5.8. Endoparasites

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Abstract

Very little research has been published regarding the prevalence and the effects of gastrointestinal parasites on native wildlife in southwestern Australia. Faecal samples from woylie populations in the Upper Warren and Karakamia were screened using microscopy for the presence of gastro-intestinal parasites. Information regarding general condition was also collected. In the Upper Warren, over 80% of all animals sampled over a ten year period were infected with at least one parasite species, and there was little difference over time in either parasite prevalence or parasite diversity. For faecal samples collected from the Upper Warren and Karakamia populations in 2006, prevalence of parasitic infection was significantly greater in Upper Warren (100%) than in Karakamia (92%), due principally to a greater prevalence of nematode larvae in the faecal samples. Preliminary data found no significant correlation between the presence of gastro-intestinal parasites and the general condition of the woylies, although the analyses were hampered by a lack of resolving power in discriminating among parasite species.

5.8.1. Introduction

There have been very few studies conducted focusing on gastro-intestinal parasitism amongst Australian wildlife in southwestern Australia. The only study of enteric parasites in native wildlife in southwestern Australia was by Adams (2003). In this study, a number of different species of native wildlife and feral cats were screened for gastro-intestinal parasites in southwestern Australia. A high prevalence of gastro-intestinal parasites was found in Australian native wildlife. Seventy two percent of animals were infected with at least one parasitic species. A number of different parasitic species were detected in these animals. The most common nematode species found were strongyle nematodes and *Strongyloides*. Many protozoan species were also detected, including *Entamoeba*, *Giardia* and unidentified coccidia species (Adams, 2003). Recently, *Blastocystis* was also detected in koomals (Parkar *et al.,* 2007).

The Woylie Conservation Research Project (WCRP) and the Australian Research Council (ARC) linkage project with DEC, provided an excellent opportunity to conduct a thorough study focusing on gastrointestinal parasites in various native species and populations in Western Australia. It would also shed light on whether infectious disease has any impact on the decline of the woylie population in Western Australia, and if so, what is the impact of gastro-intestinal parasitism and parasitic co-infections on these animals.

5.8.2. Methods

5.8.2.1. Samples

Two sets of data were available:

(1) A historical data set, consisting of records from faecal analyses of woylies from the Upper Warren, collected in 1998 (n = 63), 1999 (n=45), 2005 (n=11) and 2006 (n=51).

(2) A contemporary data set, consisting of 51 faecal samples collected from woylies in the Upper Warren in March 2006, and 60 faecal collected from woylies in Karakamia in July 2006.

5.8.2.2. Faecal analysis

All samples were fixed in 10% formalin for microscopy analysis, and 70% ethanol for analysis using molecular tools. For microscopy, all samples were concentrated using zinc-flotation and examined using light microscopy at a magnification of x100 for the presence of parasitic ova, larvae or trophozoites. Parasites were identified to the lowest possible taxonomic category.

5.8.2.3. Data analysis

Each individual woylie sample was recorded as being positive or negative for any parasitic infection, and for infection with each taxonomic category of parasites. Populations of woylies were compared for prevalence (percentage of infected hosts) and for diversity of parasite taxa, as measured by Margalef's index (d) or the Shannon-Weiner diversity index (H). For the contemporary samples from the Upper Warren and Karakamia we obtained information on sex, age (adult or subadult), weight and pes length for each woylie from the DEC database. Condition score for each woylie was calculated as a residual from the regression of log weight on log long pes length.

Differences in parameter estimates among groups were compared by Chi-square or Fisher exact tests for categorical data and by analysis of variance for continuous data. All data were checked for normality and transformed, if necessary, prior to analysis. Where multiple tests of the same hypothesis were made, a Bonferroni correction was used to obtain an experiment-wide error rate of 0.05.

5.8.3. Results

5.8.3.1. Historical data

There was a relatively stable endoparasite fauna in woylies sampled from the Upper Warren between 1996 and 2006. Overall prevalence of parasitic infection (i.e. infection with any parasite taxon) was greater than 80% in all years (Figure 5.8.1(a)) and there was little change in parasite taxon diversity over time (Figure 5.8.1(b)).

The most common parasites over all years were eggs of strongyle nematodes, with nematode larvae also seen relatively frequently, especially in 2006 (Figure 5.8.2).

Figure 5.8.1(a)

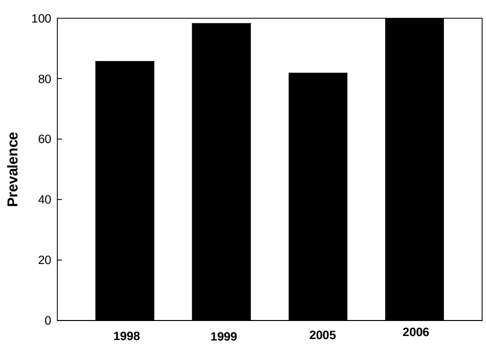

Figure 5.8.1(b)

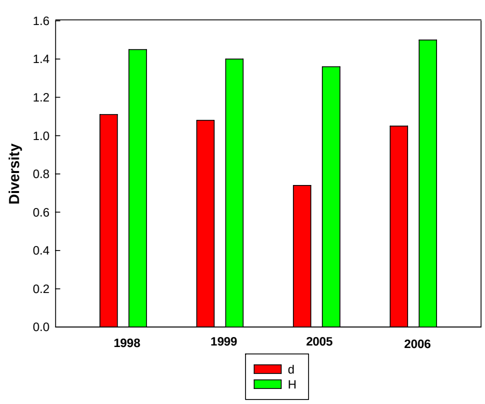

Figure 5.8.1. Prevalence of parasitic infection (a) and parasite taxon diversity in woylies from the Upper Warren over a ten year period.

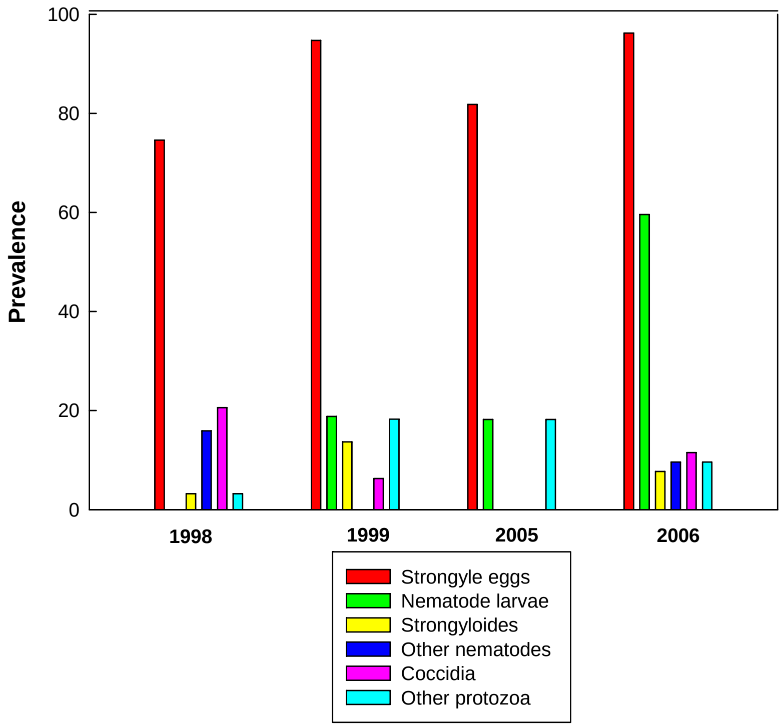

Figure 5.8.2. Prevalence of different parasite taxa in woylies from the Upper Warren over a ten year period.

5.8.3.2. Contemporary data

Ninety six percent of all woylies sample from the Upper Warren and Karakamia in 2006 were infected with at least one parasite taxon. In total, nine different taxa of gastrointestinal parasites were detected:

1. Strongyle eggs
2. Strongyloides eggs
3. Nematode larvae
4. Lungworm larvae
5. Echinonema eggs
6. Anoplocephalid eggs
7. Arthropod eggs
8. Coccidia spp
9. Entamoeba sp

Strongyle eggs were again the most prevalent taxon of parasites, with 92% of all woylies infected. There were no significant effects of either sex or age of woylies on the prevalence of any parasite taxon.

All woylies from Upper Warren were infected with at least one parasite taxon, compared with 92% from Karakamia (significant difference in prevalence, Fisher exact test, $P < 0.05$). When each parasite taxon was compared separately, both nematode larvae (60% prevalence in Upper Warren, 28% in Karakamia; Fisher exact test, $P < 0.05$ with the Bonferroni correction) and arthropod eggs (23% prevalence in Upper Warren, 0% in Karakamia; Fisher exact test, $P < 0.05$ with the Bonferroni correction) were significantly more prevalent in the Upper Warren population.

Although woylies from Upper Warren were in better condition (0.03 ± 0.02) than woylies from Karakamia (-0.03 ± 0.02), this was most likely due to the significantly smaller weights of Karakamia woylies, relative to size – a likely response to limited food resources and higher fauna densities at Karakamia (Section 4.2 Demographics). A two factor analysis of variance showed that condition was significantly affected by age (adults 0.02 ± 0.01, subadults -0.11 ± 0.04; $F = 4.31$, $P < 0.05$), but not by location or the interaction of age and location.

There were no significant effects of parasite infection (either overall or for each parasite taxon separately) on the condition of woylies from either Upper Warren or Karakamia.

5.8.4. Discussion

As expected, most woylies are infected with gastrointestinal parasites, particularly with strongyle nematodes, although a range of other nematode, cestode and protozoan parasite taxa were also found. Both the prevalence of parasite infection and the composition of the parasite fauna in the Upper Warren woylie population have changed little over the last ten years.

Woylies from the Upper Warren are infected more frequently with gastrointestinal parasites than are woylies from Karakamia, but there is no evidence that parasitic infection is associated with the condition of animals in either location. At this stage, the role of gastrointestinal parasites in woylie declines is undetermined. Some of the parasites which have been found, particularly strongyle nematodes and coccodian protozoa, have the potential to produce severe parasitic disease. Theoretical and empirical studies in other systems have shown that parasitic disease can be an important regulator of host population dynamics, at the prevalence levels which were found in this study (Cornell, 2006; Hudson *et al.,* 1998). The greater prevalence of some parasites in the Upper Warren than in Karakamia is suggestive of a regulatory role. On the other hand, there is no indication that parasitic infection reduces woylie condition, at least as measured by weight and pes length. It is possible that the effects of parasitic infection are more subtle, acting at a physiological or behavioural level that increases the susceptibility of the hosts to other mortality factors, such as predation or competition. Such indirect effects of parasitism have been reported in a number of other species of free living animals (Marcogliese, 2004)

5.8.5. Future work

One major limitation of this study has been the inability to distinguish parasite species using microscopy. This necessitated the use of higher taxon levels for analysis, which has undoubtedly reduced our ability to resolve parasitic effects upon the animals. In particular, there are almost certainly a number of different strongyle nematode species, as well as a number of unidentified coccidia in the faecal samples, undoubtedly representing new species. In order to identify these organisms, molecular tools must be used. However, considering the large number of samples to be screened, a multiplex technique is necessary in order to screen these samples more efficiently. An example of such a method is pyrosequencing, as it has both a built-in quality control and allows for the detection of mixed genotypes. It is also convenient, as there is no need for post PCR-purification, (Unemo *et al.,* 2004). The development of a pyrosequencing method is currently underway as part of Unaiza Parkar's PhD research.

After thoroughly screening the samples using molecular tools, further correlations and statistical analyses may be made based on co-infections of gastro-intestinal parasites identified to the species level, clinical signs and general health. A large number of faecal samples remain available for further investigation pending the development of results from the existing subsamples that have been analysed. These will also be made more generally available for more detailed investigations by future researchers or students.

5.8.6. Conclusion

Gastrointestinal parasite infections were more prevalent in Upper Warren than in the Karakamia woylie population, but preliminary data analysis has found no evidence of a relationship between infection and woylie condition. Nevertheless, it is possible that the differences in parasitism between populations may have important indirect effects on host mortality, which should be further investigated.

5.8.7. Acknowledgements

We would like to thank the DEC and AWC field staff and volunteers that assisted in the collection of faecal samples from the field and the associated data management.

5.8.8. References

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