
0/1 *I. australiensis*
0/1 *I. fecialis*

Human Samples = 1

- 1/1 *A. triguttatum* (100%)

Table 5.10.1. Distribution of *Rickettsia gravesii* in samples screened for the Woylie Conservation Research Project.

Location	Host Species	Number (+ve / # screened)	Tick Species
Warrup	Woylie	0/5	*I. australiensis*
	Koomal	0/1	
Winnejup	Koomal	0/1	*I. fecialis*
Corbal	Human	1/1	*A. triguttatum*
	Koomal	1/4	
	Woylie	1/8	
Dryandra	Woylie	1/3	*A. triguttatum*

Of interest is that a novel species of tick, belonging to the genus *Ixodes*, has been observed from the woylies, further work is being done to characterise it.

5.10.2.2. Salmonella

Fresh faeces or faecal swabs were collected from woylies and any other species trapped within the Upper Warren in March-April 2006. *Salmonella* tests were conducted under contract by Pathwest. A total of 233 faecal samples were screened for *Salmonella*, resulting in 19 positives from four animal species, namely woylies (5/137; Table 5.10.2), koomal (2/74; Table 5.10.3), chuditch (10/15; Table 5.10.4) and quenda (2/7; Table 5.10.5). The 19 positive samples consisted of 15 different serovars, the most numerous of which were *Salmonella* Bootle and *S*. O group 1 (Table 5.10.6).

Salmonella screening summary:

Total samples tested = 233
Total samples positive = 19
Total prevalence in medium-sized mammals = 8.15%

Woylie (positive/total sampled) = 5/137 (3.65%) 1 woylie had dual infection with 2 serovars
Koomal (positive/total sampled) = 2/74 (2.7%)
Chuditch (positive/total sampled) = 10/15 (66.7%) 1 chuditch had dual infection
Quenda (positive/total sampled) = 2/7 (28.6%) 1 quenda had dual infection

Table 5.10.2. Woylie faecal samples tested for *Salmonella* at Upper Warren forest blocks.

Location	# tested	# positives	Salmonella ID
Winnejup	10	0	
Warrup	27	1	*S.* Choleraesuis var Australia
Boyicup	4	0	
Chariup	4	0	
Balban	45	2	*S.* Bootle (47:k:1,5) / *S.* Eastbourne (9,12:eh:1,5) / *S.* Charity
Corbal	26	1	*S.* Lindern
Keninup	16	1	*S.* Infantis
Yendicup	5	0	

Table 5.10.3. Koomal faecal samples tested for *Salmonella* at Upper Warren forest blocks.

Location	# tested	# positives	Salmonella ID
Winnejup	3	0	
Warrup	6	0	
Boyicup	5	0	
Camelar	5	0	
Chariup	11	1	*S.* Sachsenwald
Balban	9	0	
Corbal	10	0	
Keninup	6	0	
Moopinup	9	1	*S.* Chester
Yackelup	6	0	
Yendicup	4	0	

Table 5.10.4. Chuditch faecal samples tested for *Salmonella* at Upper Warren forest blocks.

Location	# tested	# positives	Salmonella ID
Winnejup	1	1	*S.* Wandsbek
Warrup	2	0	
Balban	2	1	*S.* Bootle (47:k:1,5), *S.* Orion (3,10:y:1,5)
Corbal	2	0	
Keninup	7	7	*S.* Muenchen (2), *S.* Bootle, *S.* Arizona ss III, *S.* Houten. *Salmonella* O group C (6,7:c:-), *Salmonella* Orion
Moopinup	1	1	*S.* Houten (43:z4,z23:-)

Table 5.10.5. Quenda faecal samples tested for *Salmonella* at Upper Warren forest blocks.

Location	# tested	# positives	Salmonella ID
Boyicup	3		0
Camelar	3	2	*S.* Birkenhead (6,7:c:1,6), *S.* O group I (16:-:-) (both)
Moopinup	1	0	

Table 5.10.6. Distribution of *Salmonella* serovars in the Upper Warren.

Location	Number	Serovar	Host Species
Keninup	1	*S.* Arizona sub species III	Chuditch
Camelar	1	*S.* Birkenhead	Quenda*
Balban, Keninup	3	*S.* Bootle	Chuditch (2)*, Woylie*
Balban	1	*S.* Charity	Woylie
Moopinup	1	*S.* Chester	Koomal
Warrup	1	*S.* Choleraesuis	Woylie
Balban	1	*S.* Eastbourne	Woylie*
Keninup, Moopinup	2	*S.* Houten	Chuditch
Keninup	1	*S.* Infantis	Woylie
Corbal	1	*S.* Lindern	Woylie
Keninup	2	*S.* Muenchen	Chuditch
Balban, Keninup	2	*S.* Orion	Chuditch*
Chariup	1	*S.* Sachsenwald	Koomal
Winnejup	1	*S.* Wandsbek	Chuditch
Keninup, Camelar	3	*Salmonella* O group (1 and C)	Chuditch, Quenda (2)*

*co-infection with other *Salmonella* serovar

5.10.2.3. Interpretation of results

Although only four isolates of *Rickettsia* were identified from 23 ticks analysed, it is highly likely that many of the other ticks were infected as high prevalences have been found in ticks from other animal species, e.g. *Antechinus* and feral pigs, in similar habitats (Owen *et al.*, 2006). The *Rickettsia* belonged to the Spotted Fever Group of the genus, which contains many human pathogens, including at least two in Australia that cause 'Australian spotted fever' and 'Flinders Island spotted fever'. While this *Rickettsia* was not identified to the species level, recent work by the Veterinary Public Health group at Murdoch University has identified a hitherto unknown species of SFG *Rickettsia* in a wide range of native animals, tentatively named *R. gravesii*. This may be the species found in the tick from the Upper Warren although this is yet to be confirmed by DNA sequencing. Of interest is that another novel SFG species, tentatively named *'R. antechini'* was identified from ticks off *Antechinus* in Dwellingup (Owen, PhD thesis, 2007), thus a possibility exists that the SFG *Rickettsia* from the woylies is also a novel species.

With respect to *Salmonella*, the most interesting fact was the wide variety of serovars recovered, possibly reflecting the different parts of the ecosystem that the four species of animals inhabit and their dietary habits. For example, koomal, which are largely arboreal, had the lowest number of *Salmonella* strains (and prevalence) recovered, followed by woylies (which have a narrow dietary range), then quenda which are more omnivorous and then the carnivorous chuditch. The high prevalence of *Salmonella* in chuditch is speculated to reflect their dietary habits in which a range of birds, small mammals, reptiles and carrion might play a part. The large variety of serovars also reflects the widespread nature of this organism in a forest ecosystem, with possible sources of infection being faecal-contaminated food and water supplies. Although kangaroos were not sampled in these areas, a similar wide range of serovars has been found in these animals in studies carried out in other ecosystems, including southwestern Australia (unpublished data). A number of the serovars are found in human infections, with humans possibly contributing to the environmental contamination either directly or indirectly via water.

5.10.2.4. Involvement of *Rickettsia* and Salmonella in woylie declines

The possible involvement of *Rickettsia* in the woylie decline is remote, as despite being commonly associated with ticks from wildlife, there has been no evidence of these organisms affecting native animals in other habitats or ecosystems worldwide. Nevertheless, occasional infections have been documented in domestic dogs (Solano-Gallego *et al.*, 2006) and information on their possible effect on animals under severe stress is unknown.

Similarly, although *Salmonella* have also been shown to be commensals in the intestines of a wide range of native animals in Australia (Bensink *et al.*, 1991; Hart *et al.*, 1985; Thomas *et al.*, 2001), little evidence is available to support their role as pathogens and their involvement with the woylie decline is also believed to be remote, particularly as the *Salmonella* obtained were from samples collected from trapped healthy animals. This data shows a prevalence of *Salmonella* in the marsupials, however gives us no evidence of any effects they may have on their hosts' well-being.

Salmonellosis has previously been documented as both the primary cause of death (Atkinson, 1964; Mushin and Ashburner, 1964), and as a secondary invader (Winter and O'Connor, 1957) in macropods that died with enteritis from zoos or private collections. However, whilst all *Salmonella* species are capable of causing disease, they are more commonly found in the intestinal tracts of healthy, rather than sick or diseased animals (Samuel, 1983). Additionally, once introduced to a group of macropods, *Salmonella* is capable of persisting for long periods of time, but whether they are continually cycled through the population, or if particular individuals remain carriers for a long time is not known (Samuel, 1983).

Nevertheless, of the two organisms, *Salmonella* is well recognised as an important pathogen of many domestic animal species and people, particularly in animal populations under stress (dietary, climatic, etc.) and the role of the organism as a woylie pathogen should not be discounted entirely. However, a relatively high prevalence of *Salmonella* within a population makes it difficult to judge the significance of their isolation from sick or dead animals. Evidence to support the role of *Salmonella* as a pathogen however would most likely come from its isolation from the internal organs of sick or recently dead animals and the absence of other likely causes of disease.

5.10.3. Recommendations and future research

While there is probably no reason to further research these organisms for their involvement in the woylie decline, their role as pathogens for occupational and recreational groups active in native forests would support further work into their ecology and epidemiology. As the Murdoch *Rickettsia* research group is actively investigating the diversity and importance of *Rickettsia* in WA it would be appreciated if collection of ectoparasites from trapped native animals was continued, as this is a simple, non-invasive activity, which will add considerably to our understanding of this fascinating group of organisms.

While only members of the genus *Rickettsia* were targeted in the ticks, it would be of interest to investigate the presence of other potential pathogens in ectoparasites, for example *Bartonella* spp in fleas, and this may be incorporated into a PhD project started in 2007. In addition to the novel *Rickettsia* identified from Antechinus ticks, Owen (PhD thesis, 2007) also identified a novel *Bartonella* species from fleas off the same animals. Their involvement in any disease process however was not investigated. *Bartonella* species are known to cause disease occasionally in dogs and people so further investigation of these organisms in fleas from woylies may be justified.

Although it would also be of immense value to further investigate *Salmonella* in native animals from a scientific and ecological perspective, at this stage the costs involved in collection and analysis of samples prohibits this activity. However, any faecal samples sent for analysis for bacterial pathogens in the future should include screening for *Salmonella*, and all isolates recovered should be stored for further characterisation.

There are a whole host of bacterial pathogens that exist in the native Australian fauna some of which have no doubt been around since before the arrival of settlers and others which were probably introduced as a result of the introduction of non-native animals into the wild. Most of the bacterial flora, however, do not pose a significant threat other than to the individual animal as a secondary infection. However, there are a few that would be worth considering in future investigations, one is “lumpy jaw” or necrobacillosis, caused by the bacteria *Fusobacterium necrophorum* and possibly *Actinomyces* spp. and *Bacteroides* spp. in the absence of *F. necrophorum*. Lumpy jaw is usually regarded as a wound infection and is associated with the presence of a specific bacteria in large numbers on the ground or pasture. Other associated bacterial infections include *Mycobacterium* and *Clostridium tetani* (cause of tetanus), the former only recorded in captive macropidae and the latter reported sporadically in northern Australia (Speare *et al.,* 1989).

5.10.4. References

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See Note (at end of this document) “General subjects and structure for subsections reporting on results”