5.11. Identification of potential diseases and risk assessment in relation to recent woylie declines

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Abstract

A qualitative approach has been used in order to assess disease risks related to declines in woylie populations in southwestern Australia. Certain disease agents have been recognised as critical to this risk assessment including: Chlamydiales, Macropod Herpesvirus, Orbivirus, Encephalomyocarditis virus and *Neospora caninum*.

Arrangements for the analysis of these diseases are in progress. The outcomes of this investigation are expected to assist the understanding of the woylie decline dynamics.

5.11.1. Introduction

The role of disease as a contributing or determining factor in wildlife decline or extinction is being increasingly recognized (Spalding and Forrester, 1993; Daszak and Cunningham, 1999; Daszak *et al.,* 2000; Spielman, 2001). In fact, it has been suggested that disease screening should be included in wildlife monitoring program and conservation projects (Spalding and Forrester, 1993; Aguirre *et al.,* 2002).

A significant challenge for this process is the paucity of known information on diseases in wildlife and the almost complete absence of baseline data to determine what is considered as 'normal' (Spalding and Forrester, 1993). Additional complications are generated by financial constraints and practical limitations. Moreover, diagnostic techniques are not always available for the species being investigated and use of tests developed for other species is not always reliable.

Therefore, it is necessary to use a logical and scientifically based process to address these limitations and develop a realistic solution that provides a comprehensive view of this important issue.

Using models proposed in the literature, especially for wildlife translocations, we have followed a systematic approach in order to be able to establish which diseases have to be prioritized in further investigations (Spalding and Forrester, 1993; Jacob-Hoff *et al.,* 2001; Munson and Karesh, 2002; Armstrong *et al.,* 2003).

5.11.2. Methods

As a quantitative evaluation of the disease risk is not achievable due to lack of knowledge of disease prevalence and species susceptibility, a qualitative approach has been used. Jacob-Hoff *et al.* (2001) showed that the qualitative disease risk analysis is a practical and useful tool to achieve our aims. As suggested by Munson and Karen (2002), we took into consideration important diseases reported to have occurred in bettongs and other macropods known to co-habit in the same geographic area. This has been accomplished through a broad literature review and the acquisition of unpublished critical data from the Australian Wildlife Health Network (AWHN).

For the sake of brevity we omitted in this section the pathogens that are currently being investigated within the Woylie Conservation and Research Project (see relevant sections in this document). Once we created a list of diseases that we believed were important and merited

special consideration, we established specific criteria to rank them. Factors that were considered in assessing the importance of a given disease included:

- Species reported to be affected by the disease
- Known presence of the disease (in captive populations and wild populations)
- Mode of transmission
- Outcome of infection (e.g. clinical signs, pathologic lesions)
- Pathogenicity of the disease (on its own accord, in synergy with other pathogens)

A qualitative grading system was applied to these factors to determine the significance of the disease in relation to population declines, and therefore assist in guiding future directions for disease investigations for this program.

5.11.3. Results

The list of selected disease agents considered to affect woylies is summarized in Table 5.11.1-3.

Investigations into haemoparasites, endoparasites, ectoparasites, and *Toxoplasma gondii* are already in place (see relevant sections in this document) and will not be discussed further in this section. Besides, a preliminary screening for Salmonellosis has been carried out (see relevant section in this document). Thus, this agent is no longer mentioned in this section.

Diseases considered to be significant for future investigations in this program include:

- Chlamydiales
- Macropod Herpesvirus
- Macropod Orbivirus
- Encephalomyocarditis virus
- *Neospora caninum*

These pathogens are considered to have potential for rapid and widespread population declines based on the outcomes of this risk assessment process.

Arboviruses have been reported to occur in macropods, although have not been reported to have shown clinical effects of these infections. These viruses are an important potential zoonosis and therefore may also warrant further investigation.

Certain disease agents have been ranked as a low or very low priority because they have not been identified at all or rarely reported to occur in Western Australia. These include: *Clostridium tetani*, *Leptospira interrogans*, *Pseudomonas pseudomallei*, *Coxiella burnetti*, *Rickettsia rickettsii*, *Leishmania* spp, Adenoviruses and 'wobbly possum syndrome'. A number of other pathogens are considered to be opportunistic or secondary infections and unlikely to be responsible for population declines in their own right. These include: *Pseudomonas* spp, *Escherichia coli*, *Klebsiella* spp and *Clostridium piliforme* (Tyzze's disease). Other diseases, such as Lumpy jaw, Mycobacteriosis, and a number of fungal diseases, are considered chronic diseases and would result in different decline patterns and observations than those seen with the current situation.

5.11.4. Discussion

Of the diseases considered important for further investigations through this risk analysis process, Macropod Orbivirus infection would be considered a priority. Infection by Orbiviruses, especially of the Wallal and Warrego serogroups, has been reported in several species of macropods that are closely related to woylies, with serious clinical consequences and an Orbivirus, probably from the Eubanangee serogroup has been associated with a sudden death syndrome in captive tammar wallabies from several research facilities and zoological gardens in NSW and Queensland. (Table 5.11.2.) (Rose *et al.*, 1999, Daszak *et al*., 2000). With the wild populations disease prevalence rates were not able to be determined but the Kangaroo blindness syndrome was noted to be widespread over a large geographical area and with the sudden death syndrome in captive tammar wallabies there was a high disease incidence rate in some collections. Thus, woylies may be considered to be potentially susceptible to Orbivirus pathogens. In its extreme, a severe clinical form, similar to that described for other macropods, could have critical consequences in woylie populations. A number of epidemics of this disease have been reported in wild populations of macropods in Australia between 1969 and 1996, including outbreaks from the South West region of Western Australia (Hooper 1999; Hooper *et al.,* 1999). Hooper *et al.* (1999) also reported a high

seroprevalence for this virus in wild kangaroos and wallabies throughout the region. The virus has been isolated from eye and brain tissues collected from kangaroos near Albany, Esperance and Perth (Hooper *et al.,* 1999). Consequently, it can be concluded that the virus is well established in the region.

Macropod Herpesvirus (MHV) is another viral infection which is known to be present in the region. MHV has resulted in sudden death in captive populations of dorcopsis wallabies, quokkas, western grey kangaroo and woylie (Dickson *et al.,* 1980; Callinan and Kefford, 1981; Wilks *et al.,* 1981). Antibodies have been found in both wild and captive populations of macropods - a study of antibody levels to MHV by Webber and Whalley (1978) indicated a higher prevalence of antibody in captive populations, whilst the highest levels were found in a population of tammar wallabies experiencing an outbreak of MHV. High antibody levels in captive animals may reflect a higher level of virus transmission due to crowding, increased contact rates with infected animals, or to conditions of stress leading to expression of latent virus (Webber and Whalley, 1978). In this regard, the fact that woylie populations started declining when they reached the highest density would suit the ecology of this infection. However, the widespread distribution of antibody levels, as discussed by Webber and Whalley (1978), suggests that this virus has evolved with marsupial species and may be endemic in wild populations. Herpesvirus can, in some instance produce severe acute disease with mortality, especially in young animals and with some herpesviruses (e.g. Aujeszky's disease virus, herpes B virus of macaques, alcephaline herpesvirus 1) after introduction into a new species (Murphy *et al.,* 1999). Dickson *et al.* (1980) reported on an outbreak of MHV infection that resulted in the acute deaths of eight woylies, among other species, over a period of a week. This report indicates that woylies may be highly susceptible to MHV infection but with opportunistic surveillance as conducted with woylie populations, it may be difficult to detect presence of Herpesvirus disease in a population. Clinical symptoms, such as ulcerations or vesicles, may be rare and difficult to detect in field investigations. In this scenario, affected population could undergo a steep decline, with increased mortality after introduction of the virus, similar to what the available monitoring data suggests. Further investigation would initially require examination for serological evidence of MHV infection in woylie serum collection.

Chlamydiales are bacteria known to cause significant disease in other marsupials. In fact, it has been proven that they cause conjunctivitis and proliferation of the eyelid in western barred bandicoot and greater glider (Bodetti *et al.,* 2003), and conjunctivitis, keratitis, cystitis as well as infertility in koalas (Fowler *et al.,* 2003). In addition, Chlamydiales types are known to be present in the region because they have been isolated in a number of species. For example, the bacteria has been isolated from western barred bandicoot and greater bilby from Dryandra, and gilberts' potoroo from Albany (Bodetti *et al.,* 2003). However, the clinical significance in macropods and especially the possible role of this pathogen in the woylie decline is still unclear. The lack of information about this pathogen in the affected woylie populations is a significant gap in determining the importance of these agents.

Encephalomyocarditis virus is a known pathogen in rural areas in Australia and it can occur when rodents build up to plague proportions around piggeries. The disease in pigs is acute with sudden death or acute neurological signs. If woylies are susceptible to this virus, it could have serious consequences for the species. Consequently, we considered that it would be worth testing the woylie groups for exposure to this virus.

Neospora caninum is a protozoa of recent discovery (first identified in 1984) (Bjerkås *et al.,* in Reichel, 2000). It is now described world-wide and isolated from a variety of species including dogs, cattle, sheep, goats, deer and horses (Reichel, 2000). We are not aware of its clinical significance in wildlife but considering the seriousness of the clinical signs (paresis or paralysis in dogs and abortion in cattle), we believe that it would be worthwhile to further investigate the possible prevalence of this infection in woylie populations. The possibility to adapt existing serological tests to woylies is being explored.

Arboviruses comprise mosquito-born viruses including Flaviviruses (Murray Valley Encaphalitis and Kunjin are present in northern WA) and Alphaviruses (Ross River Virus and Barmah Forrest Virus are present in WA). Some of the infections caused by those viruses are important zoonoses and they are worth some special considerations. Macropods have been infected but not shown any evidence of disease with the Flaviviruses or Alphaviruses and from sero-epidemiological studies appear to be possible virus reservoirs (Russell, 2002; Old and Deane, 2005). Although it is not expected that either of these viral genuses would be causing disease problems in woylie, knowing whether this species of macropods is contributing to maintain the virus in the habitat

would have a great interest in terms of human health. Moreover, a positive serologic result could give an indication on the exposure of this species to biological vectors such as mosquitoes.

Two other viruses affecting European rabbits that are present in Western Australia, Rabbit Calicivirus and Myxomatosis virus, were also considered in relation to possible effects on woylies and were discounted. Both Rabbit Calicivirus and Myxomatosis viruses are very host specific; had been evaluated in a wide range of Australian wildlife before use for biological control of European rabbits and since release have not been found to infect animals other than rabbits.

5.11.5. Future work

Contact with Dr Cheryl Johansen, who leads the Arbovirus surveillance project at UWA, Department of Microbiology, has been established and arrangements made for an initial screening of Alphaviruses and Flaviviruses in around 200 woylie serum samples. Should the Woylie Disease Reference Council (WDRC) decide to progress with the investigation of these viruses, the testing should most likely start by the end of 2007.

Currently, there are no virus laboratory facilities available at Murdoch University for Orbivirus and possibly Herpesvirus testing. The opportunity of temporarily using existing facilities for virus neutralisation tests (VNT) for these viruses and/or collaboration with external facilities are being explored.

We are currently seeking more information regarding Chlamydiales infection in macropods and a request has been forwarded to the AWHN. This could give access to unpublished data which may provide critical information and help in deciding whether and which test should be run for this group of pathogens.

We are searching for updated epidemiological data on *Neospora caninum* in livestock and availability of serological tests, in order to discuss this issue in the next WDRC meeting.

The use of population viability analysis (PVA) integrating the results of the ongoing disease investigations has been suggested (Haydon *et al.,* 2002; Miller, 2003) and we believe that it could provide useful information to assist in the identification of the cause(s) of woylie decline as well as support management decision.

5.11.6. Conclusion

A qualitative approach, as suggested by the literature, has been used in order to assess the disease risk for a specific geographic area and for a specific species. We have been able to create a list of known diseases and, according to the available information, prioritise those considered most likely to contribute to patterns of population decline observed in woylies in southwestern Australia. One of the main strengths of this process has been the multidisciplinary collaboration. In fact, the involvement of scientists from different areas of expertise overcame the risk that a personal point of view and/or experience would become the main reasoning of the assessment.

The outcome of this collegial work is that “new” diseases in addition to those that have been already tested during the first phase of the Woylie Conservation Research Project have been highlighted on the basis of the information reported in the literature and recent outcomes of the fieldwork.

These tests may reveal if there are some association between the seroprevalence of the selected viruses in the declining population compared to the healthy population, however, it will still be necessary to detect the viruses in affected animals before a role for the virus can be clearly established. Nevertheless, we believe that this investigation will obtain valuable information in regards of the woylie decline, and also baseline data for future monitoring and management activities for woylies and other sympatric species.

The results of this assessment will be discussed during the next WDRC meetings, along with the information regarding available tests and potential collaborations. The council will come to a common agreement on how to progress in this important area of investigation.

Table 5.11.1. List of selected diseases: bacteria. *Not clearly reported in the literature. ^Not sufficient data available.

	Disease	Lumpy jaw	Mycobacterium spp	Tetanus	Salmonellae	Leptospirosis	Listeria	Clostridium piliforme (Tyzzer's disease)	Pseudomonas pseudomallei (Burkholderia pseudomallei)	Pseudomonas pyocyanea	Pseudomonas spp	Escherichia coli
	Species reported	Macropodoidea	Macropodoidea	Macropodoidea	Macropodoidea	Macropodoidea	Macropodoidea	Possums,dasyurids, Wombat, koala	Tree-kangaroos	Possums, macropods, koala	Macropodoidea	Macropodoidea
Known Presence	**Captive Pop's**	Yes	Yes, including Woylie	*	Yes	Yes	Yes	Yes	*	*	Joeys	Joeys
	Wild Pop's	*	Unknown	*	Yes, but no clinical cases	Yes	Unknown	Brushtail and ringtail possum	Yes	*	*	Unknown
	Transmission			Wounds		Rodents						
	Clinical signs		Abscesses, skin ulcers, dyspnea, neurol.	Convulsions, muscle stiffness death		Uncommon		Sudden death or diarrhoea/anorexia	Melioidosis, nasal discharge		Pneumonias	Pneumonias
	Lesions					Focal interstitial nephritis	Hepatic focal Abscesses	Necrotizing hepatitis and myocarditis	Multifocal abscesses	Smelly liquid in the pouch		
	Comments	Clinical signs would be evident. Unlikely to be responsible of a so rapid decline. Body condition should be poor.	Never noted in free-ranging. Chronic disease	Uncommon in wildlife. More common in northern Australia.	Commonly isolated from macropods.	Uncommon, no clinical disease in wildlife. No identified in WA.		Wild animals from Sydney. Potentially persistent in the soil	More common in northern Australia.	Pouch are regularly checked.	Identified only in cap. animals.	Identified only in cap. animals.
	Reference	Speare *et al.* 1989	Speare *et al.* 1989	Speare *et al.* 1989, Fowler *et al.* 2003	Speare *et al.* 1989	Speare *et al.* 1989	Canfield and Hartley 1992	Fowler *et al.* 2003	Jacob-Hoff 1993, Fowler *et al.* 2003	Fowler *et al.* 2003	Jackson 2003	Jackson 2003
Pathogenic Potential	**Likely Presence**	moderate	moderate	low	moderate/high	very low	low	low	low	low	low	low
	Independent	low	moderate	high	moderate	low	moderate	moderate	low	low	moderate	moderate
	In concert	low	moderate	nil	moderate/high	low	moderate/high	moderate/high	low	low	moderate/high	moderate/high
	Risk / Priority	low	low	low	moderate/high	low	low	low	low	low	low	low

Table 5.11.1. List of selected diseases: bacteria. Cont'd *Not clearly reported in the literature. ^Not sufficient data available.

	Disease	Klebsiella spp	Yersinia pseudotuberculosis	Coxiella burneti	Rickettsia rickettsii	Chlamydiales
	Species reported	Macropodoidea	Possums	Macropodoidea	Macropodoidea	A variety of marsupials
Known Presence	**Captive Pop's**	Joeys	*	*	*	*
	Wild Pop's	*	*	Yes	Yes	Yes
	Transmission		Rodents and birds	Ticks	Ticks	
	Clinical signs	Pneumonias	Diarrhoea			Conjunctivitis, rhinitis, pneumonia, pelvic inflammatory disease, infertility.
	Lesions		Enteritis, septicaemia, hepatic , splenic and renal abscesses			
	Comments	Identified only in cap animals.		No significance in free-ranging, no ill. Queensland and Tasmania	Queensland	Isolated from wild animals from Dryandra (WB Bandicoot) and Albany (Gil.'s Potoroo). Many carried without clinical symptoms.
	Reference	Jackson 2003	Fowler *et al.* 2003	Speare *et al.* 1989	Speare *et al.* 1989	Bodetti *et al.* 2003
Pathogenic Potential	**Likely Presence**	low	low	very low	low	moderate
	Independent	moderate	moderate/high	low	moderate/high	moderate
	In concert	moderate/high	moderate/high	low	high	low-moderate
	Risk / Priority	low	low	very low	low	moderate

Table 5.11.2. List of selected diseases: virus. *Not clearly reported in the literature. ^Not sufficient data available.

	Disease Virus	Macropod Herpesvirus	Macropod Pox Virus	Arbovirus	Ross River Virus	Encephalomyocarditis Virus EMCV	Orbivirus (Wallal and Warrego serogroup)	Orbivirus (Eubenangee serogroup)	Adenovirus	Wobbly possum syndrome, envelope RNA Virus
	Species reported	Macropodoidea; wombats	Macropodoidea	Macropodoidea	Macropodoidea	Tree kangaroo; quokka	Macropodoidea	Tammar wallaby	Macropodoidea	Brush-tail possum in NZ
Known Presence	**Captive Pop's**	Yes	*	*	Yes	Yes, Tree kangaroo	*	*	*	*
	Wild Pop's	Yes. Serologically positive	Yes	Yes. Serologically positive	Yes. Serologically positive	Unknown	Yes	*	Yes, in B. gaimardi	*
	Transmission		Skin, mosquitoes		Mosquitoes	Rodents	Culicoides spp	Culicoides spp		
	Clinical signs	Conjunct., Dyspnea, vesicles and ulceration oral cav., cloacae and genital tract, death. In wallabies cases of reduce reproduction succ. (Finnie 1980)	Resemble papillomas on tail, dorsum, lip and legs		Fever, rash, polyarthritis	Sudden death, dyspnoea	Chorioretinitis, blindness, circling	Death, muscles fasciculations	None	
	Lesions	Multifocal necrosis, intranuclear inclusion bodies in various organs. Liver lesions in Woylie (Canfield and Hartley 1992).	Eosinophilic cytoplasmic inclusion bodies in epithelial cells			Pulmonary congestion and oedema, myocarditis	Nonsupperative panuveitis, retinitis	Oedema hind limbs; Haemorrhage adductors, thorax, dors cervical area and retroperitoneally; Necrosis of lymphoid germinal centres, gastric ulceration and periacinar hepatic necrosis	Enlarge epithelial cells with intranuclear inclusion bodies	
	Comments	No clinical disease reported in wild animals, but presence of sero-positive wild animals. Clinical sings should be evident.		Viraemia but not ill	Human health risk. Macropods seem to be reservoirs		Epidemics in WA (Esperance, Albany, Perth) in 94-96 (Hooper 1999)			
	Reference	Speare *et al.* 1989; Dickson *et al.* 1980; Fowler *et al.* 2003	Speare *et al.* 1989; Fowler *et al.* 2003	Speare *et al.* 1989	Old and Deane 2005	Jackson 2003; Fowler *et al.* 2003	Jackson 2003, Fowler *et al.* 2003	Fowler *et al.* 2003	Speare *et al.* 1989; Fowler *et al.* 2003	Fowler *et al.* 2003
Pathogenic Potential	**Likely Presence**	high	low	moderate/high	moderate/high	moderate/high	moderate/high	moderate/high	low	very low
	Independent	moderate	low	low	low	moderate	moderate	moderate	moderate	moderate
	In concert	moderate/high	low	low	low	moderate	moderate/high	moderate/high	moderate	very low
	Risk / Priority	moderate/high	low	low	low	moderate/high	moderate/high	moderate/high	low	very low

Table 5.11.3. List of selected diseases: fungus, protozoa and toxicosis. *Not clearly reported in the literature. ^Not sufficient data available.

	Disease	Fungus	Candida	Dermatophytosis	Cryptococcus	Protozoa	Neospora caninum	Leishmania	Toxicosis	Fluoroacetate (compound 1080)	Phalaris staggers	Pyrollizadine alkaloids	Sodium deficiency
	Species reported		Macropodoidea	Macropodoidea	Potoroos			Red kangaroos		All. WA animals tolerate high concentration	Macropodoidea	Macropodoidea	Macropodoidea
Known Presence	**Captive Pop's**		Yes	Yes	Yes		Unknown	Yes		Yes	*	*	*
	Wild Pop's		*	Unknown	Yes		Unknown	Unknown		Yes	*	*	*
	Transmission												
	Clinical signs				Respiratory signs and meningitis		Neurologiacal signs in dogs. Abortion in cattle						
	Lesions									No specific lesions detected at autopsy.			
	Comments		Identified only in captive animals	no reported in wild animals			no reported in wild animals	Identified near Darwin.A negative blood smear doesn't allow to rule out Leish.		Macropods (particularly PY) are susceptible. Unlikely: Woylie are resistant up to 100 mg/kg.	Isolated cases	Isolated cases	related to low concentration in soil and vegetation
	Reference		Jackson 2003	Speare *et al.* 1989	Vaughan *et al.* 2005, Vaughan pers com		Reichel 2000	Rose *et al.* 2004, Spratt 2005		King *et al.* 1981, Speare et al. 1989	Speare *et al.* 1989	Speare *et al.* 1989	Speare *et al.* 1989
Pathogenic Potential	**Likely Presence**		high	high	moderate		moderate/high	low		high	low	low	very low
	Independent		low	low	moderate		^	low		very low	low	low	very low
	In concert		low	low/moderate	moderate		^	moderate		low	very low	very low	very low
	Risk / Priority		low	low	low		low	low		very low	low	low	very low

5.11.7. References

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