5.12. Disease synthesis

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Abstract

The Woylie Disease Reference Council (WDRC) has made a number of key contributions relating to diagnosing the decline in the woylie populations of southwestern Australia. These have included the identification of possible pathogenic organisms from woylies sampled from the Upper Warren and Karakamia Wildlife Sanctuary, the development of an extensive range of databases and reference materials, and furthering the knowledge of the health and diseases of this species as a whole. A summary of the disease investigations thus far is presented, and suggestions made as to what further investigations are recommended.

5.12.1. Introduction

The Woylie Disease Reference Council has made a number of valuable contributions and findings through investigations of diseases of potential concern in population declines of woylies in southwestern Australia. Results from individual and collaborative investigations have contributed to a clearer understanding of the health status of woylies, including the establishment of a disease risk assessment table, development of a haematological reference range, refinement of clinical skills in managing sick and injured woylies, and initial establishment of a pathologic reference library of disease processes found in woylies. A number of potentially pathogenic organisms have been identified in woylies sampled from the Upper Warren and Karakamia Wildlife Sanctuary, including haemoparasites (species of Trypanosomes and Piroplasms), *Toxoplasma*, ectoparasites and gastrointestinal protozoan and helminth parasites. The findings of investigations into these infectious agents will be summarised below in an attempt to pull this information together. We will also highlight where information is currently deficient, and provide recommendations for prioritising further investigations.

5.12.2. Summary of Disease Investigations

A number of potentially pathogenic organisms have been identified in woylies from the Upper Warren and Karakamia populations in Western Australia. From investigations undertaken to date, there is insufficient evidence to indicate any of these pathogens as a primary infectious agent responsible for these declines. This is largely due to the absence of evidence rather than there being evidence that does not support disease as a potential factor. Most animals (>90%) trapped in the field or submitted for necropsy were found to be in good body condition, suggesting an acute process is involved in the loss of animals. This is further supported by the rate of the decline that has been observed. Demographic and survival information suggests the declines are driven primarily by adult mortality rather than reduced fecundity, and therefore diseases affecting reproductive success appear less likely to be of concern in this situation. Findings from clinical and necropsy examinations have not identified a particular disease as a common cause of death to date. However, of the pathogens currently investigated, the evidence available is suggesting that *Toxoplasma* and *Trypanosoma* may have contributed, either directly or indirectly, to declines in the Upper Warren population. Other, yet to be identified stress factors and/or infectious agents have also been suggested for future investigation and will be prioritised in the discussion below.

Toxoplasma is a systemic protozoan parasite known to particularly affect Australian marsupials (Section 5.7 *Toxoplasma*). Investigations have shown a low seroprevalence to *Toxoplasma* infection

of 5.8% in Upper Warren populations compared to a zero prevalence in Karakamia and Dryandra. This is difficult to interpret in itself but may indicate the presence of subclinical or clinical disease in Upper Warren populations. Sequential sampling revealed a decrease in seroprevalence over time. There are various explanations for this, including the possibility that seropositive animals have died, perhaps directly or indirectly as a result of Toxoplasmosis, and further data analysis is recommended. It is possible that other pathogens (such as *Trypanosoma*) may predispose the woylie population in the Upper Warren to the pathogenic consequences of infection with *Toxoplasma*. The lack of pathological evidence of Toxoplasmosis in necropsy specimens, and the lack of knowledge of the disease processes associated with these parasitic infections in natural wild populations of marsupials, precludes any definitive conclusions at this stage. Further investigations should include examination of currently available data to assess for evidence of co-infection of these organisms and the potential subclinical effects, as well as to assess changes to serological titres within an individual over time. Evidence for the congenital transmission of *Toxoplasma* was also found, which may be significant if further research indicates that this mode of transmission is common and could serve to maintain *Toxoplasma* in wild populations without the involvement of the definitive host, the cat.

Trypanosomes are a protozoan blood parasite that can result in chronic illness with weight loss, anaemia and death. They are primarily spread via an arthropod vector. Investigations of Trypanosome infections in woylies indicated a relatively high prevalence of infection in the Upper Warren population, as compared to the Karakamia population. Further investigation, using molecular tools will be undertaken (discussed below). Investigations into the potential subclinical effects of this infection and demographic variations on prevalence using existing data are recommended.

Gastrointestinal parasite infections were more prevalent in Upper Warren than in the Karakamia woylie population, which is interesting given the higher density of woylies in Karakamia compared to Upper Warren. Preliminary data analysis has found no evidence of a relationship between infection and woylie condition. It is possible that the differences in parasitism between populations may have important indirect effects on host mortality. Further analysis of existing data to look at variations in prevalence on a demographic basis, and to investigate possible subclinical effects of infection, is recommended.

A variety of ectoparasites have been identified but their role in the decline is uncertain. Ectoparasites can, in significant numbers, be primary pathogens and result in symptoms such as anaemia. Perhaps more importantly, they can act as vectors for haemoparasites, for example Trypanosomes and *Rickettsia*. Identification of ectoparasite samples will continue, along with analysis of existing data to assess effects on fecundity and host survival. Investigations into the role of ectoparasites as vectors have also been proposed and are discussed below.

Evidence of infection with *Salmonella*, and the potential for woylies to be infected with *Rickettsia* has been found in woylies from the Upper Warren region; however the significance of these findings is likely to be low in relation to these population declines.

Thirty one woylies were submitted for necropsy. In 15 cases a cause of death could not be determined by gross or microscopic examination however specimens from cadavers were often limited due to predation/scavenging. A number of potential diseases, discussed in section 5.11, are yet to be investigated. A number of viruses have been reported from macropods in Australia, some potentially resulting in acute death (e.g. Macropod Herpesvirus). To date, investigation into viral disease in woylies has been limited and are discussed further below.

The role of toxic exposure in the population decline has not been investigated to date. Toxins found in native plants such as fluoroacetate (1080) and cardiac glycosides are possible agents, although the LD_{50} of fluoroacetate is significantly higher in woylies than for all domesticated mammals. Further investigation of the role of poisonous plants in the decline of the woylie could include examination of the gastro-intestinal contents to assess for exposure to toxic plants.

5.12.3. Future priorities

A range of areas for future research have been suggested from the previous discussions and relevant sections from Chapter 5. These will be discussed further below and recommendations made as to which are considered a priority.

Viruses

Current data is deficient with respect to potential viral diseases. Those reported to have occurred in macropods are discussed in Chapter 5 (Section 5.11 Disease and risk assessment). In particular, macropod herpesvirus and macropod orbivirus have previously resulted in disease epidemics in captive and wild macropod populations (Section 5.11). It is recommended that a priority of future research is a focus on key viral pathogens as identified in Section 5.11.

Trypanosomes

A PCR was recently developed at Murdoch University veterinary parasitological department to assay for the presence of *Trypanosoma* spp. in the blood samples of woylies. This complemented the current microscopic survey of blood smears. Blood kept in archives and future samples will be assayed by PCR for the presence of *Trypanosoma* spp.

Transfection Study

A project has been designed to monitor the effect of trypanosomes in endemic populations of woylies and naïve populations. The study will focus on methods of transmission of trypanosomes and the effect on the health of woylies.

Arthropod vectors

Murdoch University and Department of Environment and Conservation will jointly support either a summer school project or honours project to collect and classify insects from the Upper Warren area that could be potential vectors for the haemoparasites or arboviruses.

Epidemiological and ecological data analysis

More detailed analysis of parasite prevalence in relation to multiple infections/co-occurrence, different species of co-habiting host (where possible), geographic location, host demography, host condition, clinical signs of disease in the host and environmental factors operating on the host is recommended (discussed earlier in this chapter). This analysis will use the established large database and will provide valuable epidemiological information regarding these potential pathogens.

Biochemistry

Serum biochemistry provides a broad approach to disease investigations, allowing investigation of organ disease and providing some focus for future investigations. Little information is available on serum biochemistry of the woylie and such data, using serum banks already available, would complement current and future pathogen data and improve identification of the aetiological agents of disease.

5.12.4. Conclusion

A number of potential pathogenic organisms have been identified and investigated through various projects represented by the WDRC, helping to further understand various health and disease aspects of the woylie. From the analyses undertaken so far, a primary pathogenic agent has not been identified that may result in population declines observed in woylies in southwestern Australia. Preliminary data indicates a possible role for *Toxoplasma* and Trypanosomes in declining populations, however this information should be interpreted with caution and further analysis is recommended to investigate these pathogens in more detail. A number of key viral pathogens that could result in the decline patterns observed have not been investigated to date, and it is recommended that studies into these diseases be prioritised in the near future. A number of other research focuses have also been presented and discussed. Other outcomes from the work of the WDRC include establishing reference health information, initial development of a pathologic reference library of woylie diseases, and further development of clinical skills both in the field and in a hospital setting

5.12.2. References

Maxie G. 2007. “The Haematopoietic system” in the “Pathology of domestic animals” Elsevier Saunders